



Pathological Changes in Gills of Tigris Asp *Leuciscus vorax* (Heckel) Due to Parasitizing with *Paradiplozoon* sp. (Monogenoidea, Oligonchoinea, Diplozoidae) from Al-Gharraf River, Southern Iraq

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ARTICLE INFO

Article History:

Received: May 16th 2025

Accepted: July 3, 2025

Online: July 11, 2025

Keywords:

Freshwater fish,
Ectoparasite,
Iraq,
Tigris River,
Paradiplozoon

ABSTRACT

Tigris asp (*Leuciscus vorax* Heckel), locally known as "Shilik," is a native freshwater fish and an important table species for the Iraqi people. Recently, the population of this species has declined due to poor water quality, reduced water discharge, the presence of invasive species, anthropogenic impacts, and pollution. This study investigates the pathological effects of twin-worm (*Paradiplozoon* sp.) infestations on the gills of *Leuciscus vorax* (Tigris asp) from the Al-Gharraf River in Dhi-Qar Province, Iraq. Fish were collected between October and December 2023. Histopathological analysis revealed significant lesions, including necrosis of secondary lamellar epithelial cells, severe fibrosis in the primary lamellae, and vascular changes such as aneurysms. These lesions disrupted oxygen uptake, causing respiratory distress. Inflammatory cell infiltration, predominantly lymphocytes, was also observed, indicating an immune response to the infestation. The histopathological results showed fusion of gill filaments due to epithelial hyperplasia, which reduced the gill surface area and impaired respiration. Fibrotic changes and abnormal vascular formations further obstructed the respiratory surface, worsening fish health. The study recommends integrating advanced diagnostic methods, such as PCR, immunohistochemistry, and ultrasonography—for early detection and improved understanding of the parasite's lifecycle and host-parasite interactions. These tools, combined with histopathological analysis, can enhance disease detection and help manage parasitic infestations, ensuring fish health and sustainability. This report represents the first study in Iraq on the pathological effects of Diplozoidea members on the gills of freshwater fish. It is also one of the few global reports documenting the impact of this relatively large parasite group on such vital fish tissue.

INTRODUCTION

Tigris asp, or Mesopotamian asp, locally known as 'Shilik', is one of the ancient freshwater fishes whose bones appear in the fossil record (Coad, 2010). It is considered a keystone species distributed in Iran, Iraq, Syria, and Turkey (Çiçek *et al.*, 2024).

Ectoparasitic infestations in both wild and farmed fish populations, whether in freshwater or marine environments, can severely compromise the health of their hosts. These infestations are caused by a variety of organisms, including crustaceans, monogeneans, protozoans, and isopods (Shinn *et al.*, 2015; Modi & Vankara, 2021).

Parasitic disease outbreaks in fishes lead to substantial economic losses through a combination of increased mortality, higher treatment expenses, and a reduction in growth rates (Shinn *et al.*, 2015; Mishra *et al.*, 2017; Assefa & Abunna, 2018; Buchmann, 2022).

Monogenenoids are ectoparasitic organisms that exhibit a direct life cycle and high host specificity (Poulin, 1992).

Gill damage or lesions can arise from chemical, physical, and biological factors, including environmental contaminants and various biological agents. These factors trigger bodily responses such as circulatory issues, proliferative, inflammatory, and degenerative conditions, as well as congestion, telangiectasia, hemorrhage, edema, epithelial and mucous cell hyperplasia, inflammation, and necrosis (Meyers & Hendricks, 1985; Roberts, 2001).

When gill tissue is damaged, the resulting obstruction of water flow reduces the efficiency of oxygen uptake in fishes (Ojha & Hughes, 2001).

Monogenenoids feed on gill blood and do not require the specialized mouthparts found in parasitic copepods; causing damage to the delicate secondary lamellae likely requires significant effort, possibly achieved through suction or the application of digestive secretions (Weli *et al.*, 2017; Mathews *et al.*, 2018).

Parasitic activity also causes hypertrophy of mucus cells, vacuole formation, and venous sinus dilation, likely affecting osmoregulation and exacerbating respiratory and osmoregulatory failure (Rosety-Rodríguez *et al.*, 2002).

