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Original Article

Pathogenicity and systemic virulence of *Aeromonas veronii* in red-bellied pacu (*Piaractus brachypomus*): an experimental disease model

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Abstract
Aeromonas veronii is an important etiological agent of motile aeromonas septicemia (MAS) in aquaculture; however, its pathogenicity in the emerging cultured species red-bellied pacu (*Piaractus brachypomus*) remains poorly understood. Pacu fingerlings (10±2 g; *n* = 180) were intraperitoneally challenged with *A. veronii* strain BLB-01 (GenBank accession: MF370515) at concentrations ranging from 10^5 to 10^9 CFU mL^{-1}, following OECD guideline 203. The median lethal dose (LD_{50}) was estimated using probit analysis. Clinical signs, haematological and biochemical parameters, and histopathological alterations were evaluated at 96 h post-LD_{50} exposure. Probit analysis (Y = 0.591 + 2.185 $\log_{10}$ concentration; R^2 = 0.984) estimated the LD_{50} as 7.8×10^6 CFU mL^{-1} (95% CI: 5.2–11.4 ×10^6 CFU mL^{-1}). Infected fish exhibited progressive MAS symptoms over 7 days. Haematological analysis revealed significant anaemia (decreased haemoglobin and total erythrocyte count), leukocytosis (increased total leukocyte count), and thrombocytopenia (reduced platelet count). Biochemical changes included stress-induced hyperglycaemia (elevated glucose), hepatocellular damage (increased ALP, AST, and ALT), and hypoalbuminemia (reduced albumin). Histopathological examination demonstrated severe lesions, including gill lamellar fusion (2.8±0.3), hepatic vacuolation (2.9±0.2), and renal tubular necrosis (2.8±0.2). *Aeromonas veronii* exhibits high pathogenicity in pacu, with an LD_{50} of 7.8×10^6 CFU mL^{-1}, providing baseline data for challenge studies and the development of vaccines and probiotics. A combination of haematological and biochemical markers particularly increased TLC, reduced platelet count, and elevated liver enzymes offers a practical tool for rapid field-level diagnosis and surveillance of MAS, supporting improved health management in Indian pacu aquaculture.

Keywords: *Aeromonas veronii*; histopathology; LD_{50}; motile aeromonas septicemia; *Piaractus brachypomus*

1 | INTRODUCTION

Aquaculture, often called "underwater agriculture," is the fastest growing food producing sector worldwide, playing a crucial role in meeting the increasing global demand for protein (Mugimba *et al.* 2021). Aquaculture remains the fastest growing food production sector globally, surpas-

sing capture fisheries in total output for the first time in history. Global production reaching 130 million tonnes in 2022, aquaculture now contributes 57% of all aquatic food for human consumption, playing a pivotal role in food security and livelihoods, particularly in Asia, which accounts for 90% of production (FAO 2024). Disease is one of the primary constraints to aquaculture and a limiting factor for economic and socioeconomic development in India and many other countries across the world (Gundi *et al.* 2015). Among the various threats to aquaculture, infectious diseases caused by bacteria, viruses, parasites, and fungi are the most significant (Sahoo *et al.* 2020). Bacterial infections cause annual losses of more than USD 6 billion (FAO 2024), constitute a substantial proportion of freshwater fish mortalities, accounting for approximately 12.8% of reported disease incidence (Sahoo *et al.* 2020).

The red-bellied pacu (*Piaractus brachypomus*), also colloquially referred to as pacu fish, is regarded as a significant aquaculture species (Galib and Mohsin 2010, 2011). Interestingly, the species entered the Indian aquaculture sector through the 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.

Among bacterial pathogens, the genus *Aeromonas* is a major group of pathogenic bacteria that affect freshwater fishes (Pessoa *et al.* 2022). These bacteria are typically Gram-negative, rod-shaped, non spore forming, facultatively anaerobic bacteria that are ubiquitously distributed in nature (Pessoa *et al.* 2019). Among the species, *Aeromonas veronii* has emerged as a highly pathogenic bacterium responsible for severe disease outbreaks and mortalities in cultured fish, particularly in tilapia farming systems worldwide (Sundar Raj *et al.* 2019). Disease outbreaks due to *A. veronii* can result in substantial economic losses. It possesses a variety of virulence determinants, including aerolysin (aer), cytotoxic enterotoxin (act), serine protease (ser), and adhesion molecules (Aha, lip), which enable it to invade, colonize, and damage host tissues (Song *et al.* 2017). The pathogenicity of *A. veronii* is attributed to different virulence determinants, of which eerolysin was identified as the key toxin toxin involved in host tissue damage and systemic infection (Ran *et al.* 2018) Infection typically manifests as motile aeromonas septicemia (MAS), characterised by haemorrhagic lesions, behavioural abnormalities, and multisystemic pathological alterations.

Pacu is regarded as a significant aquaculture species in including Colombia, Brazil, Peru, Venezuela, Vietnam, Thailand, Malaysia, and Bangladesh (Kumar *et al.* 2018). The species possesses several valuable characteristics including hardiness, rapid growth, tolerance to a wide range of water quality parameters high quality meat, and an omnivorous feeding habit that making it an ideal candidate for aquaculture (Fresneda *et al.* 2004; Ghosh and Datta 2014). Interestingly, pacu entered the Indian aquaculture sector through the ornamental fish trade around 2003–2004 (Seshagiri *et al.* 2021). Recently, a survey conducted by ICAR- National Bureau of Fish Genetic Resources across three states (Tripura, Assam, and West Bengal) reported that of 351 farmers, 305 (approximately 87%) believed that farming pacu is profitable (Seshagiri *et al.* 2021). Though farmers appreciate the growth rate of this species, ambiguous feedback often comes about its impact on the overall pond production system. To regulate the farming of this exotic species, the Government of India issued strict biosecurity guidelines (F.No 35029/8/2011/Fy(Trade)(E10310)) (Government of India 2024). These regulations mandate that pacu culture must be restricted to controlled freshwater monoculture systems within flood-secure ponds equipped with peripheral fencing to prevent accidental escapes into open waters. Furthermore, the guidelines prohibit the release of pacu into natural water bodies and require farmers to procure seed exclusively from certified hatcheries to mitigate ecological and disease risks.

