Fish Iridoviridae: Unraveling Infection Pathways, Innovative Vaccination Strategies, and Immune Response Dynamics

Muhammad Sajid[1], Uswa Sajid[2], Uzma Rais[3], Kumail Hassan[1], Kaneez Fatima[4], Saba Khurshid[5], Nimra Zubair[5], Zaighum Abbas[6]*

[1] Faculty of Veterinary Science, University of Agriculture Faisalabad, Pakistan.
[2] Department of Aquatic Ecology and Fisheries, Universidade Federal do Pará, Brazil.
[3] Department of Zoology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.
[4] Department of Zoology, University of Baltistan Skardu, Pakistan.
[5] Department of Biological Sciences, University of Sialkot, Pakistan.
[6] *Department of Biotechnology, University of Sialkot Pakistan.

***Corresponding Author:** Zaighum Abbas
*Email: Zaighum.abbas@uskt.edu.pk

Abstract
The number of novel pathogens found and researched each year due to meteorological conditions transformation rises, and the number of diseases that are known to affect different species of fish in different parts of the world is cumulative. The Ranavirus Megalocytivirus and Lymphocystivirus, are among the viruses that produce epizootic outbreaks in freshwater, marine, and farmed fish species. These viruses belong to the family Iridoviridae. Iridoviridae family of viruses can cause serious economic problems, particularly in the aquaculture industry. As a result, vaccinations have been created in recent years, and techniques for administering them have advanced. Currently, various vaccinations are accessible to manage and avert Iridoviridae infections in falcons. Notably, two commercially available vaccinations target Red Sea bream Iridoviral illness and Iridoviruses. The elusion process linked to Iridovirus infections is generally characterized by a decrease in the levels of genetic factors related to the defense system's adaptive reaction and a systemic absence of inflammatory responses. Lastly, with an emphasis on upcoming developments in the sector, this analysis also examines trends in preventative procedures for fish vaccine schemes.

Keywords: Iridoviridae, Megalocytiviruses, Lymphocystiviruses, Ranavirus, Vaccination**.**

Introduction

These infections, which affect both farmed and wild animals and create viral reservoirs among those inhabitants, are marked by notable mortality rates (Zhang, Ke et al. 2022).To reduce this potentially harmful impact, biosecurity measures that focus on four main areas fish, diseases, environment, and personnel monitoring must be put into place. To ensure healthy stocks for fish farming, these management factors include choosing pathogen-free brood stock, optimizing feeding, enforcing strict sanitation and hygiene protocols for facilities, using approved, readily available medications, methods for detecting microbiological pathogens and disinfecting fertilized eggs, rearing water, and effluents (Abd El-Hack, El-Saadony et al. 2022). Additionally, it is critical to incorporate preventative strategies, most notably the development and administration of fish vaccinations, to enhance the resilience and overall health of the aquaculture industry against Iridovirus infections. Several aspects need to be carefully considered to create effective and cutting-edge vaccinations, such as choosing the right vaccine type, administration procedures, and appropriate vaccine antigens. Importantly, choosing immune response pathways and critically determining genes linked to virus suppression or eradication requires a thorough understanding of the fish immune response (Leiva-Rebollo, Labella et al. 2024). Because they provide insightful information about the subtle differences in immune responses among various fish species, research like transcriptome analyses is becoming more and more popular. This makes it easier to identify possible therapeutic targets for the development of vaccines (Kumar, Middha et al. 2024). In aquaculture systems, fish vaccination is an easy, affordable, and efficient way to control microbiological pathologies and prevent many diseases from re-emerging, according to several studies conducted in recent decades. Fish vaccination is the most important ecologically friendly disease control method used in aquaculture. It greatly lessens the need for antibiotics and aids in the prevention and control of viral infections (Kumar, Middha et al. 2024). Additionally, by strengthening fish immune systems and offering targeted, long-term protection against a single microbial infection by stimulating innate and adaptive immune responses, immunostimulants can improve the benefits of vaccinations. Building on these ideas, the primary objective of this article is to provide a thorough analysis of the vaccines now in use that have been created to prevent iridoviral infections in fish raised for aquaculture. It will deal with the difficulties brought about by new and re-emerging illnesses in aquaculture. It also attempts to compile the information that is currently known about fish immunological reactions to Iridovirus illnesses, the immune responses that follow vaccination, and the direction that vaccine development will go (Barnes, Rudenko et al. 2022).

