Viral Vaccines for Farmed Finfish

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Abstract

Fish viral diseases cause more damage to aquaculture due to its rapid spread causing acute mortalities and are not amenable to any treatment measures. Prophylaxis through vaccination is a reliable method for controlling viral diseases of fish. Several vaccines have been developed against many viral diseases of fish such as infectious pancreatic necrosis, Koi herpesvirus disease, infectious salmon anaemia, viral nervous necrosis, infectious haematopoietic necrosis, etc. Several of these vaccines are commercially available either as monovalent or multivalent vaccines along with other bacterial vaccines. Most of the vaccines available are inactivated injectable vaccines although recombinant and DNA vaccines are available for a few viral diseases. The commercially available fish viral vaccines are listed.

Keywords

Fish viral diseases · Vaccines · Inactivated · Recombinant · DNA vaccines · Immune response

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1 Introduction

World fish production, including both inland and marine sector, reached 179 million tonnes in the year 2018 out of which aquaculture has contributed 82 million tonnes, valued at USD 250 billion [1]. Of the 82 million tonnes of aquaculture produce, 54.279 million tonnes was contributed by finfish [1]. The new height scaled in aquaculture production in recent years is due to intensification of culture practices and diversification of cultured species. Aquaculture production has recently surpassed capture fisheries production. However, the sustainability of the growth of aquaculture is constrained by various diseases affecting farmed fish species. A trend has been observed in disease occurrence where a new disease emerges every 3–5 years causing major production losses. Such diseases are most often caused by viruses and to a lesser extent by a bacterium or a parasite [1]. The direct impact of such diseases is the loss of production due to mortality and reduced growth rate. It is estimated that the loss due to diseases exceeds 25% worldwide [2]. Among the diseases, viral diseases cause more damage as it spreads rapidly causing acute mortalities and are not amenable to any treatment measures. Viral disease management involves adoption of biosecurity protocols, good management practices, and early diagnosis to contain the spread of the disease. However, specific antiviral drugs are not readily available for most viral pathogens affecting fish. Nevertheless, as fish have a well-established adaptive immune system capable of mounting a long-term specific immune response, prophylaxis through vaccination is a reliable method for controlling viral infections.

2 History of Fish Vaccination

The first report of vaccination in fish is probably made by Snieszko et al. in 1938 (as cited by Van Muiswinkel [3]) where the authors reported protective immunity in carp against infectious dropsy caused by *Aeromonas punctata* when immunized with killed bacterin. Following this, Wilhelm Schaperclaus showed that intraperitoneal injection of fish with killed or attenuated *Pseudomonas punctata* (*Aeromonas hydrophila*) evoked protective immunity upon challenge. The first report of oral vaccination in fish is by Duff [4] in 1942 where the author demonstrated that administering fish with a chloroform-killed *Aeromonas salmonicida* through feed induced protection against furunculosis when challenged by injection or by cohabitation. Not much work was reported on fish vaccines during the next 2–3 decades, probably, because of the availability of antibiotics. Most of the earlier work on fish vaccines were for bacterial diseases of salmonids. The first vaccine for fish to be licenced was *Yersinia ruckeri* bacterins in 1976 to control enteric redmouth disease [5]. Infectious pancreatic necrosis virus is the first fish virus to be isolated in vitro using tissue culture [6]. However, the first commercially available viral vaccine was for spring viraemia of carp, an attenuated oil-adjuvant vaccine produced by a Czechoslovakian company (Bioveta) in 1982 [7]. Since then, many vaccines against bacterial and viral diseases have been reported to be successful under experimental

conditions. Many vaccines, mostly inactivated vaccines, have been licensed for use in Europe and North America as monovalent and polyvalent vaccines. The first commercially available DNA vaccine for finfish is for infectious haematopoietic necrosis virus (IHNV) marketed by Aqua Health Ltd., Novartis, licensed for use in Canada and USA [2].

3 Viral Diseases and Vaccines for Farmed Finfish

The common viral diseases of farmed finfish along with the hosts affected, causative agents, and their characteristics are given in Table 1. As vaccination is an effective method to control diseases of farmed finfish, a number of vaccines have been developed and are commercially available for the control of viral diseases of fish. The commercially available vaccines for some of the common viral diseases of fish are given in Table 2. Most of the vaccines available commercially are inactivated vaccines containing formalin- or heat-killed viruses, as they are safe to administer. Although live-attenuated vaccines have advantages such as induction of high protective immunity and low dose requirement, they are not commonly used due to the risk of reversion of virulence of the attenuated virus [25]. The level of protection offered by recombinant vaccines is often low and not reproducible [26]. Although many successful DNA vaccines against viral diseases are reported, only one DNA vaccine against infectious haematopoietic necrosis virus is available commercially because of the possible consideration of the DNA vaccinated fish as genetically modified organisms [25].

Vaccines are usually administered by intraperitoneal injections and, hence, they are used only for large-sized fishes as it is practically not possible to inject small fishes which are less than 10 g. As many viral diseases affect early stages of fish, alternate vaccination methods such as immersion and oral routes are increasingly being explored. Immersion vaccines are easy to administer especially for smaller fish in large batches at the time of stocking without causing much stress to the fish. However, administration of booster dose is a problem. Oral vaccines are easy to administer to fishes of all sizes and repeated booster doses can also be administered easily. The major concerns of oral vaccines are the stability of the antigen during the storage period and degradation of the antigen in the foregut of the fish. This problem can be overcome to a certain extent by micro- or nano-encapsulation of the antigen in particles such as chitosan, alginate, etc. [27]. However, more efforts are required to increase the efficacy of oral vaccines which is a promising method of vaccinating farmed finfish with least stress. Specialized adjuvants that help in the sustained release of antigen and improved presentation of the antigen to the antigen-presenting cells are also available for vaccine preparation. Multivalent vaccines targeting more than one pathogen are also available commercially to reduce the vaccination stress.

