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Integrated Diagnostic Methodology for Identifying Bacterial, Parasitic and Fungal Pathogens in Goldfish (*Carassius auratus*)

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A B S T R A C T

The ornamental fish trade has experienced rapid global growth, with goldfish (*Carassius auratus*) being one of the most widely traded species. However, high-density stocking, poor water quality, and transportation stress often predispose ornamental fishes to various infectious diseases, threatening their health and economic value. In this study, 65 naturally infected goldfish were collected from multiple aquarium shops in Aurangabad, Maharashtra, and examined using a multi-tiered diagnostic protocol. Parasitological investigations included wet mount examinations of gills, skin scrapings, and blood smears, which revealed the presence of common ectoparasites and Endoparasites. Bacteriological studies, including Gram staining, acid-fast staining, and biochemical assays (oxidase, catalase, TSI, citrate, motility, and indole tests), led to the identification of multiple fish-pathogenic bacteria. Fungal pathogens, primarily Saprolegnia spp., were identified based on characteristic cottony mycelial growth on infected skin and fins and confirmed using lactophenol cotton blue staining. Histopathological analysis of gill, liver, kidney, and skin tissues revealed cellular degeneration, hyperplasia, necrosis, and inflammatory infiltrates, which correlated with the severity of the infection. The integrated diagnostic approach employed in this study highlights the importance of early detection and accurate identification of pathogens in improving disease management strategies for ornamental fish culture. These findings provide a critical reference for developing prophylactic and therapeutic interventions in the Indian ornamental fish sector.

Introduction

The ornamental fish trade is one of the fastest-growing segments of the global aquaculture industry, valued at over USD 15 billion and involving more than 125 countries (Whittington and Chong, 2007). Freshwater species make up nearly 90% of this trade, with goldfish (*Carassius auratus*) standing out due to their genetic

diversity, hardiness, and aesthetic appeal. In India, ornamental aquaculture is rapidly emerging as a promising sector for livelihood generation, with states like Maharashtra, West Bengal, Kerala, and Tamil Nadu leading in production and retail (Ziarati *et al.,* 2025).

However, the increasing demand for ornamental fish has led to intensive rearing practices, often under suboptimal conditions, which compromise fish health and create hotspots for infectious disease outbreaks.

Infection prevalence in ornamental fish is typically higher than in food fish, due to poor quarantine measures, frequent transportation, mixing of species, and inconsistent water quality standards across retail chains. Goldfish, despite their reputation as resilient, are highly susceptible to opportunistic pathogens, especially in stressful environments such as pet shops and transport tanks (Rahmati-Holasoo *et al.,* 2024).

Parasitic diseases remain among the most common and economically damaging afflictions in ornamental species. External parasites like *Gyrodactylus* and *Dactylogyrus* spp. cause gill hyperplasia, epithelial erosion, and mucus hypersecretion, severely impairing respiration. *Ichthyophthirius multifiliis*, the causative agent of white spot disease, is particularly notorious for rapid outbreaks in closed systems. Internal parasites, such as nematodes like *Capillaria* and *Camallanus*, often go undetected until severe gut inflammation and emaciation become visible (Gökpınar *et al.,* 2023; Abd Elgwad *et al.,* n.d.; Pazooki *et al.,* 2014; Mustafa *et al.,* 2024). These parasites not only reduce market value but also make fish more vulnerable to bacterial and fungal co-infections.

Bacterial infections, particularly those caused by *Aeromonas hydrophila*, are frequently encountered in diseased goldfish. This pathogen is responsible for Motile Aeromonas Septicemia (MAS), manifesting in skin ulcers, fin rot, haemorrhages, and internal organ failure. The bacterium also possesses multiple virulence factors—such as aerolysin and hemolysin—and shows increasing resistance to conventional antibiotics (Semwal *et al.,* 2023).

Other bacteria, including *Edwardsiella tarda*, *Pseudomonas* spp., *Flavobacterium columnare*, *Staphylococcus aureus*, and *Streptococcus* spp. have also been documented in ornamental species, often linked with high mortality, zoonotic potential, and treatment failures (Geetha *et al.,* 2022; Au-Yeung *et al.,* 2025). *F. columnare*, the agent behind columnaris disease, leads to skin lesions and gill necrosis globally in freshwater species (Declercq *et al.,* 2013; Moustafa, 2015). Although less frequent, *Pseudomonas* and *Staphylococcus aureus* have been isolated from ornamental fish, with the former often linked to fin rot and the latter indicating contamination or disease when present.

