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ДОСЛІДЖЕННЯ ВПЛИВУ ОСНОВНИХ ВІРУСІВ РИБ НА АКВАКУЛЬТУРУ (ОГЛЯД)

Аквакультура є галуззю, що розвивається найшвидше у світі, проте стикається із серйозними проблемами, пов'язаними з хворобами, особливо вірусними інфекціями. В останні роки хвороби гідробіонтів мали величезні економічні та екологічні наслідки, а поява нових інфекцій, зокрема вірусів, становить постійну загрозу. Згідно з результатами нещодавніх досліджень, риби містять більше вірусів, ніж будь-який інший клас хребетних. Віруси риб охоплюють широкий спектр патогенів, що належать до різних родин вірусів, таких як Reoviridae, Birnaviridae, Iridoviridae та Rhabdoviridae. Ці віруси вражають види риб на різних стадіях розвитку, викликаючи як легкі, так і важкі захворювання, що часто призводить до масової смертності та значних економічних збитків. Боротьба з вірусними захворюваннями у гідробіонтів залишається складною через брак ефективних терапевтичних засобів, а відкриття потужних вірусних вакцин ще далеко не завершене. Зусилля дослідників, спрямовані на розуміння вірусного патогенезу, взаємодії вірусу та хазяїна, а також розробку вакцин, є важливими для зменшення впливу вірусів риб на сталий розвиток аквакультури. Незважаючи на ці труднощі, існує обмежена інформація про різноманітність, чисельність та еволюцію вірусів риб. В огляді наголошується на різноманітності

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вірусів риб, динаміці їх передачі, їхньому глибокому впливі на аквакультуру та профілактиці з акцентом на важливих видах риб.

Ключові слова: *аквакультура, віруси риб, патогенез, передача вірусів.*

Aquaculture is one of the booming industries globally. It is estimated that fish accounts for approximately 16 percent of the global consumption of animal protein. One of the key factors restricting aquaculture production is the presence of various diseases in commercial and economic fishes. The lack of understanding of fish viruses' abundance, diversity, and evolutionary dynamics has resulted in significant financial and environmental consequences as new aquatic pathogens emerge.

The rise in the need for seafood and the significant growth of aquaculture paves the way for the spread of emerging viruses. Following a substantial economic loss, wild and farmed aquatic populations are vulnerable to several diseases. Diverse aquatic pathogens, such as bacteria, viruses, fungi, and parasites, inhabit natural water bodies, but healthy fishes have exhibited considerable resistance.

They can also adapt to dynamic environmental changes to avoid diseases caused by pathogens. The fish become vulnerable to infections and diseases only when the level of the pathogen in the water body develops rapidly due to external factors, and the natural resistance of the fish stock cannot deal with the increasing pathogens.

Nevertheless, a comprehensive understanding of fish viruses, including their evolution and prevalence, remained limited [109]. Hence, there is a swift demand to explore the unexplored aquatic virosphere, which could hold significant practical value for aquaculture and provide facts about virus ecology and evolution.

According to a recent report, fishes harbor more viruses than any other class of vertebrates. It is indeed important to note that most RNA virus families previously believed to infect mammals have been found in bony fishes [109]. This indicates that the evolution of these viruses encompasses the whole history of the vertebrates. As fishes have an ancient evolutionary origin, it could be a probability that certain fish viruses occupy more basal phylogenetic positions than those seen in the other vertebrate hosts, like mammals, birds, and amphibians [72].

As a remarkable illustration, detecting hepadnavirus in fishes revealed their ancient roots in vertebrates, with more instances of host hopping from aquatic to terrestrial vertebrates [25]. However, there is no indication of viruses that affect fish, causing diseases or infections in humans, emphasizing the phylogenetic gap between fish and humans and the significant distinctions in cell receptors and cell types. While the presence of plant viruses in human feces suggests that viruses can be transmitted passively through food [18], consumption of raw fish has been linked to bacterial (group B Streptococcus) disease in humans [115]. However, the spreading of viruses amongst fish has been said to occur horizontally via feces, contaminated water, or unpasteurized fish products in aquaculture [65]. Effective management strategies for fish viral disea-

ses in aquaculture include stringent biosecurity measures, vaccination programs, and the development of diagnostic tools for rapid detection and early intervention. Fish viruses represent significant challenges to the aquaculture industry, necessitating comprehensive strategies for disease prevention, control, and management. Addressing these challenges requires collaborative efforts among researchers, industry stakeholders, and policymakers to ensure the long-term viability and resilience of global aquaculture production.

Fishes reveal assorted population ecologies likely to play a role in viral diversification and transmission.

Infectious pancreatic necrosis virus (IPNV)

Infectious pancreatic necrosis virus (IPNV), a non-enveloped double-stranded RNA virus belonging to the family Birnaviridae, is a subspecies of the genus *Aquabirnavirus.* This virus poses significant concern within aquaculture, particularly among salmonid farmers. It is estimated that around 80—90 % of stocks of Atlantic salmon (*Salmo salar*), young rainbow trout (*Salmo gairdnery*), and post-smolt are under colossal loss. Infectious pancreatic necrosis (IPN) was initially known as acute catarrhal enteritis by R.H. M'gonigle in 1941 but soon was changed to IPN as the histopathology of the infected brook trout (*Salvelinus fontinalis*) resembled catarrhal enteritis [77]. Necrosis of the exocrine pancreas in the infected salmon fish was the basis for its name and the sub-species type. This viral disease is seen in young fry and salmonids shortly after they absorb their yolk sac and the first feeding.

Genome analysis of the IPN virus confirmed two segments (A and B) of dsRNA around six kilobases (kb) [21]. The viral genome codes for five proteins (VP1 to VP5), three of them are structural proteins (VP1, VP2, and VP3), and the other two are known to be non-structural ones (VP4 and VP5) [27].

Segment A of the viral genome encodes a precursor polyprotein (107 kDa), giving rise to the structural (VP2 and VP3) and non-structural proteins (VP4). The other non-structural protein (VP5) is encoded in a small open reading frame at the 5 end of segment A. Segment B of the genome encodes VP1 encoding for RNA-dependent RNA polymerase protein [27].

The replication cycle of this virus takes about 24 hours at 15 °C. Seven genogroups (I-VII) according to 10 serotypes are known in this virus, and similarity is found among the serotype, genotype, and type strain. The American strains WB (West Buxton), USA, and Ja (Jasper) from Canada would constitute genotype 1, corresponding to serotypes A1 and A9 [11]. Likewise, the Danish-type strains Sp (Spajarup) and Ab (Abildt) constitute serotypes A2 and A3 genotypes 5 and 2. From Germany, Hetch (He) is a serotype of A4 and genotype 6. Meanwhile, genotype 3 comprises isolates clustering with type strains Te (Tellina, from the UK; serotype A5) and C1 (Canada 1; serotype A6). Type strains C3 and C5 (from Canada, corresponding to serotypes A7 and A8) constitute genotype four, and the seventh genotype corresponds to Japanese marine birnavirus (MaBV) [87]. The structure of the pathogen responsible for IPNV infection and its infection site is represented in Fig. 1.

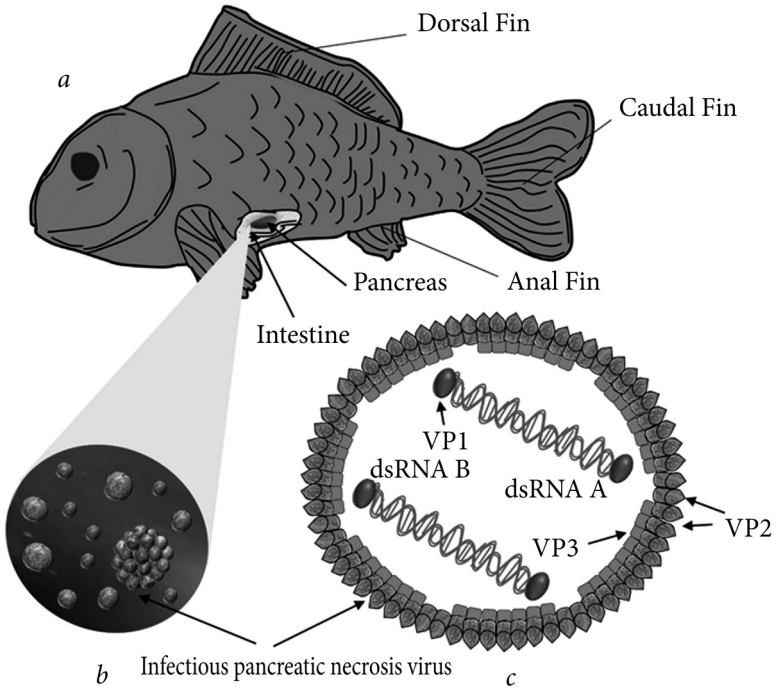

Fig. 1. Structure of pathogen responsible for IPNV infection (*a*) and its infection site (*b*)

A novel IPN virus strain that caused mortality in salmon with genetic resistance in Western Norway was identified [48]. This resulted in the recovery and assemblage of the virus's full-length genome by deep sequencing. Critical amino acid patterns that have previously been linked to virulence in the virus were shared by the newly discovered variant. However, they identified unique deduced amino acid residues in the VP2 hypervariable region. This variation differs from all other reference sequences because these particular mutations resulted in significant outcomes. Even in salmon that have been genetically determined to be resistant to IPN, the changes improve the ability of the virus to elude the defensive host mechanism more effectively. This provides new insight into the pathogenicity and evolution of IPNV variants and encourages monitoring and understanding of the behaviour of the viruses. This study contributes significantly to fish viral research by illuminating fundamental mechanisms behind viral epidemics in salmon populations.

In 2021—2022, a similar study was carried out on farmed Atlantic Salmon (*Salmo salar*). This study emphasizes the significance of using genetically resistant farmed Atlantic salmon and a robust surveillance approach to combat IPNV (Infectious Pancreatic Necrosis Virus). The study's results demonstrate the importance of specific amino acid sequences in VP2, which are critical in determining virulence. Further, it helps in the viral invasion and aiding the immune response [40].

IPNV-resistant fish appears more efficacious in controlling the disease than the vaccines. A protective immune response in salmon can show significant variations depending on their genetic background and the IPNV strains involved. The virus may withstand a temperature of about 10°C for seven months and, interestingly, several years at -20°C (some strains are sensitive to freezing and thawing, especially if the environment has a low pH). It is resistant when exposed to chloroform, ether, and glycerol while inactivated by iodophors, UV radiation, and chlorine.

The virus is diagnosed based on isolation and cell culture following a neutralization test to identify its serotype corresponding to the particular genotype. IPNV can enter a wide range of mammalian cells; however, it is most likely to be receptor-mediated. IPNV replication was not found in any mammalian cell lines investigated [90]. Macropinocytosis is the most common method of virus internalization in CHSE-214 cells (derived from a Chinook Salmon embryo). FHM, CHSE-214, BF2, RTG-2, and EPC are among the cell lines employed.

