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Fish Vaccination: Differences Between Monovalent and Polyvalent Bacterial Vaccines

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Abstract

Increasing fish farming density has led to significant challenges, such as the rapid spread of infectious diseases, including concurrent infections and immunosuppression, causing substantial economic losses, so vaccination is now recognized as a reliable and standardized method for protecting against these infectious agents. To address this need, polyvalent vaccines have taken the place of monovalent vaccines in aquaculture. Nonetheless, there is a divide among researchers, with some advocating for monovalent vaccines while others support polyvalent vaccines in aquaculture, presenting a significant challenge for the industry. The difference between monovalent and polyvalent vaccines lies in their target pathogens and scope of protection. Monovalent vaccines are designed to target a single pathogen and are very effective in preventing

a specific disease. In contrast, polyvalent vaccines target multiple pathogens simultaneously, offering broader protection. While monovalent vaccines may require multiple injections to protect against several diseases, polyvalent vaccines simplify the vaccination process by combining protection in one injection, which reduces costs and lowers stress on the fish. Consequently, polyvalent vaccines provide a more efficient and comprehensive immunization solution for fish, especially in situations where concurrent infections are common. Given the challenges outlined, this article aims to compare the immunogenicity of monovalent and polyvalent bacterial vaccines administered via oral, immersion, and injection methods in aquaculture sector.

Key words: monovalent, polyvalent, bacterial vaccine, relative percent survival, aquaculture, oral, immertion, injection

The growing demand for fish and limited wild stocks have made aquaculture vital in food production. It has become one of the most efficient and rapidly expanding sectors in recent decades (Ababouch et al., 2023; Khanzadeh et al., 2023). To meet rising fish protein needs, farming practices have become denser and more intensive (Ahmad et al., 2022). However, these conditions have increased fish susceptibility to infectious diseases (Dawood et al., 2021). Moreover, intensive fish farming raises the vulnerability of fish to infectious diseases, causing significant economic losses due to mortality and veterinary expenses (Cascarano et al., 2021; Erfanmanesh et al., 2024). Therefore, to control and treat infectious diseases, antibiotics can be utilized in fish as a means to prevent and manage fish infections (Bondad-Reantaso et al., 2023). Antibiotics can disrupt beneficial gut microorganisms, causing nutritional and immune issues (Medina-Félix et al., 2023; Shah et al., 2021). Prolonged use promotes antibiotic-resistant bacteria, posing public health risks. This resistance can spread among humans, animals, and the environment (Pereira et al., 2022). Consequently, aquaculture is moving away from antibiotics toward sustainable alternative solutions.

Vaccination is currently the most effective method for preventing the spread of diseases (Costanzo and Roviello, 2023). Vaccination is a suitable alternative to address the complications caused by the mortality of diseases after limiting the use of antibiotics in aquaculture (Barnes et al., 2022). Vaccines work by stimulating the fish's immune system to produce antibodies that protect against specific diseases (Wang et al., 2023). Vaccines against various bacterial and viral pathogens have been successfully developed and commercialized, both as monovalent (targeting a single pathogen) and polyvalent (targeting multiple pathogens) formulations (Aly et al., 2021; Erfanmanesh et al., 2023 a). While monovalent vaccines are highly effective in preventing specific diseases, fish often face concurrent infections, making multiple vaccines necessary for comprehensive protection (Mohamad et al., 2021). Polyvalent vaccines offer several advantages in this context, including simplification of the vaccination process (owing to the use of one injection instead of multiple injections), cost reduction, and reduced stress on the fish (Shoemaker et al. 2012; Mohamad et al. 2021). These vaccines provide broader protection against multiple pathogens, making them a more efficient and comprehensive immunization option for aquaculture

(Mohamad et al., 2021; Erfanmanesh et al., 2023a). Vaccines can be administered through various routes, such as oral administration, injection (either intraperitoneally or intramuscularly), and immersion (Vinay and Bedekar, 2022). The method of vaccine administration is influenced by several factors, including the nature of the pathogen, the route of delivery, the type of vaccine, labor costs, and the fish's age and size (Schulz et al., 2020).

Despite the increasing use of vaccines in aquaculture, there is disagreement among researchers regarding whether monovalent or polyvalent vaccines are more effective in immunogenicity. Additionally, there is a lack of review articles evaluating monovalent and multivalent vaccines in aquaculture. Therefore, the main purpose of this review article is to examine different types of aquatic vaccines and to compare and evaluate the immunogenicity of monovalent and multivalent bacterial vaccines used in aquaculture.

Methods

This article is a narrative review that provides findings from various studies on the effects of bacterial monovalent and polyvalent vaccines on relative survival percentage in aquaculture. The review drew upon journal databases such as PubMed, AGRICOLA, Google Scholar, Scopus, Science Direct, and Web of Science to gather relevant literature. Search terms included keywords such as monovalent vaccines, polyvalent vaccines, bacterial vaccines, vaccine types, vaccine administration methods, immune system, effect of vaccines on the immune system, antibody production, and pathogens. This review incorporated a mix of both credible and less formal English-language materials. Additionally, we examined trusted English publications, including books, journals, and online resources. Given their extensive biomedical and scientific databases, PubMed/MEDLINE and Google Scholar were chosen as the main sources for gathering information in this study. In this article, 138 articles were chosen that included the keywords monovalent and polyvalent bacterial vaccines delivered via oral, parenteral, or injectable routes. Approximately 50 distinct and reputable journals were included in this review, with Fish & Shellfish Immunology being the most commonly referenced source. The collected data were analyzed to evaluate the impact of these vaccines on immune system functionality, survival rates, and antibody titers in fish exposed to different pathogens and reared under varying conditions. This approach allows for the interpretation of findings across diverse fish species and pathogen types, providing insights into the comparative effectiveness of monovalent and polyvalent vaccines in aquaculture practices.

The role of vaccines in aquaculture

The fish farms face significant challenges from diseases that cause substantial yearly damage. In response to this problem, fish farmers have heavily relied on antibiotics to combat these disease outbreaks (Cascarano et al., 2021). However, the prolonged use of antibiotics in aquaculture not only generates significant environmental issues but also fosters antibiotic resistance in aquatic bacterial flora, ultimately exacerbating the problems faced by the aquaculture industry (Okeke et al., 2022; Okoye et al., 2022). As a result, more focus has been placed on

methods that prevent disease outbreaks in the first place. One such approach is vaccination, which works by stimulating and activating the immune system of fish against specific diseases (Du et al., 2022). The immune system's stimulation and vaccination effectiveness rely primarily on the interaction between the vaccine and the host (Gudding, 2014). However, the favorable host environment also plays a crucial role in establishing appropriate resistance (Gudding, 2014). Vaccination should complement other biosecurity measures (i.e., quarantine, disinfection, and farm hygiene, crowding and stress management, and nutrition and feed control) to achieve the best disease management approach (Padrós et al., 2022). This helps reduce the spread of infections and improve environmental conditions, which in turn enhances the host's resistance to diseases (Padrós et al., 2022). Therefore, nowadays, the vital and indispensable role of vaccination in aquaculture is abundantly clear.

A summary of the immune system in fish

The immune system of bony fish differs from mammals in several key ways. Fish lack bone marrow and lymph nodes, so their immune organs are classified as lymphoid rather than myeloid. The main lymphoid organs are the kidneys, thymus, spleen, and gut-associated lymphoid tissues (GALT), which develop during larval stages. Fish skin is covered in a protective mucus layer that acts as the first line of defense against pathogens and is part of the mucosa-associated lymphoid tissue (MALT). MALT includes skin-associated lymphoid tissue (SALT), gill-associated lymphoid tissue (GIALT), and GALT, all of which have both innate and adaptive immune defenses (Ben Hamed et al., 2021). The fish immune system has both systemic and mucosal compartments, and local immune responses can be triggered by intestinal immunization (Ben Hamed et al., 2021). All immune cells necessary for a local immune response are abundantly present in the gut mucosa. The innate immune system provides a fast, non-specific reaction to pathogens, with components like antibacterial peptides, lysozyme, lectins, acute phase proteins, and the complement system (Rauta et al., 2012). When a pathogen overcomes the body's initial innate defenses, the adaptive immune system responds by targeting the microbe with precision and establishing immunological memory (Rauta et al., 2012). This adaptive, or acquired, immunity generates a focused and durable defense against the specific pathogen, coordinated through the actions of two distinct types of lymphocytes, each responsible for activating different components of the adaptive immune response (Mashoof and Criscitiello, 2016). Teleost fish possess three immunoglobulin classes: IgM, IgD, and IgT, with IgM being the main systemic antibody and IgT predominantly found in mucosal areas (Magadan et al., 2015). Recent research highlights the significant role of T cells in combating intracellular bacterial infections and the ongoing exploration of immune system gene expression, microbiota interactions, and their implications for fish health and immunity (Nakanishi et al., 2015). Studies are also focusing on the distribution and function of T cell populations in various fish tissues, as well as the structural and genetic diversity of fish immunoglobulins.

