



Isolation, identification and immuno-pathogenesis of snakehead rhabdovirus-Indian strain (SHRV-In) in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Snakehead rhabdovirus (SHRV-In) is the most potentially destructive pathogen of snakehead, capable of causing up to 100% mortality within five days. The present study aimed to screen freshwater fishes in Tamil Nadu for the occurrence of SHRV-In and to assess its immuno-pathogenesis potential in Nile tilapia. Screening for rhabdovirus across six districts of Tamil Nadu, namely Tirunelveli, Thoothukudi, Madurai, Erode, Trichy and Thanjavur, revealed the occurrence of this virus in five districts, with five isolates recovered from snakehead and tilapia exhibiting scale loss, discolouration, dermal ulceration and haemorrhagic lesions. RT-PCR confirmed all isolates based on amplification of a 483-bp conserved region of the viral L polymerase gene. In addition, the non-virion (NV) gene, a unique feature of novirhabdovirus, was amplified and confirmed with an expected genomic region of 218 bp. Phylogenetic analysis of the conserved L polymerase region revealed 96.03% nucleotide sequence identity with the previously reported SHRV-In strain. Experimental challenge of Nile tilapia with SHRV-In-TL induced clinical and pathological alterations, including haemorrhages, scale loss, cloudy eyes, darkening, visceral congestion and abdominal fluid accumulation. Significant alterations in histopathological, haematological and immune parameters were also observed, although no mortality occurred. These findings demonstrate that SHRV-In can be detected in tilapia and induce measurable sublethal physiological and pathological responses, highlighting the need for continued surveillance and investigations into its host range, replication dynamics and epidemiological significance in freshwater aquaculture.

Keywords: infectivity; phylogenetic analysis; RT-PCR; SHRV-In; tilapia

1 | INTRODUCTION

Aquaculture, a rapidly growing industry, is pivotal in driving economic elevation and ensuring food security globally (Bjørndal *et al.* 2024). Although it is a much-demanded

food sector, the emergence and spread of diseases in aquaculture, particularly the emerging viral pathogens, has posed significant challenges to aquaculture health management. Among aquatic animal diseases,

rhabdoviruses are particularly notable as they were reported to infect 34 fish families across all major classes, including Agnatha (jawless fishes), Chondrichthyes (cartilaginous fishes), and Osteichthyes (bony fishes) (Costa and Holmes 2024). The family Rhabdoviridae includes two subfamilies (Alpharhabdovirinae and Gammarhabdovirinae), which comprise a diverse group of viruses assigned to five genera *Perhabdovirus*, *Sprivivirus*, *Scophrhavirus*, *Siniperhavivirus* and *Novirhabdovirus*. A number of rhabdoviruses such as snakehead rhabdovirus (SHRV), spring viraemia of carp virus (SVCV), hiram rhabdovirus (HIRRV), infectious haematopoietic necrosis virus (IHNV), viral haemorrhagic septicaemia virus (VHSV) and pike fry rhabdovirus (PRV) have been recorded from various marine and freshwater fishes (LaPatra *et al.* 2024).

Structurally, the rhabdoviruses are bullet- or rod-shaped but can appear filamentous and enveloped. The viral genome ranges from approximately 10 to 16 kb. It encodes six primary proteins arranged in a conserved order as nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), non-structural protein (NV) and RNA-dependent RNA polymerase (L) (Walker *et al.* 2011, 2015). Notably, the NV gene is an important component of rhabdoviruses from the genus *Novirhabdovirus* (SHRV, HIRRV, IHNV, VHSV, PRV), which are particularly pathogenic to fish. Knowing the importance of these viruses, extensive research uncovers a significant level of genetic diversity with over 100 sequenced fish rhabdovirus strains. The snakehead rhabdovirus (SHRV) initially emerged as a significant pathogen following an epizootic outbreak in snakehead fish (*Ophicephalus striatus*) in Thailand in the early 1990s (Kasornchandra *et al.* 1992). Subsequently, novel fish rhabdoviruses such as snakehead vesiculovirus (SHVV) were discovered in hybrid catfish in China (Zeng *et al.* 2014), while SHRV was recently isolated from striped snakehead fish in India, both associated with significant mortality and ulcerative skin lesions (John *et al.* 2021). Clinical manifestations of SHRV-In infection include skin darkening, surface ulceration, haemorrhages, vascular congestion and exophthalmia (John *et al.* 2021).

Due to the lack of clarity regarding the host susceptibility and pathogenicity of SHRV-In, its infectivity was assessed in three freshwater fish species, namely tilapia, grass carp, and loach. The study demonstrated that SHRV-In infection did not cause mortality in any of the tested species (Priyadharshini *et al.* 2024). However, the fact that there is very limited data on host susceptibility, pathogenic mechanisms and immune responses to SHRV-In emphasizes the critical need for comprehensive research on the occurrence and diversity of viral infections to mitigate the impact of rhabdoviral infections and ensure aquaculture sustainability. To address these gaps, this study aimed to explore the occurrence and host specificity of SHRV-In infections in farmed fish populations,

the detection of structural protein gene in isolates and the infectivity potential of the virus in tilapia.

2 | METHODOLOGY

2.1 Sample collection

Freshwater fish samples, including snakehead (*Channa striata*), tilapia (*Oreochromis niloticus*), pacu (*Piaractus brachypomus*), garra (*Garra* spp.), catla (*Catla catla*), rohu (*Labeo rohita*), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), and catfish (*Heteropneustes fossilis*) exhibiting clinical signs of viral infection associated with surface ulceration, haemorrhagic lesions and mortality were collected from fish farms in six districts of Tamil Nadu. A total of 117 fish specimens were collected from these districts over a two year period from October 2022 to September 2024. Infected samples were transported to the laboratory on ice within 24 hours.

2.2 Detection and isolation of virus using cell culture

Brain, kidney, spleen, eye and muscle tissues collected aseptically from infected fish were pooled for initial SHRV-In isolation and homogenized in L-15 medium containing 2% FBS and 1X antibiotic-antimycotic solution (Gibco, USA) at a 1:10 dilution (Sivasankar *et al.* 2017). After centrifuging the homogenate for 15 minutes at $3,000 \times g$, the cleared suspension was passed over a 0.22- μ m syringe filter. A 0.7 mL aliquot of the filtered homogenate was then inoculated into SSN1 cell lines in 25 cm² culture flasks, kept at 27°C, and examined for the progress of cytopathic effects (CPE). Upon CPE development, the virus was harvested by centrifugation and subjected to molecular confirmation.

