Research Article

Unveiling the ISKNV menace: Disease outbreak investigations in the Oscar fish (*Astronotus ocellatus*) farms of Kerala, India

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ABSTRACT

This manuscript investigates a mass mortality event that occurred among Oscar fish (*Astronotus ocellatus*) in two ornamental fish farms in Kerala, India. The affected fish displayed various clinical signs, including abnormal swimming behaviour, lethargy, damaged fins, and skin haemorrhages. Electron microscopy analysis revealed polygonal viral particles (121±9.2 *x* 113±11.1 nm) in the spleen, indicating a viral cause. The *A. ocellatus* fin (AOF) cells inoculated with the infected tissue homogenate exhibited a cytopathic effect. Furthermore, PCR analysis confirmed the presence of infectious spleen and kidney necrosis virus (ISKNV) infection in the affected fish. This study sheds light on the cause of mass mortality events in cultured Oscar fish and emphasizes the need for preventive measures to control the spread of infectious viral agents in aquaculture systems. It also suggests that ISKNV may have a broader impact on ornamental fish farms in Kerala, requiring urgent mitigation measures in the region's aquaculture industry.

Keywords: Ornamental fish culture, aquarium trade, ISKNV, virus, megalocytivirus, Oscar fish, *Astronotus ocellatus*

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INTRODUCTION

Globally, the industry of ornamental fish aquaculture is expanding. Each year, over 1.5 billion live ornamental fish from 2000 distinct species are traded internationally (Livengood and Chapman, 2007; Ploeg, 2007). This global trade involves the movement of live fish and aquaculture equipment across borders. While international trade plays a vital role in meeting the demand for fish, it also poses risks in terms of disease transmission. When live fish are transported from one country to another, they can carry pathogens, including viral pathogens such as koi herpes virus, spring viraemia of carp virus, and infectious spleen and kidney necrosis virus (ISKNV) (Ariel, 2005; Jeong et al., 2008). These pathogens may be present in the fish themselves, in the water they are transported in, or on surfaces such as nets or tanks. The introduction of infected fish or contaminated equipment into a new region can initiate disease outbreaks in previously unaffected areas. One such important transboundary virus that infects fish is ISKNV which is a large, double-stranded DNA virus belonging to the family Iridoviridae and genus Megalocytivirus (Johan and Zainathan, 2020). The ISKNV mature virion is 150–170 nm in diameter and has icosahedral symmetry (Dong et al., 2008). The World Organization for Animal Health (WOAH), formerly known as the Office International Des Epizooties (OIE), has listed ISKNV, along with its closely related yet distinct counterpart, Red Sea bream iridovirus (RSIV), as notifiable pathogens (OIE, 2022). ISKNV is known to affect over 50 freshwater and marine fish species (Jeong et al., 2008; Whittington and Chong, 2007). Furthermore, this economically devastating virus is widely spread among the countries involved in the international live ornamental fish trade. ISKNV was first isolated in *Siniperca chuatsi*, a species of Chinese mandarin fish (He et al., 2002), and since then the virus has been detected in various fish species from many countries such as Australia (Go and Whittington, 2006; Lancaster et al., 2003), Korea (Jeong et al., 2008), Japan (Tanaka et al., 2014), Malaysia (Razak et al., 2014), Germany (Jung-Schroers et al., 2016), USA (Subramaniam et al., 2016), Vietnam (Dong et al., 2017), Thailand (Baoprasertkul and Kaenchan, 2019), Ghana (Ramírez-Paredes et al., 2019), Indonesia (Sukenda et al., 2020), Brazil (Figueiredo et al., 2022), China (Zhu et al., 2021), and recently from India (Girisha et al., 2020; Swaminathan et al., 2022, 2021). The spread of ISKNV has significantly threatened the global aquaculture industry by resulting in enormous economic losses and massive mortality (He et al., 2001; Wang et al., 2007). In India, ISKNV is considered an exotic pathogen and had been reported in ornamental fishes, including Oscar fish (*Astronotus ocellatus*) (Girisha et al., 2020) and giant gourami, *Osphronemus goramy* (Swaminathan et al., 2021). Furthermore, ISKNV has caused large-scale mortalities in food fish such as wild pearlspot, *Etroplus suratensis* (Swaminathan et al., 2022).

