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Strategies and Techniques for Enhancing Fish Health and Averting Disease Outbreaks in Aquaculture Settings through the Use of Vaccination: A Review

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ABSTRACT

The fastest–growing animal food-producing agricultural sector in the world is aquaculture, which accounts for almost half of the world's food fish production.However, the expansion of high-density fish populations also brings forth a challenge–the rapid transmission and spread of infectious agents including several viral, bacterial, and parasitic diseases. Expansion and successful development of sustainable aquaculture practice and increasing production largely depends upon the prevention and control of outbreaks of several emerging and re-emerging infectious diseases which can result in economic loss, food safety hazards, and environmental hazards. Vaccination strategies have become highly effective and economical in protecting the health of fish and other aquaculture organisms from various infectious diseases such as edwardsiellosis, motile aeromonas septicemia (MAS), Tilapia Lake Virus (TiLV) disease, infectious salmon anemia (ISA), vibriosis, and white spot disease etc. An ideal vaccine is expected to be safe, effective, economical, and easily administered. Most of the available fish vaccines are empirically designed vaccines based on inactivated or live attenuated bacterin vaccines. Novel advances in the fields of immunology, biotechnology, and molecular biology have led to the development in designing novel and effective fish vaccines and the improvement of the existing vaccines to provide sufficient immune protection against diseases. This review investigates the currently available fish vaccines for use in finfish aquaculture against different infectious diseases different mode of vaccine administration with addressing pros and cons in detail. The information was collected from different secondary sources, and then compiled systematically.

KEYWORDS: Aquaculture, bacterial disease, bacterin, live vaccines

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1. INTRODUCTION

Aquaculture plays an important role in producing balanced and nutritious food in agricultural sector also provides a great contribution to food security and socio-economic development in many countries. The aquaculture practices of world have shifted from extensive to semi-intensive and intensive culture system where high-value fish species are reared at higher stocking density using commercial feeds. But there are many constraints against the sustainable development of this aquaculture sector. Among these, the major constraint causing devastating threat to the aquaculture production and causing huge loss to the farmer is disease in any type of culture methods extensive, semi-intensive and intensive aquaculture system which results in economic loss (Mishra et al., 2017; Joshna et al., 2024).

In all farms of intensive culture systems, where single or multiple species of fish are reared at high density,and also by adopting general management practices including bio-securitymanagement and water quality are critical for aquatic animal production (Bone et al., 1995). Though, there are some important challenges to develop productiveand sustainable aquaculture which are associated with all facilities above vulnerable to disease outbreaks because many pathogenic organisms which are present in the environment are opportunistic and parasitic in nature and may lead to infection, signs of disease may be found on some fish (Roberts, 1978; Woo et al., 2002; Komar et al., 2004). Appearance and outbreak of disease in aquatic animals mainly depends on the synergy between thehost, pathogen and environment. Accordingly, Control of diseases in aquaculture sector mainly relies on a combination of good management practices, use of the few approved and commercially available drugs and vaccines that prevent the infection (Nicholson, 2006; Mishra et al., 2017).

Bacterial infectious diseases are the most prevalent disease challenges in fish farming while viral diseases are more difficult to control due to the lack of anti-viral therapeutics.Lack of information on the mechanisms of viral pathogenesis and disease resistance in fish these are two main challenges in developing effective viral vaccines against viral infections. The unavailability of efficient treatment modules to control viral and bacterial diseases posed a vital demand for developing and implementing effective approaches for prevention and control of these diseases. Besides, the adverse effects of infectious diseases have also demanded the strategic development of vaccine design because indiscriminate use of antibiotics in aquaculture can raise problems of developing bacterial resistance, food safety hazards, and environmental problems. Treatment of many bacterial infections in fish using only antimicrobials is impossible.