2.3 Molecular characterization of rhabdovirus

The supernatant from infected cell cultures (TCID₅₀ 107.5 mL⁻¹) was analyzed for the confirmation of viral agents using RT-PCR. Using Trizol reagent (Life Technologies), total RNA was isolated from virus-infected SSN1 cells in accordance with the manufacturer's directions. For cDNA synthesis, 10 μ L of RNA was subjected to reverse transcription using a cDNA synthesis kit (Thermo Fisher, UK) with specific reverse primers targeting the non-structural protein (NV) and RNA-dependent RNA polymerase (L). The reactions were carried out at 25°C for 10 minutes, 37°C for 160 minutes, and 85°C for 5 minutes. The synthesized cDNA was subjected to PCR amplification for molecular confirmation of the rhabdovirus isolates. The L gene was amplified from all rhabdovirus isolates using the respective primer pair, whereas the NV gene was further analyzed in SHRV-In isolates from snakehead and tilapia. Primer sequences and cycling conditions used for amplification of target genes (L and NV) are detailed in the Table 1. The amplified products were separated by electrophoresis on 1.2% agarose gels containing ethidium bromide

and visualized using a UV gel documentation system (Bio-Rad, USA).

2.4 Genomic characterization of the L polymerase gene

To confirm the identity of SHRV-In isolate and examine its phylogenetic relatedness, the amplified conserved region of block III of the L polymerase gene fragment of the virus isolated from tilapia (SHRV-In-TL) was purified and subjected to Sanger sequencing at Eurofins Genomics India Pvt Ltd., Bangalore. Multiple sequence alignment was performed using ClustalW implemented in MEGA version 12.0.11 with default parameters. Phylogenetic analysis

was conducted using the Maximum Likelihood (ML) method based on the Tamura–Nei model, with 1,000 bootstrap replications to assess the robustness of the inferred tree topology (Tamura and Nei 1993; John *et al.* 2021). Additionally, pairwise nucleotide identity among sequences was calculated in MEGA version 12.0.11 using the p-distance method (Kumar *et al.* 2024). A total of 14 L-gene nucleotide sequences of rhabdoviruses including SHRV-In-TL, SHRV, SHRV-In, VHSV, IHNV, HIRRV, SVCV, PRV, EVEX, TiRV1, TiRV2 and HSHRV were retrieved from GenBank and used for phylogenetic analysis.

TABLE 1 Primers and PCR conditions used for gene detection work (GenBank Acc. No. AF147498).

Name	Gene	Primer sequences 5'-3'	Size (bp)	Cycles	Cycling condition
MRGSH011FW	L gene	AAAGGTCGGGGTTTGGACTAATG	483	34	95°C for 1 min, 55°C for 1 min,
MRGSH011RV	L gene	CCAGACATACTGACACAGCCCTTC			72°C for 1 min
RHABDONVFW1	NV gene	AAAGAACCGTCCAAGCACAC	218	34	94°C for 30 sec, 57°C for 45 sec,
RHABDONVRE1	NV gene	CGATTTGTGTGCCTCATTTG			72°C for 1 min

2.5 Experimental infection of SHRV-In-TL in tilapia

The pathogenicity of the virus isolated from tilapia was evaluated in healthy fingerlings (ABW: 22 g; ABL: 12.5 cm) sourced from the fish farm facility of FC & RI, Thoothukudi and acclimatized in 60 L freshwater in 100 L aquaria at 30±1°C and pH 8.0±0.2. Fish were fed commercial pellets twice daily with 20% daily water exchange. Prior to challenge, fish were confirmed negative for SHRV-In by cell culture and PCR. Fifteen fish were maintained in each of three replicate tanks for each experimental group (45 fish/group) and challenged intraperitoneally with 100 µL of viral suspension (107.5 TCID₅₀/mL), while controls received 100 µL Leibovitz-15 medium (Sivasankar *et al.* 2017). The Spearman–Kärber method (Karber 1931) was used to determine the TCID₅₀/mL of the virus before challenge. Fish were monitored for clinical and behavioral changes for 28 days. Similarly, a separate set of fish was maintained in triplicate tanks under the same experimental conditions for sampling. Blood samples and spleen, kidney, brain, and liver tissues were collected from three different fish from each replicate tank at 2, 5, 7, and 10 dpi for haematological, immunological, and histopathological analyses. Viral infection in experimentally challenged fish was re-confirmed by cell culture and RT-PCR at the end of the trial.

2.6 Collection of blood from experimental fish

At 2, 5, 7 and 10 dpi, three fish were randomly selected from each replicate tank to evaluate haematological, immunological and serum biochemical changes (Priyadharshini *et al.* 2024). A 2.5 mL sterile syringe with a 26-gauge needle containing 5% EDTA as an anticoagulant was used to draw blood samples from the caudal vein of each fish. Approximately 400–500 µL of blood was collected from each fish and divided into two vials: one with

EDTA for the immunological parameters assessment and the other without EDTA for serum collection. The clotted blood was centrifuged at 2500 × g for 5 minutes, and the serum was carefully extracted and held at -20 °C until further exploration.

2.7 Evaluation of haematological parameters

The collected blood samples were instantly evaluated for haematological parameters, including red blood cell (RBC) count, concentration of haemoglobin (HGB), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) count, total platelet (PLT) count and mean platelet volume (MPV). These parameters were measured using an automated haematological analyzer (Laboholic, China).

Evaluation of immunological parameters

Respiratory burst activity (RBA), superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) activities were determined according to Anderson and Siwicki (1995), Campa-Córdova *et al.* (2002), Takahara *et al.* (1960) and Quade and Roth (1997), respectively, with modifications to the MPO assay as described by Sahoo *et al.* (2005). RBA was assessed by the nitro blue tetrazolium (NBT) reduction assay, with absorbance measured at 540 nm. SOD activity was determined using the NBT reduction method and absorbance was recorded at 630 nm. CAT activity was estimated by monitoring the decomposition of H₂O₂ at 240 nm, while MPO activity was determined using the TMB–H₂O₂ assay with absorbance measured at 450 nm. All absorbance measurements were performed using a BioTek ELISA reader (USA).

2.8 Histopathological examination

To assess histopathological changes, tissue samples

(spleen and kidney) were collected from both experimentally infected and uninfected groups. Following the conventional histological procedures, tissues were fixed in 10% NBF fixative, embedded in paraffin wax, and then cut into 4 μm -thick tissue slices using a microtome (Thermo Shandon, UK) and stained with haematoxylin and eosin (H&E). A light microscope was employed for microscopic inspection, and a Nikon FireWire digital camera (Nikon, Japan) was used to take pictures.

