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A Review On Recent Advancement Of Aquaculture Industry Through Vaccination: It's Role In Preventing Infectious Fish Pathogens

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Abstract: Compared with the animal husbandry, Fish farming is new method and it is rapidly becoming one of the pivotal sectors in the agriculture economy across the World, driven by the ever-increasing demand for high quality animal protein at reasonable price, especially with the exponentially increasing human population. However, the development of high density fish populations also brings forth a challenge – the hasty transmission and spread of infectious disease agents in aquaculture. During the early days, when diseases appeared in aqua-farming practices, antibiotics or chemotherapeutics were used for disease treatment, and even for disease control. Preventive measures have become more and more significant as part of bio-security in aquaculture. Disease prevention by using vaccines is predominantly important in aquaculture where the number of individuals at high risk and where treatment is a challenge from practical, technical and economic points of view. Vaccination in general, a safe and cost-effectively acceptable preventive measure as it contributes to a sustainable aquaculture with low use of antibiotics. To control and prevent viral, bacterial and some extent parasitic infectious diseases, vaccination is the well-organized method. In the 1938 *Snieszko et al.*, reported very first in his published article about disease prevention using vaccines and protective immunity in carp immunized with *Aeromonas punctata*. Vaccine development technology in aqua-farming has advanced very rapidly after the invention of the inactivated *Aeromonas salmonicida* vaccine in 1942. Fish vaccination has been used in aquaculture since the 1980s to prevent infectious diseases. Now there is diverse range of vaccines commercially available in the market including live attenuated, killed/inactivated or genetically engineered vaccines for viral and bacterial diseases. Currently, most commercially available vaccines for aquatic organisms are inactivated, and the basic method of manufacturing of vaccines is still the inactivation by means of either physical or chemical methods of virulent wild-type pathogens. However, live attenuated vaccination in aquaculture practices offers numerous beneficial effects over inactivated vaccines viz., method of vaccination is simple, manufacturing costs are low, provide prolonged immunity, vaccine delivery is very rapid and low dose of immunization. In recent times, with the incessant diminution of technological price (for example, sequencing of genome, sophistication in screening of antigens, advancements in cell culture, etc), the progress of innovative antigen expression and delivery mechanisms, and the immense accretion of fundamental knowledge on mucosal immunity of fish, vaccines for aquaculture are guaranteed to see rapid improvement in the upcoming years. Advancement in developing greatly effective vaccines against mucosal immunity and subsequent adjuvants is a significant direction for forthcoming development, and improving vaccine delivery methods will certainly enable the new vaccines development.

Key words: Adjuvants, Aqua-farming, Immunization, Live attenuated vaccines, Vaccination.

Introduction:

In general, when illness set in aqua-farming operations antibiotics and chemotherapeutics were used to relieve symptoms and even disease prevention which leads to antibiotic resistance along with other side effects. To overcome these consequences, vaccination is evolving as a trustworthy and standardized method to induce immunity in host organism against bacterial and viral outbreaks. The characteristics of ideal vaccine are safe, effective, economical, and easily administered. The fish vaccination industry frequently publishes novel and innovative information on fish immunology and vaccinology, contributing to the development and improvement of vaccine formulation and efficacy (Rathor and Swain, 2024). The very initial reports were made available by *Snieszko et al.,* (1938) on defensive immunity, who used vaccine to induced immunity in carp vaccinated with *Aeromonas punctata*. Vaccine development technology in aqua-farming has rapidly advanced after the available of inactivated vaccine against *Aeromonas salmonicida* in 1942. In the year 1976 Department of Agriculture, United States provided license to the fish vaccine used to protect from enteric-red mouth disease in fish, is the first fish vaccine used to treating yersiniosis in salmonid fish and followed by a vaccine for vibriosis (Kumar *et al.*, 2015).