Local studies on the pathological effects of monogenoids on fish gills have received little attention. Rahemo and Al-Neemi (1999) reported that *Ancylodiscoides vestulensis* (*Thaparocleidus vestulensis*), attached to the secondary gill filaments of European catfish (*Silurus glanis* L.), induced epithelial cell hyperplasia. Al-Ali (1998) revealed that *Dactylogyrus* spp. infections on the gills of *Cyprinus carpio* L. and *Barbus luteus* (*Carasobarbus luteus*) caused histopathological effects, including an inflammatory response, cellular infiltration, and atrophy. Abbas (2007) recorded histopathological effects due to infection with *Ancylostidoides parasituri* in *Silurus triostegus* Heckel, including hypertrophy of gill lamella tips, fusion of some lamellae, and cell infiltration, in Al-Mashab city, northern Basrah. Al-Shaikh and Mansoor (2013) documented the presence of hyperplasia in the gill filaments of *C. carpio* infested with *Dactylogyrus minutus*. Sadiq (2017) reported a range of pathological changes in the gills of *C. carpio* infected with *Dactylogyrus* sp., including hyperplasia, blood vessel congestion and hemorrhage, disruption and degeneration of gill epithelial cells with focal necrotic areas, and immature granulomatous inflammation accompanied by clubbing and erosion within the gill filaments.

Due to the lack of previous studies on the pathological effects on the gills of fishes caused by Diplozoidae, the present investigation was conducted to enhance our understanding of this group of monogenoids and their impact on the gills of Tigris asp (*L. vorax*).

MATERIALS AND METHODS

Samples of fish were collected by local fishermen during October to December 2023. A total of 37 specimens of *Leuciscus vorax* were obtained from the Al-Gharraf River near Al-Bada'a, northern Dhi-Qar Province, across a 15 km stretch of the study area, between 46°17'45" to 46°11'64" East longitude and 31°44'60" to 31°58'74" North latitude. Fresh or live fishes were kept in cool boxes containing river water with an aerator and transported to the Fish Disease and Parasite Laboratory, Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah. The gills of the fishes were examined for ectoparasites under a dissecting microscope. Diplozoids were excised from both infected and non-infected fishes, rinsed in normal saline, and fixed in 10% buffered formalin for 24 hours. The samples were then dehydrated through ascending concentrations of ethanol, cleared in xylene, embedded in paraffin for sectioning (5 µm), and stained with haematoxylin and eosin (Østevik *et al.*, 2021).

RESULTS AND DISCUSSION

The pathological effects of *Paradiplozoon* sp. on the gills of Tigris asp were evident and included severe destruction of secondary lamellae due to necrosis of their epithelial cells, infiltration of inflammatory cells, particularly lymphocytes, in the affected gills, and severe fibrosis in the primary lamellae. Cartilaginous tissue proliferation was observed, along with irregular blood vessels and aneurysms within the fibrous tissue. Other notable alterations included epithelial hyperplasia, hemorrhaging, congestion of secondary lamellae, thickening and fusion of lamellae, and excessive mucous secretion (Figs. 3, 4).

