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An Investigation of Transboundary and Emerging Diseases in the Ornamental Fisheries Sector of Punjab, India

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ABSTRACT

The study, conducted from September, 2022 to April, 2023, investigated the prevalence of important aquatic pathogens among ornamental fish maintained and traded across selected districts of Punjab. The ornamental fish trade involves the frequent movement of live aquatic organisms across state and international borders, making it a potential pathway for the introduction and spread of exotic pests and diseases. This highlights the need for robust biosecurity measures within border control programs. To enable early and accurate pathogen detection, both DNA and RNA extraction techniques followed by molecular diagnostic assays were employed to strengthen biosecurity protocols and help safeguard the health of traded ornamental fish. A total of 106 ornamental fish samples were screened using these molecular tools, and all samples tested negative for major notifiable RNA and DNA pathogens. However, certain opportunistic infections were recorded, including bacterial infections caused by *Aeromonas hydrophila* and *Citrobacter freundii*, fungal infections such as mouth fungus and saprolegniasis, and parasitic infestations by *Lernaea cyprinacea*, occurring mainly during the winter and post-winter months and indicating seasonal susceptibility, likely linked to temperature-related stress. Overall, the findings indicate that Punjab remained free of major exotic pathogens during the study period, with ornamental fish brought in from other regions generally appearing healthy, displaying vibrant colouration, active behaviour, and high survival rates. Consequently, the ornamental fish trade in Punjab continued to grow, creating economic opportunities for breeders and traders while increasing public interest in fishkeeping across the state.

KEYWORDS: Biosecurity, ornamental fish, aquatic pathogens, disease surveillance, transboundary risk

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1. INTRODUCTION

Ornamental fishes, often called "living jewels", are valued for their diverse colouration, attractive shapes, and unique behaviours, and are kept in aquaria and garden pools for aesthetic appeal and recreational enjoyment. Over time, fishkeeping has become a globally popular hobby, further enriched by aquascaping and underwater photography (Laskar et al., 2016). Technological advancements, including underwater imaging systems, automated filtration units, and demand feeders, have improved the efficiency and appeal of fish maintenance, increasing consumer interest (Cooke et al., 2021). Consequently, the ornamental fish industry has expanded significantly, with global trade estimated at approximately US$ 18–20 billion in 2020 (Singh et al., 2023), and projected to continue growing at a compound annual rate of nearly 8% over the coming decade (Sharma et al., 2025), driven by rising demand from hobbyists, public aquaria, and research institutions worldwide. Within this global framework, India is rich in aquatic biodiversity and serves as an important reservoir of ornamental fish species (Chaudhari et al., 2023). The country hosts a variety of indigenous species, including guppies, tetras, and barbs, that are highly valued in both domestic and international markets (Khairnar and Kaur, 2021). India also imports over 300 ornamental fish species from leading exporting countries across Asia, as well as from the United States and the United Kingdom (Girisha et al., 2021), making it a significant contributor to and participant in the global ornamental fish trade, although its export share remains relatively modest compared with leading exporting nations (Parappurathu et al., 2021).The ornamental fish trade involves frequent movement of live organisms across geographical boundaries, creating pathways for infectious agents. Numerous studies indicate that this trade can transport harmful microorganisms-including bacteria, fungi, parasites, and viruses-that may affect cultured stocks and threaten native aquatic biodiversity (Rahmati et al., 2023; Tripathi et al., 2025). Such trade-linked pathogens are increasingly recognised as posing a global zoonotic risk (Syanya et al., 2025). The release of infected ornamental fish into natural water bodies can establish disease in wild populations, causing ecological imbalance and significant economic losses in fisheries and aquaculture. While trade facilitates species diversification and market growth, it simultaneously increases the risk of transboundary pathogen movement. Therefore, disease surveillance and early pathogen detection are critical components of risk management in the ornamental fish trade (Srinath and Uma, 2024; Kalaria et al., 2024) Punjab has rapidly emerged as a key centre for the ornamental fish trade in India over the past decade, primarily sourcing freshwater species from coastal states such as West Bengal and Tamil Nadu. This interstate movement heightens the risk of introducing exotic pathogens into the region. Growing public interest in aquascaping has further stimulated the local market (Khairnar and Kaur, 2018). The Guru Angad Dev Veterinary and Animal Sciences University has played a pivotal role in advancing the sector by promoting important species and best management practices through farmer awareness programs and stakeholder engagements, thereby enhancing production efficiency and regional competitiveness. Presently, over 150 aquarium retail outlets operate across the state, primarily in Ludhiana, Mohali, Jalandhar, and Amritsar (Singh et al., 2026). However, this rapid expansion, coupled with increased movement of ornamental fishes, elevates the risk of introducing and spreading exotic pathogens, posing potential threats to native aquatic fauna and ecosystem health. Therefore, this study was undertaken to screen for important aquatic pathogens in ornamental fishes and generate baseline information on disease prevalence in selected districts of Punjab.