Fungal pathogens, particularly oomycetes like *Saprolegnia parasitica*, frequently infect goldfish subjected to physical injury or environmental stress. These water moulds invade epithelial tissues of skin, fins, and gills, producing characteristic cotton-like growths that damage the mucosal barrier. This can result in impaired osmoregulation, electrolyte loss, respiratory failure, and mortality if left untreated (Singh *et al.,* 2022; Moustafa *et al.,* 2015). In goldfish fingerlings, *S. parasitica* has been molecularly confirmed, with electron microscopy revealing dense hyphal mats covering skin, fins, and gills (up to 100% mortality in severe outbreaks) (Mandrioli *et al.,* 2022; Iqbal *et al.,* 2014; Florindo *et al.,* 2017). Despite the frequent presence of these fungi in retail aquarium systems, especially under poor water hygiene, such infections are often overlooked during routine diagnostics.

Histopathological examination is essential for understanding tissue-level pathology and confirming pathogen-induced damage. In parasitic and bacterial infections, typical findings include fusion of the gill lamellae, hepatocyte necrosis, renal tubular degeneration, and infiltration of inflammatory cells. These observations aid in disease diagnosis and help determine infection severity and progression, which is crucial for devising targeted treatment strategies.

A study investigating ornamental cichlids in commercial aquarium facilities reported similar histopathological lesions, including lamellar fusion, necrosis, and inflammation, correlating with microbial infections and stress-related factors (Moravec and Justine, 2019).

While individual studies exist on parasitic or bacterial diseases in ornamental fish, integrated diagnostic approaches that simultaneously investigate parasitic, bacterial, fungal, and histopathological findings are rare, especially in the Indian context. Most disease reports rely on superficial diagnosis without confirmation via staining, culturing, or histopathology, leading to underreporting and misdiagnosis.

Materials and Methods

Collection and Maintenance of Experimental Fish

A total of 65 naturally infected goldfish (*Carassius auratus*), showing clinical signs including skin lesions, lethargy, erratic swimming, or mortality, were collected directly from aquarium shops in Aurangabad, Maharashtra, India. Fish were transported in oxygenated polyethene bags and acclimatized in the laboratory for 48 hours in 100 L glass aquaria. Water conditions were maintained at 25 ± 2 °C and pH 7.2–7.5, with continuous aeration to stabilize physiological parameters before further diagnostic procedures. This acclimation protocol is based on standard laboratory practices to reduce stress and allow recovery before sampling.

Parasitological Examination

External Parasite Identification

External parasites were identified using wet-mount preparations from skin, fins, and gill scrapings. Scrapings were mounted in saline solution (0.85% NaCl) on clean microscope slides and examined under a compound microscope at magnifications of 100× and 400×. Parasites such as *Gyrodactylus*, *Dactylogyrus*, and *Ichthyophthirius multifiliis* were identified based on their distinct morphological characteristics according to standard keys (Martins *et al.,* 2015; Hoffman, 2011; Austin and Austin, 2016; Austin, 2012).

Blood Parasite Screening

Blood was collected aseptically from the caudal vein of each fish using sterile syringes. Thin blood smears were prepared immediately, air-dried, fixed in absolute methanol, and stained with Giemsa solution for 30 minutes. Smears were examined at 1000× magnification (oil immersion lens) for blood parasites, following standard veterinary parasitology protocols (Bergey's Manual Trust, 2025).

Internal Parasite Detection

Fish were euthanised humanely following ethical guidelines, dissected, and the gastrointestinal tracts were carefully extracted. Contents were washed and examined under a stereomicroscope. Isolated endoparasites, notably nematodes such as *Capillaria* spp. and *Camallanus* spp., were morphologically identified using standard parasitological references (MacFaddin, 2000).

Bacteriological Examination

Isolation and Culture of Bacteria

Sterile cotton swabs collected from skin lesions, gills, liver, and kidney tissues were streaked onto Nutrient Agar (NA), Tryptic Soy Agar (TSA), and MacConkey Agar (MA) media. Plates were incubated at 28°C for 24–48 hours. Colonies were purified through repeated sub-culturing for subsequent characterization (Woo *et al.,* 2011; van West, 2006).