2.9 Data analysis

IBM SPSS version 26 (SPSS Inc.) was used to analyze the data. The Shapiro–Wilk test was used to assess normality, and Levene's test was used to assess homogeneity of variance. The data were considered normally distributed and homogeneous when $p > 0.05$. Where necessary, data were log-transformed before analysis. The mean $\pm$ standard deviation (SD) is used to express the results. At each sampling time point, three different fish were sampled from each tank, and their measurements were averaged to obtain a single tank-level observation. Therefore, the tank was considered the experimental unit, with three tank-level replicates per treatment group. A two-way ANOVA was performed with a significance level of $p < 0.05$ to investigate the interaction effects between two independent variables (groups and time intervals) on the dependent variables (immunological, and haematological parameters). Tukey's post hoc test was executed at a significance level of 0.05 for pairwise comparisons between

experimental groups at particular time intervals. Substantial changes ($p < 0.05$) between time periods were found by examining simple main effects within the independent variables. As the Group factor consisted of only two levels (control and infected), post hoc comparisons were not applicable. Therefore, estimated marginal means (EMMs) generated from the SPSS output were used to assess differences between the groups.

3 | RESULTS

3.1 Occurrence of SHRV-In

Screening for SHRV-In was conducted from October 2022 to September 2024 in freshwater fish farms across six districts of Tamil Nadu: Thoothukudi, Tirunelveli, Thanjavur, Trichy, Madurai and Erode (Figure 1). Five specimens, comprising four snakeheads and one tilapia, were positive for SHRV-In, showing haemorrhages, scale loss, skin ulcers and ascites (Figure 2). Infection was confirmed by cell culture and RT-PCR targeting the conserved block III region of the RNA-dependent RNA polymerase (L) gene. RT-PCR further confirmed the presence of the non-structural protein (NV) gene in all recovered isolates (Figure 3). The virus was detected in farms from Tirunelveli, Thoothukudi, Madurai, Trichy and Thanjavur, representing 4.2% of the clinically affected fish samples screened. The isolates were designated SHRV-In-TL, SHRV-In-TK, SHRV-In-MD, SHRV-In-TR and SHRV-In-TJ according to their geographical origin.

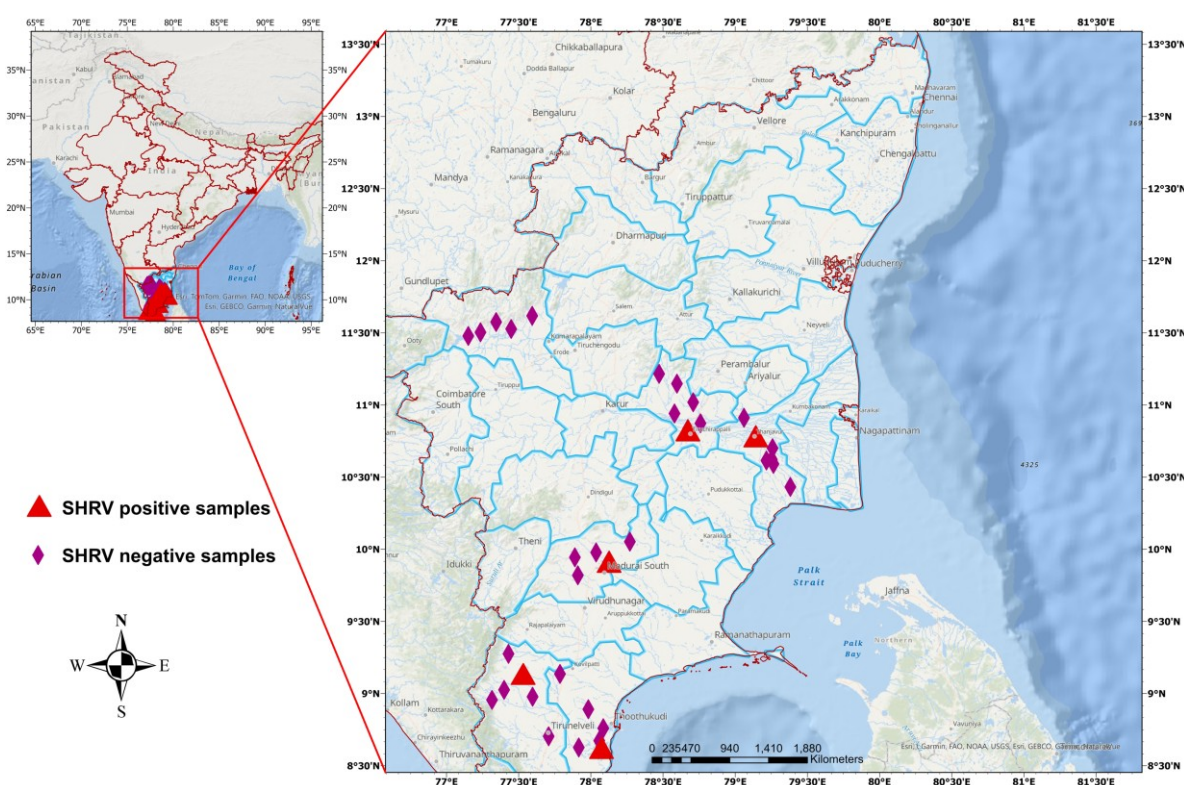


FIGURE 1 Map representing sampling region in Tamil Nadu of south India for the screening of SHRV-In from freshwater fishes.

