Fish and Shellfish Immunology 168 (2026) 110962

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Oral antiviral vaccines in aquaculture: Current status, challenges, and future prospects

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A B S T R A C T

Vaccination remains the most effective strategy for preventing viral infections in the aquaculture industry. Most commercially available antiviral vaccines are produced from attenuated viral particles and administered via intraperitoneal or intramuscular injection. While this approach has proven effective concerns on safety, and the negative impact of injection on the welfare of the farmed fish species cannot be overlooked. Therefore, research have been geared towards finding effective oral vaccine alternatives to eliminate the need for fish handling without incurring additional operational costs. However, the instability of antigenic peptides in the harsh digestive enzymatic conditions becomes a major setback to adequately stimulate optimum immune responses against viral pathogens. This article discusses progress, challenges, and future possibilities in the design and development of commercial and experimental oral vaccines for finfish and shellfish aquaculture species against viral infection.

1. Introduction

The aquaculture industry has recorded historic growth over the last decade, achieving a global production of over 130.9 million tons valued at approximately $313 billion [1]. Unfortunately, the aquaculture industry is under the threat of ever-growing challenges of infectious diseases outbreaks, which accounts for more than 10 % of annual production losses. This amounts to over $10 billions globally, underscoring the persistent impact of fish diseases on the aquaculture sector [1].

Viruses are among the deadliest pathogens responsible for about 22.6 % of cases of infectious diseases in commercial fish production; a significant bottleneck in sustainable production [2]. The severity of viral infections has been reported to be devastating in fish production facilities, with mortality rates reaching up to 100 %, while surviving fish often exhibit reduced performance. The implementation of effective health management strategies—including enhanced disease surveillance, the establishment of maximum farming density standards, the designation of sanitary zones, improved farm biosecurity protocols, the use of immunostimulants, and vaccination programs—have played a crucial role in mitigating and containing the economic losses caused by viral diseases in the aquaculture industry. For instance, the Chilean aquaculture industry introduced a national surveillance strategy to curb the incessant outbreak of infectious salmon anaemia (ISA) in the country's salmonid farms [3–6]. Among these strategies, vaccination provided a paradigm-shifting solution owing to its effectiveness and environmental friendliness in the control and prevention of fish infectious diseases, while contributing to the reduction of antibiotic usage in the aquaculture sector. However, most commercial vaccines are directed against bacterial pathogens and only a few have been developed against viruses [7,8].

1.1. Viral diseases of major impact in aquaculture

Several viral infections have been reported in the aquaculture sector. In this section, we will provide an update on prevalent and emerging viral diseases that have been documented to be major contributors to aquaculture economic losses worldwide [2,9] (Table 1).

Best practices require mandatory reporting of any suspicious clinical signs of disease or outbreak as a measure to curb the spread of notorious

This article is part of a special issue entitled: Aquaculture vaccines published in Fish and Shellfish Immunology.

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https://doi.org/10.1016/j.fsi.2025.110962
Received 1 July 2025; Received in revised form 7 October 2025; Accepted 21 October 2025
Available online 24 October 2025

viral pathogens in farmed fish species. This is because the movement of fish between aquaculture production sites and fish eggs across countries, has been identified as a key factor in the emergence and prevalence of viruses globally. The World Organization for Animal Health (WOAH) identified nine notifiable viral fish diseases: Infectious salmon anaemia (ISA), Salmonid alphavirus (SAV), Viral hemorrhagic septicemia (VHS), Infectious hematopoietic necrosis (IHN), Epizootic hematopoietic necrosis (EHN), Koi herpesvirus disease (KHVD), Spring viremia of carp (SVC), Tilapia Lake Virus (TiLV), and Red sea bream iridoviral disease (RSIVD); five of which primarily affect species such as Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), two of which affect Common carp (*Cyprinus carpio*), and the last two are common in Nile tilapia (*Oreochromis niloticus*) and Red sea bream (*Pagrus major*) [10].

The prevalence of viral diseases varies significantly according to regions and culture conditions. Some of them have a limited endemic distribution, while others are prevalent in different regions. Cases of EHN were unequivocally classified as endemic in Australia, with only isolated cases emerging in other regions, just as RSIVD and Cardiomyopathy syndrome (CMS) were reported to be restricted to the Asian and

Table 1
Viral diseases of major impact in fish aquaculture.

Disease	Affected Host	Virus	Main target organs	Geographic Distribution	Commercial Vaccine	Reference
Infectious Salmon Anaemia (ISA)	Atlantic salmon (*S. salar*), Rainbow trout (*O. mykiss*), Brown trout (*S. trutta*)	*Infectious salmon anemia virus* (*Isavirus*, family *Orthomyxoviridae*)	Blood vessels, Heart, Kidney, Liver, spleen	Norway, Chile, Canada, USA, Scotland, Faroe Islands and Ireland	Available	Aamelfot et al. [36], Thorud & Djupvik [37]
Salmonid Alphavirus Infection (SAV)	Atlantic salmon (*S. salar*), Rainbow trout (*O. mykiss*)	*Salmonid alphavirus* (*Alphavirus*, family *Togaviridae*)	Heart, Brain and Kidney	France, Croatia, Ireland, Italy, Germany, Spain, Norway, UK	Available	McLoughlin & Graham [19], Munro et al. [38], Boucher et al. [39]
Infectious Hematopoietic Necrosis (IHN)	Salmonids, including Rainbow trout (*O. mykiss*), Atlantic salmon (*S. salar*),	*Infectious hematopoietic necrosis virus* (*Novirhabdovirus*, family *Rhabdoviridae*)	Hematopoietic tissues; kidney and spleen	Austria, Belgium, Canada, Chile, China, Croatia, France, Iran, USA, Slovenia, Czech Republic, Korea, Spain, Switzerland, Taiwan, Germany, Italy, Japan, Netherlands, Poland and Slovenia	Available	Dixon et al. [30]
Viral Hemorrhagic Septicemia (VHS)	Salmonids, Pike (*Esox lucius*), Marine species	Viral hemorrhagic septicemia virus (*Novirhabdovirus*, family *Rhabdoviridae*)	Head Kidney, Heart and Spleen	Europe, North America, Asia	Not Available	Meyers & Winton [40], Mortensen et al. [41], Schlotfeldt et al. [42], Lazarte & Jung [43]
Epizootic Hematopoietic Necrosis (EHN)	Redfin perch (*Perca fluviatilis*), Rainbow trout (*O. mykiss*)	Epizootic hematopoietic necrosis virus (*Ranavirus*, family *Iridoviridae*)	Liver, Kidney, Spleen	Australia	Not Available	Langdon et al. [44]
Infectious Pancreatic Necrosis (IPN)	Salmonids, including Atlantic salmon (*S. salar*) and Rainbow trout (*O. mykiss*)	Infectious pancreatic necrosis virus (*Aquabirnavirus*, family Birnaviridae)	Pancreas, head kidney, intestine and Liver	Chile, México, USA, Canada, Spain, Italy, Norway, Turkey, Scotland, Ireland	Available	Fayaz et al. [45], Wolf et al. [46]
Cardiomyopathy Syndrome (CMS)	Atlantic salmon (*S. salar*)	*Piscine myocarditis virus* (*Totivirus-like virus*, family *Totiviridae*)	Heart	Norway, Scotland, Faroe Islands, Ireland	Not Available	Garseth et al. [47], Amin & Trasti [48]
Heart and Skeletal Muscle Inflammation (HSMI)	Atlantic salmon (*S. salar*), Rainbow trout (*O. mykiss*) and Pacific salmon (*O. kisutch*)	Piscine orthoreovirus (*Orthoreovirus*, family *Reoviridae*)	Heart and Skeletal Muscle	Norway, Scotland, Ireland, Canada, Chile	Not Available	Palacios et al. [120], Vallejos-Vidal et al. [49]
Red Sea Bream Iridoviral (RSIVD)	Red sea bream (*Pagrus major*), black porgy (*Acanthopagrus schlegeli*), Yellowfin Sea bream (*Acanthopagrus latus*)	Read seabream iridivirus (*Megalocytivirus*, family *Iridoviridae*)	Spleen, Kidney, Heart, Intestine and Gill	Japan, Hong Kong, Korea, Malaysia, Philippines, Singapore and Thailand	Available	Puspasari & Widowati [50], Inouye et al. [51]
Koi Herpesvirus Disease (KHVD)	Common carp (*Cyprinus carpio*), Koi carp (*Cyprinus rubrofuscu*)	Koi herpesvirus (*Cyprinid herpesvirus 3*, family *Alloherpesviridae)*	Gill, Kidney, Gut and Spleen	Japan, Taiwan, Indonesia, Malaysia, Hong Kong, China, Korea, Israel, USA, Canada, UK, Germany, Italy	Available	Bergmann et al. [52]
Spring Viremia of Carp (SVC)	Common carp (*Cyprinus carpio*), other cyprinids	Spring viremia of carp virus (*Sprivivirus*, family *Rhabdoviridae*)	Kidney, Liver, Spleen	USA, Canada, Brazil, China, Iran, Egypt, Russia, Hungary, Czech Republic	Available	Ashraf et al. [14], Fijan et al. [53]
Infection Tilapia like Virus (TiLV)	Nile tilapia (*Oreochromis niloticus*), Red tilapia (*Oreochromis* spp.) and Hybrid tilapia (*O. niloticus* × *O. aureus*)	Tilapia lake virus (*Tilapinevirus*, family *Amnoonviridae)*	Eyes, Brain, Liver	Colombia, Peru, Ecuador, USA, Egypt, India, Indonesia, Malaysia, Thailand, Taiwan, Philippines, Tanzania, Uganda	Not Available	Kembou-Ringert et al. [54], Eyngor et al. [55]
Viral Nervous Necrosis (VNN)	Marine fish species, including Groupers (*Ephinephelus* spp), European Sea bass (*Dicentrarchus labrax)*, Asian Sea Bass (*Lates calcarifer)*, Turbot (*Scophthalmus maximus)*	Nervous necrosis virus (*Betanodavirus*, family *Nodaviridae*)	Brain, Spinal cord and Retina	Worldwide, particularly in Asia and Mediterranean area	Available	Bandín & Souto [13], Mori et al. [56]

Northern European aquaculture industry, respectively. Since the first incidence of Heart and skeletal muscle inflammation disease (HSMI) was reported in Norway, this disease has been subsequently detected in Chile, Iceland, Canada, and the USA. In Europe, Asia, and North America, SVC is known to be prevalent, although it is less widespread compared to other diseases. Salmonid farms in Norway, Chile, and the United Kingdom are severely affected by ISA, while Infectious pancreatic necrosis (IPN), Viral nervous necrosis (VNN), SAV, IHN, VHS, KHVD, and TiLV have a broader geographical distribution, affecting multiple continents and posing a global concern [6,11,12].

While all diseases generate economic losses, there is a remarkable variation in the fatality rate recorded among most cultured fish species. Possibly due to intensive production of a narrow range of fish species in the aquaculture industry, thereby promoting sporadic spread of species-specific viral pathogens with different levels of virulence. In this context, reported losses in commercially farmed fish —particularly in the rapidly expanding salmonid industry over the past two decades— have exhibited a range of outcomes. HSMI and EHN in rainbow trout appear to have a lower economic impact compared to others, although they may be significant locally. Meanwhile, in Atlantic salmon, CMS infection leads to a substantial mortality rate because of severe damage to the fish's heart. Interestingly, significant losses in red sea bream and Atlantic salmon due to RSIVD and SAV, respectively have been widely reported, establishing host-specificity of virus-related diseases in farmed fish, as VNN is common to marine fish aquaculture [13]. Freshwater fish, especially Tilapia and Common carp, are not exempt from this menace with the emergence of TiLV in the former, and the latter being continuously ravaged by SVC [14]. The emergence of ISA, IPN, IHN, VHS, KHVD and TiLV, poses a significant threat to the sustainable expansion of the global aquaculture industry [9,15–18].

