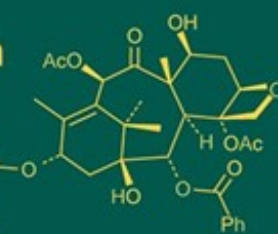
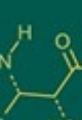
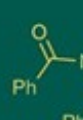


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Experimental pathogenicity of *Aeromonas caviae* strain BLB-05 in pacu fish (*Piaractus Brachypomus*) LD₅₀, hematobiochemical alterations and multi-organ pathology

Aniket Funde¹, Arun Sharma², TI Chanu³, Isha Kumari⁴, Swapnil Ananda Narsale⁵, Pallavi Bhalavey⁶, Dipalee Thombre⁷

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ABSTRACT

Aeromonas caviae poses a significant threat to emerging red-bellied pacu (*Piaractus brachypomus*) aquaculture in India, yet systematic pathogenicity data remain scarce. This study evaluated the virulence of *A. caviae* strain BLB-05 (MT973994) in pacu fingerlings through experimental intraperitoneal challenge, determining LD₅₀ via probit analysis (OECD 203). Fingerlings (10±2 g) exposed to graded doses (10⁵-10⁹ CFU/mL) exhibited dose-dependent motile *Aeromonas* septicemia, with LD₅₀ at 3.02 × 10⁷ CFU/mL yielding 50% mortality by 120 hpi, hemorrhagic ulcers and behavioral aberrations (lethargy, erratic swimming). Probit regression analysis () estimated the 96-h median lethal dose () of *Aeromonas caviae* strain BLB-05 in red-bellied pacu *Piaractus brachypomus* as (95% CI:). Experimental challenge at or above this threshold dose induced typical signs of motile aeromonas septicemia, including skin hemorrhages and severe multi-organ damage. Infected fish showed anemia (Hb 6.1 g/dL; TEC 1.27 × 10⁶/mm³), leukocytosis (TLC 49.1 × 10³/mm³), thrombocytopenia and macrocytic hypochromic erythrocytes alongside biochemical stress: hyperglycemia (85.4 mg/dL), elevated AST/ALT/ALP (122.3/76.5/157.2 U/L) and hypoalbuminemia (0.71 g/dL). Histopathology revealed gill lamellar fusion, hepatic necrosis and renal pyknosis/karyolysis with melanomacrophage aggregates. These multi-systemic lesions confirm *A. caviae*'s exotoxin-mediated pathogenesis, underscoring urgent need for surveillance, probiotics and phytotherapeutics to safeguard pacu farming sustainability.

Keywords

Keywords: *Aeromonas caviae*, *Piaractus brachypomus*, LD₅₀, Motile *Aeromonas* septicemia, Histopathology, Aquaculture pathology

Introduction

Disease remains one of the major challenges hindering the growth of aquaculture, representing a key limitation to both economic and socio-economic advancement in India and globally (Gundi *et al.*, 2015). Among freshwater aquaculture systems, bacterial pathogens alone contribute to approximately 12.8% of total disease occurrences (Sahoo *et al.*, 2020) [25]. Within this context, members of the genus *Aeromonas* have emerged as important pathogens affecting freshwater fish species (Pessoa *et al.*, 2022) [24]. In a recent study conducted in Indian freshwater aquaculture, it has been noticed that the motile aeromonads, among all bacterial pathogens, were the major pathogens causing bacterial fish diseases (66.66%). Among aeromonads, *Aeromonas hydrophila* (52.94%) was found to be involved in the maximum number of diseases, followed by *Aeromonas sobria* (26.47%), *Aeromonas veronii* (14.70%), *Aeromonas jandaei* (2.94%), and *Aeromonas caviae* (2.94%) (Sahoo *et al.*, 2020) [25]. These bacteria are Gram-negative, rod-shaped, non-spore-forming, and facultatively anaerobic microorganisms that are ubiquitously present in aquatic environments (Pessoa *et al.*, 2019) [23]. *Aeromonas caviae* is a pathogenic bacterium that poses significant economic losses to freshwater aquaculture systems, particularly affecting catfish species through its virulence factors and inherent antimicrobial resistance. Recent molecular characterization and experimental studies have confirmed that *A. caviae* induces severe histopathological lesions, hemolytic anemia, and tissue necrosis with high mortality rates, establishing it as an important pathogenic threat to farmed fish populations in aquaculture (Sharma *et al.*, 2022) [27]. *Aeromonas caviae* harboring multiple virulence genes (*fla*, *aer*, *ela*, *hly*), poses a significant threat to farmed fish species by inducing severe tissue damage, high mortality rates, and complex pathogenic mechanisms across diverse aquaculture systems (Xue *et al.*, 2022) [29].

The red-bellied pacu (*Piaractus brachypomus*), also colloquially referred to as pacu fish, is regarded as a significant aquaculture species. Interestingly, the species entered the Indian aquaculture sector through the

Corresponding Author: Arun Sharma

Aquatic Environment and Health Management (AEHM) Division, ICAR-Central Institute of Fisheries Education (ICAR CIFE), Mumbai Maharashtra, India

ornamental fish trade around 2003–2004, where it is locally known as rupchanda in states such as Tripura, Assam, and West Bengal. Recently, a survey conducted across three states reported that of 351 farmers, 305 (approximately 87%) believed that farming pacu is profitable. Though farmers appreciate the growth rate of this species, ambiguous feedback often comes about its impact on the overall pond production system. The Indian government has issued guidelines for culturing pacu in Indian aquaculture systems.

The red-bellied pacu (*Piaractus brachipomus*) has emerged as a commercially significant aquaculture species across multiple regions globally, including Colombia, Brazil, Peru, Venezuela, Vietnam, Thailand, Malaysia, and Bangladesh (Kumar *et al.*, 2018) [18]. This species exhibits several advantageous traits that position it as a promising candidate for aquaculture development, including robustness, accelerated growth rates, adaptability to diverse water quality conditions, superior meat quality, and an omnivorous dietary preference (Fresneda *et al.*, 2004; Ghosh *et al.*, 2014) [13, 14]. In India, the species was introduced through the ornamental fish trade sector around 2003–2004 and is regionally recognized as Rupchanda in states including Tripura, Assam, and West Bengal (Seshagiri *et al.*, 2021) [26]. A comprehensive survey by ICAR-NBFGR (2020) across three Indian states demonstrated that among 351 farmers surveyed, approximately 87% (305 farmers) perceived pacu farming as economically viable (Seshagiri *et al.*, 2021) [26]. While farmers acknowledge the rapid growth potential of this species, there remains considerable uncertainty regarding its broader ecological and production impacts within pond-based farming systems. Recognizing the commercial significance of pacu aquaculture, the Indian government has established guidelines for the cultivation of red-bellied pacu within domestic aquaculture systems (F.No 35029/8/2011/Fy(Trade)(E10310).

No systematic study on interaction of this species with the *Aeromonas caviae* is available. Therefore, the present study was conducted as to evaluate pathogenicity study of *P. brachypomus* against *Aeromonas caviae* in experimental fish diseases model.

2. Material and methods procurement of bacteria

The bacterial strains used in this study were *Aeromonas caviae* Strain BLB-05 (Gen Bank accession number: MF370717), obtained from the Department of Aquatic Animal Health Management, ICAR-CIFE, Mumbai. These isolates were provided on nutrient agar plates and subsequently maintained at 4 °C until further use.

2. Procurement of fish

For the present investigation, fingerling-stage red-bellied pacu (*P. brachypomus*) weighing 10 ± 2 g was sourced from a commercial hatchery (Ali Fish Farm, West Bengal, India) and transferred to ICAR-CIFE, Mumbai. Stress-mitigation and surface disinfection protocols were executed immediately via a 30-s immersion in 1% NaCl and a subsequent 2-min immersion in $2 \text{ mg L}^{-1} \text{ KMnO}_4$. Acclimatization lasted 21 days within 500 L capacity FRP tanks under uninterrupted aeration. Pre-experimental tank preparation involved overnight disinfection with KMnO_4 (4 mg L^{-1}), followed by flushing, refilling, and 48-h pre-aeration. Feeding was maintained twice daily *ad libitum* using pelleted feed (25% crude protein). Visual inspections were conducted daily to assess physical appearance, mortality, and swimming behaviour. Standard physicochemical water parameters were periodically assessed in accordance with APHA (2005) protocols, supported by a 30% static water exchange every two days to eliminate metabolic waste.

2.3 Experimental challenge for LD50

The acute toxicity of *Aeromonas caviae* in red-bellied pacu (*Piaractus brachypomus*) was evaluated following the OECD guideline 203 for static renewal bioassays (OECD, 1992). A total of 180 fish were randomly distributed into 18 tanks (10 fish per 50 L tank) under a completely randomized design. Out of these, 150 individuals were intraperitoneally injected with 0.1 mL suspensions of *Aeromonas caviae* at graded concentrations corresponding to

McFarland standards 5–8 ($T_1 = 1 \times 10^5$; $T_2 = 1 \times 10^6$; $T_3 = 1 \times 10^7$; $T_4 = 1 \times 10^8$; $T_5 = 1 \times 10^9$ CFU/mL). The control group received an equal volume of sterile PBS. Each treatment, including the control, was carried out in triplicate to ensure reliability of the results. Throughout the 96hr exposure period, fish were not provided feed and the tanks were left unsiphoned. The median lethal dose (LD_{50}) was estimated through probit analysis as described by Finney (1952) [12].