2. MATERIALS AND METHODS

2.1. Study area and surveillance program

The present study was conducted between September, 2022 and April, 2023 to screen aquatic pathogens in ornamental fishes using samples collected from selected outlets in Punjab, India, covering four geographical zones: Malwa (Ludhiana), Doaba (Jalandhar), Majha (Amritsar), and Powadh (Mohali) (Figure 1A-B). This study was part of a broader disease surveillance project implemented by the Department of Fisheries under the Pradhan Mantri Matsya Sampada Yojana, aimed at monitoring disease outbreaks in aquaculture.

2.2 . Selection of sampling sites and fish collection

A total of 20 ornamental fish outlets were selected from four districts of Punjab. In total, 106 dead ornamental fish samples, including goldfish and koi carp, were collected and transported to the laboratory. These samples showed clinical signs of gill discolouration, skin haemorrhages, and fin necrosis. The suspected fish species include goldfish, widow tetra, koi carp, rosy barb, tin foil barb, telescopic eye, gourami, iridescent shark, and angelfish (Table 1).

2.3. Preservation of samples

Collected fish samples were transported to the laboratory under controlled conditions. Upon arrival, suspected ornamental fish samples were aseptically dissected, and organs, including the kidney, gills, and tissues, were collected. These samples were preserved in sterile tubes and stored at −80°C for subsequent molecular analysis.

2.4. Parasitological examination

Parasitological investigations were performed using skin scraping and fin clipping techniques. The infected fish