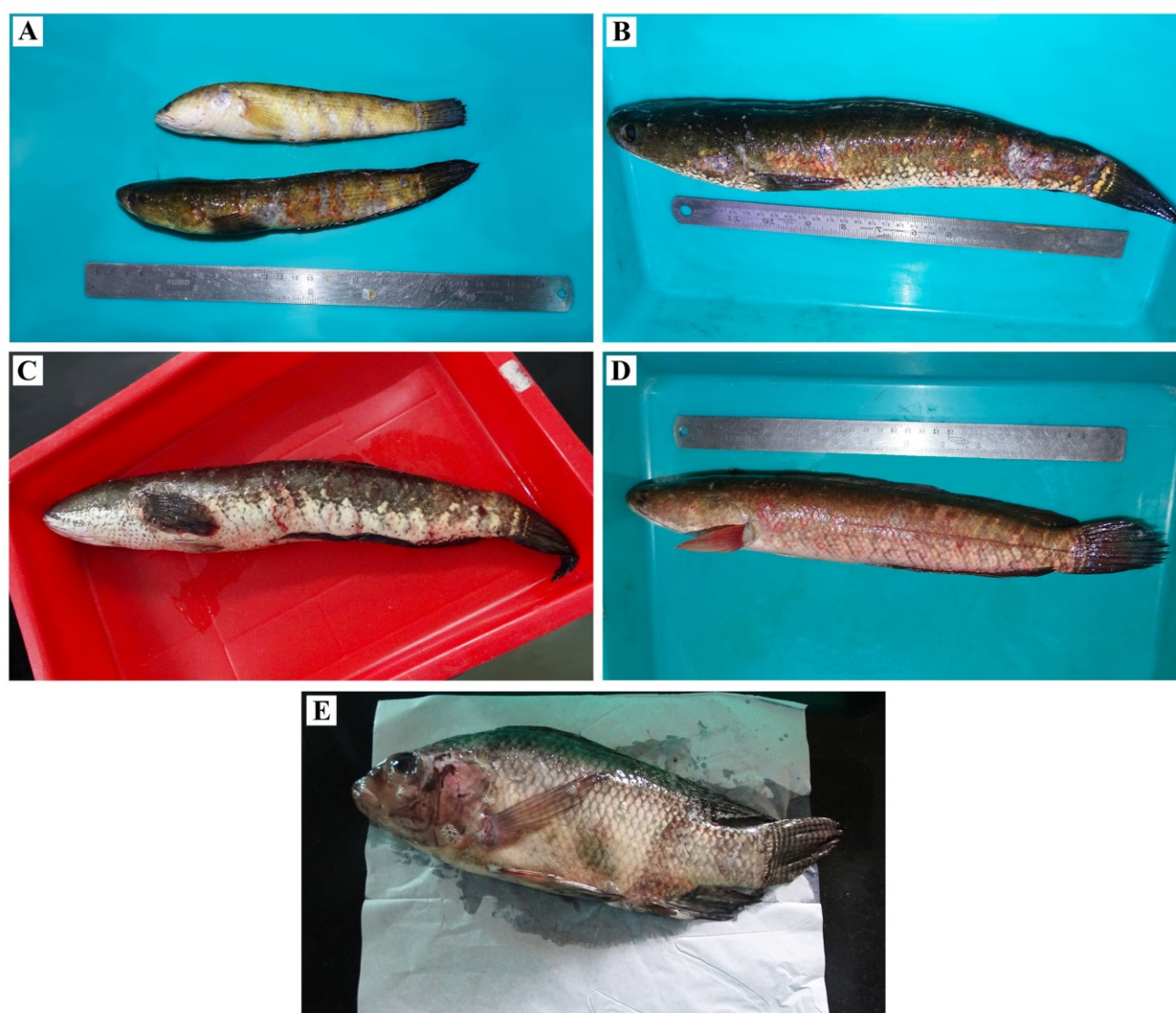


FIGURE 2 Snakeheads and tilapia infected with SHRV-In exhibiting severe haemorrhages, loss of scales, skin ulcers and ascitic fluid accumulation.

3.2 Lineage analysis of the L gene fragment

Multiple sequence alignment of the L gene sequences obtained from the gel-purified PCR amplicons with corresponding regions of reference rhabdoviruses, together with phylogenetic analysis, confirmed their close genetic relationship with members of the genus *Novirhabdovirus* within the family *Rhabdoviridae*. Pairwise nucleotide identity analysis revealed that the isolate shared 96.03% similarity with the corresponding region of snakehead rhabdovirus (SHRV-In, MT459927), 91.26% similarity with SHRV-In (PX060562), 89.15% similarity with SHRV (AF147498; NC_000903.1) and 65 to 70 % with IHNV (L40883), HIRRV (AF104985) and VHSV (Y18263). Furthermore, phylogenetic inference using the Maximum Likelihood (ML) method grouped the isolate with SHRV

strains, supporting their phylogenetic relatedness (Figure 4). The nucleotide sequence has been submitted to the GenBank database under accession number PZ136792.

3.3 Pathogenicity of SHRV-In in tilapia

Healthy tilapia fingerlings challenged intraperitoneally with a dose ($10^{7.5}$ TCID₅₀/mL) of SHRV-In-TL-propagated in cell line showed clinical signs such as skin hemorrhage, scale loss, cloudy eye and body darkening. In addition, infected fish showed congestion of internal organs with fluid accumulation in the abdominal cavity (Figure 5). However, no mortality was recorded throughout the observation period. Moreover, virus was re-isolated in SSN1 cells from spleen and kidney of experimentally infected tilapia.

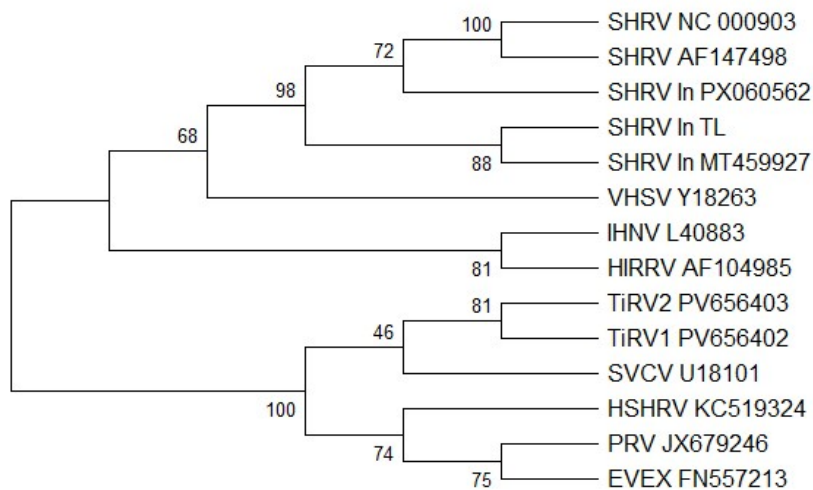
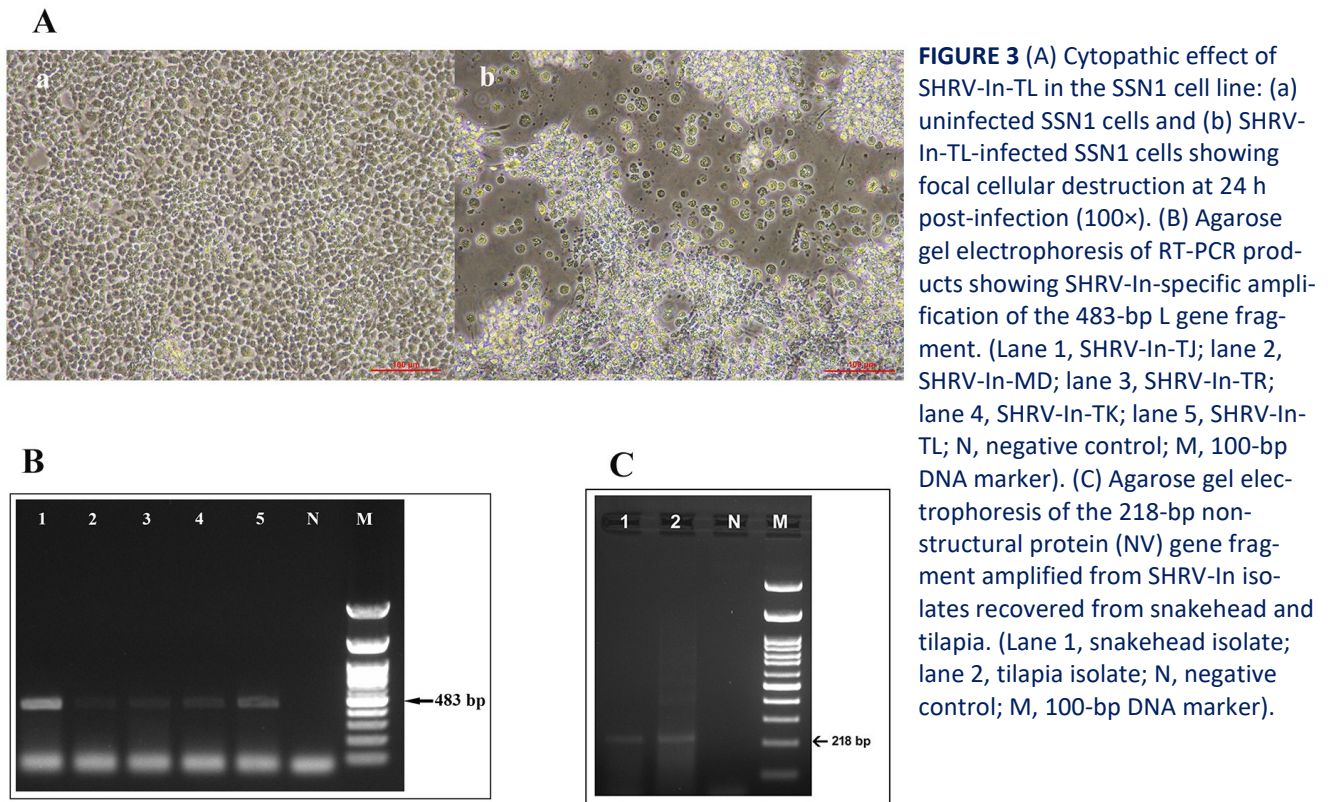