Moribund and freshly dead fish were subjected to bacteriological re-isolation under sterile conditions. Pure colonies of *Aeromonas caviae* were successfully re-isolated from the primary target organs (kidney, liver and blood) of infected fish and confirmed via PCR targeting the 16S rRNA gene, thereby fulfilling Koch's postulates. No bacteria were isolated from the control group.

After determining the LD_{50} dose by probit analysis (Finney, 1952) [12], the same dose was injected into three replicate groups, along with one triplicate control group, for subsequent sampling and analysis. The present study was approved by the Animal Ethics Committee.

2.4 Statistical methods for LD50 determination -conversion of percentage mortalities to probit and calculation of LD50

Cumulative mortality was recorded as the experimental endpoint over a 96-h exposure period. The median lethal dose (LD_{50}) was estimated through probit analysis as described by Finney (1952) [12]. Mortality data are presented in Table 1 for Pacu fish fingerlings challenged with graded concentrations of *Aeromonas caviae* ranging from 1×10^5 to 1×10^9 CFU mL^{-1} . For each concentration, 30 fish were intraperitoneally injected with a constant volume of 100 μL bacterial suspension and monitored for 96 h. The number of dead fish was recorded for each treatment group, and mortality was expressed as a percentage of the total fish exposed. The percentage mortality data were transformed into corrected mortality values. Finally, Corrected mortality percentages were subsequently transformed into probit units and bacterial concentrations were converted to their logarithmic ($\log_{10}$) values. Probit values were plotted against $\log_{10}$ bacterial concentrations, and the concentration corresponding to probit 5 (50% mortality) was determined. The LD_{50} was estimated using probit analysis following Finney (1952) [12] and this dose was used for subsequent experimental exposure with three replicate groups, while one triplicate control group, was maintained for subsequent sampling and analysis.

2.5 Sample collection

Post- LD_{50} challenge (3.02×10^7 CFU/mL, i.p.), destructive sampling was conducted across four distinct time points: 0 h (baseline), 24 h, 72 h, and 96 h post-infection, alongside an uninfected parallel control group ($n = 15$ fish per time point per group). To prevent sampling-induced physiological artifacts, entirely independent cohorts of fish were utilized for each analytical stream. At each sampling interval, 15 fish per group (5 fish per replicate) were allocated exclusively for hematological analysis via caudal vein puncture. A separate, independent cohort of 15 fish per group (5 fish per replicate) was sampled solely for serum biochemistry using non-anticoagulated syringes. Finally, a third independent cohort of 15 fish per group (5 fish per replicate) was euthanized directly for target organ excision (gills, liver, and kidney) and subsequent histopathological processing.

2.6 Behavioural changes

Following determination of the LD_{50} , the same dose was administered, and fish were subsequently observed for behavioural and clinical changes.

2.7 Blood collection and haematological parameters

Pooled blood samples were collected from five randomly selected fish in each treatment group at each sampling point. Fish were anaesthetized with Tricaine methane sulfonate (MS-222 [Sigma-Aldrich Inc., St Louis, MO, USA]) at a concentration of 100 mg L^{-1} (Pathinathan *et al.*, 2025). The blood was drawn from the caudal vein region using a 1 mL BD tuberculin syringe which was previously pre-rinsed with 2.7% EDTA. The collected blood was transferred immediately into K-2 EDTA vials (and gently shaken to ensure proper anti-

coagulant mixing to prevent clotting and haemolysis. The collected blood sample from each treatment and control was immediately analysed for haematological parameters such as viz., Total count of erythrocytes (TEC [10^6 cells μL^{-1}]), Total count of leukocytes (TLC [10^3 cells μL^{-1}]) monocytes and neutrophils were analysed using an automated blood analysers (Pentra 60C+ and Pentra XL 80,

Automated haematology analyzers, France), Haemoglobin (Hb [g dL^{-1}]) (Erdal *et al.*, 1991), Packed cell volume or haematocrit (HCT[%]) (Wintrobe 1934) were computed. The erythrocyte indices were determined from the results of the RBC count, haemoglobin (Hb), and PCV, using standard formulae in line with Dacie and Lewis (2011).

$$\text{Mean Corpuscular Volume (MCV) in femtoliters (fL)} = \frac{\text{packed cell volume (\%)} \times 10}{\text{RBC count in } 10^6/\text{mm}^3}$$

$$\text{Mean Corpuscular Haemoglobin (MCH) in picogram (pg)} = \frac{\text{Hb in g \%} \times 10}{\text{RBC count in } 10^6/\text{mm}^3}$$

$$\text{Mean Corpuscular Haemoglobin Concentration (MCHC) in grams per decilitre (g/dL)} = \frac{\text{Hb in g \%} \times 100}{\text{PCV\%}}$$

2.8 Serum collection and serum biochemical parameters

Pooled blood samples were collected ($n = 5$ per replicate) from each treatment and control group at each sampling point post infection using heparinized syringes. For serum separation, blood samples were allowed to clot naturally without the addition of any *anti*-coagulant. Once clotting occurred at room temperature, the samples were centrifuged at 3000 g for 10 min (Nuwansi *et al.*, 2019), and the serum was separated and transferred to sterile Eppendorf tubes. The samples were stored at -20°C for future biochemical analysis. Serum biochemical parameters, including aspartate aminotransferase (AST) (ERBA AST KIT [Code Number - 120204]), alanine aminotransferase (ALT) (ERBA ALT KIT [Code Number - 120207]) Alkaline phosphatase (ALP) albumin and glucose concentration were also quantified using commercial kits (ERBA KIT, India), following the provided guidelines. These hepatic Enzymatic activities were determined spectrophotometrically using a microplate reader (Spectromax Plus™, USA) at 340 nm for AST and ALT, 405 nm for ALP, 630 nm for Albumin and 505 nm for glucose. All these assays were performed in triplicate and results were expressed in UL^{-1} .

2.9 Histopathology

Gill (lamellar fusion, epithelial hyperplasia), liver (vacuolation, karyorrhexis) and kidney (necrosis, melanomacrophage [MMC] aggregates) lesions were blindly scored on a 0-3 semi-quantitative scale by two independent pathologists: 0 = no lesion; 1 = mild ($< 25\%$ affected tissue); 2 = moderate ($25\text{--}50\%$); 3 = severe ($> 50\%$). Scores from 3 sections per fish ($n = 15$ sections/organ/group) were averaged (mean $\pm$ SE). Inter-observer agreement: Cohen's $\kappa = 0.87$ (substantial). Differences analysed by Student's *t*-test ($p < 0.05$).

2.10 Statistical analysis

Data are presented as mean $\pm$ standard error (SE). Prior to parametric hypothesis testing, the underlying statistical assumptions of normality and homogeneity of variance were verified independently for each individual haematological and biochemical parameter using the Shapiro-Wilk test and Levene's test, respectively. Since all datasets satisfied these criteria ($p > 0.05$), significant differences between the control and infected groups were determined using an independent samples *t*-test. All statistical analyses were conducted at a significance level of $p < 0.05$ using SPSS software.

3. Result

3.1 Physiochemical parameters of water

The water quality parameters were monitored throughout the experimental period and the mean values recorded for each parameter across treatment groups during the present study were as follows:

Temperature of $28\text{--}30^\circ\text{C}$, pH of $7.8\text{--}8.1$, Dissolved oxygen (D.O) levels of $5.6\text{--}6.0$ mg L^{-1} , Hardness levels of $187.5\text{--}189$ mg L^{-1} , Alkalinity levels $132\text{--}137.5$ mg L^{-1} , and total ammonia nitrogen ($\text{NH}_3\text{--N}$) levels of $0.20\text{--}0.21$ mg L^{-1} . Overall, minor variations were observed, but water quality remained stable compared to the control.

3.2 Experimental infection

The Table 1 summarizes the mortality of red-bellied pacu *Piaractus brachipomus* experimentally infected with *Aeromonas caviae*. The mortality of Pacu fish fingerlings experimentally infected with *Aeromonas caviae* strain BLB-05 is summarized in Table 1. (100%) mortality was observed in the group injected with the highest concentration of 1×10^9 CFU mL^{-1} , whereas no mortality occurred in the control group. Probit regression analysis ($y = 0.591 + 2.185\log_{10}\text{concentration}$; $R^2 = 0.984$) estimated the 96-h median lethal dose (LD_{50}) to be 3.02×10^7 CFU mL^{-1} (95%CI = $2.10\text{--}4.50 \times 10^7$ CFU mL^{-1}). At or above this threshold concentration, infected fish exhibited characteristic clinical signs, including surface hemorrhages and abnormal swimming behaviour. At this concentration, fish exhibited mortality and clinical signs such as haemorrhages and abnormal swimming behaviour.

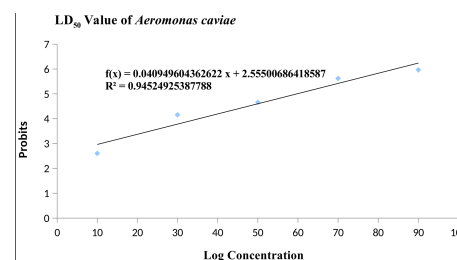


Figure 1. LD_{50} Value of *Aeromonas caviae* Strain BLB-05. Probit regression plot showing median lethal dose (LD_{50}) of *Aeromonas caviae* strain BLB-05 in *Piaractus brachipomus*. Probit regression: $y = 0.591 + 2.185\log_{10}\text{concentration}$, $R^2 = 0.984$. $\text{LD}_{50} = 3.02 \times 10^7$ CFU mL^{-1} (95%CI = $2.10\text{--}4.50 \times 10^7$ CFU mL^{-1}) at probit 5.0 (also see Table 1; Finney, 1952) [12].