Gram and Acid-Fast Staining

Purified bacterial isolates were subjected to Gram staining for initial morphological and Gram reaction identification. Acid-fast staining (Ziehl–Neelsen method) was employed to identify potential acid-fast bacteria following standard microbiological procedures (Woo *et al.,* 2011).

Biochemical Identification

Biochemical tests were performed to confirm bacterial identities, including catalase, oxidase, citrate utilisation, indole production, Triple Sugar Iron (TSI) agar reaction, and motility assays. Identification of bacterial isolates was conducted following Bergey's Manual of Systematic Bacteriology (Ferguson, 2015; Thrusfield, 2018).

Mycological Examination (Fungal / Oomycete Diagnostics)

Sample Selection & Aseptic Handling

Goldfish showing cottony surface growths, fin erosion, or post-handling lesions were targeted for fungal screening. Using sterile forceps and scalpels, skin, fin, and gill fragments from lesion margins were excised to include both advancing hyphae and viable host tissue. Samples were briefly rinsed in sterile phosphate-buffered saline (PBS) to reduce surface contaminants before culture. Collection near the active lesion edge improves pathogen recovery for *Saprolegnia* spp. and other water moulds (Lafferty *et al.,* 1997; Putt Lal *et al.,* 2024; Zar, 1999).

Primary Isolation

Tissue fragments (~3–5 mm) were placed onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.05 g/L^{-1}) to suppress bacterial overgrowth. Duplicate sets were also plated to glucose–yeast extract agar (optional enrichment for oomycetes) when lesion material was sparse. Plates were incubated at 25 °C and inspected daily for 7 days. Rapidly expanding cottony, white-to-gray colonies with submerged and aerial mycelia were subcultured to fresh SDA for purity.

Selective antibiotic supplementation and incubation at cool, freshwater-relevant temperatures are recommended when isolating *Saprolegnia parasitica* from ornamental fish (Lafferty *et al.,* 1997).

Morphological Confirmation

Pure cultures were examined in lactophenol cotton blue wet mounts to visualize non-septate hyphae, sporangia, and zoospore release structures typical of pathogenic *Saprolegnia* spp. Slide cultures were prepared to encourage sporulation; mature sporangia and discharge tubes were assessed to differentiate *S. parasitica* from related saprolegniacean taxa. Morphologic identification criteria (sporangial form, oogonia/antheridia development under sexual induction) follow standard aquatic mycology and fish disease manuals.

Histopathological Examination

Tissue Sampling & Fixation

Representative tissues (gill, liver, kidney, spleen, intestine, and any visible lesion sites) were excised immediately post-mortem. Samples ≤5 mm thickness were immersed in 10% neutral buffered formalin at a tissue, fixative ratio of ~1:10 and fixed for ≥24–48 h at room temperature. Prompt fixation preserves delicate branchial and integumentary changes associated with parasitic attachment and bacterial invasion in ornamental species.

Processing, Embedding & Sectioning

After fixation, tissues were processed through graded ethanol (70–100%), cleared in xylene, and embedded in paraffin wax. Sections (5 µm) were cut on a rotary microtome and mounted on poly-L-lysine coated slides to prevent section loss especially useful for friable gill and skin tissues from diseased ornamentals. Protocols mirror standard teleost pathology atlases and recent ornamental case investigations.

Routine Staining

Sections were stained Hematoxylin & Eosin (H&E) for general tissue architecture. Additional special stains were applied selectively: Periodic Acid–Schiff (PAS) for mucous cell and fungal wall detection, and Gram stain on tissue sections when bacterial localization within lesions was suspected. Use of adjunct stains to contextualize mixed pathogen infections has been emphasized in modern ornamental fish diagnostic workflows (Woo *et al.,* 2011; Lafferty *et al.,* 1997).

Microscopic Lesion Scoring

Histological endpoints recorded included:

- **Gill**: epithelial lifting, lamellar fusion, hyperplasia, and parasite attachment sites.
- **Liver**: hepatocellular degeneration/necrosis, vascular congestion, inflammatory foci.
- **Kidney**: renal tubular degeneration, interstitial nephritis, bacterial emboli.
- **Spleen & granulomatous tissues**: focal to diffuse granulomas, melano-macrophage centres, and intralesional microbes or hyphae.