Vaccine types

Traditional fish vaccines have mainly relied on inactivated whole organisms, although some live attenuated and subunit protein vaccines, often combined with adjuvants, have been

successfully commercialized. Most of the vaccines currently licensed for use in aquaculture are produced using traditional methods that echo the foundational principles established by pioneers like Jenner and Pasteur centuries ago (Tizard, 1999). These vaccines were administered through immersion or injection, leading to a degree of humoral immunity in the fish. In the 1990s, the introduction of modified live vaccines marked a significant advancement in aquaculture vaccination (Shoemaker et al., 2009). These newer vaccines have proven effective, contributing to higher production levels in commercial aquaculture and a reduction in the reliance on chemical therapeutics and antibiotics delivered through feed (Ma et al., 2019). Figure 1 illustrates how vaccines activate the immune system in aquaculture.

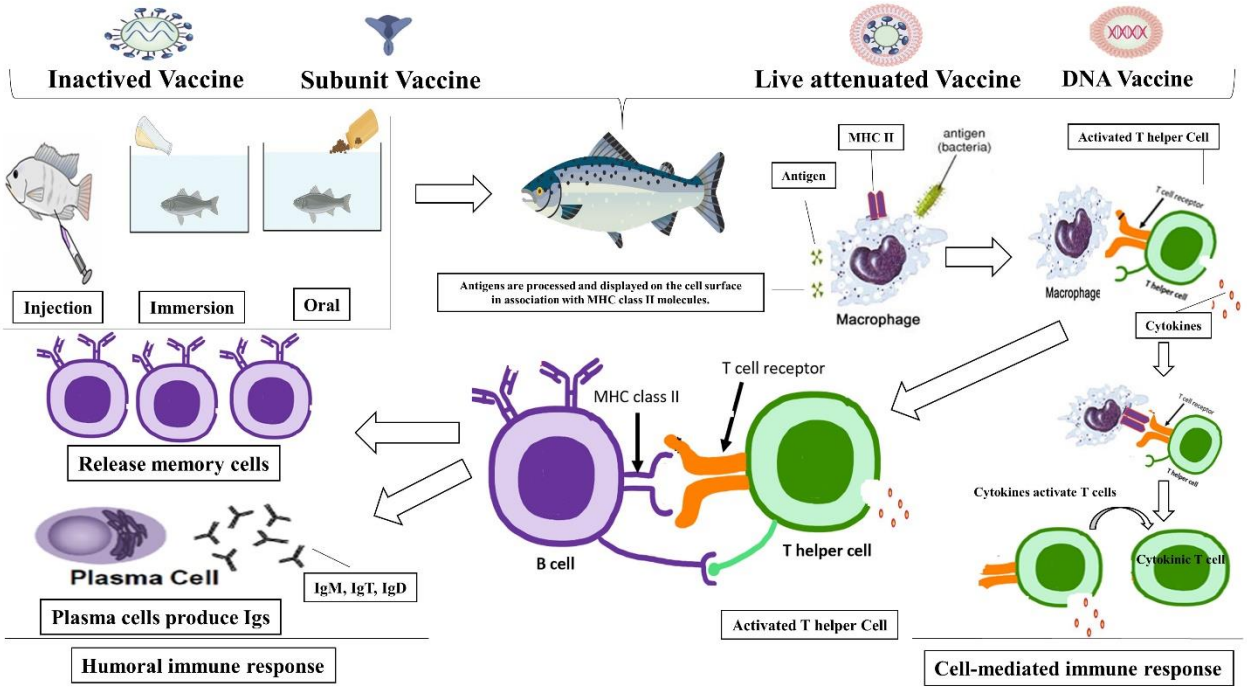

Figure 1 illustrates the types of vaccines and the mechanisms by which they activate the immune system using various administration methods in aquaculture

Inactivated vaccines

Inactivated or killed vaccines are typically derived from a pathogenic organism that has been rendered incapable of causing infection or replicating within or outside the host. This is achieved through various physical and chemical processes. Techniques such as heat treatment, ultraviolet radiation, or formalin exposure can induce these changes while preserving the antigenic properties of the microorganism (Tlaxca et al., 2015). In contrast to live vaccines, inactivated vaccines tend to be more stable in field conditions and are often less expensive to manufacture (Biering et al., 2005). These vaccines do not remain in the environment or within vaccinated fish, so they are generally safe. However, they may elicit a weaker or shorter-lasting immune response

than other vaccine types (Vishweshwaraiah and Dokholyan, 2022). The limited immunogenicity of inactivated vaccines can be linked to insufficient activation of cell-mediated immunity in fish species (Biering et al., 2005). As a result, it may be necessary to incorporate adjuvants or administer multiple booster shots to effectively stimulate protective immunity in fish. The drawbacks of inactivated vaccines include the presence of antigens that may inhibit the immune response, potential toxic reactions from adjuvants designed to enhance immunity, diminished immune responses due to protein denaturation, and systemic reactions associated with certain adjuvants (Di Pasquale et al., 2015). For example, Linh et al. (2022) reported the protective effect of a formalin-inactivated vaccine against *Streptococcus agalactiae* (through three distinct routes: immersion using a water-based vaccine, injection with an oil-based vaccine, and oral administration with a water-based vaccine) in Nile tilapia (Linh et al., 2022). Additionally, the immuno-protective effectiveness of a feed-based, whole-cell inactivated bivalent vaccine against Streptococcus and Aeromonas infections has been documented in red hybrid tilapia (Monir et al., 2021).

Live attenuated vaccines

These vaccines are composed of live, attenuated, or non-pathogenic microorganisms that can replicate and display their immunogenic properties within the host (Muktar et al., 2016). Attenuated vaccines are manufactured using an assortment of methods, including chemical alteration, heat treatment, serial passage of the pathogen in diverse heterologous systems (such as alternative host organisms, and tissue culture), as well as genetic attenuation approaches (for instance, mutation via deletion, disruption, or insertion of metabolic routes or virulence genes) (Bedekar and Kole, 2022). Live attenuated vaccines do not necessitate the use of adjuvants, as the weakened pathogen retains the replication capacity and can effectively stimulate both humoral and cell-mediated immune responses. This dual activation leads to the establishment of robust, long-lasting protective immunity within the host organism (Ma et al., 2019). For example, high protection was reported using a live attenuated vaccine against *Streptococcus agalactiae* (injection method) in Nile tilapia (Laith et al., 2019). Protection against *Aeromonas veronii* was observed in loach (*Misgurnus anguillicaudatus*) following the administration of a live attenuated vaccine via injection and immersion routes (Zhang et al., 2020).

DNA vaccines

The DNA vaccines comprise a self-replicating extrachromosomal plasmid that carries the immunogenic gene of the pathogenic agent (Heppell and Davis, 2000). This plasmid is introduced into the fish after being multiplied in bacterial cultures and purified. These vaccines can initially trigger non-specific immune responses, which are then followed by specific immunity; however, the precise mechanisms of protection offered by these vaccines in fish remain uncertain (Kurath, 2008). For example, significant protection against these infections was provided by recombinant DNA vaccines against *Streptococcus agalactiae* in Nile tilapia via oral route (Zhu et al., 2017) and DNA vaccines against *Vibrio anguillarum* in Asian seabass via intramuscular injection (Kumar et al., 2007).

Subunit vaccines

Subunit vaccines employ solely antigenic components for immunization purposes. As these vaccines lack the ability to replicate within the host, they pose no risk of pathogenicity to the host organism or non-target species (Adams et al., 2008). Biotechnological tools are employed to identify and design the gene sequence encoding the protective antigen of the pathogen. The antigenic genes are then inserted into various hosts, including prokaryotes (e.g., bacteria) and eukaryotes (e.g., cell cultures, yeast, insect cells, microalgae, and transgenic plants), which are cultured on a large scale under controlled laboratory conditions using fermentation technology to produce the antigen protein (Noonan et al., 1995; Lecocq-Xhonneux et al., 1994; Vakharia, 2005; Acosta et al., 2006). Despite extensive research on subunit vaccines, their development and commercialization in aquaculture remain limited. This is mainly because they often require adjuvants to elicit sufficient protection against certain pathogens (Ma et al., 2019). Additionally, the high production costs of recombinant vaccines make them less economically viable for many fish species, which are generally viewed as lower-value compared to other livestock in agriculture (Ma et al., 2019). In a study conducted on juvenile *Megalobrama amblycephala*, the immune response and protective efficacy of a formalin-inactivated *Aeromonas hydrophila* vaccine and a OmpA subunit vaccine, both administered via intraperitoneal injection, were evaluated. The findings indicated that both vaccines improved survival rates relative to the unvaccinated group. However, the OmpA subunit vaccine showed greater protection than the inactivated vaccine (Zhang et al., 2023).