The efficacy of a vaccine can be assessed using various parameters. Assessment of the relative percent survival (RPS) using the formula [1 − (mortality in vaccinated fish/mortality in non-vaccinated fish)] × 100 is a direct method to assess the protection offered by the vaccine. To evaluate a vaccine based on RPS, it is

Table 1 Major viral disease of farmed finfish and the causative agents

Disease	Host species	Pathogen/ classification	Viral morphology	Viral genome	References
Diseases caused by DNA viruses					
Epizootic haematopoietic necrosis	Redfin perch and Rainbow trout	Epizootic haematopoietic necrosis virus Genus: *Ranavirus* Family: *Iridoviridae*	Large (130–170 nm) enveloped icosahedral virus	Linear dsDNA, 125–127 kbp	[8, 9]
Koi herpesvirus disease	Carps and carp varieties such as Koi carp and ghost carp	Cyprinid herpesvirus 3 Genus: *Cyprinivirus* Family: *Alloherpesviridae*	Icosahedral enveloped virus 100–110 nm	dsDNA, 295 kbp	[10]
Red seabream iridoviral disease	Red seabream and more than 30 other marine cultured species	Red seabream iridovirus Genus: *Megalocytivirus* Family: *Iridoviridae*	Non-enveloped, icosahedral, 120–200 nm in diameter	dsDNA, 112 kbp	[11]
Diseases caused by RNA viruses					
Viral nervous necrosis (Viral encephalopathy and retinopathy)	Asian seabass, European seabass, turbot, halibut, Japanese parrotfish, red-spotted grouper, and striped jack	Nervous Necrosis Virus Genus: *Betanodavirus* Family: *Nodaviridae*	Icosahedral, non-enveloped, 25 nm in diameter	Two positive-sense ssRNA molecules—RNA1 (3.1 kb) and RNA2 (1.4 kb)	[12]

Infectious haematopoietic necrosis	Salmons and trout	Infectious haematopoietic necrosis virus Genus: *Novirhabdovirus* Family: *Rhabdoviridae*	Enveloped, bullet-shaped 150–190 × 65–75 nm	Non-segmented, negative-sense, ssRNA, 11 kb	[13, 14]
Viral haemorrhagic septicaemia	Rainbow trout, turbot, Japanese flounder as well as a broad range of wild freshwater and marine species	Viral haemorrhagic septicaemia virus Genus: *Novirhabdovirus* Family: *Rhabdoviridae*	Bullet-shaped measuring about 180 nm long and 70 nm in diameter	11 kb negative ssRNA	[15]
Spring viraemia of carp	Common carp, grass carp, silver carp, bighead carp, crucian carp, goldfish, etc.	Spring viremia of carp virus Genus: *Vesiculovirus* Family: *Rhabdoviridae*	Bullet-shaped, 80–180 nm in length and 60–90 nm in diameter	Negative-sense, linear ssRNA of ~11 kb	[16]
Infectious pancreatic necrosis	Rainbow trout, brook trout, brown trout, Atlantic salmon	Infectious pancreatic necrosis virus Genus: *Aquabirnavirus* Family: *Birnaviridae*	Non-enveloped, icosahedral measuring about 70 nm in diameter	Bi-segmented, dsRNA virus, Segment A: ~3.0 kbp; segment B: 2.4 kbp	[17–19]

(continued)

Table 1 (continued)

Disease	Host species	Pathogen/ classification	Viral morphology	Viral genome	References
Infectious salmon anaemia	Atlantic salmon	Infectious salmon anaemia virus Genus: *Isavirus* Family: *Orthomyxoviridae*	Enveloped virus, 100–130 nm in diameter	Eight single-stranded, negative-sense RNA segments	[20]
Pancreas disease (PD) or sleeping disease (SD)	Atlantic salmon, common dab, rainbow trout, and Arctic charr	Salmonid alphavirus Genus: *Alphavirus* Family: *Togaviridae*	Enveloped, spherical, 60–70 nm in diameter	Single-stranded, positive-sense RNA virus, with a genome of ~12 kb	[21, 22]
Tilapia lake virus disease	Wild tilapia, farmed tilapia, commercial tilapia hybrids, and giant gourami	Tilapia lake virus (TiLV) Genus: *Tilapinevirus* Family: *Amnoonviridae*	Enveloped, 55–75 nm in size	Negative-sense, single-stranded RNA virus with ten segments, 10.323 kb in total length	[23, 24]

Table 2 Commercially available viral vaccines for finfish

Name of the vaccine	Type of vaccine	Delivery method	Disease/Pathogen targeted	Target Species	Produced by
AQUAVAC® IPN Oral	Recombinant	Oral	Infectious pancreatic necrosis (IPN) virus	Atlantic salmon	MSD Animal Health www.msd-animal-health-me.com/
NORVAX® Compact PD	Inactivated vaccine	Intraperitoneal injection	Salmonid Alphavirus (SAV)/Pancreas disease	Atlantic salmon	MSD Animal Health www.msd-animal-health-me.com/
ALPHA JECT micro® 1 PD	Inactivated	Intraperitoneal injection	Salmonid alpha virus (SAV)	Atlantic salmon	PHARMAQ AS, Norway www.pharmaq.no/
Clynav	DNA vaccine	Intramuscular injection	Salmonid Alphavirus 3 (SAV)/Pancreas disease	Atlantic salmon	Elanco GmbH www.ema.europa.eu/
NORVAX® Minova 6	Inactivated, multivalent vaccine	Intraperitoneal injection	Furunculosis, classical vibriosis, coldwater vibriosis, wound disease, and infectious pancreatic necrosis (IPN)	Atlantic salmon	MSD Animal Health www.msd-animal-health-me.com/
KV3 Vaccine	Attenuated virus vaccine	Immersion and Injection	KHV disease	Common carp and Koi carp	Phibro Animal Health Corpn.