Pathogenicity studies on pacu are particularly crucial in the Indian context, where *P. brachypomus* has transitioned from ornamental trade to commercial aquaculture since 2003-2004, with 87% of surveyed farmers reporting profitability despite ambiguous pond impacts. Emerging bacterial pathogens like *A. veronii* pose significant risks amid unregulated hatcheries and releases into natural waters, potentially exacerbating economic losses (already >USD 6 billion globally from bacterial diseases) and biodiversity concerns in a sector contributing 90% of Asia's aquatic production (Singh 2018). Quantifying LD_{50} via standardized models (e.g. OECD 203) is essential for risk assessment, enabling precise challenge doses in vaccine trials (e.g. antigen optimization), probiotic efficacy tests (e.g. pathogen exclusion in gut models), and phage therapy development (e.g. reducing *Aeromonas* burden by 40–50% in synergy with liposomes). These baselines support immunoprophylaxis strategies, aligning with India's aquaculture growth needs while minimizing antibiotic reliance. Therefore, the present study was conducted to evaluate pathogenicity of *P. brachypomus* against *A. veronii* using an experimental fish diseases model.

2 | METHODOLOGY

2.1 Test chemicals and glassware

The chemicals, glassware, and plasticware used for vari-

ous analyses were obtained from Sigma (USA), SRL (India), Hi-media (India), Qualigen (India), and Merck (Germany).

2.2 Procurement of fish and acclimatisation

Pacu fish fingerlings, with an average weight of 10±2 g were procured from Ali Fish Farm, West Bengal, India. After arrival at the wet laboratory of ICAR-Central Institute of Fisheries Education, Mumbai, a standard protocol of husbandry was implemented to minimize stress and ensure proper disinfection. This included a dip in 1% NaCl solution for 30 sec, followed by a 2 min treatment in 2-ppm potassium permanganate ($KMnO_4$). The procured fish were acclimatised for 21 days in fiber reinforced plastic (FRP) tanks of 500L capacity with adequate aeration and rearing conditions Tanks used for the experimental purpose were also treated overnight with $KMnO_4$ solution (4 mgL^{-1}), flushed and cleaned with freshwater, filled, and aerated for 48 h. During this period, fish were fed ad libitum with pelleted feed of 25% crude protein twice daily. Daily fish observations were made to monitor for external and internal changes, feeding behaviour, swimming patterns, and signs of disease or stress. Water quality parameters, including temperature (°C), pH, dissolved oxygen (DO [mg $^{L-1}$]), alkalinity [mg L^{-1}], hardness [mg L^{-1}], and total ammonia-nitrogen (TAN [mg L^{-1}]), were monitored regularly according to APHA (2005) guidelines. A 30% water exchange was conducted every two days to remove excess feed and faecal matter, ensuring optimal rearing conditions for the fish.

2.3 Procurement of bacteria

The bacterial strains used in this study were *Aeromonas veronii* Strain BLB-01 (Gen Bank accession number: MF370515), 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.

2.4 Experimental design and challenge study for estimation of LD_{50}

The acute toxicity of *A. veronii* in pacu fish fingerlings was evaluated following the OECD guideline 203 for static renewal bioassays (OECD 1992). Because OECD 203 is intrinsically designed for chemical testing, specific modifications were implemented for a biological pathogen challenge. A total of 180 pacu fish fingerlings (average weight: 12±0.5 g) fish were randomly distributed into 18 tanks (10 fish per 50 L tank) under a completely randomized design provided with adequate aeration. Of these, 150 individuals were intraperitoneally injected with 0.1 mL bacterial suspensions of *A. veronii* at graded concentrations corresponding to McFarland standards 5–9 (T1 = 1×10^5; T2 = 1×10^6; T3 = 1×10^7; T4 = 2.4×10^8; T5 = 1×10^9 CFU mL^{-1}). The control group received an equal volume of sterile phosphate buffered saline (PBS). Each treatment, including the control, was carried out in triplicate (10 fish per replicate) to ensure reliability of the results. Throughout the 96 h exposure period, fish were not fed, and the tanks were left unsiphoned. It is worth noting that withholding feed and omitting tank siphoning over the 96-h experimental window represents a potential confounding influence on fish stress physiology and localized water quality. This protocol was necessary to prevent organic matter interaction with the bacterial suspension and to minimize physical handling stress during acute infection. However, because the parallel control group was subjected to the identical fasting and non-siphoning regime and maintained stable baseline haematological, biochemical, and histological profiles the severe multisystemic disruptions observed in the challenged groups can be definitively attributed to the pathogenicity of *A. veronii* rather than experimental artifacts.

2.5 Statistical methods for LD_{50} determination – conversion of percentage mortalities to probit and calculation of LD_{50}

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). Mortality data are presented in Table 1 for pacu fish fingerlings challenged with graded concentrations of *A. veronii* 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), 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.

2.6 Sample collection

Post-LD_{50} challenge (7.8×10^6 CFU mL^{-1}, 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 haematological 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.

2.7 Behavioural changes

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

2.8 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 anaesthetised 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 1ml BD tuberculinsyringe which was previously pre-rinced 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 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 analysers, 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).

Mean corpuscular volume (MCV) in femtoliters (fL) = packed cell volume (%) ×10 / RBC count in 10 6 cmm

Mean corpuscular haemoglobin (MCH) in picogram (pg) = Hb in g % ×10 / RBC count in 10 6 cmm

Mean corpuscular haemoglobin Concentration (MCHC) in grams per decilitre (g dL^{-1}) = Hb in g % ×100 / PCV%

2.9 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}.