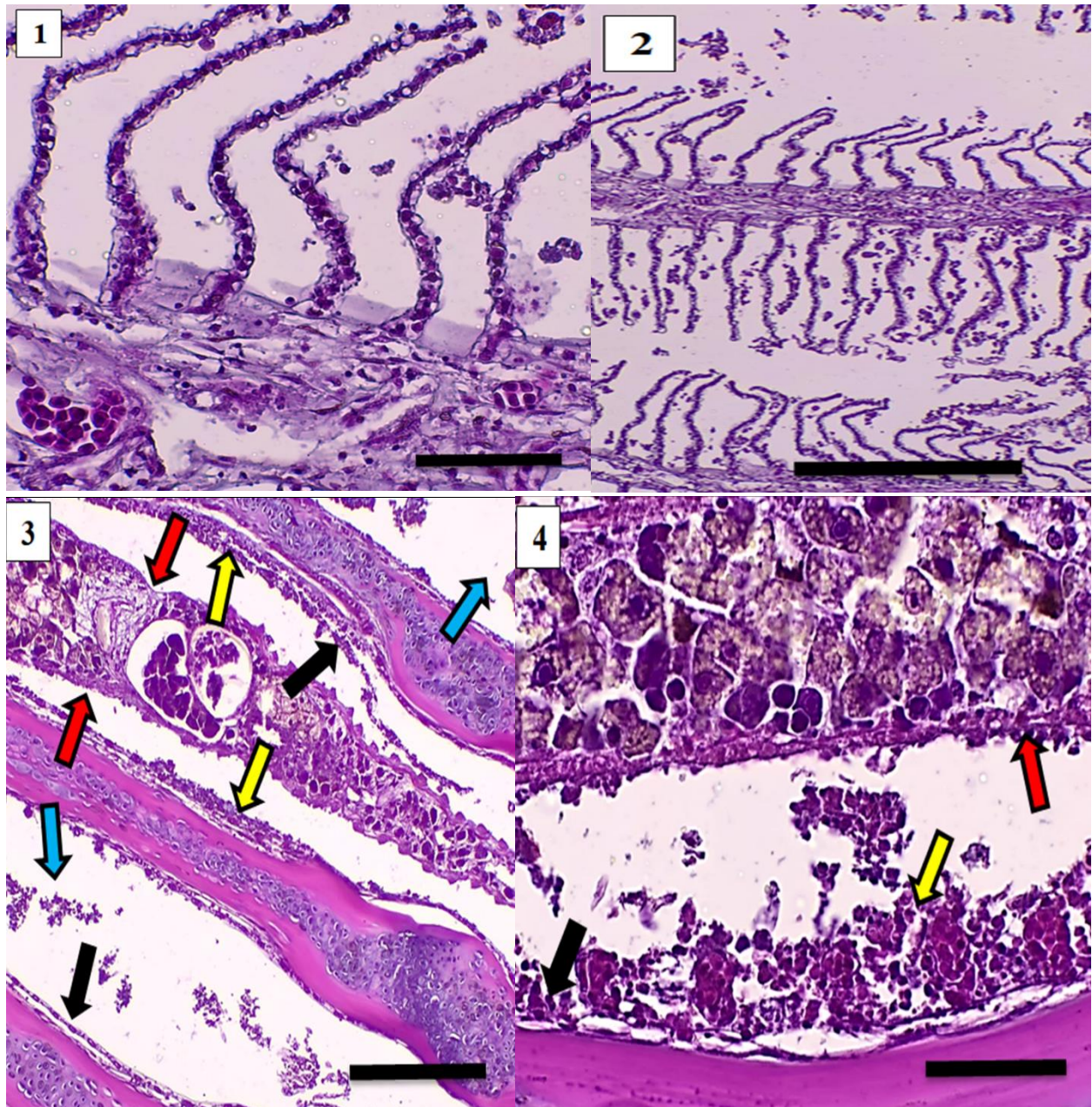
Severe fibrosis was observed in the spaces between affected primary lamellae, where collagenous fibrous tissue was present. This fibrous tissue was also attached to the gill support cartilage, connecting the structures together. Additionally, severe destruction of the epithelial lining of secondary lamellae due to necrosis was evident in the affected areas of the primary lamellae (Figs. 5, 6).

Fibrosis was especially prominent between the damaged primary lamellae, with fibrous tissue attaching to the gill-supporting cartilage. New, irregular blood vessels and aneurysms were observed within the fibrous matrix, which was notably rich in vasculature (Fig. 7).

Hyperplasia of the epithelial cells of secondary lamellae was observed, characterized by multilayering of the cell walls and the presence of inflammatory cells in the affected regions. The parasite itself was located in these damaged areas and showed signs of structural degradation, likely due to the activity of inflammatory cells within its body (Figs. 8, 9).

The pathological changes observed in the gills of fish infected with *Paradiplozoon* sp. provide valuable insight into host–parasite interactions and their detrimental impact on fish health. In this study, the gills of uninfected fish exhibited normal architecture, with intact filaments and no excess mucus secretion (Figs. 1, 2). This finding is consistent with previous work by (Smith,

2019), who reported similar characteristics in healthy fish. In contrast, parasitic infections caused significant histopathological alterations, including gill filament fusion, primarily due to hyperplasia, rapid epithelial cell proliferation, and mucus buildup (Aisyah & Andriani, 2024). These observed changes reflect the gills' physiological response to the stress induced by parasitic invasion.

