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REVIEW PAPER

Viral vaccines for farmed finfish

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Abstract Over the past decade, aquaculture has grown at an average annual growth rate of approximately 6 % worldwide despite many challenges. Viral diseases are one of the major challenges that are threatening a sustainable growth of finfish farming globally. Vaccination of farmed fish plays an important role in commercial fish farming to mitigate viral diseases. In this review, we summarized the major viral diseases that have caused serious economic losses, and emerging diseases that pose a potential threat to aquaculture. The current status of viral vaccines in farmed fish are discussed, particularly the different types of vaccines that were licensed in recent years and are now commercially available, and the routes of delivery of those vaccines including the merits and demerits of each of these delivery method. Furthermore, the article provides an overview of different experimental vaccines that have been reported in the literatures in recent years besides highlighting the future need for developing cost-effective, oral vaccines that can be easily applicable at farm level.

Keywords Finfish vaccine · Injection vaccine · Immersion vaccine · Oral vaccine · Virus-like particles · Subviral particles

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Introduction

Aquaculture is a growing and major global industry that contributes significantly to the gross national product of countries with large coastlines, especially of those countries in Asia and South America. Worldwide farm-raised food fish production reached almost 63 million tonnes with an estimated value of US$130 billion in 2011 (www.fao.org/fishery/topic/16140/en). Over the past 10 years, the contribution of capture fishery to global fish production has declined from 70.2 % in 2001 to 59.9 % in 2011. Simultaneously, aquaculture has steadily increased its contribution from 27.6 to 40.1 % at an average annual growth rate of approximately 6 % (www.fao.org/fishery/topic/16140/en). This unprecedented growth of global aquaculture has not been free of challenges and aquaculture will continue to face challenges as its expansion continues. In fact, a multitude of challenges, such as disease outbreaks and the rising cost of feed, are now threatening the sustainable growth of aquaculture.

The major causative agents of infectious diseases in finfish aquaculture include bacteria (54.9 %), viruses (22.6 %), parasites (19.4 %) and fungi (3.1 %) [70]. Although bacterial diseases are the most prevalent disease challenge in fish farming, viral diseases are more difficult to control due to the lack of anti-viral therapeutics, high susceptibility of fish during the early stages of life cycle, challenges in developing viral vaccines that are effective early in the life cycle, and paucity of information on the mechanisms of viral pathogenesis and natural resistance in wild populations of fish. As a result, periodic outbreaks of viral diseases have resulted in catastrophic losses to fish farmers around the globe and threaten the long-term sustainability of the industry [54, 109]. The emergence of disease outbreaks have been exacerbated by more intensive

aquaculture methods and operational techniques as fish farming has moved from subsistence to industrial-scale. Movement of live fish and fish eggs across countries and continents, introduction of new or improved species, and monoculture of fish at a very high density are some of the key factors that contribute to disease outbreaks [55, 77, 109, 114].

An example of the importance of viral disease to finfish aquaculture can be seen in the Chilean salmon industry. Infectious salmon anemia (ISA) disease was first detected in Chile in 1999 [57]. Between 1987 and 2004, the salmon industry in Chile underwent a rapid expansion. By 2004 production started to decline as increasing number of mortalities were recorded. During the winter of 2007, ISA epizootics occurred in grow-out farms in central Chile [19, 67]. In 2010, the Chilean salmon industry was faced with another outbreak of ISA. This epizootic took production levels from 670,000 ton in 2008 to about 100,000 ton in 2010 [97]. The estimated loss due to ISA from 2007 to 2011 was approximately US $1.0 billion. The outbreak was traced to inadequate production management methods, including failure to vaccinate fish against ISA. In the US, outbreaks of ISA devastated the salmon industry in Cobscook and Passamaquoddy Bays, Maine, USA, in 2001 resulting in depopulation of the entire area [4]. Another example of the continuing impact of fish viruses is the recent report of ISA in sockeye salmon stocks in British Columbia, Canada, where the disease had never before been observed [90].

Infectious hematopoietic necrosis (IHN) disease is another economically important fish disease that has severely impacted farmed salmonids. The first recorded outbreak of IHN occurred in 1953 in sockeye salmon hatcheries in Oregon and Washington states in the USA [60]. During 1973–1974, IHN outbreaks caused over 90 % mortalities in sockeye salmon alevins and fingerlings in the US [40]. The first natural outbreak of IHN in Atlantic salmon was reported from British Columbia, Canada, in 1992. During 1990s through early 2000, infectious hematopoietic necrosis virus (IHNV) epizootics caused mortalities as high as 77 % in smolts (<700 g) and ~50 % in harvest-sized fish [89]. In May 2012, an IHN outbreak occurred in a salmon farm off Bainbridge Island, Washington, USA, resulting in the removal of all dead or dying fish and a significant loss to the salmon industry (http://wildfishconservancy.org/resources/publications/wild-fish-runs/going-viral-ihn-outbreaks-in-puget-sound).

Viral haemorrhagic septicemia (VHS) is an economically important viral disease of farmed rainbow trout, turbot, Japanese flounder and olive flounder. Disease outbreaks due to VHS have been recorded in sea-farmed rainbow trout in France and Denmark, sea-farmed and land-based turbot farms in Germany and Scotland [46, 92]. VHS outbreaks causing high mortalities (50–70 %) in flounder have been reported from farms in Japan in early 2000 [50]. During 2007–2009, VSH outbreaks in rainbow trout were also reported from Norway [51].

There are a number of viral diseases that have caused mass mortalities among non-salmonid fish as well. The predominant non-salmonid fish important to aquaculture are carps, barbels and other members of the cyprinid family, accounting for 63 % of world aquaculture production in 2010 (Supplementary Fig. 1a). Spring viremia of carp (SVC) is an economically important viral disease of common carp (*Cyprinus carpio carpio*). The disease is endemic in Europe and causes significant mortalities in European carp [1]. In 2002, a SVC outbreak was reported in the USA. It was attributed to the importation of koi infected with the virus [45]. SVC caused mass mortalities in wild common carp at Cedar Lake, Wisconsin, USA, in 2002 and in farmed koi in Washington and Missouri states in the USA in 2004 [27, 36]. In 2006, the disease was recorded in apparently healthy common carp from Lake Ontario, Canada [34].

Another viral disease that has caused mass mortalities among members of the cyprinids worldwide includes koi herpesvirus disease (KHVD) caused by the koi herpesvirus (KHV), also known as *Cyprinid herpesvirus-3* (*CyHV-3*). In late 90s, major outbreaks of KHVD occurred in the USA, Israel, and Germany [71, 81]. In 2010, mass mortalities associated with KHVD in wild common carp were reported from Canada. Outbreaks of KHVD have also been reported from a number of Southeast Asian countries between 2005 and 2009, and KHVD has now been reported from 26 different countries [71, 81].

