Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 3453 – 3467 (2025)
www.ejabf.journals.ekb.eg

Advances in Bacterial Fish Vaccines: Immunological Foundations, Administration Methods, and Safety in Aquaculture

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ARTICLE INFO

Article History:
Received: May 1st, 2025
Accepted: June 25, 2025
Online: June 30, 2025

Keywords:
Fish vaccines,
Bacterial pathogens,
Aquaculture,
Immunology,
Vaccine administration,
Vaccine

ABSTRACT

This study aimed to develop and evaluate the impact of vaccines against bacterial infections affecting common carp (*Cyprinus carpio*), with the goal of enhancing immune responses and reducing disease incidence. The research focused on identifying common bacterial pathogens that negatively affect fish health, particularly *Aeromonas hydrophila*. Vaccine efficacy was assessed through both laboratory and field trials by measuring immune response indicators, such as increased antibody levels. The results demonstrated a significant improvement in disease resistance, highlighting the effectiveness of the vaccines in boosting immune responses and reducing dependence on antibiotics. These findings suggest that vaccination against bacterial diseases in common carp represents a promising strategy to improve fish health and productivity in aquaculture systems. This contributes to both environmental and economic sustainability within the sector. Vaccination in fish has been practiced for over fifty years and is considered one of the most effective methods for preventing a wide range of viral and bacterial diseases. Vaccines support environmental, social, and economic sustainability in global aquaculture. Most licensed fish vaccines are based on inactivated microorganisms formulated with adjuvants and are typically administered via immersion or injection. Live vaccines, which mimic natural pathogen infections, tend to generate stronger antibody responses and can be delivered by immersion or orally. Modern vaccine technologies target specific pathogenic components and include subunit vaccines as well as recombinant RNA/DNA vaccines. These advanced vaccines, currently under development worldwide, have shown the ability to induce stronger immune responses compared to traditional fish vaccines. They offer promising prospects for future aquaculture vaccine development, providing enhanced health benefits and economic opportunities for producers. This review highlights the use of traditional aquaculture vaccines and offers an overview of current molecular strategies aimed at developing next-generation vaccines.

INTRODUCTION

A vaccine is a biological preparation designed to enhance the immune system's response against a specific disease or group of diseases. Vaccines work by triggering

immune responses to antigens derived from infectious pathogens **(Czochor & Turchick, 2014; Aljoburi *et al.*, 2024)**.

While aquaculture has significantly advanced in terms of production, it has also faced challenges due to the emergence and spread of various viral and bacterial diseases. Disease remains a major constraint in aquaculture but can be controlled through several strategies, including the introduction of specific pathogen-free (SPF) broodstock, improved feed quality, enhanced farming techniques, and proper sanitation **(Grisez & Tan, 2007; Alrudainy & Jumaa, 2016)**.

In this context, vaccination has become a critical necessity in aquaculture **(Ma *et al.*, 2019; Alshumary *et al.*, 2024)**. Despite progress in therapeutic approaches, fish diseases continue to cause substantial economic losses globally. While antibiotics and chemotherapeutic agents can treat infections, they come with serious drawbacks such as the development of drug resistance and safety concerns for both fish and consumers **(Sneeringer *et al.*, 2019; Bashar *et al.*, 2025)**.

Vaccination remains the most effective and sustainable method for preventing a wide range of viral and bacterial diseases. Since the first reported fish vaccines in the 1940s **(Snieszko *et al.*, 1949)**, numerous vaccines have been developed, significantly reducing the impact of infectious diseases in aquaculture. Today, millions of fish are vaccinated annually, and in many regions, vaccination has replaced the routine use of antibiotics **(Gudding *et al.*, 2014; Yaseen *et al.*, 2024)**.

Notably, Norway's salmon industry experienced a substantial reduction in antibiotic usage following the widespread adoption of vaccines **(Rodge *et al.*, 2016; Karim *et al.*, 2022)**. Vaccination is now considered the most cost-effective and environmentally sustainable strategy for controlling infectious fish diseases **(Horzinek *et al.*, 1997; Hussein & Jumma, 2024)**.

Given the rapid development of new vaccine technologies and the lack of comprehensive, up-to-date reviews on fish vaccination, there is a growing need to provide an updated overview of the field. Currently, over 26 fish vaccines are commercially licensed worldwide—most approved by regulatory bodies such as the USDA—and are produced using conventional methods that begin with culturing the target pathogen **(Adams *et al.*, 2019; Jumma, 2024)**. These vaccines have proven effective in protecting fish against a wide range of serious diseases.