2.10 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 (25–50%); 3 = severe (>50%). Scores from 3 sections per fish (n = 15 sections / organ / group) were averaged (mean ± SE). Inter-observer agreement: Cohen's κ = 0.87 (substantial). Differences analysed by Student's t-test with an α level of significance of 0.05.

2.11 Data analysis

Data are presented as mean ± 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 | RESULTS

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 28–30°C, pH of 7.8–8.1, dissolved oxygen levels of 5.6–6.0 mg L^{-1}, hardness levels of 187.5–189 mg L^{-1}, alkalinity levels of 132–137.5 mg L^{-1}, and total ammonia nitrogen (NH3-N) levels of 0.20–0.21 mg L^{-1}. Overall, minor variations were observed, but water quality remained stable compared to the control.

3.2 Experimental infection and median lethal dose (LD_{50}) determination

The mortality of pacu fish fingerlings experimentally in-

fected with *A. veronii* is summarised in Table 1. Mortality was absolute (100%) in the group injected with $1x10^9$ CFU mL^{-1} bacterial suspensions. No mortality were observed in the control group. Probit regression analysis (Y = 0.591 + 2.185 $\log_{10}$concentration; R^2 = 0.984) estimated the 96-h median lethal dose (LD_{50}) to be 7.8×10^6 CFU mL^{-1} (95% CI: 5.2–11.4 $\times10^6$ CFU mL^{-1}) (Figure 1). At or above this threshold concentration, infected fish exhibited characteristic clinical signs, including surface haemorrhages and abnormal swimming behaviour.

TABLE 1 Probit analysis data yielding LD_{50} = 7.8×10^6 CFU mL^{-1} (95% CI = 5.2–11.4 $\times10^6$). Regression equation: y = 0.591 + 2.185 $\log_{10}$ [CFU mL^{-1}], R^2 = 0.984 (also see Figure 1). SE of LD_{50} = ± 0.16 $\log_{10}$ units.

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	7	23.3	23.3	6	4.27
1×10^7	100	30	14	46.7	46.7	7	4.92
1×10^8	100	30	28	93.3	93.3	8	6.50
1×10^9	100	30	30	100	99.17	9	7.40

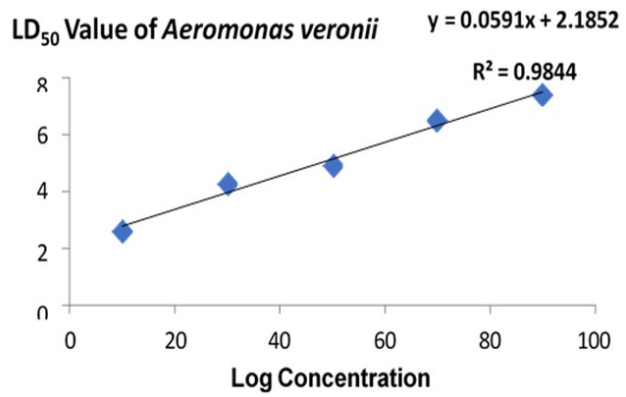

FIGURE 1 Probit regression plot showing median lethal dose (LD_{50}) of *Aeromonas veronii* strain BLB-01 (GenBank: MF370515) in *Piaractus brachypomus*. Probit regression: y = 0.591 + 2.185 $\log_{10}$ concentration, R^2 = 0.984. LD_{50} = 7.8×10^6 CFU mL^{-1} (95% CI = 5.2–11.4 $\times10^6$ CFU mL^{-1}) at probit 5.0 (also see Table 1; Finney 1952).

3.3 Behavioural changes and clinical signs

Clinical sign and behavioural alterations in *A. veronii* challenged pacu fish fingerlings progressed in a dose-dependent manner across the 7-day observation period (Figure 2A–2E). Following intraperitoneal injection at the LD_{50} dose, fish clinically normal appeared norma immediately after challenge (Figure 2A). By day 4 (stage I; Figure 2B), affected pacu began to exhibit erratic, uncoordinated and circular swimming behaviour, accompanied by mild mucus hypersecretion over the body surface and fins, indicating early irritation and activation of mucosal defences mechanisms. By day 5 (stage II; Figure 2C), clinical signs intensified, with conspicuous mucus accumulation on the skin and gills, reduced responsiveness to external stimuli, and the onset of faint cutaneous discoloration along the ventral region. On day 6 (stage III; Figure 2D), fish showed pronounced lethargy, loss of equilibrium, and distinct external lesions, including petechial to focal skin haemorrhages and perioral erythema around the mouth. By day 7 (stage IV; Figure 2E), severely affected individuals developed extensive ventral and perianal haemorrhages, marked erythema of the opercular and abdominal regions, emaciation, and moribund behaviour, which are characteristic of advanced motile aeromonas septicemia. Collectively, these clinical and behavioural manifestations suggest the high virulence and systemic nature of *A. veronii* infection in pacu.

3.4 Haematological parameters

Significant alterations in the haematological profiles were observed in fish challenged with *A. veronii* compared to the control group (Figure 3A–3G). Haemoglobin (Hb) levels showed a marked decline (*t*-test: t = 3.45, df = 28, p = 0.002) in *A. veronii*-infected fish (6.1±0.4 g dL^{-1}) relative to the control (8.9±0.3 g dL^{-1}). Packed cell volume (PCV) also significantly decreased (t = 4.12, df = 28, p < 0.001) from 28.5±1.2% in the control group to 19.8±1.0% in the infected group (Figure 3D). Similarly, the total erythrocyte count (TEC) was significantly lower (t = 3.89, df = 28, p = 0.001) in the *A. veronii* LD_{50} group ($1.28\pm0.1\times10^6$ cells μL^{-1}) compared to the control ($1.64\pm0.1\times 10^6$ cells μL^{-1}) (Figure 3E). In contrast, the total leukocyte count (TLC) exhibited a significant elevation (p < 0.05) in *A. veronii*-infected fish (48.6×10^3 cells μL^{-1}) compared to the control (27.8×10^3 cells μL^{-1}), indicating an active immune response (Figure 3F). Concurrently, platelet counts showed a significant reduction (p < 0.05) in the infected group (3.0×10^3 cells μL^{-1}) when compared to the control (5.9×10^3 cells μL^{-1}) (Figure 3B). Erythrocyte indices also changed notably: mean corpuscular volume (MCV) was significantly higher (p < 0.05) in infected fish (155.4 fL) than in the control (134.9 fL) (Figure 3D); mean corpuscular haemoglobin (MCH) increased significantly (p < 0.05)

in infected fish (35.2 pg) relative to the control (28.7 pg) (Figure 3C); and mean corpuscular haemoglobin concentration (MCHC) significantly decreased ($p < 0.05$) from 25.7% in the control to 20.4% in the infected group (Figure 3G).