Liver necrosis, caecal pancreatic acinar necrosis, and epithelial sloughing are the hallmarks of histological analysis of the fish tissue infected with the IPNV virus. Replication of the virus continues in the cell lines derived from a wide range of freshwater, marine hosts, and green turtles [76]. Being resistant to temperature, it retains its infectivity at 60°C for about an hour in fish silage.

Diagnosis is based on the clinical signs, case history, and laboratory examination. QRT-PCR and IFAT are frequently used. Furthermore, its diagnosis should be based on the diagnostic tools' ability to detect various virus strains. The gross pathology of the young infected fish comprises an empty stomach and intestinal tract. Pyloric caeca could consist of petechiae, and the body cavity may contain milky exudate. Other salient features that would help are anorexia, atypical swimming behaviour, frequent pseudo-fecal portions, uncoordinated movements, and periods of lethargy followed by a fast and atypical swim.

As mentioned earlier, though caecal epithelial sloughing, pancreatic acinar necrosis, and liver necrosis are the histological hallmarks of IPNV infection, isolation-based diagnosis in cell culture followed by antibody-based identification of the agent is recommended [92]. To meet the demand for rapid screening, quick immunological assays, like the Indirect fluorescent antibody test (IFAT) [30], and molecular-based techniques such as conventional polymerase chain reaction (PCR) have been developed [121]. Real-time reverse transcription-PCR (qRT-PCR) is commonly used for various reasons, such as its simplicity, specificity, and less time-consuming technique [13, 90].

Variants of RT PCR are widely accepted as a method to screen IPNV. Staining methods like immunoperoxidase, immunofluorescence of infected cells [30], coagglutination [60] or immunoblotting [28] have been used.

Infectious salmon anemia virus (ISAV)

Infectious salmon anemia (ISA), a disease of farmed Atlantic salmon (*Salmo salar*) initially described in 1984 in Norway, is caused by the infectious salmon anemia virus (ISAV). Rainbow trout are also known to be infected. The highest prevalence was observed in the 1990s, necessitating the implementati-

on of disease-prevention measures. Anemia, eye and skin hemorrhaging, ascites, swollen spleen, pale gills, and dark liver are common signs of diseased fish. Circulatory failure is one of the main pathways of disease pathogenesis [1].

The mucosal barrier is one of the most common sites for aquatic viruses, including ISAV, to enter the host [e.g., skin, gills, or gastrointestinal (GI) tract]. As gills are constantly exposed to water, they are believed to be ports of entry for many infectious agents [43]. A schematic representation of ISAV and its target organs is depicted in Fig. 2.

Previous studies have shown that low virulent ISAV (LVI) infects and replicates faster in the gills than highly virulent ISAV (HVI). According to the results of the immersion challenge, the method of entry and phase of early ISAV infection varied amongst isolates.

ISAV is caused by the virulent HPR-deleted strains, known as ISAV-HPR0, which may have evolved from non-virulent variants. The World Animal Health Organization and European Commission (Council Directive 2006/88/EC) have listed infections caused by ISAV as a notifiable disease. Virus outbreaks in farmed Atlantic salmon were documented in Canada, the United Kingdom, Chile, the United States, Scotland, and the Faroe Islands [75].

Most ISAV-infected fish do not survive and die during the production cycle, whereas the outbreak progresses slowly. Disease caused by ISAV is contagious, yet its vertical transmission is still a talk over, while the ability to spread horizontally is well established.

The disease can spread to other salmon sea farms by passive water transmission or using contaminated equipment, as well as fish migration and boat traffic. ISAV is a sub-type of the genus *Isavirus* belonging to the family Orthomyxoviridae. The virus has evolved into cold-water salmonid fish and thrives best at 15 °C.

It is an enveloped virus with a 100—130 nm diameter and a genome composed of eight single-stranded RNA segments with negative polarity. The virus has receptor-destroying, hemagglutinating, and fusion activities, among other things. The virus's morphological, physiochemical, and genetic features are similar to those of Orthomyxoviridae. The virus has also been designated as the type species for the new genus *Isavirus*, which belongs to the Orthomyxoviridae family.

The genome of the virus ISAV encodes at least ten proteins. Four of these are major structural proteins, including a nucleoprotein (68 kDa), a matrix protein (22 kDa) and a haemagglutinin esterase protein (42 kDa) accountable for receptor binding and neutralization. The genome segments coded with a surface glycoprotein (50 kDa) are considered to possess the fusion activity. While segments 1, 2, and 4 codes for the viral polymerases PB1, PB2, and PA, respectively. Segments 7 and 8 contain two open reading frames each. ORF1 of segment 7 codes for a nonstructural protein with interferon antagonistic properties, whereas ORF2 encodes nuclear export protein. A smaller part of the ORF1 of segment 8 encodes the matrix protein. On the other hand, larger segment ORF2 codes for RNA-binding structural protein with interferon antagonistic properties [23].

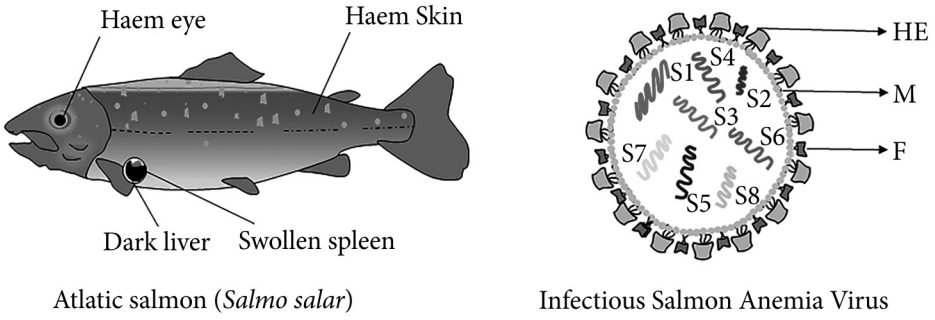

Fig. 2. Schematic representation of ISAV and its target organs

Sequence analyses showed differences between and across specific geographical areas of the isolates. Variability among the 5'-terminal ends of the HE gene has led to classifying ISAV isolates into two significant groups: the European and the North American. There are three subdivisions of the European group. The haemagglutinin gene attributes a highly polymorphic, small region (HPR) where gaps are prevalent rather than single-nucleotide substitutions. Nevertheless, no direct association between deletion patterns and the phylogenetic groups in the HPR has been observed. Still, they have been suggested to be crucial in virulence as all the ISAV-infected fishes carry these deleted regions. The virulence of these isolates, which have identical HPRs, is also influenced by other genes, resulting in significant variations in disease development and severity.

The role of ISAV components in virulence modulation in in vitro and in vivo settings was investigated [16]. It exhibits a distinct pattern from the influenza A virus, in which most segments are infected. To delve deeper, the authors created synthetic reassortant viruses using a highly virulent strain (ISAV 752_09, HPR7b) and an avirulent strain (SK779/06, HPR0) through reverse genetics. The scientists investigated the behaviour of these synthetic viruses on various cell lines by exploring their capacity for infection and replication and their influence on the in vitro cellular immune response. The findings showed that segment 5 of ISAV contributes to the observed traits and is involved in different stages of the viral cycle, influencing viral infection.

The study emphasizes the genetic compatibility between the genomic segments of HPR7b and HPR0, posing a latent risk of reassortment that could lead to a new virus with an unpredictable phenotype. This investigation sheds light on the complex interplay of viral components in determining the virulence and infectivity of ISAV, contributing valuable insights to the field of virology and potential risk assessment for emerging viruses.

The performed studies [35] aimed to investigate the genetic composition of a commercial population of Atlantic salmon that underlies resistance to ISAV. The study investigates the underlying genetic mechanism of this resistance by RNA sequencing. According to the findings, ISAV resistance has a po-

lygenic structure and moderate heritability, making it a good candidate for genome-assisted selection methods. The heart transcriptomes of genetically resistant and susceptible samples reveal an intricate response, indicating a mutual interaction between the host and pathogen that regulates the innate immune response, especially the interferon pathway. The gene TRIM25 emerges as a good option for subsequent functional investigations on ISAV resistance due to the diversity of the transcriptome profiles connected with resistance. The potential of genome editing experiments to elucidate the impact of TRIM25 in disease progression offers a chance for developing and enhancing salmon resistance to ISAV, eventually leading to improved stability, food security, and fish well-being.

ISAV propagated in cell culture was not affected by exposure to 15°C for ten days or 4°C for 14 days. Though ISAV is associated with other influenza viruses, the mechanism of viral entry is distinct. The influenza virus attaches to host cells through the sialic acid-binding domain on the viral fusion protein. Meanwhile, viral entry in ISAV occurs through a multistep mechanism. Before viral attachment and entry, the haemagglutinin esterase of ISAV forms a complex with the F fusion protein on its surface. Upon receptor binding, this complex dissociates, facilitating the endocytosis of the virus by the host cell. Freshwater lakes undergo diurnal oscillation of pH (0.2—0.4 units) and acidic lakes (pH < 6.5) resulting from photosynthetic respiration by aquatic organisms. This acidic pH environment becomes optimal for ISAV fusion. A fusogenic milieu could be generated via acid deposition from anthropogenic sources due to the acidification of Salmo salar ecosystems. At the same time, the absence of ISAV HE protein results in premature ISAV F protein rearrangement. ISAV HE: F complex protects the ISAV F protein from environmental pH fluctuations. A pH decrease could cause ISAV F protein dissociation, which would be an irreversible signal for F protein rearrangement. When exposed to a low-pH environment, the expression of F protein on the surface of cultured cells is adequate for syncytia formation. It's evident from the structures that ISAV HE does not resemble the paramyxoviral HN protein, unlike ISAV HE, which possesses receptor-binding and destroying activities. Conformational rearrangement typically occurs via receptor binding of paramyxoviral HN, transmitting a bound-state signal to the paramyxoviral F protein, triggering rearrangement and culminating in the merger of host and viral membranes. Contrastingly, receptor binding of 4-OAS fails to induce any conformational change in ISAV HE and is unlikely to trigger the activation of ISAV F for host-virus membrane fusion.

The segmented viral genome will be released into the cytoplasm upon fusion of the virus and the endosomal membrane. Subsequently, the genomic RNA of the virus is translocated into the nucleus. The viral proteins are produced in the cytoplasm and endoplasmic reticulum before being directed to the plasma membrane for assembly. Finally, the esterase activity of ISAV HE is neutralized by 4-OAS-containing receptors on the membrane, facilitating the release of the virus from the host cell as a final cycle step.

ISAV infections have no vaccinations or treatments available. Early detection is critical to managing the disease's spread. Isolation of the virus (VI), indirect immunofluorescent antibody testing (IFAT), RT-PCR, and LAMP are routinely used to detect ISAV [42, 85, 86, 88]. Mitochondrial processing peptidase (isothermal and non-PCR-based nucleic acid amplification system) combined with rolling circle amplification /Hbr amplification would aid in developing an on-site field test for the diagnosis of ISAV [79].