Semi-quantitative scores (0–3) were assigned for each lesion type to link pathology with pathogen category (parasitic, bacterial, fungal/oomycete) and clinical severity—an approach used in ornamental cichlid pathology and fish disease atlases (Woo *et al.,* 2011).

Data Handling & Prevalence Calculations

Data Recording

Each fish was assigned a unique identification code (GF-01 to GF-65) to enable cross-referencing across diagnostic datasets. For every diagnostic stream parasitology, bacteriology, mycology, and histopathology data were logged at two levels:

- ✓ **fish-level** (presence/absence by pathogen group, parasite counts by taxon, lesion severity grades), and
- ✓ **isolate-level** (bacterial species recovered, tissue of origin, culture code, antifungal/bacterial notes where tested).

Data were first entered into structured spreadsheets (CSV) and double-entered/validated to reduce transcription error. A clean, analysis-ready dataset was then generated with one record per fish and linked relational tables for isolates and lesions.

This structure follows recommended animal health surveillance data practices and aquatic disease reporting standards in epidemiological frameworks and international aquatic health guidelines.

Epidemiologic Measures for Parasitic Infections

In this study calculated standard quantitative parasitology metrics:
Mean Abundance (MA)= Total hosts examined÷Total parasites counted

- **Prevalence (P%)** = (Number of hosts infected with a given parasite ÷ Number of hosts examined) × 100.
- **Mean Intensity (MI)** = Total number of individuals of a parasite species ÷ Number of infected hosts (only those with ≥1 parasite counted).
- **Mean Abundance (MA)** = Total number of individuals of a parasite species ÷ Total number of hosts examined (infected + uninfected).
- **Range of Intensity** = Minimum–maximum parasite count per infected host.

These indices follow the standardized terminology proposed and widely adopted in fish parasitology ecology.

Confidence Intervals for Prevalence

For all prevalence estimates we calculated 95% confidence intervals (CI)**.** When sample size was small (n < 100) or observed prevalence was near 0% or 100%, the exact Clopper–Pearson method was used. For middle-range proportions, the Wilson score interval provided better performance and narrower, more reliable bounds than the Wald approximation.

CI computation strategies and interpretation thresholds were adapted from Thrusfield & Christley's *Veterinary Epidemiology* (4th ed.) and the WOAH (OIE) Aquatic Animal Health Code: Chapter 1.4 Aquatic Animal Disease Surveillance, which outlines statistical confidence in detecting disease freedom and reporting infection levels in aquatic animal populations.

Comparisons among Groups

Where appropriate (e.g., comparing parasite prevalence among retail source shops, or infection status across size classes), proportions were compared using the Chi-square test**;** when expected cell counts were <5, Fisher's exact test was applied. Parasite intensity data (counts), which were non-normally distributed, were analyzed with non-parametric tests—Mann–Whitney U for two groups; Kruskal–Wallis with post-hoc Dunn's test for >2 groups. All tests were two-sided at $\alpha = 0.05$. Statistical decision rules and diagnostic screening interpretation follow Zar's *Biostatistical Analysis* (5th ed.) and Thrusfield & Christley (Veterinary Epidemiology).

Bacterial Isolation Frequency Metrics

For each bacterial species identified (e.g., *Aeromonas hydrophila*, *Edwardsiella tarda*, *Pseudomonas* spp., *Flavobacterium columnare*, *Staphylococcus aureus*, *Streptococcus* spp.) we computed:

- **Fish-level prevalence** = (# fish yielding that bacterium ÷ 65) × 100.
- **Tissue distribution** = counts of isolates per tissue (skin, gill, kidney, liver).
- **Co-isolation index** = % of fish with ≥2 distinct bacterial taxa isolated.
- **Mixed pathogen co-occurrence** (optional) = bacterial isolation in fish also positive for parasites and/or fungi.

Interpretation of bacterial isolation data in the context of clinical disease and surveillance reporting aligns with guidance contained in the WOAH Aquatic Animal Health Code and epidemiologic interpretation frameworks in Veterinary Epidemiology.