(continued)

Table 2 (continued)

Name of the vaccine	Type of vaccine	Delivery method	Disease/Pathogen targeted	Target Species	Produced by
ALPHA JECT micro® 6	Inactivated, multivalent	Intraperitoneal injection	*A. salmonicida*, *Vibrio salmonicida*, *Listonella anguillarum*, *Moritella viscosa* and IPN	Atlantic salmon	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® 6-2	Oil adjuvant vaccine	Injection	Furunculosis, Vibriosis, Coldwater vibriosis, Winter sore, IPN	–	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT micro® 7 ILA	Oil adjuvant vaccine	Injection	Furunculosis, Vibriosis, Coldwater vibriosis, Winter sore, IPN, ISA	–	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® 2-2	Inactivated	Intraperitoneal injection	Furunculosis, IPN	Atlantic salmon	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT micro® 1 Noda	Inactivated	Intraperitoneal injection	Viral nervous necrosis	European seabass	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® micro 2	Oil adjuvant vaccine	Injection	IPN, Salmon Rickettsial Septicaemia (SRS)	–	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® micro 3	Oil adjuvant vaccine	Injection	Vibriosis, IPN, SRS	–	PHARMAQ AS, Norway www.pharmaq.no/

Icthiovac® VNN	Inactivated	Intraperitoneal injection	Viral nervous necrosis	European Sea bass	Hipra, Spain www.hipra.com/
ALPHA JECT® IPNV-Flavo 0,025	Oil adjuvant vaccine	Intraperitoneal	IPN, Flavobacteriosis	–	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® micro 1 ISA	Inactivated	Intraperitoneal injection	Infectious salmon anaemia	Atlantic salmon	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® 4-1	Oil adjuvant vaccine	Injection	Furunculosis, Vibriosis, IPN, SRS	–	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® 5-1	Oil adjuvant vaccine	Injection	Furunculosis, Vibriosis, IPN, ISA, SRS	–	PHARMAQ AS, Norway www.pharmaq.no/
ALPHA JECT® 1000	Oil adjuvant vaccine	Injection	IPN	–	PHARMAQ AS, Norway www.pharmaq.no/
BLUEGUARD® IPN + SRS + As + Vo + ISA INJECTABLE	Multivalent	Injectable	*Piscirickettsia salmonis*, *Vibrio ordalii*, *A. salmonicida*, *IPNV*, *ISAV*	Atlantic salmon	Centrovet www.centrovet.virbac.com/
Apex-IHN®	DNA vaccine	Intramuscular injection	*Infectious haematopoietic necrosis*	Salmonids	Aqua health Ltd (Novartis) [2]
AQUAVAC® IridoV	Oil-adjuvant vaccine	Intraperitoneal injection	*Iridovirus*	Tilapia and Asian sea bass	MSD Animal Health (Intervet) www.aquavac-vaccines.com/

important to select a challenge model that best reflects the natural route of infection [28]. In case of non-lethal viruses, the ability of the vaccine to protect fish from histological changes following challenge with virulent virus can also be used to assess the efficacy of a vaccine. Measurement of specific antibodies in serum/mucous by ELISA is another direct method of assessing the efficacy of a vaccine, provided, anti-fish antibody is available. Serum neutralization test or competitive ELISA can also be used to quantify the antibodies if anti-fish antibodies are not available.

3.1 Epizootic Haematopoietic Necrosis

Epizootic haematopoietic necrosis (EHN), an OIE-notifiable disease, is a systemic disease characterized by extensive visceral tissue damage leading to mortality [29]. The disease is caused by epizootic haematopoietic necrosis virus (EHNV) belonging to the genus *Ranavirus* within the sub-family *Alphairidovirinae* and family *Iridoviridae* [30]. ENHV is an enveloped icosahedral virus assembled in the cytoplasm and released by budding, measuring 130–170 nm, and has up to 36 major polypeptides [8]. Its genome comprises of dsDNA of size 125–127 kbp [9]. Fish species susceptible to this virus are redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*). Experimentally, the virus can infect many Australian native species including Murray cod (*Maccullochella peelii*), Macquarie perch (*Macquaria australasica*), and Murray River rainbowfish (*Melanotaenia fluviatilis*) [31]. The virus is shed from infected tissue and disintegrating carcass [15]. The disease affects all age groups, although the clinical signs are more severe in juveniles and fingerlings. The target organs of the virus are kidney, spleen, and liver. Isolation of the virus in cell culture and antibody-capture ELISA are used for targeted surveillance [15]. EHN is endemic to Australia but similar viruses like European catfish virus (ECV), European sheatfish virus (ESV) and Santee-Cooper ranavirus have been reported in other countries associated with diseases of fish and frogs [32]. The clinical signs of the disease include distended abdomen, petechial haemorrhage at the base of fin and gills, and multifocal necrosis of haematopoietic tissue of kidney and spleen with enlargement of kidney and spleen. No report of vaccine against EHN is available and no commercial vaccines are available for this disease.

