Journal of Cell and Tissue Research Vol. 18(2) 6441-6450 (2018)
(Available online at www.tcrjournals.com)
ISSN: 0973-0028; E-ISSN: 0974-0910

Review Article

RECENT ADVANCES IN THE DEVELOPMENT OF TROPICAL FISH VIRAL VACCINES: A REVIEW

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Received: May 9, 2018; Accepted: July 12, 2018

Abstract: Infectious diseases have led to the most devastating problem in aquaculture sectors. Viral diseases are one of the major challenges to aquaculturists because it is very difficult to control once they occur in the system. Outbreaks of the viral disease lead to a greater economic loss in aquaculture production due to various improper farm managements. Prevention is the only way to control disease incidence caused by viral pathogens. Incorrect use of antibiotics and chemical drugs in aquaculture is associated with several deleterious side effects. Instead of using antibiotics and chemical drugs, vaccination is an effective tool in controlling, preventing, protecting and recovery of fish from virus diseases in cultured fish contributing to sustainability in the aquaculture sector. Therefore, the development of viral vaccines against emergent tropical viral diseases is crucial for promoting successful aquaculture production. The present review was based on different vaccines developed against tropical fish viruses.

Key words: Fish virus, Viral vaccines

INTRODUCTION

Aquaculture is one of the world's fastest growing industries in fish production. It is also an important sector contributing significantly to the world economy. In comparison to terrestrial farm animals and plants, aquatic animals require more attention in order to monitor and manage their health. Both farmed and wild fish are most susceptible to the various viral pathogens. Regrettably, viral diseases have been more difficult to control due to lack of knowledge about pathogenesis and its virulent nature. Particularly, intensive aquaculture has brought more disease problems which leading to great economics losses.

Many viral pathogens have been reported to cause mass mortalities of the fish population in tropical cultured fish. major viral pathogens in aquaculture include Rhabdoviruses infectious hematopoietic necrosis virus (IHNV) infecting non-salmonids including European eel, herring, cod, sturgeon, pike, shiner perch and tube snout; spring viramia of carp virus (SVCV) mainly infects in common carp; viral haemorrhagic septicaemia virus (VHSV) can be caused infection in flounders, eels, mimmichiog, stickleback, brown trout and striped bass in Canada and Epizootic ulcerative syndrome rhabdoviruses in striped snakeheads and a freshwater eel in Northern Thailand and Myanmar]; Betanodavirus infecting over 40 species including barramundi, Japanese parrotfish, turbot, European seabass, redspot grouper and striped jack; Reovirus mainly causing potential lethal infection in grass carp in china; Birnavirus – infectious pancreatic necrosis virus (IPNV) infection

from several fish species including tropical fishes such as Giant snakehead, Snakehead and eye-spot barb; Sand goby virus isolating from sand goby with ulcer disease reared in freshwater cages in Thailand. Picorna-like virus affecting grouper culture in Thailand; Koi herpes virus (KHV) is a highly virulent disease to common carp in US, Indonesia, Japan, Israel, Singapore, Philippines, Hong Kong, Thailand and Korea. Another major group causing lethal infections belong to Iridoviruses: [Megalocytivirus-Infectious spleen and kidney necrosis virus (ISKNV) causing infection particularly in cichlids, gouramis and poeciliids; Red sea bream iridovirus (RSIV) causing mortality in cultured juvenile red sea bream in Japan, White sturgeon iridovirus group (WSIV) in white sturgeon in North America and Canada and Russian sturgeon in northern Europe; Lympocystis virus causing infection to seabass in Thailand, Singapore and Malaysia; Ranavirus- Epizootic hematopoietic necrosis virus (EHNV) outbreaks typically involve juvenile Redfin Perch and farmed Rainbow Trout, Santee-Cooper ranavirus (SCRV) infecting largemouth bass, Singapore grouper iridovirus (SGIV) causing disease in brown-spotted grouper, Largemouth Bass Virus (LMBV) infecting largemouth bass and striped bass, Koi ranavirus (KIRV) infecting koi in India [1]. More recently, a new virulent ranavirus isolated from marine ornamental fish has been reported in India [2]. Significant losses of cultured and wild populations of fish occur every year due to viral diseases across the world. Disease prevention approach by an effective vaccination method is the superlative practice for successful aquaculture production in a sustainable manner.