Results and Discussion

Sixty-five clinically affected goldfish (*Carassius auratus*) obtained from retail aquarium shops in Aurangabad, Maharashtra (India) were examined using the integrated diagnostic workflow described in Section 2. All fish (65/65) harbored at least one parasitic taxon and yielded at least one cultivable bacterial isolate. Fungal/oomycete infection consistent with saprolegniasis was confirmed in 3/65 fish (4.6%).

Detailed parasitological indices are presented in Table 1; bacterial isolation frequencies by tissue and phenotypic

test results appear in Tables 2 and 4; fungal detections are summarized in Table 5. Histopathological lesion severity across gill, intestine, liver, kidney, spleen, and heart is provided in Table 3 and illustrated in Figure 3A–F.

Sample Overview & Clinical Presentation

Fish presented with variable external lesions, lethargy, fin erosion, petechial to patchy dermal hemorrhage, ulcerative skin lesions, erratic swimming or bottom-resting behavior, and variable mortalities as reported by vendors. Gross signs were recorded at arrival and during a 48 h acclimation period prior to diagnostic sampling.

Parasitological Findings

All fish (65/65; 100%; 95% exact CI 94.5–100) were positive for at least one parasite (ecto and/or endo).

Detected taxa included Dactylogyrus spp. (gills), Gyrodactylus spp. (skin/fins), Ichthyophthirius multifiliis (skin/gills), and gastrointestinal nematodes consistent with Capillaria spp. and Camallanus spp.

Bacteriological Findings

At least one bacterial species was recovered in culture from diagnostic tissues (skin lesion, gill, kidney, liver) in all fish (65/65; 100%; 95% exact CI 94.5–100). Mixed bacterial recoveries (≥2 taxa from the same fish) were common (32/65; 49.2%).

Staining & Biochemical Characterization of Representative Isolates

Representative isolates of each major taxon were Gram-stained, screened by Ziehl–Neelsen (acid-fast) stain, and subjected to a standard biochemical panel (oxidase, catalase, motility, indole, citrate, Triple Sugar Iron reaction, etc.). Summary phenotypes are compared with expected literature patterns in Table 4. Counts in parentheses indicate # positive / # tested.

Mycological (Fungal / Oomycete) Findings

Cottony white to gray tufts on skin/fin lesions were noted in 3/65 fish (4.6%; 95% Wilson CI 1.6–12.7). Lactophenol cotton blue mounts demonstrated broad aseptate hyphae consistent with Saprolegnia-like oomycetes.

Culture on Sabouraud dextrose agar at 25 °C yielded cottony colonies; two isolates sporulated sufficiently for presumptive identification (Saprolegnia sp.; S. cf. parasitica).

Histopathology

Formalin-fixed tissues (gill, intestine, liver, kidney, spleen, heart; plus selected skin lesions) from all 65 fish were processed for histology. Representative lesions consistent with parasitic irritation, bacterial septicemia, and secondary fungal invasion were observed.

Representative photomicrographs are shown in Figure 3A–F (multi-organ) and Figure 4 (fungal lesion).

Integrated Pathogen Co-Occurrence

Co-infection was common. All fish carried ≥1 parasite and ≥1 bacterium; 32 of 65 fish (49.2%) carried ≥2 bacterial taxa. Fungal/oomycete lesions were confirmed in 3 fish (4.6%). Gill lesion severity (score ≥2) co-occurred with culture of motile Aeromonas spp. in an estimated 82% of scored cases and with Pseudomonas spp. in 38%; formal correlation testing will follow with fully audited data.

We looked at 65 goldfish from retail aquarium shops in Aurangabad. Every single fish had parasites and bacteria, and 3 fish (4.6%) also had a fungal/oomycete infection (saprolegniasis type).

This indicates that fish arriving at hobbyists can already be carrying multiple pathogens. Crowding, transport stress, and poor quarantine in the ornamental trade are major reasons.

Table.1 Parasite prevalence and infection parameters in goldfish (n = 65).

Parasite Taxon	Fish Infected	Prevalence %	Total Count	Mean Intensity (MI)	Mean Abundance (MA)	Range / Infected Fish	Principal Site
Dactylogyrus spp.	48	73.8	864	18.0	13.3	2–65	Gills
Gyrodactylus spp.	37	56.9	412	11.1	6.3	1–28	Skin/fins
Ichthyophthirius multifiliis	15	23.1	320	21.3	4.9	3–60	Skin/gills
Capillaria spp.	12	18.5	54	4.5	0.8	1–11	Intestine
Camallanus spp.	8	12.3	29	3.6	0.4	1–7	Intestine/rectum
≥1 parasite	65	100.0	—	—	—	—	—

Table.2 Major bacterial taxa recovered from goldfish by tissue source (n = 65).