In this situation, fish vaccination has become the most important, easy, and effective approach to prevent and control infectious diseases in fish. Vaccination is a process by which a protective immune response is induced in animals by administering preparation of antigens derived from pathogens and made non-pathogenic by means of heat or other ways. Fish represent the lowest but diverse group of vertebrates (Sahoo et al., 2021; Bedekar and Kole, 2022), possess both innate and adaptive immune systems for defense mechanism (Secombes and Wang, 2012). Vaccines stimulate fish's immune response and increase protection against diseases. Several significant progresses have been made in developing effective fish vaccines. But until now, only a few vaccines are commercially available against infectious viral and bacterial diseases for treating only economically important fish species. Prophylactic treatment and good management practices can usually prevent or reduce the susceptibility of fish/shrimp to disease. Although antibiotics can overcome bacterial diseases, consumer health and food safety issues prevent their use in aquaculture. Therefore, vaccination is the only best alternative to combat bacterial and viral diseases.Fish vaccination was started in 1942 against *Aeromonas salmonicida* infection. Advancing vaccination is the most important and the prior approaches for prevention and control of infectious diseases of fish. In aquaculture, vaccination is an important aspect that has been regarded as an efficient treatment method for the prevention of a wide variety of bacterial as well as viral diseases (Ma et al., 2019). Protection at stock level can be achieved through vaccination. Clarke et al. (2013) and Assefa and Abunna (2018) documented the development of vaccines for several fish species including trouts, salmons, tilapia seabream, sea bass, yellowtail catfish (*Ictalurus punctatus*), and Vietnamese catfish (*Pangasianodon hypophthalmus*) etc. (Su et al., 2021). A bivalent DNA vaccine developed against Infectious pancreatic necrosis (IPNV) could induce significant protective immune responses in rain bow trout as reported by Xu et al. (2017). Recent developments in vaccines and vaccinology provide significant breakthroughs in identifying new vaccine candidates to more effectively combat fish pathogens, including viral, bacterial, and parasitic agents.

2. VACCINES

Vaccines are various preparations of antigen derived from specific pathogenic organisms that are rendered non-pathogenic. They stimulate the immune system both innate and adaptive and increase the resistance to disease from subsequent infection by specific pathogen Mohamad et al., 2021). Vaccination is a preventive measure that protects fish against a future disease and the associated cost due to morbidity, mortality and therapeutics treatment. A vaccine only protects against specific disease. For example, a vaccine against *Streptococcusiniae* infection will protect the vaccinated fish against *Streptococcus iniae* but it will not protect the fish against *Vibrio anguillarum*. The first report on fish vaccination was done by David C. B Duff and he is regarded as "Father of fish vaccination". A vaccine can be either water or oil based. Typically, injection vaccines are oil based as the oil provides adjuvant qualities. This means that the oil increases the effectiveness of the vaccine as well as the duration of the desired protection. The choice of vaccine depends on the particular case. It will depend on whether protection can be obtained, the duration of the protection possible verses the required duration, the final cost of the vaccine in relation to the benefit to the farmer and the registration limitation imposed by authorities in the countries where the vaccine is marketed.

3. TYPES OF FISH VACCINE FORMULATION

3.1. Bacterins

Inactivated vaccines are the most of the bacterial vaccines that are used in aquaculture which are obtained from a broth culture of a specific strain(s) that are subjected to subsequent formalin inactivation (Toranzo et al., 1997). The immune system of fish/shrimp gets activated with interaction of bacterins which in result leads to production of antibodies (i.e., the humoral immune responses) (Roy, 2011). Whereas with some vaccine acceptable levels of protection are achieved with aqueous formulations administered by injection or immersion, for other bacterins, such as those devised for Salmonids against *Aeromonas salmonicida subsp. salmonicida*, an acceptable level of protection can only be achieved by immunization with oil-adjuvanted bacterins delivered by injection (Toranzoet al., 2009). Different inactivating agents variably affect the efficacy of inactivated vaccine and duration of protective immunity produced post-vaccination. Earlier study reported that when b-propiolactone (BPL), binary ethylenimine (BEI), formaldehyde and temperature were used as inactivating agents for infectious haematopoietic necrosis virus (IHNV) in rainbow trout (*Oncorhynchus mykiss*), the BPL inactivated IHNV whole virus vaccine illustrated maximum efficacy comparatively (Anderson et al., 2008; Tang et al., 2016). The best results have been obtained with bacterins that comprised of both bacterial cells and extracellular products.The formalin-killed cells of *Pseudomonas anguilliseptica* could play an important role in immunization of olive flounder against these bacteria (Jang et al., 2014). Earlier study has raised doubts about the effectiveness of bacterin-killed vaccines in controlling bacterial kidney disease (BKD), pointing out the lack of understanding of the vaccine's potential and its virulence mechanisms (Delghandi et al., 2020).Studies suggested that inactivated virus vaccine using antigen ALV405 of SAV is capable of protecting the salmonid fishes from infection of Pancreas disease (PD) efficiently either with usage as a single vaccine candidate or as polyvalent vaccine (Karlsen et al., 2012; Jang et al., 2014).