Developing a vaccine is a crucial technique for controlling outbreaks by safeguarding the stock or inhibiting the spread of ISAV.

Atlantic salmon paramyxovirus

Paramyxoviruses belonging to the Paramyxoviridae family were initially isolated from adult Chinook salmon *Oncorhynchus tshawytscha*, returning to Oregon's coastal rivers in 1982 and 1983 [123]. Salmonid fry treated at a high titer value do not show any signs of virus replication or mortality, even though these isolates multiply slowly in fish cell lines and can result in persistently infected cultures [69].

ASPV was initially isolated in Norway from an inflamed gill of a fish, a disease with a multifactorial etiology that results in significant losses in farmed Atlantic salmon (*Salmo salar* L.) [67].

Viruses of the Paramyxoviridae family exhibit pleomorphism, with sizes ranging from 150— 300 nm. They possess an enveloped, single-stranded, negative-sense RNA genome of 15—19 kb and are classified into Paramyxovirinae and Pneumo-virinae. *Morbillivirus*, *Rubulavirus*, *Avulavirus*, *Respirovirus*, and *Henipavirus* are the five genera that make up the Paramyxovirinae virus family [14, 68].

The complete genome sequence of ASPV, the first paramyxovirus isolated from teleost fish to be characterized at the molecular level, was described by K. Falk et al. [32]. Paramyxoviruses have also been isolated from finfish species other than Pacific salmon.

A paramyxovirus caused buccal—opercular hemorrhaging and increased mortality in turbot, *Scophthalmus maximus* in Spain [17]. Additionally, a potential paramyxovirus was discovered in the gills of koi, a species of common carp, *Cyprinus carpio* [12]. ASPV has been genetically analyzed and shown to be closely related to the members of the *Respirovirus* genus [32,89].

The ASPV was discovered in Atlantic salmon in 1995. It is a single species within the *Aqua paramyxovirus* genus, characterized by a negative-sense RNA and linear genome comprising six non-overlapping genes. It is enveloped, spherical virions with around 150 nm diameter. When fish are infected, it causes proliferative gill inflammation (PGI) and respiratory issues, propagating mainly within fish farms through contact with infected specimens. Virion attaches itself to the host's cell surface and merges with the plasma membrane, releasing its ribonuclear into the cytoplasm for replication. The virus exits the host cell through budding, enabling it to infect other neighbouring cells and propagate further. ASPV's impact on proliferative gill inflammation is profound, resulting in symptoms that include cell death, a proliferation of gill tissue,

reduced growth rate, and mortality among the affected fishes. This virus has inflicted significant losses on Norwegian aquaculture since the 1980s with increased outbreaks. Given the detrimental effects on aquaculture, implementing PCR would be beneficial for the rapid, accurate detection of the virus in the aquaculture industry.

The complete genome of ASPV, isolated from Atlantic salmon with proliferative gill inflammation (PGI), has been studied. With an RNA genome that is 16,965 nucleotides long, it has six non-overlapping genes that encode the nucleocapsid, phospho-, matrix, fusion, hemagglutinin-neuraminidase, and large polymerase proteins, respectively. These genes are organized in the following order: 3'- N - P/C/V - M - F - HN - L -5'. Gene junctions within the virus exhibit high conservation, characterized by transcription start and stop signal sequences containing intergenic regions. The pathogenesis of paramyxovirus is depicted in Fig. 3.

Infectious hematopoietic necrosis virus

Infectious hematopoietic necrosis virus (IHNV), an economically significant pathogen in aquaculture, induces clinical abnormalities and mortality in various salmonid species, including rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon. It is the primary agent responsible for infectious hematopoietic necrosis.

Virus outbreaks affecting young fish have led to substantial economic losses (up to 90 % or more in fry) [94]. The first case of IHNV infection in blueback salmon brood was reported in the 1950s [105]. The virus has been reported in Austria, Belgium, Korea, China, Chile, Croatia, Switzerland, Russia, Czech Republic, France, Germany, Spain, Iran, Japan, Netherlands, Taiwan, Canada, Poland, Italy, Slovenia, and the USA.

Some of the species of fish highly susceptible to the virus include Chinook salmon (*O. tshawytscha*), steelhead trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), rainbow trout, sockeye salmon (*O. nerka*), chum salmon (*O. keta*), coho salmon (*O. kisutch*), masu salmon (*O. masou*), and biwa trout (*O. rhodurus*) [26].

IHNV belongs to the family Rhabdoviridae, with the genus *Novirhabdovirus*, and was initially isolated from sockeye salmon [122].

The virus is enveloped and has a negative-sense single-stranded RNA; it has a well-defined shape and the structure of a bullet. Its virion size is approximately 150—190 nm long and 65—75 nm wide. The genome of the RNA virus is about 11 kilobases and encodes six viral proteins: nucleoprotein (N), matrix protein (M), polymerase-associated phosphoprotein (P), non-structural protein (NV), glycoprotein (G), and RNA-dependent RNA polymerase (L) [64]. The presence of non-structural protein is distinctive, leading to the establishment of an individual genus, *Novirhabdovirus*, within the Rhabdoviridae family with IHNV as its type species and the Western Regional Aquaculture Centre (WRAC) isolate (Genbank Accession L40883 for sequence) as the type strain.

The N protein interacts with the viral RNA genome to form a ribonucleoprotein (RNP) complex, which coils into a bullet-shaped structure. L and P pro-

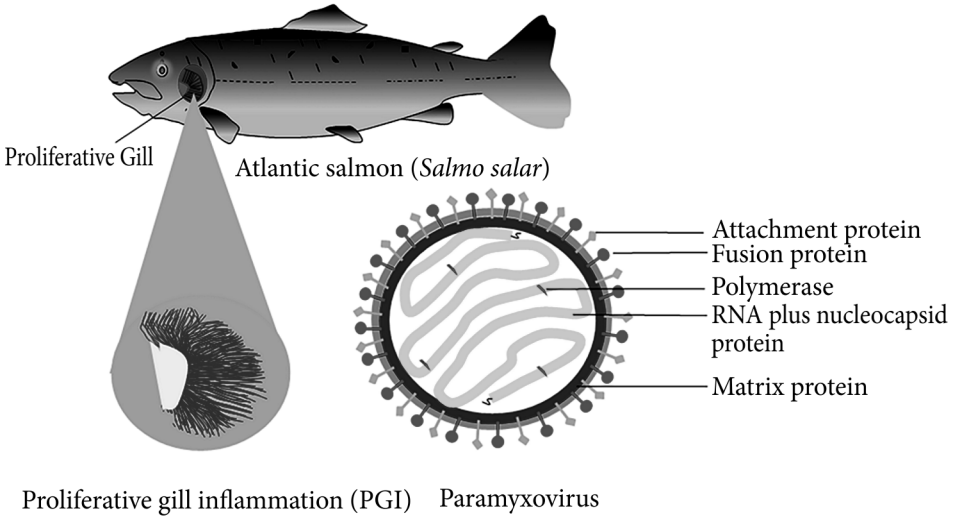

Fig. 3. Pathogenesis of paramyxovirus

teins also associate themselves with the RNP, playing a significant role in genome replication and transcription of viral mRNAs. The M protein lines the inner surface of the host-derived envelope, facilitating the cementing of the RNP and envelope, thereby packing them into a bullet-like shape. Further, M protein inhibits host protein synthesis and induces apoptosis. The NV protein is crucial in the pathogenicity of the virus [117]. A pictorial representation of the hematopoietic necrosis virus and its target organ is depicted in Fig. 4.

Since there are no effective treatment alternatives for IHNV, timely detection plays a pivotal role in preventing and managing the transmission of infectious diseases.

The primary identification of IHNV is based on examining prognostic factors and behavioral changes in infected fish, which seem to be simple to recognize and provide presumptive evidence of infection.

Gross and microscopic examinations, chemical pathology, electron microscopic analysis, and tissue imprinting are other reliable ways to detect and identify IHNV infection. Infected fish become lethargic and exhibit irregular swimming patterns such as erratic whirling, flashing, and spiral swimming. Apart from these, changes in the physical appearance include opaque feces casts, darkening of the skin color, mucoid and pale gills, exophthalmia, and petechial haemorrhages could be noted [33].

In addition, other pathological symptoms of IHNV-infected fishes include pale internal organs, for instance, liver, spleen, and kidney; bloated belly with gelatinous substance; exophthalmia; petechial hemorrhages in the muscles and tissues surrounding the organs of the body cavity; and deformities on the spine. Microscopic pathological signs include degenerative necrosis in hematopoietic tissues, the digestive tract, spleen, pancreas, and liver [107]. Among these, sple-

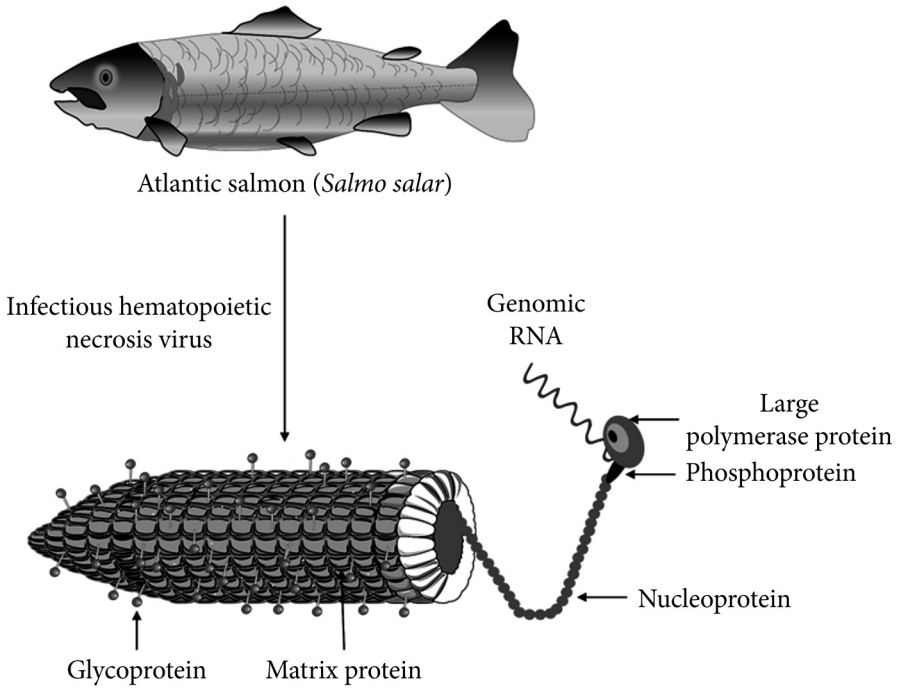

Fig. 4. Pictorial representation of hematopoietic necrosis virus and its target organ

nic and renal hematopoietic tissues exhibit the most severe impact of IHNV infection. Therefore, the virus's cytopathic effect (CPE) is most prominently observed in tissue imprints prepared from the spleen and kidney.