Type of fish virus vaccines and their delivery methods

A vaccination is a biologically produced product intended to stimulate the natural and adaptive immune systems in response to a specific antigen found in or generated from the pathogen that causes the disease, improving protection against a particular infectious agent (Bedekar, Kole et al. 2022). As of right now, 26 approved fish vaccines are offered for sale in the global market. These vaccines are suitable for a variety of fish species and provide defense against a variety of fish pathogens, including some viral families like birnaviruses, alphaviruses, orthomyxoviruses, iridoviruses, and rhabdoviruses. There are several ways to deliver vaccines, such as by injection, oral, or submersion (Irshath, Rajan et al. 2023). Several factors, including the infectious agent, the way of infection, the fish's life phase, labor expenses, immunological memory level, and vaccine production processes, influence the choice of the most effective approach. Since the rate and severity of the natural immune reaction differ based on the vaccination route, the delivery method selection may affect the immune system reaction and the degree of immunity against the target pathogen. By coating, spraying, or encapsulating the fish vaccine, oral immunization introduces it into the diet (Mondal and Thomas 2022). According to Plant and LaPatra, this approach has benefits including low-stress levels, ease of use, and safety when applied to fish of various sizes and developmental stages (Leiva-Rebollo, Labella et al. 2024). Furthermore, oral vaccination is an easy way to increase immunity while the fish are growing out in cages or ponds. The level of protection against disease is increased by oral vaccinations, especially those that contain inactivated entire antigens and the booster shot for the initial vaccination. Compared to immersion immunization, injection techniques more specifically, intraperitoneal (IP) or intramuscular (IM) routes offer benefits. They give fish longer-lasting protection and just need a little quantity of antigen (Ben Hamed, Tapia-Paniagua et al. 2021). When compared to immersion approaches, IP injection is thought to be the most effective and productive method of immunizing fish, frequently utilizing adjuvants for increased protection. This method has been used to give recent vaccinations. On the other hand, because IM administration offers a longer length of protection for DNA immunization, fish farmers choose it. A disadvantage of injection techniques is that stress-induced fish mortality after immunization occurs more frequently (Wang, Ji et al. 2020). Fish can be effectively immunized against microbial infection through immersion vaccination, particularly when using live, attenuated, or vector formulations. We can use a dip or a bath to perform this procedure. Because of its efficiency, speed, ease of use, low stress levels, and affordability, immersion is frequently used and advised for smaller fish. The brief duration of fish immunity, which can last anywhere from three to twelve months, is a drawback though, as booster doses are frequently required (Rathor and Swain 2024). Infectious suspensions that have been subjected to chemical or physical treatment are the source of inactivated. These substances maintain their antigenicity while preventing microbial nucleic acid replication. These vaccines require high inoculation dosages and may cause potentially dangerous reactions due to immune-enhancing adjuvants, despite being very affordable, easily prepared, and demonstrating great stability of immunogenicity in field circumstances (Miccoli, Manni et al. 2021). Furthermore, proteins that have undergone denaturation may become less immunogenic, resulting in weakened or transient immunity. Adjuvants or repeated booster shots may be required to solve this. The live microorganisms used in attenuated vaccinations are incapable of causing a productive infection. Because they are easier to enter hosts, provide humoral and cellular protection, and proliferate more readily than inactivated vaccines, they tend to promote stronger immune responses. They do, however, have a limited shelf life, inadequate thermal stability, and the potential to convert to infectious forms and infect immunocompromised people (Kumar, Middha et al. 2024). Many methods, including gene deletion, natural selection, serial rounds in cell line cultures, and reverse genetics, are used to produce avirulent strains of viruses for attenuated vaccines. In Israel, there is currently only one KoVax commercial vaccination that is based on a live-cultured virus. It treats the koi herpesvirus (KHV) disease in carp and is given intraperitoneally, orally, or submerged. Since subunit vaccines cannot multiply within hosts, they exclusively use immunological components of pathogens and pose no threat to humans or non-target species.These elements can be retrieved directly from the pathogen using in silico analysis, or they can be produced using recombinant expression vectors like yeast, Escherichia coli, insect cells, and cabbage worms. These vaccinations frequently need adjuvants or several booster shots to provide long-term immunity since they may cause a weakened immune response (Bedekar, Kole et al. 2022). DNA vaccines are advantageous and safe since they only require the immunogenic portions of the pathogen. They are composed of plasmids carrying specific antigenic genes. They also have additional benefits such as the capacity to co-administer multivalent vaccinations, flexible, scalable, and cost-competitive production, and durability in storage because the plasmid DNA's chemical stability is increased. Adjuvants are not necessary for DNA vaccines to successfully stimulate humoral and cellular responses (Priya and Kappalli 2022). Fish viruses such as rhabdoviruses, togaviruses, birnaviruses, and Iridovirus have all been the targets of DNA vaccine development. To fight pancreatic illness, a DNA recombinant vaccine carrying the puK-SPDV-poly2#1 plasmid, which encodes several SAV-3 proteins, has been authorized in EU. Surprisingly, studies show that the mode of vaccination delivery influences the immune system's reaction to DNA vaccines considerably, with gastrointestinal and immersion methods showing better immunological protection for fish (Kibenge 2024). Compared to conventional fish vaccinations, more recent RNA-based vaccines provide several benefits. They are more immunogenic, more easily broken down by regular cellular functions, and safer because they don't pose a risk for infection or insertional mutagenesis. There are now two main categories of RNA-based vaccines: self-amplifying mRNA and conventional, non-replicating mRNA. Fish pathogen antigens are substituted for virus structural protein genes in self-amplifying RNA vaccines, which may protect against a range of fish viral illnesses. Aquaculture is paying more attention to mucosal vaccinations because of their extended immunity durations. They might trigger defense mechanisms at mucosal surfaces, preventing the virus from replicating in its original location (Sivakumar, Punniyakotti et al.). A subset of

vaccinations known as "live vectors" express immune-related antigens, such as those associated with the intestinal mucosa, using genetically altered non-pathogenic viruses as carriers. The capacity of live vector vaccines to efficiently induce antigen expression in vivo and hence enhance humoral, cellular, and mucosal immunization is a major advantage. More and more research is being done on nanoparticles as possible aquaculture vaccination candidates. Because of their small size, they can travel throughout the body through the circulatory system and enter target cells like capillaries. In addition to improved immune activation without booster doses, nano vaccines have other benefits. Since they don't need to be kept in a cold chain, they make distribution and storage easier and come with fewer expenses and logistical difficulties than regular vaccinations (Bedekar and Kole 2022).

Iridoviridae: taxonomy and host range

The enormous double-stranded DNA, or dsDNA, viruses of the family Iridoviridae have dimensions ranging from 120 to 200 nm with icosahedral symmetry. Species of this family infect vertebrates, such as amphibians, reptiles, and bony fish, and have a wide host range. Guanine-cytosine (GC) content, protein and nucleotide sequence belonging, phylogenetic relatedness, illness symptoms, and antigenicity are the traits that set the genera apart (Koonin, Dolja et al. 2020).

Megalocytiviruses

The infectious spleen and kidney necrosis virus (ISKNV) and the scale drop disease virus (SDDV) are the two species that make up the genus Megalocytivirus. A sizable cluster of ISKNV species is also present, consisting of three genotypes further separated into clades I and II, for a total of six clades. Strains from the SDDV species are included in the second cluster (Fusianto 2021). Two recent findings are the isolation and classification of an unnamed three-spined stickleback (TSIV) and an SDDV-close European chub Iridovirus. It has been suggested that they belong to the genus MCVs as species. Megalocytiviruses, often called "inclusion body-bearing cells," cause cells in sick organs and tissues, including the kidney, spleen, gastrointestinal tract, and gills, to proliferate (Zhu, Duan et al. 2021). These are frequently fatal systemic diseases. Hypertrophied cells with large granular and basophilic in nature inclusion that push the nucleus and increase the cytoplasm are known as inclusion body-bearing cells. Fish disease manifests as lethargy, aberrant body coloring, gill petechiae, and histologic alterations in the digestive tract, gills, and spleen. All of this could lead to the mortality of fish, especially ornamental species, in clean water and marine habitats, both wild and farmed. Because of its high mortality rates which may occasionally exceed 100% during controlled infection and epizootics in confined fish populations, this genus is gaining more recognition. (Kayansamruaj 2020).