Table 1. Probit analysis data yielding $\text{LD}_{50} = 3.02 \times 10^7$ CFU mL^{-1} (95%CI = $2.10\text{--}4.50 \times 10^7$). Regression equation: $y = 0.591 + 2.185\log_{10}[\text{CFU mL}^{-1}]$, $R^2 = 0.984$ (also see Figure 1). SE of $\text{LD}_{50} = \pm 0.16\log_{10}$ units.

Table 1. Dose-Dependent Mortality Response of red-bellied pacu (*P. brachypomus*) Challenged with *Aeromonas caviae* (BLBM-05)

Bacterial concentration (CFU mL ⁻¹)	Injection volume (μL)	Total fish (n)	Number dead	Mortality (%)	Corrected mortality (%)	log ₁₀ concentration	Probit value
1 × 10 ⁵	100	30	0	0.0	0.0	5	2.60
1 × 10 ⁶	100	30	5	16.7	16.7	6	4.03
1 × 10 ⁷	100	30	12	40.0	40.0	7	4.75
1 × 10 ⁸	100	30	25	83.3	83.3	8	5.97
1 × 10 ⁹	100	30	30	100.0	99.17	9	7.40

LD₅₀ = 3.02 × 10⁷ CFU mL⁻¹ (95% CI = 2.10–4.50 × 10⁷). Regression equation: $y = 0.591 + 2.185 \log_{10} [\text{CFU mL}^{-1}]$; R² = 0.984. SE of LD₅₀ = ±0.16 log₁₀ units.

3.3 Clinical sign & behavioral changes

Experimental challenge of red-bellied pacu *Piaractus brachypomus* with *Aeromonas caviae* via intraperitoneal injection at the median lethal dose (LD₅₀) of 3.02 × 10⁷ CFU/ml /fish, determined by probit analysis following Probit Analysis (Finney, 1952) [12] method, induced fulminant motile *Aeromonas* septicemia (MAS) with 50% cumulative mortality by 120 hours post-infection (hpi). At baseline (0 dpi, panel A), fish displayed normal silvery-reddish integument with intact skin and fins, indicative of healthy morphology. By 4 dpi (Stage I, panel B), an early circumscribed erythematous lesion emerged on the dorsal surface, characterized by localized hyperaemia and subtle epidermal desquamation without ulceration. Progression to Stage II at 6 dpi (panel C) revealed expansion of the red lesion with pronounced dermal edema and incipient ulceration, accompanied by fin margin fraying and mild exophthalmia in affected individuals. Advanced

Stage III at 8 dpi (panel D) exhibited overt ulcerative dermatitis with deep tissue erosion, haemorrhagic crusting, and secondary muscle exposure, consistent with *Aeromonas*-induced motile *Aeromonas* septicemia (MAS) pathology. These gross lesions align with virulence-mediated extracellular enzyme activity, including hemolysins and proteases, reported in *Aeromonas* challenges of Amazonian characins.

3.4 Behavioural alterations

In parallel, infected fish exhibited dose- and time-dependent behavioural aberrations, including lethargy, anorexia and surface respiration from 4 dpi onward, escalating to erratic swimming, loss of equilibrium, and clustering near tank walls by 6–8 dpi. Such neurobehavioral changes reflect systemic toxemia and hypoxia secondary to gill haemorrhages, as documented in experimental *Aeromonas hydrophila* infections of *P. brachypomus*. No morbidity occurred in un-injected controls, confirming injection-elicited pathogenesis.

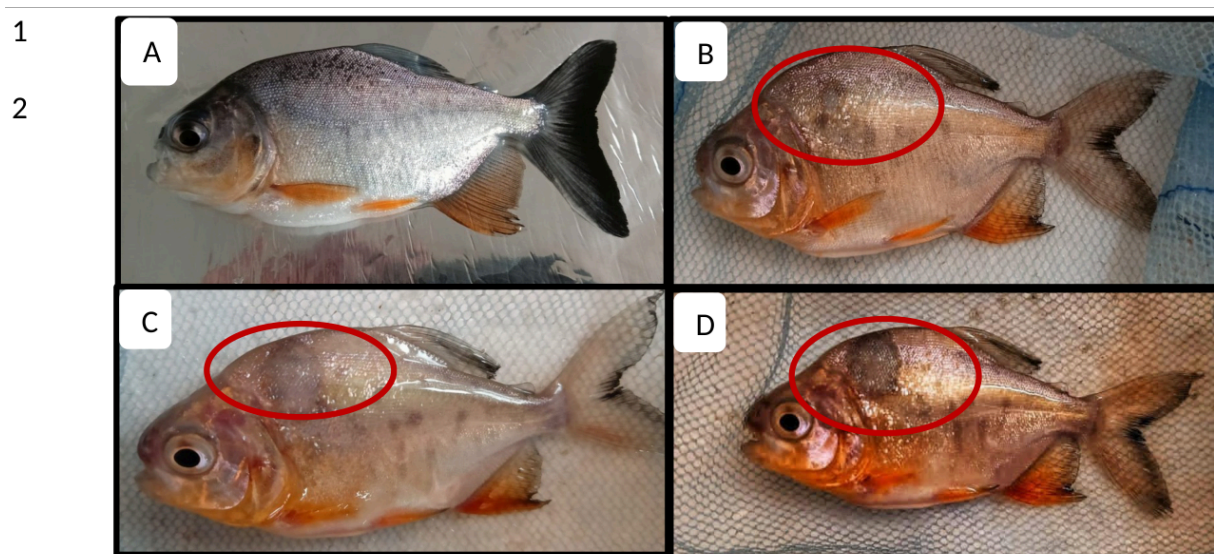


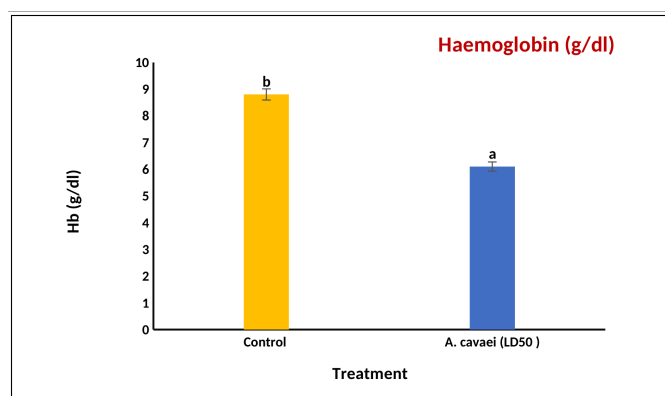
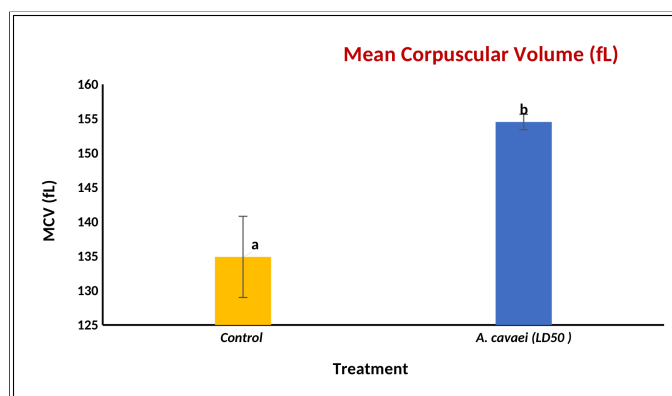
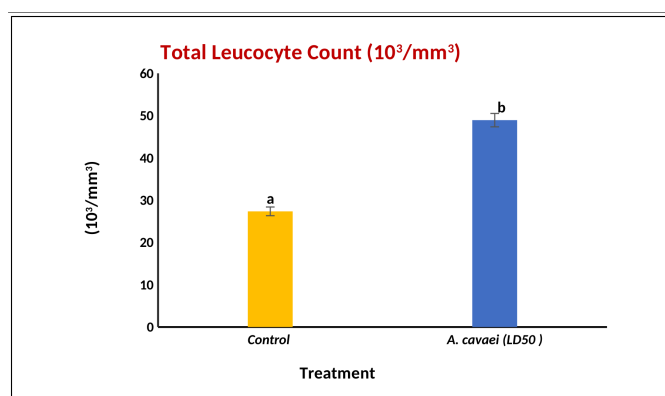
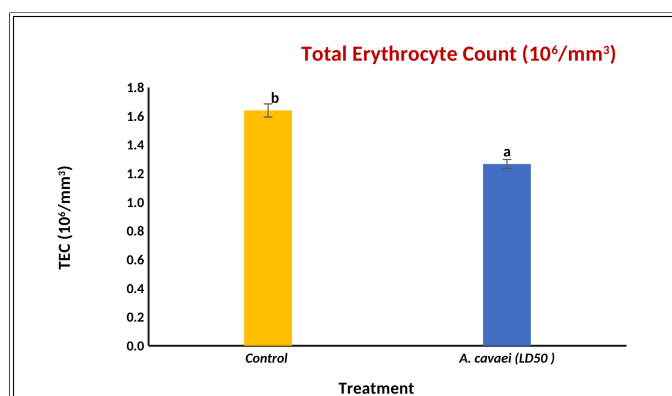
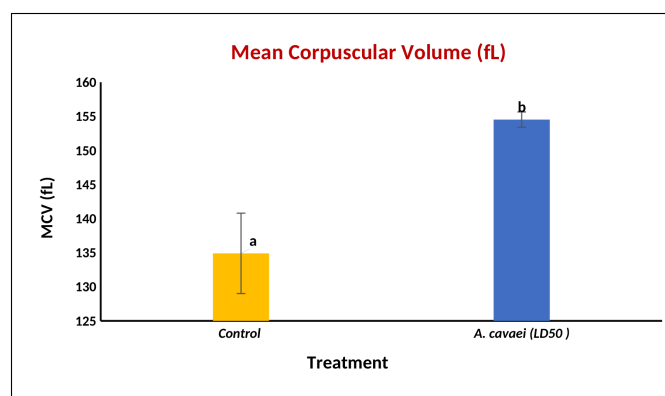
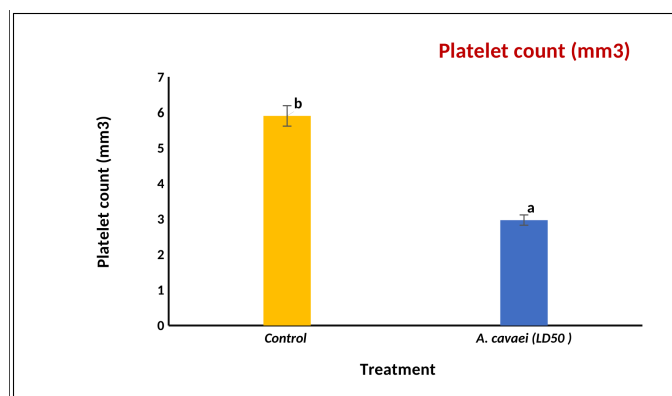
Figure 2. Progression of *Aeromonas caviae* infection in *Piaractus brachypomus* following experimental injection: (A) At the time of injection (baseline, healthy appearance); (B) 4th day post-injection (Stage I, early dorsal lesion indicated); (C) 6th day post-injection (Stage II, progressing red lesion); (D) 8th day post-injection (Stage III, advanced ulcerative lesion)