The global ornamental fish trade is about USD 18-20 billion, whereas it is about ~ 60.9 million USD in India and export of ornamental fish is growing at the rate of 11.6 % per annum. Under the Pradhan Mantri Matsya Sampada Yojana scheme of Indian government, special attention is given to the ornamental fish aquaculture industry with an economic stimulus of about ~ 70.2 million USD (Department of Fisheries, Government of India, 2020). Hence it is anticipated that the ornamental aquaculture sector of the country will experience significant growth in the upcoming years. However, the ornamental fish industry in India also faces significant challenges in the form of disease problems as they can cause significant losses and affect the quality of the fish produced.

In this manuscript, we present a case study of different incidences of diseases that occurred in two different Oscar fish hatcheries located in Chalakudy and Perumbavoor, in the state of Kerala, India. Oscar fish, a member of the family Cichlidae, is highly valued as an ornamental fish in the global market due to its colouration (Staeck and Linke, 1995). It is one of the most popular cichlids, widely bred in several nations worldwide, and is a favorite among aquarium enthusiasts (Hochwartner et al., 2010; Rahmati-holasoo et al., 2010). There are about 421 ornamental fish hatcheries in Kerala, India, and among them, 109 hatcheries are involved in the breeding and rearing of Oscar fish (Department of Fisheries Kerala, 2022). After the outbreak of ISKNV in giant gourami in Kerala, India (Swaminathan et al., 2022), our lab routinely performs targeted active surveillance for ISKNV under National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) project. In both cases reported in this paper, the infected fish exhibited clinical signs and a high mortality rate before succumbing to the infection without any improvement after conventional treatments. The primary objective of this study was to comprehensively investigate and identify the specific causative agent responsible for the occurrence of both disease outbreaks.

MATERIALS AND METHODS

Case

Two fish farmers reported the occurrence of mass mortality in Oscar fish juveniles and brood stock cultured in several indoor cement concrete cisterns/tanks between June and August 2022. Farm-1 is located in Chalakudy, Thrissur district and Farm-2 is located in Perumbavoor, Ernakulam district of Kerala, India. In both of these farms the gross clinical signs of the affected fish were documented, and samples were collected for further diagnosis. Likewise, mass mortality among different life stages of Oscar was documented in about 5 farms in the districts of Thrissur and Ernakulam in Kerala during the period of June – August 2022 (Fig. 1). However, samples could not be obtained from these 5 farms for diagnosis.

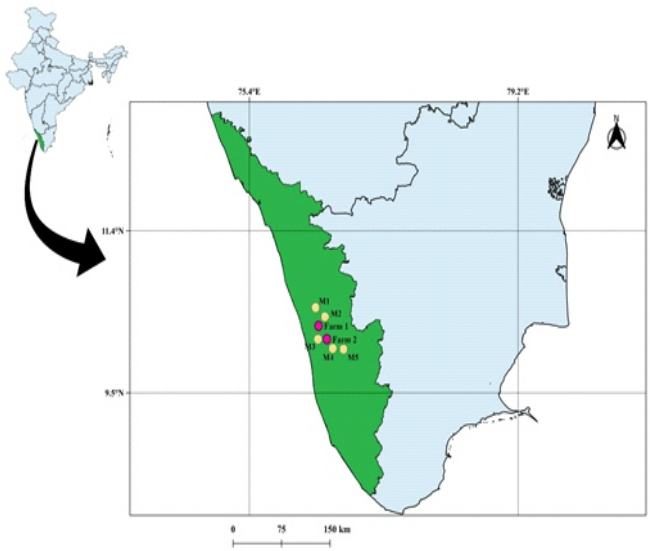

Figure 1. Map showing the farm location of the disease outbreak in Kerala, India. Farm-1 (Pink dot) is located in Chalakudy, Thrissur district, Kerala, India, and Farm-2 (Pink dot) is located in Perumbavoor, Ernakulam district, Kerala, India. The M1-M5 locations (yellow dots) are the sites of fish farms that witnessed mass mortality among Oscar fish during the period of June to August 2022. Unfortunately, no samples could be obtained for diagnosis at the time.