3.2 Koi Herpesvirus Disease

Koi herpesvirus disease (KHVD) is a highly contagious disease affecting common carp and its varieties like koi carp and ghost carp causing significant mortality [33]. It is an OIE-notifiable disease. Koi herpes virus disease is caused by koi herpesvirus (KHV) also called as cyprinid herpes virus-3 (CyHV-3) which belongs to the family *Alloherpesviridae* and the genus *Cyprinivirus*. CyHV-3 is the type species of the genus *Cyprinivirus* which also contains the species CyHV-1 and CyHV-2. It is an

enveloped virus measuring 100–110 nm with a dsDNA genome of size 295 kbp [10]. Surviving fish become persistent carriers for long time [34] and the virus is shed through faeces, urine, gills, and gill mucus [15]. The virus is transmitted usually horizontally through water and vectors, although vertical transmission cannot be ruled out. Gills and skin are the major portals of entry of the virus [35, 36]. The disease has a 100% morbidity rate and mortality ranges from 70% to 80% [33, 37].

Control of the disease is by following strict biosecurity measures and introduction of disease-free fish into the culture system. Many researchers have reported the protective effect of recombinant and DNA vaccines expressing different genes encoding the antigenic proteins of the pathogen. Boutier et al. [38], described the development of a recombinant attenuated vaccine against KHV for mass vaccination of carps through immersion route which induced protective mucosal immune response at portals of pathogen entry. A DNA vaccine expressing the glycoprotein gene offered better protection to common carp (*Cyprinus carpio*) [39, 40]. A DNA construct expressing ORF149 of KHV coupled to single-walled carbon nanotubes (SWCNTs) when injected intramuscularly resulted in better immune response, higher RPS, and longer protection [41]. However, oral administration or single intramuscular injection of DNA vaccine expressing ORF25 did not confer protection against KHV when challenged by immersion or cohabitation, although multiple doses induced strong protection [28]. Constructs expressing multiple antigenic proteins such as ORF25, ORF148, ORF149, ORF81, ORF72, and ORF92 could trigger a protective immune response [28]. A siRNA targeting DNA polymerase gene of KHV could inhibit the in vitro viral replication in common carp brain cells. However, its potential to inhibit viral replication in vivo needs to be established [42]. A live-attenuated vaccine has been licenced for emergency use in Israel [15].

3.3 Red Seabream Iridoviral Disease

Red seabream iridoviral disease (RSIVD) is an OIE notifiable disease of farmed red seabream, *Pagrus major*, and several other farmed marine fishes [43] causing up to 100% mortality. The disease was first reported in Japan in 1990 [44] and since then, the disease has spread to many East and South-East Asian countries viz. China, Hong Kong, Korea, Malaysia, Philippines, Singapore, Thailand [15], and India [45]. The disease causes mortality ranging between 0% and 100% depending on the age, size, species of the affected fish, water temperature, and other culture conditions. The disease outbreaks are mostly observed in summer when the water temperature is above 25 °C [15]. The disease is caused by red seabream iridovirus (RSIV) belonging to the genus *Megalocytivirus* under the family *Iridoviridae*. The virus is icosahedral measuring 120–200 nm in diameter. The genome is made up of dsDNA of about 112 kbp and is highly methylated [11]. Infectious spleen and kidney necrosis virus (ISKNV), turbot reddish body iridovirus (TRBIV), and rockbream iridovirus (RBIV) are closely related viruses having high sequence similarity with RSIV. Further investigation needs to be carried out to decide whether these viruses should be included under RSIV. The clinical signs include lethargy, anaemia,

petechiae on the gills, and enlargement of the spleen [44, 46]. The target organs of the virus are gills, intestine, kidney, spleen, and heart. The disease spreads horizontally through water.

A formalin-inactivated vaccine is commercially available in Japan for RSIVD for red seabream (*P. major*), striped jack (*Pseudocaranx dentex*), Malabar grouper (*Epinephelus malabaricus*), and orange-spotted grouper (*Epinephelus coioides*) [47, 48]. This is the first viral vaccine for marine fish [49]. The vaccine when administered intraperitoneally to juvenile red seabream, resulted in higher survival upon challenge [47] and the antigen could not be detected in the spleen of the vaccinated group [48]. This vaccine did not offer protection to the genus *Oplegnathus*, as it is highly susceptible to RSIV [49]. However, another formalin-inactivated vaccine could offer significant protection to yellowtail (*Seriola quinqueradiata*), amberjack (*Seriola dumerili*), kelp grouper (*Epinephlelus moara*), striped jack (*P. dentex*), and spotted parrotfish (*Oplegnathus punctatus*) [50]. A recombinant vaccine consisting of formalin-killed *Escherichia coli* expressing the 351R capsid protein of RSIV expressed in fusion with GAPDH of *Edwardsiella tarda*, when injected intraperitoneally resulted in significantly higher survival on challenge [51]. A DNA vaccine consisting of plasmids encoding the major capsid protein (MCP) and a transmembrane domain against RSIV could provide better protection to red seabream than the unvaccinated control fish [52]. Small interfering RNAs (siRNAs) targeting the MCP gene of RSIV could inhibit the replication of RSIV in vitro, in a cell culture system, indicating the potential of siRNA in protecting cultured fish against RSIVD [53]. A commercial oil-adjuvanted vaccine is available for tilapia and Asian seabass for intraperitoneal injection.