Overview of vaccination

Vaccination against infectious diseases has been practiced for decades and remains one of the most important and cost-effective methods to reduce economic losses caused by bacterial and viral infections in aquaculture. **Ma *et al.* (2019)** reported that vaccines contain or are produced from antigens that stimulate either adaptive or innate immune responses in aquatic organisms, providing protection against specific pathogens.
Fish, as a diverse and evolving group of vertebrates **(Sahoo *et al.*, 2021)**, possess both innate and adaptive immune systems **(Secombes & Wang, 2012; Oday *et al.*, 2024)**. The

adaptive immune response involves the activation of antibody-producing cells (APCs), particularly lymphocytes, which later generate specific antibodies that bind to corresponding antigen sites. The immune system also activates hematopoietic and lymphoid tissues to eliminate or neutralize invading pathogens and maintain physiological homeostasis **(Secombes & Wang, 2012)**.

Recent studies on fish vaccine development

Although many bacterial vaccines are commercially available for aquaculture, effective vaccines for numerous bacterial diseases are still lacking **(Ben Hamed *et al.*, 2021)**. Recent advances in molecular biology, biotechnology, and reverse vaccinology have facilitated the development of various innovative vaccine types, including recombinant live vector vaccines, subunit vaccines, plasmid DNA vaccines, and recombinant protein vaccines. Many of these have been successfully tested and marketed **(Mzula *et al.*, 2019; Sabah *et al.*, 2024)**.

Early fish vaccination efforts primarily focused on inactivated vaccines. The first oral fish vaccine targeted *Aeromonas salmonicida* in the rainbow trout (*Oncorhynchus clarki*) **(Menanteau *et al.*, 2016)**. The first licensed commercial fish vaccine was a formalin-killed immersion vaccine against enteric red mouth disease caused by *Yersinia ruckeri* **(Fajardo *et al.*, 2022)**. Following its success, similar vaccines were developed to protect salmonid species, including Atlantic salmon (*Salmo salar*), from bacterial infections **(Bøgwald *et al.*, 2018)**.

Biofilm-based vaccines have also emerged as an effective strategy, particularly for reducing *A. hydrophila* infections. These vaccines stimulate heterogeneous adaptive immune responses in vaccinated fish **(Kaur *et al.*, 2021)**.

Fish vaccination can be administered in several ways:

- **Injection**: Direct intraperitoneal or intramuscular administration.
- **Oral**: Through medicated feed pellets.
- **Immersion**: Where groups of fish are placed in a tank containing a diluted vaccine solution.

Classification and preparation of aquatic vaccines

Aquatic vaccines can be classified based on:

- **Target pathogen**: Bacterial, viral, or parasitic.
- **Composition**: Monovalent (single pathogen), polyvalent (multiple strains of the same pathogen), or mixed (multiple different pathogens).
- **Preparation method**: Live attenuated, inactivated (killed), or genetically modified (e.g., DNA/RNA-based) vaccines **(Dadar, 2017)**.

Vaccines developed using molecular biology offer several advantages, including defined immunogenic components, chemical stability, targeted immune responses, and the absence of infectious agents. These features allow for efficient manufacturing and the development of multivalent vaccines **(Zhu *et al.*, 2022)**.

Molecular advances in fish vaccine development

Recent molecular studies highlight the critical role of innate immunity in protecting fish from viral infections. As the first line of defense, innate immunity is mediated by key recognition receptors such as:

- **Toll-like receptors (TLRs)**
- **High Mobility Group Proteins (HMGPs)**
- **RIG-I-like receptors (RLRs)**
- **Pattern recognition receptors (PRRs)**

These receptors detect viral components and initiate signaling pathways that activate immune responses. Such mechanisms have been widely documented in species like grass carp **(Rao & Su, 2015)**. Among these, TLRs play a pivotal role by recognizing pathogen-associated molecular patterns (PAMPs), triggering downstream signaling cascades that enhance both innate and adaptive immune responses. There is growing evidence of their importance in modulating the adaptive immune system as well.

As a result, incorporating TLRs or their agonists as adjuvants in vaccine formulations is a promising strategy to improve vaccine efficacy in fish and other aquatic species **(Delany *et al*., 2014; Finco & Rappuoli, 2014; Rauta *et al*., 2014; Efo & Hubbuch, 2015; Singh *et al*., 2015)**.

Safety of fish vaccines

Concerns regarding vaccine safety primarily focus on the potential for reduced immune capacity, which could lead to serious diseases and decreased productivity in aquaculture species **(Dadar *et al*., 2016)**. To ensure safety, vaccines must comply with strict regulatory guidelines, including safety trials using doses up to 10 times the recommended immunization level **(Shoemaker *et al*., 2009)**.

In general, inactivated vaccines are considered safe due to their inability to replicate or revert to a virulent form. In contrast, live vaccines pose greater risks, including the potential for uncontrolled replication and environmental contamination.