Sampling

The moribund fish juveniles from both cases (body length: 7.5 - 15 cm; body weight: 66 - 120 g) and broodfish (body length: 18.6 - 22.1 cm; body weight: 160 - 274 g) were sampled from both the fish farms and transported to our disease diagnostic laboratory. Samples were collected from moribund fish at two different Oscar fish hatcheries. Scrapings of the skin and gills were taken for parasitological examination, while internal organs were sampled for virological and bacteriological studies. The spleen and kidney tissues were collected and stored in 99% ethanol, RNA later, and L-15 medium with 2% Fetal Bovine Serum (FBS), respectively, for subsequent molecular and virological diagnostics. Additionally, Oscar's gill, kidney, and spleen were fixed in 2.5% glutaraldehyde for transmission electron microscopy (TEM) investigation. The disease outbreaks reported in this study occurred in natural cases. Hence no approval of research ethics committees was required to accomplish the goals of this study.

Disease investigation of infected fish and virus isolation

Observation of clinical symptoms was carried out, and wet mount preparations of scrapings from collected infected fish gills and skin surfaces were examined microscopically for the presence of parasites. For bacterial isolation, swabs of kidney, liver, brain, and spleen tissues were inoculated individually onto tryptone soya agar (TSA) (HiMedia, India) plates and incubated at 28 °C for 24-72 h. Furthermore, pooled gill, brain, liver, spleen, and kidney tissues from individual outbreak samples were processed into a tissue homogenate using the conventional technique for infecting the cell culture monolayer. To prepare the samples, sterile Leibovitz's 15 (L-15) medium was used, supplemented with 1000 IU/mL penicillin, 1000 g/mL streptomycin, and 2.5 g/mL amphotericin B (Life Technologies), without fetal bovine serum (FBS). Pooled tissues were briefly homogenized in the media and then subjected to three cycles of freeze-thawing, alternately, to release the virus particles from the cells. The homogenate was centrifuged at 300 *xg* for 30 min at 4 °C, and the filtrate was passed through a 0.22 µm filter (Millipore, Carrigtwohill, Ireland). In a 25-cm^2 flask (Nunc, Roskilde, Denmark), the filtrate (500 µL) was inoculated onto a confluent monolayer of the *A. ocellatus* fin (AOF) cell line (Kumar et al., 2019) and incubated at 28 °C. Moreover, cells were also inoculated with 500 µL of the medium in the control flask. Cells were examined every day under an inverted microscope (Nikon, Japan) for any cytopathic effect (CPE) until 15 days post-inoculation (dpi). After the observation of CPE,AOF cells and the cell culture media were collected for ISKNV and other known viruses using PCR. Further, virus quantitation was done in the affected tissues homogenates to determine the 50% tissue culture infective dose (TCID50). Briefly, growing media was used to prepare tenfold (10^{-1} to 10^{-10}) serial dilution and was transferred to a well with monolayer of AOF cells (seeding density of 1×10^4 cells per mL) in a 48-well microtiter plate starting with the highest dilution. Two wells containing 100 µL of cell suspension and 100 µL of L-15 with 2% of FBS served as the control. The plates were incubated at 28^0C and all the wells were examined daily for the appearance of cytopathic effect for 2 weeks. The TCID50 values were computed according to Reed and Muench, 1938.

Ultrastructural examination of affected tissue

Transmission electron microscopy (TEM) examination of the spleen and kidney tissues was conducted after fixing them with 2.5% glutaraldehyde. Briefly, the diseased gill, spleen, and kidney tissues were post-fixed in a solution of 2.0% osmium tetroxide prepared in a 0.1 M phosphate buffer, and the fixation process lasted for 1 hour at a temperature of 4 °C. Subsequently, the tissues underwent a dehydration process using gradually increasing concentrations of ethanol before being embedded in araldite CY212. To obtain ultra-thin sections measuring between 60 and 70 nm, a Leica Ultracut UCT microtome was employed for cutting purposes. These sections were then mounted on copper grids and treated with uranyl acetate and alkaline lead citrate for staining. Ultrastructural changes in the processed samples were observed using a transmission electron microscope (Tecnai T12 Spirit transmission electron microscope) operating at 60 kV.