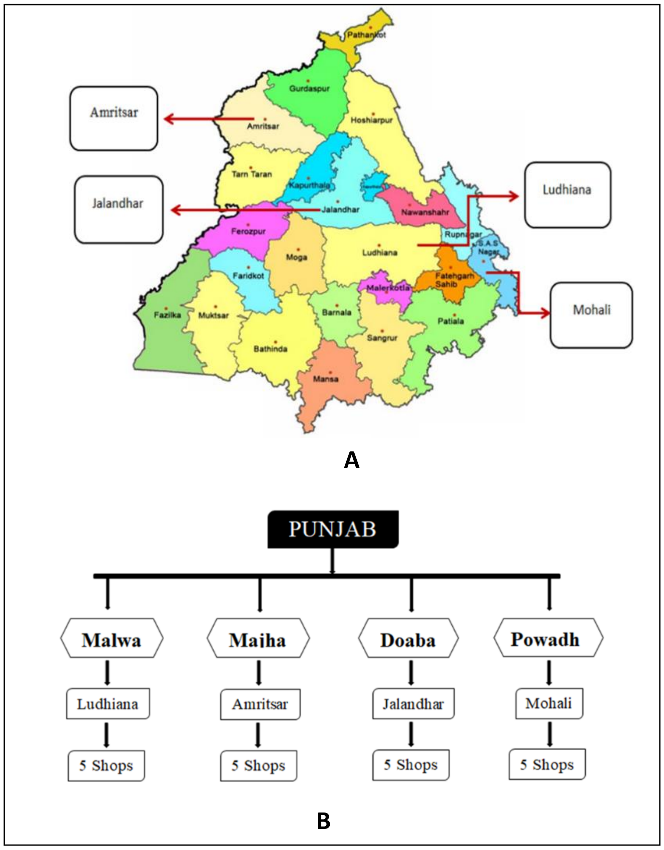

Figure 1: Location of study (A) and number of outlets from selected districts (B)

exhibited abnormal external signs, including excessive mucus secretion, respiratory distress, and sluggish movement. The parasites were transferred to grease-free slides and then air-dried using sterilized forceps. The slides were then observed under a LEICA DM 3000 LED microscope to identify parasites without staining.

2.5. Genomic DNA and RNA extraction

Total Genomic DNA was isolated using the Hipura® mammalian genomic DNA purification kit (HIMEDIA, India). RNA extraction from samples was performed using the TRIzol-based method (Adel et al., 2021). Subsequently, complementary DNA (cDNA) was synthesized using the Hi-c DNA synthesis kit (Thermo Fisher Scientific, India).

2.6. Pathogen identification using MALDI-TOF-MS

The isolated pathogen was also confirmed using a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS). The analysis was conducted at the Department of Veterinary Microbiology, College of Veterinary Sciences, GADVASU, Ludhiana.

2.7. PCR amplification and agarose gel electrophoretic analysis of pathogens

PCR assays were carried out to screen the infected fish samples for various DNA and RNA of OIE-listed pathogens using specific primers to confirm the causative agents. The details of primers and cycling conditions used in this study are given in Table 2. The PCR assay for detecting

Table 1: Details of fish samples collected for screening of pathogen by PCR assay

Place of sampling	Sampling period	Suspected fish species collected	No. of ornamental fish outlets sampled	No. of ornamental fish samples screened	Pathogens* screened by PCR	Pathogens detected (No. of positive samples)
Ludhiana	September-November and January-March	Gold fish, Widow tetra, Milky carp, Rosy barb, koi carp, Red cap fish	5	30	SVCV, TLV, EUS, RSIV, ISKNV, KHV, *A. hydrophila*	*A. hydrophila* (14)
Amritsar	September-November and January-March	Koi carp, Gold fish, Tiger fish, Gourami, Widow tetra, Angel fish, Freshwater shark	5	22	SVCV, TLV, EUS, RSIV, ISKNV, KHV, *A. hydrophila*	Negative
Jalandhar	September-November and January-March	Telescopic eye, Freshwater shark, Tin foil barb, Gourami, Koi carp, Gold fish	5	28	SVCV, TLV, EUS, RSIV, ISKNV, KHV, *A. hydrophila*	Negative
Mohali	September-November and January-March	Rosy barb, Koi carp, Widow tetra, Black widow, Cichlids, Telescopic eye	5	26	SVCV, TLV, EUS, RSIV, ISKNV, KHV, *A. hydrophila*	Negative

SVCV: Spring viremia of carp virus; TLV: Tilapia lake virus; EUS: Epizootic ulcerative syndrome; RSIV: Red sea bream iridovirus; ISKNV: Infectious spleen kidney and necrosis virus; KHV: Koi herpes virus