Cell culture following diagnosis of the virus by immunological and molecular techniques is considered the gold standard for the diagnosis of IHNV [15]. It can be detected by observing the development of viral cytopathic effects in cell lines such as fathead minnow under a phase-contrast microscope [22, 26]. Further confirmation tests by molecular method, serological, or a combination of both become mandatory when CPE is observed under an electron microscope [94]. On the other hand, serological diagnostic methods such as virus neutralization test (VNT) and enzyme-linked immunosorbent assay (ELISA) remain necessary for confirming IHNV infection. Based on PCR and loop-mediated isothermal amplification (LAMP) technologies, molecular diagnostic approaches are generally considered more sophisticated than serological procedures due to their enhanced detection sensitivity. Serological diagnosis seldom uses monoclonal or polyclonal antibodies that specifically bind to the pathogen. A classic viral neutralization test (VNT) is time-consuming, taking about 2-8 weeks to complete. However, it is considered a method for detecting IHNV without sacrificing the fish [53]. Other rapid tests based on recognition of viral antigen including the direct and indirect fluorescent antibody tests (FAT/IFAT) [4], western blotting [101], immunohistochemistry [29] and ELISA [61] have been developed successfully. APIC staining and

FAT/IFAT are frequently used to detect IHNV-infected fishes via fixed tissue sections or immunostaining of tissue imprints. It has been reported that APIC assay can detect IHNV in fixed tissues that are up to a year old [29].

Likewise, dot blotting, western blotting, and ELISA have been used to confirm the presence of IHNV by detecting viral components using antibodies that distinctly bind to the viral antigens. To further enhance the serological detection of IHNV, conducted an advanced screening method using flow cytometry to select recombinant antibodies that could serve as potential universal diagnostic reagents was conducted [127].

Apart from particular antibodies, alkaline phosphatase or biotin-labeled nucleic acid hybridization probes could be used to identify the genomic materials of IHNV [41]. Employing molecular diagnosis in clinical microbiology laboratories speeds the identification and detection of IHNV. Reverse transcriptase PCR could often be used to detect N and G genes of IHNV [62]. Compared to RT-PCR, qRT-PCR is also widely utilized and has been proven to have higher sensitivity and a lower risk of contamination [26, 94]. Among its capabilities, it efficiently quantifies viral genome transcripts, making it a valuable tool for determining the health status of an infected fish [95].

RT-droplet digital PCR (RT-ddPCR) was used to identify IHNV quantitatively as an alternative to qRT-PCR [55]. In addition, multiplex RT-PCR (mRT-PCR) has been developed to detect major viruses that infect rainbow trout, including IHNV.

IHNV has an impact on both wild and hatchery-raised salmonids [104]. Research has been implemented for the earlier three decades to develop safe and effective vaccines to combat the disease [71, 103]. Over five years, LaPatra et al. [71]. demonstrated that passive immunity to one strain of IHNV immunized other rainbow trout against all other IHNV variants. It has been shown [102] that two neutralization-resistant attenuated IHNV mutants (RB-1 and 193-110-4) provided significant protection against wild-type IHNV in rainbow trout, resulting in a high survival rate. As a biotechnological innovation, a series of living recombinant IHNV was created using a reverse genetic technique.

Investigations have shown that the N protein sequence has been discovered to play a role in the attenuation of IHNV pathogenicity. Further, N and G sequence alternations provide some protection and immunity [70, 104].

Vaccination is a substitute for fish farmers who can afford to spend more money to protect fish from IHNV infection. Oral vaccinations that are possibly low-cost and have few reports of success are still promising, but they are unsuitable for farmers with limited resources. To date, only Apex-IHN®, a DNA vaccination, has been licensed in USA and Canada. Despite having exceptional defensive efficiency, the application of DNA vaccination is now highly restricted. Therefore, it is essential to support research on DNA vaccine safety.

Infectious Spleen and Kidney Necrosis Virus

ISKNV, a *Megalocytivirus* with a broad host range, was the second virus to cause spontaneous infections in zebrafish, resulting in clinical illness. However, no fatality was reported [113]. It was initially isolated from mandarin fish

in 1998 (*Siniperca chuatsi*) [44]. Megalocytiviruses have been confirmed to infect over 50 distinct fish species, posing a severe threat to the aquaculture industries of China, Japan, Australia, and Southeast Asia [59, 81, 120]. The ISKNV virus was formerly exclusively detected in Singapore, Taiwan, China, and Korea [54, 116], but it was later discovered in the United States [126], Australia [81], Japan [116], and Germany, via fish transportation [59]. Since India imports a wide variety of ornamental fish [36], the potential for viral infection through the imported live fish cannot be disregarded, despite the uncertain pathway of entry. ISKNV was found in *Carassius auratus*, *Astronotus ocellatus*, *Mikrogeophagus ramirezi*, *Pterophyllum scalare*, *Poecilia sphenops*, *Cyprinus carpio koi*, *Maylandia lombardoi*, *Trichopodus trichopterus*, *Poecilia reticulata*, and *Puntius titteye* [37]. Interestingly, among 10 ISKNV-positive fish species studied, three species — *Maylandia lombardoi*, *Puntius titteye*, and *Cyprinus carpio koi* were discovered for the first time globally. Ramírez Paredes et al. ISKNV was discovered in farmed tilapia in Africa [97].

Chloriridovirus, *Megalocytivirus*, *Iridovirus Lymphocystivirus*, and *Ranavirus* are members of the Iridoviridae, a Group I (dsDNA) virus family that comprises five genera recognized by the Eighth Report of the International Committee on the Taxonomy of Viruses (ICTV) [19]. *Lymphocystivirus*, *Megalocytivirus*, and *Ranavirus* are three genera known to infect fish. Iridoviruses have a terminally redundant genome and are circularly permuted, which makes them distinctive among eukaryotic organisms [51]. The iridoviruses infecting fishes notably display highly methylated genomes [118]. An invasive pathogen infection known as Red Sea bream *Iridovirus* (RSIV) was observed in Asian Seabass from India [37]. A pictorial representation of the infectious spleen and kidney necrosis virus with their target organs is given in Fig. 5.

Megalocytiviruses were further separated into genera [66] based on their major capsid protein (MCP) and ATPase gene, identifying infectious spleen and kidney necrosis virus (ISKNV) (Genotype I and II), Red Sea bream iridovirus (RSIV) (Genotype I and II) and turbot reddish body iridovirus (TRBIV) (Genotype III). ISKNV, a newly emerged viral disease, is causing immense economic losses in the ornamental fish sector [46, 125]. Furthermore, transporting live fish for trade across national borders raises the risk of exotic infections. Initially, ISKNV was isolated in Chinese mandarin fish (*Siniperca chuatsi*), which manifests symptoms such as lethargy, aberrant swimming, diminished body coloration, and white gills [45], which leads to considerable economic losses [38]. Subsequently, it spread to other countries such as Malaysia, Singapore, Korea, Australia, Indonesia, and Germany [38, 59, 111, 112].

Livebearers, cichlids, and gourami species are some of the ornamental fishes that are highly vulnerable to ISKNV [126], and surprisingly, few fish species that appeared healthy and had no clinical symptoms were also carrying the ISKNV [54]. As a result of these findings, it is clear that the ISKNV manifests either symptomatic or asymptomatic cases in ornamental fish. India currently classifies ISKNV as an exotic pathogen that needs to be discovered and reported.

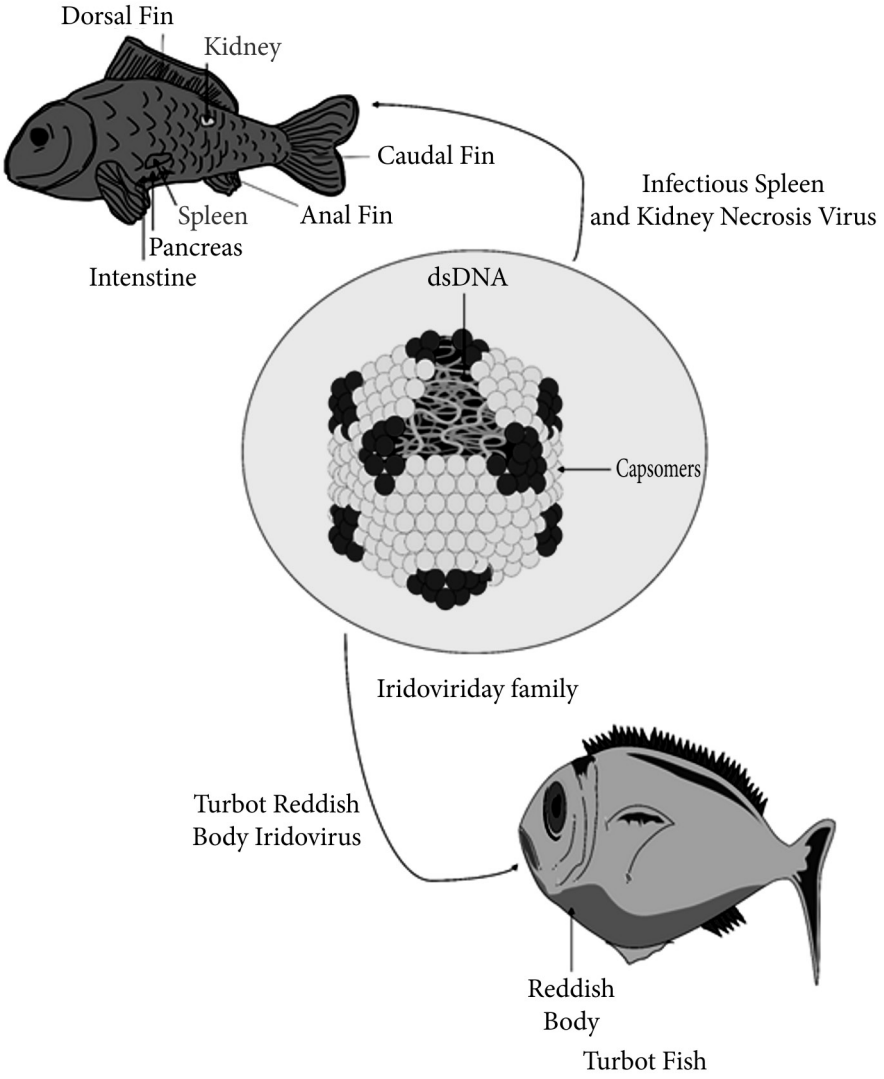

Fig. 5. Pictorial representation of infectious spleen and kidney necrosis virus and Red Sea bream iridovirus with their target organs

Red Sea bream iridoviral disease (RSIVD)

Red Sea bream iridoviral disease is caused by the Red Sea bream iridovirus (RSIV), a double-stranded DNA Icosahedral virus with a 120-240 nm diameter (RSIVD). The *Megalocytivirus* genus belongs to the iridoviridae family, which also contains 56 *Iridovirus*, *Ranavirus*, *Lymphocystivirus*, and *Chloriridovirus* [52]. Megalocytivirus is categorized into RSIV genotype I and genotype II, ISKNV, and turbot reddish body iridovirus (TBIV) [34, 66]. Other Asian countries where RSIV has been found include Taiwan, China, Hong Kong, Korea, Japan, Malaysia, Singapore, and Thailand. Many marine and brackish finfish

species, including *Lates calcarifer*, are known to be infected [99, 124]. The symptoms encompass fatigue, sluggish locomotion, and dilated basophilic 63 cells in vital organs such as the heart, gills, liver, spleen, and kidney [49, 84], with a 100 % fatality rate. The initial incidence of RSIV infection in native Indian Asian seabass has been documented [37]. Infected fish show anaemia in their gills and an enlarged spleen with multiple expanded cells propagating the virus. Several similar viral infections affecting cultured fish in Asia have been documented, including infectious spleen and kidney necrosis virus (ISKNV) documented in mandarin fish (*Siniperca chuatsi*) in China [44], rock bream iridovirus found in striped beakfish (*Oplegnathus fasciatus*) cultures in Korea [58] and turbot reddish body iridovirus (TRBIV) observed in turbot (*Scophthalmus maximus*) in China [108, 110]. The iridovirus contains a genome of double-stranded DNA of about 110 kbp length, enclosed within an icosahedral virion capsid ranging in diameter from 140 to 200 nm [52]. The phylogenetic analysis of the major capsid protein (MCP) gene divides the ISKNV species into three genotypes: the RSIV genotype (found in marine fish), the ISKNV genotype (present in both freshwater and marine fish), and the TRBIV genotype (found primarily in marine flatfish) [39, 66].