PCR Amplification of ISKNV and Sequencing

Nucleic acids (DNA and RNA) were extracted from the infected fish spleen, kidney tissues, and infected AOF cells using Gene JET RNA purification kit (Thermo Scientific, Lithuania) and DNAeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. cDNA was synthesized by using the Verso cDNA kit (Thermo Scientific). All the infected fish tissue samples, and the AOF cells that showed CPE were tested for the detection of cyprinid herpesvirus (CyHV-2) (Engelsma et al., 2013), spring viraemia of carp virus (SVCV) (Stone et al., 2003), koi ranavirus (KIRV) (George et al., 2015), koi herpesvirus (KHV) (Bercovier et al., 2005), and carp oedema virus (CEV) (Oyamatsu et al., 1997). Further, RSIV and ISKNV (Kurita et al., 1998) were screened by PCR using gene-specific primers. Moreover, the samples underwent screening for ISKNV, wherein a specific region of the major capsid protein (MCP) gene, spanning 1362 base pairs, was targeted (GenBank accession MK778086). The screening was carried out using primers ISKNVF (5′-ATGTCTGCAATCTCAGGTGC- 3′) and ISKNVR (5′- TTACAGGATAGGGAAGCCTG- 3′). The PCR components included 1x GoTaq flexi buffer (Promega, USA), 2.5 mM $MgCl_2$, 1mM dNTP mix, 1.25 units of GoTaq DNA Polymerase (Promega, USA), 50 pmol of both primer and 50 ng of template DNA. PCR amplification was conducted using a thermocycler with a total of 35 cycles. Each cycle consisted of a denaturation step at 94 °C for 30 seconds, followed by an annealing step at 55 °C for 30 seconds, and an extension step at 72 °C for 30 seconds. A final extension step of 10 seconds at 72 °C was conducted. The resulting PCR products were subsequently examined using a 2% agarose gel containing ethidium bromide (EtBr) and visualized utilizing a Bio Imaging System (Bio-Rad, USA). Applied Biosystems 3730 XL capillary sequencer (Agrigenome, Kerala, India) was used to sequence the PCR products.

Phylogenetic analysis of ISKNV

The sequences of ISKNV obtained from Farm-1 and Farm-2 samples were compared alongside with the other ISKNV and iridovirus MCP gene sequences currently available in GeneBank using NCBI-BLAST. The molecular phylogenetic analysis of ISKNV was performed in MEGA by version 10.2.6 (Kumar et al., 2018).

Multiple sequence alignment was performed using MUSCLE aligning the nucleic acid sequences of the MCP gene of ISKNV generated in the present study and other representatives retrieved from the NCBI database. The best-fit model was determined to be Kimura 2-parameter model with invariant sites (K2+I). The phylogenetic tree was constructed using the Maximum likelihood method using the K2+I model with 1000 bootstraps. The tree was visualized using iTOL (Letunic and Bork, 2021).

RESULTS

Examination of moribund fish

Two Oscar fish farms in Kerala India reported mass morality during June to August 2022. Farm-1 from Chalakudy, Thrissur district of Kerala reported a mortality of about 60-70% of the stock in the month of June 2022, and subsequently, a 100% mortality was recorded in July 2022. Water temperature during mass mortalities ranged from 25 to 28 °C. Later in the middle of July 2022, a similar case was reported from farm-2 located at Perumbavoor, Ernakulam district of Kerala. The mortalities of juveniles and adults were ranging between 30-40% in July 2022 and a 100% mortality was observed in the month of August 2022. The water temperature during this period ranged between 25 and 30 °C. In both cases, in the beginning, dozens of fish died in all the cement tanks daily. Consequently, these losses increased and resulted in 100% cumulative mortalities in both fish farms. Interestingly, neither of the fish farms had any previous cases of viral disease outbreaks or mass mortality. The affected fish exhibited abnormal swimming behaviour, lethargy, excess mucous on the skin, damaged fins, loss of scales, and skin hemorrhages (Fig. 2). Similar clinical signs were also documented by the aqua farmers in the 5 other Oscar fish farms that experienced the mass mortality during the same period in that region. Moreover, during necropsy the affected fish exhibited enlarged kidney and spleen.