FIGURE 4 Phylogenetic relationship inferred using the Maximum Likelihood method and Tamura-Nei model of nucleotide substitutions and the tree with the highest log likelihood (-3,565.93) is shown. The percentage of replicate trees in which the associated taxa clustered together (1,000 replicates) is shown next to the branches.

3.4 Haematological parameters alteration

Haematological parameters were significantly altered following experimental infection with SHRV-In-TL. RBC counts exhibited a transient decline at 7 dpi and subsequently increased at 10 dpi, although no significant differences were observed compared to controls. Concomitantly, haemoglobin levels, MCV, and MCH were significantly reduced at 7 dpi. Haematocrit values mirrored this trend, with a significant decrease at 7 dpi followed by recovery at 10 dpi. Notably, MCHC was significantly reduced in infected fish at 7 dpi, despite no significant

changes in RBC counts subsequently. WBC counts initially declined, followed by a significant increase at 5 dpi, and subsequently decreased at 7 dpi and then increased at 10 dpi. Platelet counts were significantly reduced at 7 dpi, with subsequent recovery. However, no significant differences in MPV were observed throughout the experiment. Overall, SHRV-In-TL infection significantly alters haematological parameters in infected fish for up to 7 days, then reverts to normal trends compared to the uninfected group (Figure 6).

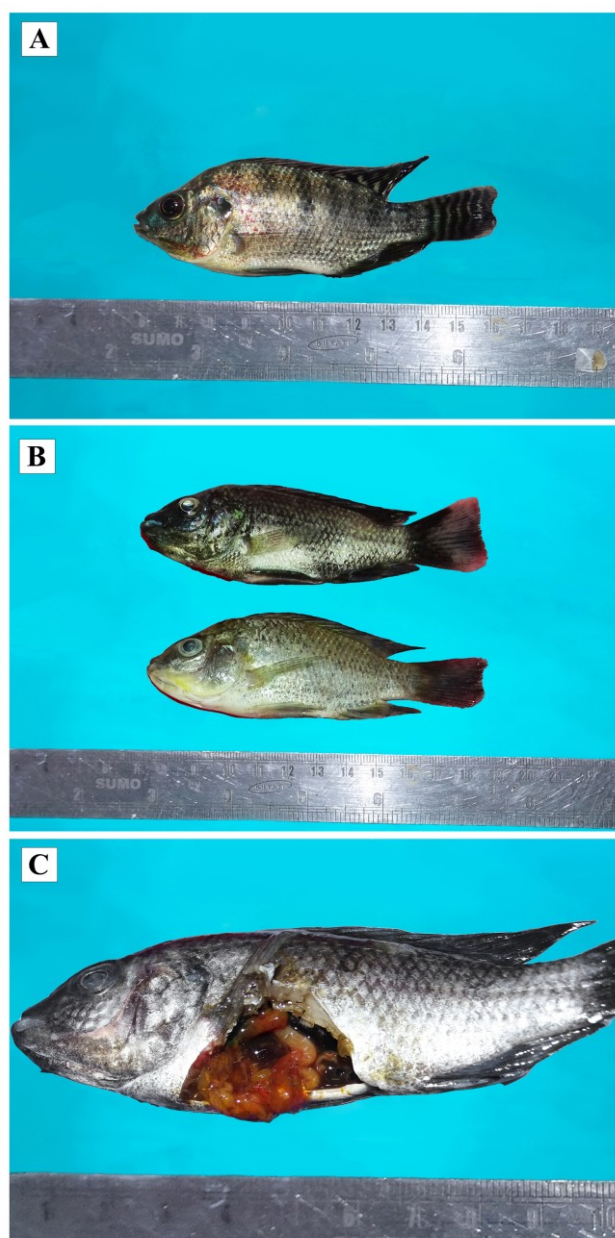


FIGURE 5 Experimentally challenged fish with (A) skin hemorrhage, (B) scale loss, cloudy eye and body darkening (C) congestion of internal organs with fluid accumulation in the abdominal cavity.

3.5 Immune parameters alteration

Infected fish showed a substantial increase ($p < 0.05$) in RBA levels at 5 dpi, but then a drop in comparison to the control group. Afterwards, an increase in RBA was observed at 7 and 10 dpi. Activity of SOD was significantly higher ($p < 0.05$) at 5 dpi in infected fish but declined at 7 and 10 dpi. Unexpectedly, CAT activity showed a significant increase ($p < 0.05$) at 7 dpi and slightly reduced at 10 dpi in the infected group. Similar to SOD level, the MPO activity displayed a significant decrease in the infected group at 7 and 10 dpi (Figure 7).