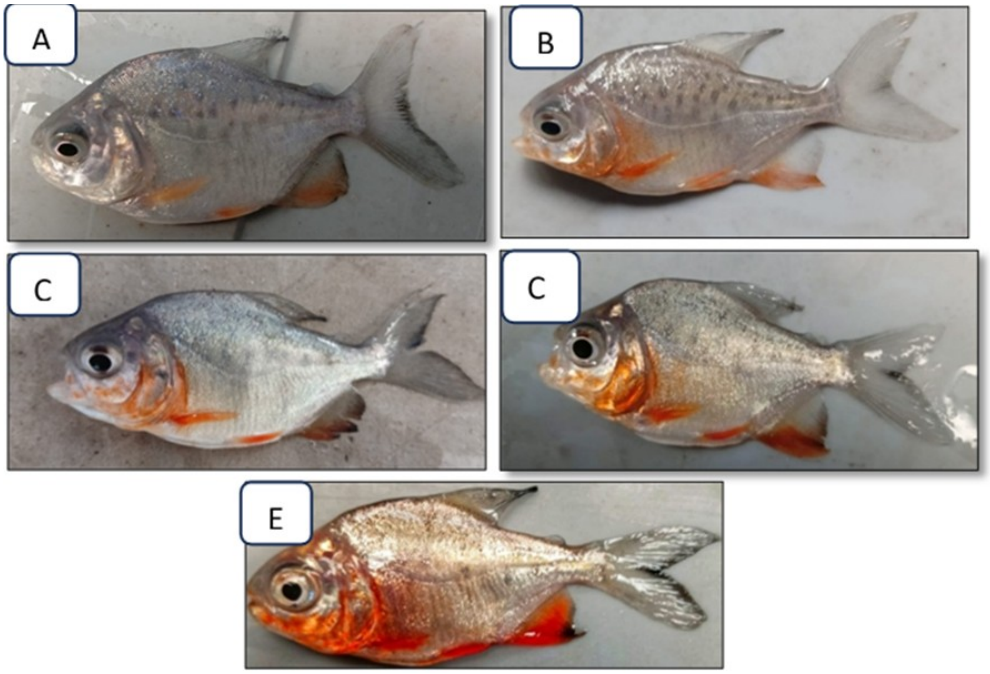

FIGURE 2 Clinical progression of *Aeromonas veronii* infection in *Piaractus brachypomus*. 2A = healthy fish at the time of injection (day 0); 2B = day 4 post-injection (stage I); 2C = day 5 post-injection (stage II); 2D = day 6 post-injection (stage III); 2E = day 7 post-injection (stage IV), showing advanced external lesion and discolouration.

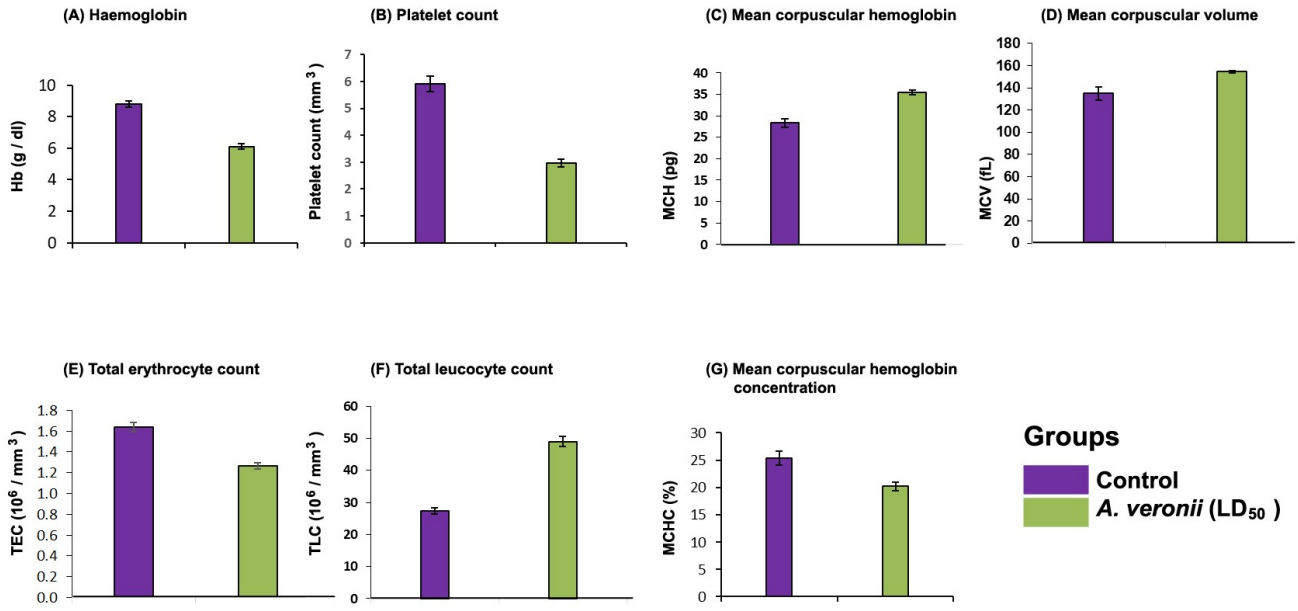

FIGURE 3 Comparative haematological parameters in *Aeromonas veronii*–infected *Piaractus brachypomus* versus control fish at 96 h post-infection. A = haemoglobin (Hb), B = platelet count; C = mean corpuscular haemoglobin (MCH); D = mean corpuscular volume (MCV); E = total erythrocyte count (TEC); F = total leucocyte count (TLC) and G = mean corpuscular haemoglobin concentration (MCHC) are presented. Values represent mean ± SE ($n = 5$ per replicate), with asterisks indicating significant differences ($p < 0.05$) between infected and control groups.