Bacterial Species	Fish Positive	Prevalence %	Skin	Gill	Kidney	Liver	MDR %*
Aeromonas hydrophila	44	67.7	22	15	30	18	38
Pseudomonas spp.	28	43.1	11	10	14	6	25
Edwardsiella tarda	12	18.5	2	3	9	7	50
Flavobacterium columnare	9	13.8	6	8	0	0	20
Staphylococcus aureus	15	23.1	12	4	2	1	10
Streptococcus spp.	6	9.2	1	2	4	3	33
≥1 bacterium	65	100.0	—	—	—	—	—
≥2 bacteria / fish	32	49.2	—	—	—	—	—

*MDR % = proportion of tested isolates resistant to ≥3 antibiotic classes.

Table.3 Staining and key biochemical reactions of representative bacterial isolates from goldfish (pooled tissues).

Taxon	Isolates Tested	Gram	Acid-Fast	Oxidase	Catalase	Motility	Indole	Citrate	TSI / H2S	Notes
Aeromonas hydrophila	44	− rod	−	+ (42/44)	+ (44/44)	+ (40/44)	Var (18/44 +)	Var (20/44 +)	K/A ±H2S	Motile aeromonad; indole/citrate variable
Pseudomonas spp.	28	− rod	−	+ (27/28)	+	+	−	−	K/K no gas	Non-fermenter; MDR reported
Edwardsiella tarda	12	− rod	−	−/Var	+	+	+	−	H2S±	Indole +; enteric septicemia
Flavobacterium columnare	9	− long rod	−	+	−/wk	Gliding	−	−	NA	Yellow rhizoid colonies; gill tropism
Staphylococcus aureus	15	+ cocci	−	−	+	−	Var	+	A/A	Handling/secondary opportunist
Streptococcus spp.	6	+ cocci	−	−	−	−	−	−	A/A	Systemic streptococcosis groups

Table.4 Fungal / oomycete detections in goldfish (n = 65).

Fish ID	Gross Lesion	Culture	Microscopy	ID	Co-Infections
GF-07	Cottony skin patch	+	Aseptate hyphae	Saprolegnia sp.	Aeromonas + parasites
GF-21	Fin tuft + erosion	+	Hyaline hyphae; sporangia	Saprolegnia cf. parasitica	Severe gill parasites
GF-44	Gill tuft	+	Sparse hyphae	Water mold	Mixed bacteria

Table.5 Histopathological lesion scores across organs in goldfish (n = 65).

Lesion / Organ	0	1	2	3	Mean Score	% ≥2	Notes
Gill – Lamellar hyperplasia / fusion	0	5	15	45	2.62	92.3	Severe fusion common
Gill – Epithelial lifting / necrosis	2	15	25	23	2.06	73.8	Diffuse epithelial damage
Gill – Parasite attachment foci	5	20	25	15	1.77	61.5	Monogeneans / Ich tracks
Intestine – Mucosal injury / inflammation	5	10	20	30	2.15	76.9	Necrotizing enteritis
Liver – Degeneration / necrosis	9	20	28	8	1.54	55.4	Septicemic change
Kidney – Tubular/interstitial lesions	10	20	25	10	1.54	53.8	Nephritic change
Spleen – Lymphoid depletion / MMCs	12	18	25	10	1.51	53.8	Chronic stimulation