Figure 2. Mass mortality of Oscar fish in Kerala, India. (A) Dead juvenile Oscar fish with excessive mucous production and loss of scales. (B) Infected Oscar fish showing enlarged spleen and haemorrhages in the body cavity.

Parasitological and bacteriological examination

In both cases, parasites were not found on the gills, body surfaces, and other internal organs. The bacteriological examination of the infected fish did not reveal the presence of any pathogenic bacteria in the kidney, liver, brain, and spleen tissues of the affected fish.

Ultrastructural examination of the affected tissue

Electron microscopic observation revealed vacuolation and numerous polygonal viral particles consistent with iridovirus were observed in the spleen and kidney cells. The mean (mean ± standard deviation) diameter of the complete virions was 121±9.2 nm (n=20) from opposite vertices and 113±11.1 nm (n=20) from opposite faces. Viral particles were naked with an electron-dense core surrounded by a translucent zone and an outer nucleocapsid layer (Fig. 3).

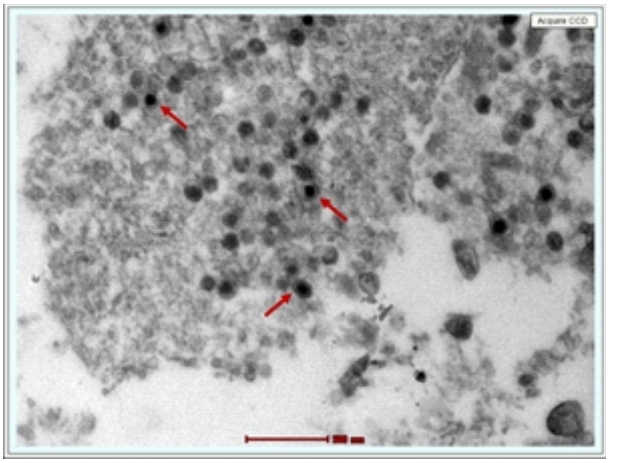

Figure 3. Numerous polygonal viral particles with an electron-dense core surrounded by a translucent zone and an outer nucleocapsid layer visualized in the spleen tissue of the affected fish using a transmission electron microscopy (TEM).

Viral replication in cell culture

CPE was observed in the AOF cell line inoculated with pooled tissue homogenate of the affected fish. Morphological changes like vacuolation, shrinkage, and rounding were observed in the cells at 1–2 dpi. The severity of CPE increased greatly at 3 dpi and numerous rounding cells began to detach from the flask after 5 dpi (Fig. 4). The viral titer on the seventh day was $10^{5.52}$ TCID50 mL^{-1} and $10^{6.11}$ TCID50 mL^{-1} from farm-1 and farm-2 respectively.

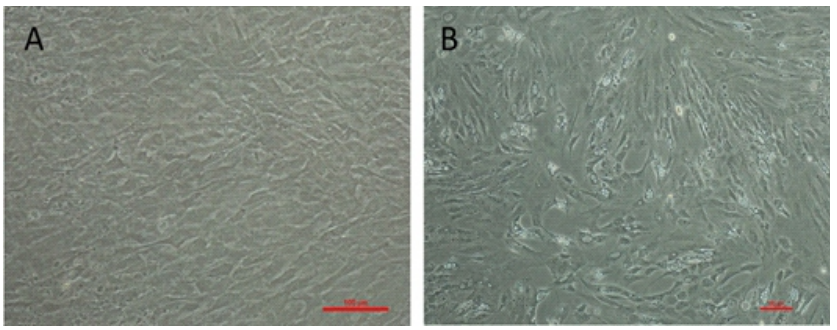

Figure 4. The cytopathic effect (CPE) in AOF cells following infection with ISKNV (A) Uninfected AOF cells at 2 dpi. (B) AOF cells infected with ISKNV showing vacuolation in cells and shrinkage and rounding at 2 dpi.