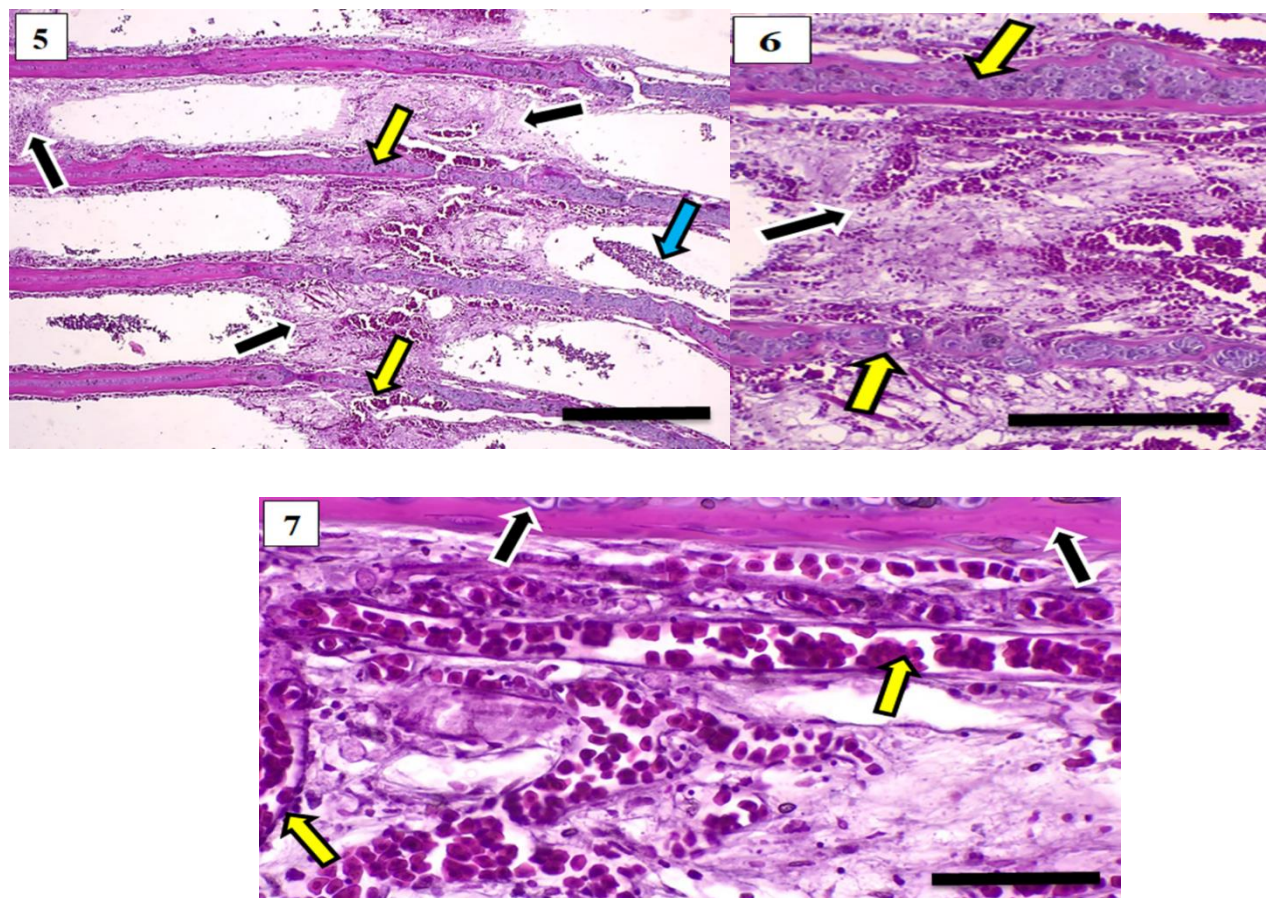


Figs. 1 and 2. Photomicrograph of normal gills. H & E. Scale bar, Fig. (1)= 400 μ m and Fig. (2) = 100 μ m.