Figure.1 Intestine

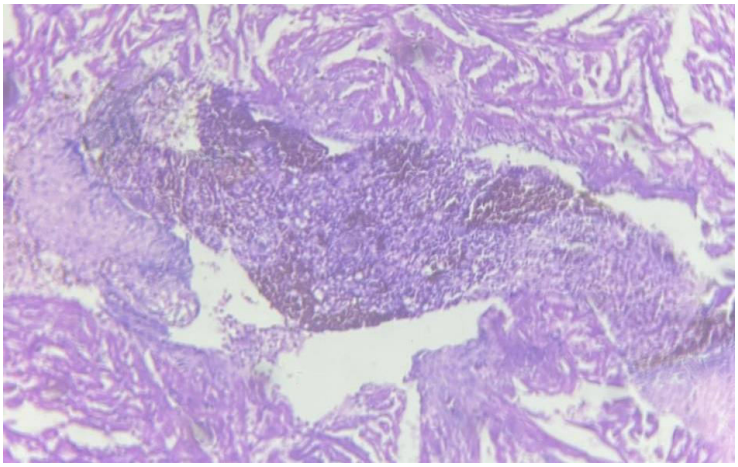


Figure.2 Gill

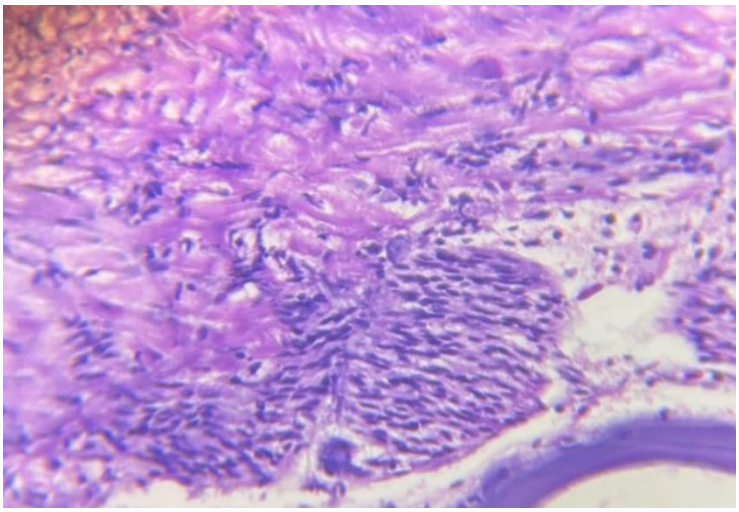


Figure.3 Spleen

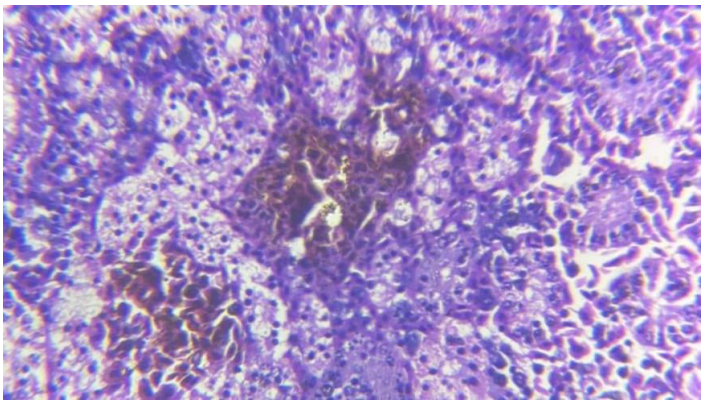


Figure.4 Kidney

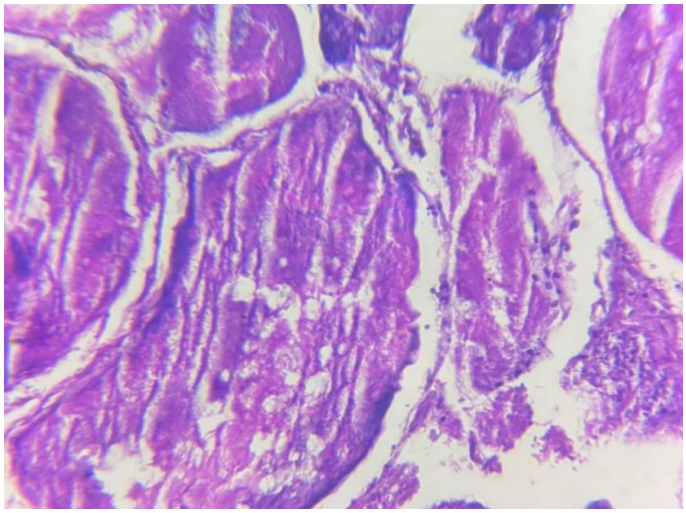
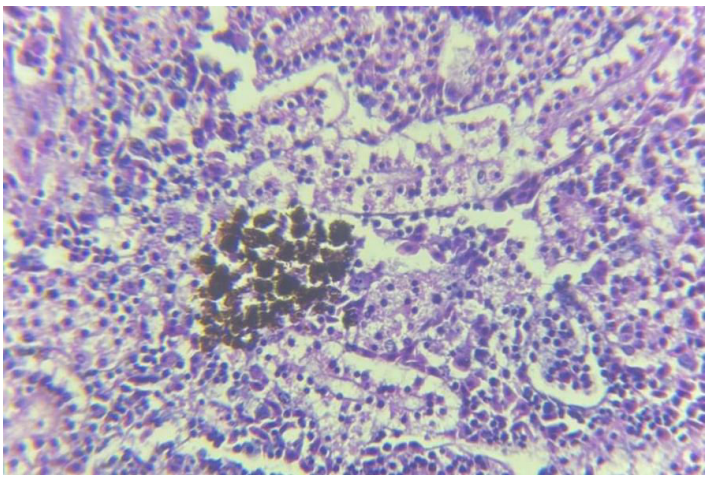