3.5 Biochemical parameters

A significant alteration in serum biochemical profiles was observed in red-bellied pacu *Piaractus brachipomus* challenged with *Aeromonas caviae* compared to the control group. Serum glucose levels exhibited a marked elevation ($p < 0.05$) in the infected group (85.4 mg/dl) relative to the control (72.3 mg/dl). Similarly, alkaline phosphatase (ALP) activity significantly increased ($p < 0.05$) in *Aeromonas caviae* challenged fish (157.2 U/L) compared to the control (41.3 U/L), while alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly elevated ($p < 0.05$) in the infected group (76.5 U/L and 122.3 U/L, respectively) versus the controls (38.5 U/L and 46.7 U/L). In contrast, serum albumin levels showed a significant reduction ($p < 0.05$) in infected fish (0.71 g/dl) compared to the control (0.89 g/dl). All values represent mean $\pm$ SE, with statistical significance determined at $p < 0.05$. Statistical analysis demonstrated significant differences between the challenged group and the control across all parameters ($p < 0.001$, $n = 15$).

3.6 Haematological parameters

A significant alteration in the haematological profile was observed in fish challenged with *Aeromonas caviae* compared to the control group. Haemoglobin levels showed a marked decline ($p < 0.05$) in *A. caviae* -infected fish (6.1 g/dl) relative to the control (8.9 g/dl), while total erythrocyte count (TEC) significantly decreased ($p < 0.05$) in the *A. caviae* LD₅₀ group ($1.27 \times 10^6/\text{mm}^3$) compared to the control ($1.64 \times 10^6/\text{mm}^3$). In contrast, total leucocyte count (TLC) exhibited a significant elevation ($p < 0.05$) in *A. caviae* -infected fish ($49.1 \times 10^3/\text{mm}^3$) versus the control ($27.8 \times 10^3/\text{mm}^3$), indicating an active immune response, and platelet counts showed a significant reduction ($p < 0.05$) in the infected group ($3.0 \times 10^3/\text{mm}^3$) relative to the control ($5.9 \times 10^3/\text{mm}^3$). Erythrocyte indices also changed notably, with mean corpuscular volume (MCV) significantly increasing ($p < 0.05$) in *A. caviae* -infected fish (154.5 fL) compared to the control (134.9 fL), mean corpuscular haemoglobin (MCH) elevating significantly ($p < 0.05$) in infected fish (35.2 pg) relative to the control (28.7 pg), and mean corpuscular haemoglobin



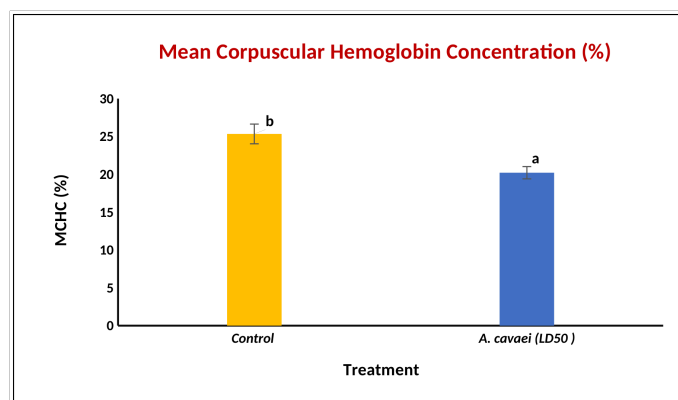


Figure 3. Comparative haematological parameters in *Aeromonas caviae* infected *Piaractus brachyomus* versus control fish at 96 h post-infection. Values represent mean $\pm$ SE ($n = 5$ per replicate), with asterisks indicating significant differences ($p < 0.05$) between infected and control groups.

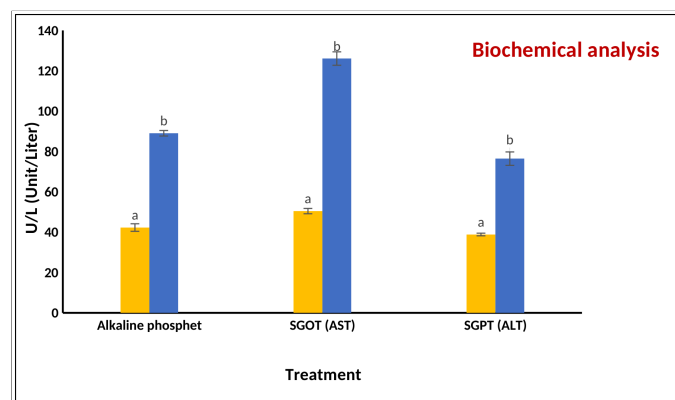
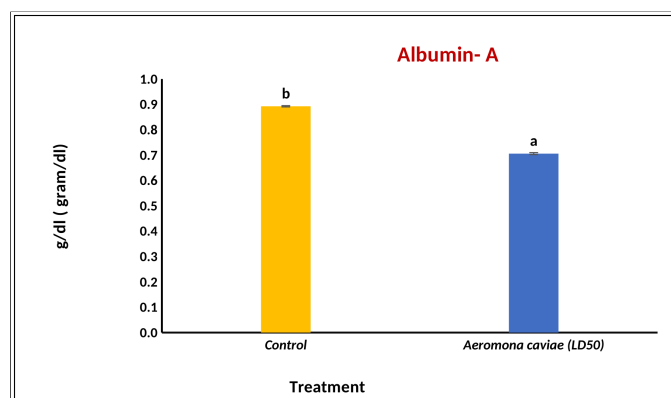
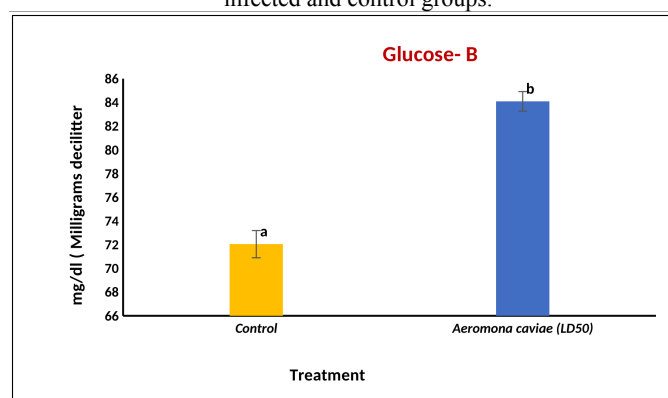


Figure 4. Comparative analysis of key serum biochemical parameters showing significant modulation in *Aeromonas veronii* (LD₅₀)-infected *Piaractus brachyomus* versus control. A = serum Albumin; B = glucose; C = serum levels of alkaline phosphatase (ALP), SGOT (serum glutamic-oxaloacetic transaminase), and SGPT (serum glutamic-pyruvic transaminase) were measured in control fish and fish injected with *Aeromonas caviae* at the median lethal dose (LD₅₀). Biochemical enzyme activities were markedly elevated in *Aeromonas caviae* -infected fish compared to controls, reflecting hepatic stress and systemic physiological response to bacterial infection. Values represent mean $\pm$ SE ($n = 5$ per replicate), with different letters on the top of the column indicating significant differences ($p < 0.05$) between infected and control groups.



centration (MCHC) decreasing significantly ($p < 0.05$) in the *A. caviae* group (20.3%) versus the control (25.7%). All values are presented as mean $\pm$ SE, with statistical significance at $p < 0.05$. All haematological alterations in the infected group, including reduced Hb ($p = 0.002$) and elevated TLC ($p < 0.001$), were statistically significant compared to the control group ($n = 15$).22



3.7 Multisystemic histopathological alterations

Histopathological analysis of tissues from red-bellied pacu (*Piaractus brachyomus*) experimentally infected with *Aeromonas caviae* strain BLB-05 at the LD₅₀ dosage revealed profound, statistically significant ($p < 0.001$) architectural disruptions across all target organs compared to uninfected controls (Table 2). Semi-quantitative lesion severity scoring confirmed severe tissue damage (> 2.4 on a 3-point scale) across gills, liver, and kidney (Table 2).