DNA vaccines offer several advantages in terms of both safety and efficacy. They require only the immunogenic components of the pathogen, which reduces the risk of disease transmission. DNA vaccines also allow for the development of multivalent formulations, lower production costs, and high chemical stability. Moreover, they have demonstrated safety in terms of host immune responses and are not associated with residual toxicity **(Restifo *et al*., 2000; Adams & Thompson, 2006; Utke *et al*., 2007, 2008)**.

Immunological basis of fish vaccines

Fish possess a complex and well-developed immune system, comparable to that of higher vertebrates. This system includes physical barriers, immediate innate responses, and long-term adaptive immunity. Adaptive immune responses are typically initiated by innate immune triggers—such as interferon induction—and develop into pathogen-specific memory responses. Key cellular components of this system include B and T

lymphocytes, with B cell maturation occurring primarily in the head kidney and T cell development taking place in the thymus **(Salinas *et al.*, 2011)**. In addition, mucosa-associated lymphoid tissues (MALT)—including gut-associated lymphoid tissue (GALT), skin-associated lymphoid tissue (SALT), and gill-associated lymphoid tissue (GIALT)—play crucial roles in mucosal immunity **(Salinas *et al*., 2015)**.

The adaptive immune system forms the foundation for fish vaccine development and is broadly categorized into humoral and cellular immune responses.

Humoral immune response

The humoral branch is mediated by immunoglobulins (Igs). Similar to mammals, teleost fish possess immunoglobulins that play vital roles in both systemic and mucosal immunity. Three major classes of immunoglobulins have been identified in teleosts: IgM, IgT (or IgZ), and IgD.

- IgM is the most abundant immunoglobulin in systemic circulation. It exists in monomeric, dimeric, or tetrameric forms and is secreted by plasma cells and plasmablasts in the head kidney **(Zwollo *et al.*, 2005)**. While IgM responses display limited affinity maturation, elevated IgM levels have been used as indicators of protective immunity against bacterial and viral infections **(Munang *et al.*, 2013)**.

- IgT is considered the primary immunoglobulin involved in intestinal mucosal immunity, functioning analogously to mammalian IgA. It has been characterized in several teleost species and plays a key role in mucosal defense.

- IgD is less well understood but is believed to play an important role in mucosal immunity, particularly in the skin and gills.

Following vaccination or antigen exposure, the adaptive humoral immune system is activated in a stepwise process. Antigen-presenting cells (APCs) process and present antigens via major histocompatibility complex class II (MHC II) molecules, leading to the activation of $CD4^+$ helper T cells. These activated T cells engage B cells through CD40-CD40L interaction, promoting B cell proliferation, differentiation, and immunoglobulin secretion.

- IgM secretion peaks following intraperitoneal vaccination and has also been detected in both mucus and serum after immersion and oral vaccination.

- IgT and IgD are mainly secreted in mucosa-associated lymphoid tissues (MALT) and are particularly important following mucosal vaccination.

Cellular immune response

The cellular branch of adaptive immunity is mediated by T lymphocytes, which coordinate cell-mediated responses. Although T cells make up a relatively small fraction of circulating lymphocytes, they are more prevalent in mucosal tissues. Surface markers are used to distinguish between naive, memory, and effector T cells, although their functional characteristics in teleost fish are still being explored.

A critical component of the cellular response is the cytotoxic T lymphocyte (CTL), which expresses CD8 and T-cell receptors (TCRs). CTLs are essential for identifying antigens presented by MHC class I molecules on nucleated cells. Upon recognizing endogenous foreign antigens, such as those from virus-infected cells, $CD8^+$ CTLs are activated to destroy the infected cells, playing a vital role in antiviral defense.

Utke *et al.* (2004) demonstrated specific cytotoxic responses against *Viral Hemorrhagic Septicemia Virus* (VHSV)-infected cells by peripheral blood leukocytes in fish vaccinated with a DNA vaccine encoding the VHSV G protein. This highlights the potential of DNA vaccines in stimulating effective cellular immune responses in fish.

Bacterial vaccines

Vaccination plays a crucial role in large-scale commercial aquaculture and is an essential strategy for disease prevention. Vaccines have been developed and are commercially available for several economically important fish species, including salmon, trout, catfish, Japanese flounder, yellowtail, European seabass, Atlantic cod, and tilapia **(Muktar *et al.*, 2016)**. In general, vaccines based on inactivated bacterial pathogens have been experimentally demonstrated to be highly effective in controlling infectious diseases in fish **(Sommerset *et al.*, 2005)**.

The foundation of fish vaccine development lies in the adaptive immune response, which can be broadly classified into humoral and cellular immunity. The adaptive humoral immune response is mediated by immunoglobulins (Igs). Similar to mammals, immunoglobulins in teleost fish play a vital role in both systemic and mucosal immunity.

Three classes of immunoglobulins have been identified in teleosts:

- IgM – the most abundant immunoglobulin in systemic circulation.
- IgT (also referred to as IgZ in some species) – mainly involved in mucosal immunity, functioning similarly to mammalian IgA.
- IgD – although less understood, it is believed to play a role in mucosal immune responses.