3.2. Live attenuated vaccines

Vaccines that have been prepared using live microorganisms (bacteria, viruses) that have been grown in culture and those no longer have the properties that cause significant diseases are called as live attenuated vaccines. Such kinds of vaccines potentially have many advantages in aquaculture. If the vaccinated fish shed the vaccine strain an effective dissemination of the antigen in the population would take place overfor an extended period. They also have the advantage that is to stimulate the cellular branch of the immune system (Toranzo et al., 2009; Shoemaker et al., 2009; Wang et al., 2014). A live vaccine commercially available under the name "Renogen" and licensed by Novartis in South Africa (S.A.) has been reported for preventing BKD (Toranzo et al., 2009). Further Mohd-Aris et al. (2019) successfully developed a genetically attenuate the *V. harveyi* strain *MVh-vhsV. harveyi* mutant by protease deletion, as a candidate live-attenuated vaccine against vibriosis in *Epinephelus sp.* The strain *MVh-vhs* was shown to be safe when tested in the host, suggesting that the attenuation of virulence-associated protease *MVh-vhs* decreases the virulence properties.

Some live vaccines have been tested experimentally: *Aeromonas salmonicida, Edwardsiellatarda, E. ictaluri.* Beforeallowing the usage of these vaccines in to the field problems concerning safety, persistence in the fish and in the environment, reversion to virulence, risk of spreading to non-target animals including wild fish, among others, must be resolved (Munangandu et al., 2015; Loessner et al., 2012).Besides killed vaccines, live-attenuated vaccines are under strong consideration to be commercialized as fish vaccines due to their advantages. At present, only an *E. ictaluri* attenuated live vaccine has been licensed in the USA to be used by bath in 9days old fish to prevent ESC of catfish (Toranzo et al., 2009) (Table 1).

3.3. DNA vaccines

Vaccines that compose a particular portion of genetic material that can be incorporated into the animal, after being incorporated into the animal it has an ability to produce an antigen i.e., particular immune-stimulating portion of a pathogen, continuously, thus providing an "internal" source of vaccine material (Roy, 2011). These vaccines have theoretical advantages over conventional vaccines: in mammals, the specific immune response after DNA vaccination encompasses antibodies; T-helper cells and cytotoxic cells. Before administering DNA vaccines in commercial purpose in aquaculture safety for the fish, environment and consumer have to be taken

Table 1: Commercially available vaccines against major infectious bacterial diseases of finfish