Lymphocystiviruses:

Lymphocystis disease viruses 1-4 (LCDV1-4) are the four species that comprise the genus Lymphocystivirus. The wart-like lesions brought on by LCDV infection can affect more than 100 species of freshwater and marine fish, usually showing up on the fish's external surface.

(Volpe, Errani et al. 2023). These lesions, which are mostly on the skin but can occasionally occur in internal organs, are composed of clusters of single infected cells. High viral morbidity can occur, and in certain situations, a large number of these lesions might make it difficult for infected fish to move around and feed, which can indirectly increase their mortality. The duration of viral propagation in fish varies greatly and is sensitive to temperature, ranging from one week to nine months (Volpatti and Ciulli 2022).

Ranaviruses

The diverse pathogens in the genus Ranavirus can infect lower animals systemically. The WOAH defines ranaviruses as notifiable fish and amphibian diseases, in part due to their wide host range (Hick, Becker et al. 2024). Numerous factors, such as host range, phylogeny, nucleotide sequence identity, and genomic and protein properties, can be used to differentiate between different species of Ranaviruses. The diverse pathogens in the genus Ranavirus are able to infect lower vertebrates systemically. The WOAH defines ranaviruses as notifiable fish and amphibian diseases, in part due to their wide host range. Numerous factors, such as host range, phylogeny, nucleotide sequence identity, and genomic and protein properties, can be used to differentiate between different species of Ranaviruses (Qin, Munang'andu et al. 2023). It has been speculated that ranaviruses have zoonotic mode of transmission having crossed from fish to higher vertebrates such as amphibians and reptiles. This possibility may be because reptiles often share their environment with susceptible fish and amphibians. Ranavirus illnesses can be contracted by touch, injection, or contamination of water or soil, or by consuming water contaminated by infected cold-blooded vertebrates through predation (Rosa, Botto et al. 2022). Concerning the species, virus, and host's age and condition, End of European Perch outbreaks can be more harmful in some species than in others. Due to their rapid spread, ranaviruses attack vulnerable wild and domesticated host animals, resulting in elevated rates of disease and death. Fish infections are commonly characterized by loss of buoyancy, anorexia, redness, hemorrhages, inflamed gills, and destruction of the hematological tissue as well as additional cells and organs (Abbas, Hafeez-ur-Rehman et al. 2023).

Immune responses of infected and vaccinated fish to Iridoviridae

Iridoviridae infection and immune response in fish

Iridoviruses, like many other infections, use reactive oxygen species to get past host and macrophage antiviral barriers, converting these immune cells into vectors for the persistence and propagation of the virus (Liao, Huang et al. 2022). Two

rhabdoviruses, for example, can be inhibited by the Japanese flounder parasite Paralichthys olivaceus's Mx, but not the Red Sea bream iridoviral virus (RSIV). The megalocytivirus TGIV and the ranaviruses ECV and EHNV are not susceptible to the antiviral effects of the Mx proteins obtained from Senegalese sole of barramundi and rainbow trout, Oncorhynchus mykiss (Bedekar and Kole 2022). Remarkably, it is found that at least three Mx isoforms successfully stop the gilthead seabream, the iridoviruses ECV and LCDV-Sa, the lymphocystivirus LCDV-Sa, and the Rhabdovirus VHSV from replicating. Here, it is shown for the first time that a teleost Mx molecule effectively prevents DNA viral infection. Therefore, each fish species' unique virus susceptibility may be determined by how well the teleost IFN/Mx response functions (Hick, Becker et al. 2024).

Thus, after megalocytivirus TGIV infection, a considerable proportion of phagocytic a basophilic and eosinophilic mononuclear leukocytes have been found to have TGIV genomic DNA in their nuclei. All vertebrate iridoviruses share TGIV's characteristic viral immune evasion and propagation mechanism, which is undoubtedly a complex and controlled strategy for subduing and taking advantage of the host defense cells. It is also the case that iridoviruses encode genes that impede the host immune system (Liu, Chen et al. 2018). Multiple potential genes that may aid in suppressing host immunity have been found through sequence analysis. In particular, infection with EHNV and FV3 caused the proinflammatory genes tnfα and il1ß to be expressed, while ECV and DFV caused the immunosuppressive gene transforming growth factor-beta (tgfß) to be temporarily upregulated. Furthermore, ß2-microglobulin genes and apoptotic components were also increased to some extent by all ranaviruses. These genes are essential for surface MHC class I expression and, consequently, for the function of cytotoxic T-cells. This implies that these viral infections may also initiate the adaptive immune response (Zhou, Fu et al. 2022). A class of non-coding RNA known as microRNAs (miRNAs) is essential to many biological processes. They still have a little known function in fish cellular immunity and viral infection mechanisms. The function of miR-124 in orange-spotted grouper infected with SGIV and the ensuing host immune responses have also been studied. Following SGIV infection, grouper miR-124 expression was markedly up-regulated. While miR-124 does not influence virus entry, it may have an impact on viral gene expressions and SGIV-induced cytopathic effects (CPEs). In general, grouper miR124 targets p38a mitogen-activated protein kinase (MAPK) and JNK3 (Jun N-terminal kinase), which may enhance viral propagation and suppress fish immune response(Jancovich, Qin et al. 2015). In grouper spleen cells, transcriptome analysis was also carried out to identify the molecular processes triggered by Ranavirus SGIV infection (Jancovich, Qin et al. 2015). SGIV infection triggered more than 100 DEGs, but the most important ones were the MAPK signaling pathway, which is linked to SGIV-induced cell death, and the cytoskeleton signaling route, which is implicated in the rounding of cells during CPE in infected cells. Additionally, during viral infection, there was an upregulation of the MAPK gene c-Jun, which is implicated in virus assembly and replication. The bulk of DEGs implicated in the immunological response, however, were down-regulated after SGIV infection, which may indicate a potential immune evasion mechanism for the virus (Guo, Wang et al. 2022).