It is well documented that existing as well as emerging viral diseases pose a serious threat to fish farming globally. As fish farming grows worldwide, there is a growing need to develop comprehensive health management practices that would include identifying the etiologic agent of the diseases, developing improved surveillance and disease diagnostic methods, developing novel and effective vaccines and anti-viral therapeutics, and more rigorously deploying existing modern technologies to disease management in aquaculture which, to date, has been underserved by advancing science.

Viral diseases of farmed finfish

Among finfish, carps, barbels and other cyprinids constitute 63 % of world aquaculture production with an estimated value of US $34 billion (Supplementary Fig. 1). Grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), Indian carp (*Catla catla*), common carp (*Cyprinus carpio*), bighead carp (*Hypophthalmichthys*

nobilis), and crucian carp (*Carassius carassius*) are the predominant cyprinid species cultivated worldwide (Fishstat: ftp://ftp.fao.org/FI/STAT/summary/default.htm). Tilapia and other cichlids as well as salmonid are the two other major finfish groups in commercial cultivation (Supplementary Fig. 1). Among all the commercially cultivated species, Atlantic salmon (*Salmo salar*) is the highest per fish and total value fish species that is cultivated at industrial-scale. It had a worldwide production reaching approximately 1.4 million tonnes in the year 2010 and a measured value of approximately US $7.82 billion (Fishstat: ftp://ftp.fao.org/FI/STAT/summary/default.htm). The increase in aquaculture production has been accompanied by an increase in trade and movement of live fish and fish products across countries. According to the Food and Agricultural Organization (FAO), world trade in fish and fishery products is estimated at US $102 billion in 2008 (http://www.fao.org/docrep/013/i1820e/i1820e00.htm). Spread of viral diseases through international trade is now a proven threat to aquaculture. Movement of animals showing no apparent clinical signs or carrying sub-clinical infection, asymptomatic carrier hosts, and shipment of contaminated/infected eggs are threats to the sustainable growth of finfish farming [84].

Cultivated and wild finfish species are susceptible to an array of viral pathogens. A list of DNA and RNA viruses that infect finfish is provided in Tables 1 and 2. Among these viral diseases, seven are listed as reportable finfish viral diseases by the Office Internationale des Epizooties (OIE) in 2013 (http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2013/). The OIE list of aquatic animal diseases is a dynamic list that changes over time as new diseases are listed and some previously listed diseases are removed. Diseases that are included in the OIE list are deemed by the OIE to pose a risk of spread through commercial trade of fish and fish products.

Three out of the seven OIE listed viral diseases are caused by DNA viruses viz., epizootic haematopoietic necrosis (EHN), KHVD, and red sea bream iridovirus disease (RSID), while the remaining four diseases are caused by RNA viruses, IHNV, ISAV, SVCV, and VHSV. There are a number of other viral diseases that are not included in OIE list yet cause major losses in commercial aquaculture. These non-OIE listed diseases are either recently delisted because they are enzootic in all the regions where susceptible fish are cultivated or occur naturally or a very recently identified disease for which the worldwide geographic prevalence as well as the potential threat is not yet known. For example, infectious pancreatic necrosis (IPN) was removed as an OIE notifiable disease of aquatic animals in 2005 but IPN continues to cause losses to the aquaculture industry in Europe [15, 86]. Examples of recently identified diseases include cadiomyopathy syndrome (CMS) [43], and hemorrhagic kidney syndrome (HKS) involving Toga-like virus in Atlantic salmon [11]. Another example of a recently identified virus that has been implicated in increased mortality in barbel fry (*Barbus barbus*) in Hungary includes a circovirus (single-stranded DNA containing virus) although a relation between the presence of the virus and clinical manifestations has yet to be established [65]. As more improved diagnostic methods are developed to meet the needs of the burgeoning aquaculture industry, new diseases are likely to be discovered that may otherwise be enzootic in wild fish populations [75]. These newly discovered diseases can potentially cause major losses if they are introduced in commercial aquaculture operations where a single species of fish is cultivated at a very high density providing stress and conditions that enable rapid disease transmission.

Vaccination of farmed fish

The aquaculture industry has responded to viral diseases in a variety of ways, but none of them is entirely adequate for the challenge that must be met to enable sustainable aquaculture. Fish health management has become, and will remain, a critical component to disease control and is invaluable to improved harvests and sustainable aquaculture [54]. In general, implementing efficient health management tools, such as increased disease surveillance, improved farm biosecurity protocols, vaccination regimes, use of immunostimulants and other tools, have helped to mitigate and contain most losses due to viral and other diseases. Among different disease management strategies, vaccination has proved to be a very effective way of protecting fish from viral and bacterial diseases. Several current reviews summarized these efforts [14, 24, 35, 42, 98]. A number of viral vaccines have been developed in finfish, as well as improved techniques for delivery of these vaccines, at affordable prices (Table 3). Effective and economical vaccines are helping to improve the profit outlook for the finfish industry. The majority of the commercial vaccines are targeted at the high value salmonid industry with vaccines against diseases such as IPN, ISA, IHN, and pancreatic disease (PD). However, there are also commercially available vaccines against diseases that attack other finfish, such as SVC (that infects carp, European catfish, and rainbow trout), viral nervous necrosis (VNN; that infects sea bass and sea bream), and KHVD (that infects common carp, koi, goldfish, ornamental catfish and sturgeon among many other species).

As aquaculture is expected to supply an increasingly higher proportion of the high quality protein needed for the earth's rising population, the degree of intensification of industrial aquaculture management methods will put