Table 2. Semi-quantitative lesion severity scores (0–3 scale) in gill, liver, and kidney of *Aeromonas caviae* -infected versus control red-bellied pacu (*Piaractus brachyomus*) at 96 h post-infection

Organ	Lesion	Control (Mean $\pm$ SE)	Infected (Mean $\pm$ SE)	<i>t</i> -value	<i>p</i> -value
Gill	Lamellar fusion	0.1 $\pm$ 0.1	2.8 $\pm$ 0.3	12.5	< 0.001
Gill	Epithelial hyperplasia	0.0 $\pm$ 0.0	2.6 $\pm$ 0.4	9.8	< 0.001
Liver	Vacuolation	0.2 $\pm$ 0.1	2.9 $\pm$ 0.2	15.2	< 0.001
Liver	Karyorrhexis	0.0 $\pm$ 0.0	2.7 $\pm$ 0.3	11.9	< 0.001
Kidney	Tubular necrosis	0.1 $\pm$ 0.1	2.8 $\pm$ 0.2	14.3	< 0.001
Kidney	MMC aggregates	0.3 $\pm$ 0.2	2.4 $\pm$ 0.4	7.6	< 0.001

Values are presented as mean $\pm$ SE; $n = 15$ sections/organ/group. Statistical analysis was performed using Student's *t*-test; $df = 28$.

Gill

Control gills exhibited intact primary filaments with regular secondary lamellae, pristine epithelium, and unobstructed interlamellar spaces (H&E; $\times 20$). In contrast, infected gills displayed multifocal degenerative and reactive lesions, leading to a significant increase in secondary lamellar fusion scores (2.8 ± 0.3 vs control 0.1 ± 0.1 ;

$t = 12.5$, $p < 0.001$) and epithelial hyperplasia (2.6 ± 0.4 vs control 0.0 ± 0.0 ; $t = 9.8$, $p < 0.001$) (Table 2). Pathological alterations included lamellar curling (thin arrows), shortening (thick arrows, Fig. I; yellow arrow, $\times 100$), and complete lamellar destruction (stars) with epithelial lifting and fusion (arrowheads, Fig. B; $\times 40$). Prominent desquamation of epithelial cells (yellow arrows, Fig. C; $\times 100$), clubbing of lamellar tips (yellow arrows, Fig. D; $\times 100$), and hyperplasia

(Fig. G; $\times 100$) reflected a proliferative response to bacterial exotoxins and severe hypoxic stress. Secondary lamellar curling (green arrow, Fig. E; $\times 100$) further compromised respiratory efficiency. Furthermore, infected tissues showed marked infiltration of inflammatory cells (yellow arrows, Fig. B) alongside intravascular erythrocytic alterations, including poikilocytosis with elliptocytes (green arrows, Fig. H; $\times 100$), partial lysis (green arrow), and hemolytic debris (yellow arrow, Fig. F; black arrow for intact erythrocytes; $\times 10$

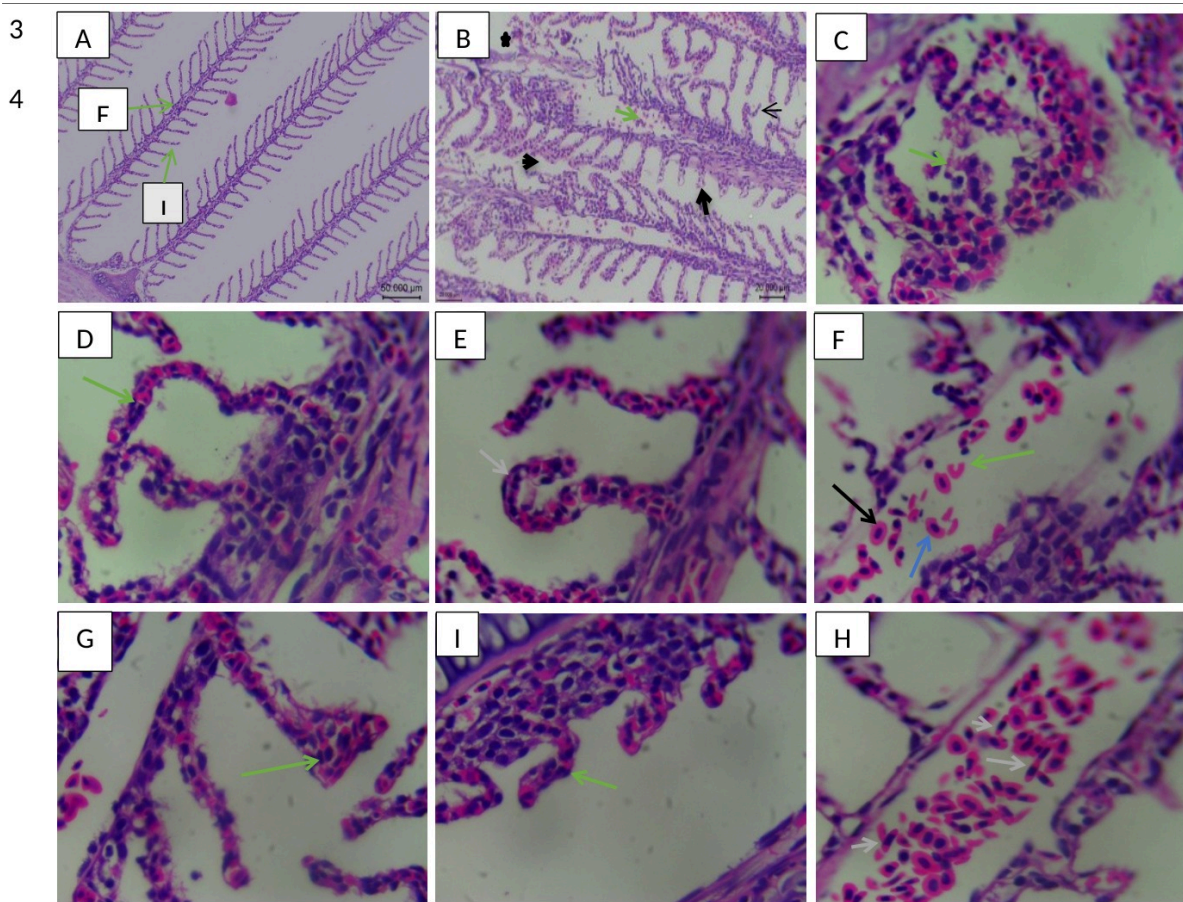


Figure 5. Gill histopathology (H&E). (A) Control: normal filaments (F) and lamellae (L) ($\times 20$; fusion score 0.1 ± 0.1 , hyperplasia score 0.0 ± 0.0). (B) *A. caviae*-infected (LD_{50}): severe fusion (2.8 ± 0.3 , $p < 0.001$) and hyperplasia (2.6 ± 0.4 , $p < 0.001$). Lamellar curling (thin arrow), shortening (thick arrow), destruction (star), fusion (arrowhead), and inflammatory infiltration (yellow arrow) ($\times 40$); (C) Desquamation (yellow arrow) ($\times 100$); (D) Tip clubbing (yellow arrow) ($\times 100$); (E) Lamellar curling (green arrow) ($\times 100$); (F) Erythrocytes (black arrow), partial lysis (green arrow), and hemolytic debris (yellow arrow) ($\times 100$); (G) Hyperplasia ($\times 100$); (H) Poikilocytic elliptocytes (green arrows) ($\times 100$); (I) Lamellar shortening (yellow arrow) ($\times 100$). Scale bar = $50\mu\text{m}$ (see Table 2).

Kidney

Uninfected kidneys exhibited normal glomerular and tubular architecture, characterized by well-defined renal corpuscles and uniform tubular epithelium (H&E; $\times 20$). Infected kidneys displayed hallmark lesions of acute bacterial nephritis, with a significant elevation in tubular necrosis scores (2.8 ± 0.2 vs control 0.1 ± 0.1 ; $t = 14.3$, $p < 0.001$) and melanomacrophage center (MMC) aggregate density (2.4 ± 0.4 vs control 0.3 ± 0.2 ; $t = 7.6$, $p < 0.001$) (Table 2). Pyknosis, marked by shrunken, hyperchromatic nuclei (yellow arrows in Figs. B and D; H&E; $\times 100$), indicated early cellular necrosis due to bacterial exotoxins. Prominent MMC aggregates (yellow arrow in Fig. C; H&E; $\times 40$) signified heightened phagocytic activity and cellular debris removal in response to persistent infection. Advanced tubular necrosis and vascular disruption were evidenced by karyolysis

with pale, dissolved chromatin (blue arrow in Fig. D; H&E; $\times 100$) alongside widespread hemorrhage (star in Fig. D). Severe hemorrhagic congestion with dilated vessels and extravascular erythrocytes (green arrow in Fig. E; H&E; $\times 40$) underscored endothelial damage and septicemic spread.