Observable clinical signs remain a useful, quick presumptive diagnostic approach in distinguishing viral diseases from other infectious outbreaks in farmed species. Changes in the physiology and behaviour of fish hint at the possibility of a viral infection. Gill paleness is commonly associated with ISA and CMS; petechial haemorrhages on the gas exchange organ could be SVC and RSIVD; extensive gill haemorrhages and lesions are caused by VHS and KHVD, respectively. Several viruses, including IHNV, IPNV, VNN, and EHV, have been reported to cause changes in the pigmentation of the fish skin and loss of scales due to TiLV in tilapia. Eye-related problems such as exophthalmia and haemorrhage due to IHN, TiLV, SVC and sunken eyes caused by KHVD. With extensive bleeding on the fin due to VHS, fin rot in rainbow trout is usually due to EHN. Severe disruption of vital internal organs, such as the liver and spleen, by ISA, CMS, VHS, SVC, VNN, and RSIVD significantly alters the fish's abdominal conformation with a distended abdomen. Behavioural changes due to abnormal swimming patterns caused by damage to the nervous system due to IPN, VHS, VNN; anorexia and lethargy are some of the common features of IPN, VNN, EHN, and RSVID in affected fish species [19–25]. This is an indication of the complexity of viral infections in affecting the optimum physiological activities of infected fish through multisystemic tissue damages.

Each virus exhibits distinct tissue tropisms. ISAV targets endothelial cells in Atlantic salmon, leading to systemic anaemia, while IPNV primarily affects pancreatic acinar cells in salmonids, also involving kidney and intestinal tissues. The Piscine orthoreovirus (PRV), a virus that commonly causes HSMI, primarily targets cardiac and skeletal muscle and shows tropism for erythrocytes in Atlantic salmon. Similarly, the tropism of SAV is variable, it can cause necrosis in the pancreas and cardiac and skeletal myopathies in salmonids —infecting pancreatic exocrine cells in Pancreas Disease (PD) in Atlantic salmon, and skeletal muscle and heart tissue in Sleeping Disease (SD) in rainbow trout, as seen in Cardiomyopathy Syndrome (CMS) especially in Atlantic salmon. Hematopoietic tissues, the kidney and spleen, are the main targets of IHN and EHN viruses. VHSV exhibits a broad tropism across endothelial cells in multiple organs, especially in salmonids, such as Rainbow trout or Atlantic salmon. Detection of SVCV at a high level in the liver, kidney, spleen, gills, and brain of common carp is a reflection of its systemic impact on the affected fish. An extensive tropism of *Tilapinevirus* notably affects the brain, eyes, and liver of farmed tilapia; meanwhile, monotrophic cyprinid herpesvirus mainly targets gills of common carp. The nervous system of the European sea bass in the Mediterranean region is specifically affected by VNNV, showing particular tropism for the brain and retina [26–32].

1.2. Vaccination in fish disease management

Understanding the specific characteristics, distribution, prevalence, and economic impact of these viral diseases is critical for developing effective prevention and control strategies, including the implementation of robust biosecurity measures and the development of vaccines [33]-preferably administered through less stressful methods, such as oral delivery. In this context, vaccination emerges as a fundamental tool for the prevention and control of viral diseases in aquaculture [34,35]. This prophylactic strategy does not only protect individual fish, but it also helps to ensure the sustainability of the aquaculture ecosystem by lowering disease prevalence and the need for chemical treatments such as antibiotics. Among the different vaccine delivery strategies in fish, oral vaccination stands out as a promising, practical, and stress-reducing method. Oral vaccination minimizes handling and stress by allowing mass immunization of huge populations of fish through their daily feed, in contrast to injectable vaccines that require individual fish handling, a process that can be time-consuming, expensive, and stressful. The simplicity of its administration makes it a highly appealing choice for the aquaculture industry.

1.3. Types of vaccines in aquaculture

To date, 46 commercial antiviral vaccines are available for aquaculture, including various multivalent formulations (Table 2). Most of these, targeted commercially valuable species such as salmonids, and are designed to protect them against diseases caused by IPNV, ISAV, SAV and IHNV. In addition, three vaccines have been licensed against VNNV: one for sevenband grouper in Japan and two for sea bass in the Mediterranean market. Furthermore, a commercial vaccine against the Koi herpesvirus disease (KHVD) is available for common carp, and another has been developed for tilapia, targeting the ISKN virus. Therefore, there are no commercial vaccines available for several other high-impact viral diseases, such as HSMI, SVC, TiLV and VHS.

Vaccines can be classified according to antigen administration systems: 1) Replicative antigen delivery system: live attenuated vaccines, DNA vaccines, vector viral vaccines and RNA vaccines; 2) Non-replicative antigen delivery system: inactivated whole-cell vaccines, subunit vaccines, peptide vaccines, virus-like particles (VLPs), among others [57]. Despite the availability of different options, inactivated viral vaccines continue to play a dominant role in the global aquaculture vaccine market. Currently, approximately 87 % of antiviral vaccines licensed for fish are inactivated, with only three subunit vaccines (7 %), two DNA vaccines (4 %), and one attenuated vaccine (2 %) (Table 2).

1.3.1. Inactivated viral vaccines

Inactivated or killed vaccines use physical, chemical or radiation methods to inactivate disease-causing pathogens, which lose or attenuate their ability to infect or replicate in or outside the host, without compromising the antigenicity of the pathogen. The term *killed* is often used for bacterial vaccines, while *inactivated* is more commonly used for viral vaccines [58,59]. The antigen-presenting cells of the host recognize, process, and present the foreign structural proteins of the inactivated virus and subsequently activate its immune system, primarily through the humoral immune response [57]. As these vaccines typically do not trigger a complete cellular immune response, they induce weaker immunity compared to live vaccines. Therefore, they require appropriate adjuvants and/or boosters to sustain protective immunity over

Table 2
Commercially available antiviral vaccines for fish.

Type of Vaccine	Disease	Antigen	Administration route	Commercial name	Host	Company	Country
Inactivated	Infectious pancreatic necrosis (IPN)	IPNV	i.p.	ALPHA JECT® 1000	Atlantic salmon/ rainbow trout	Pharmaq AS	Chile
			i.p.	Blueguard® IPN inyectable	Atlantic and Pacific salmon, rainbow trout and Chinook salmon	Virbac	Chile
			Immersion	IPE-VAC® INMERSIÓN	Atlantic and Coho salmon, rainbow trout and Chinook salmon	Veterquimica S.A.	Chile
				IPE-VAC®2 INMERSIÓN			
			i.p.	Ipe-Vac® Microdosis			
Inactivated	Salmon pancreas disease (SPD)	SAV	i.p.	Norvax® Compact PD	Atlantic salmon	MSD Animal Health	Ireland, UK
		SAV3	i.p.	ALPHA JECT® micro 1 PD	Atlantic salmon	Pharmaq AS	Norway, Ireland, UK
Inactivated	Infectious salmon anemia (ISA)	ISAV	i.p.	ALPHA JECT® micro 1 ISA	Atlantic salmon	Pharmaq AS	Chile
Inactivated	Viral nervous necrosis (VNN)	RGNNV	i.p.	ALPHA JECT® micro 1 Noda	European Sea bass	Pharmaq AS	Spain, Italy, Croatia, and Greece
		VNNV	i.p.	ICTHIOVAC® VNN	European Sea bass	HIPRA	Spain, Italy, France, Greece, and Turkey
		RGNNV	i.p.	Nisseiken VNN	Sevenband and longtooth Grouper	Nisseiken Co., Ltd.	Japan
Inactivated	Iridoviruses, Infectious spleen and kidney necrosis (ISKN)	Iridovirus	i.p.	AQUAVAC® IridoV	Tilapia, Asian sea bass and japanese yellowtail	MSD Animal Health	Asian
Inactivated	Infectious spleen and kidney necrosis (ISKN)	ISKNV-I	i.p.	formalin-killed ISKNV-I	Mandarin fish	Yuyeah Bio-Tech	China
Inactivated Polyvalent	IPN/ISA	ISAV, IPNV, *P. salmonis, A. salmonicida, V. ordalli*	i.p.	PROVIDEAN® AQUATEC 5	Atlantic salmon	Tecnovax S.A.	Chile
Inactivated Polyvalent	IPN/ISA	ISAV, IPNV, *P. salmonis, V. ordalli*	i.p.	PROVIDEAN® AQUATEC 4	Atlantic salmon	Tecnovax S.A.	Chile
Inactivated Trivalent	IPN	IPNV, *P. salmonis, V. ordalli*	i.p.	PROVIDEAN® AQUATEC 3	Atlantic salmon/ rainbow trout	Tecnovax S.A.	Chile
Inactivated Bivalent	IPN	IPNV, *P. salmonis*	i.p.	PROVIDEAN® AQUATEC 2	Atlantic and Coho salmon, rainbow trout	Tecnovax S.A.	Chile
			i.p.	AGROVAC® IPN-SRS	Salmonids	AGROVET	Chile
Inactivated Polyvalent	IPN/ISA	ISAV, IPNV, *P. salmonis, A. salmonicida, V. ordalli*	i.p.	AGROVAC® 4+ISA	Salmonids	AGROVET	Chile
Inactivated Bivalent	IPN	IPNV, *P. salmonis*	i.p.	ALPHA JECT® micro 2	Atlantic and Pacific salmon, rainbow trout	Pharmaq AS	Chile
Inactivated Trivalent	IPN	IPNV, *P. salmonis, V. ordalli*	i.p.	ALPHA JECT® micro 3	Atlantic salmon	Pharmaq AS	Chile
Inactivated Polyvalent	IPN/ISA	IPNV, ISAV, *A. salmonicida, V. ordalli*	i.p.	ALPHA JECT® micro 4-2	Atlantic salmon	Pharmaq AS	Chile
Inactivated Polyvalent	IPN	IPNV, *M. viscosa, Aliivibrio salmonicida, V. anguillarum, A. salmonicida*	i.p.	ALPHA JECT® micro 6	Atlantic salmon	Pharmaq AS	Norway, Ireland, UK and Faroe Islands
Inactivated Polyvalent	IPN/ISA	IPNV, ISAV, *M. viscosa, A. salmonicida, V. anguillarum*	i.p.	ALPHA JECT micro® 7 ISA	Atlantic salmon	Pharmaq AS	Canada, Norway and Faroe Islands
Inactivated Bivalent	IPN	IPNV, *Flavobacterium psychrophilum*	i.p.	ALPHA JECT® IPNV-Flavo 0,025	Atlantic salmon	PharmaqAS	Chile

(*continued on next page*)

Table 2 (*continued*)