3.6 Histopathological changes in tilapia

Histopathological examination revealed varying degrees of tissue damage and necrosis consistent with viral infection in the spleen and kidney of experimentally infected tilapia compared to control. The histopathology of spleen displayed vacuolation of melanomacrophage centres, widespread focal necrosis and haemorrhages. In the kidney, extensive damage to the tubular architecture was evident, including degeneration of tubules, shrinkage of glomerular, vacuolation and necrosis. Histopathology of all the tissues revealed the cells in the control groups had normal structural patterns (Figure 8).

4 | DISCUSSION

The global aquaculture industry faces significant challenges from emerging and re-emerging viral pathogens, predominantly rhabdoviruses. These viruses infect a variety of aquatic hosts, particularly fish, and are responsible for substantial economic losses due to mass mortalities. Prominent examples include IHNV, VHSV, HIRRV, SHRV, SVCV and PRV, which have been reported to infect several species such as rainbow trout, mandarin fish, eels, cyprinids and snakehead (Liu *et al.* 2019). In this study, we screened commercially important freshwater fish species from Tamil Nadu, including snakehead, tilapia, pacu, garra, catla, grass carp, rohu, common carp, and catfish, for the presence of SHRV-In using cell culture and RT-PCR techniques. Fish samples were collected in this study based on disease signs including haemorrhages, dermatitis with scale loss, ulcerations and discoloured white patches, similar to other rhabdovirus infections documented previously (Kasornchandra *et al.* 1992; Zeng *et al.* 2014).

The presence of SHRV-In in cell culture supernatants was confirmed by amplification of the 483 bp target genomic sequence inside the block III segment of the rhabdovirus L polymerase gene, consistent with previous findings (John *et al.* 2021). Our results revealed a 4.2% prevalence of SHRV-In infection across 117 samples, detected in both snakehead and, notably, tilapia from five districts in Tamil Nadu (Tirunelveli, Madurai, Trichy, Thanjavur, and Thoothukudi). Although the incidence of SHRV-In infection is low, the study reveals that it has spread throughout Tamil Nadu. Previously, Soliman *et al.* (2008) found the first time for the occurrence of rhabdovirus (SVCV) from tilapia in Egypt and the Middle East area. Further, our prevalence data suggest that snakehead may be the most susceptible host for SHRV-In. Notably, this study represents the first incidence of rhabdovirus infection in farmed tilapia. Therefore, effective surveillance for the occurrence of SHRV-In is to be implemented because there may be chances for widespread distribution across India.

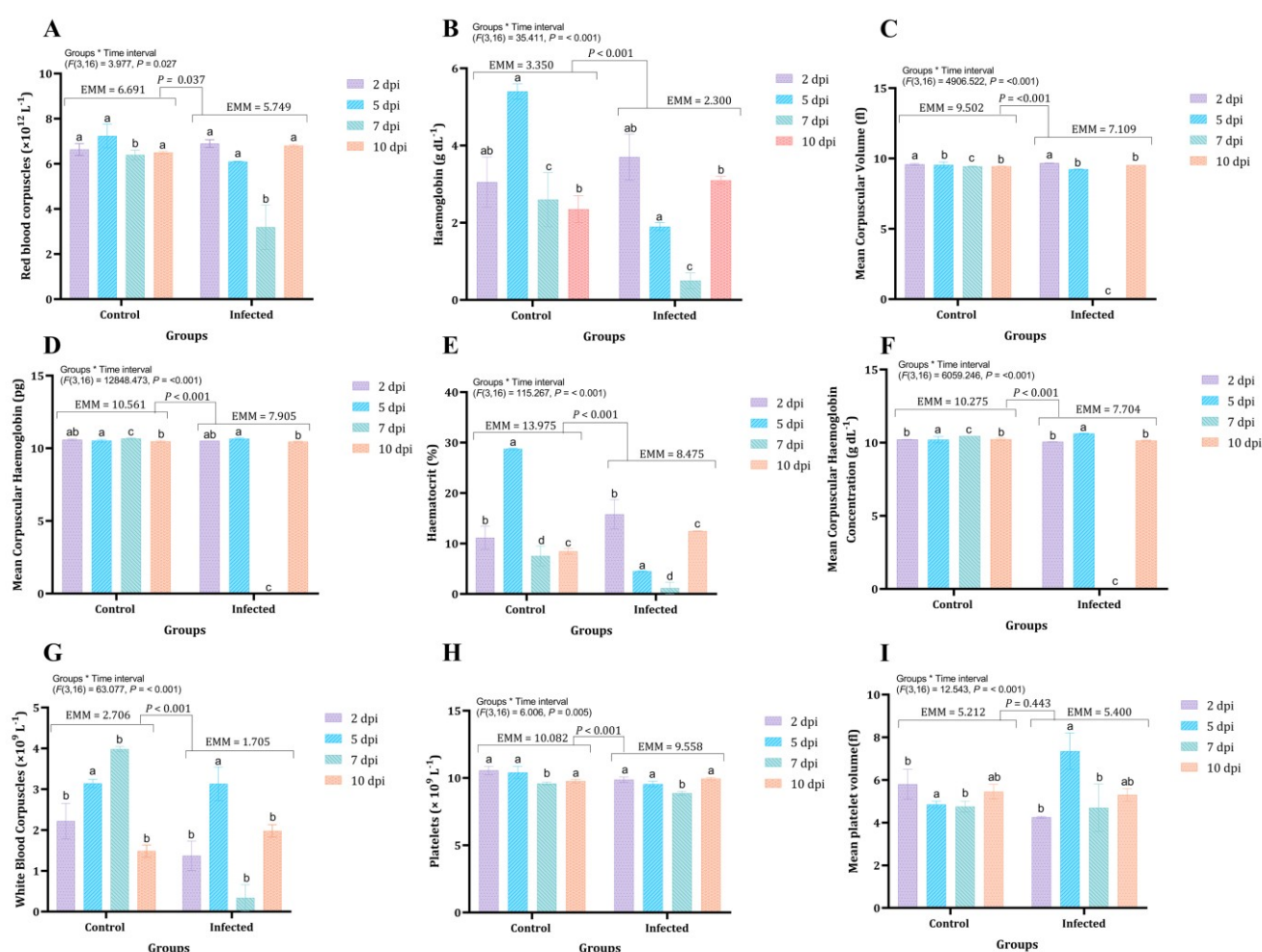


FIGURE 6 Haematology parameters of tilapia challenged with SHRV-In. (A) RBC counts, (B) haemoglobin, (C) MCV, (D) MCH, (E) haematocrit, (F) MCHC, (G) WBC, (H) PLT and (I) MPV. EMM: Estimated marginal means indicate the significant differences between the “Groups”. The different alphabets (a,b,c ...) indicate the significant differences between time intervals as determined by Tukey’s post hoc test ($p < 0.05$).