Monovalent vaccines

Monovalent vaccines in aquaculture are designed to target a single, specific pathogen or disease-causing agent (Zhang et al., 2021). This allows for a more focused and effective approach to disease prevention, as the immune response is tailored to the target pathogen, providing more targeted and efficient protection compared to broad-spectrum vaccines (Zhang et al., 2021). Meanwhile, monovalent vaccines have a simpler composition and are generally more cost-effective for the producer as they only contain antigens from a single pathogen. This can make their development and production process more streamlined and efficient compared to more complex polyvalent vaccines (Ben Hamed et al., 2021). For instance, protection has been reported using monovalent vaccines against *Streptococcus iniae* and *Flavobacterium covae* in Asian seabass (Tumree et al., 2024), as well as against *S. agalactiae* and *S. iniae* in Asian seabass (Lan et al., 2021).

Polyvalent vaccines

Polyvalent vaccines are a comprehensive solution for protecting fish against a wide range of infectious diseases. These vaccines contain antigens from multiple pathogens in a single formulation, allowing for broad and comprehensive protection against various diseases (Mondal & Thomas, 2022). The use of polyvalent vaccines simplifies the vaccination process and helps to ensure that fish are effectively safeguarded against a wide range of potential infectious threats.

Polyvalent vaccines offer a practical and cost-effective solution for aquaculture by reducing the need for multiple injections (Mohamad et al., 2021). This innovative approach simplifies the vaccination process, making it more efficient and accessible for fish farmers. When discussing polyvalent vaccines, some important points should be considered. Making multiple antigens in polyvalent vaccines can result in more impurities and higher production costs. On the other hand, choosing the right antigens is also important because both antigenic and non-antigenic parts can interact synergistically or antagonistically, ways. Therefore, finding the best combination is necessary to get an effective immune response and make the most of the vaccine's potential (Ben Hamed et al., 2021). For example, Nile tilapia received 60% protection from polyvalent vaccines against streptococcosis, lactococcosis, and enterococcosis (Abu-Elala et al., 2019). Aly et al. (2021) found that fish (*Sparus aurata*) groups vaccinated with polyvalent bacterins showed survival rates of 91.75% for the *V. parahaemolyticus* O11:K40 and *V. alginolyticus* combination, and 75% for the *V. parahaemolyticus* O3:K6 and *V. alginolyticus* combination (Aly et al., 2021). Figure 2 compares monovalent and polyvalent vaccines in aquaculture.

Feature	Monovalent Fish Vaccine 	Polyvalent Fish Vaccine 
Definition	Targets a single fish pathogen or strain	Targets multiple pathogens or strains affecting fish
Example in Aquaculture	Vaccine against Aeromonas	Vaccine against Aeromonas + Vibrio + Streptococcus
Advantages	Strong immune response to one pathogen - Simple to formulate and administer	Broader protection - Fewer injections - Economical for large farms
Disadvantages	No protection against co-infections - May require multiple vaccinations	Risk of antigenic competition - More complex to produce and test
Immune Specificity	High specificity, efficient for endemic disease	Covers multiple threats, ideal for polymicrobial environments
Application Suitability	Best for targeted control or low-pathogen diversity settings	Best for high-density farming with varied pathogens
Cost and Logistics	Lower cost per dose, simple storage	Higher production cost, complex cold-chain needed
Side Effects	Usually fewer due to simple antigen load	Possible increased reactions or reduced response to some components

Figure 2 illustrates the advantages and disadvantages of monovalent and polyvalent vaccines in aquaculture.

Vaccine administration route

Vaccines are typically administered in aquaculture by three methods: injection, immersion, and orally, each having its own advantages and disadvantages (Figure 3).

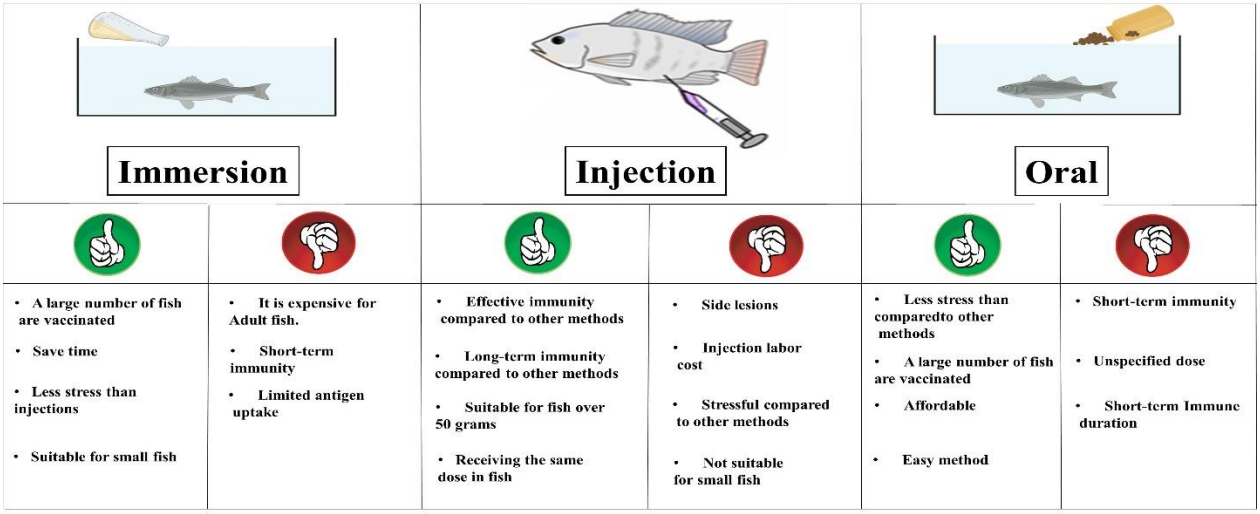

Figure 3 illustrates the advantages and disadvantages of injectable, immersion, and oral vaccines.

Oral

Oral vaccination in fish is an attractive method for inducing immune responses against pathogens, especially in aquaculture settings. This approach involves delivering antigens through the fish's gastrointestinal tract, which triggers a series of processes that ultimately lead to the activation of the immune system (Lee et al., 2021). The appeal of oral vaccines lies in their ease of administration, reduced stress for the fish, and the ability to vaccinate fish of various sizes, making mass vaccination feasible (Radhakrishnan et al., 2023). Compared to injections, oral vaccine administration is easier to administer, cost-effective, and less stressful for the fish, making this method ideal for immunizing large numbers of fish regardless of size (Radhakrishnan et al., 2023). Oral vaccines generally contain antigens that must be safeguarded against degradation in the challenging conditions of the gastrointestinal tract. In fish, the stomach environment can be particularly detrimental to these antigens, causing them to break down before they can reach the gut-associated lymphoid tissue (GALT), which is crucial for triggering immune responses (Mutoloki et al., 2015). Upon oral administration, oral vaccines introduce antigens, such as inactivated pathogens or subunit proteins, that are primarily absorbed in the gastrointestinal tract. This process heavily relies on specialized immune cells, like microfold cells (M cells) and dendritic cells. M cells are essential for transporting antigens through the intestinal epithelium, enabling access to the immune tissues located beneath (Kwong et al., 2023). After the antigens are presented to T cells in the GALT, these T cells become activated. This activation triggers the differentiation of T cells into effector cells, which then travel to different locations throughout the body, bolstering the immune response. In the subsequent phase, T cells lead to the activation of B cells. Following this activation, the B cells differentiate into plasma cells that generate specific antibodies, mainly immunoglobulins IgM, IgD, and IgT/Z, which play a vital role in mucosal immunity. The antibodies produced contribute to establishing both local and systemic defenses against pathogens.