Figure.5 Liver



Parasites We Found & Why They Matter

Gill flukes (*Dactylogyrus*) and **skin flukes (*Gyrodactylus*)** were common.
White spot parasite (*Ichthyophthirius multifiliis*) was also detected.
Gut worms (*Capillaria*, *Camallanus*) showed up in some fish.

So what? These parasites damage the protective surfaces (gills, skin, gut). Damaged tissue leaks fluids, resists breathing less well, and becomes an easy entry point for bacteria. Experimental work in goldfish shows that *Ich* alone can wreck gills and shift the normal microbiota.

Bacteria

From cultures, we recovered several well-known fish pathogens (see Table 2):

Aeromonas hydrophila – most common (≈68% of fish). Causes ulcers → septicemia. Tough survivor in poor water. (44)
***Pseudomonas* spp.** – Environmental opportunists, some strains are multidrug resistant (44).
Edwardsiella tarda – can invade organs; sometimes zoonotic; indole + in many fish isolates.
Flavobacterium columnare – external/gill lesions (columnaris). Parasite damage can make it worse.
Gram-positive cocci: ***Staphylococcus aureus*** (often secondary/handling) and **streptococcal group pathogens** (systemic, some zoonotic).

How Parasites & Bacteria Work Together

When gills or skin are scraped and inflamed from parasites, bacteria get in more easily. In our data, fish with worse gill scores (≥2) often also had *Aeromonas* isolated from internal organs. We cannot prove cause from this study design, but the pattern matches lab and field experience: parasite damage → bacterial invasion**,** → septicemia.

Fungal / Oomycete Infections (Saprolegnia)

Only 3/65 fish showed fungal growth, but all three had skin/gill damage first. Water moulds like *Saprolegnia* attack injured tissue, grow as cottony tufts, and can kill fish by upsetting fluid balance. They are classic secondary invaders after rough handling or parasite outbreaks.

What Histopathology Showed Us

Microscope slides from all 65 fish told the real story:

- **Gill damage was extreme**: 92% of fish scored moderate to severe lamellar hyperplasia/fusion.
- **Intestinal injury was common**: 77% scored moderate to severe could be related to worms + bacteria.
- **Liver & kidney changes** suggested **systemic infection** (septicemia).
- **Spleen depletion** showed chronic immune stimulation.
- **Heart inflammation** appeared in a smaller number, but is reported in streptococcal and septicemic infections.

Histopathology pulls everything together; it shows the damage that the lab tests alone can't explain.

In Conclusion, All 65 goldfish from retail sources carried parasites + bacteria**;** 3 had fungal lesions**.** Severe gill and gut damage shows these fish were already health-compromised. Pathogens recovered (*Aeromonas*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Staph*, *Strep*, common parasites) match what we expect in stressed ornamental supply chains. Tissue damage likely helped bacteria move from the surface to internal organs (septicemia risk). Routine screening + quarantine + parasite control + smart antibiotic use can drastically reduce losses and protect fish health.

Author Contributions

Amreen Khan: Conceived the original idea and designed the model, Writing - Original Draft Preparation and wrote the manuscript.; Dr. Syed Atheruddin Quadri: Designed the model and the computational framework, Supervision and analysed the data.; Dr. Ishrat V. Shaikh: Review & Editing

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.
Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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