Table 1 A list of viral diseases impacting farmed finfish

Common name of the disease	Causal agent	Host	Prevalence	OIE status[a]	Reference
Diseases caused by DNA viruses					
Carp pox (also known as fish papilloma virus)	*Cyprinid herpesvirus-1 (CyHV-1)*, also known as Carp pox virus (CPV)	Common carp	USA, Europe, Russia, Japan, Malaysia, Korea, Israel	–	[22, 41, 88]
Herpesviral hematopoietic necrosis	*Cyprinid herpesvirus-2 (CyHV-2,* Goldfish herpesvirus (GHV)	Goldfish	USA, Japan, Taiwan, Australia	–	[22, 37, 41, 52]
Koi Herpes (previously known as carp interstitial nephritis and gill necrosis virus, CNGV)	*Cyprinid herpesvirus-3 (CyHV-3,* Koi herpesvirus (KHV)	Common carp, koi	Japan, Taiwan, Indonesia, Malaysia, Thailand, Singapore, Hong Kong, China, Korea, Israel, US, Canada, UK, Germany, Italy	OIE Listed	[6, 22, 41, 49], OIE, 2012[b]
Channel catfish virus (CCV), (also called channel catfish herpesvirus)	*Ictalurid herpesvirus (IcHV-1)*	Channel catfish	US, Mexico	–	[22, 41, 91]
Onchorhynchus masou virus disease (OMVD)	*Onchorhynchus masou* virus (OMV), also known as nerka virus Towada Lak, Akita and Amori Prefecture, NeVTA,coho salmon herpes virus, CHV, rainbow trout kidney virus, RKV, yamame tumor virus, YTV, coho salmon tumor virus, CSTV/COTV, rainbow trout herpesvirus, RHV)	Sockey salmon, masu salmon, chum salmon, coho salmon and rainbow trout.	Japan	–	[113], OIE 2012[b],
Infectious spleen and kidney necrosis (ISKN)	Infectious spleen and kidney necrosis virus (ISKNV)	Genotype I infects mainly marine fish, Genotype II infects freshwater fish, and Genotype III infects mainly flatfish	China	–	[44, 88]
Red seabream iridoviral disease (RSID)	Red sea bream iridovirus (RSIV)	Red sea bream, black porgy, amberjack, norther bluefin tuna and many other cultured marine fish in the orders Perciformes and Pleuronectiformes	East and South East Asian countries (Japan, China, South Korea, Malaysia, Philippines, Singapore, Thailand)	–	OIE 2012[b]
Lymphocystis disease (LCD)	Lymphocystis disease virus (LCDV)	Infects both freshwater and marine species, LCDV-1 infects flounder, plaice, LCDV-2 infects dab	Worldwide occurring in both warm and coldwater fish of marine and freshwater environment	–	[18, 91]
Epizootic hematopoietic necrosis (EHN)	Epizootic hematopoietic necrosis virus (EHNV)	Redfin perch, rainbow trout, macquarie perch, silver perch, mosquito fish, mountain galaxis	Australia	OIE Listed	OIE 2012[b]
Iriodovirus of freshwater fish: European catfish virus, ECV, European sheatfish virus, ESV, Largemouth bass virus, LMBV, White sturgeon iridovirus, WSIV	Iridovirus	European catfish, European sheatfish, largemouth bass, white sturgeon, sea bass, milk fish	Europe, India	–	[3, 91]

Table 1 continued

Common name of the disease	Causal agent	Host	Prevalence	OIE status[a]	Reference
Diseases caused by RNA viruses					
Pancreas disease (PD), (also known as sleeping disease, SD)	Salmon alphavirus, previously known as sleeping disease virus (SDV)	Atlantic salmon, Rainbow trout	Norway, Scotland, Ireland, France, Italy, Spain	–	[70]
Viral encephalopathy and retinopathy (VER) (also known as viral nervous necrosis, VNN)	Betanodavirus with four genotypes, striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV), and red-spotted grouper nervous necrosis virus (RGNNV)	Infects both freshwater and marine species, stripped jack, tiger puffer, Atlantic halibut, Atlantic cod, flounder, Asia n sea bass, clown fish, European sea bass, grouper,	All continents except South America	–	[8, 12, 80, 91, 92], OIE 2012[b],
Viral hemorrhagic septicemia (VHS)	Viral hemorrhagic septicemia virus (VHSV)	Infects 80 fish species, examples of susceptible farmed species include rainbow trout, turbot, Japanese flounder	North America, Europe, Asia	OIE Listed	[95, 96], OIE 2012[b]
Infectious hematopoietic necrosis (IHN)	Infectious hematopoietic necrosis virus (IHNV)	Salmon, Trout, Char, Cod, Pike, Sturgeon	Russia, China, Japan, Korea, Iran	OIE Listed	[24], OIE 2012[b],
Spring viremia of carp (SVC)	Spring viremia of carp virus (SVCV) (also called pike fry rhabdovirus, PFRV, tench rhabdovirus, TenRV)	Common carp, pike, European catfish or wels, rainbow trout.	Most European countries, Canada, China, Iran, Egypt	OIE Listed	[1], OIE 2012[b]
Infectious salmon anemia (ISA)	Infectious salmon anemia virus (ISAV)	Atlantic salmon, Coho salmon, Pollock, Cod, Brown trout, Sea Trout, Grouper	Norway, Scotland, UK, Faroe Islands, USA, Canada, Chile	OIE Listed	[56, 82], OIE 2012[b],
Infectious pancreatic necrosis (IPN)	Infectious pancreatic necrosis virus (IPNV)	Infects both freshwater fish and marine fish; Salmon, rainbow trout, halibut, cod, carp, goldfish	Europe, US, Canada, India, Japan	–	[5, 10, 74, 83]
Cadiomyopathy syndrome (CMS)	*Piscine myocarditis virus*	Atlantic salmon	Norway, Scotland, Canada	–	[43]
Hemorrhagic kidney syndrome, "Toga-like" virus	*Cutthroat trout virus*	Cutthroat trout	USA, Canada	–	[10]
Heart and skeletal muscle inflammation (HSMI)	*Piscine reovirus (suspected)*	Atlantic salmon	Norway, Scotland	–	[32, 59]

"–" Not included in the OIE list of fish viral diseases in 2013

[a] OIE Listed fish viral diseases in 2013 (http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2013/)

[b] OIE 2012. OIE Manual of Aquatic Disease Diagnostics, http://www.oie.int/international-standard-setting/aquatic-manual/access-online/, Chap. 2.3.1, Viral haemorrhagic septicaemia, Chap. 2.3.1, Epizootic haematopietic necrosis, Chap. 2.3.4, Infectious haematopoietic necrosis, Chap. 2.3.5, Infection with infectious salmon anemia virus, Chap. 2.3.6, Koi herpesvirus disease, Chap. 2.3.7, Red sea bream iridioviral disease, Chap. 2.3.8, Spring viremia of carp, Chap. 2.3.10, *Onchohynchus masou* virus disease, Chap. 2.3.11,Viral encephalopathy and retinopathy

additional strains on the health of animals within the system (as well as having the potential to impact wild fisheries that are linked indirectly). As these intensive farming techniques increasingly dominate the industry, the potential for negative impacts on the environment will also increase. The high density culturing of fish in freshwater, brakish-water and seawater aquaculture has already demonstrated that effective disease management is a key to profitability. Current vaccination methods are primarily limited to larger fish and focused on getting the fish to a marketable size using a single vaccination cycle (least cost method). This approach is really a race between disease incidence and harvest that does little to reduce viral loads in the overall system and the environment. Ideally, vaccines should