Although oral vaccination offers several benefits, there are still challenges to overcome, especially concerning the stability of antigens in the harsh gastric conditions of fish (Parra et al., 2015; Yu et al., 2020). Antigens may be broken down before they reach the immune induction sites, potentially reducing the vaccine's effectiveness (Goncalves et al., 2022). The instability of antigens in the fish's stomach is one of the factors contributing to the lower immunogenicity and relative percent survival (RPS) observed with the oral vaccination method compared to the immersion and injection routes. To address this challenge, recent efforts in the field have focused on using encapsulation and the incorporation of excipients, such as bacterial spores from *Bacillus subtilis*, to improve oral vaccine stability and efficacy (Yao et al., 2019). In one study, using alginate/chitosan composite microspheres encapsulating a recombinant protein of *S. iniae*, a 60% RPS was reported in channel catfish (Wang et al., 2018).

Immersion

Immersion vaccination is a cutting-edge approach utilized mainly in aquaculture to protect fish from particular pathogens. This method entails placing fish in a vaccine solution, enabling the antigens to penetrate through their skin and mucosal layers. Immersion vaccination simulates a natural infection, promoting the swift absorption of antigens (Tammas et al., 2024). Research indicates that antigens can be found in various tissues, such as the skin, gills, and internal organs, within a short period, ranging from minutes to hours after exposure to a vaccine (Bøgwald and Dalmo 2019; Kitiyodom et al., 2021). Dip vaccination immerses fish in water with a high concentration of vaccine antigens for a short period, typically lasting from one to several minutes. In contrast, bath vaccination exposes fish to a more diluted vaccine antigen preparation over a longer time (Muñoz-Atienza et al., 2021). Fish can receive booster vaccinations through dip or bath methods to enhance their immune response and increase protection against targeted pathogens (Muñoz-Atienza et al., 2021). Immersion vaccination often requires large quantities of antigen, yet the resulting protection levels may be suboptimal. This is likely due to the limited amount of antigen that reaches the immune effector sites in mucosal tissues during this vaccination method (Sudheesh & Cain, 2016). The effectiveness of immersion vaccination can vary, and it may not provide the same level of protection as other vaccination methods in certain situations. The efficacy of immersion vaccines in fish can be influenced by a multitude of variables, including the vaccine dose, duration of immersion, antigen uptake (Bøgwald and Dalmo, 2019), adjuvant (Kole et al., 2018), environmental factors like temperature and osmolarity, fish size and age (Soto et al., 2014), vaccination strategies, mucosal integrity (Yun et al., 2019), and the characteristics of the challenge pathogen (Sudheesh and Cain, 2017).

The main immune response elicited by immersion vaccination is mucosal immunity. Fish have mucosa-associated lymphoid tissues (MALT) that react to antigens taken up through their skin and gills. This immune reaction typically involves the generation of specific immunoglobulins, such as IgM and IgT, which are essential for neutralizing pathogens at mucosal surfaces (Kitiyodom et al., 2021; Gong et al., 2021). Although the mucosal response is important, immersion vaccination can also trigger a systemic immune response. Studies have demonstrated

that following immersion, fish can generate antibodies in their serum, signifying a more extensive immune activation. For example, research involving tilapia vaccinated with a mucoadhesive nanovaccine revealed notable increases in serum antibody levels as well as enhanced expression of immune-related genes in the spleen and head kidney (Kitiyodom et al., 2021). Immersion vaccination has the potential to stimulate the production of diverse immune molecules, such as antimicrobial peptides and pro-inflammatory cytokines (Gong et al., 2021). These molecules bolster the fish's capacity to fight off infections and contribute to both specific and non-specific immunity (Gong et al., 2021). While immersion vaccination offers several benefits, it also faces challenges, including variable immune responses and challenges in precisely quantifying the vaccine dose absorbed by each fish. Furthermore, the immersion vaccination has a shorter duration of immunity that is often insufficient for many cultured fish species, necessitating booster doses. Moreover, its application to larger fish is impractical due to extended time requirements, higher costs, induced stress, and difficulties with administering immune-stimulating agents and adjuvants (Mondal and Thomas, 2022). Research shows that the vaccine is most effective when administered via injection, then immersion, and finally the oral route (Adams, 2019). To enhance the effectiveness of this vaccination approach, it is crucial to optimize vaccine formulations and delivery methods (Bøgwald and Dalmo, 2019).

Injection

Vaccination by injection is the most prevalent method for delivering vaccines to fish. This approach has proven to be highly effective in stimulating both humoral and cellular immune responses in the target species (Dadar et al., 2017). This method is less suitable for small fish as it requires specialized injection equipment and can cause significant handling stress. Additionally, there is an increased risk of fungal infections following vaccination (Thim et al., 2014). To minimize risks during the vaccination process, fish must be fasted for 24-48 hours and anesthetized prior to injection. It is also essential to choose a needle length appropriate for the fish's size to ensure safe and effective vaccination (Ben Hamed et al., 2021). Injectable vaccines can be administered to fish either intramuscularly (IM) or intraperitoneally (IP) (Corbeil et al., 2000). The primary adverse effects linked to the injection vaccination method can be categorized into three groups: (a) inflammation and abdominal lesions, (b) growth retardation and spinal deformities, and (c) systemic autoimmunity (Spickler & Roth, 2003). The duration of protection provided by this method is longer compared to the immersion method. In this method, the vaccine is not diluted and is enhanced with additional substances such as adjuvants, bacterial cells, carriers, bacterial antigens, and other components that are not typically included in standard immunization methods (Embregts & Forlenza, 2016). Injection vaccines are typically administered via Intraperitoneal (IP) injection is the most widely used and effective method for administering vaccines, as it provides a concentrated dose and supports a sustained immune response. In contrast, intramuscular (IM) injection, although less common, is often used for DNA vaccines and can be conducted manually or with automated devices. However, IM injections may induce stress in fish, which, if not properly managed, could result in increased mortality (Mondal and Thomas, 2022).

Immunization of fish using oral monovalent and polyvalent vaccines

Oral vaccines primarily target the gastrointestinal mucosa, especially the gut-associated lymphoid tissue (GALT), which is rich in immune cells (Hayat et al., 2021). Table 1 shows the immunogenicity of monovalent and polyvalent vaccination using oral vaccines. For example, a study evaluating the efficacy of a bivalent vaccine against *Streptococcus iniae* and *Aeromonas hydrophila* in tilapia. The report demonstrated 82% survival against *S. iniae* and 77% survival against *A. hydrophila* in the vaccinated group compared to the control group (Monir et al., 2022). In a separate study, administration of a *Lactococcus garvieae* vaccine in rainbow trout (*Oncorhynchus mykiss*) induced immunogenicity and achieved a 50% survival rate (Romalde et al., 2004). Tumree et al. (2024) conducted a comparison of the effectiveness of monovalent versus bivalent vaccines targeting *Streptococcus iniae* and *Flavobacterium covae* infections in Asian seabass. The results illustrated that the monovalent vaccines provided higher survival rates, with 33.8% against *S. iniae* and 23.1% against *F. covae*. In contrast, the bivalent vaccines had lower survival rates of 10.5% against *S. iniae* (*S. iniae* + *F. covae*) and 11.3% against *F. covae* (*S. iniae* + *F. covae*). In a separate study, Mohamad et al. (2021) reported notable survival rates in Asian sea bass challenged with multiple pathogens after receiving a polyvalent vaccine. The study recorded survival rates of 75% against *V. harveyi*, 80% against *S. agalactiae*, and 80% against *A. hydrophila* (Mohamad et al., 2021). In another study, the efficacy of a bivalent vaccine against *S. agalactiae* and *A. hydrophila* was evaluated in *Oreochromis* sp. (red hybrid tilapia). The vaccinated fish exhibited survival rates of 80% against *S. agalactiae* and 90% against *A. hydrophila* (Mohd Ali et al., 2023). In a separate study, an oral monovalent vaccine developed from *V. harveyi* was evaluated for its immunogenicity in *Epinephelus* sp. against vibriosis. The vaccine induced a robust immune response, providing protection rates of 75% against *V. harveyi*, 85% against *V. parahaemolyticus*, and 70% against *V. alginolyticus*. The combined vaccine containing *V. harveyi*, *V. parahaemolyticus*, and *V. alginolyticus* also provided a 70% survival rate (Mohamad et al., 2022). According to Table 1, oral administration of monovalent vaccines resulted in RPS ranging from 23.1% to 100%, while polyvalent oral vaccines achieved RPS values between 11.3% and 93.17%. These variations depended on the specific bacterial pathogens and fish species involved. The results indicated no significant difference in RPS between monovalent and polyvalent oral vaccines. Therefore, farmers may choose to vaccinate using either monovalent or polyvalent vaccines based on farm management practices and disease history. Additionally, oral vaccination causes little to no stress to the fish, making the choice between monovalent and multivalent vaccines less critical in this regard. The primary advantage of polyvalent oral vaccines lies in cost reduction, as farmers can administer a single combined vaccine instead of multiple separate ones.