Immune response of fish to Iridoviridae vaccination

Table 1, 2, and 3 list the several vaccines developed to prevent or reduce illnesses caused by viruses in the Alphairidovirinae subfamily. Nowadays, there are only few commercially available vaccines, such as the forma-lin-killed RSIVD vaccine in Japan and AQUAVAC® IridoV in Singapore. It has been established that the AQUAVAC® IridoV vaccine confers immunity against iridoviruses (Leiva-Rebollo, Labella et al. 2024).

Megalocytivirus vaccines

Red sea bream mortality was decreased by a vaccination against RSIV infection that was inactivated with formalin(Min, Kim et al. 2024). The increased immune response unique to fish was the cause of this protective effect. The identical vaccine, virus, and fish host were used in a later investigation, which found that this led to higher plasma levels of neutralizing antibodies and improved MHC class I expression. When fish were immunized with the pure formalin-inactivated vaccine or its protein derivatives, this happened. Only the fish who received the entire vaccination, nevertheless, were able to withstand the virus challenge. This implies that, rather than serum-neutralizing antibodies, cell-mediated immunity was responsible for the fish's survival (Yoshimizu, Kasai et al. 2016). A formalin-inactivated RSIV vaccination was administered to rock bream specimens at different doses together with different formulations of the megalocytivirus vaccine, either with or without aluminum hydroxide as an adjuvant. The neutralizing antibody titers did not differ, according to the results. Temperature and vaccine dosage affected fish survival rates as well as vaccine efficacy (SIVASANKAR 2018). It was investigated how well three viral capsid proteins worked as subunit vaccinations against RSIV infection. Juvenile red sea bream was immunized intraperitoneally (IP) with recombinant formalin-killed Escherichia coli cells producing these capsid proteins. Next, they experienced an RSIV challenge infection. The survival rates of fish immunized with 351R were significantly higher than those of the unvaccinated control group. Co-expression of the fusion protein 351-R with the bacterial enzyme glyceraldehyde 3-phosphate dehydrogenase increased survival rates and neutralizing antibody levels, indicating increased resistance to RSIV infection. The RSIV ORF 055L was cloned into a plasmid containing a CMV promoter to produce a DNA vaccine (Matsuyama, Sano et al. 2018). They evaluated the efficacy of the pcDNA-055 DNA vaccine in producing neutralizing antibodies against RSIV by using virus-infected BF-2 cell cultures during the study. In a different investigation, red sea bream was used to test a DNA vaccine that contained a plasmid expressing the MCP and an ORF of RSIV. In fish that received vaccinations, MHC class I transcript expression rose. The authors had previously used a formalin-inactivated RSIV vaccination to generate a similar pattern of expression. A vaccination against TRBIV in turbots was created using formalin-inactivation. The fish developed a high level of

neutralizing antibodies after receiving the vaccine intramuscularly (IM). The vaccine's subcutaneous and bath administration, according to the authors, significantly decreased fish mortality (Tafalla, Bøgwald et al. 2013).
Furthermore, a formalin-killed cell-cultured vaccine against ISKNV was developed, shielding over 90% of mandarin fish that received the vaccination (Liang, Zhang et al. 2021). Fish that received vaccinations exhibited primarily IgM-mediated immunity. Additionally, it was found that six proteins were strong ISKNV immunogens that only interacted with sera antibodies. The outcomes of a live attenuated immunization against ISKNV in mandarin fish with mutated genes were made public. When challenged with ISKNV after receiving vaccines, fish exhibited a dependent-on-dose reaction to vaccine protection, achieving a full recovery at higher doses. The outcomes of a live attenuated immunization against ISKNV in mandarin fish with mutated genes were made public (Throngnumchai, Jitrakorn et al. 2021). Fish that had received vaccinations and were challenged with ISKNV showed a dose-dependent response to vaccine protection, with 100% survival at higher doses. Furthermore, the vaccination produced neutralizing antibody responses, primarily IgM, that were anti-ISKNV specific. For ISKNV, the megalocytivirus's MCP gene was cloned into the prokaryotic expression vector pBV220. Juvenile mandarin fish were vaccinated by IP using recombinant MCP and an adjuvant, which led to the development of lymphocytes and high serum levels of specific antibodies. The dose-dependent nature of the immune response was found.
(Zeng, Pan et al. 2021). The ISKNV mcp gene was cloned into the eukaryotic expression vector pcDNA3.1 in a different investigation by Chinese perch injected intraperitoneally (IM) with pcM mixed with QCDC adjuvant elicited an immunological response. Genes belonging to the type I IFN system, such as Mx, Viperin, IRAK1, and IRF-7, showed increased expression levels at 6 hours after immunization. However, a second peak in Mx and IRF-7 gene expression was observed 21 days later (He, Shen et al. 2023). Furthermore, a noteworthy elevation in IgM levels was observed. At 28 days after vaccination, the Chinese perch that received a pcMCP augmented with adjuvant vaccine showed an 80% relative percentage survival (RPS). Mandarin fish were immunized via immersion using single-walled carbon nanotubes (SWCNTs), a potential carrier of the ISKNV-DNA vaccine (Leiva-Rebollo, Labella et al. 2024). In comparison to a carrier, the immune response was significantly stronger in fish vaccinated with SWCNTs-pcDNAMCP. Fish immunized with SWCNTs-pcDNA-MCP had an RPS of 82.4% after 14 days, whereas fish immunized with naked pcDNA-MCP had an RPS of just 54.2%. An additional investigation using the SWCNT-based subunit vaccination technology (SWCNT-MCP), which codes for the ISKNV MCP gene (Zhao, Xiong et al. 2020). When compared to fish vaccinated solely with MCP, young mandarin fish vaccinated via immersion showed a greater and more enduring immunological response. A live vector vaccine called BacMCP was created using baculovirus technology. It is driven by a CMV promoter and contains the MCP coding sequence of ISKNV. Large-mouth bass that received the BacMCP vaccination showed overexpression of genes linked to immunity. The method of vaccination and fish size affected the vaccine's effectiveness, which was 100% in smallmouth bass (Qin, Munang'andu et al. 2023).

Table 1. Global Fish vaccines against Megalocytivirus.