Status of vaccines and vaccination of farmed fish; A vaccine may be defined as a preparation of microorganisms or their antigenic components which can induce protective immunity against the appropriate pathogenic microorganisms but which does not itself cause disease [3]. Vaccination of farmed fish plays an important role in commercial fish farming in order to mitigate diseases caused by specific pathogens. There are several diseases which might be controlled by vaccination [4]. During the last two decades vaccination has become established as an important method of preventing infectious diseases in farmed fish. The complete positive effect of vaccination is reduced mortality in farmed fish. Vaccination is also important for the future of the fish farming industry that contributes to a sustainable biological production with negligible consumption of antibiotics. Immunization of fish can be done by one of three major modes such as injection preferably intraperitoneally, immersion, usually by dipping the fish in a diluted vaccine solution and oral administration of the vaccine. Generally, injection method is better when compare to immersion and oral administration [5].

In general, the fish vaccine is consists of three major categories, namely, killed whole cell vaccine, live-attenuated vaccine and recombinant DNA-based vaccines [5]. Viruses must be required cultures of fish cells for their replication because the cost of vaccines development based on inactivated viruses is usually too high to make this strategy economically viable [6]. Efficacy of these vaccines can be improved by using adjuvants, immunostimulants or vaccine carriers. The successful development of bacterial vaccines has led to a decrease in the use of antibiotics in aquaculture. Several vaccines against bacterial diseases are developed and used in aquaculture worldwide [7]. The introduction of effective vaccines is most important in salmonids and other cultivable species like sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) [7]. Several efficient and cheap vaccines against various infectious diseases caused by pathogenic microorganisms like bacteria and virus have been developed and confirmed experimentally. There are several commercial vaccines available for the major fish viral diseases in which majority of the viral vaccines are based on inactivated virus and target salmonid viruses like infectious pancreatic necrosis virus (IPNV), infectious salmon anaemia virus (ISAV), and salmon alphavirus (SAV) [8]. The knowledge about different vaccines and their protective immunity is to great extent based on challenge or field experiments.

Vaccination against SVCV: Spring viraemia of carp virus (SVCV) is the causative agent of spring viraemia of carp (SVC). SVC was reported in various regions like China, Iran and Northen hemisphere [9]. The SVCV is a rhabdoviral pathogen that cause infection to both wild and culture fishes, but it mainly affects common carp (*Cyprinus carpio*) in European aquaculture. SVCV has been reported as most important virus disease of ornamental and also wild and farmed carp [10]. Natural infections of SVCV were reported from various cyprinid fish including koi (*Cyprinus carpio koi*), goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*), silver

carp (*Hypopthalmichthys molitirix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), orfe (*Leucisucus idus*), tench (*Tinca tinca*) and bream (*Abramis brama*) [11,12]. Experimental infections were also reported in other cyprinid species included roach (*Rutilus rutilus*) whilst zebra fish (*Danio rerio*) and the golden shiner (*Notemigonus crysoleucas*) have been infected with SVCV by intraperitoneal injection [12,13].

A mixture of 10 SVCV DNA vaccine plasmids containing partial or complete glycoprotein (G) gene fragments from the European SVCV reference strain (Fijan-Genogroup Id) has been tested in carp [14]. The majority of treatment groups had elicited little protection with RPS values of "11 to 33%. A DNA vaccine with an SVCV G gene from a North American isolate has designed and tested in four trial experiments [15]. In order to test the vaccine efficacy, a reliable challenge model was developed by testing the susceptibility of different fish host species to the North American SVCV that induced rapid and reproducible infections in the host. All the experiment trial studies indicated that the pSGnc DNA vaccine provides protection in vaccinated fish against challenge at low, moderate and high virus doses of the homologous virus. The non-vaccinated controls and mock construct vaccinated fish encountered high cumulative mortalities ranging from 70 to 100% [15]. DNA vaccines have been found to be very efficient against novirhabdovirus disease in salmonids and it also induced rapid and long lasting protection [16].