3.4 Viral Nervous Necrosis

Viral nervous necrosis (VNN), also called as viral encephalopathy and retinopathy (VER), is an acute viral disease affecting more than 120 species of marine, brackish water, and freshwater fishes belonging to 30 families [54]. The disease causes up to 100% mortality in larval and early juvenile stages. Adult fish when infected are asymptomatic, but become carrier of the virus. Affected fish exhibit dark or pale colouration with abnormal swimming such as circling, darting, belly up, and whirling [54]. The disease is caused by nervous necrosis virus (NNV) belonging to the genus *Betanodavirus* under the family *Nodaviridae*. NNV is a non-enveloped virus of size 25 nm with a bi-segmented single-stranded positive-sense genome [12]. The RNA-1 segment is about 3.1 kb long coding for the RNA-dependant RNA polymerase of size 110 kDa while the RNA-2 segment is about 1.4 kb long coding for the single capsid protein of the virus of size 42 kDa [12, 55]. The betanodaviruses are classified into four genotypes based on the phylogenetic analysis of T4 variable region of RNA-2: tiger puffer nervous necrosis virus (TPNNV), striped jack nervous necrosis virus (SJNNV), barfin flounder nervous necrosis virus (BFNNV), and red-spotted grouper nervous necrosis virus (RGNNV) [55]. The virus

is classified into three serotypes based on serum neutralization test and optimal growth temperature [56]. The virus is transmitted horizontally through infected fish and water and vertically through egg and milt [57, 58]. The practical way to control the disease is to use disease-free brooders and vaccinate them with a potent vaccine against NNV [59–61].

The first report of vaccine against NNV was that of Húsgag et al. [62], where a recombinant vaccine against SJNNV produced specific immune response and protection in turbot, *Scophthalmus maximus* and Atlantic halibut, *Hippoglossus hippoglossus*. Since then, several reports of experimental trials with different forms of vaccine have been reported. Inactivated vaccine administered through intraperitoneal injection gives the best immune response in terms of specific serum antibody titre [59, 63]. Among the inactivating agents, formalin inactivation results in higher antibody production compared to β-propiolactone and heat treatment [63], while UV inactivation could only provide partial protection [64]. Vaccination of broodstock with inactivated vaccine results in the transfer of maternal antibodies to the eggs and larvae and prevents vertical transmission of the virus [60]. Pakingking Jr. [61] demonstrated that annual vaccination of Asian seabass with an inactivated NNV vaccine is the practical way to prevent the vertical transmission of NNV to offspring. The immune response elicited by the inactivated vaccine is directly proportional to the antigen dose administered. However, antigen dose greater than 200 μg had a negative effect on serum antibody titre [65].

Recombinant capsid protein expressed in prokaryotes could produce neutralizing antibodies and offer protection to fish challenged with NNV [65–69]. To overcome the drawbacks of injection vaccine, recombinant protein can be administered orally [70]. The recombinant protein can also be expressed in plants for immunizing fish [71]. Live NNV can be used as vaccine when administered intramuscularly to fish and maintained at natural seawater temperature (17 °C) [72, 73]. However, use of live virus has the risk of infection when the water temperature rises to optimal level for virus replication.

A DNA vaccine expressing the viral capsid protein when injected intramuscularly to Asian seabass juveniles was reported to produce 77.33% RPS, after intramuscular challenge with betanodavirus [74]. No cross-protection between SJNNV and RGNNV has been observed [75], as both the genotypes belong to different serotypes. Two inactivated vaccines against VNN are commercially available: ALPHA JECT micro® 1 Noda marketed by PHARMAQ AS, Norway and Icthiovac® VNN marketed by Hipra, Spain. Both are recommended for European seabass for intraperitoneal administration.

3.5 Infectious Haematopoietic Necrosis

Infectious haematopoietic necrosis (IHN) is a viral disease of salmonids, such as salmon and trout, caused by infectious haematopoietic necrosis virus (IHNV), a negative-sense single-stranded, RNA virus. The disease is an OIE-notifiable disease. IHNV belongs to the genus *Novirhabdovirus* under the family *Rhabdoviridae*. It is a

bullet-shaped virus measuring 150–190 nm in length and 65–75 nm in width [13]. The genome consists of a non-segmented, negative-sense, single-stranded RNA genome of size ~11 kb encoding six proteins: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L) [14]. Kidney, spleen, and other internal organs are the target organs of the virus and the virus is excreted through urine, ovarian fluids, and mucus. The virus enters through the gills and the bases of fins [76]. The disease is more prevalent in fry stages and, adult fish are relatively resistant. The disease spread horizontally through infected fish and water, while vertical transmission through eggs has also been reported [77]. The disease is prevalent in the northern hemisphere in North America, Europe, and Asia [15]. Cumulative mortality may reach up to 95% in acute cases and varies with species, temperature, culture conditions, and virus strain [76]. The control measures include adoption of strict biosecurity measures and disinfection of fertilized eggs.

An inactivated autogenous vaccine and a DNA vaccine have been licensed for commercial use in North America [15]. The DNA vaccine against IHNV is commercially available in Canada and USA and no outbreak of IHNV has been reported in the vaccinated fish [78]. Many other researchers have patented their inventions on DNA vaccines for IHNV. Vaccination through injection results in stress to the fish and, hence, other routes of administration have been explored. Ballesteros et al. [79], demonstrated the dose-dependent increase in immune response and protection offered by the orally administered DNA vaccine expressing the glycoprotein (G) of IHNV, encapsulated into alginate microspheres. Fish can also be vaccinated through nasal route where the nasal mucosal immune system is stimulated. The use of the nasal route for vaccination in rainbow trout, where a live-attenuated IHNV vaccine was administered through nares, has been demonstrated to be safe [80–82].