Sl. No.	Target disease	Target pathogen	Target fish species	Type of vaccine	Product name	Route of administration
1.	Bacterial kidney disease (BKD)	*Renibacterium salmoninarum*	Salmonids	*Arthrobacter davidanieli*	live culture Elanco: Renogen	Injection
2.	Edwardsiellosis/ Enteric septicaemia of catfish (ESC)	*Edwardsiella ictalurid*	Catfish spp., that is, channel catfish, freshwater catfish, striped catfish, brown bullhead, Danio spp.	*Edwardsiella ictaluri*, a virulent live culture	MSD Animal Health: Aqua Vac-ESC™	Immersion
3.	Vibriosis, ISA, Wound disease	*Vibrio anguillarum, V. salmonicida, Aeromonas salmonicida* subsp. *Salmonicida*	Salmonids	*Vibrio anguillarum, serotypes* O1 *and* O2α, *V. salmonicida* and *Aeromonas salmonicida* subsp. *salmonicida, inactivated*	Pharmaq: Alpha Ject5200	Injection
4.	Infectious salmon anaemia (ISA), Furunculosis, Vibriosis	*Aeromonas salmonicida, Vibrio anguillarum, V. ordalii*	Salmonids	*Aeromonas salmonicida, Vibrio anguillarum serotypes* I and II, *V. ordalii and V. salmonicida serotypes* I and II, inactivated	Forte VII	Injection
5.	Yersiniosis/ Enteric red mouth (ERM)	*Yersinia ruckeri* (Hagerman strain), inactivated	*Yersinia ruckeri* (Hagerman strain), inactivated	*Yersinia ruckeri* (Hagerman strain), inactivated	MSD Animal Heal th:AquaVac®ER;A quaVac®ERMOral	Immersion/ Oral
6.	Vibriosis	*Vibrio anguillarum, V. ordalii*	Rainbow trout, European	*V. anguillarum* 01 and (*V. ordalii*), inactivated 02a	MSD Animal Health: AQUAVAC® Vibrio Oral	Oral
				(*Listonella*) *Vibrio anguillarum* (biotype I and II), *V. ordalii*, inactivated	MSD Animal Health; AquaVAC®Vibrio	Injection
7.	Flavobacteriosis/ Columnaris disease/Rainbow trout fry syndrome	*Flavobacterium columnare*	Cyprinds, Salmonids, Catfish, Carp, Trout, Perch, Tilapia	*Flavobacterium columnare*, attenuated bacterin Immersion attenuated	Fry Vacc 1 and 2	Immersion

into consideration. As the DNA-sequence encodes only a single microbial gene, there should be no possibility of reversion to virulence, which is a critical factor in relation to environmental safety in aquaculture (Nicholson, 2006). It has been demonstrated that DNA vaccination induces a strong and protective immunity to some viral infections in fish, particularly the Rhabdoviruses infecting rainbow trout and Atlantic salmon, and also for channel catfish herpes virus infection (Nusbaum et al., 2002). A study by Muiswinkel et al. (2018) revealed that new DNA vaccines containing the glycoprotein of the SVC virus have been developed, with formulations administered either by injection or orally. These vaccines have shown great promise in preventing this infectious disease and protecting both young fish and carp production. Further, Liu et al. (2022) revealed that OMPs-based DNA vaccines can elicit immune responses and enhance immune protection in zebrafish against *A. hydrophila* infection withhigher survival rate of zebrafish.

3.4. *Polyvalent vaccines*

Majority of thefish vaccines developed, have been designed to target individual infections caused by specific pathogens. However, developing a polyvalent vaccine that addresses multiple pathogensat a time would streamline application and reduce the workload compared to other vaccination methods. Economically, using a single vaccine to address multiple diseases is more cost-effective than purchasing separate vaccines for each condition. This is the ideal vaccine formulation method which protects animal against the majority of the diseases to which a particular fish species is susceptible (Busch, 1997; Erfanmanesh et al., 2023). An earlier study reported that a feed-based polyvalent vaccine could elicit strong innate and adaptive immune responsesin Asian seabass (*Lates calcarifer*) against vibriosis, streptococcosis, and motile aeromonad septicemia offering a comprehensive solution and promising approach for effective, large-scale fish immunization in the aquaculture industry (Mohamad et al., 2021). In addition, these polyvalent vaccines must cover all the main serotypes of each pathogen existing in a particular geographical area. However, care must be taken in the formulation of polyvalent vaccines because the problem of antigen competition can occur, especially when these vaccines are administered by injection.

4. MODES FOR THE APPLICATION OF VACCINES

Fish vaccines are typically administered through various methods, such as oral, injection (intraperitoneal or intramuscular), or immersion (Adams et al., 2008). Determining the most effective administration route depend on factors such as the type and pathogenicity of pathogen, the fish age, size and immune status, vaccine production methods, labour costs etc (Yanong and Erlacher-Reid, 2012). Vaccine administration mainly varies with temperature, fish species and the level of infection andthe chosen delivery method can affect both the immunological response and the level of protection against the pathogen. Vaccination must be performed within a certain minimum period before the risk of their exposure to pathogen (Mondalet al., 2022). There are three major modes for the application of vaccines.