Type of Vaccine	Disease	Antigen	Administration route	Commercial name	Host	Company	Country
Inactivated Polyvalent	IPN/ISA	IPNV, ISAV, *P. salmonis, A. salmonicida, V. ordalli*	i.p.	ALPHA JECT® 5-1	Atlantic salmon	Pharmaq AS	Chile
Inactivated Polyvalent	IPN	IPNV, *M. viscosa, A. salmonicida, V. anguillarum, A. salmonicida*	i.p.	ALPHA JECT® 6-2	Atlantic salmon	Pharmaq AS	Norway and Faroe islands
Inactivated Polyvalent	IPN	IPNV, *A. salmonicida, V. anguillarum, A, Aliivibrio salminicida, M. viscosa*	i.p.	Pentium Forte Plus vet	Atlantic salmon	Elanco GmbH	Norway
Inactivated Bivalent	IPN	IPNV, *P. salmonis*	i.p.	Birnagen Forte® 2	Atlantic and Pacific salmon, rainbow trout	Elanco Chile S. A.	Chile
				Blueguard® SRS + IPN	Atlantic and Pacific salmon, Chinook salmon	Virbac	Chile
Inactivated Trivalent	IPN	IPNV, *P. salmonis*, *R. salmoninarum*	i.p.	BEKA-PLUS® 3	Salmonids	Veterquimica S.A.	Chile
Inactivated Bivalent	IPN	IPNV, *P. salmonis*	i.p.	Rickemune Plus®	Salmonids	Veterquimica S.A.	Chile
Inactivated Trivalent	SPD/IPN	SAV, IPNV, *A. salmonicida*	i.p.	AquaVac® PD3	Atlantic salmon	MSD Animal Health	UK, Ireland
Inactivated Polyvalent	IPN	IPNV, *M. viscosa, A. salmonicida, V. anguillarum, A. salmonicida*	i.p.	AQUAVAC® 6	Atlantic salmon	MSD Animal Health	UK, Ireland
Inactivated Polyvalent	IPN/SPD	IPNV, SAV, *M. viscosa, Aliivibrio salmonicida, V. anguillarum, A. salmonicida*	i.p.	AQUAVAC® PD7	Atlantic salmon	MSD Animal Health	Norway
Inactivated Polyvalent	IPN/ISA	ISAV, IPNV, *P. salmonis*, *V. ordalli*	i.p.	PROVIDEAN® AQUATEC 4	Atlantic salmon	Tecnovax S.A.	Chile
Inactivated Polyvalent	IPN/ISA	ISAV, IPNV, *P. salmonis*, *A. salmonicida, V. ordalli*	i.p.	PROVIDEAN® AQUATEC 5	Atlantic salmon	Tecnovax S.A.	Chile
Inactivated Polyvalent	IPN/ISA	ISAV, IPNV, *A. salmonicida, V. salmonicida, V. anguillarum, M. viscosa*	i.p.	PENTIUMFORTE PLUS ILA®	Atlantic salmon	MSD Animal Health	Chile
Inactivated Polyvalent	IPN	IPNV, *A. salmonicida, V. anguillarum,V. A. salmonicida, M. viscosa*	i.p.	Norvax® Minova 6	Atlantic salmon	MSD Animal Health	Norway, UK
Inactivated Polyvalent	ISA	ISAV, *A. salmonicida,* V. *salmonicida, V. anguillarum, V. ordalli*	i.p.	FORTE V II™	Salmonids	Elanco Canada Limited	USA
Attenuated	Koi Herpesvirus Disease (KHVD)	KHV	Immersion	KV3	Common Carp	Phibro Animal Health	Israel, Indonesia
DNA	SPD (SAV3)	SPDV DNA plasmid	i.m.	CLYNAV™	Atlantic salmon	Elanco GmbH	EU countries, Ireland, UK and Norway
DNA	Infectious hematopoietic necrosis (IHN)	Glicoprotein G IHNV	i.m.	Apex-IHN®	Salmonids	Elanco Aqua Health	Canada
Subunit	IPN	VP2 & VP3 IPNV	Oral	AQUAVAC® IPN Oral	Atlantic salmon	MSD Animal Health	Canada, Norway, USA and Chile
Subunit Bivalent	IPN	IPNV, *P. salmonis*	i.p.	AQUAVAC® SARISTIN 2	Atlantic and Coho salmon, rainbow trout	MSD Animal Health	Chile
Subunit/ Inactivated Polyvalent	ISA/IPN	ISAV (subunit), IPNV (inactivated), *P. salmonis*, *A. salmonicida, V. ordalli*	i.p.	BlueGuard® IPN + SRS + AS + VO + ISA	Atlantic salmon	Virbac	Chile

i.p., intraperitoneal injection; i.m., intramuscular injection.

time [57,59].

Inactivated virus vaccines were the first approach to fish vaccine development and continue to be the most widely used, as they are the standard against which other vaccines are evaluated [6]. Alpha Jects Micro 1 ISA (Novartis), Blueguard® IPN injectable (Virbac), Norvax® Compact PD (MSD Animal Health) and ICTHIOVAC®VNN (HIPRA) are examples of such vaccines, targeting ISA, IPN, PD (SAV), and NNV, respectively. In addition, many multivalent vaccines are inactivated and can contain up to seven pathogens at a time (Table 2).

1.3.2. Live-attenuated vaccines

An attenuated vaccine is made by reducing the virulence of a pathogen by different attenuation methods, including chemical or heat treatment, continuous passaging in heterologous systems, and genetic attenuation via targeted mutations such as gene deletions, disruptions, or insertions affecting specific metabolic pathways or virulence factors [57]. Despite its reduced pathogenicity, the attenuated pathogen retains the ability to replicate and elicit an immune response within the host. Attenuated viruses usually undergo replication by taking advantage of the host's protein synthesis system to produce adequate viral particles sufficient to induce robust humoral and cell-mediated immune responses. This often reduces the need for booster vaccinations and adjuvants while promoting long-lasting immunity with a rapid onset, ultimately providing enhanced immune protection [60]. Another advantage of attenuated vaccines is the ease of administration through the natural route of infection, considering their ability to withstand the harsh acidic and enzymatic conditions of the stomach when administered orally [59]. In aquaculture, there is only one commercially available attenuated vaccine against Koi herpesvirus (KHV) in carp, which is administered by immersion (Table 2).

Nevertheless, live attenuated vaccines hold significant potential as prophylactics in the aquaculture industry. However, their limited adoption may be attributed to the associated potential risks, including challenges related to storage and transportation, short shelf life, the risk of infection in immunocompromised hosts, and the possibility of mutations leading to the reversion of virulence [58].

1.3.3. DNA vaccines

The mechanism of action of DNA vaccines involves the delivery of plasmids containing one or more genes of interest into the host, aiming for the expression of antigenic proteins using the transcriptional machinery of the host cells [61]. Expressed antigenic proteins are recognized by the host immune system as foreign, stimulating cascades of immune responses and thereby eliminating the risk of inadvertent infection [57].

DNA vaccines against viral fish diseases, specifically Rhabdovirus, have proven to be effective with two vaccines currently available for aquaculture, namely APEX-IHN (Elanco Aqua Health) launched in 2005, for the protection of Atlantic salmon against IHNV in British Columbia and CLYNAVTM (Elanco GmbH) marketed in 2017 for use in the European Union (EU) against salmon pancreas disease virus (PD) (Table 2). However, DNA vaccination is yet to achieve sufficient protective efficacy, primarily due to low transgene expression and/or immunogenicity, suboptimal immune responses, and the rapid degradation of delivered plasmid DNA, among other factors [62]. Although the major barrier to these vaccines is the various legal restrictions on their use in edible fish, which hinder licensing and commercialization in many countries [57]. It is well recognized that DNA vaccines are not yet fully developed and the safety concerns—particularly regarding potential long-term persistence and genomic integration—remain uncertain in the aquaculture industry.

1.3.4. Subunit vaccines

Subunit vaccines utilize protein recombinant technology to express only the immunogenic region of a pathogen in a heterologous host, from which the immunogenic antigen (recombinant peptide or protein) is purified and incorporated into the vaccine formulation. By including only the antigenic component, subunit vaccines eliminate the risk of replication or disease transmission to the host or non-target species, ensuring high safety and minimal adverse effects [57,58]. The most common production systems include bacteria—*Escherichia coli*—as well as yeast, insect cells, microalgae, and transgenic plants, among others [57].

Subunit vaccines offer broad applicability and are particularly suitable for pathogens that are difficult to culture. They can provide protection against homologous pathogens as well as multiple infections by incorporating antigens from different pathogens. Importantly, subunit vaccines can be produced in a highly characterized form and accommodate non-natural components [58]. However, subunit vaccines may induce a weaker immune response compared to live or inactivated whole-cell vaccines. This limitation arises from the restricted number of immunogenic components, which reduces immune system stimulation, as well as the absence of replication and exposure to multiple antigens that whole-cell vaccines provide. Consequently, the use of adjuvants or booster immunizations is often required to enhance and sustain protective immunity [8].

Examples of efficient subunit vaccines for farmed fish species, include the vaccine based on the fused VP2-VP3 protein of *IPNV* (MSD Animal Health) and the *ISAV* vaccine (Virbac), which contains the recombinant hemagglutinin-esterase protein and is currently available in a multivalent format (Table 2).

1.4. Methods of vaccine administration

In aquaculture, vaccines are administered via three main routes: injection (intraperitoneal or intramuscular), oral and immersion (Table 3). An appropriate vaccination route is critical and is normally determined by the developmental phase of the fish. In salmonids, immersion vaccination is typically employed at the fry stage, whereas injection or oral administration is more frequently used for small juveniles. Common practice requires administration of oral vaccine boosters following primary immunization by immersion or injection, especially after transferring juvenile smolt fish to sea cages. Despite their application individually or in combination, these immunization strategies generally fail to elicit a robust immune response, as available marketed vaccines often offer insufficient protection for farmed fish. An effective vaccine must provide long-lasting immunity via administration routes, be cost-effective, and practical to administer—such as oral or immersion—while minimizing handling stress and promoting animal welfare.

1.4.1. Injection administration

Intraperitoneal (i.p.) and intramuscular (i.m.) injections are the most common vaccination methods in aquaculture. They ensure uniform dosing and provide long-lasting protection [58]. Vaccination requires fish of appropriate size—typically juveniles over 20 g—and involves fasting, sedation, removal from water, and rapid manual or automatic injection using needles or pistols with dose control. This process is stressful and demands a trained workforce to ensure proper administration and dose accuracy, to minimize injection-site injuries [63].

These vaccines often include oil-based adjuvants to boost immune response, which may cause side effects such as adhesions, melanosis, peritonitis, or localized inflammation. Due to the small size and underdeveloped immune systems of fry, injection vaccination is unsuitable in early life stages, despite being invasive and stressful, injection remains the most effective method for antigen delivery. It enables precise dosage, use of purified antigens, and combinations with other vaccines [6]. Nonetheless, there is increasing demand for practical, cost-effective alternatives that maintain fish health and welfare, supporting sustainable aquaculture.

Table 3
Main vaccine administration routes used in Aquaculture. Adapted from Refs. [57,58,63].