PCR, sequencing, and phylogenetic analysis

PCR screening of the affected tissues from all the fish samples was negative for SVCV, KHV, KRIV, RSIV, and CEV in the infected fish. However, ISKNV was confirmed in the gill, liver, spleen, and kidney tissues collected from the affected Oscar fish. Furthermore, ISKNV was also positive in the CPE observed AOF cells. The nucleic acid sequences of the MCP gene, which were amplified from infected Oscar fish samples obtained from Farm-1 and Farm-2, have been deposited into the NCBI GenBank database with the respective accession numbers of OP473927 and OQ615295. The MCP gene sequences in this study showed high similarity (< 99%) to that of other reported MCP gene sequences in NCBI databases. The phylogenetic analysis showed that the ISKNV sequences generated in this study clustered together with the ISKNV-I genotype, while the ISKNV-I and ISKNV-II genotypes were separated into distinct clades (Fig. 5).

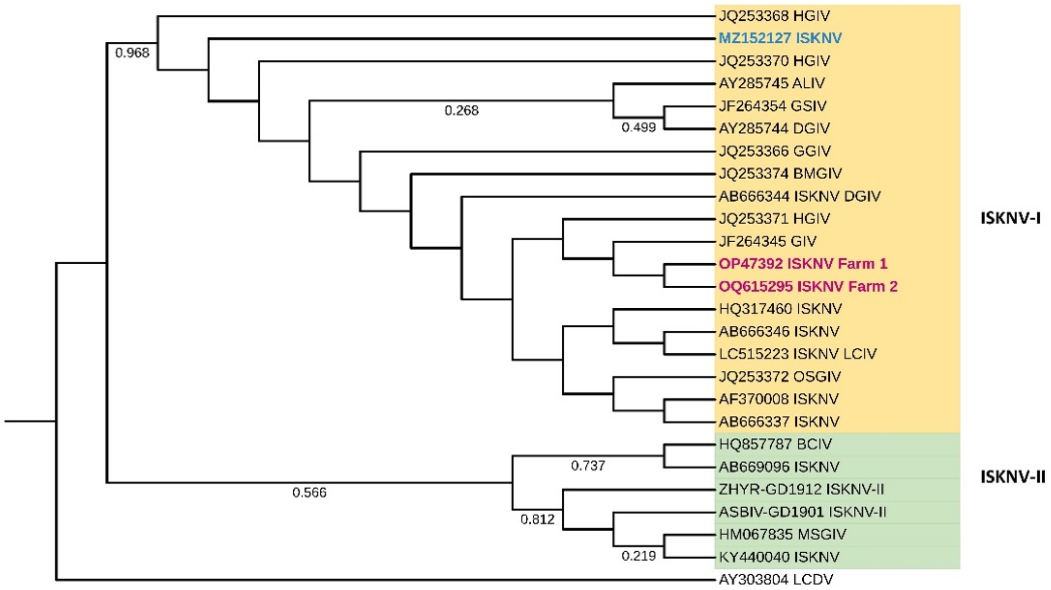

Figure 5. Phylogenetic tree of MCP gene of ISKNV. The nucleotide sequences generated in the present study (OP473927 Farm-1 and OQ615295 Farm-2) are represented in red font clustered with other ISKNV-I genotype sequences. Furthermore, ISKNV isolate from giant gourami reported in 2021 from Kerala India (MZ152127 ISKNV) (Swaminathan et al., 2021) represented in blue font also belongs to the ISKNV-I genotype. Sequences highlighted in yellow and green represent the ISKNV-I and ISKNV-II genotypes, respectively. The MCP gene of the lymphocystis disease virus (NCBI accession number AY303804) was used as an outgroup for the analysis.