The increased intensive aquaculture practices are profoundly associated with outbreaks of infectious diseases. This is because of increased rearing densities of organisms favor to outgrow a wide range of microorganisms. Among them, Viruses and Bacteria are the main causative agents which are responsible for infectious illnesses outbreaks in the field of aquaculture (Vincent *et al.*, 2019). The furthermost prevalent bacterial infections affecting cultured fish are *Aeromonas hydrophila, Streptococcus agalactiae, S. iniae*, *Vibrio alginolyticus, V. anguillarum,* and *V. parahaemolyticus (*Hossain *et al.,* 2022; Velazquez-Meza *et al.,* 2022). Among them *Aeromonas hydrophila* is the most common causative pathogen creates outbreaks in aquaculture. Disease causing agents creating a great loss to the aquaculture practices. However, by introducing specific-pathogen-free (SPF) brood stock, provide feed optimization, improvement in husbandry techniques, and good sanitation (Grisez and Tan, 2007), these organisms can be controlled at some level. Although, for disease treatment, antibiotics or chemotherapeutics are using, there are some clear drawbacks including resistance to the drugs and concerns regarding safety (Sneeringer *et al.,* 2019). In recent years, vaccination is a significant practice in aquaculture. Vaccination is considered as an effectual treatment option for the prevention of a wide range of bacterial and viral diseases (Ma *et al.,* 2019) and this is contributes to sustainable aquaculture in terms of environmental, social, and economical concerns.

Vaccination plays a crucial role in commercial basis of fish farming on large scale fish industry and it is a main reason for the success of salmon, trout, channel catfish, European sea bass and sea bream, tilapia and Atlantic cod. The ability of fish vaccines to induce a protective immunity either containing or producing an antigenic substances (Sommerset *et al.,* 2005). In general, administration of vaccines are in the form inactivated or live attenuated pathogens. This antigenic substance then induces an innate and/or acquired immune response within the organism against that specific pathogen. In the 20th century researchers focused on fish vaccinology and fish immunology has increased all over the World and it is one of the main concern in aquaculture. Several review articles pronounced the history, advancements, types, and administration routes of fish vaccines, as well as the prospects and challenges of developing vaccines in aquaculture (Sudheesh and Cain, 2017). And some researchers also focused on the use of adjuvants and immune-stimulants in vaccines of fish, along with delivery mechanisms. In addition some reviews have focused on current vaccine applications for large-scale aqua-farming practices, and future trends for inactivated, live-attenuated, and DNA vaccines (Brudeseth *et al.,* 2013). Given the development of new and innovative technology and a lack of research reviews on recent ground-breaking fish vaccine technologies, there is a need for a comprehensive overview of where the field is currently. This review aims to offer comprehensions into the current status of vaccination in aquaculture and modes of administration.

Review on current status of Bacterial and Viral vaccines:

Vaccines have been emerged as an effective means for control of pathogens; the use of vaccines against bacterial diseases in fish has successfully cut down the use of antibiotics in aquaculture (Kar *et al.,* 2022). In comparison to other biosecurity procedures, vaccination has a greater potential to avoid from illness, mortality, and preventive measures, and moreover, attractive effect more quickly than the other strategies (Midtlyng, 2001). Fish vaccines are generally in inactivated form or live attenuated form. Inactivated vaccines for fish generally comprise of heat killed or formalin destroyed bacterial virulent particles. The manufacture of inactivated bacterial antigens with oil adjuvants are enhancing antibody responses to a sufficient level for effectual and long-term protection. Vaccines against red mouth disease and vibriosis are whole cell immersion vaccines (Sommerset *et al.,* 2005). In recent days a lot more research is going on for developing vaccines against bacterial infections that significantly affect the aquaculture industry. Mostly, these vaccines are manufacturing by inactivating whole –cell formulations. For instance, a simple inactivated bacterin vaccine is found to be effective against vibriosis. In addition, use of live attenuated vaccines found to be more efficient in controlling bacterial diseases. Moreover, current research is focused in development of recombinant protein based vaccines. For example, *Piscirickettsia salmonis* bacterins have been replaced by recombinant vaccines (Kuzyk *et al.,* 2001).