Methods	Characteristics	Type of Immunity	Advantages	Disadvantages
Injection	Vaccine is administered manually or automatically by intraperitoneal (i.p.) or intramuscular (i.m.) injection	Humoral Systemic immunity	- Exact vaccine dose can be administered - High systemic immune response with prolonged protection - The use of adjuvants enhances vaccine efficacy - Multivalent vaccines can be administered with a single injection - A smaller vaccine dose can provide sustained protection	- Causes stress and potential external injuries in fish, even with anesthesia - May cause damage at the injection site or visceral adhesions in organs - High operational workload and elevated costs - Can only be used for fish over 20 g in size - Not cost-effective for low-value fish
Immersion	Fish are vaccinated in the tank or transferred to a tank containing the vaccine for a defined period	Mucosal (MALT)	- Less stress due to reduced handling - Relatively easy to operate - Simple immunization method - Suitable for small fish, but larger fish can also be vaccinated - Fish can be vaccinated in mass - Multivalent vaccines can be administered	- Requires a high vaccine dose - Lower immune response compared to injection vaccination - Can only be applied to concentrated fish populations - Requires booster measures
Oral	Vaccines are incorporated into feed by extrusion or mixed with feed (pellets) and administered orally to activate intestinal mucosal immunity	Mucosal (GALT) Humoral Systemic immunity	- Simple, safe, and easy immunization method - Avoids stress and handling-related injuries - Fish of all sizes can be vaccinated - Fish can be vaccinated in mass - No additional operational costs in farmed fish, reducing expenses	- Lower immune response compared to injection vaccination - The antigen may be destroyed in the digestive tract, requiring encapsulation for protection - Requires a high vaccine dose

1.4.2. Immersion administration

Immersion vaccination involves bathing or dipping fish in a vaccine solution within a timeframe, allowing antigens uptake through the skin, gills, and/or gut. This method triggers mucosal and systemic immune activities with a limited humoral response [6,58,63]. It is widely used for mass vaccination of young fish, from small fry (<0.5 g) to juveniles, which are difficult to handle.

Classification of immersion vaccination is into two categories based on concentration and period of exposure. The first category usually requires a high vaccine concentration over a short period of exposure, while the other category involves a higher vaccine dilution rate for a longer treatment period of vaccinated fish. This approach reduces handling, stress, mechanical damage, and labour costs compared to injection methods.

Drawbacks of immersion include the need for large volume of vaccine solution, along with poor dose control. Even more, effectiveness depends on factors such as dilution rate, fish behaviour, vaccine mixing, and solubility. These variables can reduce antigen uptake, resulting in weaker immunity and shorter protection periods [6,58,63,64]. Additionally, this method is impractical for larger fish due to the higher antigen requirement [6].

Therefore, to improve efficacy, variables such as antigen type, fish size, temperature, dose, and immersion conditions must be optimized. Advanced research in adjuvant development, booster strategies, and new vaccine formulations offer promising improvements for immersion vaccination [58,63].

1.4.3. Oral administration

Oral vaccination is the safest and easier method of vaccine administration, as it can be delivered to numerous fish in the feed, with antigens targeting the immune components of the mucosa in the gastrointestinal tract. Oral vaccination does not require handling of the fish, it does not induce stress, loss of appetite, or post-handling mortality. Additionally, it can be administered to fish of different sizes, including early life stages. Compared to other vaccination strategies, oral vaccines eliminate the need for complex vaccination logistics, additional labour, and operational costs at aquaculture facilities. Importantly, it does not negatively affect growth, meat quality, or fish welfare, as it can be deployed as an immune booster to maintain or enhance protection after immersion or injection vaccination [6]. For these reasons, oral vaccines are considered the most attractive option, given the exponential and intensive growth of global aquaculture, which demands safer, more practical, and cost-effective immunization methods that can be applied on a large scale.

Nevertheless, oral vaccines present certain challenges in terms of efficacy, like other vaccination methods. Similar to immersion vaccines, large amounts of antigen is required to achieve effective immunity, whilst the vaccine dose per fish can vary— and thus, the level of immunization per individual— since it is not possible to ensure that all fish consume the same amount of feed and receive an equal antigen dose.

Additionally, mucosal-targeted vaccines have been reported to induce oral immune tolerance, a state of immune unresponsiveness to antigens, leading to suppression of both cellular and humoral immune responses. In higher vertebrates, antigen tolerance has been associated with factors such as low-dose administration, which promotes regulatory T-cell induction, and high doses, which lead to lymphocyte anergy [65]. Repeated and prolonged administration of low doses of a particular antigen, especially to young fish with underdeveloped immune system, low water temperatures, antigen type, administration regimens, and genetic factors are contributory factors to immunological tolerance in fish [66,67]. Furthermore, the physicochemical barriers of the gut-associated lymphoid tissue (GALT) that protect against pathogen invasion can also limit antigen bioavailability, thereby reducing the chances of the oral vaccine reaching the activation site in the hind intestine to initiate local immunization. This low bioavailability may also contribute to the induction of immune tolerance [67]. Cases of immune tolerance have been reported in juvenile fish species such as carp and trout, where repeated low-dose exposure to antigenic proteins resulted in tolerance [66–69]. However, studies have also shown that repeated oral booster immunizations increase immunoglobulin M (IgM) levels in farmed salmon after injectable vaccines or primo vaccinations [68]. Additionally, an oral recombinant vaccine produced in *E. coli* against nodavirus (VNNV) in juvenile sea bass significantly increased VNNV-specific IgM levels and achieved a 100 % survival rate after challenge [70].

When formulating an oral vaccine, it is important to ensure the antigen withstand high temperatures and pressures used in feed manufacturing. Moreover, the antigen must be protected from degradation by stomach acids before reaching the posterior intestine, where it can be processed by immune cells [63,71,72]. To enhance the efficacy of oral vaccines, various encapsulation materials, including alginates, chitosan, liposomes, polymer microspheres, and biofilms, have been developed to protect the antigen from stomach biodegradation. For example, an encapsulated recombinant subunit oral vaccine expressed in yeast (*Micromatrix*™ cationic polysaccharide matrix) against ISAV in Atlantic salmon demonstrated significant protection against the virus [73]. Research is also increasingly focused on exploring the novel use of non-pathogenic microorganisms as a carriers of exogenous antigenic components in insects (halo worms, larvae), probiotics (*Lactobacillus*, yeast), plants (*Chlamydomonas*) and viral vectors, in oral vaccines for fish [74–76]. Significant efforts are underway to develop and improve oral vaccines using biotechnological tools to protect antigens from the acidic environment of the fish stomach, increase the vaccine stability, and extend the duration and level of mucosal protection against highly persistent infectious diseases.

1.5. Commercial and experimental oral antiviral vaccines

Among the commercial oral vaccines licensed for use in aquaculture, only three are directed against viral pathogens: a subunit vaccine for the prevention of infectious pancreatic necrosis virus (IPNV) (Aquavac® IPN Oral), a subunit vaccine against infectious salmon anaemia virus (ISAV) (Blueguard® ISAV Oral), and an inactivated vaccine against IPNV (Blueguard® IPN Oral). These formulations have been marketed either in liquid form or as freeze-dried powders for incorporation into fish feed to initiate oral immunisation of fish weighing more than 5 g. All are recommended for both primary and booster immunizations following injectable vaccination, with different administration schedules and doses according to the supplier (Table 4). Most studies on oral vaccines focused on salmonid species (42 %), mainly against IPNV, VHSV, ISAV and IHNV; cyprinids (28 %) against GCRV, KHV and SVCV; sea bass and groupers (24 %) against VNN (VNNV and RGNNV); and other marine species such as flounder and rock bream against HIRRV, RBIV, among others.

Majority of research efforts in oral vaccine development are focused on recombinant vaccines (65 %), followed by DNA-based vaccines (24 %), and to a lesser extent, inactivated and attenuated vaccines (11 %) (Tables 5 and 6). Production of experimental recombinant vaccines (Table 5) commonly employed bacterial expression systems, with *E. coli* being the most widely used [70,77–83], followed by *Lactobacillus* [84–88], *Lactococcus lactis* [89,90], and *Bacillus subtilis* [91–93]. Yeast-based systems constitute the second most common group, predominantly using *Saccharomyces cerevisiae* [73,78,94–100] and *Komagataella pastoris* [101,102]. Finally, plant-based expression systems have also been explored, including *Nicotiana tabacum* [103,104], *Nicotiana benthamiana* [80], *Chlorella vulgaris* [105] and *Oryza sativa* [106], among others.

As shown in Table 6, all reviewed studies on DNA, inactivated, and attenuated experimental vaccines employed encapsulation strategies to protect the antigens from gastric degradation. Alginate [107–113], chitosan [113–118], liposomes [119], and polymeric microspheres such as PLGA [120,121] and PEG [122] are the predominate encapsulation materials deployed. In the case of recombinant vaccines, only a limited number of studies report the use of additional encapsulation materials, such as sodium alginate [89,123], chitosan–alginate combinations [85,88], or other polymeric microspheres [73]. In most cases, the expression system itself serves as the delivery vehicle, such as yeasts, bacteria like *Lactobacillus*, or plants. Some studies have also explored the use of *Artemia nauplii* as a delivery platform [77,96].

Mode of administration of oral vaccines are largely dependent on their nature and dosage. Experimented oral vaccines are usually

Table 4
Authorised oral fish vaccines that have been used in aquaculture.

Type of Vaccine	Disease/virus	Vaccine	Company	Encapsulation/vehicle	Protocol	Administration protocol	Daily dose (mg/kg fish)	Total dose (mg/kg fish)
Viral subunit	Infectious pancreatic necrosis/IPNV	Aquavac® IPN Oral	MSD Animal Health	*Pichia Pastoris* recombinant	Initial immunization (≥5 g) and booster	5-day delivery 5-day rest 5-day delivery	0,02 ml/fish/day	n.d
Viral inactivated	Infectious pancreatic necrosis/IPNV	Blueguard® IPN Oral	Virbac	Bioadhesive cationic polysaccharide (MicroMatrix™)	Initial immunization (≥10 g) and booster	10 days	6 mg/fish/day	2000 mg/kg fish (in 30 g fish)
Viral subunit	Infectious salmon anemia/ ISAV	Blueguard® ISAV Oral	Virbac	Bioadhesive cationic polysaccharide (MicroMatrix™)	Initial immunization (≥10 g) and booster	10 days	6 mg/fish/day	1500 mg/kg fish (in 40 g fish)

Table 5
Experimental recombinant oral vaccines against fish viral diseases.