Table 1. Comparison of the survival rate of monovalent and polyvalent oral vaccines in fish

Diseases	Pathogens	Fish	Types of vaccines	Mono/polyvalent	Effects	RPS (%)	References
Lactococcosis	*L. garviae*	*Onchorrynchus mykiss*	Formalin-killed cells (FKCs)	Monovalent	-	*L. garviae* 50%	(Romalde et al., 2004)
Aeromonasis and Pseudomonas	*A. hydrophila* + *A. sobria* + *A. caviae* + *Pseudomonas fluorescens*	*Oreochromis* sp.	FKCs	Mono and Polyvalent	-	*A. hydrophila* 63%, *A. sobria* 86%, *A. caviae* 66%, *P. fluorescens* 70%,	(Osman et al., 2009)
Salmon rickettsial septicaemia (SRS)	*P. salmonis*	*Salmo salar*,	*P. salmonis* PS2C field strain in MicroMatrix	Monovalent	↔Weight gain, ↑ *P. salmonis* antibodies in the intestinal mucosa	*P. salmonis* 85-90%	(Tobar et al., 2011)
Enteric Redmouth Disease (ERM)	*Yersinia ruckeri*	*O. mykiss*	Commercial AquaVac ERM (MERCK Animal Health)	Monovalent	↔ Antibody titer	*Y. ruckeri* 100%	(Villumsen et al., 2014)
Enteric septicaemia of catfish	*Edwardsiella ictaluri*	*Ictalurus punctatus*	Live attenuated	Monovalent	↑ Antibody titer, ↓FCR	*E. ictaluri* 82.6–100%	(Wise et al., 2015)
SRS	*P. salmonis* + ISA virus	*S. salar*, *Oncorhynchus mykiss*,	Commercial vaccines	Mono and Polyvalent	↑ Antibody titer, anti-SRS	-	(Tobar et al., 2015)

		and *Oncorhyn chus kisutch*			and anti-ISAv specific IgM		
yersiniosis	*Y. ruckeri*	*Salmo salar*	Bacterins	Monovalent	↔ Antibody titer	*Y. ruckeri* 51%	(Ghosh et al., 2016)
Epizootic ulcerative syndrome	*A. veronii*	*Cyprinus carpio*	Recombinant OmpAI	Monovalent	↑Serum and mucus IgM, lysozyme, SOD, IL-10, IL-1β, IFN-γ, TNF-α	*A. veronii* 66.7%	(Zhang et al., 2018)
Streptococcosis	*S. iniae*	*I. punctatus*	Recombinant protein in alginate/chitosan	Monovalent	↑ Antibody titer, lysozyme, total protein, , IFN-γ, TNF-α, CD4L-2	*S. iniae* 60%	(Wang et al., 2018)
Lactococcosis / Streptococcosis	*L. garviae* + *S. iniae*	*O. mykiss*	FKCs + coated bacteria (Film)	Bivalent	↔Bactericidal activity, lysozyme, NBT, ↑IL-6, IgM, Antibody titer,	*L. garviae* 72.21%, *S. iniae* 85%	(Halimi et al., 2018)
Edwardsiellosis	*E. tarda*	*Labeo rohita*	DNA in chitosan	Monovalent	↑ Antibody titer, lysozyme, NBT, MPO	*E. tarda* 81.82%	(Kole et al., 2018)
Edwardsiellosis	*E. tarda*	Channel and Hybrid Catfish	Live attenuated	Monovalent	-	*E. tarda* 61.9-80.6%	(Chatakondi et al., 2018)

SRS	*P. salmonis*	*S. salar*	Providean Aquatech 1 Anasac in alginate	Monovalent	↔Growth parameters, ↑IgM	-	(Sotomayor-Gerding et al., 2020)
Streptococcosis and Aeromonasis	*S. iniae* + *A. hydrophila*	*O. niloticus* × O. *mossambicus*	FKCs	Mono and bivalent	↑ Lysozyme, phagocytic activity and, serum antibody (IgM)	*S. iniae* 70%, *S. iniae* + *A. hydrophila* against *S. iniae* 80%, *A. hydrophila* 63.33%,*S. iniae* + *A. hydrophila* against *A. hydrophila* 76.67%, *S. iniae* + *A. hydrophila* against both *S iniae* and *A. hydrophila* 76.67%	(Monir et al., 2020)
Lactococcosis	*Lactococcus garvieae*	*Mugil cephalus*	Biofilm + FKCs	Monovalent	↑ Phagocytic activity, IgM titres, TNF-α, TLR2, IL-1β (Biofilm)	Biofilm vaccine 77%, FKCs vaccine 18%	(Su and Chen, 2021)

Streptococcosis and Aeromonasis	*S. iniae* + *A. hydrophila*	*O. niloticus* × O. *mossambicus*	FKCs	Mono and bivalent	↑IL-8, IgM, IFN-γ (spleen, head kidney and hindgut) in Polyvalent	*S. iniae* 71.11%, *A. hydrophila* 64.44%, *S. iniae* + *A. hydrophila* against *S. iniae* 82.22%, *S. iniae* + *A. hydrophila* against *A. hydrophila* 77.78%	(Monir et al., 2021)
Vibriosis, Streptococcosis and Aeromonasis	*Vibrio harveyi* + *A. hydrophila* + *S. agalactiae*	*Lates calcarifer*	FKCs	Polyvalent	↑CCL4, dendritic cells, C3, MHC I ,β-actin, and Lysozyme	75 % against *V. harveyi*, 80% against *S. agalactiae*, and 80% against *A. hydrophila*	(Mohamad et al., 2021)
Vibriosis	*V. harveyi*	*Epinephelus* sp.	FKCs	Monovalent	↑ Antibody level of IgM against *V. harveyi*, Antibody level of IgM against *V. parahaemolytic*	*V. harveyi* 75%, *V. parahaemolyticus* 85%, *V. alginolyticus* 70%, *V. harveyi* + *V. parahaemolyt*	(Mohamad et al., 2022)

					us, Antibody level of IgM against *V. alginolyticus*	*icus* + *V. alginolyticus* 70%	
Streptococcosis and Aeromonasis	*Streptococcus iniae* + *Aeromonas hydrophila*	*Oreochromis* sp.	FKCs	Bivalent	↑ CD4, MHC-I, MHC-II, IgT, C-type lysozyme, IL-1β, TNF-α, and TGF-β	*S. iniae*: 82%, *A. hydrophila*: 77%	(Monir et al., 2022)
Aeromonasis	*A. hydrophila* + *A. veronii*	*Heteropneustes fossilis*, *Clarias batrachus* and *pangasius pangasius*	FKCs	Bivalent	↑ Lymphocytes, monocytes, granulocytes counts, and antibody (IgM) titre	*H. fossilis* 91.24, *C. batrachus* 88.09, *p. pangasius* 93.17 against *A. hydrophila* and *A. veronii*	(Rahman et al., 2022)
Aeromonasis	*A. salmonicida*	*Labrus bergylta*	FKCs	Monovalent	↑ MHC II and IgM post challenge with *A. salmonicida* (kidney and spleen)	*A. salmonicida* 66-72%	(Papadopoulou et al., 2022)
Streptococcosis and Columnaris	*S. iniae* + *Flavobacterium covae*	*L. calcarifer*	FKCs	Bivalent	↑IgM, bactericidal activity	*S. iniae*: 75.70%, *F.*	(Meachasompop et al., 2023)

					against *S. iniae*, lysozyme, gene expression of IgM, IgT, and MHCIIα, ↔growth parameters	*covae:* 77.78%	
Streptococcosis and Aeromonasis	*S. agalactiae* +*A. hydrophila*	*Oreochromis sp.*	FKCs	Bivalent	↔Growh parameter, ↑ IgM and lysozyme	*S. agalactiae* 80%, *A. hydrophila* 90%	(Mohd Ali et al., 2023)
Streptococcosis and Columnaris	*S. iniae* + *F. covae*	*L. calcarifer*	FKCs	Mono and Polyvalent	-	*S. iniae*: 33.8%, *F. covae*: 23.1%, *S. iniae* + *F. covae against S. iniae* : 10.5%, *S. iniae* + *F. covae against F. covae*: 11.3%	(Tumree et al., 2024)

Symbols: ↑: Significant increase, ↓: Significant decrease, ↔: Ineffective. RPS: Relative Percent Survival. FCR: Feed Conversion Ratio, IgM: Immunoglobulin M, TNF-α: Tumor necrosis factor-alpha, TLR2: Toll-like receptor 2, IL-1β: interleukin-1 beta, SOD: Superoxide Dismutase, IL-10: Interleukin-10, IFN-γ: Interferon-Gamma, IL-8: Interleukin-8, CCL4: Chemokine (C-C motif) ligand 4, C3: Complement Component 3, MHC I: Major Histocompatibility Complex Class I, IgT: Immunoglobulin T, TGF-β: Transforming Growth Factor-beta.