Figs. 3 and 4. Photomicrograph of gills of infested Tigris asp with *Paradiplazoon* sp. with different magnification, showing severe destruction of secondary lamellae of due to necrosis of epithelial

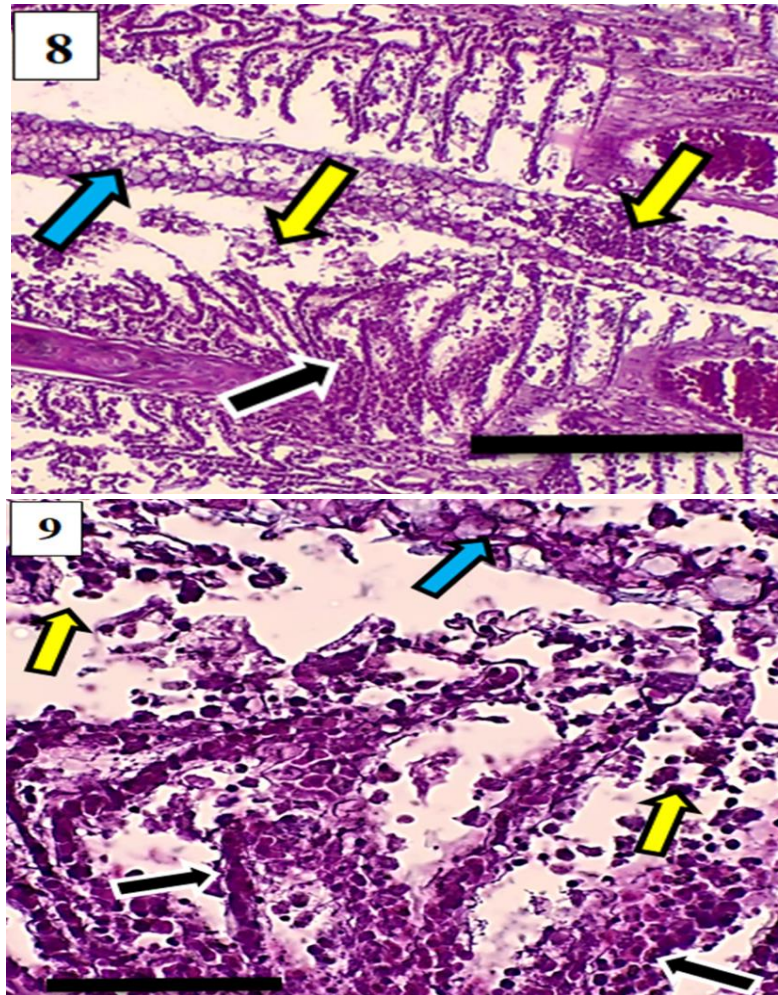
Pathological Changes in Gills of Tigris Asp *Leuciscus vorax* (Heckel) Due to Parasitizing with *Paradiplozoon* sp. (Monogeneoidea, Oligonchoinea, Diplozoidae) from Al-Gharraf River Southern Iraq

cells (black arrow) of these lamellae that caused by pressure produced by the presence of large parasite (red arrow) between primary lamellae spaces. Also, infiltration of inflammatory cells (yellow arrow) especially lymphocytes were observed. However, the severe destruction of secondary lamellae were observed in adjacent primary lamellae (blue arrow) that lied near the affected lamellae lead to fusion in lamellae. H & E. Scale bar, Fig. (1)= 200 μ m and Fig. (2)= 100 μ m.



Figs. 5 and 6. Photomicrograph of gills of *L. vorax* infected with *Paradiplozoon* sp. with different magnification; showing severe fibrosis (black arrow) was observed in the spaces between the affected primary lamellae, where the collagen fibrous tissue observed in primary lamellae, also the fibrous tissue attached in gill support cartilage (yellow arrow), connecting them together. However, the debris of the parasite (blue arrow) was observed adjacent to the fibrous tissue. Also, the severe destruction of epithelial of secondary lamellae due to necrosis of these cells was observed in the affected primary lamellae. H & E. Scale bar, Fig. (3)= 400 μ m and Fig. (4)= 200 μ m.