Liver

Control liver tissues displayed normal hepatic architecture with distinct polygonal hepatocytes arranged in cord-like patterns, prominent spherical central nuclei, and intact sinusoids (H&E; $\times 20$). Conversely, liver sections from *A. caviae*-infected fish revealed extensive hepatocellular damage, supported by a statistically significant increase in cytoplasmic vacuolation scores (2.9 ± 0.2 vs control 0.2 ± 0.1 ; $t = 15.2$, $p < 0.001$) and nuclear karyorrhexis (2.7 ± 0.3 vs control 0.0 ± 0.0 ; $t = 11.9$, $p < 0.001$) (Table 2).

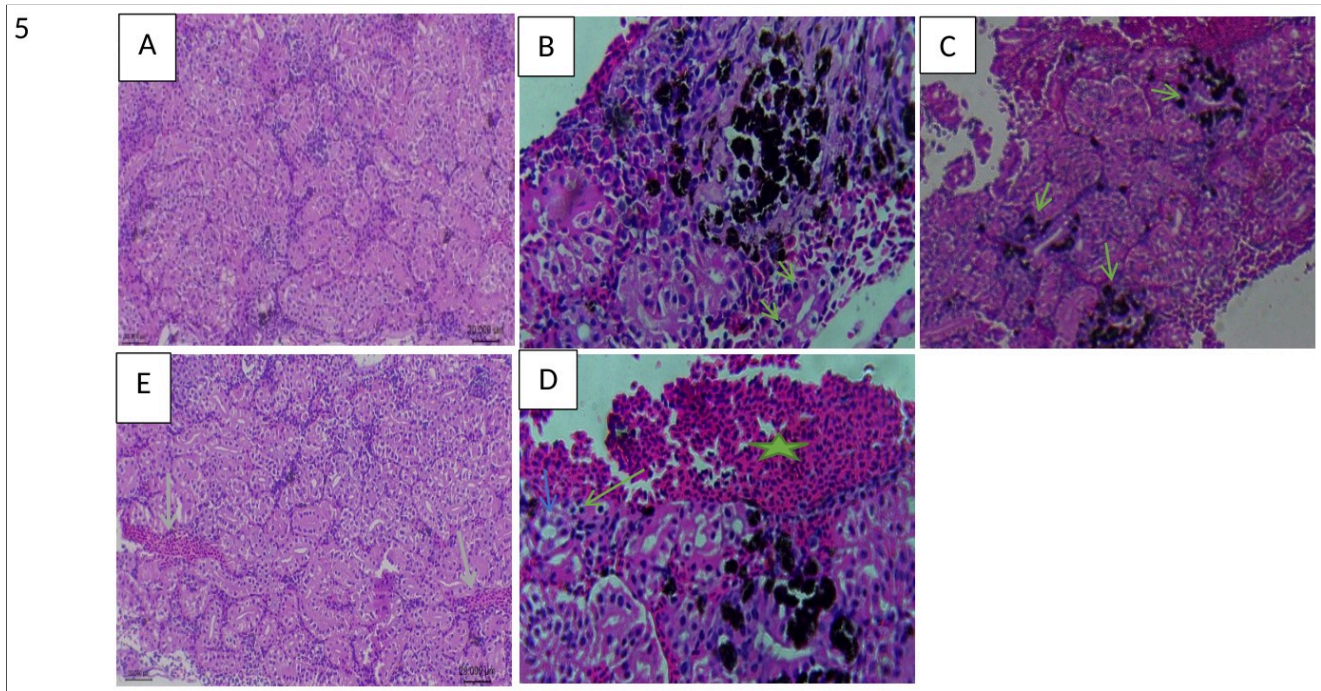


Figure 6. Kidney histopathology (H&E). (A) Control: normal architecture ($\times 20$; tubular necrosis score 0.1 ± 0.1 , MMC aggregate score 0.3 ± 0.2). (B–E) *A. caviae*-infected (LD_{50}): severe tubular necrosis (2.8 ± 0.2 , $p < 0.001$) and MMC aggregate density (2.4 ± 0.4 , $p < 0.001$). (B) Pyknosis of cells (yellow arrow) ($\times 100$); (C) Melanomacrophage centre (yellow arrow) ($\times 40$); (D) Pyknosis (yellow arrow), karyolysis (blue arrow), and haemorrhage (star) ($\times 100$); (E) Haemorrhagic congestion (green arrow) ($\times 40$). Scale bar = $50\mu\text{m}$ (see Table 2).

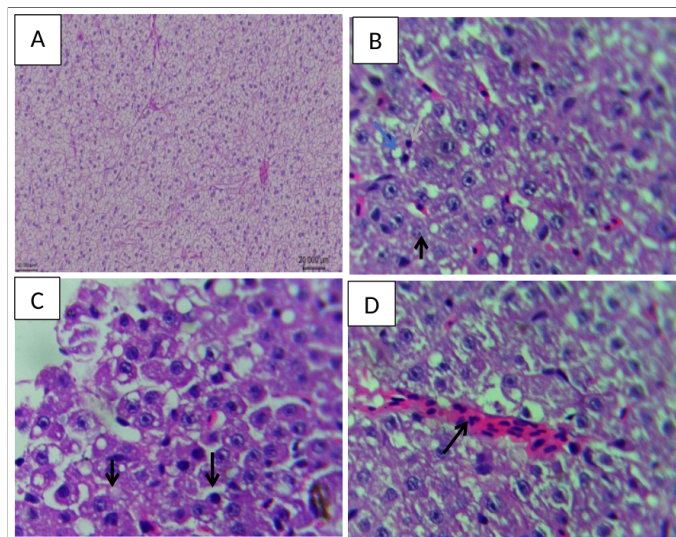


Figure 7. Liver histopathology (H&E). (A) Control: normal hepatic architecture ($\times 20$; vacuolation score 0.2 ± 0.1 , karyorrhexis score 0.0 ± 0.0). (B–D) *A. caviae*-infected (LD_{50}): severe vacuolation (2.9 ± 0.2 , $p < 0.001$) and karyorrhexis (2.7 ± 0.3 , $p < 0.001$). (B) Pyknosis of cells (green arrow), karyorrhexis (yellow arrow), and cytoplasmic vacuoles (blue arrow) ($\times 100$); (C) Pyknosis (yellow arrow) and karyolysis (green arrow) ($\times 100$); (D) Severe haemorrhagic congestion (yellow arrow) ($\times 100$). Scale bar = $50\mu\text{m}$ (see Table 2).

Infected hepatic parenchyma exhibited diffuse lipid and hydropic vacuolation, cytoplasmic degeneration, and disruption of hepatic cords. Nuclear alterations were widespread, characterized by marked pyknosis with condensed hyperchromatic nuclei, karyorrhexis (fragmented nuclei), and focal karyolysis (dissolved chromatin). Additionally, severe vascular pathologies including sinusoidal congestion, localized hemorrhages with extravasated erythrocytes, and focal parenchymal necrosis were prominent, confirming acute toxic liver injury and septicemic hepatic failure induced by bacterial exotoxins.

Discussion

Pathogenicity and Median Lethal Dose (LD_{50}) Determination

In the current study, probit analysis yielded $LD_{50} = 3.02 \times 10^7 \text{CFU mL}^{-1}$ (95%CI = $2.10\text{--}4.50 \times 10^7 \text{CFU mL}^{-1}$), Probit regression: $Y = 0.591 + 2.185 \log_{10} \text{concentration}$, $R^2 = 0.984$ for *A. caviae* strain BLB-05 in *P. brachypomus*, suggesting that *A. caviae* exerts significant pathogenic effects in closely related characids. While this indicates an intermediate level of virulence compared to the more hyper-virulent *A. veronii* ($7.8 \times 10^6 \text{CFU mL}^{-1}$) previously reported in pacu, it demonstrates comparable lethal toxicity to *A. hydrophila* strains (1.8×10^6 to $4.5 \times 10^7 \text{CFU mL}^{-1}$) documented in South American characids (Claudio et al., 2019; Fadel et al., 2025) [7, 11].

A clear progression of behavioural and clinical abnormalities was observed in red-bellied pacu *Piaractus brachypomus* following LD_{50} challenge with *Aeromonas caviae*. During the early phase of infection, fish displayed reduced activity, loss of coordinated swimming, erratic movement, and excessive mucus secretion, indicating the onset of physiological stress and epithelial irritation. As the infection progressed, more severe external manifestations became evident, including skin discoloration, perioral erythema, scale loss, ulcerative lesions, and hemorrhagic patches, all of which are typical symptoms associated with motile *Aeromonas* septicemia (MAS) (Harikrishnan et al., 2010). Similar behavioral disturbances accompanied by systemic tissue damage have been reported by Chandrarathna et al. (2018) [4] in zebrafish experimentally infected with *Aeromonas* species, where progressive organ necrosis and septicemic conditions were observed. These comparable findings highlight the invasive and systemic nature of *Aeromonas* infections across different fish hosts. The overall consistency of behavioural alterations along with hematological, biochemical, and histopathological disturbances observed in the present study further confirms that *A. caviae* infection leads to rapid systemic dissemination, causing multi-organ dysfunction and demonstrating its significant pathogenic potential in pacu culture systems.