Disease/Virus	Antigen/Expression system/ Vehicle	Fish weight/length	Dose	Administration protocol	Reference
Viral nervous necrosis/RGNNV	pET24a-NNV-CVP/*E. coli*/*Artemia nauplii*	Grouper Larvae/1 cm	10^8 CFU/ml of *E. coli*-VP + 10^7 CFU/ml GFP strain	Oral (*A. nauplii*): 2 days (18 & 19 dph)	Lin et al. [77]
Viral nervous necrosis/RGNNV	RGNNV-CP VLPs/*S. cerevisiae*	The convict grouper/63–88 g	50 μg RGNNV-CP/VLPs/fish	Oral gavage: 1 day	Wi et al. [94]
Viral nervous necrosis/RGNNV	NNV-CP (rCoat)/Tobacco chloroplasts	Seven-band grouper/3-month-old	5 μg or 10 μg rCoat	Oral (in-feed): 1 day Booster: every Monday at 2-week (4 times)	Cho et al. [104]
Viral nervous necrosis/RGNNV	OSGNNV-CP VLPs/*E. coli*	Orange-spotted grouper/ Trial 1: 0.1 g Trial 2: 0.11 g	Trial 1: 20 μg/g FBW Trial 2: 25, 50, 100, 200 μg/g FBW	Trial 1: Oral (in-feed): 1 day (twice). Booster: day 15 (twice). Trial 2: Oral (in-feed): 1 day Booster: day 7, 14 and 21	Chien et al. [79]
Viral nervous necrosis/RGNNV	NNV; strain It/411/96, genotype RGNNV/*E. coli*	European seabass/ 10–12 g	10^{10} CFU/g feed	Oral (in-feed): 3 consecutive days Booster: day 14	González-Silvera et al. [70]
Viral nervous necrosis/RGNNV	pYEG-αMCS-optRGNNV-CP/ *S. cerevisae*	Convict grouper/ 15–17 cm	Trial 1: Freeze-dried with or without cell disruption: 50 mg/ fish Trial 2: Freeze-dried with cell disruption: 50 mg/fish	Trial 1: Oral gavage: 1 day Boosters: days 9, 16 and 23 Trial 2: Oral (in-feed): 1 day Boosters: days 7, 14 and 21	Cho et al. [95]
Viral nervous necrosis/RGNNV	NNV-CNP (RGNNV/SJNNV strain SpSs IAusc160.03)/*E. coli*	Senegalese sole/ 15.2 g	Dose 1: 50 μg/fish Dose 2: 500 μg/fish	Oral gavage: 1 day Gavage booster: day 14	Thwaite et al. [82]
Viral nervous necrosis/RGNNV	RGNNV-CP VLPs/*N. tabacum*	Sevenband grouper/25.8 g	200 μg/fish RGNNV-CP-VLP	Oral (in-feed): 5 consecutive days	Nakahira et al. [103]
Rock bream iridovirus/RBIV	rMCP/*P. pastoris* SMD1168	Rock bream/7–8 g	1×10^8 cells in 2.5 g of feed/fish	Oral (in-feed): 1 day Booster: 3 times at 1-week intervals.	Seo et al. [101]
Rock bream iridovirus/RBIV	rMCP/*O. sativa* cv. Dongjin	Rock bream/10 g	1 μg of rMCP/g FBW 3 μg of rMCP/g FBW	Oral (in-feed): 1 day Booster: every Monday at 1 week (3 times)	Shin et al. [106]
Viral hemorrhagic septicemia/ VHSV	rVHSV-ΔNV-EGFP	Olive flounder/4–5 g	10^4, 10^5 or 2×10^5 PFU rVHSV-ΔNV-EGFP/fish	Oral gavage: 1 day Gavage booster: 2 wpi (same dose)	Kim et al. [124]
Viral hemorrhagic septicemia/ VHSV	VHSV glycoprotein gene/*Chlorella vulgaris* PKVL7422	Olive flounder/ 41,3 g	0.36 mg VHSV-G/g feed	Oral (in-feed): 5 consecutive days Booster: days 15–19	Kim et al. [105]
Viral hemorrhagic septicemia/ VHSV	CTB-fused rVHSV-GP1 or GP2/ *E. coli* and *N. benthamiana*	Olive flounder/ 40–50 g	5 μg rGP/*E.coli* (ip) + 30 % w/w powder (30 μg/fish) (Oral)	Ip administration: 1 day Oral booster (in feed): every Monday at 2-week (4 times)	Kim et al. [80]
Viral hemorrhagic septicemia/ VHSV	VHSV glycoprotein gene/*L. lactis*	Rainbow trout/7,0 g	10^8 & 10^{10} CFU/g *L. lactic* NZ3900/PNZ-G	Oral (in-feed): 7 consecutive days Booster: days 15–21	Naderi-Samani et al. [90]
Viral hemorrhagic septicemia/ VHSV	VHSV glycoprotein G + trout Interferon-gamma/*E. coli*	Rainbow trout/ Trial 1: 1–2 g Trial 2: 110 g Trial 3: 30 g	Trial 1: 1,1 g/kg feed Trial 2: 30 mg/kg fish Trial 3: 125 mg/kg fish (41,5 mg/ kg fish/day)	Oral (in-feed): 20 consecutive days Trial 2: Oral gavage: 1 day Trial 3: Oral gavage: 3 consecutive days	Aceituno et al. [83]
Infectious pancreatic necrosis/ IPNV	pPG-612-VP2 and pPG-612-CK6-VP2/*L. casei* 393	Rainbow trout/ 11.5 g	10^9 CFU/mL	Oral (in-feed): 1 day Booster: day 32	Duan et al. [86]
Infectious pancreatic necrosis/ IPNV	pPG-612-AHA1-CK6-VP2/*E. coli* and *L. casei*	Rainbow trout/10 g	10^{10} CFU/ml	Oral gavage: 3 consecutive days Booster: days 31–33	Chen et al. [81]
Grass carp hemorrhagic disease/GCRV	GCRV segment 6 (S6)/B. subtilis WB600/*B. subtilis* spores	Grass carp/23 g	10^{-3} μg protein/g fish (10^{12} spores/g diet	Oral (in-feed): 8 weeks	Jiang et al. [91]
Grass carp hemorrhagic disease/GCRV	GCRV-VP7/*B. subtilis* strain 168/ *B. subtilis* spores	Grass carp/50 g	1.0×10^{10} spores/fish	Oral (in-feed): 1 day Booster: day 7	Sun et al. [93]
Grass carp hemorrhagic disease/GCRV	GCRV rVP35-VP4/Bac-to-Bac baculovirus	Grass carp/18 g	0,02 μg/g/day (protein/fish)	Oral (in-feed): 6 weeks	Mu et al. [125]
Grass carp hemorrhagic disease/GCRV	GCRV II VP56 + Flagellin B/ *E. coli*/Sodium alginate	Grass carp/15 g	150 mg/Kg SA-VP56-3/FlaB 200 mg/kg SA-VP56-3/FlaB	Oral (in-feed): 28 consecutive days	Xu et al. [123]
Grass carp hemorrhagic disease/GCRV II	GCRV-HF/*B. subtilis* GC5/*B. subtilis* spore	Grass carp/15–20 g	2×10^9 spores/fish/d	Oral (in-feed): 3 consecutive days Booster: days 21–23	Chen et al. [92]
Hirame novirhabdovirus/ HIRRV	HIRRV G protein/*L. lactis* + sodium alginate (feed)	Flounder/35 g	10^9 CFU/g diet	Oral (in-feed): 7 consecutive days Booster: days 29–35	Zhao et al. [89]
Koi herpesvirus disease/CyHV-3	CyHV-3 pORF65/*S. cerevisiae*/*A. nauplii*	Common carp/18 dph	200 Artemia/carp (2×10^3 Artemia/mL + 100 μL dose)	Oral gavage: 7 consecutive days Booster: days 15–21 & 29-35	Ma et al. [96]

(*continued on next page*)

Table 5 (*continued*)

Disease/Virus	Antigen/Expression system/ Vehicle	Fish weight/length	Dose	Administration protocol	Reference
Koi herpesvirus disease/CyHV-3	CyHV-3 ORF131/EBY100 *S. cerevisiae*	Jian carp/10 g	1.6×10^9 CFU/fish	Oral gavage: 1 day Booster: day 14 & 28	Liu et al. [97]
Herpesviral hematopoietic necrosis/CyHV-2	CyHV-2 pYD1-ORF25/EBY100 *S. cerevisiae*	Gibel carp/25 g	1.3 mg/fish EBY100/pYD1-ORF25	Oral (in-feed): 3 consecutive days Booster: days 18–20	Dong et al. [98]
Herpesviral hematopoietic necrosis/CyHV-2	CyHV-2 pYD1-ORF132/EBY100 *S. cerevisiae*	Crucian carp/ 15–18 g	2×10^9 yeast cell EBY100/pYD1-ORF132	Oral gavage: 7 consecutive days. Oral gavage booster: days 14–21	Wang et al. [100]
Koi Herpesvirus disease/KHV	pYG-KHV-ORF81/*L. rhamnosus*/ Chitosan-alginate capsules	Koi carp/50 g	$\sim 10^{11}$ CFU/g pYG-KHV-ORF81/ *L. rhamnosus* CIQ249	Oral (in-feed): 3 consecutive days Booster: days 14–16 & 28-30	Huang et al. [88]
Spring viremia of carp virus/ SVCV and Koi herpesvirus disease/KHV	SVCV isolate FY413 and KHV isolate CX729/*L. plantarum*	Common carp/500 ± 50 g Koi carp/350 ± 50 g	10^9 CFU/g pYG-G-ORF81/Lp	Oral (in-feed): 3 consecutive days 1st booster: days 11–13 2nd booster: days 28–30	Cui et al. [84]
Spring viremia of carp Virus/ SVCV	pUG-SVCV-G/*L. plantarum*/ Chitosan-alginate microcapsule	Common carp/200 g	3.3×10^9 CFU/g pYG-SVCV-G/LP	Oral (in-feed): 3 consecutive days Booster: days 14–16 & 28-30	Jia et al. [85]
Infectious salmon anemia Disease/ISAV	F protein + surface HE from ISAV/ *S. cerevisiae*/Cationic polysaccharide matrix	Atlantic salmon/40 g	6 mg/fish/day	Oral (in-feed): 10 consecutive days	Caruffo et al. [73]
Infectious hematopoietic necrosis virus/IHNV	IHNV strain Sn1203/*E. coli* and *S. cerevisiae*	Rainbow trout/5 g	66.3 μg/fish EBY100/pYD1-G/ *S. cerevisiae,* 93.4 μg/fish EBY100/pYD1-bi-G/*S. cerevisae*	Oral gavage: 1 day Booster: day 32	Zhao et al. [78]
Largemouth bass ranavirus/ LMBV	GS115-pW317-MCPD/*P. pastoris*	Largemouth bass/ 24,6 g	10^7 or 10^8 CFU GS115-pW317-MCPD	Oral (in-feed): 14 consecutive days	Yao et al. [102]
Largemouth bass ranavirus/ LMBV	MCP gene sequence of LMBV + *E. coli* B subunit heat-labile enterotoxin/*S. cerevisiae*	Largemouth bass/ 25 g	1x10 [7] CFU/g feed	Oral (in-feed): 7 consecutive days	Zhang et al. [99]

incorporation in fish feed, as well as through oral gavage, within a vaccination window of 1–7 days which is often followed by one or two oral boosters administered at 7, 14, and 21 days, and in some cases up to 30 days, after the initial immunization (Tables 5 and 6).

The correlates of protection (CoP) and the main results of functional assays from experimental studies are included in Supplementary Tables 1 and 2

1.6. Adjuvants for oral anti-viral vaccines

A combination of adjuvants with antigenic proteins becomes a necessity to improve immune response, minimize side effects, and promote long-lasting protection in vaccinated fish [128]. Therefore, a careful choice of the best-fit adjuvant is crucial to a successful vaccine development. Adjuvants are substances or formulations that promote vaccine efficacy by enhancing the host's response to vaccine antigens through the activation of essential immune signals. Different classes of signals are promoted by adjuvants following the interaction between antigenic molecules and expressed pattern recognition receptors (PRRs) on immune cells have been well reported. Foremost is the Type 0 signal, which is associated with pathogen-associated molecular patterns (PAMPs) of viruses, namely unmethylated CpG DNA, ssRNA or dsRNA, stimulating the corresponding toll-like receptors (TLRs) of innate immune cells. Subsequent aluminium salts, oil/water emulsions, microparticles, and lipid-based adjuvants facilitate the activation of Type 1 signal, promoting prolonged antigen delivery to professional antigen-presenting cells (APCs), thereby improving antigen uptake. Activation of adaptive immunity by polarized APCs, known to promote the differentiation and activation of T and B cells through the release of paracrine cytokines and chemokines that lead to Type 2, is reportedly mediated by saponin-based immune-stimulating complexes (ISCOMs) adjuvants. As an improvement to this pathway, adjuvants that specifically regulate antigen-specific T-helper cell proliferation have been proposed as the Type 3 signal. Finally, Type 4 adjuvant-activated signal would be related to homing signals in the treatment of tumor and chronic infections [129,130]. Adopting this kind of strategy in developing adjuvants for fish vaccine could help to target mucosa-associated lymphoid tissue (MALT) cells against specific viral infection.