Table 2 Characteristics of viruses infecting farmed finfish

Viruses	Taxonomic classification (Family, genus, species, strains)	Virion morphology and size	Viral genome	Antigen involved in neutralizing antibodies[a]	Key references
DNA viruses-double stranded					
Carp pox virus (CPV)	*Alloherpesviridae,* Genus *Cyprinivirus*, *Cyprinid herpesvirus-1 (CyHV-1)*	Enveloped, icosahedral	dsDNA, 291 kb	ND	[22, 41]
Goldfish herpes virus (GHV)	*Alloherpesviridae*, Genus *Cyprinivirus*, *Cyprinid herpesvirus-2 (CyHV-2)*	Enveloped, icosahedral	dsDNA, 290 kb	ND	[22, 41]
Koi herpes virus (KHV)	*Alloherpesviridae,* Genus *Cyprinivirus*, type species *Cyprinid herpesvirus-3 (CyHV-3)*	Enveloped, icosahedral, 170–200 nm diameter	dsDNA, 295 kb	Putative antigen-a protein encoded by the ORF 68, a myosin homolog	[6, 7, 22, 41, 110], OIE, 2012[a]
Channel catfish virus (CCV)	*Alloherpesviridae,* Genus *Cyprinivirus*, *Ictalurid herpesvirus (IcHV-1)*	Enveloped, icosahedral, 175–200 nm	dsDNA, 134 kb	ND	[22, 41, 91]
Onchorhynchus masou virus (OMV)	*Herpesviridae, Onchorhynchus masou* virus (OMV)	Enveloped virion, 200 ± 24 × 240 ± 23 diameter	dsDNA	ND	[113], OIE 2012
Infectious spleen and kidney necrosis virus (ISKNV)	*Iridoviridae,* Type species of the genus *Megalocystivirus*	Non-enveloped, icosahedral, 150 nm diameter	dsDNA, 111 kb	ND	[44]
Red sea bream iridiovirus (RSIV)	*Iridioviridae*	Non-enveloped, icosahedral, 200–240 nm diameter	dsDNA, 112 kb	ND	[28, 61, 91], OIE 2012
Lymphocystis disease virus (LCDV-1)	*Iridoviridae, Lymphocystivirus,* 1 confirmed (LCDV-1) and 1 tentative (LCDV-2) species in this genus	Icosahedral, ~198–227 nm in diameter (LCDV-1), 200 nm (LDV-2), fringe of fibril-like external protrusion ~2.5 nm in length	dsDNA, 102.6 kb (LCDV-1), ~ 98 kb (LCDV-2)	ND	[10, 18]
Epizootic hematopoietic necrosis virus (EHNV)	*Iridoviridae,* Genus *Ranavirus, species* Epizootic hematopoietic necrosis virus	Non-enveloped, icosahedral, 175 nm diameter	dsDNA, 127 kb, virus Replicates in both nucleus and cytoplasm with intracytoplasmic assembly	ND	[20], OIE, 2012
RNA viruses-single stranded					
Salmon alphavirus (SAV)	*Togaviridae, Alphavirus,* three subtypes-SAV1, SAV2 and SAV3	Enveloped, spherical, 64–66 nm diameter	One, +ve sense, ssRNA, ~12 kb	E2	[70]
Viral nervous necrosis virus (VNNV)	*Nodaviridae, Betanodavirus,* includes four genotypes: striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV), and red-spotted grouper nervous necrosis virus (RGNNV)	Non enveloped, icosahedral, 25–30 nm diameter	Two, +ve-sense, ssRNA, 3.1 kb RNA1 and 1.42 kb RNA2	C	[91, 93], OIE, 2012

Table 2 continued

Viruses	Taxonomic classification (Family, genus, species, strains)	Virion morphology and size	Viral genome	Antigen involved in neutralizing antibodies[a]	Key references
Viral hemorrhagic septicemia virus (VHSV)	*Rhabdoviridae, Novirhabdovirus,* four genotypes: Genotype I (with several sublineages), Genotype II, Genotype III and Genotype IV	Enveloped, bullet shaped ~70 × 180 nm	One, −ve sense, ssRNA, ~11 kb	G	[30, 31], OIE, 2012
Infectious hematopoietic necrosis virus (IHNV)	*Rhabdoviridae, Novirhabdovirus,* Three genogroups based on geographical distribution in Pacific Northwest of North America- Genogroup Upper (U-Northwest coast, Oregon to Alaska genogroup in Sockeye salmon), Genogroup Middle (M-Idaho genogroup in rainbow trout), and Genogroup Lower (L-California genogroup in Chinook salmon)	Enveloped, bullet shaped ~170 × 80 nm	One, −ve sense, ssRNA, ~ 11 kb	G	[13, 25], OIE, 2012
Spring viremia of carp (SVCV)	*Rhabdoviridae, Vesiculovirus*, include four Genogroups-Genogroup I, Genogroup II, genogroup III and Genogroup IV	Enveloped, bullet shaped, 60–90 nm diameter and 80–180 nm long	One, −ve sense, ssRNA, ~11 kb	G	[1, 91], OIE 2012
Infectious salmon anemia (ISAV)	*Orthomyxoviridae, Isavirus,* ISAV is the type species of the genus *Isavirus*, two genotypes—(European and North American), and two strains identified—non-pathogenic strains, "highly polymorphic region" (HPRO), that contains a full-length surface envelope glycoprotein haemagglutinin-esterase (HE), and a pathogenic strain, HPR, that has a deletion of 35 amino acids in the HE protein	Enveloped, polymorphic, 100–130 nm diameter with 10–12 nm surface projections	Eight, −ve sense, ssRNA, ranging in size from 1.0 to 23 kb, total size 14.3 kb	HE	[82], OIE 2012
RNA viruses-double stranded					
Infectious pancreatic necrosis virus (IPNV)	*Birnaviridae, Aquabirnavirus,* two serogroups-A and B; serogroup A contains 9 serotypes and serogroup B contains 1 serotype	Non enveloped, icosahedral, 60 nm size	Two, dsRNA, ~3.1 kb and ~2.8 kb	VP2	[10, 74]
Piscine myocarditis virus (PMCV)	*Totiviridae, Giardiavirus*		One, linear, dsRNA, ~6.7 kb	ND	[43]

ND not determined, *E2* fusion protein, *C* capsid protein, *G* glycoprotein, *HE* hemaglutinin-esterase, *VP2* viral protein 2