Oral vaccination is an effective and this strategy has shown a successful induction of the antiviral response against viral diseases in different fish species [17]. According to that oral vaccines must pass safely through the stomach and should be digested in the anterior segment of the intestine. Therefore, development of efficient vectors for delivery of vaccine antigens could offer a useful approach to vaccination against SVCV [18].

Vaccination against VHSV and IHNV: Viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV) are two different viral species that belong to the *Rhabdoviridae* family. Young fishes are more susceptible to the IHNV and infected fishes were shown to have impairment of osmotic balance in connection with oedema which leads to mortality. Inactivated vaccine was initially attempted against VHSV and IHNV. Inactivated viral vaccine can be made by β-propiolactone treatment and it has found to be effective in inducing long-term protection when delivered by intra-peritoneal injection [19,20]. Inactivated vaccine delivery by bath immersion is not considered suitable for mass delivery in young fish due to the insufficient significant protection of fish against virus challenge [21].

Several approaches have already been tested to get fully attenuated viruses that could be used as safe live-attenuated vaccines. Live attenuated viruses by cell culture passages were developed to obtain a thermoresistant VHSV strain [22] and several IHNV strains [23,24]. An oral vaccine for immunization of rainbow trout (*Oncorhynchus mykiss*) has been developed against VHSV [25]. The attenuated VHSV strain as a vaccine was used to examine efficacy of this delivery method in three animal challenge experiments *in vivo*. Animals challenge studies were carried out against highly virulent VHSV for six weeks after vaccination and mortality was also recorded. The experiments showed that virus is released from the vaccine preparation delivered orally, subsequently penetrated the gut mucosa and led to higher expression levels of MHC class II and CD4 mRNAs when compared to control guts. Indirect immune fluorescence test also developed to detect VHSV vaccine in the gut [25]. Although, the live attenuated VHSV or IHNV strains are not used commercially, because of their reversion frequency to pathogenic wild-type virus [26]. Biacchesi who has found that recent advances in reverse genetics make it possible to manipulate VHSV and IHNV RNA genomes to introduce targeted mutations including nucleotide substitution, gene deletion, heterologous gene exchange and additional gene insertion, with the goal to attenuate the virus and use it as a gene vector [27].

Attenuated *Aeromonas salmonicida* has been tested as a vector for the expression of VHSV and IHNV G protein fragments when used to immunize fish by bath immersion or spray were moderately protected against homologous challenge [28]. The DNA vaccine against IHNV has provided significant protection when administered through either waterborne or injection in rainbow trout [29, 30]. Intraperitoneal injection provided lower levels of protection when compared with intramuscular injection and gene gun immunization with DNA vaccine against IHNV and VHSV in rainbow trout

[30,31]. Lorenzen et al. have been reviewed DNA vaccines based on the glycoprotein genes of the salmonid rhabdoviruses VHSV and IHNV which were demonstrated to be very efficient in inducing a protective immune response against the respective diseases in rainbow trout [32]. During 2005, DNA vaccine against IHNV for Atlantic salmon (APEX-IHN; Novartis Animal Health Canada Inc.) was licensed and commercialized in Canada [33]. The licensed DNA vaccine against IHNV in Atlantic salmon is developed for commercial usage in Canada. Replicating vaccine could be one of the ideal vaccine for fish in terms of cost, protective efficacy and ease administration, where other efficacious vaccines such as killed virus and DNA vaccine which are not yet suitable for mass delivery in young fish [21].