3.5 Biochemical parameters

A significant alteration in serum biochemical profiles was observed in Pacu fish fingerlings challenged with *A. veronii* compared to the control group (Figure 4A– 4C). Serum glucose levels exhibited a marked elevation ($p < 0.05$) in the infected group (92.6 mg dL^{-1}) compared to the control group (71.4 mg dL^{-1}) (Figure 4B). Similarly, alkaline phosphatase (ALP) activity increased significantly ($p < 0.05$) in *A. veronii*-challenged fish (157.2 U L^{-1}) compared to the control (41.3 U L^{-1}) (Figure 4B). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities also significantly increased ($p < 0.05$) in the infected group (119.3 U L^{-1} and 167.4 U L^{-1}, respectively) compared to the controls (39.5 U $L^{-1}$and 49.3 U L^{-1}) (Figure 4B). In contrast, serum albumin levels showed a significant reduction ($p < 0.05$) in infected fish (0.55 mg dL^{-1}) compared to the control (0.88 mg dL^{-1}) (Figure 4A).

Aeromonas veronii infection induced severe multisystemic pathology confirmed by semi quantitative scoring (all $p < 0.001$; Table 2). Gills exhibited lamellar fusion (2.8±0.3 vs. 0.1±0.1), epithelial hyperplasia (2.6±0.4 vs. 0.0±0.0), epithelial lifting, and erythrocyte lysis (Figures 5A–5G). Kidney displayed tubular necrosis (2.8±0.2 vs. 0.1±0.1) and prominent MMC aggregates (2.4±0.4 vs. 0.3±0.2) (Figure 6B–6D). Liver showed vacuolation (2.9±0.2 vs. 0.2±0.1), karyorrhexis (2.7±0.3 vs. 0.0±0.0), and haemorrhage (Figure 7B–7C).

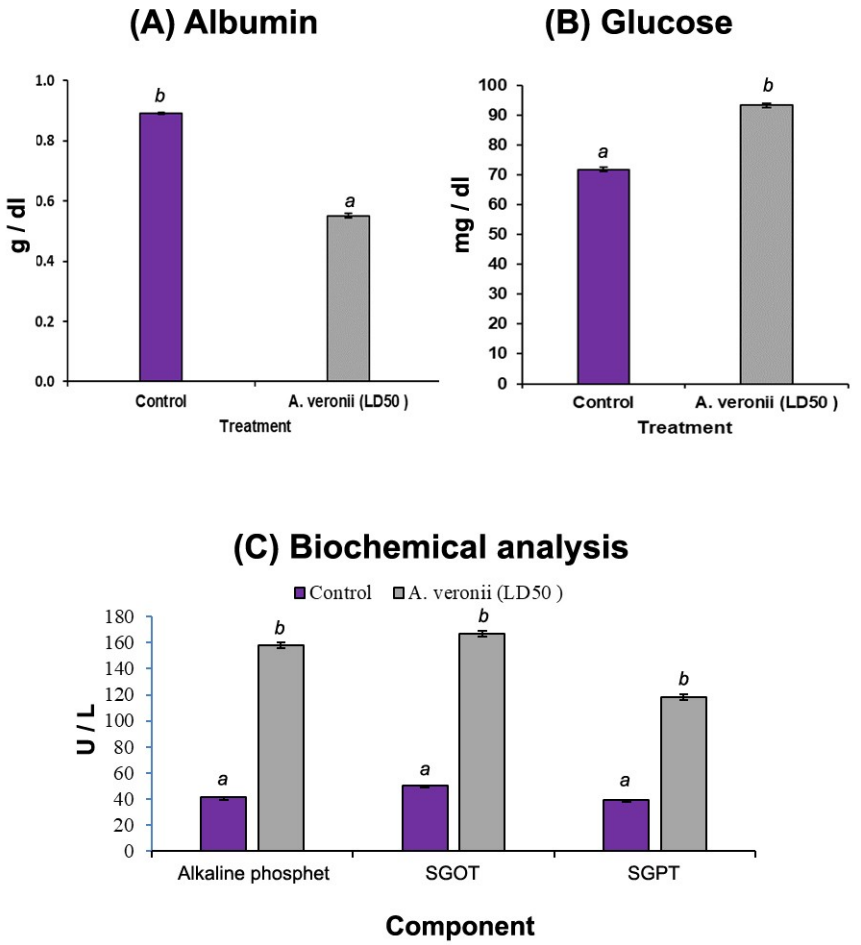

FIGURE 4 Comparative analysis of key serum biochemical parameters showing significant modulation in *Aeromonas veronii* (LD_{50})-infected *Piaractus brachypomus* versus control. A = serum Albumin; B = glucose; C = serum levels of alkaline phosphatase (ALP), SGOT (serum glutamic-oxaloacetic transaminase), and SGPT (serum glutamic-pyruvic transaminase) were measured in control fish and fish injected with *A. veronii* at the median lethal dose (LD_{50}). Biochemical enzyme activities were markedly elevated in *A. veronii*-infected fish compared to controls, reflecting hepatic stress and systemic physiological response to bacterial infection. Values represent mean ± 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.