Fig. 7. Photomicrograph of gills of *L. vorax* infected with *Paradiplozoon* sp., showing severe fibrosis was observed in the spaces between the affected primary lamellae. Note the fibrous tissue attached in gill supporting cartilage (black arrow). Also, the new irregular blood vessels and aneurism (yellow arrow) were observed within the fibrous tissue, where the fibrous tissue was rich in blood vessels. H & E. Scale bar =100 μ m.



Figs. 8 & 9. Photomicrograph of gills of *L. vorax* infected with *Paradiplozoon* sp. with different magnification,, showing hyperplasia of epithelial cells (black arrow) of secondary lamellae, where the multi-layers of cell wall of secondary lamellae and presence of inflammatory cells (yellow arrow) in the affected secondary lamellae. Also, the parasite was observed in the affected area, where the parasite (blue arrow) was destroyed by activity of inflammatory cells (yellow arrow) inside the parasite. H & E. Scale bar, Fig. 6 =200 μ m and Fig. 7=100 μ m.

Analysis of gill histopathology showed that the parasite uses specialized suckers to clamp onto the gill lamellae. This allows it to absorb nutrients while simultaneously exacerbating structural damage to the gill (Alsafy *et al.*, 2025). This finding corroborates earlier studies that documented similar abnormalities in parasitized fish, including secondary filament hyperplasia, filament fusion, and aneurysms. Such fusions severely impair gill function, limiting the fish's ability to breathe and absorb oxygen, ultimately compromising its health and survival.

In *Tigris asp*, infection with *Paradiplozoon* sp. was associated with Epitheliocystis, a prominent disease characterized by epithelial hyperplasia and excessive mucus production. In severe cases, this condition causes diffuse hyperplasia, lamellar fusion, and overproduction of mucus, all of which disrupt normal gill function (Blandford *et al.*, 2018). These findings confirm

the critical importance of healthy gills for fish well-being and reveal the significant long-term consequences parasitic infections can have on fish health and aquaculture productivity.

Fish infested with *Dactylogyrus* often exhibit symptoms such as unilateral swimming behavior, lethargy, and erosion of gill filaments (**Moyses *et al.*, 2015**). The current study supports these findings, with histopathological examinations confirming secondary filament hyperplasia and filament fusion—hallmark features of *Dactylogyrus* infestation. (**Paul & Sahoo, 2024**) also reinforced these observations, highlighting the host specificity of *Dactylogyrus* species and recommending several control strategies for managing these infections in aquaculture systems.

Histopathological observations in the present study included epithelial hyperplasia, lamellar fusion, purulent bronchitis, and the presence of aneurysms. These results are consistent with pathological alterations previously documented in parasitized fish. Severe stress induced by parasitic infections can lead to vascular changes in infected fish. Damage to pillar cells promotes increased blood flow within the lamellae, resulting in congestion or aneurysm formation (**Rosety-Rodriguez *et al.*, 2002**). These vascular disturbances further compromise gill function and overall fish health.

The epithelial hyperplasia and fusion of secondary lamellae observed in infected gills appear to serve as adaptive defenses against parasitic invasion. These responses aim to minimize the immediate effects of the parasite and preserve gill function. However, as (**Woo & Buchmann, 2012**) noted, although these changes may provide short-term protection, they often result in ongoing structural damage that eventually impairs the fish's capacity to withstand prolonged parasitic stress.

In conclusion, the pathological alterations observed in the gills of fish infected by parasites reflect the complex interaction between host and parasite. Although the gill mounts defense mechanisms to counter parasitic stress, the resulting damage can significantly impact fish health. This highlights the importance of implementing effective control measures in aquaculture to mitigate the detrimental effects of parasitic infections.

Epitheliocystis, a condition frequently observed in various teleost fish species, is often detected incidentally. However, in cases of hyperinfection, it can become severe, characterized by intense parasitic proliferation and a robust immune response that results in diffuse epithelial hyperplasia, branchial lamellar fusion, and excessive mucus production (**Blandford *et al.*, 2018; Smith, 2019; Zhou *et al.*, 2025**). Infestation by *Dactylogyrus* species also causes notable gill damage, such as epithelial hyperplasia, aneurysm formation, and necrosis of secondary lamellae. These pathological effects compromise respiration and may result in suffocation (**Paul & Sahoo, 2024**).