The recombinant G proteins of VHSV and IHNV have been produced using several prokaryotic and eukaryotic systems which include *Escherichia coli*, *Caulobacter crescentus*, *Yersina ruckeri*, yeast and baculovirus/insect cells. Recombinant G protein is also not suitable for mass delivery to fish fry due to the cost of production and route of delivery. Although, there was complexity in expressing a G protein with the correct antigenic structure it was found to induce protective immunity in fish [21]. Recombinant IHNV and VHSV developed by deletion of the NV gene (ΔNV) resulted in irreversible attenuation of virus pathogenicity in rainbow trout [34,35], yellow perch [36] and Japanese olive flounder [37]. In those studies, viruses were highly attenuated which exhibited low levels of mortality when the viruses were injected intraperitoneally. Biacchesi and Brémont have suggested that genetic immunization appears to be highly efficient for vaccination against both VHSV and IHNV but, an improved delivery system is required before this method could gain widespread use [21].

Encapsulated pIRF1A-G vaccine in alginate microspheres and orally administered to rainbow trout shown to be effectively protecting the vaccine from degradation in the fish stomach and ensure early delivery of the vaccine to the hindgut. In comparison to the injection route of vaccine administration, the oral route required approximately 20-fold more plasmid to induce the expression of significant levels of IHNV G transcripts in vaccinated fish [38].

Vaccination against GCRV: Grass carp (*Ctenopharyngodon idellus*) is an important freshwater aquaculture species widely cultured in Asian countries. An efficient and economic prophylactic measure against GCRV is the most important to improve the production of grass carp desired for the carp farming industry [39]. A novel candidate subunit vaccine has been developed against grass carp reovirus Guangdong strain (GCRV-GD108) isolated in Guangdong province, China [40]. The study showed that all provide protection against virus infection (47-82%) and the relative percent survival reached 82% in the group immunized with recombinant protein (rVP4) a dose of 3 μg/g (protein/fish weight). The expression level of IgM in head kidney of grass carp was also significantly increased in immunized groups than in blank control [40]. The study clearly suggests that the rVP4 can induce a strong immune response in fish. Wang *et al*. have investigated the protective immunity against grass carp reovirus in grass carp induced by DNA vaccination using single-walled carbon nanotubes (SWCNT) as delivery vehicles [39]. To enhance the efficacy of a vp5 DNA vaccine against GCRV in juvenile grass carp, a novel SWCNTs-pEGFP-vp5 DNA vaccine linked vp5 recombinant in the form of plasmid pEGFP-vp5 and ammonium-functionalized SWCNTs was prepared using a chemical modification method in this study. SWCNTs-pEGFP-vp5 vaccine was significantly enhanced the antibody levels, immune-related genes, and relative percentage survival in immunized fish [39]. Moreover, they have found good immune protective effect in both groups immunized through intramuscular injection and bath administration.

Vaccination against Betanodavirus:This virus comes under the family of *Nodaviridae* and is the etiological agent of viral nervous necrosis (VNN), also known as viral encephalopathy and retinopathy (VER). The nodavirus was reported to infect both freshwater and marine fishes [41]. Betanodavirus infects several fish species with high mortality up to100% across the world. Commercial vaccines against NNV are currently unavailable, although experimental vaccination using several approaches has shown high protection. To date, different types of vaccines have been developed and tested against NNV, including those made with inactivated virus, virus-like particles (VLPs), recombinant C protein and synthetic peptides from the C protein. An effective protection against NNV is probably depended upon proper stimulation of B- and T- cells [42]. Vaccination trials using *Escherichia coli* -

expressed nodavirus capsid protein have enhanced survival and presence of virus-neutralising antibodies after immunization. Such vaccination strategy could be useful for fish infected by NNV at grown-out stages [43,44].