TABLE 2 Semi-quantitative lesion severity scores (0–3 scale) in gill, liver, and kidney of *Aeromonas veronii*-infected versus control *Piaractus brachypomus* at 96 h post-infection ($n = 15$ sections/organ/group; student's *t*-test: $df = 28$).

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

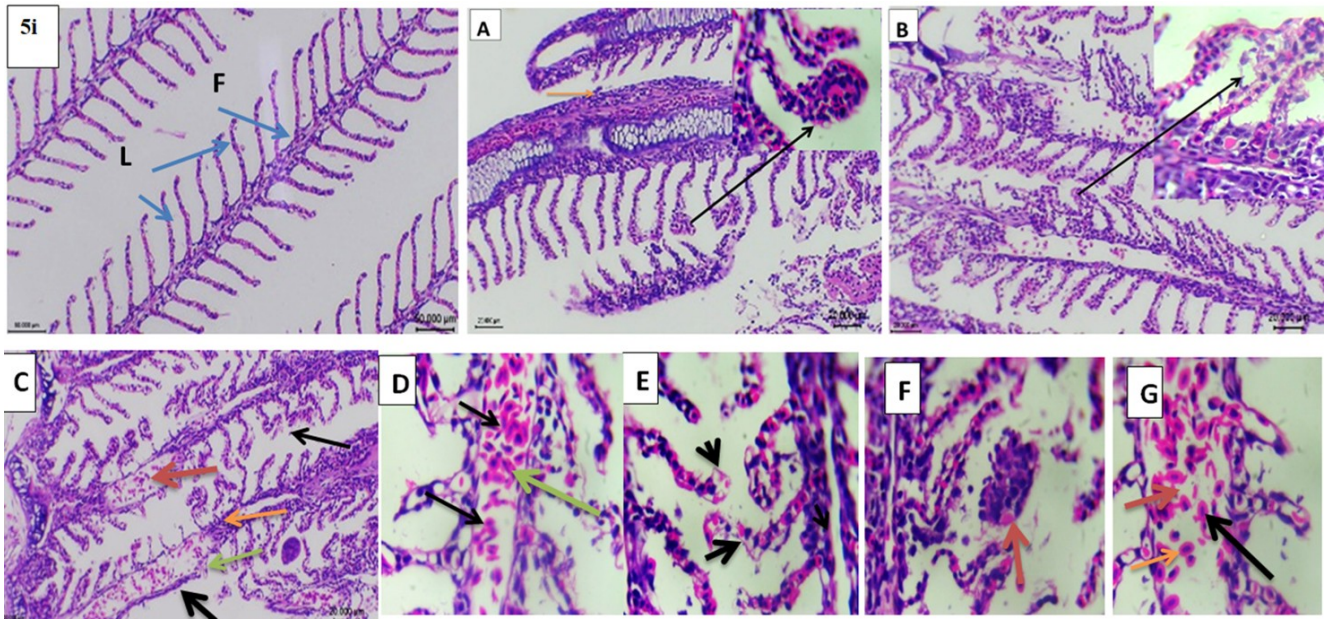

FIGURE 5 Gill histopathology (H&E, ×40). Control (5i): normal primary filaments (F) and secondary lamellae (L); lamellar fusion score 0.1±0.1 and epithelial hyperplasia score 0.0±0.0. Infected (A–G): severe lamellar fusion (2.8±0.3), epithelial hyperplasia (2.6±0.4), epithelial lifting, curling, and clubbing of lamellae, along with erythrocyte lysis. Scale bar = 50 µm (see Table 2 for details).