Monogenean infestation can lead to severe histopathological damage in fish gills. A study by (**Elbakoosh, 2023**) observed that infestation by *Gotocotyla secunda* resulted in necrosis, erosion of gill filaments, and reduction of the respiratory surface area in *Scomberomorus commerson*. These effects significantly impair the fish's respiratory efficiency, potentially leading to serious health issues or mortality. Monogenean flukes attach to the gill epithelium using specialized clamps, which often cause lamellar clubbing and direct damage to the epithelium and

underlying blood vessels. Consequently, the host exhibits epithelial proliferation and fusion of secondary lamellae, further impeding gill function (Suliman *et al.*, 2021).

Parasitic infestations induce both physiological and mechanical alterations, including enhanced cellular proliferation, immunosuppression, growth abnormalities, and behavioral changes (Igeh & Avenant-Oldewage, 2020). They also cause mechanical damage to the gills, such as lamellar fusion, desquamation of epithelial cells, hypertrophy, and in some cases, tissue replacement. The penetration of monogenean hooks leads to localized hemorrhage, necrotic lesions, and shedding of the lamellar epithelium, as reported in *S. commerson* (El-lamie *et al.*, 2022). Histopathological analysis in this study revealed marked lymphocyte infiltration, lamellar clubbing, epithelial sloughing, and hypertrophy—particularly at the lamellar tips. These immune responses, including macrophage and mucous globule infiltration, are essential in combating parasitic infections (Buchmann & Lindenstrøm, 2002) and were similarly observed in the current study (Figs. 8 and 9).

Further analysis revealed extensive monogenean-induced damage, including lamellar desquamation, partial to complete fusion, and significant lymphocytic infiltration. These findings align with those reported by (Adawy *et al.*, 2016), who documented epithelial hyperplasia, inflammation, hemorrhaging, and gill tissue destruction due to monogenean attachment. Ultrastructural analysis showed advanced gill damage, such as complete loss of secondary lamellae and desquamation, accompanied by pronounced immune activity.

Infections by *Dactylogyrus* also led to epithelial hyperplasia, partial lamellar fusion, telangiectasia, goblet cell hyperplasia, destruction of gill lamellae, and hemorrhaging (Urdes & Hangan, 2023). Lastly, *Paradiplozoon* infestations caused pronounced epithelial hyperplasia and fibrotic tissue development at parasite attachment sites, leading to blockage of gill filaments and impaired respiratory function (Purivirojkul, 2012). This fibrosis reduces oxygen uptake, causing hypoxia and threatening fish survival, especially under intensive aquaculture conditions (Bennett & Bennett, 2001; Overstreet & Hawkins, 2017).

CONCLUSION

The present study reveals that deep infestation by *Paradiplozoon* sp. inflicts significant damage on the gills of *Leuciscus vorax*. The observed pathological changes, such as necrosis, epithelial hyperplasia, fibrosis, and vascular alterations, underscore the complex nature of the host–parasite relationship. These lesions severely impair gill function, which is critical for respiration and overall fish health. As a result, infected fish become more susceptible to secondary infections, ultimately jeopardizing their survival. This highlights the critical need for effective management of parasitic infestations in both wild and farmed fish populations.

To advance our understanding of this parasite, future research should employ molecular and immunological tools such as PCR, immunohistochemistry, and serological assays to elucidate the parasite's lifecycle, mechanisms of host interaction, and immune responses. Additionally, ultrasonography offers a promising method for the early detection of gill lesions. By integrating these advanced diagnostic techniques with traditional histopathological analysis, we can improve

disease detection and management strategies, ultimately safeguarding fish health in both natural and aquaculture settings.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Dr. Ali A. Al-Darwesh and Dr. Hutheyfa A. H. Al-Salih from the College of Veterinary Medicine, University of Kufa, for their valuable assistance and insightful advice throughout the course of this study.

Ethical approval

All ethical guidelines concerning the care and handling of fish, as issued by national and international organizations, were strictly followed in this study.

Conflict of interest

The authors declare that they have no conflict of interest.

Contributions of authors

M.H.K.A., Collect the fishes, wrote the draft of manuscript and editing the manuscript.
A.H.A, propose the subject, confirm identification of the parasites, editing the manuscript.

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