Formalin inactivated NNV has been studied in juvenile sevenband grouper, *Epinephelus septenfaciatu* [45]. Intraperitoneally immunized juvenile sevenband grouper with inactivated NNV showed lower mortalities than control fish. Nishizava et al., have investigated live NNV immunization in combination with poly-IC against RGNNV infection in sevenband grouper [46]. Horizontal infection of NNV in vaccinated fish has been evaluated with live NNV to naïve fish in a cohabitation experiment [47]. The mortality rate of 10.5% was observed in the vaccinated fish indicating protective immune response from the challenge with homologous NNV.

Humpback grouper (*Cromileptes altivelis*) vaccinated with the mixture of recombinant C proteins from three Japanese isolates from the red-spotted grouper NNV was found to induce with a protective immune response following challenge with an Indonesian NNV isolate [48]. Lin et al. have also revealed that an *Artemia*-encapsulated recCP induced a protective immune response in orange-spotted grouper [49].

Investigation on the immunogenicity of betanodavirus VLPs and the protection against VNN in the European sea bass (*Dicentrarchus labrax*) has been demonstrated by Thiéry et al. [50]. Enzyme-linked immunosorbent assay and seroneutralization have also been performed on plasma from fish vaccinated with betanodavirus VLPs via intramuscular injection. VLPs elicited the synthesis of specific antibetanodavirus antibodies with neutralizing activity. Furthermore, fish vaccinated with VLPs showed protection against the live virus in a challenge study. The control of VNN through a practical vaccination mainly depends upon various parameters including administration route, effective dose and duration of protection. Due to their inherent nature of VNN, VLP-based vaccines could provide an efficient, safe, and economically viable strategy to control viral nervous necrosis in cultivable and other fish species [50]. Recombinant capsid protein is a promising candidate in vaccine development because they can be administered along with oil adjuvant [51]. *V. anguillarum* based vaccine [52] has been found to induce stronger and earlier immune response in addition to higher RPS when delivered orally through *Artemia* to orange-spotted grouper (*Epinephelus coioids*) [42].

Vaccination against IPNV: Infectious pancreatic necrosis virus (IPNV) is an aquatic birnavirus and is one of the most widely distributed viruses affecting most of the farmed finfish. The virus is responsible for the infectious pancreatic necrosis disease which causes high mortality in hatched salmonid fish. Frost and Ness have in Atlantic salmon vaccinated with Norvax® Protect- IPNV (NP-IPN) against IPNV and proved to be successful in the prevention of IPN in Atlantic salmon post-smolts [53]. Expression of IPNV VP2-VP3 fusion protein in *Lactobacillus casei* and its immunogenicity were investigated in rainbow trout [54]. The *Lactobacillus casei* as a system to express VP2-VP3 fusion protein and immunizing rainbow trout through oral dosing of antigen showed a 10-fold reduction in viral load compared with the control group. Min *et al*., have found that the strong potential of oral administration of an IPNV-live bacteria vaccine, even though protection was not complete [17]. When juvenile salmon are transferred from fresh water to seawater it has led to high economic losses worldwide [27], because of that the majority of commercial IPNV vaccines are developed with the aim to protect salmonid fish in the post-molt stage in seawater [16].

IPNV proteins have the powerful antagonistic properties against type I IFN induction in Atlantic salmon [55]. They investigated the effect of individual IPNV protein on IFNα1 induction. The study indicated that IPNV has developed multiple mechanisms to inhibit induction of IFNα1 transcription. In addition, they found that VP1 strongly activated IFNα1 transcription [55]. The oral pcDNA-VP2 vaccine has been developed against IPNV in rainbow trout [38]. The VP2 gene of IPNV encoded in an expression plasmid and encapsulated in alginate microspheres was used for the development of oral DNA vaccination in fish. A real-time RT-qPCR analysis revealed that lower levels of IPNV-VP4 transcription in rainbow trout survivors among vaccinated and challenged fish compared with the control virus group at 45 days post-infection. The strong expression level of immune-related genes includes IFN; cytokine/interleukin-related genes, such

as IL8, IL10, IL11; adaptive immune genes such as MHC1, IgM and IgT was detected in survivors from the virus control (carrier) group than in those from the vaccinated group [38].