[a] OIE 2012 See Table 1 footnote for the detail reference

Target: Major protein involved in neutralization: *E2* fusion protein, *C* capsid protein, *G* glycoprotein, *HE* hemaglutinin-esterase, *VP2* viral protein 2

provide protection for the entire life of the fish and be cost-effective. A more effective approach will have to increasingly rely on earlier vaccination (while the fish are juveniles and/or small in size), booster vaccinations to provide longer lasting protection, and rapid response vaccines to control the spread of disease and decrease any negative impacts of aquaculture on the environment (e.g., wild fish stocks). The following section provides an overview of the

Table 3 Commercially available vaccines against viral diseases in farmed finfish

Virus	Vaccine type	Antigen	Delivery Route	Vaccine name	Producer	Licensed for use in country
DNA Viruses						
Koi herpes virus (KHV)	Attenuated viral vaccine	Attenuated virus	Immersion or injection	KV-3 (also known as Cavoy)	KoVax Ltd., Jerusalem, Israel	Israel, USA
Iridovirus	Inactivated viral vaccine	Inactivated virus	Intraperitoneal injection	AQUAVAC® IridoV	Merck Animal Health, USA	Singapore
Red sea bream iridiovirus	Inactivated viral vaccine	Inactivated virus	Intraperitoneal injection	Killed iridovirus vaccine, inactivated iridovirus-streptococcicosis-vibriosis combined vaccine	The Research Foundation for Microbial diseases of Osaka University, Japan	Japan
RNA viruses						
Salmon alphaviruses (SAV)	Inactivated viral vaccine	Inactivated virus	Intraperitoneal injection	PD Norvax® Compact PD	Pharmaq AS, Norway Intervet-International BV, The Netherlands	Norway, Chile, UK
Infectious hematopoietic necrosis virus (IHNV)	DNA Vaccine	Recombinant G protein	Intramuscular injection	APEX-IHN	Aqua Health Ltd., Novartis, Canada	Canada
Spring viremia of carp virus (SVCV)[a]	Subunit vaccine	Recombinant G protein in baculovirus expression system	Intraperitoneal injection	??	Pharos, S. A., Belgium	Belgium
	Attenuated viral vaccine	Attenuated virus	Immersion	??	??	China
Infectious salmon anemia virus (ISAV)	Subunit vaccine	Recombinant hemagglutinin esterase protein	Oral	Centrovet, Chile	Centrovet, Chile	Chile
	Inactivated viral vaccine	Inactivated virus (Monovalent)	Intraperitoneal injection	Alpha Jects® Micro-1 ISA, Pharmaq AS, Norway	Alpha Jects® Micro-1 ISA, Pharmaq AS, Norway	Chile, Finland, Ireland, Norway
	Inactivated viral vaccine	Inactivated virus (Multivalent)	Intraperitoneal injection	FORTE VI, Aqua Health Ltd., Novartis, Canada	FORTE VI, Aqua Health Ltd., Novartis, Canada	Canada
	Inactivated viral vaccine	Inactivated virus (Multivalent)	Intraperitoneal injection	Microtek International Inc., British Columbia, Canada	Microtek International Inc., British Columbia, Canada	Canada
Infectious pancreatic necrosis virus (IPNV)	Subunit vaccine	VP2 and VP3 capsid proteins	Oral	AquaVac® IPN Oral	Merck Animal Health, New Jersey, USA	Canada
	Subunit vaccine	VP2 protein (Trivalent SRS/IPNV/*Vibrio*)	Intraperitoneal injection	SRS/IPNV/*Vibrio*	Microtek International Inc., British Columbia, Canada	Canada, Chile
	Subunit vaccine	VP2 capsid protein	Intraperitoneal injection	Norvax®Minova -6	Intervet-International BV, The Netherlands	??
	Inactivated viral vaccine	Inactivated IPNV (Monovalent)	Intraperitoneal injection	Alpha Jects® 1000	Pharmaq AS, Norway	Chile, Norway, UK
	Inactivated viral vaccine	Inactivated IPNV	Intraperitoneal injection	IPNV	Centrovet, Chile	Chile
	Inactivated viral vaccine	Inactivated IPNV	Intraperitoneal injection	Birnagen Forte	Aqua Health Ltd., Novartis, Canada	Canada

?? Information not available

[a] An inactivated viral vaccine against SVCV delivered via intraperitoneal injection was commercially available until 2007 and sold by Bioveta, Czech Republic. This vaccine is currently not available

current status of viral vaccines in finfish covering the different types of vaccines available, delivery routes and future directions in vaccine development.

Type of vaccines available

Historically, the first vaccines were based on inactivated (by heat or chemical) viruses. Inactivated virus vaccines are still a major portion of the overall vaccine supply (Table 3). Viruses that caused mild infections but conferred cross-protection against more virulent viral infections were also developed early on in vaccinology. Recent advances in fish genomics and immunology, as well as knowledge from viral pathogenesis studies in fish, are likely to enhance the development of vaccines and antiviral drugs for finfish and aquaculture specifically. With these and developments in improved and powerful scientific tools, new variations in the types of vaccines available are playing an increasingly important role in fish health management. These efforts are focused on producing the ideal vaccine economically, which must induce long lasting protection starting at an early age, prevent carrier formation, confer long lasting protection, and be effective against a large number of viral serotypes or viruses. Ideally, vaccines will also allow the differentiation of a vaccinated fish from an infected (or previously infected) fish to aid in epidemiology and disease surveillance/control. These attributes of the ideal vaccine are most likely to be met either by a recombinant subunit vaccine or by an inactivated viral vaccine, as a live attenuated vaccine could potentially lead to carrier formation.

Inactivated viral vaccines

Use of inactivated wild type virus as the antigen to induce an immune response was the earliest approach to fish vaccines and is still a reliable standard by which other vaccines are evaluated. The Alpha Jects® Micro 1 ISA (Novartis) and Alpha Jects® 1,000 vaccines (Table 3) are examples of this type of vaccine, targeting ISA and IPN, respectively. Inactivated viral vaccines induce strong immune responses as they retain the virus's surface exposed antigens as well as the inactivated genomic component, both of which are important for strong induction of an immune response. However, the use of inactivated viruses as a vaccine is hampered since some fish viruses are not easily culturable, such as SalHV3, a herpesvirus of Atlantic cod [66] and lymphocystis virus [115], making the production of vaccines for these viruses based on whole inactivated virus difficult, if not impossible. Alternative methods are necessary for vaccine production to lower the expense of production as well as to enable production of vaccines targeting non-culturable viruses (Table 4).