Rapid transmission and high mortality rates in infected fish populations threaten the aquaculture fishery sector. Electron microscope, monoclonal antibody technique, immuno-fluorescent antibody tests (IFAT), stained imprints or tissue sections, PCR, and multiplex PCR approaches have been developed [63, 83].

However, when the vaccine cost is too high compared to farmed fish's market value, vaccination is occasionally avoided. Even though certain RSIVD-resistant strains of Red Sea bream have been established using marker-assisted selection paired with DNA-based family selection [106] widespread implementation across vulnerable species in aquaculture will be time-consuming. In complement to vaccines and disease-resistant fish strains, it is crucial to employ strategies for identifying the infection routes and sources to prevent outbreaks. Horizontal transmission through water streams is recognized as the primary way of infection for RSIVD [106]. Fish that resist RSIVD [50], as well as latently infected fish with reduced viral replication at lower temperatures [91], wild fish, and bivalves near fish farms [56] are believed serve as virus carriers or vectors.

It belongs to the Alloherpesviridae [119] family of aquatic herpesviruses [47]. The koi herpesvirus (KHV) (Cyprinid herpesvirus (CyHV) 3) is an emerging viral pathogen affecting common carp and koi carp [82]. Koi herpesvirus disease (KHVD) has been observed in common carp and koi, and their hybrids, with more severe mortality [8]. The spherical to pleomorphic envelope of Koi herpesvirus (KHV), also called cyprinid herpesvirus 3 (CyHV-3), encompasses particles ranging from 180—200 nm. The genome of 295 kbp is the largest known in the order Herpesvirales, harboring at least 156 ORFs. Currently, the NCBI database has 9—11 whole genomic sequences of KHV, the majority of which are from viruses isolated in cell culture [7]. The virus's DNA molecule comprises a base region flanked by 22-kb repeating portions known as the left

and right repeats [5]. The protein is encoded by the genome's 156 open reading frames (ORFs), from which eight are found in duplicate regions. Among these, ORF2, ORF22, ORF25, and gene families TNFR and RING constitute five gene families encoded by KHV's genome. Specifically, six ORFs (ORF25, ORF26, ORF27, ORF65, ORF148, and ORF149) are responsible for encoding membrane glycoproteins of KHV, with their proteins have been detected in mature virions [96]. ORF25 and ORF26 encode glycoprotein type 1 membrane characterized by a hydrophilic transmembrane terminal, a polypeptide segment in the NH2 region of the transmembrane domain, and an exposed COOH terminal part in the cytoplasmic section.

The infection is influenced by age, water, the biological state of the fish, temperature, and several other stressors. Infected fish are known to be more liable to disease in colder water (around 13°C), especially during their earlier stages of development. The virus is transmitted horizontally and indirectly through sediments, water, mud, sea organisms [93], and planktons [80]. Following acute infection and mortality, the remnant population can act as a reservoir for the virus, facilitating its propagation [100].

KHV is effective in a wide range of carp species, including goldfish, koi carp, grass carp, ghost carp, and ornamental catfish, can harbor the infection [93, 100]. Investigations have revealed that freshwater shrimp, goldfish (*Carassius auratus*) [9] a variety of Cyprid, Ictalurid, Percid and Esocid fish [31], grass carp (*Ctenopharyngodon idella*), Crucian carp (*Carassius carassius*), tench (Tinca tinca) [78], rainbow trout (*Oncorhynchus mykiss*) [73] and Prussian carp (*Carassius gibelio*), brown bullhead (*Ameiurus nebulosus*) have also been found to be carriers of the KHV [6].

KHV is currently detected using a variety of diagnostic methods, such as virus isolation in a susceptible cell line, histopathology, ELISA, and PCR [98]. A pictorial representation of Red Sea bream Iridovirus with their target organs is depicted in Figure 5.

Clinical manifestations and cytology. Temperature affects susceptibility to ISKNV transmission, with infection occurring when temperatures exceed 20 °C [46]. ISKNV infection in experimental zebrafish has been associated with clinical symptoms, including inflammation, irregular swimming, colorless or hyperemic gills, low appetite, lethargy, petechial bleeding, and respiratory difficulties. Histopathology revealed the presence of hypertrophied virus-infected cells, along with megalocytes featuring enlarged nuclei characterized by marginated chromatin, basophilic, granular cytoplasm, distributing across various organs such as kidney, spleen, dermis, and lamina propria of the intestine, as well as in various other tissues. Additionally, necrosis, inflammation, and degenerative lesions were noted along with lymphohistiocytic dermatitis, granulomatous polyserositis, and hepatic granulomatous inflammatory infiltrates [10].

Clinical manifestations of primary infections in various fish species encompass malnutrition, flaring, petechiae, whitish or swollen gills, drowsiness, gloomy or pale skin tone, blisters and shattered fin rays, a gaping mouth, erratic motility [59], swollen and dark renal tissue, inflated liver with petechiae, a pale

heart, and splenomegaly are the instances of external lesions. The histopathological observations consist of widespread necrosis in the renal hematopoietic tissue and spleen, along with the identification of enlarged, basophilic virus-infected cells (megalocytes) in various tissues such as cranial connective tissues, kidney, endocardium, and spleen [46].

Real-time PCR stands out as the foremost sensitive diagnostic method for detecting ISKNV. ISKNV was first identified through clinical history and histological examination, later confirmed by electron microscopy [46]. In zebrafish tissues, ISKNV detection can also be achieved through immunohistochemistry employing the monoclonal antibody M10, which has been previously utilized to identify RSIV [10].

Turbot Reddish Body iridovirus (TRBIV)

The turbot reddish body iridovirus (TRBIV) is a novel piscine iridovirus that can cause high mortality in turbot (*Scophthalmus maximus*) cultivated in northern China. TRBIV (turbot reddish body iridovirus) is one of the most common viral infections in farmed turbot, with a mortality rate of up to 40 %. This virus is distinct from the Red Sea bream iridovirus (RSIV) in Japan, the Singapore grouper iridovirus (SGIV) in Singapore, and the infectious spleen and kidney necrosis virus (ISKNV) in China [108, 110].

A conventional PCR technique for identifying TRBIV presence in cultured turbot has been deviced [108]. Traditional PCR, on the other hand, has several disadvantages, including the requirement for a rapid thermal cycler, lack of specificity, and low amplification efficiency [114]. To overcome these limitations in viral diagnostics, a loop-mediated isothermal amplification (LAMP) technique was developed to detect TRBIV. LAMP, a recently developed single-tube technology, can amplify a few DNA copies to over a billion in less than 1 hour, exhibiting excellent specificity, speed, and efficiency in nucleic acid amplification under isothermal conditions. The reaction involves a DNA polymerase with high strand displacement activity and four primers targeting six unique sequences on the target DNA. Amplification of the target gene is achieved within an hour at temperatures ranging from 60 to 65°C [88]. As a result, this method can achieve high specificity in viral detection within a short period without requiring expensive equipment. At the same time, LAMP was used to detect RSIV and RGIV [74]. However, this method has yet to be employed to detect TRBIV specifically. This study employs a sensitive and rapid diagnostic approach to detect TRBIV in turbot.

Dwarf Gourami iridovirus (DGIV)

It belongs to the Iridoviridae family, specifically the genus Ranavirus. DGIV primarily affects Dwarf Gouramis, causing symptoms such as abnormal swimming behavior, loss of appetite, lethargy, bloating, and color changes. Dwarf Gourami (*Trichogaster lalius*) is found in India, Pakistan, Bangladesh, and Nepal in the lowland Ganges and Brahmaputra basins. The University of Sydney's [38] reported the identification of an iridovirus in ornamental gourami's in Australia, as well as the experimental sensitivity of Murray cod (*Maccul-*

lochella peelii) to DGIV infection. The possibility of exotic iridoviruses being brought into Australia via imported ornamental fish, as well as the susceptibility of native Australian fish, is highlighted by these findings. An irido-like virus was discovered in diseased dwarf gourami (*Trichogaster lalius*) brought to Australia from Singapore in 1988 [2]. After a tropical fish trader imported infected Orange chromide cichlid (*Etroplus maculatus*) to Canada from Singapore in 1989, a similar iridovirus disease was discovered in the fish [3]. Eastern India and Bangladesh are home to the dwarf gourami, a freshwater fish. The chromide cichlid is a freshwater fish found in India and Sri Lanka. They are widely used as ornamental fish. The iridovirus infection was also discovered in African lampeye (*Aplocheilichthys centralis*) and dwarf gourami shortly after their importation from Singapore by tropical fish dealers. The African lampeye, a freshwater fish initially from Sierra Leone, Nigeria, and Cameroon, was brought to Singapore and has been cultured there since around 1990, most of which are grown on Sumatra Island, Indonesia. Diseased fish of African lampeye exhibited symptoms consistent with those seen in iridovirus infections of dwarf gourami [2]; orange chromide cichlid [3], brown-spotted groupers, *Epinephelus tauvina* [20], and *E. Malabaricus* [24], sea bass, *Lateolabrax* sp. [57]; *Seriola quinqueradiata*, and striped jack, *Caranx delicatissimus* [49] (92), *Siniperca chuatsi* [44]; and striped beakperch *Oplegnathus fasciatus* [58].

This virus can spread rapidly among fish in aquariums or fish farms, leading to significant mortality rates if not properly managed. Prevention strategies include maintaining good water quality, avoiding overcrowding, and quarantining new fish before introducing them into established aquariums. Unfortunately, there's no specific treatment for DGIV, so prevention and quarantine measures are crucial to control its spread.