Vaccination against koi herpes virus (KHV): Koi herpesvirus (KHV) is a severe threat to the common carp and ornamental koi industries with very high mortality. KHV was assigned to the genus - *Cyprinivirus*, species - Cyprinid herpesvirus-3 (CyHV-3) on the basis of homology to other cyprinid herpesvirus by the International Committee on the Taxonomy of Virus (ICTV).

Attenuated live vaccine appears to be the most appropriate method for mass vaccination of carp. Attenuated vaccine candidates can be produced by successive passages in cell culture [56]. The vaccine strain candidate was further attenuated by UV irradiation to increase the mutation rate of the viral genome [56,57]. Live attenuated vaccines potentially have many advantages resulting in the balanced system and responses involving both humoral and cellular immune system. The attenuated virus was isolated by 20 serial passages of the Israeli CyHV-3 isolates in koi fin cell. Harvested viruses have induced the disease in a small percentage of naïve fingerlings following injection and bathing [56, 58]. The attenuated virus clone has efficiently protected the immunized fish against challenge infection for long periods [56].

Field vaccination has been performed by immersion of tens of thousands of juvenile fish at a minimal age of 3 months in Israel [59]. Fish are collected from ponds, weighed and introduced to large vaccination tanks in which fish are held at a density 20–30% w/v and are supplemented with oxygen. As per the biomass of fish, the vaccine is poured into the tanks and allowed to incubate for 40–60min. Fish are then transferred to ponds where the water temperature is within the permissive range, for at least 5 days and then they are placed back in the earthen ponds [59]. Recently, the live attenuated strain of CyHV- 3 (produced by Kovax Ltd.) is the only licensed vaccine available in Indonesia and the US where it serves as a prophylactics treatment to prevent this deadly disease [60]. The vaccine has been licensed in Israel since 2005 and has been widely used in both ornamental and edible carp. Following field studies performed in Indonesia, the vaccine has been licensed for use in carp fingerlings since 2010 [60].

The formalin inactivated CyHV-3 vaccine candidates have been described by Yasumoto et al. [61]. The efficacy of vaccine determined by exhibiting 70% protection through feed by 21 days challenge experiment. The formalin inactivated CyHV-3 vaccine can be used for oral immunization in fish food [62]. Miyazaki et al. have developed an improved liposome-vaccine containing formalin-killed KHV antigens within the liposomal membrane compartment and examined its ability to the stimulate immune response in common carp by oral administration [63]. Carp immunized by 3 day oral administrations of the liposome-KHV vaccine showed 77% survival against a challenge with KHV while unvaccinated control fish showed 10% survival and the relative percent survival (RPS) was 74% [63]. An investigation of vaccination efficacy period against herpesviral haematopoietic necrosis (HVHN) caused by infection with cyprinid herpesvirus 2 (CyHV-2) in goldfish *Carassius auratus* has been carried out by Ito and Maeno [64]. CyHV-2 inactivated with formalin (0.1%, v/v) for 2 days at 4^{o}C was prepared to investigate the vaccine efficacy in goldfish. RPS) values of the vaccinated-4w (after 4 weeks) and 8w (after 8 weeks) groups showed 42.5% and 57.6%, respectively. The study suggested that the efficacy period of the vaccine is at least 8 weeks and a booster shot showed a tendency to enhance the protection against CyHV-2 in goldfish [64].

Vaccination against CCV: Channel catfish virus (CCV) is a herpesvirus responsible for serious infection in fry and fingerlings of channel catfish (*Ictalurus punctatus*) in North America, Russia and Honduras. The virus has caused serious economic losses of channel catfish (*Ictalurus punctatus*) [65]. This viral infection is most common during summer months. CCV vaccine development has had some disadvantages due to their ability to return to a virulent form, difficulties in delivering vaccine to eggs and large numbers of small fish, the reluctance of licensing authorities and the cost of development and production [66].