Among the rhabdovirus genes, the RNA-dependent RNA polymerase (L) gene is one of the most highly conserved genomic regions and is considered a reliable molecular marker for phylogenetic reconstruction and viral classification (Bourhy *et al.* 2005). In the current study, RT-PCR analysis of SHRV-In-TL isolate infected cell culture supernatants using specific gene primers revealed the presence of the block III region of L polymerase gene at the expected size (483 bp), which was used for sequencing (Bourhy *et al.* 2005; John *et al.* 2021). Additionally, non-virion (NV) was amplified and confirmed with an expected genomic region of 218 bp in SHRV-In-TL isolate, as it is a unique feature of *Novirhabdovirus*. Similar to these SHRV-In isolates, other rhabdoviruses, VHSV and IHNV genomes possess a tiny non-structural NV protein that is situated between the G and L genes (Ammayappan and Vakharia 2011). The presence of the NV gene indicates that the virus is a member of the *Novirhabdovirus* genus,

similar to VHSV and IHNV (Biacchesi *et al.* 2017; Baillon *et al.* 2020). Sequence alignment in the present study further revealed that the SHRV isolated from tilapia share over 96.03 %, 91.26% and 89.15 % amino acid similarity with the previously reported SHRV strains from India (SHRV-In, MT459927; PX060562) and USA (SHRV, NC_000903.1; AF147498), respectively (Wattanavijarn *et al.* 1986; Kasornchandra *et al.* 1992; John *et al.* 2021). These findings support the classification of the virus isolates within the SHRV genotype and reflect the evolutionary stability of this virus. This study also focused on the pathogenicity of SHRV-In identified in tilapia from Tirunelveli District, Tamil Nadu. Since no natural mortality was observed in farmed tilapia, an artificial infectivity model with SHRV-In-TL was conducted to understand the disease dynamics, pathogenesis and mortality pattern in order to prove Koch’s postulates.

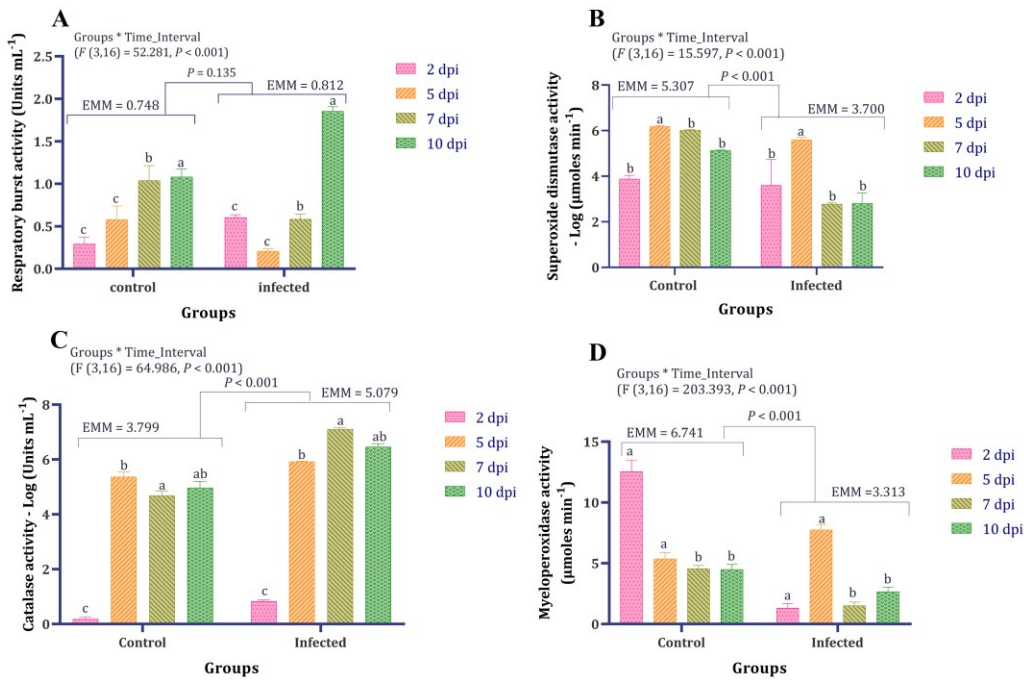


FIGURE 7 Immunological parameters of tilapia challenged with SHRV-In. (A) RBA, (B) SOD, (C) CAT and (D) MPA. EMM: Estimated marginal means indicate the significant differences between the “Groups”. The different alphabets (a,b,c ...) indicate the significant differences between time intervals as determined by Tukey’s post hoc test ($p < 0.05$).

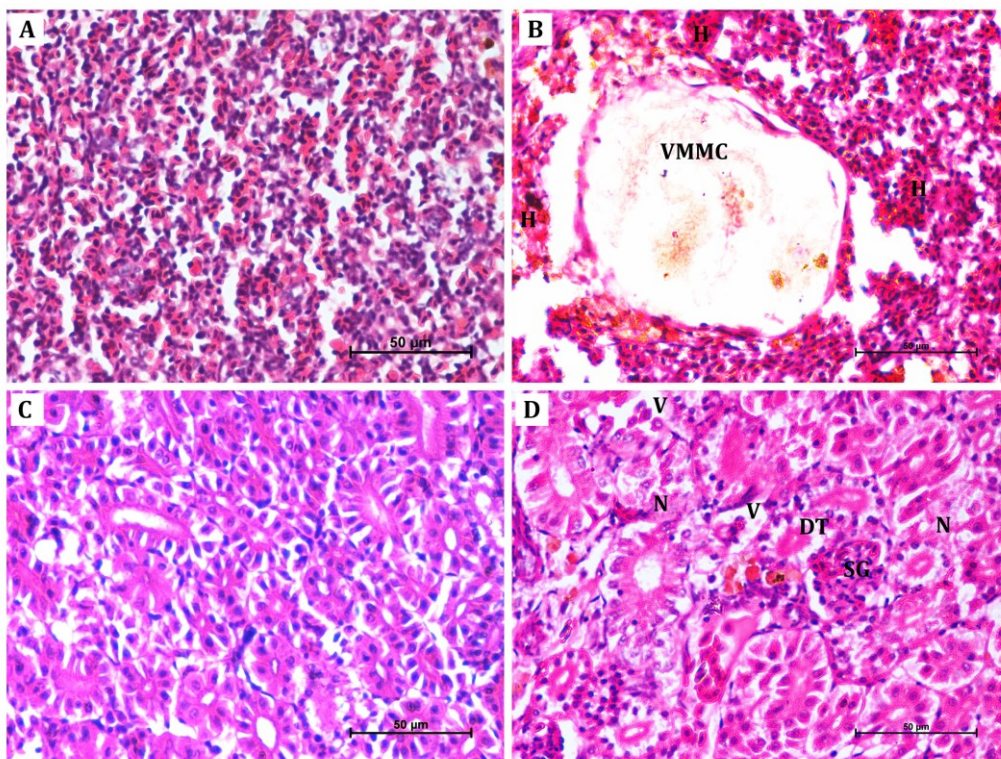


FIGURE 8 Histopathological changes in tilapia challenged with SHRV-In (a) control spleen, (b) infected spleen, (c) control kidney and (d) infected kidney. (VMMC – vacuolated melanomacrophage centre; H – haemorrhage; V – vacuolation; N – necrosis; DT – degeneration of renal tubules; SC – shrinkage of glomerular cells).