Conclusion

As an energetic contributor to global protein and a growing economy, aquaculture faces challenging issues that call for a thorough knowledge of the underlying causes of fish viruses. The sector has been facing numerous constraints and challenges which are complex and multifaceted. This review emphasizes the need to investigate the diversity, prevalence, and evolution of viruses in fish, laying the foundation for adaptable management approaches and responsive prevention strategies. It also sheds light on the critical need to understand fish virus diversity, abundance, and evolution. A thorough understanding of the etiological agents of major fish viruses is required for the development of effective preventive measures and responsive management protocols. These viral challenges highlight the critical need for collaborative efforts in diagnostics, research, and intervention strategies. Collaboration between industry stakeholders, policymakers, and researchers is critical for implementing proactive measures, mitigating risks, and strengthening the aquaculture system's resilience to viral diseases. A comprehensive approach is essential to secure the future of aquaculture and its ability to provide a consistent and abundant supply of fish protein to a growing global population.

Literature Cited

1. Aldrin, M., T.M. Lyngstad, A.B. Kristoffersen et al. 2011. Modelling the spread of infectious salmon anaemia among salmon farms based on seaway distances between farms and genetic relationships between infectious salmon anaemia virus isolates. *J. R. S. Interface* **8**(62): 1346—1356.

2. Anderson, I.G., H.C. Prior, B.J. Rodwell & G.O. Harris. 1993. Iridovirus like virions in imported dwarf gourami (*Colisa lalia*) with systemic amoebiasis. *Australian Veterinary Journal* **70**(2): 66—67.

3. Armstrong, R.D. & H.W. Ferguson. 1989. Systemic viral disease of the chromide cichlid *Etroplus maculatus*. *Dis. Aquat. Organ.* **7**: 155—157.

4. Arnzen, J.M., S.S. Ristow, C.P. Hesson & J. Lientz. 1991. Rapid fluorescent antibody tests for infectious hematopoeitic necrosis virus (IHNV) utilizing monoclonal antibodies to the nucleoprotein and glycoprotein. *J. Aquat. Anim. Health* **3**(2): 109—113.

5. Bayliss, S.C., D.W. Verner-Jeffreys, K.L. Bartie et al. 2017. The promise of whole genome pathogen sequencing for the molecular epidemiology of emerging aquaculture pathogens. *Frontiers in Microbiology* **8**: 121.

6. Bergmann, S.M., M. Cieslak, D. Fichtner et al. 2016. Is there any species specificity in infections with aquatic animal herpesviruses. The Koi Herpesvirus (KHV): An Alloherpesvirus Model. *Fisheries and Aquaculture Journal* **7**(169): 2.

7. Bergmann, S.M., Y. Jin, K. Franzke et al. 2020. Koi herpesvirus (KHV) and KHV disease (KHVD) — a recently updated overview. *Journal of Applied Microbiology* **129**(1): 98—103.

8. Bergmann, S.M., J. Sadowski, M. Kiełpiński et al. 2010. Susceptibility of koi×crucian carp and koi×goldfish hybrids to koi herpesvirus (KHV) and the development of KHV disease (KHVD). *J. Fish. Dis.* **33**(3): 267—272.

9. Bergmann, S.M., H. Schütze, U. Fischer et al. 2009. Detection of koi herpes virus (KHV) genome in apparently healthy fish. *Bulletin of the European Association of Fish Pathologists* **29**(5): 145—152.

10. Bermúdez, R., A.P. Losada, de A.M. Azevedo et al. 2018. First description of a natural infection with spleen and kidney necrosis virus in zebrafish. *J. Fish. Dis.* **41**(8): 1283—1294.

11. Blake, S., J.Y. Ma, D.A. Caporale et al. 2001. Phylogenetic relationships of aquatic birnaviruses based on deduced amino acid sequences of genome segment A cDNA. *Dis. Aquat. Organ.* **45**(2): 89—102.

12. Body, A., F. Lieffrig, G. Charlier & A. Collard. 2000. Isolation of virus-like particles from koi (*Cyprinus carpio*) suffering gill necrosis. *Bulletin of the European Association of Fish Pathologists* **20**(2): 87—88.

13. Bowers, R.M., S.E. Lapatra & A.K. Dhar. 2008. Detection and quantitation of infectious pancreatic necrosis virus by real-time reverse transcriptase-polymerase chain reaction using lethal and non-lethal tissue sampling. *Journal of Virological Methods* **147**(2): 226—34.

14. Brunt, A.A., R.G. Milne, T. Sasaya et al. 2005. Family Paramyxoviridae. Pp. 655—671 in: *Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses* / Ed. by C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, & L. A. Ball. Elsevier Academic Press, London.

15. Burbank, D.R., T.R. Fehringer & L.V. Chiaramonte. 2017. Comparison of selected nonlethal samples from adult steelhead for detection of infectious hematopoietic necrosis virus using cell culture. *J. Aquat. Anim. Health* **29**(2): 67—73.

16. Cárdenas, M., S. Michelson, D.R. Pérez et al. 2022. Infectious salmon anemia virus infectivity is determined by multiple segments with an important contribution from segment 5. *Viruses* **14**(3): 631.

17. Cepeda, C., C. Rivas, J. Lamas et al. 1993. Pathogenicity study of a new viral agent (paramyxovirus) isolated from turbot (samandrag). Pp. 651—655 in: *Fourth National Congress on Aquaculture, Pontevedra, Illa de Arouas, Galicia, Spain.*

18. Chau, M.L., S.L. Chen, M. Yap et al. 2017. Group B Streptococcus infections caused by improper sourcing and handling of fish for raw consumption, Singapore, 2015—2016. *Emerging Infectious Diseases* **23**(12): 2002.

19. Chinchar, V.G., S. Essbauer, J.G. He et al. 2005. *Family iridoviridae.*

20. Chua, F.H., M.L. Ng, K.L. Ng et al. 1994. Investigation of outbreaks of a novel disease,'Sleepy Grouper Disease', affecting the brown-spotted grouper, *Epinephelus tauvina* Forskal. *J. Fish. Dis.* **17**(4): 417—427.

21. Coulibaly, F., C. Chevalier, B. Delmas & F.A. Rey. 2010. Crystal structure of an Aquabirnavirus particle: insights into antigenic diversity and virulence determinism. *J. Virol.* **84**(4): 1792—1799.

22. Crane, M.S. & L.M. Williams. 2008. Viruses of salmonids: virus isolation in fish cell lines. *Australian and New Zealand Standard Diagnostic Procedures. Sub-Committee on Animal Health Laboratory Standards.* http://www.scahls.org.au/_data/assets/pdf_file/0008/1522691/Virus_Isolation. pdf. 2008.

23. Cunningham, C.O., A. Gregory, J. Black et al. 2002. A novel variant of the infectious salmon anaemia virus (ISAV) haemagglutinin gene suggests mechanisms for virus diversity. *Bulletin of the European Association of Fish Pathologists* **22**(6): 366—374.

24. Danayadol, Y. 1997. Iridovirus infection in brown-spotted grouper (*Epinephelus malabaricus*) cultured in Thailand. *Diseases in Asian Aquaculture* 67—72.

25. Dill, J.A., A.C. Camus, J.H. Leary et al. 2016. Distinct viral lineages from fish and amphibians reveal the complex evolutionary history of hepadnaviruses. *J. Virol.* **90**(17): 7920—7933.

26. Dixon, P., R. Paley, R. Alegria-Moran & B. Oidtmann. 2016. Epidemiological characteristics of infectious hematopoietic necrosis virus (IHNV): a review. *Veterinary Research* **47**(1): 1—26.

27. Dobos, P. 1995. The molecular biology of infectious pancreatic necrosis virus (IPNV). *Annual Review of Fish Diseases* **5**: 25—54.

28. Dopazo, C.P. & J.L. Barja. 2002. Diagnosis and identification of IPNV in salmonids by molecular methods. *Molecular Diagnosis of Salmonid Diseases* 23—48.

29. Drolet, B.S., J.S. Rohovec & J.C. Leong. 1993. Serological identification of IHNV in fixed tissue culture cells by alkaline phosphatase immunocytochemistry. *J. Aquat. Anim. Health* **5**: 265—269.

30. Espinoza, J.C. & J. Kuznar. 2002. Rapid simultaneous detection and quantitation of infectious pancreatic necrosis virus (IPNV). *Journal of Virological Methods* **105**(1): 81—85.

31. Fabian, M., A. Baumer & D. Steinhagen. 2013. Do wild fish species contribute to the transmission of koi herpesvirus to carp in hatchery ponds? *J. Fish. Dis.* **36**(5): 505—514.

32. Falk, K., W.N. Batts, A. Kvellestad et al. 2008. Molecular characterisation of Atlantic salmon paramyxovirus (ASPV): a novel paramyxovirus associated with proliferative gill inflammation. *Virus Research* **133**(2): 218—227.

33. Fish viruses and bacteria: pathobiology and protection. 2017. / Ed. by P.T. Woo & R.C. Cipriano. CABI.

34. Fu, S., G. Qu, S. Guo et al. 2011. Applications of loop-mediated isothermal DNA amplification. *Applied Biochemistry and Biotechnology* **163**(7): 845—850.

35. Gervais, O., A. Barria, A. Papadopoulou et al. 2021. Exploring genetic resistance to infectious salmon anaemia virus in Atlantic salmon by genome-wide association and RNA sequencing. *BMC Genomics* **22**(1): 1—4.

36. Girisha, S.K., K.B. Kushala, M.S. Nithin et al. 2021. First report of the infectious spleen and kidney necrosis virus (ISKNV) infection in ornamental fishes in India. *Transboundary and Emerging Diseases* **68**(2): 964—972.

37. Girisha, S.K., T.G. Puneeth, M.S. Nithin et al. 2020. Red Sea bream iridovirus disease (RSIVD) outbreak in Asian seabass (*Lates calcarifer*) cultured in open estuarine cages along the west coast of India: First report. *Aquaculture* **520**: 734712.

38. Go, J., M. Lancaster, K. Deece et al. 2006. The molecular epidemiology of iridovirus in Murray cod (*Maccullochella peelii peelii*) and dwarf gourami (*Colisa lalia*) from distant biogeographical regions suggests a link between trade in ornamental fish and emerging iridoviral diseases. *Molecular and Cellular Probes* **20**(3—4): 212—222.

39. Go, J., T.B. Waltzek, K. Subramaniam et al. 2016. Detection of infectious spleen and kidney necrosis virus (ISKNV) and turbot reddish body iridovirus (TRBIV) from archival ornamental fish samples. *Dis. Aquat. Organ.* **122**: 105—123.

40. Godoy, M., M.J. Kibenge, M. Montes de Oca et al. 2022. Isolation of a New Infectious Pancreatic Necrosis Virus (IPNV) Variant from Genetically Resistant Farmed Atlantic Salmon (*Salmo salar*) during 2021—2022. *Pathogens* **11**(11): 1368.