3.6 Viral Haemorrhagic Septicaemia

Viral haemorrhagic septicaemia (VHS) is an OIE-notifiable viral disease of farmed rainbow trout in Europe [83]. In addition to rainbow trout, a large number of marine and freshwater fish are susceptible to the disease. The disease has been reported in about 80 species of fish in the Northern hemisphere, including North America, Europe, and Asia. VHS is caused by VHS virus (VHSV), an enveloped bullet-shaped virus measuring about 70 × 180 nm belonging to the genus *Novirhabdovirus*, within the family *Rhabdoviridae*. The genome is a negative-sense, single-stranded RNA of size approximately 11 kb [15]. The genome (3′ N-P-M-G-NV-L-5′) encodes six proteins: a nucleoprotein, N; a phosphoprotein, P; a matrix protein, M; a glycoprotein, G; a non-virion protein, NV, and a polymerase, L [84]. The neutralizing antibodies are targeted against the G protein and, hence, G protein is often the target for developing sub-unit or DNA vaccines [25]. The VHSV isolates have been grouped into four genotypes based on the genomic sequences. VHSV causes disease and mortality in all life stages. However, the disease is more common in young fish not previously infected [15]. The target organs of the virus are

kidney, heart, and spleen. In chronic cases, high virus titre is observed in brain [85]. Fish surviving the infection becomes the carrier of the virus. Virus is shed through urine and reproductive fluids, although there is no evidence of vertical transmission. Transmission is horizontal through contact with contaminated fish or water. Mortality is low at lower temperatures but the cumulative mortality is high. Although disease outbreaks occur during all seasons, it is more common during spring and when the water temperature is fluctuating [15]. Young rainbow trout fry is more susceptible with mortality reaching close to 100%. Control of the disease is by periodic surveillance and stamping out carrier fishes.

Currently, no commercial vaccine is available for VHS. A thermoresistant live-attenuated VHSV strain-induced high levels of protection [86]. However, the vaccine is not commercially available, probably, because the vaccine did not perform well under field conditions [25]. Another live-attenuated viral vaccine administered orally along with polyethylene glycol resulted in significant protection against VHSV [87]. Attempts were made to produce recombinant/sub-unit vaccine by expressing the G protein in *E. coli* [88], or *A. salmonicida* [89]. However, these recombinant/sub-unit vaccines did not offer protection due to the lack of post-translational modifications in the recombinant proteins produced in the prokaryotes. There are many reports of development of successful DNA vaccines against VHSV [90–94]. However, the efficacy of these vaccines was tested against homologous virus. Prevalence of many genotypes and the limited protection offered against heterologous strains are the disadvantages for commercial use of these vaccines. Further, DNA vaccines are not being approved in Europe, as the DNA-vaccinated fish may be considered as genetically modified organism [25]. An inactivated VHSV vaccine conjugated with poly lactic-co-glycolic acid (PLGA) nanoparticles, when administered through immersion route followed by a booster dose by oral route gave 73.3% RPS [95].

3.7 Spring Viraemia of Carp

Spring viraemia of carp (SVC) is an acute viral disease of carps and some other cyprinid and ictalurid species causing haemorrhagic and contagious viraemia [15]. It causes significant mortality during spring [16]. This disease is OIE-notifiable and is caused by SVC virus (SVCV), a member of the genus *Vesiculovirus* under the family *Rhabdoviridae* in the order *Mononegavirales*. The virus is enveloped and bullet-shaped, measuring approximately 80–180 nm in length and 60–90 nm in diameter. The genome of the virus is non-segmented, single-stranded negative-sense RNA consisting of 11,019 nucleotides encoding five proteins: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), and an RNA-dependent RNA polymerase (L). The non-virion (NV) gene found in between the G and L genes in *Novirhabdovirus* genus of *Rhabdoviridae* family is missing in this virus [16]. Based on the partial G gene sequence, SVCV isolates are classified into four genotypes, 1a, 1b, 1c, and 1d. Isolates from North America and Asia belong to genotype 1a, while isolates from Europe belong to genotypes 1b, 1c, and 1d [96].

Carps less than 1 year are more susceptible to the virus, although all age groups can be affected with mortality ranging from 1% to 40% [15]. Liver and kidney are the target organs of the virus, while lower virus titre is observed in spleen, gills, and brain [97]. Virus is transmitted horizontally, although vertical transmission through eggs has not been ruled out. Disease outbreaks usually happen in spring in temperate countries when the temperature ranges between 11 and 17 °C, and mortalities are less at temperatures below 10 °C and above 22 °C [98]. Control measures to prevent disease outbreaks include adopting strict biosecurity measures and reducing stocking density during winter and early spring. In farms with environmental control, raising water temperature above 19 °C will prevent disease outbreaks [15].

Very few published reports are available on vaccination against SVCV. An inactivated oil-adjuvanted vaccine comprising two strains of SVCV was commercialized by a Czechoslovakian company (Bioveta) in 1982 [7]. This has been the first viral vaccine for fish; however, the vaccine is currently unavailable. The DNA vaccines for SVCV are not as efficacious as that of *Novirabdovirus* DNA vaccines [25]. A DNA vaccine expressing the full-length glycoprotein (G) gene of SVCV could produce 48% RPS on challenge with heterologous strain of SVCV in common carp [99], while 50–88% RPS was recorded in koi carp [100]. Embregts et al. [101], reported up to 100% protection in European common carp (*C. carpio carpio*) vaccinated with experimental DNA vaccine against SVCV by intramuscular route. However, the same vaccine when administered orally by alginate encapsulation method did not offer any protection [102]. Recombinant sub-unit vaccines conjugated to single-walled carbon nanotubes could induce specific and non-specific immune parameters and protect common carp vaccinated through immersion route suggesting the potential of single-walled carbon nanotubes for use in immersion vaccines [103]. No commercial vaccines are currently available for SVCV.