Artificial challenge of tilapia fingerlings with SHRV-In by intraperitoneal injection induced observable pathological signs such as skin haemorrhages, scale loss, cloudy eye, body darkening and congestion of internal organs with fluid accumulation in the abdominal cavity. Although the virus did not cause mortality, the existence of clinical signs of SHRV-In in tilapia resembles with previous reports

of the virus infectivity in snakehead (John *et al.* 2021). Severity of infection followed by mortality might have been due to several factors, including the potential of the viral strain, environmental settings, host type and life stage (Dixon *et al.* 2016). Also, it has been previously documented that the infection with SHRV-In might be host-specific (Priyadharshini *et al.* 2024). Besides, the intrinsic

genomic variation might also have influenced the replication and infectivity of the viral strain. Furthermore, other rhabdovirus such as VHSV and IHNV can establish persistent infections, often characterized by asymptomatic carrier states, frequently associated with neural tissues (St-Hilaire *et al.* 2001; Hershberger *et al.* 2010). Similar to rhabdoviruses, nodaviruses can also establish latent infections in various hosts often without any apparent clinical symptoms (Hegde *et al.* 2003; Gomez *et al.* 2006). This observation is consistent with findings from studies on betanodavirus in farmed turbot (*Psetta maxima*), where the viral isolate was able to replicate in host tissues but did not induce clinical signs of viral encephalopathy and retinopathy (VER) or cause mortality (Oliveira *et al.* 2013).

One of the most accepted and popular techniques for assessing the physiological and health status of fish is the haematological examination (Docan *et al.* 2018; Fazio 2019). Findings of elevated white blood cell counts and decreased platelet counts, hematocrit, hemoglobin, MCV, and MCH in tilapia infected with SHRV-In align with observations in other RNA viral infections of fish. For instance, *Betanodavirus* infection in mullet has been shown to induce significant reductions in RBC counts, hematocrit, and hemoglobin, indicative of severe anemia (Ghiasi *et al.* 2016). Previously, Priyadharshini *et al.* (2024) documented a decrease in the amount of hemoglobin, HCT, MCV, MCH and MCHC in snakeheads after experimental challenge with SHRV-In. These haematological alterations may result from a combination of factors, including direct viral effects on blood cells, immune-mediated destruction of erythrocytes, and potentially impaired erythropoiesis.

Successful pathogens can establish long-term infections by evading host immune responses (Sorci *et al.* 2013). This strategy may contribute to their survival and transmission within host populations. In our study, the initial rise in RBA (a measure of oxidative stress), most likely results from the host's immune system becoming activated and releasing reactive oxygen species (ROS). This is further supported by the elevated SOD activity at 5 dpi, suggesting the host's attempt to neutralize the increased oxidative stress. The fish may be employing antioxidant defense mechanisms, such as elevated SOD activity, to mitigate oxidative damage. A second peak in RBA observed at 10 dpi suggests a renewed period of oxidative stress. Overproduction of ROS can harm biological constituents, including DNA, proteins and lipids (Kannan and Jain 2000). However, the increased level of CAT may counteract this oxidative damage. MPO is essential for the killing of bacteria and other pathogens. In this study, decreased MPO levels in the infected group could increase the susceptibility of individuals with persistent viral infections to secondary bacterial or fungal infections, which can further complicate the disease course.

Substantial tissue damage was found in the spleen, and kidney of experimentally infected tilapia on histo-

pathological examination, which is consistent with earlier findings (John *et al.* 2021; Alkshab and Taha 2021). Other rhabdoviruses including VHSV and IHNV could also cause histopathological changes, including haemorrhages, focal necrosis, cytoplasmic vacuoles, necrotic hepatocytes, severe splenic degeneration and extensive damage to kidney tubules and glomeruli (OIE 2019). Despite such histological alterations in different organs, the infected tilapia did not exhibit severe clinical signs. Similar observations of viral detection in tissues in the absence of prominent clinical signs have also been observed in other rhabdovirus cases. For instance, VHSV existed in the rainbow trout brain tissues for over 400 days without clinical signs (Neukirch 1986). Also, in VHSV, necrotic lesions in the kidney, spleen and liver often healed in the survivors, but low levels of virus particles persist in nerve tissue without active pathology in Pacific herring (Hershberger *et al.* 2010). On the other hand, IHNV was also detected in the brain tissue of sockeye salmon after 9 months post-challenge (Garver *et al.* 2013).

5 | CONCLUSIONS

This study reports the first natural occurrence of SHRV-In in tilapia in India. Targeted protein fragments and genetic linkage indicate that the SHRV-In isolated from tilapia is molecularly identical to SHRV-In isolated from snakehead, although complete genome sequencing is needed for definitive confirmation. While experimental infection caused no mortality, significant histopathological, hematological, and immunological changes indicated host responses to infection. Tilapia may therefore serve as a reservoir host and contribute to viral persistence and transmission, particularly in multispecies and polyculture systems. Therefore, continuous surveillance, strict biosecurity, and improved awareness are essential to limit the spread of SHRV-In in aquaculture.

ETHICAL APPROVAL

The experimental study was conducted following the Government of India's guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for experimentation on fish, 2021.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Priyadharshini: Investigation, methodology, project administration, software and writing-original draft preparation. Sivasankar: Conceptualization, investigation, methodology, project administration, supervision and writing-review and editing. Chrisolite and Rani: Supervision, validation and writing-review and editing. Subash: Method-

ology, data curation, software and supervision. Vijayamitharaj: Supervision and validation. Mohamed Mansoor, Magesh Kumar Palraj and Selvamagheswaran: Investigation and methodology.

AI-USE STATEMENT

The authors declare that AI tools were used solely for language editing and grammar refinement. The authors take full responsibility for the accuracy and scientific content of the 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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