Pathogen (Megalocytivirus)	**Vaccine Type**	**Territory**	**Fish Species**	**Antigen**	**Route**	**References**
ISKNV	Subunit	Thailand	Mandarin fish	ORF117, MCP, ORF054	Intraperitoneal	(Throngnumchai, Jitrakorn et al. 2021)
	Inactivated	China	Nile tilapia	MCP	Intraperitoneal	(Zhang, Duan et al. 2020)
	DNA	China	China Perch	MCP	Immersion	(Zhao, Zhang et al. 2020)
	Recombinant	China	Mandarin fish	MCP	Immersion	(Jung, Nikapitiya et al. 2017)
TGIV	Recombinant	China	Grouper	E.coli	Immersion	(Zhang, Liu et al. 2021)
RSIV	Recombinant Live vector	Japan	Rock sea Bream	ORF18R, ORF 351R	Intraperitoneal	(Shimmoto, Kawai et al. 2010)
	Live	Korea	Rock sea Bream	Rearing Temperature	Intramuscular	(Oh, Oh et al. 2014)

Lymphocystivirus vaccines

An investigation was conducted into the expression of a DNA vaccine against LCDV in a Japanese flounder. In fish that received this vaccination, tumor growth was inhibited. Eventually, a DNA vaccination against LCDV was developed, which was placed in PLGA microcapsules to stop nucleases from denaturing DNA in the Japanese flounder's digestive system. For up to 24 weeks, the microencapsulated vaccination dramatically raised the serum of particular antibodies against LCDV (Leiva-Rebollo, Gémez-Mata et al. 2023). They also created PLGA nanoparticles to encapsulate the LCDV vaccine that was designed for Japanese flounders' oral immunization. The fish's immune response was boosted by the nanoparticle vaccine through an increase in antibody, superoxide dismutase, and lysozyme levels as well as the activation of phagocytosis (Radhakrishnan, Vaseeharan et al. 2023). Additionally, a novel DNA vaccination against LCDV-Sa was created. The mcp gene was cloned into a plasmid to create the vaccine, which was then administered intraperitoneally

(IM) to gilthead seabream specimens. This vaccination causes the upregulation of genes involved in the inflammatory process, which is indicative of an immunological response; also, it causes the formation of particular neutralizing antibodies, which is indicative of a humoral immune response The gilthead seabream subjected to an experimental challenge appears to have its infection progression regulated by the vaccination's unique modulation of the immune response (Zheng, Sun et al. 2006).

Table 2. Global Fish vaccines against Lymphocystivirus

Pathogen Lymphocystivirus	Vaccine Strain	Fish Species	Antigen	Route	Territory	References
LCDV3	DNA	Gilthead Sea bream	MCP	Intramuscular	Spain	(Huang, Huang et al. 2011)
LCDV2	DNA	Japanese flounder	MCP	Intramuscular	China	(Leiva-Rebollo, Gémez-Mata et al. 2023)
	Inactivated	Japanese flounder	WCIV	Intraperitoneal	Korea	(Jang, Kim et al. 2011)

Ranavirus vaccines

There haven't been many studies or developments of Ranavirus vaccines to yet. More than 90% of the immunized fish in the orange-spotted grouper vaccination experiment, which used two inactivated Ranavirus SGIV formulations, survived (Ford, Brookes et al. 2022). Pro-inflammatory cytokine and IFN-stimulated gene production suggested that both vaccines elicited a general antiviral immune response. Activating MHC class I and cytokine genes, as well as producing particular serum antibodies, the vaccine produces distinct humoral and cellular immune responses one month following vaccine administration. A bivalent vaccination against the Ranavirus GIV that was formalin-killed and formulated in water-in-oil was created (Ma, Cheng et al. 2022). The vaccine had a protective effect against the Ranavirus infection when it was injected intraperitoneally (IP) into juvenile groupers. The largemouth bass virus (LMBV)-mcp gene was cloned and inserted into the pCDNA3.1(+)-fag plasmid to construct a DNA vaccination. The spleen, head kidney, and liver of the largemouth bass vaccination group revealed markedly elevated expression of the mx, tnfα, il1β, and il8 genes. During the immunization period, a high titer of neutralizing antibodies specific to LMBV was developed by all fish that received the DNA vaccine (Kai, Chang et al. 2024).

Table 3. Global Fish Vaccines against Ranavirus

Pathogen Ranavirus	Vaccine Strain	Fish Species	Antigen	Route	Territory	References
SGIV	Inactivated	Grouper	WCIV	Intraperitoneal	China	(Ou-yang, Wang et al. 2012)
	DNA	Grouper	ORF19R	Intramuscular	China	(Yu, Zheng et al. 2019)
LMBV	subunit	Largemouth bass	MCP	Immersion	China	(Jia, Guo et al. 2020)
	DNA	Largemouth bass	MCP	Hypodermic	China	(Yi, Zhang et al. 2020)
	Recombinant-live Vector	Largemouth bass	MCP	Oral	China	(Yao, Zhang et al. 2022)

Conclusions

Iridovirus immunity is complex and multifaceted, and it is likely regulated by species and stage of development specialization, as the evidence presented in this review clearly shows. Additionally, these studies highlight important gaps in our understanding of the immune system responses that these viruses elicit as well as possible weaknesses in the host's ability to produce strong defenses that are capable of managing and eliminating such infections. Special attention should be paid to the various very efficient strategies used by the family Iridoviridae to evade host immune components. In addition to facilitating spread and extending the host range, this encourages viral persistence. Adaptive immune system suppression and a systematic reduction in inflammatory responses are the outcomes of most infections, according to the data currently available on the immune response elicited by Iridovirus infection. These findings suggest that the virus might be employing specific strategies to evade the host immune system's identification. Studying these immune defense pathways may aid in designing new vaccination tactics against diverse viruses from the Iridoviridae family. The aquaculture industry's needs cannot be fully met by the approved vaccines now in use, and there is a severe shortage of fish virus vaccines. Thus, greater research into the creation of highly potent aquatic vaccines is needed in order to meet

aquaculture needs. Future studies on iridovirus vaccines will concentrate on comprehending the mechanism underlying mucosal immunity and how it relates to systemic immunity.