4.1. *Oral vaccination*

With oral vaccination, the vaccine is either mixed with the feed coated on the top of the feed or bio encapsulated. When vaccines are used as a top dressing in feed, a coating agent is usually applied, either to prevent leaching of the antigen from the pellets or toprevent breakdown of the antigen in the acidic environment of the fish stomach. For sensitive antigen, various micro encapsulation methods are being evaluated and tested.Bio encapsulation is used where fish fry is to be vaccinated. In this case, live feed, such as *Artemia nauplii*, Copepods or rotifer, are incubated in a vaccine suspension after which they are fed to the fry. Since these live organisms are non-selective filter feeders, they will accumulate the antigen in their digestive tract and as such, transform themselves into living microcapsules. An earlier study conducted on oral vaccines against IPNV reported that genetically engineered recombinant *Lactobacillus casei* provided promising protection in salmonids (Hua et al., 2021). Recently, a new feed-based, whole-cell oral polyvalent vaccine was developed to combat vibriosis in Asian sea bass (*Lates calcarifer*). This vaccine shows promise as a candidate for large-scale immunization of fish in aquaculture (Mohamad et al., 2021).

Oral vaccination has the advantage that it is a very easy vaccine administration method with no stress to the fish. However, oral vaccines have a very short-term stability once mixed with the feed. In most cases, only limited protection can be obtained and the duration of protection can be rather short. Moreover, although oral vaccination is the preferred method from a fish farmer's perspective, at present there are few, if any, effective oral vaccines in the market (Radhakrishnan et al., 2023).

4.2. *Immersion vaccination*

Epithelium of skin and gills has mechanism to protect fish in a broad as well as in a specific way. Immersion vaccination works on the ability of mucosal surface is to recognise pathogens they had been come into contact with. When fish are immersed in water containing the diluted vaccine, the suspended antigen from the vaccines may be absorbed by the skin and gills. Then, specialised cells, such as antibody secreting cells, present in the skin and gill epithelium will be activated and will protect the fish when fish are exposed to the live pathogen at a later stage (Bogwald and Dalmo, 2019).

There are two types of application method of vaccine in this immersion vaccination they are dip and bath. In dip vaccination, the fish are immersed for a very short duration, usually 30 seconds, in a highly concentrated vaccine solution, usually 1 part vaccine product to 9 parts water. With bath vaccination, fish are exposed to for a longer period, usually 1 to several hours, in a lower concentration of vaccine (Figure 1).

Of the two alternatives, dip vaccination is more widely used

Figure 1: Immersion vaccination

since it facilitates faster vaccination of large number of fish. Immersion vaccination is widely used for vaccination of fry from 1 to 5 g (Komar et al., 2004). A recently conducted study examined the effectiveness of ERM immersion vaccines against the pathogen *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*), focusing on both biotypes 1 and 2 with serotype O1. The results demonstrated that both biotypes can protect the fish from infection(Yang et al., 2021). The limitations of emergency vaccination are that the duration of immunity is not very long and a booster vaccination is required when disease prevails over longer periods. Also, the method is impractical for larger size fish due to cost effectiveness and the stress that could be induced by vaccination (Muktar et al., 2018; Zanuzzoetal., 2020).

4.3. Injection vaccination

Initially, fish farmers may not follow the injection vaccination method as theyresult in causing stress resulting from the handling and injection of the fish will cause high mortality. Before injecting vaccine to fish it should be exposed to minute level of anaesthetic agents which prevent heavy movement of fish while injecting and allows the vaccine to penetrate into the body and also prevents mechanical injuries resulting in faster recovery. When injection vaccination is performed properly, mortality immediately after vaccination should not exceed 0.25%. Injection vaccines can be administered by intramuscular or intraperitoneal injection, but the latter is by far the most common (Figure 2). As intraperitoneal injection vaccination involves depositing the vaccine in the abdominal cavity, it is important that the needle should penetrate the targeted abdominal wall of fish by 1 to 2 mm. Short needles might deposit the vaccine in the musculature and cause inflammation and a bad immune response.