Immunization of fish using immersion monovalent and polyvalent vaccines

Immersion vaccination involves submerging fish in a solution containing vaccine antigens, allowing direct absorption through the skin and gills (Muñoz-Atienza et al., 2021). Table 2 shows the immunogenicity of monovalent and polyvalent vaccination using immersion vaccines. For example, researchers investigated an immersion polyvalent vaccine targeting Streptococcosis, Lactococcosis, and Enterococcosis in Nile tilapia. The fish exhibited a survival rate of less than 40% after being challenged with these bacteria. This indicates the polyvalent vaccine's limited effectiveness by immersion method (Abu-Elala et al., 2019). Evans et al. (2004) studied a monovalent Streptococcosis vaccine targeting *S. agalactiae* in tilapia. They showed that the *S. agalactiae* vaccine provided a 35% survival rate in Nile tilapia (Evans et al., 2004). In another study, killed *S. iniae* immersion vaccination combined with multiple puncture in rainbow trout achieved a 60% survival rate. This was while vaccination without multiple punctures reduced the survival rate to 20% (Nakanishi et al., 2002). This study demonstrates that the killed *S. iniae* immersion vaccine is effective in improving survival rates in rainbow trout when used in conjunction with multiple punctures. Meanwhile, Hoare et al. (2017) found that a polyvalent vaccine against *F. psychrophilum* resulted in an 84% survival rate in rainbow trout fry following exposure to the pathogen (*F. psychrophilum*). In a separate study, the effect of a polyvalent streptococcosis/lactococcosis and yersiniosis vaccine was evaluated in rainbow trout. The result showed that the polyvalent immersion vaccine for streptococcosis/lactococcosis and yersiniosis demonstrated survival rates of 50%, 70%, and 60% in rainbow trout (Erfanmanesh et al., 2023a). In a separate study, Khunrang et al. (2023) investigated the impact of a monovalent *S. agalactiae* vaccine administered via immersion at various concentrations in Nile tilapia. They found that the vaccine resulted in survival rates of 86.67% at a concentration of 10^6 CFU per fish, 83.33% at 10^8 CFU per fish, and 76.67% at 10^{10} CFU per fish (Khunrang et al., 2023). Furthermore, Lan et al. (2024) reported a survival rate of 63% in Asian seabass using a novel approach that combines oxygen nanobubbles, chitosan, and vibriosis vaccine through the immersion method (Lan et al., 2024). In a separate study, the effect of a polyvalent streptococcosis/lactococcosis and yersiniosis vaccine was evaluated in rainbow trout. The result demonstrated that the polyvalent immersion vaccine targeting streptococcosis/lactococcus and yersiniosis showed survival rates of 56.6%, 53.3%, and 66.6% in rainbow trout, respectively (Erfanmanesh et al., 2023b). In addition, Cao et al. (2024) evaluated a bivalent recombinant vaccine designed to protect against both *S. agalactiae* and *S. iniae* through immersion in tilapia fish. They found that the RPS was 75% following a challenge with *S. agalactiae* and 72.4% after exposure to *S. iniae* in the tilapia (Cao et al., 2024). According to Table 2, RPS following immersion vaccination with monovalent vaccines ranged from 20% to 100%, while RPS for polyvalent vaccines administered by immersion ranged from 57% to 100%. These results suggest that polyvalent vaccines generally perform better than monovalent vaccines, depending on the bacterial species and fish type. However, immersion vaccination is recommended primarily for fish weighing 3 to 50 grams, as it is not cost-effective for larger fish. Additionally, antigen uptake through the skin, gills, and mouth may be uneven among fish, which can affect the uniformity of the immune response.

Table 2. Comparison of the survival rate of monovalent and polyvalent immersion vaccines in fish

Diseases	Pathogens	Fish	Types of vaccines	Mono/polyvalent	Effects	RPS (%)	References
Vibriosis/ Pasteurellosis	*V. anguillarum*+ *Pasteurella piscicida*	*Dicentrarchus labrax*	FKCs	Bivalent	-	*V. anguillarum* 100%, *P. piscicida* ≃ 95%,	(Gravninge n et al., 1998)
Streptococcosis	*S. iniae*	*O. mykiss*	FKCs	Monovalent	-	*S. iniae* (multiple puncture/imme rsion 60%, immersion 20%)	(Nakanishi et al., 2002)
Streptococcosis	*S. agalactiae*	*O. niloticus*	FKCs	Monovalent	-	*S. agalactiae* 3.6×10^5: 34%, 1.7×10^6: 35%	(Evans et al., 2004)
vibriosis	*V. vulnificus* (strains CECT4604 and CECT5198)	*Anguilla anguilla*	FKCs	Bivalent	↑Antibody titer (skin mucus), ↑ lysozyme,	*V. vulnificus* 57%	(Esteve-Gassent et al., 2004)
Edwardsiellosis	*E. ictaluri*	*Pangasanodon*	FKCs	Monovalent	-	*E. ictaluri* 47%	(Thinh et al., 2009)

		hypophthalmus					
Streptococcosis	*S. iniae*	*Morone chrysops* × *Morone saxatilis*	Live attenuated	Monovalent	-	*S. iniae* (41-100%)	(Locke et al., 2010)
Edwardsiellosis	*E. tarda*	*S. maximus*	Live attenuated	Monovalent	↑IL-1β- IFN-γ, MHC I, IgM, CD8	*E. tarda* 35.7-63.3%	(Xiao et al., 2013)
Edwardsiellosis and Vibriosis	*E. tarda* + *V. anguillarum*	*S. maximus*	vaccine-containing microspheres	Mono and bivalent	↑NBT, ↑Antibody titer (against *E. tarda* and *V. anguillarum*)	Bivalent against *E. tarda* 78.8% and against *V. anguillarum* 93.5%	(Wang et al., 2013)
Francisellosis	*Francisella noatunensis* subsp. Orientalis	*O. niloticus* × *Oreochromis mossambicus*	FKCs	Monovalent	↑Antibody titer (25 and 30 °C),	*F. noatunensis* (fry 50% 30 °C , fingerlings 35% 30 °C)	(Soto et al., 2014)
Edwardsiellosis	*E. tarda*	*Paralichthys olivaceus*	FKCs	Monovalent	↑Serum and mucus antibody titer,	*E. tarda* (10^6 CFU/ml: 30min 9%, 60min 26%, 90min 35%), (10^7 CFU/ml: 30min 35%, 60min 48%,	(Du et al., 2015)

						90min 65%), (10^8 CFU/ml: 30min 52%, 60min 78%, 90min 74%), (10^9 CFU/ml: 30min 70%, 60min 56%, 90min 45%)	
Bacterial Coldwater Disease (BCWD)	*F. psychrophilum*	*O. mykiss* (fry)	FKCs	Polyvalent (Two strains of *F. psychrophilum* from trout and one recovered from Atlantic salmon)	↔Seum IgM, ↑serum IgT, gene expression ↑IL-1β (head-kidney), IgM, IgT (hind-gut), TLR-2 (skin), ↓IL-1β (gill), CD8α (head kidney), CD4-1 and CD8α (spleen)	*F. psychrophilum* 84%	(Hoare et al., 2017)
Motile Aeromonas septicemia (MAS)	*A. hydrophila*	*Ictalurus furcatus* × *Ictalurus punctatus*	FKCs	Monovalent	-	*A. hydrophila* 90-91%	(Shoemaker et al., 2018)
Staphylococcosis	*Staphylococcus aureus*	*Labeo rohita*	FKCs	Monovalent	-	*S. aureus* 68% after 30 day, 76% after 60 day	(Deepalakshmi and GB, 2018)