Attenuated virus vaccines

Vaccines based on live attenuated virus have been applied extensively in humans, such as the chicken pox vaccine. Attenuated virus vaccines are based on live virus that have been selected for cross reactivity (a less virulent virus that elicits an immune response to the target virus), genetically modified to attenuate the virus, and/or cultivated under conditions that disable viral virulence. As a result, the attenuated virus replicates in the target host albeit at a much lower rate compared to the wild type virus and has no or reduced clinical signs. Attenuated viral vaccines typically provoke a strong and sustained immune response to the target disease. So far, there is only one commercial vaccine that is based on attenuated virus in aquaculture. This vaccine is against KHVD and is based on attenuated *Cyprinid herpesvirus-3 (CyHV-3)* which is also known as Koi herpesvirus (KHV) and as carp interstitial nephritis and gill necrosis virus [85].

Subunit vaccines

Subunit vaccines are another class of vaccines that have emerged with the advent of molecular biology. Production of specific viral proteins ("viral subunits") using a recombinant protein expression system allowed the rapid production of focused vaccines based on a single viral antigen or small number of viral antigens. Molecular tools allowed the high expression of the most highly antigenic proteins of the target virus in bulk and subsequent delivery of these highly purified preparations as a vaccine. Initial work with subunit vaccines was not successful due to the rapid degradation of the protein during processing, delivery, or in the animals. However, rapid advances were made to stabilize the antigens and many subunit vaccines were developed (Table 3). Highly successful examples of subunit vaccines are the IPNV VP2-based vaccine from Microtek International and the ISAV recombinant hemagglutinin esterase gene from Centrovet (Table 3).

While some subunit vaccines were found to be very effective others were not as effective in vaccine form. A variety of approaches were developed to improve the antigenic response of the animals. Formulation of the vaccines was the first focus and relied on improved stabilization of the proteins, addition of chemicals that induced the immune system generally or poised the immune system for a better immune response (e.g., adjuvants), multiple antigens included in the vaccine (like the Trivalent subunit vaccine from Microtek; Table 3), and different formats to both improve the display of the

Table 4 A list of experimental vaccines reported in the literature to address viral diseases in aquacultured finfish

Virus	Vaccine type	Antigen	Delivery route	Detail	Host	Reference
AHNV	Protein and DNA	Capsid	IM	Protein protected while DNA did not	Atlantic halibut	[99]
GCRV-GD108	Recombinant protein	VP4 (capsid protein)	Injection	Purified protein injected	Grass carp	[103]
IPNV	DNA	VP2	Oral	Alginate microspheres protect the pDNA	Rainbow trout	[23]
IPNV	Recombinant bacterium	VP2/VP3 capsid protein	Oral	*Lactobacillus casei* transformed with plasmids containing IPNV capsid genes	Rainbow trout	[73]
IPNV	Recombinant protein	VP2 based SVP	IM	Foreign epitope expressed on surface of SVP	Rainbow trout	[26]
IPNV	DNA	Segment A polyprotein	IM and In vitro cell culture	VLP formation; VP2, VP3, VP4 observed	Rainbow trout	[21]
ISAV	DNA	Hemagglutinin-esterase (HE)	IM	SAV replicon engineered to express HE	Atlantic salmon (*Salmo salar*)	[111]
SAV	DNA	SAV-replicon	In vitro testing	dsRNA production of protein	Fish cell lines	[78]
VHSV	dsRNA	G-Protein	In vitro testing	RNAi protection	Fish cell culture (EPC and CHSE-214)	[58]
VHSV	DNA	G-protein	In vitro testing	Removal of all viral regulatory sequences	Fish cell lines	[69]
VHSV	Inactivated virus	Whole virus	IM	Mixture of squalene and aluminum hydroxide adjuvant	olive flounder (*Paralichthys olivaceus*)	[108]
VNN	Inactivated virus	Whole virus	IM	Formalin inactivated RGNNV	Brown-marbled grouper (*Epinephelus fuscogutattus*)	[79]

GCRV-GD108 grass carp reovirus Guangdong 108 strain, *RGNNV* red spotted grouper nervous necrosis virus, *VHSV* viral hemorrhagic septicemia virus, *VNN* viral nervous necrosis virus

antigens to the immune system and prevent their rapid degradation in the animal.

Virus-like particles (VLPs) and subviral particles (SVPs)

One of the subunit vaccine formats is the virus-like particles (VLPs) or subviral particles (SVPs). The intrinsic ability of viral structural proteins to self-assemble into particles (VLPs or SVPs) that mimic the native virus in both size and processing by the host have led to the development of a class of subunit vaccines based on VLPs [17, 33, 38]. VLPs have been expressed in bacteria, yeast, transgenic plants and cell culture. A number of human vaccines have been produced using this technology, such as the Gardasil® vaccine (Merck). Recently, an effort has been made to extend this approach to production of vaccines for fish and shellfish [47, 94]. In order to develop a VLP-based vaccine against IPNV, Shivappa and colleagues engineered production of VLPs using vectors expressing the two IPNV capsid proteins, VP2 and VP3, either as a polyprotein or separately [94]. When expressed, these capsid proteins formed VLPs that were similar in size to the native virus and induced a strong immune response in salmon [69, 94]. A more recent paper described production of an IPN vaccine based on the VP2 protein of IPNV [2]. In this study, a subunit-based particle was made that was smaller (22 nm) than the native virus (60 nm) but induced a strong anti-IPNV response in rainbow trout, and this particle was referred to as a SVP. In a follow up study, these authors demonstrated that a foreign epitope (*c-myc*) could be expressed on the subviral particle and induce an increased immune response to IPNV as well as to the foreign epitope [24]. Further research has led to the successful display of the ISAV hemagglutinin epitope on the surface of this IPNV VP2-based subviral particle (Dhar et al. 2012, unpublished data). Three other vaccines based on the VP2 capsid protein of IPNV are already marketed—IPNV (licensed in Chile, Centrovet, Chile), Norvax (Intervet-International BV, The Netherlands), and SRS/IPNV/*Vibrio* (licensed in Canada and Chile, Microtek International Inc., British Columbia, Canada).

Recombinant DNA vaccines

Recombinant DNA vaccines involve injecting an organism with histone-free ("naked") DNA representing a gene of the pathogen itself. The gene represents a viral encoded protein which is usually under the control of a strong promoter in the fish system being targeted by the vaccine. The vaccine is administered by intramuscular injection or by gene gun bombardment of the epidermis. Upon delivery, the naked DNA is taken up by the muscle cells and the recombinant viral antigen protein is strongly expressed. The antigen presenting cells, such as the dendritic cells at the site of injection, present the viral antigen via the MHC class I molecules to lymphocytes in the lymph nodes [29]. In higher vertebrates, it has been shown that DNA-based vaccines have an immunological advantage over other vaccination approaches because DNA-based vaccines induce strong and long-lasting humoral and cell-mediated immunity similar to those provided by live attenuated vaccine but without the risk of inadvertent infection. DNA vaccines promise to provide a strong tool for aquaculture [64]. A DNA vaccine against IHNV was the first effective DNA vaccine tested in fish (Table 3). Subsequently, DNA vaccines were tested against a number of fish viruses, including IPNV [9, 72], VNNV [99], and SVCV [106]. So far only one DNA vaccine, a vaccine against IHN, has been approved for use in Canada. However, this vaccine is not approved in Europe and the US for commercial application due to safety concerns.