DISCUSSION

ISKNV was first detected in India in around 2018-19 from the states of Karnataka and Kerala (Girisha et al., 2020; Swaminathan et al., 2022). This virus was reported from asymptomatic exotic ornamental fishes from the state of Karnataka, India (Girisha et al., 2020). Moreover, two different disease outbreak incidences of ISKNV were observed in Kerala, India. Firstly, the mass mortality of farmed giant gourami (*O. goramy*) (Swaminathan et al., 2021) was caused due to ISKNV outbreak leading to huge economic losses. Secondly, a massive 'Fish Kill Incident' of wild pearl spot (*E. suratensis*) in Peechi Dam located in the Western Ghats biodiversity hotspot (Swaminathan et al., 2022). This

was the first report of ISKNV from a fish species endemic to India and this incidence highlights the threat posed by this virus on the indigenous fish species. Subsequently, in 2022, we encountered disease outbreaks in two different Oscar fish farms in Kerala, India and the diseased fish were collected and subjected to etiological investigation. The affected fish exhibited gross external clinical symptoms like abnormal swimming behavior, lethargy, excess mucous on the skin, damaged fins, loss of scales, and skin hemorrhages. The present study confirms that ISKNV is the main pathogen responsible for both the disease outbreaks in Oscar fish farms with high morbidity and mortality. The confirmation was done through various methods such as virus isolation in cell culture, TEM analysis, and molecular studies. The clinical manifestation of this disease was similar to the previous reports of ISKNV outbreaks (Fusianto et al., 2021; Huang et al., 2021; Kerddee et al., 2021; Subramaniam et al., 2014; Yanong and Waltzek, 2011; Zhu et al., 2021). Furthermore, the pattern of infection and disease progression observed in this study is almost consistent with the ISKNV outbreak happened in 2019 at a giant gourami fish farm in Kerala, India were the morality reached up to 95% in the affected stock (Swaminathan et al., 2021).

Interestingly, both of these fish farms are located at least 35 km apart and they do not have any common source of water or any other means of direct physical contact. In addition, there were reports of similar mortality patterns in five other Oscar fish farms in the region. However, we were unable to obtain samples for diagnosis, making it difficult to confirm ISKNV as the cause of these mass mortalities. Nonetheless, there exists a chance that ISKNV may be responsible for these incidents. ISKNV may have occurred in these fish farms due to various factors, including the introduction of infected fish, contaminated equipment, aerosols, biological vectors or carriers, as well as the potential release of virus-contaminated water from the affected fish farm into natural canals. Furthermore, the occurrence of ISKNV in Oscar fish farms indicates that the virus might have widely spread throughout the region within a span of four years following the first reported incidence of ISKNV in Kerala, India. Hence qualitative and quantitative assessments are needed to estimate the exact distribution and prevalence of ISKNV in the region. The ISKNV virus can be broadly classified into two different genotypes based on the sequence of its major capsid protein (MCP) gene (Fu et al., 2011; Kurita and Nakajima, 2012; Zhu et al., 2021). Both the ISKNV isolates from Farm-1 and Farm-2 in the present study belong to genotype-I (Fig. 5). Similarly, the ISKNV isolated from giant gourami from Kerala India also belongs to the same genotype (Swaminathan et al., 2021).

ISKNV is known to infect and cause disease in more than 50 fish species, dwelling in freshwater, brackish water, and seawater environment (Jung-Schroers et al., 2016). For a long period of time, ISKNV was endemic to the Southeast Asian region. However, the international trade movement of fishes between countries is the major reason for the spread of ISKNV to other parts of the world, particularly among ornamental fishes (Jeong et al., 2008; Whittington and Chong, 2007). For example, in Germany ISKNV outbreak was reported from imported ornamental fishes such as Angelfish (*Pterophyllum altum*) and Platy (*Xiphophorus maculates*) (Jung-Schroers et al., 2016). A similar scenario was also observed in South Korea where ISKNV was reported from 10 different species of imported ornamental fishes (Jeong et al., 2008). Furthermore, the ability of ISKNV to infect food fishes like tilapia and pearl spot threatens food security and livelihood in many developing countries (Figueiredo et al., 2022; Swaminathan et al., 2022). The global supply chain of ornamental fish trade might have been a possible entry route for ISKNV in India. The Indian

ornamental fish industry involves a two-way trade (both import and export) in the global market (Seair Exim Solutions, 2021). In addition, the domestic ornamental fish trade involves an inter-state supply chain across India. Hence this global and domestic transboundary movement of ornamental fish may potentially favour the spread of ISKNV in India. Undoubtedly, the transboundary movement of fish, the high infectivity rate, and the wide host range of ISKNV make it a potential threat to the endemic fishes of India. This further threatens the food security and fish biodiversity of the country. There are no detailed studies related to the susceptibility of different indigenous fishes to ISKNV and research on this area is the need of the hour to evaluate the risk posed by this exotic virus.