41. González, J.M. & M.A. Moran. 1997. Numerical dominance of a group of marine bacteria in the alpha-subclass of the class Proteobacteria in coastal seawater. *Applied and Environmental Microbiology* **63**(11): 4237—4242.

42. Gunimaladevi, I., T. Kono, S.E. Lapatra & M. Sakai. 2005. A loop mediated isothermal amplification (LAMP) method for detection of infectious hematopoietic necrosis virus (IHNV) in rainbow trout (*Oncorhynchus mykiss*). *Archives of Virology* **150**(5): 899—909.

43. Gustafson, L.L., S.K. Ellis, M.J. et al. 2007. Hydrographics and the timing of infectious salmon anemia outbreaks among Atlantic salmon (*Salmo salar L.*) farms in the Quoddy region of Maine, USA and New Brunswick, Canada. *Preventive Veterinary Medicine* **78**(1): 35—56.

44. He, J.G., S.P. Wang, K. Zeng et al. 2000. Systemic disease caused by an iridovirus like agent in cultured mandarinfish, *Siniperca chuatsi* (Basilewsky), in China. *J. Fish. Dis.* **23**(3): 219—222.

45. He, J.G., S.P. Weng, Z.J. Huang & K. Zeng. 1998. Identification of outbreak and infectious diseases pathogen of *Siniperca chuatsi*. *Acta Scientiarum Naturalium Universitatis Sunyatseni* **5**: 74—77.

46. He, J.G., K. Zeng, S.P. Weng & S.M. Chan. 2002. Experimental transmission, pathogenicity and physical—chemical properties of infectious spleen and kidney necrosis virus (ISKNV). *Aquaculture* **204**(1—2): 11—24.

47. Hedrick, R.P., O. Gilad, S. Yun et al. 2000. A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *J. Aquat. Anim. Health* **12**(1): 44—57.

48. Hillestad, B, S. Johannessen, G.O. Melingen & H.K. Moghadam. 2021. Identification of a new infectious pancreatic necrosis virus (IPNV) variant in atlantic salmon (*Salmo salar L.*) that can cause high mortality even in genetically resistant fish. *Frontiers in Genetics* **12**: 2172.

49. Inouye, K., K. Yamano, Y. Maeno et al. 1992. Iridovirus infection of cultured Red Sea bream, *Pagrus major*. *Fish Pathology* **27**(1): 19—27.

50. Ito, T., Y. Kawato, Y. Yoshiura et al. 2014. Potential infectivity of the virus re-isolated from surviving Japanese amberjack (*Seriola quinqueradiata*) after experimental infection with Red Sea bream iridovirus. *Bulletin of the European Association of Fish Pathologists* **34**: 17—24.

51. Jakob, N.J., K. Müller, U. Bahr & G. Darai. 2001. Analysis of the first complete DNA sequence of an invertebrate iridovirus: coding strategy of the genome of Chilo iridescent virus. *Virology* **286**(1): 182—196.

52. Jancovich, J.K., V.G. Chinchar, A. Hyatt et al. 2012. Family Iridoviridae. Pp. 193—210 in: *Virus taxonomy: classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses.* / Ed. by A.M.Q. King, M.J. Adams, E.B. Carstens & E.J. Lefkowitz. Elsevier, Amsterdam.

53. Jenčič, V., P. Hostnik, I. Toplak & A. Grilc Fajfar. 2014. Utility of the viral neutralisation test for detection of antibodies to infectious haematopoietic necrosis virus in rainbow trout (Oncorhynchus mykiss). *Bulletin of the European Association of Fish Pathologists* **34**(5): 175—181.

54. Jeong, J.B., H.Y. Kim, L.J. Jun et al. 2008. Outbreaks and risks of infectious spleen and kidney necrosis virus disease in freshwater ornamental fishes. *Dis. Aquat. Organ.* **78**(3): 209—215.

55. Jia, P., M.K. Purcell, G. Pan et al. 2017. Analytical validation of a reverse transcriptase droplet digital PCR (RT-ddPCR) for quantitative detection of infectious hematopoietic necrosis virus. *Journal of Virological Methods* **245**: 73—80.

56. Jin, J.W., K.I. Kim, J.K. Kim et al. 2014. Dynamics of megalocytivirus transmission between bivalve molluscs and rock bream *Oplegnathus fasciatus*. *Aquaculture* **428—429**: 29—34.

57. Jung, S., T. Miyazaki, M. Miyata et al. 1997. Pathogenicity of iridovirus from Japan and Thailand for the Red Sea bream *Pagrus major* in Japan, and histopathology of experimentally infected fish. *Fisheries Science* **63**(5): 735—740.

58. Jung, S.J. & M.J. Oh. Iridovirus-like infection associated with high mortalities of striped beakperch, *Oplegnathus fasciatus* (Temminck et Schlegel), in southern coastal areas of the Korean peninsula. *J. Fish. Dis.* **23**: 223—226.

59. Jung-Schroers, V., M. Adamek, P. Wohlsein et al. 2016. First outbreak of an infection with infectious spleen and kidney necrosis virus (ISKNV) in ornamental fish in Germany. *Dis. Aquat. Organ.* **119**(3): 239—244.

60. Kamei, Y., M. Yoshimizu, Y. Ezura & T. Kimura. 1988. Effects of environmental water on the infectivities of infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV). *Journal of Applied Ichthyology* **4**(1): 37—47.

61. Kim, W.S., M. Mochizuki, T. Nishizawa & M. Yoshimizu. 2008. Detection of specific antibodies against infectious hematopoietic necrosis virus from rainbow trout sera by ELISA using two novirhabdoviruses. *Fish Pathology* **43**(3): 112—116.

62. Knüsel, R., S.M. Bergmann, K. Einer Jensen et al. 2007. Virus isolation vs RT PCR: which method is more successful in detecting VHSV and IHNV in fish tissue sampled under field conditions? *J. Fish. Dis.* **30**(9): 559—568.

63. Koesharyani, I., D. Roza, K. Mahardika et al. 2001. Marine fish and crustacean diseases in Indonesia. Pp. 5—7 in: *Manual for fish disease diagnosis. II. Gondol Marine Research for Mariculture and Japan International Cooperation Agency* / Ed. by K. Sugama, K. Hatai & T. Nakai. Bali.

64. Kurath, G.A. & J.C. Leong. 1985. Characterization of infectious hematopoietic necrosis virus mRNA species reveals a nonvirion rhabdovirus protein. *J. Virol.* **53**(2): 462—468.

65. Kurath, G. & J. Winton. 2011. Complex dynamics at the interface between wild and domestic viruses of finfish. *Current Opinion in Virology* **1**: 73—80.

66. Kurita, J. & K. Nakajima. 2012. Megalocytiviruses. *Viruses* **4**: 521—538.

67. Kvellestad, A., B.H. Dannevig & K. Falk. 2003. Isolation and partial characterization of a novel paramyxovirus from the gills of diseased seawater-reared Atlantic salmon (*Salmo salar* L.). *J. Gen. Virol.* **84**(8): 2179—2189.

68. Lamb, R.A. & G.D. Parks. 2007. Paramyxoviridae: The viruses and their replication. Pp. 1449—1496 in: *Fields Virology*. / Ed. by B.N. Fields, D.N. Knipe & P.M. Howley. Philadelphia, Lippincott Williams & Wilkins.

69. Lannan, C.N., C.K. Arakawa, J.R. Winton & J.L. Fryer. 1989. Persistent infections of fish cell lines by paramyxovirus isolates from Chinook salmon (*Oncorhynchus tshawytscha*). Pp. 309—316 in: *Viruses of Lower Vertebrates*, Springer.

70. LaPatra, S.E., C. Evilia & V. Winston. 2008. Positively selected sites on the surface glycoprotein (G) of infectious hematopoietic necrosis virus. *J. Gen. Virol.* **89**(3): 703—708.

71. LaPatra, S.E., K.A. Lauda & G.R. Jones. 1994. Antigenic variants of infectious hematopoietic necrosis virus and implications for vaccine development. *Dis. Aquat. Organ.* **20**: 119.

72. Lauber, C., S. Seitz, S. Mattei et al. 2017. Deciphering the origin and evolution of hepatitis B viruses by means of a family of non-enveloped fish viruses. *Cell Host & Microbe* **22**(3): 387—399.

73. Matras, M., M. Stachnik, E. Borzym et al. 2019. Potential role of different fish species as vectors of koi herpesvirus (CyHV-3) infection. *Journal of Veterinary Research* **63**(4): 507.

74. Mao, X.L., S. Zhou, D. Xu et al. 2008. Rapid and sensitive detection of Singapore grouper iridovirus by loop mediated isothermal amplification. *Journal of Applied Microbiology* **105**(2): 389—397.

75. Mardones, F.O., A.M. Perez & T.E. Carpenter. 2009. Epidemiologic investigation of the re-emergence of infectious salmon anemia virus in Chile. *Dis. Aquat. Organ.* **84**(2): 105—114.

76. Mertens, P.P., P.B. Jamieson & P. Dobos.1982. In vitro RNA synthesis by infectious pancreatic necrosis virus-associated RNA polymerase. *J. Gen.Virol.* **59**(1): 47—56.

77. M'gonigle, R.H. 1941. Acute catarrhal enteritis of salmonid fingerlings. *Transactions of the American Fisheries Society* **70**(1): 297—303.

78. Michel, B., G. Fournier, F. Lieffrig et al. 2010. Cyprinid herpesvirus 3. *Emerging Infectious Diseases* **16**(12): 1835.

79. Millard, P.J., L.E. Bickerstaff, S.E. LaPatra & C.H. Kim. 2006. Detection of infectious haematopoietic necrosis virus and infectious salmon anaemia virus by molecular padlock amplification. *J. Fish. Dis.* **29**(4): 201—213.

80. Minamoto, T., M.N. Honjo, H. Yamanaka et al. 2011. Detection of cyprinid herpesvirus-3 DNA in lake plankton. *Research in Veterinary Science* **90**(3): 530—532.

81. Mohr, P.G., N.J. Moody, L.M. Williams et al. 2015. Molecular confirmation of infectious spleen and kidney necrosis virus (ISKNV) in farmed and imported ornamental fish in Australia. *Dis. Aquat. Organ.* **116**(2): 103—110.

82. More, S., A. Bøtner, A. Butterworth et al. 2017. Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): *Salmonella* infection in poultry with serotypes of animal health relevance (*S. pullorum, S. gallinarum and S. arizonae*). *EFSA Journal* **15**(8): 4891, 34 pp.

83. Nakajima, K., Y. Maeno, M. Fukudome et al. 1995. Immunofluorescence test for the rapid diagnosis of Red Sea bream iridovirus infection using monoclonal antibody. *Fish Pathology* **30**(2): 115—119.

84. Nakajima, K., Y. Maeno, K. Yokoyama et al. 1998. Antigen analysis of Red Sea bream iridovirus and comparison with other fish iridoviruses. *Fish Pathology* **33**(2): 73—78.