Additional research is also required on adjuvants, effective dosages, vaccine material coatings and carriers, and the processing of antigens for vaccine manufacture. The length of the immunization, the quantity of booster shots, and the physiological and development stages of the fish are other crucial factors that need to be taken into account. Enhancing the fish vaccine assessment method also requires identifying alterations in the quantities of genes and proteins, as well as in the antibodies and cellular reactions of the immunized fish. For the best possible vaccine design, the right viral antigens must be chosen. The rapid advancement of omics, including functional, proteome, metabolome, and genome, in addition to genome editing technologies, may yield significant new insights into the genomes of fish iridoviruses, the mechanisms of infection, and the identification of genetic targets for highly effective vaccinations. It is obvious that vaccinations based on nucleic acids, including DNA, mRNA, and live vector vaccines, will be crucial in avoiding infectious viral illnesses in aquaculture.

References

1 Abbas, F., M. Hafeez-ur-Rehman, N. Mubeen, A. Bhatti and I. Daniel (2023). Parasites of Fish and Aquaculture and their Control. Parasitism and Parasitic Control in Animals: Strategies for the Developing World, CABI GB**:** 248-266.

2 Abd El-Hack, M. E., M. T. El-Saadony, H. F. Ellakany, A. R. Elbestawy, S. S. Abaza, A. M. Geneedy, A. F. Khafaga, H. M. Salem, A. H. Abd El-Aziz and S. J. M. P. B. Selim (2022). "Inhibition of microbial pathogens in farmed fish." **183**: 114003.

3 Barnes, A. C., O. Rudenko, M. Landos, H. T. Dong, A. Lusiastuti, L. H. Phuoc and J. J. R. i. A. Delamare-Debouteville (2022). "Autogenous vaccination in aquaculture: A locally enabled solution towards reduction of the global antimicrobial resistance problem." **14**(2): 907-918.

4 Bedekar, M. K. and S. Kole (2022). DNA Vaccines for fish. Advances in fisheries biotechnology, Springer**:** 289-336.

5 Bedekar, M. K. and S. Kole (2022). Types of vaccines used in aquaculture. Fish immune system and vaccines, Springer**:** 45-63.

6 Bedekar, M. K., S. J. V. D. M. Kole and V. V. f. V. D. Protocols (2022). "Fundamentals of fish vaccination." 147-173.

7 Ben Hamed, S., S. T. Tapia-Paniagua, M. Á. Moriñigo and M. J. T. J. A. R. Ranzani-Paiva (2021). "Advances in vaccines developed for bacterial fish diseases, performance and limits." **52**(6): 2377-2390.

8 Ford, C. E., L. M. Brookes, E. Skelly, C. Sergeant, T. Jordine, F. Balloux, R. A. Nichols and T. W. J. V. Garner (2022). "Non-Lethal Detection of Frog Virus 3-Like (RUK13) and Common Midwife Toad Virus-Like (PDE18) Ranaviruses in Two UK-Native Amphibian Species." **14**(12): 2635.

9 Fusianto, C. K. (2021). Detection and control of infectious spleen and kidney necrosis virus in aquaculture.

10 Guo, X., W. Wang, Q. Zheng, Q. Qin, Y. Huang, X. J. F. Huang and S. Immunology (2022). "Comparative transcriptomic analysis reveals different host cell responses to Singapore grouper iridovirus and red-spotted grouper nervous necrosis virus." **128**: 136-147.

11 He, J.-h., W. Shen, D. Han, M. Yan, M. Luo, H. Deng, S. Weng, J. He and X. J. J. o. V. Xu (2023). "Molecular mechanism of the interaction between Megalocytivirus-induced virus-mock basement membrane (VMBM) and lymphatic endothelial cells." **97**(11): e00480-00423.

12 Hick, P., J. Becker and R. Whittington (2024). Iridoviruses of fish. Aquaculture virology, Elsevier**:** 139-164.

13 Huang, Y., X. Huang, Y. Yan, J. Cai, Z. Ouyang, H. Cui, P. Wang and Q. J. B. g. Qin (2011). "Transcriptome analysis of orange-spotted grouper (Epinephelus coioides) spleen in response to Singapore grouper iridovirus." **12**: 1-12.

14 Irshath, A. A., A. P. Rajan, S. Vimal, V.-S. Prabhakaran and R. Ganesan (2023). "Bacterial pathogenesis in various fish diseases: recent advances and specific challenges in vaccine development." Vaccines **11**(2): 470.

15 Jancovich, J. K., Q. Qin, Q.-Y. Zhang and V. G. Chinchar (2015). "Ranavirus replication: molecular, cellular, and immunological events." Ranaviruses: Lethal pathogens of ectothermic vertebrates: 105-139.

16 Jancovich, J. K., Q. Qin, Q.-Y. Zhang and V. G. J. R. L. p. o. e. v. Chinchar (2015). "Ranavirus replication: molecular, cellular, and immunological events." 105-139.

17 Jang, H. B., Y. R. Kim, I. S. Cha, S. W. Noh, S. B. Park, M. Ohtani, J. Hikima, T. Aoki and T. S. Jung (2011). "Detection of antigenic proteins expressed by lymphocystis virus as vaccine candidates in olive flounder, Paralichthys olivaceus (Temminck & Schlegel)." Journal of fish diseases **34**(7): 555-562.

18 Jia, Y.-J., Z.-R. Guo, R. Ma, D.-K. Qiu, Z. Zhao, G.-X. Wang, B. J. F. Zhu and S. Immunology (2020). "Immune efficacy of carbon nanotubes recombinant subunit vaccine against largemouth bass ulcerative syndrome virus." **100**: 317-323.

19 Jung, M.-H., C. Nikapitiya, T.-N. Vinay, J. Lee, S.-J. J. F. Jung and S. Immunology (2017). "Rock bream iridovirus (RBIV) replication in rock bream (Oplegnathus fasciatus) exposed for different time periods to susceptible water temperatures." **70**: 731-735.

20 Kai, Y.-H., C.-K. Chang, H.-Q. Li, W.-H. Chen, S.-C. Chi and Y.-C. J. A. Wu (2024). "Immune responses in giant groupers after vaccination with inactivated bivalent vaccine against betanodavirus and ranavirus." 741179.

21 Kayansamruaj, P. (2020). "Project Title Isolation and characterization of iridoviruses isolated from farmed Asian sea bass and grouper in Thailand."

22 Kibenge, F. S. (2024). Classification and identification of aquatic animal viruses. Aquaculture virology, Elsevier**:** 3-47.