Fish viral diseases cause more damage to aquaculture due to its rapid spread causing acute mortalities and are not amenable to any treatment measures. Prophylaxis through vaccination is a reliable method for controlling viral diseases of fish. Viral disease outbreaks are more difficult control as there is no availability of anti-viral therapeutics and information regarding the viral mechanisms especially how they affects the organisms. Further, manufacturing a fish virus vaccine is certainly a very challenging and time-taking process as they have many subtypes or serotypes, and vaccines derived from one subtype are typically less effective against other subtypes (Crane and Hyatt, 2011). Moreover, mutations can occur rapidly when the virus reproduces in the infected fish (Stepien *et al.,* 2015), especially with the RNA virus. Major viral diseases in aqua-farming include Infectious Pancreatic Necrosis (IPN), Viral Hemorrhagic Septicemia (VHS), Koi herpesvirus disease (KHVD), and Infectious Hematopoietic Necrosis (IHN), Epizootic haematopoietic necrosis (EHN), Red seabream iridoviral disease, Viral nervous necrosis (VNN), also called as viral encephalopathy and retinopathy (VER). Koi herpesvirus disease (KHVD) is a highly contagious disease affecting common carp and its varieties like koi carp and ghost carp causing significant mortality (Haenen *et al.,* 2004). The first successful vaccination experiments against these diseases were done by administering vaccines, both avirulent and attenuated strains (Fryer *et al.,* 1976; Hill *et al.,* 1980). As on today, there are 26 licensed fish vaccines available globally to protect against viral diseases caused by families such as rhabdoviruses, birnaviruses, and orthomyxoviruses. These vaccines are used in variety species of fish such as salmon, trout, channel catfish and tilapia (Mandal and Thomas, 2022). The most common types of vaccines used in aquaculture are inactivated injectable vaccines. However, other types of vaccines, such as attenuated and DNA vaccines are also used to address viral diseases (Du *et al.,* 2022).

Recent innovative techniques in advancements of vaccine development:

There is much advancement in aquaculture vaccine development in recent years, including Nucleic acid based vaccines, sub unit vaccines, and recombinant vector based vaccines etc. These vaccines use DNA or RNA to encode the antigen of interest. They can activate both cellular and humoral immunity, and are effective against intracellular pathogens. Technological advancements have helped us understand fish immunological mechanisms, which have led to better vaccine administration (Jose Priya and Kappalli, 2022). Vaccines are available for many fish species, including Atlantic salmon, catfish, grouper, rainbow trout, common carp, koi carp, and tilapia. However, there are still challenges to overcome, such as vaccine degradation, oral tolerance, and stressful environments (Rathor and Swain, 2024). The cost of vaccines is also a concern, and the route of administration may not be optimal for some vaccines. Advancements in technical and scientifically expertise have fast-tracked the development process of aquaculture vaccines in current years. It is enable us an enhancing number of available choices for the protection of organisms in aqua-farming against pathogens, marking a important milestone in aquaculture vaccinology.

1. **Subunit vaccines:**

Using recombinant technology, subunit vaccines are manufacturing where only the immunogenic target regions of a pathogen are isolated, purified and used in vaccine formulation such as viral capsid proteins and bacterial glycoproteins, and are generally safer than antibiotics which are used for controlling fish diseases in aquaculture. These vaccines are in general effective against bacterial diseases of fishes. Many of the sub-unit vaccines are prepared by expressing the sub-unit protein in Escherichia coli-based prokaryotic expression system. For instance, for example, the most successful used subunit vaccine is Merck Animal Health has produced an IPNV peptide subunit vaccine for infectious pancreatic necrosis (IPN) in Norway (Ma *et al.,* 2019). Infectious salmon anemia vaccine encompassing recombinant hem-agglutinin-esterase protein is existing as an oral vaccine in the trade name of Centrovet in the Chile. Baculovirus and yeast expression systems were used for the vaccine against viral haemorrhagic septicaemia and IHNV (Biering *et al.,* 2005). There is no commercially available subunit vaccine in aquaculture. The major drawback in this type of vaccines is environmental safety. Subunit vaccines, as they contain only fragments of the pathogen, may not always be able to produce a strong immune response.