Streptococcosis, Lactococcosis, Enterococcosis	*S. agalactiae* + *S. iniae* + *L. garviae* + *Enterococcus faecalis*	*O. niloticus*	FKCs	Polyvalent	↔ Agglutination titers, ↑Microagglutination, blood parameter, biochemical, lysozyme	Less than 40% (*S. agalactiae*, *S. iniae*, *L. garviae*, *E. faecalis*)	(Abu-Elala et al., 2019)
Edwardsiellosis	*E. ictaluri*	*Pangasius hypophthalmus*	Live attenuated	Monovalent	-	*E. ictaluri* 89.29-90%	(Triet et al., 2019)
BCWD	*F. psychrophilum*	*O. mykiss*	Live attenuated	Monovalent	↑ Specific anti- *F. psychrophilum* antibody titres (After 2, 4, 6, and 8 weeks post vaccination),	*F. psychrophilum* 51-72%	(Ma et al., 2019)
Streptococcosis and Vibriosis	*S. iniae* + *V. harveyi*	*L. calcarifer*	FKCs	Monovalent and bivalent	↔ Growth parameters, cholesterol and triglyceride, ↓glucose (monovalent *S. iniae)*, ↑ calcium (monovalent *S. iniae),*↔ bactericidal activity, ↓ ACH50 (monovalent *V.*	*S. iniae* 20% (movovalent), *S. iniae* 30% (bivalent), *V. harveyi* 30% (monovalent) *V. harveyi* 40% (bivalent)	(Mohammadi et al., 2021)

					harveyi, ↑ lysozyme (bivalent *S. iniae and V. harveyi*)		
Streptococcosis and Motile Aeromonads Septicaemia	*S. agalactiae + A. hydrophila*	*O. niloticus*	FKCs	Bivalent	↑ Antibody titre (against *S. agalactiae* and *A. hydrophila* after 1, 2 and 3 weeks)	The challenged bacteria is not available 60.86-64.55%	(Sugiani et al., 2021)
Streptococcosis/lactococcosis and yersiniosis	*S. iniae + L. garvieae + Y. ruckeri*	*O. mykiss*	FKCs	Polyvalent	↑ %WG, WBC, % Hct, Complement, ↔ lysozyme, ↑ antibody titers against streptococcosis, a gainst lactococcosis and against yersiniosis (20,40 and 60^{th})	*S. iniae* (50%), *L. garvieae* (70%) and *Y. ruckeri* (60%)	(Erfanmanesh, et al., 2023a)
Streptococcosis	*S. agalactiae*	*O. niloticus*	FKCs	Monovalent	↑ Antibody titre, gene expression of Ig, blood parameters,	*S. agalactiae* (10^6 CFU/fish 86.67, 10^8 CFU/fish 83.33, 10^{10} CFU/fish 76.67)	(Khunrang et al., 2023)
Streptococcosis / Lactococosis and Yersiniosis	*S.iniae + L.garvieae,*	*O. mykiss*	FKCs	Polyvalent	↑ Antibody titre (against *S.iniae, L.garvieae,* and *Y.*	*S.iniae* 56.6%, *L.garvieae*	(Erfanmanesh, et al., 2023b)

	+ *Y. ruckeri*				*ruckeri*), Complement, lysozyme,	53.3% *and Y. ruckeri* 66.6%	
Streptococcosis	*S.iniae*	*L. calcarifer*	Heat-killed	Monovalent	↑ IgM levels against *S. iniae*, gene expression of MHCI, MHCII, CD8, *and* CD4, TNFα, IL-1β, IgM-like, IgT-like, and IgD-like	-	(Vinh et al., 2023)
Vibriosis	*V. harveyi*	*L. calcarifer*	Heat-inactivated	Monovalent	↑ Antibody titre (serum, mucus and gut), gene expression of IL-1β (head kidney 7, 21, 28 day(s)), IL-1β (spleen 14, 21 day (s)), TNF-α (head kidney 21, 28 day(s)), TNF-α (spleen 14, 21 day (s)), CD4 (head kidney 28 day(s)), CD4 (spleen 7, 14, 28)), CD8 (head kidney 7, 14, 28	*V. harveyi* 83%	(Lan et al., 2024)

					day(s)), CD8 (spleen 7, 14, 21)), IgM (head kidney 28)		
Vibriosis	*V. harveyi* +Chitosan + Oxygen nanobubbles	*L. calcarifer*	Heat-inactivated	Monovalent	↑ Antibody titre (serum and mucus), IgM (serum and mucus), gene expression of IL-1β, TNF-α, CD4, CD8, IgT (gill, head kidney, spleen)	*V. harveyi* 63%	(Lan, et al., 2024)
Streptococcosis and Vibriosis	*S.iniae,* + *V. harveyi*	*L. calcarifer*	FKCs	Mono/bivalent	↔ Blood parameters, biochemical parameters, NBT, lysozyme, complement, bactericidal, ↑ antibody titre (*S.iniae,* and *V. harveyi*)	*S.iniae* 66.6%, *V. harveyi* 76.6%,	(Erfanmanesh et al., 2024)
Streptococcosis	*S.iniae* + *S. agalactiae*	Tilapia	Recombinant (nanocarrier)	Bivalent	↑ Serum antibody levels until 4 weeks, ↑ serum acid phosphatase, alkal	*S. agalactiae* 75% and *S. iniae* 72.4%	(Cao et al., 2024)

					ine phosphatase, lyso zyme, and total antioxidant capacity, ↑ immune-related genes (IL1β, IL-6, CD8α, CD4-1, IgM, MHC I, MHC II, IFN-γ, TNF-α)		

Symbols: ↑: Significant increase, ↓: Significant decrease, ↔ : Ineffective. RPS: Relative Percent Survival. NBT (Nitroblue Tetrazolium), IgM: Immunoglobulin M, TNF-α: Tumor necrosis factor-alpha, TLR2: Toll-like receptor 2, IL-1β: interleukin-1 beta, SOD: Superoxide Dismutase, IL-10: Interleukin-10, IFN-γ: Interferon-Gamma, IL-8: Interleukin-8, CCL4: Chemokine (C-C motif) ligand 4, C3: Complement Component 3, MHC I: Major Histocompatibility Complex Class I, IgT: Immunoglobulin T.

Immunization of fish using injection monovalent and polyvalent vaccines

Table 3 shows the immunogenicity of monovalent and polyvalent vaccination using oral vaccines. For instance, Hoare et al. (2019) studied a polyvalent vaccine for *F. psychrophilum* (three serotypes) in rainbow trout, comparing formulations with and without the adjuvant montanide. They found that the vaccine containing adjuvant achieved a 100% RPS, while the version without the adjuvant demonstrated an RPS of 76-88% (Hoare et al., 2019). In a separate study, researchers examined an aqueous and adjuvant bivalent vaccine that included *A. hydrophila* and *L. garvieae* in rainbow trout. They reported that both the aqueous and adjuvanted bivalent vaccines achieved 100% RPS against *L. garvieae* and *A. hydrophila* at day 30 post-vaccination. The adjuvanted bivalent vaccine had an RPS of 95.3% for both pathogens at the same time point (Bastardo et al., 2012). In addition, the bivalent vaccine against *A. salmonicida* and *E. tarda* in turbot showed an RPS of 77.1% (Su et al., 2023). Queiróz et al. (2024) examined the impact of a monovalent injectable vaccine against *S. agalactiae* in Nile tilapia. They reported RPS of 71%, 93%, 94%, 70%, 86%, and 67% at 15, 30, 150, 180, 210, and 300 days post-vaccination (dpv), respectively (Queiróz et al., 2024). In a separate study, the efficacy of killed autogenous polyvalent vaccines against *V. harveyi, V. alginolyticus*, and *S. iniae* was evaluated in Asian seabass. The reports showed that the injected polyvalent vaccine resulted in an 82.14% RPS after challenge with the aforementioned bacteria (Ahangarzadeh et al., 2023). In addition to other studies, a study evaluated the Streptococcosis/Lactococcosis and Yersiniosis polyvalent vaccine administered intravenously to rainbow trout. The results showed that the polyvalent vaccine exhibited strong immunogenicity and RPS of 80% for *S. iniae*, 90% for *L. garvieae*, and 80% for *Y. ruckeri* after challenge in rainbow trout (Erfanmanesh, et al., 2023a). Furthermore, a bivalent vaccine against Streptococcus and vibriosis was evaluated in Asian seabass by injection. The vaccine was reported to induce favorable immunogenicity. The RPS was also recorded in Asian seabass after challenge with *S. iniae* and *V. harveyi*, at 83.3% and 86.6%, respectively (Erfanmanesh et al., 2024). Moreover, the monovalent live-attenuated *Streptococcus* vaccine increased the immune response and survival rate to 82.4% after challenge with *S. iniae* in Nile tilapia (El-daim et al., 2024). According to Table 3, monovalent injectable vaccines provided an RPS ranging from 70% to 94%, while polyvalent vaccines showed an RPS between 51.56% and 100%. This indicates that vaccination via injection, whether monovalent or polyvalent, can be chosen based on the fish species, farming conditions, and disease history. Table 3 also demonstrates that both types of vaccines elicit strong immunogenicity and effective RPS against the bacterial diseases mentioned. Given that polyvalent injectable vaccines require only a single-dose injection and are cost-effective, they are recommended as a preferred vaccination approach.