Injection vaccination has a number of major advantages

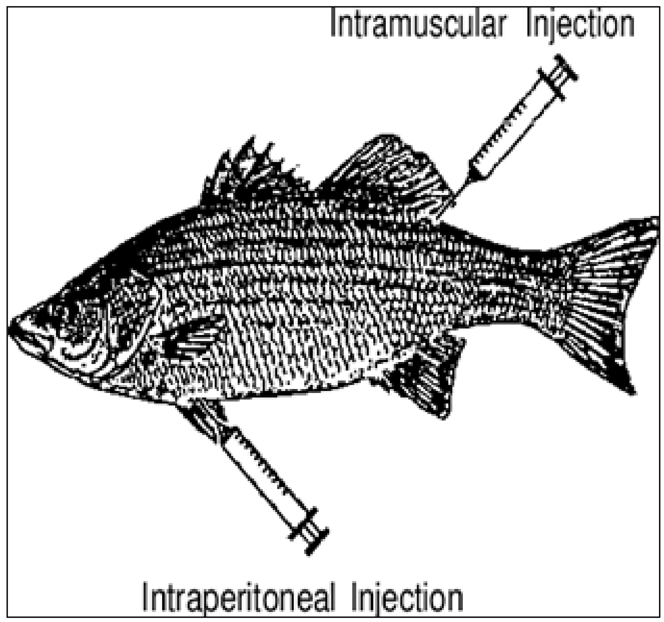

Figure 2: Injection sites in fish

that makes it a preferred vaccination method. Injection vaccination provides for a long duration of protection, i.e. for over a year, and it allows for multiple antigens is to be combined in a single vaccine and, therefore, in a single administration. In addition, the fish farmer is assured that every fish in the population has received the vaccine and at the correct dose. The injection volumes for fish are usually 0.1 or 0.2 ml and give protection throughout the production cycle of most farmed species. Injections are in general superior to any other vaccine application method; however, a practical point of view, they can only be applied to fish of 10 g or more.Generally, the injectable vaccines are reported to provide an excellent protection against bacterial infections like vibriosis (Magnadottir, 2010). A recent study of Erfanmanesh et al. (2023) revealed that though applying polyvalent vaccines by injection and immersion method has significant effects on immune protection and survival rate of fish against yersiniosis disease. However, the injection method is more effective and more suitable than the immersion method.

5. VACCINES USED IN AQUACULTURE

Currently, there are many commercial vaccines available against infectious bacterial and viral diseases of fish for using in aquaculture. The first commercialized fish vaccines were bacterial vaccine, introduced in the USA in late 1970s. These vaccines were inactivated whole-cell immersion vaccines and proved efficient in preventing many bacterial diseases. Advances in biotechnology and immunology has led to development and commercialization of many other fish vaccines like DNA vaccines, Nano vaccines, subunit vaccines, genetically modified vaccines and Polyvalent vaccines Shefat et al. (2018) shown in (Table 1). Modified live *Edwardsiella ictaluri* vaccine, produced in 2000 is the first licensed bacterial live vaccine in aquaculture. Inactivated bacterin vaccines and live attenuated vaccines have been proved efficient by immersion of fish. Simple inactivated bacterin vaccines works well against vibriosis but other bacteria are more difficult to control. Polyvalent vaccines, for Salmonids incorporating different *Vibrio* species and *Aeromonas salmonicida* as an antigen, are also available. DNA vaccines also were employed experimentally as safe live vaccines with a high level of success against Furunculosis but their approval for use in the field has not yet been forthcoming (Xuting Wang et al., 2002). Muiswinkel et al. (2018) reported that new DNA vaccines containing glycoprotein of SVC virus, including formulations introduced via injection or oral route, have been developed that are proven to be very promising in preventing infectious disease and protecting the young fishes as well as the carp production.Further, a recent study revealed that vaccination with a DNA vaccine (pcDNA3.1–ORF10) resulted in a high survival percentage in Tilapia sp., indicating that it could induce protective immunity in tilapia and may be a potential vaccine candidate for controlling diseases caused by TiLV (Yu et al., 2022). Viral diseases are more difficult than bacterial infectious diseases to control due to the lack of anti-viral therapeutics and effective viral vaccines. The World Organization for Animal Health has listed certain viral diseases as catastrophe for large scale aquaculture industry such as Epizootic Hematopoietic Necrosis (EHN), Koi Herpes Virus Disease (KHVD), Infectious Hematopoietic Necrosis Virus (IHNV), Spring Viremia of Carp (SVC) and Viral Haemorrhagic Septicaemia (VHS). Large numbers of research trials have been conducted but only a few viral vaccines are licensed. Currently available commercial viral vaccines for aquaculture are inactivated virus vaccines or recombinant protein vaccines (Table 2). No live attenuated vaccines are currently licensed for using in aquaculture, only one DNA vaccine against IHN (Infectious hematopoietic necrosis) disease is available. Inactivated viral vaccines are effective at high dose if delivered by injection, but cost-effective inactivated viral vaccines are difficult to develop where live viral vaccines showed good results in fish. The lack of effective viral vaccines is one of the main problems facing fish vaccinology (Dhar, 2014).