Haematological alterations

Haematological parameters are widely recognized as sensitive indicators of physiological stress and host immune responses during bacterial infections in fish. In the present study, infection with *Aeromonas*

caviae resulted in significant reductions in haemoglobin (Hb) concentration and total erythrocyte count (TEC), suggesting the development of anemia in experimentally challenged fish. Such reductions may arise from bacterial hemolysin activity, erythrocyte membrane damage, or suppression of erythropoiesis under systemic infection, findings that align with previous reports in fish affected by *Aeromonas* infections (Copatti *et al.*, 2019; Khalil *et al.*, 2017) [1, 9]. In contrast, infected fish exhibited a significant increase in total leukocyte count, indicating stimulation of host immune defenses in response to bacterial invasion. Leukocytosis is a typical response during acute infections, reflecting mobilization and proliferation of immune cells aimed at combating Gram-negative bacterial pathogens (Esteban, 2012; Claudiano *et al.*, 2019) [10, 7]. A pronounced reduction in thrombocyte count was also observed, suggesting systemic septicemic conditions. Thrombocytopenia during bacterial infections may result from toxin-mediated damage to hematopoietic tissues or increased utilization of thrombocytes during inflammatory and hemorrhagic processes, a pattern commonly reported in *Aeromonas*-induced septicemia (Zhao *et al.*, 2025) [30]. Furthermore, alterations in erythrocyte indices, including increased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) alongside reduced mean corpuscular hemoglobin concentration (MCHC), indicate the development of macrocytic-hypochromic anemia. Such hematological profiles are typically associated with infection-induced stress, cellular swelling, or compensatory erythropoietic responses during systemic disease (Copatti *et al.*, 2019) [9]. Collectively, these hematological disturbances reflect severe physiological stress, immune activation, and hematopoietic disruption resulting from *A. caviae* infection, thereby supporting the systemic nature and pathogenic impact of the bacterium in pacu under experimental conditions.

Serum biochemical alterations

The significant elevation in serum glucose levels observed in *Aeromonas caviae*-infected red-bellied pacu *Piaractus brachipomus* compared with the control group reflects a typical physiological stress response induced during bacterial infection. Such hyperglycemic conditions are generally mediated through cortisol release, which stimulates glycogenolysis and gluconeogenesis to meet increased metabolic energy demands during immune activation and disease progression (Bojarski *et al.*, 2025) [3]. Activation of the hypothalamic–pituitary–interrenal (HPI) axis during bacterial invasion results in mobilization of glucose reserves, a phenomenon frequently reported in fish exposed to pathogenic stress. Similar elevations in blood glucose have been documented in several fish species challenged with *Aeromonas* spp., confirming its reliability as an indicator of physiological stress during infection (Harikrishnan *et al.*, 2011; Chen *et al.*, 2019) [17, 5]. A pronounced increase in serum alkaline phosphatase (ALP) activity was also recorded in infected fish, suggesting hepatic and biliary stress associated with systemic bacterial infection. Elevated ALP activity is commonly linked with hepatobiliary dysfunction and tissue damage resulting from septicemic conditions. During infection, cellular disruption of hepatocytes and biliary epithelial tissues results in leakage of this enzyme into circulation, a response similarly observed in *Aeromonas*-infected freshwater fishes such as crucian carp and rainbow trout (Liu *et al.*, 2022) [21]. The observed increase in ALP levels in the present study corresponds with histopathological evidence of hepatic degeneration, reinforcing its usefulness as a biochemical marker for diagnosing aeromoniasis in cultured fish (Chen *et al.*, 2022) [6]. Conversely, serum albumin levels declined markedly in infected fish compared to controls, indicating hypoproteinemia resulting from impaired hepatic protein synthesis and increased vascular permeability during systemic infection. Bacterial toxins and inflammatory mediators are known to damage vascular and hepatic tissues, leading to protein leakage and enhanced protein catabolism under acute inflammatory conditions (Harikrishnan *et al.*, 2011) [17]. Similar reductions in albumin concentration have been reported in fish suffering from *Aeromonas*-associated skin and systemic infections (Svobodová *et al.*, 2006; Liu *et al.*, 2022) [28, 21]. Reduced albu-

min levels may further compromise osmotic regulation and immune protein transport, thereby aggravating disease severity in infected pacu populations (Liu *et al.*, 2024) [19]. Marked increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were also observed following *A. caviae* infection, indicating severe hepatocellular damage and membrane disruption. These transaminases are commonly referred to as leakage enzymes, released into circulation when hepatocytes undergo necrosis or cellular injury. Comparable elevations in these enzymes have been reported in fish experimentally infected with pathogenic *Aeromonas* species, including Nile tilapia and other freshwater models (Farag *et al.*, 2021). The higher AST relative to ALT observed in infected fish further suggests involvement of extrahepatic tissues such as muscle and kidney, findings that correspond with the tissue damage detected histopathologically in the present study (Akani *et al.*, 2016) [2]. Collectively, these biochemical disturbances confirm the systemic pathogenic impact of *A. caviae* in pacu and demonstrate patterns consistent with bacterial septicemia reported across global aquaculture systems (Chen *et al.*, 2019) [5].

Multisystemic histopathological alterations

Histopathological examination in the present study revealed extensive, statistically significant tissue damage in red-bellied pacu (*Piaractus brachipomus*) experimentally infected with *Aeromonas caviae* strain BLB-05, confirming the development of severe systemic septicemia. Semi-quantitative lesion severity scoring (0–3 scale) at 96 h post-infection demonstrated a marked, statistically significant ($p < 0.001$) increase in pathological alterations across all evaluated organs compared to the control group (Table 2).

Among the examined organs, gill tissues exhibited prominent pathological alterations. Infected fish showed a significantly higher score for secondary lamellar fusion (2.8 ± 0.3 vs control 0.1 ± 0.1 ; $t = 12.5$, $p < 0.001$) and epithelial hyperplasia (2.6 ± 0.4 vs control 0.0 ± 0.0 ; $t = 9.8$, $p < 0.001$) (Table 2). Other branchial changes included epithelial lifting, lamellar clubbing and curling, desquamation, inflammatory cell infiltration, hemorrhagic deposits, and erythrocyte lysis. Such lesions substantially reduce respiratory surface area and compromise gas exchange efficiency while simultaneously disrupting mucosal protective barriers, thereby increasing susceptibility to further pathogenic invasion. Similar gill pathologies have been reported in pacu and other teleosts experimentally infected with pathogenic *Aeromonas* species, indicating that branchial tissues are primary targets during septicemic infections (Carraschi *et al.*, 2012).

The liver of infected fish showed pronounced pathological changes characterized by cytoplasmic vacuolation (2.9 ± 0.2 vs control 0.2 ± 0.1 ; $t = 15.2$, $p < 0.001$) and severe karyorrhexis (2.7 ± 0.3 vs control 0.0 ± 0.0 ; $t = 11.9$, $p < 0.001$) (Table 2). Associated lesions included nuclear pyknosis, hemorrhage, and vascular congestion. These alterations indicate acute hepatocellular degeneration and necrosis resulting from bacterial toxin action and systemic inflammatory responses. Since the liver plays a central role in metabolism, detoxification, and immune regulation, hepatic impairment significantly contributes to physiological imbalance during infection. Comparable hepatic degeneration has been documented in teleost fishes infected with *Aeromonas* species, where systemic infection leads to hepatotoxic effects and metabolic disruption (Carraschi *et al.*, 2012).

Renal tissues also exhibited severe structural damage, highlighted by significant tubular necrosis (2.8 ± 0.2 vs control 0.1 ± 0.1 ; $t = 14.3$, $p < 0.001$) and increased melanomacrophage center (MMC) aggregate formation (2.4 ± 0.4 vs control 0.3 ± 0.2 ; $t = 7.6$, $p < 0.001$) (Table 2). Other changes included nuclear pyknosis, karyolysis, and hemorrhagic congestion, indicating severe impairment of excretory and hematopoietic functions. The head kidney in teleost fishes functions as a major hematopoietic organ; therefore, its degeneration directly contributes to haematological disturbances observed during infection. Additionally, the significant increase in MMC density reflects enhanced phagocytic activity, heme recycling, and cellular debris pro-

cessing associated with chronic inflammation and tissue degradation (Claudio *et al.*, 2019) [7].

The severe systemic pathological changes observed in *P. brachypomus* can be directly attributed to the coordinated action of *A. caviae* virulence factors. Bacterial hemolysins (*hly*, *aer*) and extracellular proteases (*ela*) released during systemic proliferation account for the extensive erythrocyte lysis, vascular injury, and parenchymal tissue necrosis seen across the gills, liver, and kidney. Furthermore, flagellar assembly proteins (*fla*) likely facilitated rapid host colonization and tissue invasion, leading to fulminant septicemia and severe multi-organ dysfunction.