Routes of vaccination

Vaccination for aquatic species has three major routes of delivery: injection [intraperitoneal (IP) and intramuscular (IM)], immersion, and oral (per os). Most current vaccines are delivered predominantly by IP injection (Table 3). Each delivery route has its own advantages and limitations. These are discussed in the following sections.

Delivery by injection

Currently, the most commonly used method for vaccination is via injection of the vaccine. This is usually done manually using a needle and IP or IM injection (Table 3). Alternative injection methods using devices such as compressed air have also been tested but are not in general use. Injection requires that the fish be of fairly large size and, even with automation, injection is stressful on the fish and labor intensive. These problems have led to the development of a number of semi-automated injection methods where the fish are forced through a chute or enclosed space and, as they are wedged in are autoinjected with the vaccine. Automated methods are not in general use and tend to increase the amount of lesions at the site of injection.

Vaccination by injection has a number of definite advantages. Injection provides the most direct delivery of antigen to the immune system through IM and IP injection. The vaccine can be concentrated and delivered in the presence of adjuvants and other beneficial compounds (e.g., carriers, bacterial antigens/bacterial cells, and etc.) that could not be delivered by other methods. The antigens are protected in highly purified formats and can be easily stored in refrigerated form since they are so concentrated.

However, injection as the route of vaccination also has substantial issues that make the search for effective alternative vaccination methods worthwhile, and this is especially true for aquaculture. The major issue with injectable vaccines is that they cannot be economically delivered multiple times in the production cycle—due to logistical issues and high cost. They are normally delivered at the beginning of grow-out and the protection is intended to span the entire grow-out cycle (until harvest).

The high cost of labor is another major barrier for any injectable vaccine. Automation has been developed to reduce the labor costs. All injection has the potential to cause damage at the site of injection, such as melanization and tissue adhesion damage in interperitoneal injections [53] and abdominal lesions [39]. The new automated methods have increased chances of injection-induced damage relative to other injection methods as trained medical personnel are not utilized in the injection. Mortalities are at least partially associated with the increased stress and handling of fish during injection.

Injection cannot be carried out early in the life of the fish (juvenile and pre-smolt fish) due to the small size of these fish. As a result, there is a window for pathogen infection for unvaccinated fish from larval to just before grow-out where increases in titer occur before these fish can be vaccinated by injection. It should also be noted that the immune systems of young fish are underdeveloped and could require multiple vaccinations to induce sufficient protection against the target pathogen.

Delivery by immersion

Delivery of a vaccine by immersion of the fish in a solution containing the vaccine is proved to be a gentle and safe way of vaccine delivery. The vaccine is delivered both to the skin and all mucosal surfaces accessible to the surrounding liquid, which contains the antigen (vaccine). Immersion is particularly effective for induction of mucosal immunity but less so for humoral immunity. Immersion as a route of vaccination is generally limited to smaller fish due to the large volumes of the immersion liquid or dip needed with large fish (which equates to larger amounts of

antigen/vaccine needed for immunization). Improvements in the efficacy of immersion vaccination have been obtained through the use of ultrasound and the use of a hyperosmotic solution for the immersion medium.

Immersion vaccination is particularly suitable for smaller fish, which helps to provide the possibility of life-long immunization. Juveniles and/or small fish can be immunized in large batches and the handling of small fish is kept to a minimum; thus, both stress and associated mortalities are reduced. Immersion is particularly suited to induction of a mucosal immune response [62, 76]. This is an inexpensive and low-stress method that requires little labor relative to injection as a method of vaccination. Since injection cannot be used for small fish either oral or immersion delivery method will be a more preferred route.

However, immersion does not provide as strong an induction of the humoral immune responses as injection vaccination. Fish must be collected and immersed in the antigen-containing dip which can be time consuming and expensive with larger fish. It is also not easy to deliver adjuvants and other immune stimulating compounds in an immersion system. Additionally, the vaccine has to be disbursed in a dilute solution for the immersion step and significant vaccine can be lost during the delivery process (i.e., stay in the immersion fluid and be lost when the vaccination is over). The need to suspend the vaccine in the aqueous medium also puts constraints on the type of material that may be in the vaccine such as solubility and stability.

Oral or *per os* delivery

Oral delivery is conceptually a very simple and elegant way to deliver vaccines and is both natural and non-invasive. It mimics the natural feeding of the fish and allows high concentration antigen to be delivered directly to the digestive tract.

Oral delivery can be accomplished with fish of any age, and could be produced inexpensively (and theoretically passed on to the consumer). Oral delivery does not require fish to be handled separately and, therefore, reduces the overall cost of the vaccination and any mortality associated with handling of the fish as seen in the case of injectable vaccine. With this reduction in labor and cost, oral vaccines can be used repetitively in the life of an aquacultured fish. Oral vaccines can be delivered to juvenile fish just developing humoral immunity, to fish prior to release in the grow-out cages, and even during grow-out in response to looming viral outbreak. In addition to Oral vaccines can be delivered to fish at very low cost and without stress on the fish. Delivery of oral vaccines to fish which have already been immunized by immersion or injection as a booster vaccine can provide additional protection not achievable by a single vaccination [104, 105].

Nonetheless, oral immunization is not a panacea and has significant hurdles to overcome before it is widely accepted in the industry. Induction of humoral immunity is not as strong as injection vaccination with current strategies. Antigen must be packaged and shelf-stable for inclusion in feeds or treatments for rapid delivery of oral vaccines at any site where aquaculture is practiced. In addition, maintaining antigen stability is especially challenging under high temperature and high humidity conditions that are common in many countries in Asia and the tropics.

Encapsulation of oral vaccines

One of the major challenges in developing oral vaccine is to preserve the antigen. This is a two-fold problem. First, the antigen needs to be stable in the vaccine formulation that must, by definition, be put into a feed or feed supplement and delivered to the fish. This feed or feed supplement would most desirably be made in advance by the vaccine maker and be very stable on the shelf. Such an oral vaccine would be much bulkier than injectable vaccines, contain a mixture of materials that might destabilize the antigen (e.g., minerals, enzymes, and etc.), and would be less simple to store refrigerated (due mostly to mass). Second, the antigen must be stable in the aquatic medium in which it is to be delivered and in the gut of the fish so that it is delivered in a form ready to induce a suitable immune response. The antigen would most preferably be poised or directed to the gut surface in order to facilitate this immune response.