Table 2: Details of primers and cycling conditions used in this study

Sl. No.	Pathogen/ Disease	Primer	Sequence (5'-3')	Cycling conditions					References
				Denaturation	Annealing	Extension	No. of cycles	Product (bp)	
1.	Koi herpes virus	TK-F TK-R	GGG-TTA-CCT-GTA-CGA-G CAC-CCA-GTA-GAT-TAT-GC	95°C for 30 seconds	52°C for 30 seconds	72°C for 1 min	35	409 bp	Mawardi et al. (2019)
2.	*Aphanomyces invadans*	B073F B0638R	CTT-GTG-CTG-AGC-TCA-CAC-TC ACA-CCA-GAT-TAC-ACT-ATC-TC	95°C for 30 seconds	56°C for 45 seconds	72°C for 2 min 30 seconds	34	564 bp	Ho et al. (2023)
3.	Spring viremia of carp virus	SVCV-F1 SVCV-R2	TCT-TGG-AGC-CAA-ATA-GCT-CAR-RTC AGA-TGG-TAT-GGA-CCC-CAA-TAC-ATH-ACN-CAY	95°C for 1 min	55°C for 1 min	72°C for 1 min	35	714 bp	Park et al. (2025)
4.	Tilapia lake virus	NE1 NE2	TAT-GCA-GTA-CTT-TCC-CTG-CC TTG-CTC-TGA-GCA-AGA-GTA-CC	94°C for 40 seconds	58°C for 40 seconds	72°C for 40 seconds	30	491 bp	Kembou Tsofack et al. (2017)
5.	Red sea bream iridovirus	4-F 4-R	CGG-GGG-CAA-TGA-CGA-CTA-CA CCG-CCT-GTG-CCT-TTT-CTG-GA	94°C for 30 seconds	58°C for 1 min	72°C for 1 min	30	568 bp	Kim et al. (2024)
6.	Infectious spleen and kidney virus	1F 1R	CTC-AAA-CAC-TCT-GGC-TCA-TC GCA-CCA-ACA-CAT-CTC-CTA-TC	94°C for 30 seconds	58°C for 1 min	72°C for 1 min	30	570 bp	Kim et al. (2024)
7.	*A. hydrophila*	AHF3A AHF3B	ATC-AAT-GGT-ACC-GAG-CGG-GTA CGA-AGA-AGG-TGG-CCA-GAA-TC	95°C for 30 seconds	65°C for 30 seconds	72°C for 2 min 30 seconds	34	264 bp	He et al. (2025)

SVCV, TLV, EUS, RSIV, ISKNV, KHV, and *A. hydrophila* was performed separately using a common PCR program. PCR products were analyzed by electrophoresis using 0.8% agarose gel stained with ethidium bromide. The amplified products were visualized under ultraviolet transillumination and documented for further interpretation.

3. RESULTS AND DISCUSSION

3.1. Collection and clinical observations of suspected ornamental fish

During the surveillance period, suspected ornamental fish samples were collected from selected outlets across Punjab, India, covering four geographical zones: Malwa (Ludhiana), Doaba (Jalandhar), Majha (Amritsar), and Powadh (Mohali). The highest number of disease-suspected ornamental fishes, including koi carp, goldfish, widow tetras, and silver barbs, was recorded in December, 2022. Clinically, infected koi carp exhibited ulceration of the body surface with scale loss, fin necrosis, haemorrhages, and gill discolouration. Behavioural abnormalities, including lethargy, surface swimming, loss of equilibrium, and disorientation were also observed at multiple outlets. Representative clinical signs observed during the study are presented in Figure 2A–D. These symptoms were consistent with previously reported disease conditions in ornamental fishes (Badhusha et al., 2022), necessitating molecular confirmation.

3.2. Molecular screening of viral pathogens

All suspected samples were screened for major DNA viruses (RSIV, ISKNV, and KHV) and RNA viruses (SVCV and