CCV as a vaccine vector for the channel catfish industry was investigated by inserting the *Escherichia coli lacZ* gene into the CCV genome and evaluating the immune response to the foreign gene product in catfish exposed to the recombinant vaccine candidate [67]. Antibody response was developed to the inserted foreign gene product which peaked at

approximately 15–20 days post-infection when channel catfish fingerlings were immersion exposed to CCVlacZ. The anti-β-galactosidase response was also significantly enhanced when the fingerlings were re-exposed to the virus 20 days after the initial exposure [67]. Protective immune response with early and late transcripts of the *Ictalurid herpesvirus* I (IHV-1) by DNA vaccination in channel catfish has been investigated by Nusbaum et al. [68]. The experimental study indicated that the single injections of DNA expression constructs containing ORF 59, encoding the envelope glycoprotein, or ORF 6, encoding a presumptive membrane protein, have found to be induced the strongest resistance to challenge compared to uninjected, PBS injected or vector injected groups [68].

Harbottle et al. evaluated the DNA vaccination against channel catfish virus (CCV) by comparing 11 encoded genes, multiple doses, co-delivery of DNA vaccines and the resultant immune responses, as well as making a direct comparison with previously published DNA vaccines for CCV [69]. The polymerase chain reaction was developed to amplify the open reading frame (ORF) for each gene, cloned into pcDNA3.1/V5/His-TOPO and expression level of the predicted molecular weight proteins was also confirmed in cell culture. Each vaccine was injected intramuscularly and evaluated by immersion challenge. Unfortunately, they did not found to offer significant protection in any experiment, including groups receiving multiple constructs [69].

Vaccination against LCDV: Lymphocystis disease virus (LCDV) is an iridovirus that affects more than 25 marine fish species [70]. Lymphocystis virus was genetically classified into two different species; LCDV-1, which occurs in flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*), whereas LCDV-2 is usually found in dab (*Limanda limanda*) lesions [71,72]. Lymphocystis viruses cause severe disease, mortality and economic losses in farmed fish and ornamental fish in wild as well as hatcheries.

Immune response of DNA vaccine has been investigated against lymphocystis disease virus and subsequently analysed the expression profiles of immune related genes after vaccination of Japanese flounder (*Paralichthys olivaceus*) [73]. After DNA vaccination, RT-PCR showed that significant changes in the expression of selected genes include MHC class I α, MHC II α, T cell receptor (TCR), tumour necrosis factor (TNF), tumour necrosis factor receptor (TNFR), Mx, interleukin (IL)1β, CXC and IL8R [73]. Zheng et al. reported that the construction of a vaccine against LCDV using nucleic acid vaccination technology [74]. A fragment of the major capsid protein encoding gene from an LCDV isolated from China (LCDV-cn) was cloned into a eukaryotic expression vector pEGFP-N2, yielding a recombinant plasmid. The recombinant plasmid was then inoculated into Japanese flounder via two routes (intramuscular injection and hypodermic injection) at three doses (0.1, 5, and 15 μg). Vaccine administration T-lymphopoiesis in different tissues and antibodies raised against LCDV was evaluated. These studies indicated that the recombinant plasmid induced unique humoral or cell-mediated immune responses depending on the inoculation route and conferred immune protection [73, 74].

Vaccination against ISKNV: Infectious spleen and kidney necrosis virus (ISKNV) is the type species of genus *Megalocytivirus* in the family *Iridoviridae*. The formalin-killed cell-cultured (FKC) vaccine has been successfully developed against ISKNV [75]. Fish immunized with the FKC vaccine were recorded greater than 90% protection against virulent ISKNV in the immuno-protection experiments. Sera derived from the immunized fish appear to be significantly inhibited the virus infection both *in vitro* and *in vivo*. IgM purified from the immunized fish sera has shown efficient neutralization effects *in vivo* and it strongly suggests that antibody-mediated immunity may play an important role in the FKC vaccine [75].