Currently, vaccines are available for some economically important bacterial and viral diseases, like there is a Salmon pancreas disease vaccine available under a PMA. Economically important fish species such as Atlantic salmon, rainbow trout, seabass, sea bream (*Sparus aurata*), barramundi (*Lates calcarifer*), tilapia, turbot (*Scophthalmus maximus* L.), yellowtail (*Seriola quinqueradiata*) and gold-striped amberjack (*Seriola dumerili*), striped jack (*Pseudocaranx dentex*) and channel catfish (*Ictalurus punctatus*) (Sommers et al., 2005). But unlike all the other Salmon vaccines designed for administration in a single injection this has to be given separately from any other injectable vaccine. To date there is not yet any vaccine available for trout. There are also some other bacterial and viral diseases of fish against which no vaccines have been developed yet. Novel advances in Biotechnology, and Immunology can lead to effective vaccine design and development against many pathogenic disease (Ellis, 1988; Dadar et al., 2017; Shefat et al., 2018).

Development of fish vaccines is a challenging task, due to a variety of pathogens, hosts, and the uniqueness of host-susceptibility to each pathogen. Major limitations in fish vaccine developments are less understanding of fish immunology, many vaccines unlicensed, not cost effective (expensive) and stressful on administration. It is hoped that, in near future vaccine developments may promote from the increased knowledge of the fish immune system and knowledge of pathogen and virulence mechanisms which helps in development of live vaccines, improved DNA vaccines, sub unit vaccines, polyvalent and monovalent vaccines, improved adjuvants and Oral delivery systems. New vaccination strategies, aquaculture expansion and disease investigation centre should be initiated.

6. SAFETY OF FISH VACCINE

Oneof the majorconcerns associated with vaccine is fish safety, especially due to their potentially poor immunogenicity, which can result in severe diseases and reduced production in vaccinated fish (Dadar et al., 2017). Vaccines must comply with safety guidelines, including tests using ten times the immunizing dose (Shoemaker et al., 2009). Inactivated or killed vaccines, are considered to be safe for marine animals. However, there are concerns of regaining the pathogenicity regarding the use of modified live vaccines. Further the DNA vaccines are seeming to be safe

Table 2: Commercial vaccines available against major infectious bacteria land viral diseases of fish