Studies on probiotics [70,77,78,84–86,89–93,95–101,105], chitosan [88,109,113–118], sodium alginate [108–110,112,123,131], and PLGA [120,121] are among the most researched adjuvants for oral vaccines against viral diseases in fish (Table 5). The growing preference for probiotics, including lactic acid bacteria (LAB) and yeast, as vaccine carriers and adjuvants can be attributed to their ability to evade the gastrointestinal barriers to outcompete pathogenic microbes in the host's gut colonization. This system ensures delivery of intact antigens within a short time to enhance the activation of innate immunity [90,132–134]. As demonstrated in the research of Naderi-Samani et al. [90], *Lactococcus lactis* NZ3900 was successfully transformed with antigenic G-protein from viral haemorrhagic septicemia virus (VHSV) and orally vaccinated rainbow trout fry using this probiotic-based vaccine. At five days post-vaccination (dpv), transformed bacteria were still detected in the fish's gut with a higher relative percentage of survival (RPS) of 78 % recorded in the challenge test. Interestingly, an increase in the IgM titer value from 35 dpv to 60 dpv correlated with a decline in the viral load, suggesting continuous activation of the humoral system against VHS. Similarly, the use of *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus casei* 393 against SVCV [85], KHV [88] and IPNV [87], respectively, have been tested in common carp and rainbow trout. The use of bacterial (*Bacillus subtilis*) spores as oral vaccine vehicle and adjuvant is trending in the experimental vaccination of carp species [91–93,97]. To achieve this, transformed *B. subtilis* with VP4 major capsid protein from grass carp reovirus (GCRV) were stimulated to sporulate to generate spore-encapsulated vaccines [91]. Following the challenge test, the 8-week orally vaccinated grass carp had a lower RPS of 47 % the gut mucosal IgZ/T and serum IgM peaked at weeks 2 and 4, respectively, and declined thereafter. Against the same disease, Sun et al. [93] developed two groups of *B. subtilis* spore with VP7 of GCR. Of

Table 6
Experimental DNA, Inactivated, and Attenuated oral vaccines against fish viral diseases.

Type of Vaccine	Disease/Virus	Antigen/Vehicle or encapsulation	Fish/weight	Dose	Administration protocol	Reference
DNA	Infectious pancreatic necrosis/IPNV	IPNV VP2/alginate microspheres	Rainbow trout/ 1 g Brown trout/ 1.5 g	10 μg pcDNA-VP2/fish	Oral gavage: 1 day	De las Heras et al. [108]
DNA	Infectious pancreatic necrosis/IPNV	IPNV VP2/alginate microspheres	Rainbow trout/ 1 g	10 μg/fish	Oral gavage: 1 day	Ballesteros et al. [109]
DNA	Infectious pancreatic necrosis/IPNV	IPNV VP2/alginate microspheres	Rainbow trout/ 1.5 g	10 μg/fish/day	Oral (in-feed): 3 consecutive days	Ballesteros et al. [110]
DNA	Infectious pancreatic necrosis/IPNV	IPNV VP2/alginate microspheres	Rainbow trout/ 1.5 g	10 μg pcDNA-VP2/fish	Oral gavage: 1 day	Ballesteros et al. [111]
DNA	Infectious pancreatic necrosis/IPNV	IPNV VP2/Chitosan-tripolyphosphate (CS-TPP-pcDNAVP2) and alginate microparticles (alg-pcDNAVP2)	Rainbow trout/ 3 g	10 and 25 μg/fish	Oral (in-feed): 1 day Booster: day 15	Ahmadivand et al. [113]
DNA	Infectious pancreatic necrosis/IPNV	VP2 capsid protein/cationic liposome DOTAP	Atlantic salmon/Trial 1: 5 g Trial 2: 20 g	Trial 1: 0.2, 0.6 and 1 mg pDNA/kg fish/day Trial 2: 1 and 2 mg pDNA/kg fish/day	Trial 1: Oral (in-feed): 7 consecutive days Trial 2: Oral (in-feed): 5 consecutive days	Reyes et al. [119]
DNA	Infectious hematopoietic necrosis/IHNV	IHNV glycoprotein/alginate microspheres	Rainbow trout/ 3–4 g	Trial 1: (1) 10 μg/fish, (2) 20 μg/fish (3) 25 μg/fish, (4) 50 μg/fish Trial 2: (1) Oral group: 100 μg/fish (2) Injected group: 5 μg/fish	Trial 1: (1) and (3) oral gavage: 1 day (2) and (4) Booster 15 dpiv Trial 2: (1) oral gavage: 1-day (2) im injection	Ballesteros et al. [112]
DNA	Infectious hematopoietic necrosis/IHNV	IHNV G/PLGA	Rainbow trout/ 5 g	Group 1: 22 μg/fish Group 2: 43 μg/fish Group 3: 1 μg/fish (control)	Group 1: Oral (in-feed): 4 consecutive days Group 2: Oral (in-feed): 8 consecutive days Group 3: Intramuscularly injected	Adomako et al. [120]
DNA	Viral nervous necrosis/VNNV	RNA2 capsid protein nodavirus/ chitosan–tripolyphosphate nanoparticles	Asian sea bass/ 10–15 g	100 μg/ml plasmid complex/fish	Oral (in-feed): 1 day	Vimal et al. [117]
DNA	Viral nervous necrosis/VNNV	NNV (genotype RGNNV/Chitosan	European sea bass/6 g	10 μg/fish	Oral (in-feed): 2 consecutive days	Valero et al. [118]
DNA	Grass carp hemorrhagic disease (GCHD)/ GCRV	VP4 and VP56 genes of GCRV & adjuvants: grass carp interferons (IFNs: IFN1, IFN3, IFNγ2)/PLGA	Grass carp/15 g	20 μg/fish	Oral (in-feed): 3 consecutive days Booster: days 8, 9 and 10	Jiang et al. [121]
DNA	Infectious salmon anemia Disease/ ISAV	ISAV 752 strain + Alphavirus replicon (adjuvant)/Chitosan	Atlantic salmon/40 & 70 g	7 μg of DNA and 1×10^5 $TCID_{50}$ of virus/fish	Oral (in-feed): 7 consecutive days	Rivas-Aravena et al. [116]
Inactivated	Viral hemorrhagic septicemia/VHSV	VHSV (F1Wa05 strain)/chitosan nanoparticle (CNPs-IV)	Olive flounder/ Trial I: 10 g & Trial II: 12 g	CNPs-IV with l ml/fish (1×10^8 $TCID_{50}$ virus) nanospheres mixed with 5 % (w/w of feed)	Trial I: Oral (in-feed): 2 consecutive days & Oral/ Oral: Booster 14 days after 1st oral delivery: 2 days Trial II: Immersion/oral: In-feed booster 14 days after 1st immunization: 2 consecutive days	Kole et al. [114]
Inactivated	Viral hemorrhagic septicemia/VHSV	Trivalent vaccine: VHSV (F1Wa05 strain) (IV), *S. parauberis* serotype I (SP1DS strain) (Sp) and *Miamiensis avidus* (YS2 strain) (Sc)/chitosan-PLGA	Olive flounder/ 11.2 g	VHSV: $6.5 \times 10^{8.8}$ virus/fish Sp: 4.32×10^9 CFU/ fish Sc: 2×10^5 ciliates/ fish	Oral (in-feed): 15 consecutive days	Kole et al. [115]
Attenuated	Viral hemorrhagic septicemia/VHSV	VHSV (strain ATT 150)/polyethylene glycol (PEG)	Rainbow trout/ 40–80 g	Group 1: 100 μg/fish (4.8 TCID/fish) Group 2: 100 μg/fish (5.8 TCID/fish) Group 3: 5 μg/fish (1 %; 4.7 TCID/fish) and 50 μg/fish (10 %; 5.7 TCID/fish)	Oral PEG-particles: 3 consecutive days	Adelmann et al. [122]
Inactivated	Viral nervous necrosis/VNNV	HGNNV strain/nano-encapsulated (Alarvita Biolife Company)	Orange-spotted grouper/0.18 g	1. Bath: 5×10^{-5} $TCID_{50}$/ml 2. Oral: 60,000 artemia mix with BEI-	1. Bath: 60 min 2. Oral (Vaccine–Artemia mix): 3 consecutive days	Kai et al. [126]

(*continued on next page*)

Table 6 (*continued*)

Type of Vaccine	Disease/Virus	Antigen/Vehicle or encapsulation	Fish/weight	Dose	Administration protocol	Reference
				inactivated NNV at 10^8 $TCID_{50}$/10 ml		
Inactivated	Viral nervous necrosis/VNNV	RGNNV (A betanodavirus SGEhi00 strain)/Capsaicin	Sevenband grouper/Exp. 1: 10 g and Exp. 2: 45 g	Exp 1: Pellet Feeding: 10^8 $TCID_{50}$/fish/day. Anal Intubation: 0.1 mL of vaccine/fish/day Exp 2: Capsaicin-Enhanced Feed: 0.5 mg capsaicin/fish/day	Exp 1: Oral (in feed): 7 consecutive days Anal Intubation: twice at one-week intervals Exp 2: Oral Capsaicin-Enhanced Feed: 7 consecutive days	Gaafar et al. [127]
Inactivated	Infectious pancreatic necrosis/IPNV	IPNV (SP strain rNVI-15PTA)/alginate	Atlantic salmon/200 g	1st oral boost: ~2.2 × 10^9 $TCID_{50}$/kg fish 2nd oral boost: ~1.5 × 10^9 $TCID_{50}$/kg fish	1st oral boost (in feed): one year post ip vaccination: 7 days 2nd boost (in feed): 7 weeks post 1st boost: 7 days	Chen et al. [107]

Note for Tables 5 and 6 dpc: days post challenge; dpv/dpi: days post vaccination or immunization; hpiv: hours post initial vaccination; hpb: hours post booster vaccination; hpc: hours post challenge; wpc: weeks post challenge; wpv/wpi: weeks post vaccination or immunization; DD: Degree Days; RPS: Relative percentage survival; ELISA: Enzyme-linked immunosorbent assay; FBW: Feed Body Weight; dph: days-post-hatching; HK: Head kidney; Abs: antibodies; ip: intraperitoneal.

which spore vaccines, whose growth were arrested in the gastrointestinal tract of grass carp, had doubling RPS of 50.66 and 56.73 % compared to fish vaccinated with a non-germination-arrested spore vaccine treatment with a lower RPS of 28.09 %. This suggests that the dormancy state of the spore could have a significant impact on the timing and release of the encapsulated antigenic viral particles in the delivery of the immunostimulatory substance to the targeted gut immune cells. Therefore, further studies are required in this direction to enhance the potential of spore vaccine usage in fish disease management.