Table 3. Comparison of the survival rate of monovalent and polyvalent injection vaccines in fish

Diseases	Pathogens	Fish	Taypes of vaccines	Mono/polyvalent	Effects	RPS (%)	References
Streptococcosis	*S. agalactiae* (five strains NK_1, $N_{17}O$, $N_{14}G$, N_3M and N_4M)	Broodstock of *O. niloticus*	FKCs	Polyvalent	↑ Leucocyte, phagocytic activity (PA), respiratory burst (RB), and lysozyme activity, ↑ Antibody titer (NK_1, $N_{17}O$, $N_{14}G$, N_3M and N_4M)	*S. agalactiae* (against five strains): 5^{th} day: 77.78%, 10^{th} day:72.36%, 15^{th} day:62.20%, 20^{th} day: 51.56%	(Nakanishi et al., 2002)
Aeromonads Septicaemia, pseudomonasis, Enterococcosis	*A. hydrophila* + *P. aeroginosa* + *E. durans*	*O. niloticus*	FKCs	Polyvalent	↑ hematological parameters, ↔ glucose, Serum agglutination titer against (*A. hydrophila*, *P. aeroginosa* and *E. durans*), ↔ anti-microbial activity against *A. hydrophila* and	-	(Silva et al., 2009)

					E. durans, ↑ anti-microbial activity against *P. aeroginosa* and *E. coli*		
Aeromonads Septicaemia, , Lactococcosis	*A.hydrophila* (QSP 1.1 and QSP 10.1) + *L. garvieae* (TW446.B3)	*O. mykiss*	FKCs	Bivalent	↑ Antibody titre	Aqueous bivalent against *L. garvieae* (100%) and against *A.hydrophila* (100%), Adjuvanted bivalent against *L. garvieae* (95.3%) and against *A.hydrophila* (95.3%)	(Bastardo et al., 2012)
BCWD, IPNV	*F. psychrophilum* +*A. salmonicida*+ *Listonella anguillarum* O1 and O2, *Moritella viscosa*+ *Vibrio*	*O. mykiss*	FKCs	Bivalent (*F. psychrophilum* + IPNV) and polyvalent (*F. psychrophilum* + *A.*	↑Antibody titer (bivalent and polyvalent)	Bivalent 77.5%, polyvalent 87%	(Fredriksen et al., 2013)

	salmonicida+ infectious pancreatic necrosis virus (IPNV)			*salmonicida*+ *Listonella anguillarum* O1 and O2, *Moritella viscosa*+ *Vibrio salmonicida*)			

Streptococcosis and iridoviral	Iridovirus + *S.iniae*	*Pagrus major*	FKCs	Bivalent	-	IP against RSIV+25℃ + post 3 d:69%, IP against *S.iniae* +25℃ + post 3 d:90%, IP against RSIV+15℃ + post 10 d:88%, IP against *S. iniae* +15℃ + post 14 d:89%, IM against RSIV + post 10 d:100%, IM against RSIV + post 20 d:65-96%, IM against *S.iniae* + post 14 d:92-100%	(Thanasaksiri et al., 2018)

BCWD	*F. psychrophilum*	*O. mykiss*	FKCs	Polyvalent (serotype Fd; AVU-2T/13, serotype Th; and AVU-3S/13, serotype FpT) + adjuvant (Montanide ISA 760VG)	↑ Antibody titre	*F. psychrophilum* + adjuvant 100%, homologous 76%, heterologous 88%	(Hoare et al., 2019)
Streptococcosis / Lactococosis and Yersiniosis	*S.iniae* + *L.garvieae* + *Y. ruckeri*	*O. mykiss*	FKCs	Polyvalent	↑ WG (%), WBC, hemoglobin, lysozyme, complement, ↑ Antibody titre (against *S.iniae*, *L.garvieae*, and *Y. ruckeri*)	*S.iniae* 80 %, *L.garvieae* 90 % *and Y. ruckeri* 80 %	(Erfanmanesh, et al., 2023a)
Vibriosis and Streptococcosis	*V. alginolyticus* + *V. harveyi* + *S. iniae*	*L. calcarifer*	FKCs	Polyvalent	↑Antibody titer against *V. harveyi*, *V. alginolyticus* and *S. iniae,* ↑ lysozyme, ↔ complement, ↑ total Ig, total protein	Against *V. alginolyticus* (76.99-84.61%), against *V. harveyi* (82.14-85.71%), against *S. iniae*	(Ahangarzadeh et al., 2023)

						(60.87-69.56%)	
Furunculosis, Edwardsiellosis	*A. salmonicida* + *E. tarda*	*Scophthalmus maximus*	FKCs	Bivalent	↑Antibody titer against *A. salmonicida* and *E. tarda 3 and 4 weeks post vaccination*, lysozyme, TLR2, IL-1β, CD4, MHCI, and MHCII genes in the liver, spleen and kidney	77.1% against *A. salmonicida* + *E. tarda*	(Su et al., 2023)
Streptococcosis / Lactococosis and Yersiniosis	*S.iniae* + *L.garvieae*, + *Y. ruckeri*	*O. mykiss*	FKCs	Polyvalent	↑ Antibody titre (against *S.iniae*, *L.garvieae*, and *Y. ruckeri*), Complement, lysozyme,	*S.iniae* 70%, *L.garvieae* 60% *and Y. ruckeri* 76.6%	(Erfanmanesh, et al., 2023b)
Streptococcosis and Vibriosis	*S.iniae*, + *V. harveyi*	*L. calcarifer*	FKCs	Mono/bivalent	↔ Blood parameters, ↑biochemical parameters, ↔NBT, ↑lysozyme, complement,	*S.iniae* 83.3%, *V. harveyi* 86.6%,	(Erfanmanesh et al., 2024)

					↔bactericidal, ↑ antibody titre (*S.iniae*, and *V. harveyi*)		
Streptococcosis	*Streptococcus pyogenes*	*O. niloticus*	FKCs	Monovalent	↑Total protein, ↑GSH, DPPH, ABTS, CAT, GR, SOD, ↔ MPO, NO, GST,GPx, ↑some hematological parameters	-	(Nasr-Eldahan et al., 2024)
Streptococcosis	*S.iniae*	*O. niloticus*	Live-attenuated	Monovalent	↑ Lysozyme activity, Serum bactericidal activity, Acid phosphatase, Alkaline phosphatase, NO	*S.iniae* 82.4%	(El-daim et al., 2024)
Streptococcosis and Iridoviral disease	PISCIVAC™ Irido Si and *S. iniae*	*L. calcarifer*	FKCs	bivalent	-	14 day post challenge with ISKNV CU ($10^{0.55}$ TCI $D_{50,}$ 86-100%) and	(Thanasaksiri et al., 2024)

						(*S. iniae* 7.7×10^6 CFU, 100%)	
Streptococcosis	AQUAVAC® STREP Sa (*S. agalactiae*)	*O. niloticus*	FKCs	Monovalent	↑ Anti-*S. agalactiae* IgM levels until day 150,	*S. agalactiae* 15 day post challenge 71 %, 30 day *post* challenge 93%, 150 day *post* challenge 94%, 180 day *post* challenge 70%, 210 day *post* challenge 86% and 300 day *post* challenge 67%	(Queiróz et al., 2024)
Vibriosis	*V. Anguillarum*	*O. niloticus*	Heat-inactivated	Monovalent	-	*V. Anguillarum* 73.3-86.6%	(Manimaran & Kannabiran, 2024)

Symbols: ↑: Significant increase, ↓: Significant decrease, ↔: Ineffective. RPS: Relative Percent Survival. WG: weight gain.

Conclusion

The choice between monovalent and polyvalent vaccines in aquaculture depends on factors like efficacy, cost, and pathogen diversity. Monovalent vaccines offer targeted protection against a single pathogen but may require multiple doses, increasing labor and stress. In contrast, polyvalent vaccines combine antigens to protect against several pathogens in one injection, simplifying management and improving fish welfare. Moreover, the polyvalent vaccines can have synergistic or antagonistic antigen interactions affecting vaccine effectiveness. Ultimately, given the similar immunogenicity of monovalent and multivalent vaccines, the choice between them varies depending on the fish species, pathogens, and farm conditions, and should be made based on the farm's disease history and environmental factors.

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