Both of these issues have been addressed by encapsulation techniques to varying degrees. However, additional research is required in this area to improve oral vaccination. The recently introduced ISAV vaccine from Centrovet uses a proprietary microencapsulation system designed to protect the SVP-based ISAV hemagglutinin esterase antigen from destruction [104]. While the composition of this encapsulation matrix was not disclosed by these authors, other research has been on-going using materials such as alginate and chitosan to improve oral delivery of vaccines in fish [100–102, 107] and work on this area continues.

Bioenpsulation has been exploited to developing a better delivery method for vaccines and other materials in aquaculture. An example is the use of *Artemia* to deliver vaccines in larval and juvenile stages has been described for cultured koi, *Cyprinus carpio* [85]. However, one of the limitations of using *Artemia* for bioencapsulation is that it does not lend itself to scale-up delivery to larger fish and, therefore, alternative bioencapsulation methods for delivery of subunit vaccines are required. These can be based on the expression host used for production of the antigen (e.g.,

recombinant bacteria or yeast). An example is the Centrovet ISAV vaccine where the HE antigen is expressed in yeast that are then microencapsulated in a proprietary formulation [104]. The use of a host taken for antigen production and targeting of the antigen to the gut uptake sites is also a possibility. The host organism could be engineered to express targeting moieties on the cell surface that are recognized by the fish gut and presented to the immune system.

Future direction

Current commercial vaccines are predominantly delivered via injections. The magnitude of modern aquaculture, its reliance on intensive methods, and introduction of many types of fish to mass production indicate that vaccines will become more important to the industry. There is significant research in process to develop vaccines based on other routes of delivery, especially oral, that are less stressful, more flexible, and provide cost-savings in the vaccine, labor and outcome. A brief description follows but, by necessity, will not cover all of the research underway in the area.

Experimental vaccines

To date, only a few of the vaccines tested in the laboratory have made it into the market place for commercial application so far (Table 3), and there remains an enormous opportunity for bringing new vaccines into the market place and developing more efficacious vaccines. For example, an IPNV DNA vaccine expressing VP2 antigen and delivered orally provided almost 80 % relative percent survival (RPS) upon challenge by an infectious homologous virus in rainbow trout fry of 1–2 g size [23]. Currently, there is no commercially available vaccine against diseases caused by VHSV and VNNV. However, a number of recent studies have reported candidate vaccines against these two diseases. For example, a DNA vaccine expressing the G-protein of VHSV delivered via intramuscular injection provided 100 % RPS in trout and flounder [16, 63]. Similarly, VNNV vaccines based on inactivated virus, recombinant capsid protein, and a DNA vaccine expressing the viral capsid protein that are delivered either by intramuscular injection or via immersion have been reported [48, 99, 112]. An oral vaccine against ISAV is now commercially available [104] (Centrovet Ltd., Chile, Table 3), however, its efficacy in preventing ISAV infection under field conditions is not yet publically available.

The potential for oral vaccines to contribute to fish health is tremendous. Their simple delivery and low-cost production methods associated with oral vaccines is a strong incentive to further develop this method of vaccine delivery in aquaculture. It is obvious from Table 3 that oral vaccination has yet to contribute significantly to the overall vaccine pool available for aquaculture. The ISAV vaccine from Centrovet is the single oral vaccine commercially available.

This slow rise in commercial oral vaccines in aquaculture is due to major hurdles to production of oral vaccines. Foremost of these hurdles is the stability of the immune stimulating agent in the gut and its ability to successfully induce a strong humoral immune response upon oral delivery. Mucosal immunity is important as oral vaccines are strong inducers of mucosal immunity, however, a complete vaccine should induce innate, mucosal and humoral immune responses. Oral vaccines should be amenable to all types of vaccines such as subunit vaccines, attenuated virus based vaccines, nucleic acid vaccines and inactivated virus based vaccines.

One of the most promising delivery systems for subunit and nucleic acid-based vaccines are recombinant organisms that do not require viral culturing and purification of antigens (or nucleic acids). A number of expression systems have been considered, but using yeast as a biofactory for vaccine production provides strong advantages over other systems: (1) The production of biologics in yeast is well understood in the industry and veterinary vaccine manufacturers are likely to subscribe to a system that does not require additional infrastructural modifications. (2) Since many yeast species and strains have generally regarded as safe status (GRAS), the regulatory barrier for vaccines made in yeast tend to be lower than other manufacturing platforms such as bacteria or insect-cell based system. (3) The cost of vaccine production in yeast-based manufacturing system is likely to be lower as the cost of virus purification from yeast will be lower than that of for insect cells. (4) The yeast expression system is a genetically well known and, thus, it is amenable to the genetic modification needed for high expression of the vaccine antigen protein. (5) In fish feed, whole yeast is used as an immunostimulant. Therefore, vaccine made in yeast not only has general immune stimulatory benefit but also provides specific immunity against target pathogen(s).

Vaccination has already been proved as an important management tool for profitable aquaculture. While the current methods predominantly depend on injection for delivery, there is a need to develop alternative methods to reduce the cost of vaccination and provide a convenient means to vaccinate smaller fish as well as boost protection provided by injectable vaccination for stronger and longer lasting protection. Oral vaccines are now only beginning to emerge as an alternative to injection vaccines. The recent introduction of an ISA oral vaccine in salmonids (Table 3) may signal a shift toward a more robust development of alternative vaccination strategies for aquaculture. Novel technologies are under development that will continue to

provide innovative tools for improved vaccines in aquaculture and beyond. A prime example can be found in shrimp work where foreign antigens are displayed on the baculovirus viral surface to produce strong immune responses in shrimp [87].

Today, among a wide array of finfish species that are farmed worldwide, carps and other cyprinids constitute almost 63 % in terms of total aquaculture production in the world and 43 % in terms of measured value (Supplementary Fig. 1). However, currently most of the commercially available vaccines are targeted against high-value salmonids that constitute only about 6 % in terms of total global production and 17 % in terms of measured value of the product (Supplementary Fig. 1). This clearly signals a market opportunity for developing vaccines against non-salmonid species, especially considering that carps and other non-salmonids are increasing farmed at a high density in Asia and elsewhere in the world to mitigate the growing demand. So it is likely that in the coming years there will be a number of viral vaccines being developed and marketed against diseases in cyprinids and other non-salmonids.

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