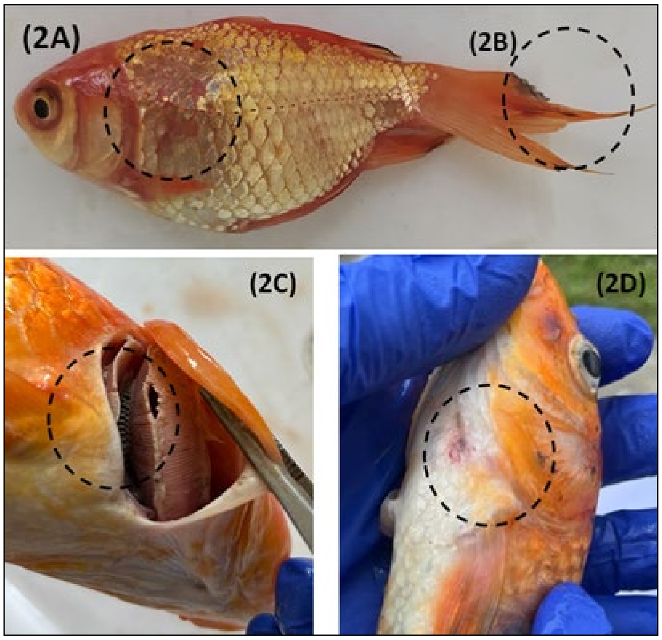

Figure 2: A: Loss of scales; B: Necrosis of fins; C: Discolouration of gills; D: Haemorrhage on body surface

TiLV) using PCR with pathogen-specific primers. The amplification profiles for DNA viruses are presented in Figure 3A–C, while RNA virus screening results are shown in Figure 4A–B. All samples tested negative for the targeted viral pathogens. Previous studies reported ISKNV infection in commonly traded ornamental fish species (Girisha et al., 2021); however, no such infection was detected in the present study. Similarly, although clinical signs resembling KHV infection were observed, PCR results confirmed the absence of KHV. Earlier reports documented KHV outbreaks in Indian koi carp with high mortality (Badhusha et al., 2022), emphasizing the importance of molecular

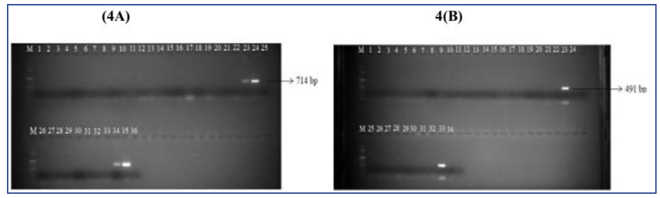

Figure 4: Detection of SVCV-lane M: 100 bp DNA ladder; Lane 1-22: -ve samples; Lane 23 and 24: +ve controls; Lane 25: -ve control; Lane M: 100 bp DNA ladder; Lane 26-33: -ve samples; Lane 34 and 35: +ve control; Lane 36: -ve control (A) TLV-M: 100 bp DNA ladder; Lane 1-22: -ve samples; Lane 23: +ve controls; Lane 24: -ve control; Lane M: 100 bp DNA ladder; Lane 26-32: -ve samples; Lane 33: +ve control; Lane 34: -ve control (B)

diagnostics. None of the samples tested positive for RSIV, despite its occurrence in cultured Asian seabass (*Lates calcarifer*) with significant mortality (Sumithra et al., 2022). Seasonal stress plays a critical role in viral disease outbreaks. During winter, low temperatures increase susceptibility to infections such as SVCV (Hu, 2024), which has been associated with economic losses (Souto et al., 2024). However, winter-collected samples in the present study tested negative for SVCV. Conversely, tilapia lake virus (TiLV), typically associated with higher temperatures (22–32°C), affects vital organs such as the brain, liver, and eyes (Aich et al., 2022). Despite sampling during summer, all samples tested negative for TiLV, indicating the absence of major transboundary viral pathogens in the region during the study period.