23 Koonin, E. V., V. V. Dolja, M. Krupovic, A. Varsani, Y. I. Wolf, N. Yutin, F. M. Zerbini, J. H. J. M. Kuhn and m. b. reviews (2020). "Global organization and proposed megataxonomy of the virus world." **84**(2): 10.1128/mmbr. 00061-00019.

24 Kumar, A., S. K. Middha, S. V. Menon, B. PAITAL, S. Gokarn, N. Meghana, B. Rakshith, M. C. Harish, S. P. Mugunthan and S. M. Kantwa (2024). "Zoonosis and Vaccination for Fish Health Managment."

25 Kumar, A., S. K. Middha, S. V. Menon, B. Paital, S. Gokarn, M. Nelli, R. B. Rajanikanth, H. M. Chandra, S. P. Mugunthan and S. M. Kantwa (2024). "Current Challenges of Vaccination in Fish Health Management." Animals **14**(18): 2692.

26 Kumar, A., S. K. Middha, S. V. Menon, B. Paital, S. Gokarn, M. Nelli, R. B. Rajanikanth, H. M. Chandra, S. P. Mugunthan and S. M. J. A. Kantwa (2024). "Current Challenges of Vaccination in Fish Health Management." **14**(18): 2692.

27 Leiva-Rebollo, R., J. Gémez-Mata, D. Castro, J. J. Borrego and A. M. J. F. i. I. Labella (2023). "Immune response of DNA vaccinated-gilthead seabream (Sparus aurata) against LCDV-Sa infection: relevance of the inflammatory process." **14**: 1209926.

28 Leiva-Rebollo, R., A. M. Labella, J. Gémez-Mata, D. Castro and J. J. Borrego (2024). "Fish Iridoviridae: infection, vaccination and immune response." Veterinary Research **55**(1): 88.

29 Leiva-Rebollo, R., A. M. Labella, J. Gémez-Mata, D. Castro and J. J. J. V. R. Borrego (2024). "Fish Iridoviridae: infection, vaccination and immune response." **55**(1): 88.

30 Liang, H., L. Zhang, X. Fu, Q. Lin, L. Liu, Y. Niu, X. Luo, Z. Huang and N. J. V. Li (2021). "Development of a double-antibody sandwich ELISA for rapid detection of the MCP antigen concentration in inactivated ISKNV vaccines." **9**(11): 1264.

31 Liao, W., L. Huang, S. Han, D. Hu, Y. Xu, M. Liu, Q. Yu, S. Huang, D. Wei and P. J. V. Li (2022). "Review of medicinal plants and active pharmaceutical ingredients against aquatic pathogenic viruses." **14**(6): 1281.

32 Liu, X., N. Chen, X. Gao, Y. Zhang, X. Li, Y. Zhang, X. Bing, H. Huang and X. Zhang (2018). "The infection of red seabream iridovirus in mandarin fish (Siniperca chuatsi) and the host immune related gene expression profiles." Fish & shellfish immunology **74**: 474-484.

33 Ma, H., C. Cheng, Y. Deng, G. Liu, S. Fan, J. Feng and Z. J. A. Guo (2022). "Effective protection against Ranavirus-type grouper iridovirus and Vibrio harveyi by priming a bivalent GrouperVAC-Irido-R-Vh followed by boosting a monovalent V. harveyi vaccine." **560**: 738455.

34 Matsuyama, T., N. Sano, T. Takano, T. Sakai, M. Yasuike, A. Fujiwara, Y. Kawato, J. Kurita, K. Yoshida and Y. Shimada (2018). "Antibody profiling using a recombinant protein–based multiplex ELISA array accelerates recombinant vaccine development: case study on red sea bream iridovirus as a reverse vaccinology model." Vaccine **36**(19): 2643-2649.

35 Miccoli, A., M. Manni, S. Picchietti and G. Scapigliati (2021). "State-of-the-art vaccine research for aquaculture use: The case of three economically relevant fish species." Vaccines **9**(2): 140.

36 Min, J.-G., G.-H. Kim, C.-H. Kim, W.-J. Kwon, H.-D. Jeong and K.-I. J. V. Kim (2024). "Evaluation of Formalin-Inactivated Vaccine Efficacy against Red Seabream Iridovirus (RSIV) in Laboratory and Field Conditions." **12**(6): 680.

37 Mondal, H. and J. J. A. i. Thomas (2022). "A review on the recent advances and application of vaccines against fish pathogens in aquaculture." **30**(4): 1971-2000.

38 Oh, S.-Y., M.-J. Oh and T. Nishizawa (2014). "Potential for a live red seabream iridovirus (RSIV) vaccine in rock bream Oplegnathus fasciatus at a low rearing temperature." Vaccine **32**(3): 363-368.

39 Ou-yang, Z., P. Wang, X. Huang, J. Cai, Y. Huang, S. Wei, H. Ji, J. Wei, Y. Zhou, Q. J. D. Qin and C. Immunology (2012). "Immunogenicity and protective effects of inactivated Singapore grouper iridovirus (SGIV) vaccines in orange-spotted grouper, Epinephelus coioides." **38**(2): 254-261.

40 Priya, T. J. and S. J. V. Kappalli (2022). "Modern biotechnological strategies for vaccine development in aquaculture–prospects and challenges." **40**(41): 5873-5881.

41 Qin, P., H. M. Munang'andu, C. Xu and J. J. V. Xie (2023). "Megalocytivirus and other members of the family iridoviridae in finfish: a review of the etiology, epidemiology, diagnosis, prevention and control." **15**(6): 1359.

42 Radhakrishnan, A., B. Vaseeharan, P. Ramasamy and S. J. A. I. Jeyachandran (2023). "Oral vaccination for sustainable disease prevention in aquaculture—an encapsulation approach." **31**(2): 867-891.

43 Rathor, G. S. and B. Swain (2024). "Advancements in Fish Vaccination: Current Innovations and Future Horizons in Aquaculture Health Management." Applied Sciences **14**(13): 5672.

44 Rosa, G. M., G. A. Botto, A. T. Mitra, J. S. de Almeida, M. Hofmann, W. T. Leung, A. P. A. de Matos, M. F. Caeiro, E. Froufe and A. J. B. C. Loureiro (2022). "Invasive fish disrupt host-pathogen dynamics leading to amphibian declines." **276**: 109785.