We could observe large scale mortalities in the affected animals in the present study (100 % cumulative mortality) and also from the previous reports of ISKNV incidences from Kerala, India (Swaminathan et al., 2022, 2021). The high pathogenicity in the ISKNV isolated in these outbreaks could be indirectly linked to the temperature fluctuations during monsoon season in Kerala, India. Fishes are cold-blooded, and hence their physiological and immunological responses are temperature dependent (Le Morvan et al., 1998). Temperature modulation plays a prominent role in the manifestation of various fish diseases, with the pathogen's virulence being closely linked to a specific temperature range. While most host-pathogen systems maintain a delicate balance between the host's defences and the pathogen's invasiveness, this equilibrium can be easily disrupted by rapid fluctuations in water temperature (Roberts, 2012). Sometimes the infection could be asymptomatic with no apparent clinical signs (Girisha et al., 2020) and a clinical manifestation can occur during the disturbance in the physiological equilibrium of the host. Hence, it is plausible to hypothesize a robust correlation between ISKNV and fluctuation in water temperature. However, further studies are needed to validate this hypothesis. Furthermore, asymptomatic fishes can also act as a carrier for further spread of the virus.

The state of Kerala in India has about 421 small and medium scale ornamental fish hatcheries and farms. These hatcheries and farms handle both exotic and endemic ornamental fish species. Many of these hatcheries were either shutdown or not fully functional during the COVID pandemic lockdowns. However, after post COVID lockdowns many of the farmers from Kerala, India procured exotic ornamental fish brooders from the fish importers to rejuvenate their hatchery operations. Many times, these ornamental fish brooders are imported clandestinely into the country without quarantine or screening of pathogens. After post-COVID lockdowns, the ornamental fish industry is undergoing a revamp, and there is an increasing momentum in the intra-and inter-state trade of live ornamental fish. Hence, it is plausible to hypothesize that the range and prevalence of ISKNV could expand in the coming years in India. The spread of ISKNV could have a significant impact on the ornamental fish trade in this region, as the virus is likely to be transmitted through live ornamental fish trade to other parts of the country. To control the spread of this virus, it is necessary to implement proper screening, quarantine and biosecurity measures by the ornamental fish farmers. Moreover, the risk of ISKNV spread among the wild fish that can lead to their population decline could not be neglected. Hence a multidisciplinary approach is required to comprehensively assess the clinical relevance and infection risks of this economically significant disease.

CONCLUSION

The present study highlights the devastating impact of ISKNV, as evidenced by the outbreak that occurred in Oscar fish farms in Kerala, India. Moreover, it underscores the need for a comprehensive survey to determine the current prevalence of the virus in the region. Such a survey would also help to better understand the impact of abiotic factors such as temperature on the course of the disease. By conducting a wider investigation, it is possible to gain valuable insights that will inform strategies for preventing future outbreaks and mitigating the impact of the virus on the ornamental aquaculture industry. In addition, it is crucial to implement thorough screening procedures for brooders and to maintain strict quarantine and biosecurity measures at the level of individual farms. Moreover, there is an urgent need for research to understand the genetic variations between ISKNV isolates in the region that may help to develop effective vaccines against the virus. By taking these steps, it is possible to minimize the risk of disease transmission and prevent the spread of infections. Therefore, it is essential that fish farmers and industry professionals remain vigilant in their efforts to maintain high standards of hygiene and disease prevention. By better understanding the nature of ISKNV and developing measures to mitigate its spread, we can contribute towards a more sustainable and resilient future for aquaculture.

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DECLARATION OF COMPETING INTERESTS

The authors declared no potential conflicts of interest.

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