3.3. Bacterial infections

Despite negative viral screening, bacterial infections were detected. *Aeromonas hydrophila* infection was confirmed in koi carp (*Cyprinus carpio koi*) and goldfish (*Carassius*

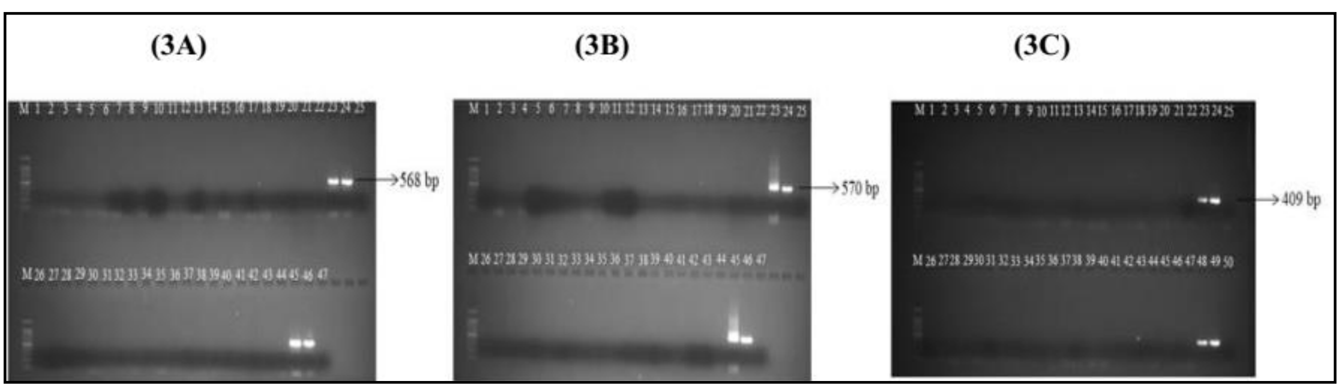

Figure 3: Detection of RSIV-Lane M: 100 bp DNA ladder; Lane 1-22: -ve samples; Lane 23 and 24: +ve controls; Lane 25: -ve control; Lane M: 100 bp DNA ladder; Lane 26-44: -ve samples; Lane 45 and 46: +ve controls; Lane 47: -ve control (A) ISKNV-Lane M: 100 bp DNA ladder; Lane 1-22: -ve samples; Lane 23 and 24: +ve controls; Lane 25: -ve control; Lane M: 100 bp DNA ladder; Lane 26-44: -ve samples; Lane 45 and 46: +ve controls; Lane 47: -ve control (B) KHV-Lane M: 100 bp DNA ladder; Lane 1-22: -ve samples; Lane 23 and 24: +ve controls; Lane 25: -ve control; Lane M: 100 bp DNA ladder; Lane 26-47: -ve samples; Lane 48 and 49: +ve controls; Lane 50: -ve control (C)

auratus), presenting with haemorrhagic lesions, ulcerations, scale loss, tail rot, and abdominal dropsy (Figure 2A–D). PCR amplification showing a specific 264 bp product confirmed infection in 14 samples, as illustrated in Figure 5A. Additionally, *Citrobacter freundii*, an opportunistic pathogen, was detected in goldfish from Ludhiana. Environmental stress is a major contributing factor to bacterial disease outbreaks (Cardoso et al., 2021). Factors such as poor water quality, handling stress, and overcrowding likely contributed to these infections.

3.4. Fungal infections

Fungal infections were predominantly observed during the winter months (November, 2022 to February, 2023). Mouth fungus caused by *Chondrococcus columnaris* was characterised by white cotton-like growth around the mouth, while saprolegniasis caused extensive cotton-like growth on the skin and fins. These infections were often associated with secondary complications due to parasitic infestations or bacterial infections. All samples were screened for Epizootic Ulcerative Syndrome (EUS), and PCR results confirmed the absence of the pathogen, as shown in Figure 5B.