5. Conclusion

This study establishes *Aeromonas caviae* (strain BLB-05) as a highly virulent pathogen in red-bellied pacu (*Piaractus brachypomus*), with an LD₅₀ of 3.02×10^7 CFU/mL inducing fulminant motile *Aeromonas* septicemia (MAS). Experimental challenges revealed dose-dependent mortality, progressive ulcerative dermatitis, behavioral aberrations, and systemic multi-organ dysfunction, corroborated by severe haematological disruptions (anemia, leukocytosis, thrombocytopenia), biochemical stress markers (hyperglycemia, elevated AST/ALT/ALP, hypoalbuminemia), and histopathological lesions in gills, liver, and kidneys. These findings highlight *A. caviae*'s potent virulence via exotoxins, hemolysins, and proteases, underscoring its threat to emerging pacu aquaculture in India. Proactive surveillance, strain-specific probiotics, and phototherapeutic interventions are recommended to mitigate economic losses and support sustainable farming of this high-value species.

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Author contribution

A.F. writing—original draft preparation; A.S. -conceptualization, data analysis, and editing; T.I.C., N.P., I.K., and S.A.N., P.B, writing review and editing. All authors have read and approved the final manuscript.

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Data availability

- The author stated that the present work has not been previously published, nor is it before another journal for consideration.

Conflict of interest

Authors declare no conflict of interest

Ethics statement

This study complies with India's animal welfare regulations. The animals were treated in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All research protocols were approved by the Institutional Biosafety Committee (CIFE/IBSC/2022-23) at the ICAR-Central Institute of Fisheries Education, Mumbai.

References

1. Abou Khalil NS, Abd-Elkareem M, Sayed AH. *Nigella sativa* seed protects against 4-nonylphenol-induced haematotoxicity in *Clarias gariepinus* (Burchell, 1822): Oxidant/antioxidant rebalance. *Aquaculture Nutrition*. 2017;23(6):1285-1293.
2. Akani NP, Gabriel UU. Enzymatic responses of *Clarias gariepinus* (Burchell, 1822) exposed to sub-lethal concentrations of an oilfield wastewater. *Journal of Applied Biology & Biotechnology*. 2016;4(5):026-032.
3. Bojarski B, Witeska M, Kondera E. Blood biochemical biomarkers in fish toxicology—a review. *Animals*. 2025;15(7):965.
4. Chandrarathna HPSU *et al.* Outcome of co-infection with opportunistic and multidrug resistant *Aeromonas hydrophila* and *A. veronii* in zebrafish: Identification, characterization, pathogenicity and immune responses. *Fish & Shellfish Immunology*. 2018;80:573-581.
5. Chen F *et al.* Isolation, identification and characteristics of *Aeromonas veronii* from diseased crucian carp (*Carassius auratus gibelio*). *Frontiers in Microbiology*. 2019;10:2742.
6. Chen P *et al.* *Aeromonas veronii* infection remarkably increases expression of lysozymes in grass carp (*Ctenopharyngodon idellus*) and injection of lysozyme expression cassette along with QCDC adjuvant significantly upregulates immune factors and decreases cumulative mortality. *Microbial Pathogenesis*. 2022;169:105646.
7. Claudio GS *et al.* Hematological and immune changes in *Piaractus mesopotamicus* in the sepsis induced by *Aeromonas hydrophila*. *Fish & Shellfish Immunology*. 2019;87:91-100.
8. Claudio GS *et al.* Hematological and immune changes in *Piaractus mesopotamicus* in the sepsis induced by *Aeromonas hydrophila*. *Fish & Shellfish Immunology*. 2019;87:91-100.
9. Copatti CE, Baldisserotto B, de Freitas Souza C, Garcia L. Protective effect of high hardness in pacu juveniles (*Piaractus mesopotamicus*) under acidic or alkaline pH: Biochemical and haematological variables. *Aquaculture*. 2019;502:250-257.
10. Esteban MA. An overview of the immunological defenses in fish skin. *ISRN Immunology*. 2012:853470.
11. Fadel A, Mahmoud MA, Abdelsalam M, Eissa ESH, Sherif AH. *Aeromonas veronii* infection in cultured *Oreochromis niloticus*: prevalence, molecular and histopathological characterization correlated to water physicochemical characteristics, with the protective autochthonous probiotic. *Aquaculture International*. 2025;33:298.
12. Finney DJ. *Probit Analysis*. 3rd edn. Cambridge: Cambridge University Press; 1952.
13. Fresneda A, Lenis G, Agudelo E, Olivera Ángel M. Espermiación inducida y crioconservación de semen de cachama blanca (*Piaractus brachypomus*). *Revista Colombiana de Ciencias Pecuarias*. 2004;17(Suplemento):46-49.
14. Ghosh S, Datta MK. Farming of red-bellied pacu (*Piaractus brachypomus*) in India with special reference to West Bengal. *Fish. Chimes*. 2014;34(2&3):32-39.
15. Government of India. Guidelines to culture the red-bellied pacu fish in Indian aquaculture system (F.No 35029/8/2011/Fy(Trade) (E10310). 2024.
16. Gundi VAKB, Bogireddy D, Vundru AK, Arthala PK, Vadela MB, Karri S, Allam US, Gujjula MS, Kodali VP. Microbial pathogens in aquaculture: a review of emerging threats. *Academic Biology*. 2025;3:doi:10.20935/AcadBiol7814.
17. Harikrishnan R, Rani MN, Balasundaram C. Haematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Fish Physiology and Biochemistry*. 2011;38(1):67-76.
18. Kumar A, Pradhan PK, Das PC, Srivastava SM, Lal KK, Jena JK. Growth performance and compatibility of pacu, *Piaractus brachypomus* with Indian major carps in polyculture system. *Aquaculture*. 2018;490:236-239.
19. Liu W *et al.* Identification of *Aeromonas veronii* as the pathogen associated with massive mortality in bronze gudgeon (*Coreius heterodon*). *Animals*. 2024;14(16):2440.

20. Liu W *et al.* Identification of *Aeromonas veronii* as the pathogen associated with massive mortality in bronze gudgeon (*Coreius heterodon*). *Animals*. 2024;14(16):2440.
21. Liu Y, Chen Q, Li Y, Bi L, Jin L, Peng R. Toxic effects of cadmium on fish. *Toxics*. 2022;10(10):622.
22. Pandey V, Bhat RAH, Chandra S, Tandel RS, Dubey MK, Sharma P, Gehlot B, Dash P, Joshi R. Clinical signs, lethal dose and histopathological lesions in grass carp, *Ctenopharyngodon idella* experimentally infected with *Edwardsiella tarda*. *Microbial Pathogenesis*. 2021;161:105292.
23. Pessoa RBG, de Oliveira WF, Santa Clara Marques D, dos Santos Correia MT, Maciel de Carvalho EVM, Coelho LCBB. The genus *Aeromonas*: A general approach. *Microbial Pathogenesis*. 2019;130:81-94.
24. Pessoa RBG, Oliveira WF, Correia MTS, Fontes A, Coelho LCBB. *Aeromonas* and Human Health Disorders: Clinical Approaches. *Frontiers in Microbiology*. 2022;13:868890.
25. Sahoo PK, Mahapatra KD, Sahoo L, *et al.* Fish disease research in India: Present status and future strategies. *Indian Journal of Fisheries*. 2020;67(Special Issue):1-20.
26. Seshagiri B, Kumar A, Pradhan PK, Sood N, Kumar U, Satyavati C, Sravanti Y, Prasoon K, Ghosh A, Kantharajan G, Basheer VS, Chakrabarti PP, Das PC, Lal KK, Jena JK. Farming practices and farmers' perspective of a non-native fish red-bellied Pacu, *Piaractus brachipomus* (Cuvier, 1818) in India. *Aquaculture Research*. 2021;56(3):199-210.
27. Sharma A, Chanu TI, Nayak SK, Jahageerdar S, Krishna G. Pathogenesis of *Aeromonas caviae* in *Clarias magur*. *Microbial Pathogenesis*. 2022;169:105662.
28. Svobodová Z *et al.* *Aeromonas* causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): clinical pathology, haematology, and biochemistry. *Acta Veterinaria Brno*. 2006;75(2):171-177.
29. Xue M, Xiao Z, Li Y, Jiang N, Liu W, Meng Y, Fan Y, Zeng L, Zhou Y. Isolation, Identification and Characteristics of *Aeromonas caviae* from Diseased Largemouth Bass (*Micropterus salmoides*). *Fishes*. 2022;7(3):119.
30. Zhao LC *et al.* The deficiency of virulence factor AerA in *Aeromonas veronii* alleviates intestinal injury and microbiota disorders in largemouth bass (*Micropterus salmoides*). *Fish & Shellfish Immunology*. 2025;161:110281.

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Authors

Aniket Funde

Aquatic Environment and Health Management (AEHM) Division, ICAR–Central Institute of Fisheries Education (ICAR CIFE), Mumbai Maharashtra, India

Arun Sharma

Aquatic Environment and Health Management (AEHM) Division, ICAR–Central Institute of Fisheries Education (ICAR CIFE), Mumbai Maharashtra, India

TI Chanu

Aquaculture Division, ICAR–Central Institute of Fisheries Education (ICAR-CIFE), Mumbai Maharashtra, India

Isha Kumari

COFS, CCS Haryana Agricultural University Hisar, India

Swapnil Ananda Narsale

Aquatic Environment and Health Management (AEHM) Division, ICAR–Central Institute of Fisheries Education (ICAR CIFE), Mumbai Maharashtra, India

Pallavi Bhalavey

COFS, CCS Haryana Agricultural University Hisar, India

Dipalee Thombre

National Facility for Biopharmaceuticals, Khalsa college, Matunga, Mumbai, Maharashtra, India