3.8 Infectious Pancreatic Necrosis

Infectious pancreatic necrosis (IPN) is a viral disease of salmonids affecting young fry of rainbow trout (*Salmo gairdnery*) and post-smolt of Atlantic salmon (*Salmo salar*) resulting in close to 100% mortality [104]. The disease is characterized by anorexia and abnormal corkscrew and erratic swimming [104]. The disease is caused by IPN virus (IPNV), a non-enveloped, icosahedral bi-segmented dsRNA virus [17] measuring around 70 nm [18]. The virus is the type species of the genus *Aquabirnavirus* under the family *Birnaviridae*. IPNV is the first fish virus to be isolated in vitro using tissue culture [6]. This is one of the most widely prevalent viruses infecting most of the farmed fishes causing high mortality in juvenile salmon when they are transferred from freshwater to seawater [25]. The genome consists of two segments—A and B. Segment A is of size 2962–3097 bp and segment B is around 2400 bp [19]. RNA-A codes for a polyprotein and a non-structural protein, VP5. The polyprotein is cleaved to generate VP2 (capsid protein), VP3, and VP4. RNA-B codes for VP1 which is the RNA-dependent RNA polymerase [105]. Based

on the sequence of VP2, IPNV has been classified into six genogroups comprising of ten serotypes [106]. Fish which recover from the infection become life-long asymptomatic carriers [107]. The virus is transmitted horizontally through water and vertically through eggs. The virus enters through gills, intestinal epithelium, and skin [19].

Several attempts have been made to develop vaccines against IPNV. However, attempts to protect fish at early stages against IPNV were not successful because the fish were not immunocompetent when they were the most susceptible to IPNV [25]. Inactivated whole-virus vaccine could protect Atlantic salmon better than sub-unit or DNA vaccine [108]. Recombinant vaccines containing capsid protein (VP2) of IPNV either alone or as a fusion protein with VP3 when injected to rainbow trout elicited increased levels of specific IgM and reduced viral load in challenged fish [109, 110]. Immature viral particles (provirus) of IPNV triggered neutralizing antibodies when injected to rainbow trout fry [111]. Virus-like particles of IPNV have also been demonstrated to be an excellent candidate for protecting fish against IPNV when administered intraperitoneally [112]. However, as administration of vaccine by injection is stressful and is not feasible for small fry, many oral vaccines have been developed which could protect salmonids from IPNV. A live-vector vaccine containing the coding region of VP2 of IPNV when administered by immersion route resulted in 88.24% RPS [113]. Live-recombinant *Lactobacillus casei* expressing the VP2 when administered orally to rainbow trout resulted in high neutralizing antibodies and low viral load after challenge [114, 115]. Many researchers have developed DNA vaccines containing plasmid constructs expressing VP2 of IPNV for administration through injection or orally through feed after conjugating with alginate microspheres or chitosan and tripolyphosphate nanoparticles [116–122]. The efficacy of the DNA vaccines was dose-dependent [121] eliciting specific IgM response, low viral load on challenge, and upregulation of various immune-related genes. Recombinant live virus has also been reported to protect rainbow trout from IPNV. A recombinant vaccine with IHNV as the backbone vector expressing VP2 of IPNV provided better protection and survival when challenged with IPNV and IHNV demonstrating the potential of recombinant viruses to protect fish against two or more viruses [123]. Many commercial vaccines, either as monovalent vaccine or as multivalent vaccine along with other inactivated bacterial and viral pathogens, are available against IPN for salmonids (Table 2).

3.9 Infectious Salmon Anaemia

Infectious salmon anaemia (ISA) is a systemic viral disease of salmonids, affecting mainly Atlantic salmon (*S. salar*), characterized by severe anaemia. It is an OIE-listed disease. The disease is caused by ISA virus (ISAV), the type species of the genus *Isavirus* under the family *Orthomyxoviridae*. Two variants of the virus exist, the highly polymorphic region (HPR)-deleted pathogenic ISAV, and the non-pathogenic HPR0 (non-deleted HPR) ISAV. HPR deleted ISAV is associated with clinical disease, while the HPR0 ISAV is not associated with clinical disease in

Atlantic Salmon [124]. ISAV is an enveloped virus measuring 100–130 nm in diameter and the genome consists of eight single-stranded, negative-sense RNA segments [20] coding for at least ten proteins [125] out of which four are major structural proteins. The haemaglutinin-esterase (HE) protein is one of the most variable proteins and is responsible for the receptor-binding activity and generates neutralizing antibodies. Hence, HE protein is commonly used for developing sub-unit or DNA vaccines [25, 126, 127]. The major route of infection is through gills and to a lesser extent through skin and intestine. The primary target for the virus is endothelial cells lining the blood vessels of gills, heart, liver, kidney, and spleen [128]. Diseased fish exhibit severe anaemia, haemorrhage, and necrosis in several organs [15]. The virus is excreted though urine, faeces, and mucus. The disease affects all life-stages and outbreaks are common in seawater cages. The disease has been reported in Norway, Canada, the United Kingdom, the Faroe Islands, the USA, and Chile [125]. Morbidity and mortality are typically low and the daily mortality ranges from 0.5% to 1%. The cumulative mortality also remains low, although in severe outbreaks mortality may exceed 90% over several months [15]. Control of ISA is through early diagnosis and culling all fish in the affected cages. Disinfection of eggs is an important control measure.

Many vaccines against ISAV have been licensed for use in Norway, Chile, Ireland, Finland, and Canada [25]. Many of them are inactivated, injectable vaccines either monovalent or multivalent with the addition of inactivated pathogens, usually pathogenic bacteria. An oral vaccine against ISA containing a recombinant hemagglutinin-esterase and fusion protein as antigens induced high level of specific IgM antibodies and protection in Atlantic salmon [129]. Inactivated vaccines, when injected along with adjuvants, elicited higher immune response and protection in a dose-dependent manner [130]. Subsequent oral administration of one or more booster doses of vaccines is beneficial and results in high level of specific serum IgM [131]. Several oral immunizations are required in the field to maintain high antibody levels and protection. A salmonid alphavirus-based replicon vaccine expressing HE of ISAV protected Atlantic salmon against the viral challenge [132, 133].