2. **Nucleic acid based vaccines:**

Nucleic acid vaccines are made from either DNA or RNA that encodes an antigen, have several benefits and are considered a promising technology for disease control for cultivable fish species in aquaculture. Nucleic acid vaccines can capable to induce both cell mediated and humoral mediated immune response and the most efficient delivery route for these vaccines, at present is IM injection. Administration of these has shown no adverse effects in the vaccinated fish to date. (Lorenzen and LaPatra, 2005). They are considered safe to administer because they can't revert to a pathogenic state. Furthermore, DNA vaccines usually constructed as multivalent, and provide protection or cross-protection by using gene coding for multiple antigens in the plasmid design (Biering and Salonius, 2014). DNA vaccines are more effective against viral infections, and especially efficient against fish rhabdoviruses, as they usually utilize the same cellular mechanics that a virus utilizes once they enter a host cell (Hølvold *et al.,* 2014). DNA vaccination involves the delivery of plasmid DNA encoding a vaccine antigen to the host (Heppell and Davis, 2000) now available in the market are effective against intracellular pathogens like *Mycobacterium marinum* and Infectious haematopoietic necrosis (IHN) virus. In Canada, one DNA vaccine is commercially available for infectious hematopoietic necrosis virus (IHNV) (David *et al.,* 2005). In 2017, Europe government given license to DNA vaccine, SAV-3 which is used against Salmonid alphavirus. The interaction between the early innate and subsequent adaptive response after DNA vaccination is not fully understood (Tammas *et al.*, 2024). DNA vaccines offers several advantages, including overall safety of administration, speedy production, and suitable for making vaccines to combat a wide range of aquatic disease causing agents, particularly intracellular ones. Due to its relative novelty, the vaccination technology is challenged with both customer acceptance, governing and legal scrutiny that impede the extensive application of DNA vaccines in the field of aquaculture (Jose Priya and Kappalli, 2022). There are two major RNA-based vaccines, are distinguished by the translational capacity of the RNA: conventional, non-amplifying mRNA and self-amplifying mRNA (i.e., replicons) (Cho *et al.,* 2017). RNA vaccines are several advantageous as they are non-infectious and degenerated by routine cellular processes, and has no possible risk of infection or insertional mutagenesis. The alphavirus genome is basis for the most currently used RNA vaccines. Earlier research reports have proven that alphaviral RNA vaccines are more effective in activating antigen specific immune responses, particularly cellular responses, in comparison with conventional plasmid DNA vaccines (Ma *et al.*, 2019).

3. **Recombinant vector vaccines:**

Recombinant vector vaccines are live virus vectors that can be used in aquaculture to protect against pathogenic infections in aquaculture. For instance, examples of recombinant vector vaccines in aquaculture including viral nervous necrosis (VNN) vaccine used on species like milkfish, grey mullet, and mangrove red snapper. Another recombinant vaccine is oral recombinant vaccine developed to protect salmon from infectious salmon anemia virus (ISAV) (Leong *et al.,* 1997). In this type of vaccines, antigenic genes are transferred into live virus vectors and then into the recipient host which in turn express the encoded protein of another pathogenic microorganism, as the vaccine antigen (Adams *et al.*, 2008). The manufacturing of these sub unit vaccines are based on self-assembling capability of structural proteins of virus with the similarity of a native virus has bring about in the development virus-like particles (VLPs) (Dhar *et al.,* 2014). The recombinant vector vaccine antigens can able to stimulate both humoral and cellular immune responses. The vector dynamically replicate inside the host cells, stimulating the immune system like an adjuvant. The improved baculovirus expression system approached for rapid expression of abundant recombinant proteins (VLPs) and it is an economical and effectual method for producing heterologous proteins (Bedekar *et al.*, 2022). VLPs may be produced incompetent hosts such as bacteria, fungi or plant and also produced by genetic recombination of dissimilar virus-producing chimera. Now a days, only a few investigational VLPs-based vaccines were established, for example, vaccine for infectious pancreatic necrosis (IPN), in which the IPNV protein capsid VP2 expressed in yeast self-assembles of VLPs into sub-viral particles (SVPs) and trigger immunological response in Rainbow trout (Dhar *et al.*, 2010). Other examples including vaccine for Atlantic cod NNV (ACNNV) for seabass, where the coat protein was expressed in plant, *Nicotiana benthamiana* (Marsian *et al.*, 2019), vaccines against grouper nervous necrosis for orange-spotted grouper (Chien *et al.*, 2018) and viral nervous necrosis for European seabass (Thiéry *et al.*, 2006).

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