45 Shimmoto, H., K. Kawai, T. Ikawa, S. i. J. M. Oshima and immunology (2010). "Protection of red sea bream Pagrus major against red sea bream iridovirus infection by vaccination with a recombinant viral protein." **54**(3): 135-142.

46 Sivakumar, S., P. Punniyakotti, K. Kavitha and P. Saravanan Concepts and Types of Vaccines. Fish Vaccines, CRC Press**:** 49-63.

47 SIVASANKAR, P. (2018). HOST IMMUNE RESPONSES TO RANAVIRUS INFECTION IN MARINE AND FRESHWATER FISHES, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu Dr. J ….

48 Tafalla, C., J. Bøgwald, R. A. J. F. Dalmo and S. Immunology (2013). "Adjuvants and immunostimulants in fish vaccines: current knowledge and future perspectives." **35**(6): 1740-1750.
49 Throngnumchai, B., S. Jitrakorn, P. Sangsuriya, S. Unajak, P. Khunrae, H. T. Dong, V. Saksmerprome, T. J. P. E. Rattanarojpong and Purification (2021). "Refolded recombinant major capsid protein (MCP) from Infectious Spleen and Kidney Necrosis Virus (ISKNV) effectively stimulates serum specific antibody and immune related genes response in Nile tilapia (Oreochromis niloticus)." **184**: 105876.
50 Volpatti, D. and S. J. A. P. Ciulli (2022). "Lymphocystis virus disease." 201-216.
51 Volpe, E., F. Errani, L. Mandrioli and S. Ciulli (2023). "Advances in viral aquatic animal disease knowledge: the molecular methods' contribution." Biology **12**(3): 466.
52 Wang, Q., W. Ji and Z. Xu (2020). "Current use and development of fish vaccines in China." Fish & shellfish immunology **96**: 223-234.
53 Yao, J.-Y., C.-S. Zhang, X.-M. Yuan, L. Huang, D.-Y. Hu, Z. Yu, W.-L. Yin, L.-Y. Lin, X.-Y. Pan and G.-l. J. F. i. I. Yang (2022). "Oral vaccination with recombinant Pichia pastoris expressing iridovirus major capsid protein elicits protective immunity in largemouth bass (Micropterus salmoides)." **13**: 852300.
54 Yi, W., X. Zhang, K. Zeng, D. Xie, C. Song, K. Tam, Z. Liu, T. Zhou, W. J. F. Li and S. Immunology (2020). "Construction of a DNA vaccine and its protective effect on largemouth bass (Micropterus salmoides) challenged with largemouth bass virus (LMBV)." **106**: 103-109.
55 Yoshimizu, M., H. Kasai, T. Aoki, M. Ototake, M. Sakai, T.-S. Jung, J.-i. Hikima, N. Okamoto, T. Sakamoto, A. J. E. o. L. S. S. P. U. N. E. Ozaki, Scientific and C. Organization (2016). "Prevention and treatment of diseases caused by fish pathogens."
56 Yu, N.-T., X.-b. Zheng, Z.-X. J. F. Liu and S. Immunology (2019). "Protective immunity induced by DNA vaccine encoding viral membrane protein against SGIV infection in grouper." **92**: 649-654.
57 Zeng, R., W. Pan, Y. Lin, J. He, Z. Luo, Z. Li, S. Weng, J. He and C. J. I. Guo (2021). "Development of a gene-deleted live attenuated candidate vaccine against fish virus (ISKNV) with low pathogenicity and high protection." **24**(7).
58 Zhang, Q.-Y., F. Ke, L. Gui, Z. J. W. B. Zhao and Security (2022). "Recent insights into aquatic viruses: Emerging and reemerging pathogens, molecular features, biological effects, and novel investigative approaches." **1**(4): 100062.
59 Zhang, W., C. Duan, H. Zhang, S. Weng, J. He and C. J. A. Dong (2020). "Widespread outbreaks of the emerging mandarinfish ranavirus (MRV) both in natural and ISKNV-FKC vaccinated mandarinfish Siniperca chuatsi in Guangdong, South China, 2017." **520**: 734989.
60 Zhang, Z., G. Liu, J. Liu, B. Zhu, G. Wang, F. J. F. Ling and S. Immunology (2021). "Epitope screening of the major capsid protein within grouper iridovirus of Taiwan and the immunoprotective effect with SWCNTs as the vaccine carrier." **117**: 17-23.
61 Zhao, Z., Y. Xiong, C. Zhang, Y.-J. Jia, D.-K. Qiu, G.-X. Wang, B. J. F. Zhu and S. Immunology (2020). "Optimization of the efficacy of a SWCNTs-based subunit vaccine against infectious spleen and kidney necrosis virus in mandarin fish." **106**: 190-196.
62 Zhao, Z., C. Zhang, Q. Lin, N.-q. Li, Z.-B. Huang, M. Zhao, X.-Z. Fu, G.-X. Wang, B. J. F. Zhu and s. immunology (2020). "Single-walled carbon nanotubes as delivery vehicles enhance the immunoprotective effect of an immersion DNA vaccine against infectious spleen and kidney necrosis virus in mandarin fish." **97**: 432-439.
63 Zheng, F.-R., X.-Q. Sun, H.-Z. Liu and J.-X. Zhang (2006). "Study on the distribution and expression of a DNA vaccine against lymphocystis disease virus in Japanese flounder (Paralichthys olivaceus)." Aquaculture **261**(4): 1128-1134.
64 Zhou, Y., H.-C. Fu, Y.-Y. Wang, H.-Z. Huang, X.-Z. Fu and N.-Q. Li (2022). "The dynamic immune responses of Mandarin fish (Siniperca chuatsi) to ISKNV in early infection based on full-length transcriptome analysis and weighted gene co-expression network analysis." Fish & Shellfish Immunology **122**: 191-205.
65 Zhu, Z., C. Duan, Y. Li, C. Huang, S. Weng, J. He and C. J. A. Dong (2021). "Pathogenicity and histopathology of infectious spleen and kidney necrosis virus genotype II (ISKNV-II) recovering from mass mortality of farmed Asian seabass, Lates calcarifer, in Southern China." **534**: 736326.