Protective immunity of recombinant major capsid protein of ISKNV has been studied against iridovirus disease in mandarin fish [76]. In this study, the gene encoding the major capsid protein (MCP) is a predominant structural component of the iridovirus particles was cloned into a temperature induction prokaryotic expression vector pBV220 and a recombinant protein was detected about 50 kDa in molecular weight. The juvenile mandarin fish were vaccinated with recombinant protein re-natured by dialysis with recombinant MCP emulsified with ISA 763 adjuvant by intraperitoneal injection. The MCP injected (50 μg/fish) group has shown significantly greater survival than the others following challenge infection with ISKNV. According to these Fu et al. [76] suggested that humoral immunity and cellular

immunity of mandarin fish are induced by recombinant MCP and the best immune dose was 50 µg/fish.

Similarly, protective immunity against ISKNV has been developed by immunization with DNA plasmid containing MCP gene in Chinese perch *Siniperca chuatsi* [77]. The protective immune response was induced by intramuscular injection of Chinese perch with pcMCP combined QCDC adjuvant. The expression levels of type I IFN system genes including IRF-7, IRAK1, Mx and Viperin shown to be up-regulated at 6 h and it reached a peak at 48 h. Chinese perch vaccinated with pcMCP added QCDC adjuvant has shown that the relative percent survival (RPS) is 80% in a challenge trial on the 28th day post-vaccination. Real-time PCR has also revealed that the levels of viral load in the dead fish of the vaccinated group were significantly higher than those in mock-vaccinated fish [77].

Vaccination against RSIV: Red seabream iridovirus (RSIV) has caused significant economic losses in aquaculture. This disease was first observed in red sea bream (*Pagrus major*) aquaculture in Shikoku Island, Japan, in 1990 [78]. The protective effect of immunization of juvenile red seabream, *Pagrus major*, with DNA plasmids encoding the viral major capsid protein (MCP) and an open reading frame (ORF) which contains a transmembrane domain was investigated against red seabream iridovirus (RSIV) [79]. At the 15th day post-vaccination, the expression level of the MHC class I transcript was significantly upregulated in the DNA-vaccinated fish and the relative level of expression was maintained until the 30th day post-vaccination [79]. The relative percentage survival (RPS) values of DNA vaccinated fish were in the range of 42.8 to 71.4% in two experimental runs and these were significantly different from the control groups. Oh et al. [80] have developed a live RSIV vaccine in rock bream by the concept of the viral multiplication in fish is downregulated by maintaining fish at far from optimum temperatures at the onset of disease. Rock bream inoculated with RSIV were reared at 21 – 30 °C in which mortality rate was e" 90%, while no mortality was observed in fish that received an RSIV inoculation and were reared at d"18°C. The fish surviving the RSIV infection at low rearing temperature were strongly protected from rechallenge with homologous RSIV and the study suggested that a positive effect of a live RSIV vaccine for rock bream.

CONCLUSION

Prevention of virus diseases outbreaks are the major challenges for aquaculture sectors. Antibiotics and other chemical substances are sometimes used to control various diseases in aquaculture, but they can produce resistance in pathogenic and non-pathogenic microorganisms which are a threat both to the environment and even to human and animal health. Therefore, the effective control of infectious viral diseases without chemicals is more and more important in the cultivation of tropical aquatic organisms. Good husbandry practices and health management in which vaccination is an indispensable tool for disease control in aquaculture. Better understanding of the specific disease pathogenicity, cellular and mucosal immune system of different fish species, adjuvants, immunostimulants and antigen delivery system would help to the development of successful fish viral vaccines in aquaculture. The effective vaccination of tropical fishes against various viral diseases has been investigated experimentally, but successful vaccines have not been developed at the farm level. Therefore, the fish pathologist, biotechnologist, immunologist and the vaccinologist should be involved with cooperation among themselves to achieve effective fish vaccines development.

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