Another probiotic used for vaccination trials is yeast, mainly *Saccharomyces cerevisiae* [78,94–100,135–138], although in some studies *Pichia pastoris* [101,102,139] were used. A comparative study of disrupted and non-disrupted *S. cerevisiae* expressing capsid protein from red-spotted grouper nervous necrosis virus (RGNNV) in an oral gavage experiment by Cho et al. [95] indicated that research fish vaccinated with the former had a higher RPS of 96.5 % as against the latter with an RPS of 75 %. In a related study, artemia was used to deliver cyprinid herpesvirus-3 (CyHV-3) antigenic protein expressed on *S. cerevisiae* as a vaccine adjuvant in an oral gavage trial conferred up to 61 % RPS in the experimental fish [96]. Other authors transformed *S. cerevisiae* EBY100 strain to express ORF132 of CyHV-2 [100] and major capsid protein (MCP) of largemouth bass ranavirus (LMBV) [99]. These oral vaccines were administered to Prussian carp and largemouth bass to achieve RPS of 64 % and 73 %, respectively. Seo et al. [101] and Embregts et al. [139] demonstrated the efficacy of methylotrophic yeast, *Pichia pastoris*, as an expression vector for rock bream iridovirus (RBIV) antigenic capsid protein with subsequent bioencapsulation in live diets (*Daphnia magna* and *Brachionus plicatilis*) of flounder, carp and trout larvae to orally deliver the vaccine to the young fish. With the peak serum IgM reached at 28 dpv to achieve a 41.6 % survival rate in the lab-infected largemouth bass with LMBV, the diet-administered yeast (*P. pastoris*) expressed recombinant protein could channel the pathway to novel antiviral therapeutic solutions by optimizing the vaccine production process.

Some of these probiotics has been also encapsulated for oral vaccination using chitosan-alginate microcapsules [85,88,123], although chitosan has been used alone to encapsulate other types of oral vaccines, like DNA vaccines [118,140], inactivated virus [114,116] or subunit vaccines [123]. Chitosan is an exceptional encapsulation agent due to its non-toxicity, biodegradability, and strong mucoadhesive properties, which are key to effectively activating mucosal immune responses through oral immunization [141,142]. The colonization of transformed *Lactobacillus rhamnosus* expressing KHV ORF81 in the intestine of koi carp after 20 days of oral delivery through chitosan-alginate capsules makes evident its suitability in safe delivery of probiotic-expressed vaccines against the highly acidic gastric (pH 1.5) and hypertonic intestinal conditions (9 % NaCl, 0.5 % bile salts) [88]. A survival rate of 85 % was achieved in the fish fed with encapsulated probiotic vaccine-treated diet compared to less than 20 % survival rate in fish fed with only encapsulated probiotic, and 100 % mortality rate in the control group, which emphasized the efficacy of chitosan-alginate as adjuvant. Although Rivas-Aravena et al. [116] study revealed that Atlantic salmon fed with chitosan-encapsulated inactivated ISAV showed a lower survival rate of 39 %. In contrast, the same encapsulated vaccine with alphavirus replicon conferred a higher survival rate of 77 % in the vaccinated fish.

Furthermore, scientists have explored the use of encapsulated DNA vaccine against RGNNV in European sea bass [118]. Despite achieving a 45 % survival rate due to significant upregulation of cell-mediated cytotoxic and interferon activities in this assay, the inability of this novel therapeutic to enhance the activities of antigen-specific neutralizing antibodies raises questions on its potential to stimulate long-term immunity. In another trial, a chitosan-coated oral DNA vaccine expressing immunogenic capsid protein of turbot reddish body iridovirus (TRBIV) developed by Zheng et al. [140], enhanced the survival rate of the vaccinated fish by 68 %, with anti-TRBIV antibody were detected after 90 days. In another trial, Largemouth bass larvae were vaccinated against *Micropterus salmoides* rhabdovirus (MSRV) with chitosan-alginate microspheres encapsulating MSRV G2 protein. Serum antibodies peaked at 21 days post-vaccination in encapsulated and non-encapsulated groups; however, the highest antibody titer was reported in the encapsulated group. Also, infection challenge test showed significant differences in the RPS of the encapsulated (41.9, 48.4 and 54.8 %) and non-encapsulated group (25.8 %), demonstrating the improvement in vaccine efficacy by the adjuvant action of chitosan alginate microspheres [123].

Although several studies have demonstrated the immunocompatibility of chitosan as a vaccine carrier and adjuvant, cases of suboptimal immunostimulatory activities of chitosan-coated vaccines raised concerns. Variation in the experimental conditions due to absence of a standardized protocol in the encapsulation production of chitosan-capsuled vaccines might have contributed to the differences in physicochemical properties in terms of the molecular weight and degree of acetylation of the chitosan particles. According to Collado-González and Esteban [143], it is recommended to maintain chitosan at a 10–15 % deacetylation degree and between 50 and 100 kDa molecular weight. Furthermore, the modes of administration of the experimented chitosan-vaccines were quite different: 60 % of the reviewed studies adopted immersion vaccination, while the remaining 40 % applied oral

vaccination at varying doses for diverse fish species at various stages of development under heterogeneous rearing conditions. These multilayered conditions complicate the assessment of the isolated effects of chitosan on vaccine effectiveness against viral infections in fish. The effectiveness of chitosan in ensuring the safe delivery of antigen to mucosal-associated lymphoid tissues cannot be questioned. Future research should focus on enhancing the timing of antigen release by chitosan in carnivorous and herbivorous fish species and assess its interactive influence on fish humoral immunity activation to optimize non-invasive vaccination strategies against viral infection in the aquaculture industry.

In a similar context, sodium alginate, a polymer of β-D-mannuronic acid and α-L-guluronic acid extracted from brown algae cell walls, has been considered as a suitable therapeutic encapsulation substance due to its ability to enhance innate immunity, disease resistance and growth of fish [128]. Research has been geared towards assessing the effectiveness of sodium alginate as an encapsulation agent in orally delivered antiviral vaccines in rainbow trout [108–112,131], Atlantic salmon [107], Flounder [89], common carp [144] and grass carp [123]. However, major setbacks to the adoption of this substance in commercial vaccine development could be its poor encapsulation capability and side effects on the digestive system because of its low biocompatibility [145,146].

Poly(lactic-co-glycolic) acid polymer (PLGA) is one of the most widely explored synthetic nano-encapsulation polymers in the field of biomedicine. Its functionality as a vaccine carrier and adjuvant is credited to its exceptional characteristics, which facilitate a long-lasting immune response by increasing antigen delivery time in a slow-releasing manner. Trials on the delivery of inactivated virus and DNA vaccines using PLGA nanoparticles have been reported in the studies of Kole et al. [114](2019) and Jiang et al. [121](2023), respectively. Jiang et al. [121] orally vaccinated grass carp for three days (primo-vaccination) with booster vaccination administered for three days thereafter using a PLGA-coated plasmid DNA vaccine coding for antigenic capsid proteins of GCRV and interferon cytokine to boost the survival of the infected fish by almost 70 % compared to the treatments without cytokine. Therefore, exhibiting the potential of antiviral cytokines such as interferon and other cytokines used as an adjuvant in vaccine development, as reviewed by Guo and Li [147]. To our knowledge, apart from Jiang et al. [121], most of the studies on the application of PLGA as adjuvant in vaccine were focused on injection-based vaccines [123,148–150] and mostly aimed at bacterial diseases [151–156].

1.7. Oral antiviral prophylaxis in crustaceans

While oral viral vaccines for finfish have shown considerable success in controlling viral infections, similar advancements are being made in the development of oral vaccines for other commercially important aquatic species, such as shrimp. In shrimp aquaculture, progress has been focused on developing oral prophylaxis against viral diseases, including White Spot Disease (WSD), Yellow Head Disease (YHD), and White Tail Disease (WTD) (Table 6). Among them, the white spot syndrome virus (WSSV), the causative agent of WSD, is one of the deadliest pathogens affecting shrimp, resulting in 100 % mortality within 7 days [157]. Yellow Head Virus (YHV), which causes YHD, leads to yellow discoloration of the cephalothorax and gills in infected shrimp, resulting in rapid mortality, with rates reaching 100 % within 3–9 days in acute cases [158]. *Macrobrachium rosenbergii* nodavirus (MrNV) is responsible for a deadly WDT infection in freshwater prawns, especially impacting the post-larvae of *M. rosenbergii* with mortality rates reaching 100 % [159]. These viruses are highly contagious and virulent, capable of spreading rapidly and causing large-scale fatalities that result in substantial economic losses for producers. prompting urgent actions for the development of effective vaccines and preventive strategies. Oral vaccines offer advantages in terms of ease of administration and cost-effectiveness, making them a promising non-invasive approach for disease prevention in commercial aquaculture species. Table 7 provides an overview and summarizes the current progress of antiviral prophylaxis in shrimp.

1.7.1. Classes of crustacean vaccines

Subunit Vaccines: Subunit vaccines using viral proteins (e.g., VP28) or baculovirus vectors have shown moderate protective effects. For example, a combination of VP19 and VP28 provided 50 % protection in giant tiger shrimp (*Penaeus monodon*), whereas VP28 alone offered limited protection (30 %) [160]. The capsid protein of MrNV provides 55–70 % protection in *M. rosenbergii* [161]. Despite promising results, their effectiveness remains moderate.

Inactivated Vaccines: Inactivated vaccines have demonstrated more promising results. In *P. monodon*, inactivated vaccines administered via feed coating resulted in 77.6 % survival rate at 3 dpv and 46.3 % survival at 45 dpv [162]. In *Fenneropenaeus indicus*, inactivated vaccines provided 100 % survival at 5 and 10 dpv, highlighting their potential for short-term protection [163]. Long-term efficacy, however, requires further investigation.

DNA Vaccines: DNA vaccines, including plasmid DNA of immunogenic viral particles transformed in biological agents or encapsulated in chitosan nanoparticle carriers, have shown potential in activating crustacean immune system. For instance, Ning et al. [164] successfully administered a diet-based DNA vaccine to red swamp crayfish (*Cambarus clarkii*) against WSSV using bioengineered *Salmonella typhimurium* with plasmid vector (pDNA3.1) encoding for WSSV envelop protein (VP28), indicating the effectiveness of bacteria as a vaccine vehicle in shellfish. Another study reported an outstanding protection of shrimp challenged with WSSV post vaccination with chitosan-encapsulated vaccine [165].

Double-stranded RNA (dsRNA) Vaccines: Another important gene-based vaccine solution being explored in crustacean antiviral therapy are the dsRNA vaccines. These novel vaccines consist of short interfering RNAs (siRNAs) that target virus RNA genetic factors (e.g., Rab7, YHV protease) to disrupt their translation into viral proteins in the host system, thereby preventing viral infection and proliferation. Feed-coated dsRNA vaccines administered to *Litopenaeus vannamei* have been reported to drastically reduce mortality rate caused by viral diseases [166,167].