Sl. No.	Name of vaccine	Species vaccinated	Disease
1.	Arthrobacter vaccine	Salmonids	Columnaris disease
2.	*Aeromonas salmonicida* Bacterin	Salmonids	Furunculosis
3.	*Yersinia ruckeri* Bacterin	Salmonids	Yersiniosis
4.	*Edwardsiella ictalurii* vaccine	Catfish	Edwardsiellosis
5.	*Flavobacterium columnare* vaccine	Channel catfish, Salmonids, FW species	Columnaris disease
6.	*Vibrio salmonicida* Bacterin	Salmonids	Coldwater vibriosis
7.	*Listonella anguillarum* vaccine	Salmonids, seabass, yellowtail	Vibriosis
8.	*Vibrio anguillarum*-Ordalii	Salmonids, rainbow trout	Vibriosis
9.	*Vibrio anguillarum-salmonicida* Bacterin	Salmonids	Vibriosis
10.	*Edwardsiella ictaluri* Bacterin	Channel catfish, Japanese flounder	Enteric septicemia
11.	Free-cell *Aeromonas hydrophila* vaccine	Indian major carps	Dropsy
12.	*Streptococcus agalactiae* vaccine/ *Streptococcus iniae* Vaccine	Tilapia	Streptococcosis
13.	Enteric red mouth (ERM) vaccine	Salmonids	Enteric redmouth disease
14.	*Aeromonas hydrophila* vaccine	Salmonids	Motile aeromonas septicemia
15.	Carp erythrodermatitis	Carpspecies	Erythrodermatitis
16.	*Piscirickettsia salmonis* vaccine	Salmonids	piscirickettsiosis
17.	*Flavobacterium psychrophilum* vaccine	Salmonids, FW species	Flavobacteriosis
18.	*Renibacterium salmoninarum* Vaccine	Salmonids	Bacterial kidney disease
19.	Infectious hematopoietic necrosis virus vaccine	Salmonids	Infectious hematopoietic necrosis
20.	Infectious pancreatic necrosis virus vaccine	Salmonids	Infectious pancreatic necrosis
21.	Infectious salmon anemia vaccine	Salmonids	Infectious salmon anemia
22.	Spring viremia of carp vaccine	Common carp	Spring viremia of carp
23.	Koi herpes virus (KHV) vaccine	Koicarp	Koi herpesvirus disease
24.	Betanoda virus	Grouper	Betanodavirus disease
25.	Carp erythrodermatitis	Carp	Erythrodermatitis
25.	Grass carp hemorrhage disease vaccine	Grass carp	Grasscarp haemorrhage disease
26.	Nodavirus vaccine	Seabass	Viral nervous necrosis
27.	Pancreas disease virus vaccine	Salmonids	Pancreas disease

and advantageous since they only require an immunogenic part of the pathogen. Additionally, offering benefits such as the potential for co-administration of multivalent vaccines, low-cost production, storage stability due to the increased chemical stability of plasmid DNA, and quick modification of DNA sequences to target new pathogen mutants. Furthermore, DNA vaccines are safe in terms of disease transmission and interaction with live attenuated vaccines, which is not always achieved with recombinant proteins. They do not requireadjuvants to enhance both cell-mediated and humoral immune responses effectively (Restifo et al., 2000; Utke et al., 2008). Moreover,they do not require oil adjuvants, which can produce side effects as seen with polyvalent oil-adjuvant vaccines (Dadar et al., 2017). The Veterinary Biologics and Biotechnology Division of the Canadian Food Inspection Agency authorized Apex-IHN®, a DNA vaccine, for use against IHN, and it has also been approved in the USA (Lorenzen and LaPatra, 2005). This vaccine is effective against other fishviral infections, such as viral haemorrhagic septicaemia (VHS) in trout as well as Pacific and Atlantic salmon, and triggers both adaptive and innate immune responses in various fish species (Garver et al., 2005; Holvold et al., 2014; Aida et al., 2021). Likewise, Clynav, a DNA recombinant vaccine developed by Elanco Animal Health, has been approved in the EU and Norway for use against pancreas disease in salmonids (Aida et al., 2021) ensuring safety.The routes of vaccine administration, ambient conditions, and the variability in biochemical and serological characteristics of pathogens remain significant obstacles in developing an effective and safe commercial vaccine (Ben et al., 2021).

7. CONCLUSION

In case of aquaculture, vaccination reduces the use of antibiotics and protects fish from infectious diseases avoiding the risk of drug resistance. Most of the fish vaccines are for high-value fresh water and marine fish species to prevent bacterial and viral diseases. Currently available vaccines are based on simple empirically developed inactivated pathogens. Limited knowledge on immune systems of fish, further vaccines against intracellular bacterial and viral pathogens are one of the big challenges for the coming years.

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