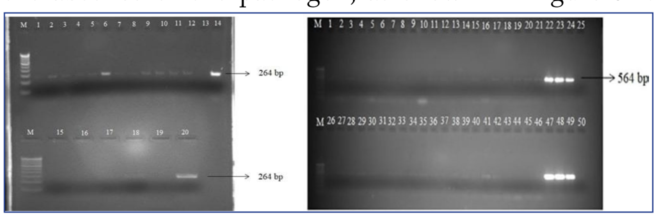

Figure 5: Detection of *A. hydrophila*-Lane M: 100 bp DNA Ladder; Lane 2-12: +ve sample; Lane 13:-ve control; Lane14: +ve control; Lane M: 100 bp DNA ladder; Lane 15-18: -ve samples; Lane 19: -ve control; Lane 20: +ve control (A); Detection of EUS-Lane M: 100 bp DNA ladder; Lane 1-21 -ve samples; Lane 22; 23 and 24: +ve controls; Lane 25: -ve control; Lane M: 100 bp DNA ladder; Lane 26-46: -ve samples; Lane 47; 48 and 49: +ve controls; Lane 50: -ve control (B)

Although no EUS cases were detected, previous reports have documented its occurrence in Indian estuarine systems (Sumithra et al., 2020). The absence of EUS in this study is encouraging but warrants continued surveillance.

3.5. Parasitic infestations

Parasitic infestations, particularly *Lernaea cyprinacea*, were more prevalent in late winter (February–March 2023). They were observed in black molly (*Poecilia sphenops*) and swordtail (*Xiphophorus helleri*) of the Poecillidae family, and in goldfish (*Carassius auratus*) and koi carp (*Cyprinus carpio*) of the Cyprinidae family, across all trading outlets in Punjab (Table 3). These infestations adversely affected fish health and aesthetic value (Figure 6A–C). Fish exposed to environmental stressors such as crowding, poor nutrition, and fluctuating water quality are more susceptible to parasitic infections (Ahmed et al., 2020). Parasites can disrupt physiological balance and increase mortality risk (Sivasankar et al., 2025). Treatment included antiparasitic drugs (Paracidol-FW, Paracide-F, Parasite-S), physical removal and chemical treatments (formalin and potassium permanganate). These interventions were effective in controlling infestations.

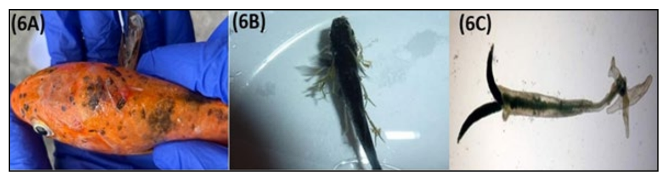

Figure 6. Infected koi carp (A) and black molly (B) with *Lernea cyprinacea* (C)

3.6. Implications for biosecurity and disease management

The findings indicated that the ornamental fish sector in Punjab remained free of major emerging and transboundary viral diseases during the study period. However, the occurrence of bacterial, fungal, and parasitic infections highlighted the influence of environmental stress and management practices on fish health. These observations underscore the importance of preventive biosecurity measures, including quarantining newly introduced fish, maintaining optimal water quality, and conducting routine health monitoring. Strengthening awareness and training programs for ornamental fish traders is essential to improve disease management practices and support sustainable industry growth.

Table 3: Prevalence of parasite infection in ornamental fish in selected districts of Punjab

Sl. No.	Districts	No. of suspected ornamental fish samples	No. of infected fish samples				Total No. of infected fish	Prevalence (%)
			Gold fish	Koi carp	Molly	Sword tail		
1.	Ludhiana	30	2	4	5	4	15	50.00
2.	Amritsar	22	-	2	-	-	02	9.09
3.	Jalandhar	28	3	5	-	-	08	28.57
4.	Mohali	26	1	3	-	-	04	15.38

4. CONCLUSION

The present study provided baseline evidence that major OIE-listed viral pathogens were absent from ornamental fish traded in Punjab. Nonetheless, the detection of bacterial, fungal, and parasitic infections underscores the need for continuous surveillance and improved management practices. Strengthening biosecurity frameworks and stakeholder awareness will be critical to safeguarding aquatic health and ensuring the long-term sustainability of the ornamental fish industry.

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