85. Nérette, P., I. Dohoo & L. Hammell. 2005. Estimation of specificity and sensitivity of three diagnostic tests for infectious salmon anaemia virus in the absence of a gold standard. *J. Fish. Dis.* **28**(2): 89—99.

86. Nerette, P., I. Dohoo, L. Hammell et al. 2005. Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anaemia virus. *J. Fish. Dis.* **28**(2): 101—110.

87. Nishizawa, T., S. Kinoshita & M. Yoshimizu. 2005. An approach for genogrouping of Japanese isolates of aquabirnaviruses in a new genogroup, VII, based on the VP2/NS junction region. *J. Gen.Virol.* **86**: 1973—198.1.

88. Notomi, T., H. Okayama, H. Masubuchi et al. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28**(12): E63.

89. Nylund, S., M. Karlsen & A. Nylund. 2008. The complete genome sequence of the Atlantic salmon paramyxovirus (ASPV). *Virology* **373**(1): 137—148.

90. Ørpetveit, I., T. Küntziger, H. Sindre et al. 2012. Infectious pancreatic necrosis virus (IPNV) from salmonid fish enters, but does not replicate in, mammalian cells. *Virol. J.* **9**(1): 1—6.

91. Oh, S.Y., M.J. Oh & T. Nishizawa. 2014. Potential for a live Red Sea bream iridovirus (RSIV) vaccine in rock bream *Oplegnathus fasciatus* at a low rearing temperature. *Vaccine* **32**(3): 363—368.

92. OIE (World Organisation for Animal Health). Manual of diagnostic test for aquatic animals. 2006. 5th edition. OIE, Paris.

93. *OIE (World Organisation for Animal Health). Aquatic Animal Health Code.* 2017. OIE, Paris. Available from: http://www.oie.int/eng/normes/fmanual.

94. *OIE (World Organization for Animal Health). Salmonellosis.* 2018. OIE, Paris. Available at: OIE Terrestrial Manual 2018 — Chapter 3.9.8 Salmonellosis. Accessed 23 April 2019.

95. Overturf, K., S. LaPatra & M. Powell. 2001. Real time PCR for the detection and quantitative analysis of IHNV in salmonids. *J. Fish. Dis.* **24**(6): 325—333.

96. Rakus, K., P. Ouyang, M. Boutier et al. 2013. Cyprinid herpesvirus 3: an interesting virus for applied and fundamental research. *Veterinary Research* **44**(1): 1—6.

97. Ramírez Paredes, J.G., R.K. Paley, W. Hunt et al. 2021. First detection of infectious spleen and kidney necrosis virus (ISKNV) associated with massive mortalities in farmed tilapia in Africa. *Transboundary and Emerging Diseases* **68**(3): 1550—1563.

98. Rathore, G., G. Kumar, T.R. Swaminathan & P. Swain. 2012. Koi herpes virus: a review and risk assessment of Indian aquaculture. *Indian J. Virol.* **23**(2): 124—133.

99. Red Sea bream iridoviral disease. Pp. 251—261 in: WOAH (World Organisation for Animal Health). Manual of Diagnostic. Tests for Aquatic Animals. 2012.

100. Reed, A.N., T. Putman, C. Sullivan & L. Jin. 2015. Application of a nanoflare probe specific to a latency associated transcript for isolation of KHV latently infected cells. *Virus Research* **208**: 129—135.

101. Ristow, S.S., J. De Avila, S.E. Lapatra & K. Lauda. 1993. Detection and characterization of rainbow trout antibody against infectious hematopoietic necrosis virus. *Dis. Aquat. Organ.* **15**: 109—114.

102. Roberti, K.A., J.S. Rohovec & J.R. Winton. 1998. Vaccination of rainbow trout against infectious hematopoietic necrosis (IHN) by using attenuated mutants selected by neutralizing monoclonal antibodies. *J. Aquat. Anim. Health* **10**(4): 328—337.

103. Romero, A., S. Dios, M. Bremont et al. 2011. Interaction of the attenuated recombinant rIHNV-Gvhsv GFP virus with macrophages from rainbow trout (*Oncorhynchus mykiss*). *Veterinary Immunology and Immunopathology* **140**(1—2): 119—129.

104. Rouxel, R.N., C. Tafalla, E. Mérour et al. 2016. Attenuated infectious hematopoietic necrosis virus with rearranged gene order as potential vaccine. *J. Virol.* **90**(23): 10857—10866.

105. Rucker, R.R., W.J. Whipple, J. Parvin & Ch.A. Evans. 1953. A contagious disease of salmon possibly of virus origin. *Bulletin* **76**: 35—46.

106. Sawayama, E., S.-I. Kitamura, K. Nakayama et al. 2019. Development of a novel RSIVD-resistant strain of Red Sea bream (*Pagrus major*) by marker-assisted selection combined with DNA-based family selection. *Aquaculture* **506**: 188—192.

107. Schipp, M. 2012. *Aquatic animal diseases significant to Australia: identification field guide.* Fourth Edition. Canberra, Australian Government Department of Agriculture, FAF.

108. Shi, C.Y., K.T. Jia, B. Yang & J. Huang. 2010. Complete genome sequence of a Megalocytivirus (family Iridoviridae) associated with turbot mortality in China. *Virol. J.* **7**(1): 1—9.

109. Shi, M., X.D. Lin, X. Chen et al. 2018. The evolutionary history of vertebrate RNA viruses. *Nature* **556**(7700): 197—202.

110. Shi, C.-Y., Y.-G. Wang, S.-L. Yang et al. 2004. The first report of an iridovirus-like agent infection in farmed turbot, *Scophthalmus maximus*, in China. *Aquaculture* **236**: 11—25.

111. Song, J.Y., S.I. Kitamura, S.J. Jung et al. 2008. Genetic variation and geographic distribution of megalocytiviruses. *The Journal of Microbiology* **46**(1): 29—33.

112. Subramaniam, K., M. Shariff, A.R. Omar & M. Hair Bejo. 2012. Megalocytivirus infection in fish. *Reviews in Aquaculture* **4**(4): 221—233.

113. Sukenda, S., G. Lila, J.M. Zairin et al. 2020. Identification of giant gourami iridovirus (GGIV): a new infectious spleen and kidney necrosis virus (ISKNV) from natural outbreak in cultured *Osphronemus goramy*. *Aquaculture International* **28**(3): 1069—1082.

114. Sun, Z.F., C.Q. Hu, C.H. Ren & Q. Shen. 2006. Sensitive and rapid detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in shrimps by loop-mediated isothermal amplification. *Journal of Virological Methods* **131**(1): 41—46.

115. Tan, S., Y. Lin, K. Foo et al. 2016. Group B Streptococcus serotype III sequence type 283 bacteremia associated with consumption of raw fish, Singapore. *Emerging Infectious Diseases* **22**(11): 1970.

116. Tanaka, N., T. Izawa, M. Kuwamura et al. 2014. The first case of infectious spleen and kidney necrosis virus (ISKNV) infection in aquarium maintained mandarin fish, *Siniperca chuatsi (*Basilewsky). *Japan Journal of Fish Diseases* **37**(4): 401—405.

117. Thoulouze, M.I., E. Bouguyon, C. Carpentier & M. Brémont. 2004. Essential role of the NV protein of Novirhabdovirus for pathogenicity in rainbow trout. *J. Virol.* **78**(8): 4098—4107.

118. Wagner, H., D. Simon, E. Werner et al. 1985. Methylation pattern of fish lymphocystis disease virus DNA. *J. Virol.* **53**(3): 1005—1007.

119. Waltzek, T.B., G.O. Kelley, M.E. Alfaro et al. 2009. Phylogenetic relationships in the family Alloherpesviridae. *Dis. Aquat. Organ.* **84**(3): 179—194.

120. Wang, Y.Q., L. Lü, S.P. Weng et al. 2007. Molecular epidemiology and phylogenetic analysis of a marine fish infectious spleen and kidney necrosis virus-like (ISKNV-like) virus. *Archives of Virology* **152**: 763—773.

121. Williams, K., S. Blake, A. Sweeney et al. 1999. Multiplex reverse transcriptase PCR assay for simultaneous detection of three fish viruses. *Journal of Clinical Microbiology* **37**(12): 4139—4141.

122. Wingfield, W.H., J.L. Fryer & K.S. Pilcher. 1969. Properties of the sockeye salmon virus (Oregon strain). *Proceedings of the Society for Experimental Biology and Medicine* **130**(4): 1055—1059.

123. Winton, J.R., C.N. Lannan, D.P. Ransom & J.L. Fryer. 1985. Isolation of a new virus from chinook salmon (*Oncorhynchus tshawytscha*) in Oregon USA. *Fish Pathology* **20**(2—3): 373—380.

124. Wu, Y.C., Y.H. Kai & S.C. Chi. 2013. Persistently betanodavirus-infected barramundi (*Lates calcarifer*) exhibit resistances to Red Sea bream iridovirus infection. *Developmental & Comparative Immunology* **41**(4): 666—674.

125. Wu, S.Q., X.H. Li, H.J. Pan & Z.B. Huang. 1997. Research on the pathogen of the outbreak-infective disease of *Siniperca chuatsi*. *Journal of Fisheries of China* **21**: 56—60.

126. Yanong, R.P. & T.B. Waltzek. 2010. *Megalocytivirus infections in fish, with emphasis on ornamental species.* University of Florida EDIS, Florida.

127. Xu, L.M., J.Z. Zhao, M. Liu et al. 2016. High throughput screening of recombinant antibodies against infectious hematopoietic necrosis virus from a combinatorial antibody library. *Aquaculture* **460**: 32—36.

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UNVEILING THE IMPACT OF KEY MAJOR FISH VIRUSES ON AQUACULTURE: A COMPREHENSIVE OVERVIEW

Aquaculture is the world's fastest-expanding industry, yet faces major challenges from aquatic diseases, especially viral infections. In recent years, aquatic diseases have had massive economic and environmental consequences, and the emergence of novel aquatic infections, particularly viruses, poses a constant threat. According to recent discoveries, fishes harbor more viruses than any other vertebrate class. Fish viruses encompass a wide array of pathogens belonging to diverse viral families such as the Reoviridae, Birnaviridae, Iridoviridae, and Rhabdoviridae. These viruses infect various fish species at different developmental stages, causing diseases ranging from mild to severe, often resulting in mass mortalities and substantial economic losses. Addressing viral diseases in aquatic species remains challenging due to the scarcity of effective therapeutics available, and the discovery of potent viral vaccines for delivery in aquatic systems remains a long way off. Research efforts focusing on understanding viral pathogenesis, host-virus interactions, and vaccine development are essential for mitigating the impact of fish viruses on aquaculture sustainability. Despite these challenges, there exists limited information on the diversity, abundance, and evolution of fish viruses. This review emphasizes the diversity of fish viruses, their transmission dynamics, and their profound implications for aquaculture and prevention with a keen focus on important fish species.

Keywords: *aquaculture, fish viruses, pathogenesis, virus transmission.*