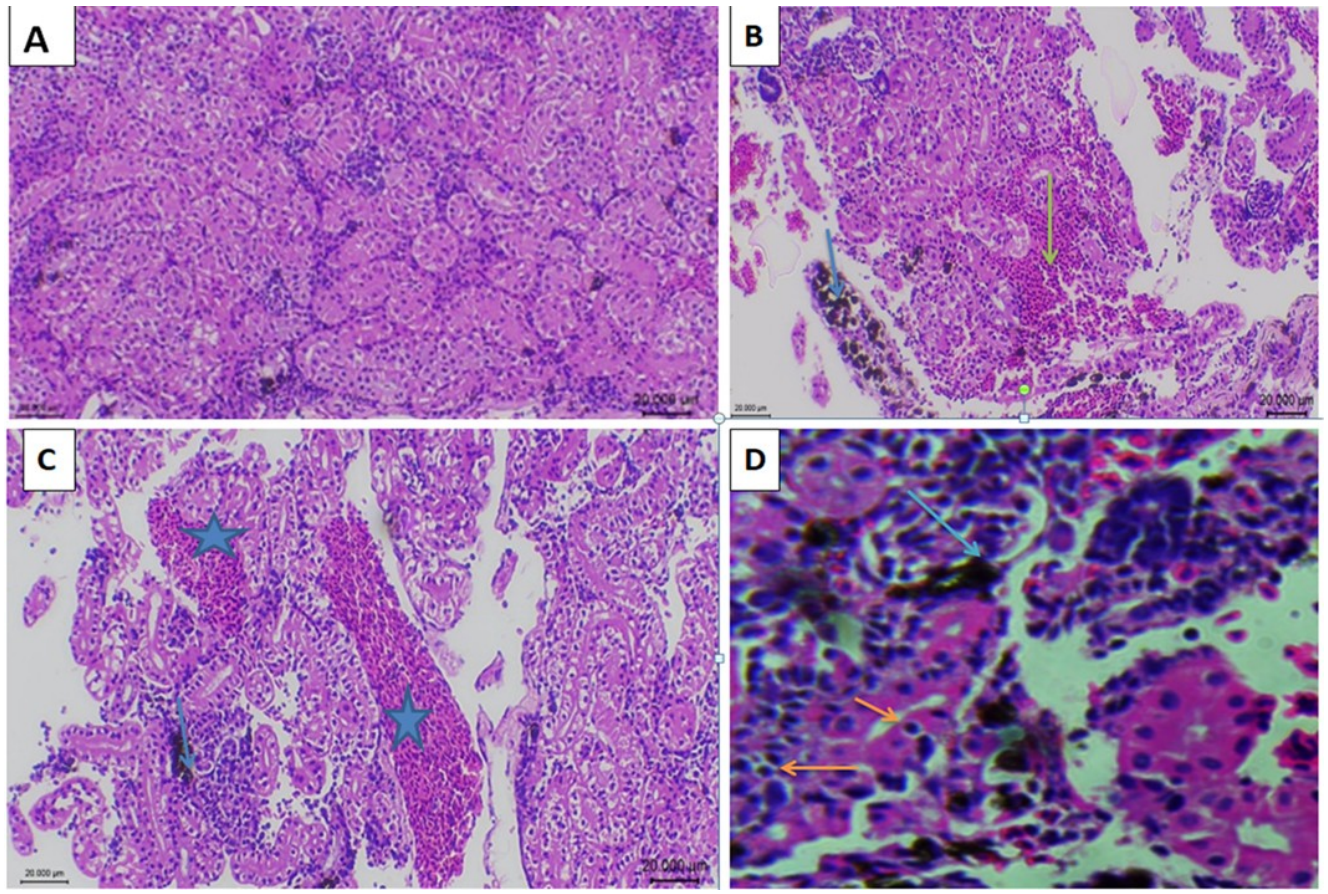

FIGURE 6 Kidney histopathology (H&E, ×40). Control (A): normal renal tubules and glomeruli; tubular necrosis score 0.1±0.1 and melanomacrophage center (MMC) score 0.3±0.2. Infected (B–D): extensive tubular necrosis (2.8±0.2), haemorrhagic congestion, and prominent MMC aggregates (2.4±0.4). Scale bar = 50 µm (see Table 2 for details).

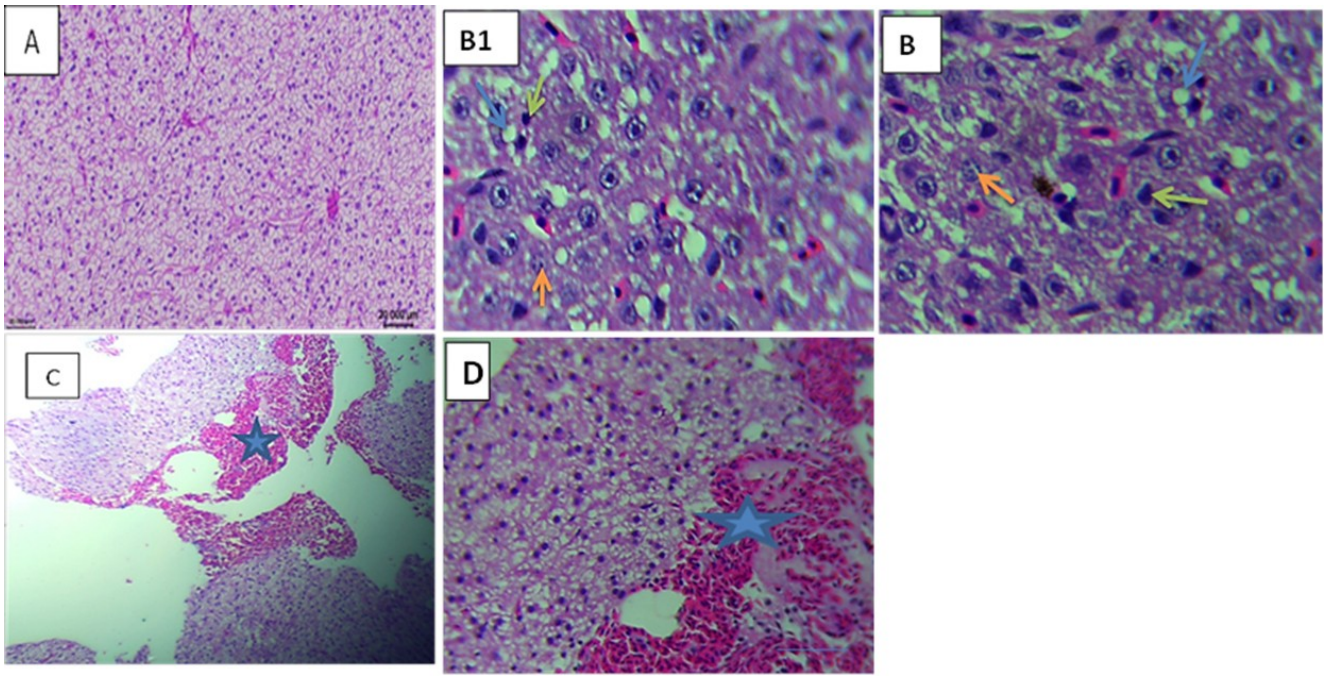

FIGURE 7 Liver histopathology (H&E, ×40). Control (A): normal hepatocytes with central vein; vacuolation score 0.2±0.1 and karyorrhexis score 0.0±0.0. Infected (B–C): severe hepatocellular vacuolation (2.9±0.2), karyorrhexis/pyknosis (2.7±0.3), and parenchymal haemorrhage. Scale bar = 50 µm (see Table 2 for details).

4 | DISCUSSION

4.1 Pathogenicity and median lethal dose (LD_{50}) determination

In the current study, probit analysis yielded LD_{50} = 7.8×10^6 CFU mL^{-1} (95% CI = 5.2–11.4 $\times10^6$ CFU mL^{-1}), probit regression: Y = 6.24 + 1.89X, R^2 = 0.98 for *A. veronii* in *P. brachypomus*, suggesting that *A. veronii* exerts even stronger pathogenic effects in closely related characids. This indicates a substantially higher virulence compared to related studies, being approximately 4.3-fold greater than *A. hydrophila* (1.8×10^6 CFU mL^{-1}) reported in pacu *Piaractus mesopotamicus* and 2.9-fold higher than *A. veronii* (2.7×10^6 CFU mL^{-1}) documented in tilapia *Oreochromis niloticus* (Claudiano *et al.* 2019; Fadel *et al.* 2025).