3.10 Pancreas Disease or Sleeping Disease

Pancreas disease (PD) or sleeping disease (SD) is an OIE-listed disease caused by infection with salmonid alphavirus (SAV) in Atlantic salmon, rainbow trout, common dab [21], and Arctic charr [22]. The disease is a systemic viral disease characterized by necrosis of exocrine pancreatic tissue, cardiomyocytic necrosis, and white skeletal muscle degeneration [15]. SAV is an enveloped spherical virus measuring 60–70 nm in diameter belonging to the genus *Alphavirus* and family *Togaviridae*. The genome consists of a positive-sense, single-stranded RNA of approximately 12 kb. The genome codes for eight proteins, four of which are non-structural proteins (nsP1–nsP4), and the remaining four are capsid glycoproteins (E1, E2, E3 and 6K). Glycoprotein E2 induces neutralizing antibodies [21] and,

hence, is an ideal candidate for developing sub-unit or DNA vaccines. Based on the sequences of protein E2 and nsP3, SAV isolates have been divided into six genotypes, SAV1–SAV6 [134]. Gills and intestinal track are the portals of entry. Although the predilection sites of the virus are not known, in acute cases, high titre of the virus is found in kidney, heart, blood, and several other organs. Virus is shed through mucus and faeces. Disease outbreaks are influenced by environmental factors, and increase in water temperature favours the virus [135]. The disease affects all life-stages in both freshwater and seawater [136]. The virus persists in the infected fish for several months and is transmitted horizontally through water [137].

A BEI inactivated SAV-1 vaccine administered to Atlantic salmon provided protection against cohabitant challenge with the wild-type virus [138]. Xu et al. [139], compared the efficacy of inactivated whole-virus vaccine with sub-unit vaccine and DNA vaccine expressing E1 and E2 spike proteins of salmonid alphavirus sub-type 3 (SAV-3) and concluded that the immunogenicity of inactivated whole virus was superior than sub-unit and DNA vaccines. Karlsen et al. [140], also demonstrated the near-total protection of Atlantic salmon immunized with inactivated SAV3 genotype. Two inactivated vaccines are commercially available for Atlantic salmon.

3.11 Tilapia Lake Virus Disease

Tilapia lake virus (TiLV) disease is a new and emerging disease, first observed in Israel in 2009 in tilapia [141]. Since then, the disease has spread to many countries in Asia, Africa, and America [142]. The disease is caused by TiLV, classified under the genus *Tilapinevirus*, family *Amnoonviridae*, and order *Articulavirales* [143]. TiLV is enveloped with icosahedral symmetry measuring 55–75 nm [141]. The genome consists of ten segments of linear, negative-sense, single-strand RNA of about 10.323 kb total length [23, 141]. The disease is characterized by high mortality during hot summer months with more than 80% mortality. The clinical signs include black discoloration, skin abrasions, and ocular degeneration. Histological changes observed include congestion of the internal organs such as kidney and brain with foci of gliosis and perivascular cuffing of lymphocytes in the brain cortex and ocular inflammation [141]. The disease is restricted to tilapines including wild, farmed, and hybrid tilapia and giant gourami [24, 141]. Other fishes co-habitated with infected tilapia or experimentally injected with TiLV did not exhibit clinical signs [24, 144]. No reports on vaccine development or commercial vaccines for TiLV are available.

4 Immune Response to Vaccines

The innate immune molecules such as interferon are induced rapidly in response to vaccination. Subsequently, the adaptive immune system of fish comes into play when the fish encounters a pathogen or after immunization. The B lymphocytes,

upon antigen presentation, differentiate into plasma cells and secrete IgM which are found in the serum and mucus of gills, skin, and intestine. IgM is the major immunoglobulin of fish. The specific serum IgM level is maximum following intraperitoneal vaccination compared to other routes of vaccination. IgM is also secreted into the mucus when immunized by immersion or by oral routes. In addition to mucosal IgM, systemic IgM is also produced, although at a lesser magnitude, upon immersion and oral vaccination. IgT, an intestinal immunoglobulin, equivalent to IgA of mammals but, phylogenetically distant from IgA, is secreted in the intestine upon exposure to antigens [145]. IgD is also a mucosal immunoglobulin, the transcripts of which are upregulated many folds in the gills upon immersion-vaccination in fish [146] suggesting that this immunoglobulin might play an important role in the mucosal immunity. Several factors are known to affect the modulation of fish immune response. In general, the adaptive immune response increases with age [147] and temperature [148] and decreases with chronic stress [149].

5 Concluding Remarks and Future Outlook

Vaccination of finfish has a clear advantage of reducing the impact and loss due to diseases, reducing the use of chemotherapeutants, besides providing long-term protection. Hence, vaccination of fish is an important tool in fish health management. At present, most of the vaccines available commercially are for salmonids which account for 6.2% of the total aquaculture production. However, cyprinids contribute about 62.7% to the total production and, this shows the huge potential for the development of vaccines for carps and other cyprinids. Even if vaccination can marginally improve the survivability of fish, it can offset the vaccine and vaccination costs. Optimization of vaccine dose for protection of fish, development of anti-fish antibody for seromonitoring of vaccinated fish, establishment of protective antibody titre required to withstand a natural infection, and improved vaccine delivery methods for mass vaccination are the areas which require increased attention in the field of fish vaccinology.

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