Recombinant Protein Vaccines: Recombinant protein viral protein expressed in *Saccharomyces cerevisiae*, have demonstrated high efficacy in combating viral infections in shrimp farming. Whiteleg shrimp (*Penaeus vannamei*) orally vaccinated with purified recombinant WSSV VP28 protein were fully protected from WSSV infection in a challenge test, with the detection of the VP28 protein in the eyestalk and hepatopancreas of the experimental fish [168]. Other studies indicated that using *Bacillus subtilis* and chitosan nanoparticles as a vaccine vehicle for recombinant proteins in crustaceans significantly enhances immune response, resulting in improved protection against viral infections [165,169].

1.7.2. Dosing of vaccine in crustaceans

Vaccine efficacy is dose-dependent and can be influenced by the age and weight of experimental animals. In the case of juvenile shrimp, which are typically smaller and younger, their immune systems may be more responsive to vaccines, leading to higher survival rates. To illustrate this, juvenile *Fenneropenaeus indicus* (average body weight of 3–4 g) showed optimal protection at a dose of 0.025 g IVP (dry weight) per feed [163]. Similarly, juvenile *Penaeus japonicus* (average body weight (abw) of 2–3 g) exhibited the highest survival rate of 92.8 % when administered a dose of 50 μg recombinant VP28 per day for 14 days, with protection decreasing after the vaccination period. In contrast, the adult larger shrimp (abw of 12–15 g) exhibited a lower survival rate, within a narrow range of 73.6 %–76.9 % at the same dosage, indicating a reduction in the vaccine effectiveness in comparison to the smaller shrimp, suggesting that immune response may vary with developmental

Table 7
Application of oral antiviral prophylaxis in invertebrates.

Type of vaccine	Disease/ Virus	Antigen/Vehicle or expression system or encapsulation	Specie/Weight	Dose	Administration protocol	Reference
Recombinant	WSD/ WSSV	VP19 and VP28/*E. coli* inactivated	*Penaeus monodon*/1 g	~10^8 bacteria/0,02 g x feed pellet	Feed coating: 7 consecutive days. 1 time/day	Witteveldt et al. [160]
Recombinant	WSD/ WSSV	VP28-VP24 fusion protein/*S. cerevisiae*	*Penaeus vannamei*/ 0.3 g	EBY100/pYD1-VP28-VP24 mixed with feed: 1:40 (μg/μg)	Feed coating: 7 consecutive days. 1 time/day	Lei et al. [168]
Recombinant	WSD/ WSSV	VP28/Baculovirus	*Penaeus monodon*/ 10–12 g	1 × 10^8 pfu/shrimp x g	Feed coating: 7 consecutive days. 1 time/day	S y Kwang et al. [173]
Recombinant	WSD/ WSSV	VP28/*Chlorella vulgaris*	*Litopenaeus vannamei*/4.5 g	5 mg of VP28-expressing *C. vulgaris*/meal	Feed coating: 7 consecutive days. 3 times/day	Kim et al. [174]
Recombinant	WSD/ WSSV	VP19 and VP28/inactivated *E. coli*	*Procambarus clarkii*/ 15 g	2 μg VP19 or VP28 protein/shrimp·g/day	Feed coating: 25 consecutive days. 1 time/day	Jha et al. [175]
Recombinant	WSD/ WSSV	VP28/Silkworm pupae	*Procambarus clarkii*/ 20–25 g	10, 50, 100 and 200 g silkworm pupae/feed kg	Feed coating: 28 consecutive days. 2 times/day	Wei and Yang [176].
Recombinant	WSD/ WSSV	VP26 and VP28/*E. coli*	*Marsupenaeus japonicus*/3.7 g	10 μg rVP26 or rVP28 g^{-1} of shrimp/day	Feed coating: 15 consecutive days. 1 time/day	Satoh et al. [177]
Recombinant	WSD/ WSSV	VP28/*Brevibacillus brevis*	*Penaeus japonicus*/ 2–3 g & 12–15 g	1, 10 and 50 μg rVP28/ day	Feed coating: 14 consecutive days. 1 time/day	Caipang et al. [170]
Recombinant	WSD/ WSSV	VP28/*B. subtilis*	*Fenneropenaeus chinensis*/25 g	10^{10} spores/0.01 g pellets	Feed coating: 7 consecutive days. 1 times/day	Fu et al. [169]
Recombinant	WSD/ WSSV	VP19 and VP28/Freund's Complete Adjuvant	*Litopenaeus vannamei*/6–8 g	0.1 mg VP19 or VP28/ shrimp/day	Feed coating: 14 consecutive days. 1 time/day	Choi et al. [178]
Recombinant	WSD/ WSSV	VP28/Liposome	*Marsupenaeus japonicus*/6–7 g	25 μg rVP28/day	Feed coating: Trial 1: 3 consecutive days. Trial 2: 7 consecutive days. 1 time/day	Mavichak et al. [179]
Recombinant	WSD/ WSSV	VP28/Chitosan tripolyphosphate	*Litopenaeus vannamei*/2–4 g	25 μg rVP28 g/feed	Feed coating: 20 consecutive days. 1 times/day	Taju et al. [165]
Recombinant	WTD/ MrNV	MrNV-CP-RNA-2 capsid protein (VLPs)/Sf-9 insect cells/cod liver oil	*Macrobrachium rosenbergii*/90 mg	10 μg MrNV/shrimp	Feed coating: 30 and 60 consecutive days. 3 times/day	Citarasu et al. [161]
Inactivated	WSD/ WSSV	WSSV formalin-inactivated/ commercial binder	*Penaeus monodon*/ 5–8 g	1.75 × 10^6 DNA copies/ day/shrimp	Feed coating: 7 consecutive days. 1 time/day	Sudheer et al. [180]
Inactivated	WSD/ WSSV	WSSV formalin-inactivated/cod liver oil	*Penaeus monodon*/ 10–15 g	50 μg vaccine/shrimp	Feed coating: 14 consecutive days. 1 time/day	Faisan and Amar [181].
Inactivated	WSD/ WSSV	WSSV (MCCV101) formalin-inactivated/commercial binder	*Fenneropenaeus indicus*/3–4 g	0.025, 0.05, 0.075 and 0.1 g IVP/g feed	Feed coating: 7 consecutive days. 1 times/day	Bright Singh et al. [163]
DNA	WTD/ MrNV	MrNV-CP-RNA-2 capsid protein/*E. coli* + cod liver oil (binder)	*Macrobrachium rosenbergii*/12 g)	1 mg DNA plasmid/g feed	Feed coating: 20 and 40 consecutive days. 3 times/day	Citarasu et al. [182]
DNA	WSD/ WSSV	VP28/*Salmonella typhimurium*	*Cambarus clarkii*/ 35–40 g	10^9 CFU/crayfish	Feed coating: Feed twice at 3-day intervals	Ning et al. [164]
DNA	WSD/ WSSV	VP28/Chitosan nanoparticle	*Penaeus monodon*/ 8–10 g	1 mg DNA plasmid/10 g feed	Feed coating: 7 consecutive days. twice/day	Rajeshkumar et al. [183]
dsRNA	YHD/ YHV	dsRab7 & dsYHV/*E. coli* HT115	*Litopenaeus vannamei*/250–300 mg	1.5 × 10^{10} CFU *E. coli*/ piece agar (2 pieces/day)	Feed coating: 9 consecutive days. 1 time/day	Sanitt et al. [167]
dsRNA	YHD/ YHV	Hairpin dsRNA-YHV/ *Chlamydomonas reinhardtii*	*Penaeus vannamei*/ 0.1 g	1 × 10^8 cells/g-feed	Feed coating: 5 consecutive days. 5 times/day	Somchai et al. [166]

Note: dpv: days post vaccination; dpi: days post infection; WSD: White Spot Disease; WSSV: White Spot Syndrome Virus; WTD: White Tail Disease; MrNV: *Macrobrachium rosenbergii* nodavirus; YHD: Yellow Head Disease; YHV: Yellow head virus; BW: Body weight.

stage [170]. The effectiveness of the vaccine could be optimized by adjusting the dosage or method of administration depending on the size and age of the shrimp population being treated. The method of administration also plays a pivotal role in vaccine efficacy. Two primary methods—feed coating and bath immersion—have been widely investigated. Bath immersion demonstrated higher short-term survival rates, while feed coating offers practicality for large-scale applications [162].

Although significant progress has been made in the development of crustacean vaccines, substantial challenges remain in optimizing oral vaccines for shrimp. Currently, research on oral vaccines against shrimp viruses remains predominantly at the experimental phase, with several critical areas requiring further refinement before translation into applicable solutions in the aquaculture industry. One of the key challenges in developing vaccines for shrimp is the unique nature of their immune system. Unlike fish and other vertebrates, crustaceans do not possess an adaptive immune system with antibodies or memory cells. Instead, they rely on their innate immune system, which is based on mechanisms such as phagocytosis, antimicrobial peptides, and the recognition of pathogen-associated molecular patterns (PAMPs). This innate immune response is generally rapid but lacks the ability to "recall" previous encounters with pathogens, which is a hallmark of adaptive immunity in vertebrates [171]. However, recent research has suggested that crustaceans may possess a form of innate immune memory, known as "immune priming", which allows shrimp to mount a stronger immune response after initial exposure. While distinct from adaptive immunity, this form of immune memory plays a crucial role in providing long-term protection and can be leveraged in vaccine development [172]. Given this difference in immune function, vaccine strategies for shrimp must focus on enhancing their innate immune responses, as they do not rely on adaptive immune mechanisms such as antibody production. The optimization of oral vaccines for shrimp must account for the unique characteristics of their immune systems, ensuring that vaccines stimulate strong and lasting innate immune responses. These include enhancing vaccine delivery systems to improve carrier efficacy, assessing long-term immune protection, and developing polyvalent formulations that target multiple pathogens. Continued research into vaccine design and administration strategies is essential to maximize their efficacy in commercial shrimp farming.

The correlates of protection (CoP) and the main results of functional assays from experimental studies in invertebrates are included in Supplementary Table 3.

1.8. Challenges and future perspectives

Oral vaccination is a practical and scalable strategy for disease prevention in aquaculture. However, several challenges must be addressed to fully explore its potential. One key limitation is the typical shorter duration of immunity compared to injection-based vaccines, necessitating the current reliance on booster doses to maintain protection. The development of more potent oral vaccines—particularly those incorporating advanced adjuvants and ligand-targeting technologies—may enhance antigen uptake and immune activation in mucosal tissues, reducing the need for frequent boosters. Furthermore, combining oral vaccination with other immunization strategies, such as primovaccination via injection followed by oral boosters, offers a promising integrated approach to prolong immunity while minimizing handling stress. Addressing mucosal tolerance, a major risk of oral vaccination due to prolonged or excessive exposure to antigens, remains critical. Advances in vaccine formulation technology, including encapsulation and targeted delivery systems, are expected to improve the stability and efficacy of oral vaccines. Together, these innovations point toward a future where oral vaccination becomes a cornerstone of sustainable, welfare-focused health management in the aquaculture industry.

Credit author statement

Aceituno P, Ordóñez-Grande B, García-Ordoñez M, Liang, X, Okeleye O, Ji J: literature review and original draft preparation. Rojas M and Roher N: Writing, Reviewing and Editing.

Funding

This work was supported by grants from the Spanish Ministry of Science, European commission and AGAUR funds to NR (PID2021-126710OB-C21 MINECO/FEDER and 2021-SGR-00068 AGAUR). MR and PA were funded by the National Agency for Research and Development (ANID)/Scholarship Program/Doctorado Becas Chile/2019/72200332/72200480.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2025.110962.

Data availability

No data was used for the research described in the article.

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