4.2 Haematological profile disruption

The observed haematological alterations are consistent with septicemic progression. Anaemia, reflected by decreased haemoglobin and total erythrocyte count, is likely attributable to aerolysin-mediated haemolysis and splenic sequestration of erythrocytes. Elevated total leukocyte count indicates activation of innate immune responses, particularly neutrophil mobilization against Gram-negative bacterial endotoxins. Concurrent thrombocytopenia suggests increased platelet consumption, possibly associated with disseminated intravascular coagulation during systemic infection (Esteban 2012; Ran *et al.* 2018).

4.3 Serum biochemical alterations and hepatic stress

Biochemical responses further highlight systemic physiological disruption. Elevated glucose levels reflect stress-induced hyperglycaemia mediated via activation of the hypothalamic–pituitary–interrenal (HPI) axis. Increased activities of liver enzymes—ALP and ALT—indicate cholestasis and hepatocellular injury caused by bacterial toxins, while elevated AST suggests more generalized tissue damage, including muscle and cardiac involvement. Reduced albumin levels signify impaired hepatic synthetic function and increased vascular permeability during endotoxemia (Harikrishnan *et al.* 2011a, 2011b).

4.4 Multisystemic histopathological alterations

Histopathological examination, supported by semi-quantitative lesion scoring (all lesions significantly different at $p < 0.001$ compared to control), revealed extensive multisystemic damage indicative of severe *A. veronii* septicemia, with mean lesion scores ranging from 2.4 to 2.9 (on a 0–3 scale).

Gill tissues exhibited near complete secondary lamellar fusion (2.8±0.3 vs. 0.1±0.1 in controls), along with marked epithelial hyperplasia (2.6±0.4), epithelial lifting and desquamation, lamellar curling and clubbing, and erythrocyte lysis accompanied by inflammatory cell infiltration. These alterations suggest severe impairment of respiratory function and disruption of primary mucosal defences, consistent with *Aeromonas*-induced gill pathology reported in pacu (Carraschi *et al.* 2012). Liver sections demonstrated pronounced hepatocellular vacuolation (2.9±0.2 vs. 0.2±0.1), along with nuclear degeneration (karyorrhexis and pyknosis; 2.7±0.3) and parenchymal

haemorrhage. These findings are characteristic of toxin-mediated cellular damage and hypoxia-induced necrosis, aligning with previous reports of *Aeromonas*-associated hepatotoxicity in teleost fishes (Carraschi *et al.* 2012). Kidney tissues showed extensive tubular necrosis (2.8±0.2 vs. 0.1±0.1), karyolysis, interstitial haemorrhage, and significant accumulation of melanomacrophage centers (2.4±0.4 vs. 0.3±0.2). These pathological changes indicate acute renal dysfunction and an active chronic inflammatory response, which are typical features of Gram-negative bacterial septicemia (Claudiano *et al.* 2019). Collectively, the high lesion severity scores across multiple organs confirm the systemic pathogenicity and virulence of *A. veronii* in pacu fish fingerlings.

The temporal tracking of haematological and biochemical markers observed in this study provides a conceptual baseline for understanding the kinetics of *A. veronii* infection in *P. brachypomus*. For instance, the marked elevation of serum transaminases (AST and ALT) within the first 24 h post-infection suggests a potential physiological window where early hepatic stress might be detected. In an experimental setting, this corresponds to the phase prior to the onset of severe, irreversible tissue necrosis observed at 72 h and 96 h.

While these coordinated physiological shifts point toward a theoretical timeline for clinical monitoring, we emphasize that these parameters cannot yet be treated as definitive diagnostic thresholds or prescriptive therapeutic windows for field applications. Because this study was conducted under controlled laboratory conditions using a single challenge route (i.p.), further field validation studies are strictly required to evaluate how water quality fluctuations, mixed infections, and natural horizontal transmission routes alter these biomarker kinetics. Consequently, these proposed intervals should be interpreted cautiously as preliminary indicators intended to guide future clinical validation and diagnostic framework development.

5 | CONCLUSIONS

The current study demonstrated that experimental infection with *Aeromonas veronii* at LD_{50} concentrations induced significant alterations in haematological and biochemical profiles of pacu fish fingerlings. These pathogens triggered classical signs of motile aeromonas septicemia, including behavioural abnormalities, external lesions, and systemic physiological disruption. The major haematological indicators, such as haemoglobin, total erythrocyte count, and thrombocytes count reduction, alongside elevated leukocyte counts and altered red blood cell indices (MCV, MCH, MCHC), provided evidence of infection-induced anaemia, immune response, and impaired haemopoiesis. Simultaneously, the significant increase in serum glucose, ALP, AST, and ALT levels, as well as decline in albumin concentrations, shows metabolic stress and hepatic damage in pacu. These integrated responses have exhibited the pathological severity associated with *Aeromonas* infections in pacu and emphasizes the importance of routine blood profiling in early diagnosis and disease surveillance in aquaculture. This study highlights the need for stringent biosecurity measures, timely disease diagnosis, and targeted therapeutic strategies to mitigate *Aeromonas*-related losses in *P. brachypomus* aquaculture. Future research should explore the development of the standard health management protocol, immunoprophylaxis, and the development of disease resistance variety to enhance host resilience against *Aeromonas* spp. infections in commercially important fish species, *P. brachypomus*.

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ETHICAL APPROVAL

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 (Ref. CIFE/IBSC/2022-23) at the ICAR-Central Institute of Fisheries Education, Mumbai.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Investigation, Writing Original Draft: Aniket Funde; Conceptualization: Arun Sharma; Formal Analysis: Megha K. Bedekar; Methodology: T. I. Chanu; Editing- Gowtham Kumar E.; Data Curation: Haseena Shaik; All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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