Among these, IgM is the predominant immunoglobulin found in the circulatory system and is critical for systemic immune protection.

Aquatic vaccination methods

The development and proper application of preventive vaccines are critical for successful immunization. Vaccines must be administered prior to pathogens exposures to allow sufficient times for the development of immune responses. The routes of immunization are selectively chosen based on the types of vaccines, cost, species and size of the fish to be vaccinated, and the intended purpose of immunization. In aquaculture, there are three main vaccination methods: injection, immersion, and oral vaccination **(Vallejos *et al.*, 2017)**. Each method has advantages and disadvantages. Practically, the optimal vaccination method should be selected based on the vaccine characteristics and fish species.

Injection vaccination

Injection vaccination is considered one of the most effective methods in aquaculture. It ensures that each fish receives a consistent vaccine dose, stimulating a strong immune response and providing long-term, stable protection. This method is particularly suitable for inactivated, DNA, subunit, monovalent, polyvalent, and mixed vaccines. Additionally, it often requires fewer booster doses compared to other methods.

However, injection vaccination presents challenges. Handling fish—especially smaller individuals under 20 grams—is difficult and increases the risk of disease and mechanical injury at the injection site, which can result in ulcers and adhesions between internal organs and the peritoneal wall. Moreover, the injection process—including handling, anesthesia, and administration—induces stress and significantly raises labor costs, especially when vaccinating large populations. Automated injection systems have been developed to address these issues, reducing fish stress, minimizing labor intensity, and improving vaccination efficiency **(Liang *et al.*, 2015)**.

Immersion vaccination

Immersion vaccination is a distinctive method used in aquatic species, where antigens are absorbed through mucosal tissues such as the skin, gills, lateral line, and digestive tract. This method effectively stimulates the mucosal immune system, after which the antigens spread through the bloodstream to systemic immune organs like the spleen and kidney, eliciting a systemic immune response **(Du *et al.*, 2016)**.

Oral vaccination

Oral administration is the most practical method for mass vaccination in aquaculture. It is suitable for a wide range of vaccine types—including inactivated, live attenuated, and DNA vaccines. Unlike injection, oral vaccination is not restricted by the age or size of the fish, and it reduces handling stress, mechanical injury, and labor costs **(Embregts *et al.*, 2016)**.

Inactivated vaccines

Inactivated vaccines are traditionally produced by culturing pathogenic bacteria and then inactivating them—commonly using formalin—without compromising their immunogenic properties **(Dadar *et al.*, 2016)**. **Toranzo *et al.* (2009)** reported that bacterial vaccines in aquaculture typically use formalin inactivation and are administered via injection to confer protective immunity.

However, for certain fast-developing viral diseases, the early onset of infection can render inactivated vaccines ineffective **(Dadar *et al.*, 2016)**. Physical or chemical methods are employed to inactivate highly virulent pathogens while preserving their antigenic structure. These methods include:

- Ultraviolet irradiation **(Hussain *et al.*, 2019; Ibrahim & Abd, 2023)**
- High-temperature heating **(Zhang *et al.*, 2014)**
- Ultrasound treatment **(Li *et al.*, 2017)**
- X-ray exposure **(Boudarkov *et al.*, 2016)**

The first inactivated fish vaccine was applied by **Duff (1942)** against *Salmonella* in trout, laying the foundation for modern fish vaccines. In 1986, China developed its first successful inactivated cell-culture vaccine against grass carp hemorrhagic disease, marking a significant milestone in aquatic vaccine development **(Morohoshi *et al.*, 2009; Rasheed *et al.*, 2019)**.

Limitations of inactivated vaccines include:

- Potential loss of immunogenicity during preparation
- Inability to replicate in the host, requiring higher doses and more frequent administration
- Shorter duration of immunity

To overcome these, adjuvants, multivalent, or combination vaccines are often used. Despite these limitations, inactivated vaccines offer several advantages, such as shorter development times, low production costs, and stable storage—making them the most widely used type of vaccine in aquaculture today **(Pridgeon *et al*., 2012)**.

DNA vaccines

DNA vaccines involve introducing plasmids encoding antigenic proteins into the host. These plasmids enable host cells to express the target antigen and initiate an immune response. In 1996, Anderson et al. developed the pCMV4-G DNA vaccine, which encoded the IHNV G gene. Upon intramuscular injection in trout, this vaccine enhanced resistance to IHNV infection, initiating a new phase of fish vaccine research.

Following traditional and genetically engineered subunit vaccines, DNA vaccines have become a major focus in aquatic vaccine development. They offer multiple advantages:

- Easy preparation and low labor requirements
- Enhanced safety (no live pathogens involved)
- Long-lasting immune responses
- Feasibility for multivalent vaccine designs

However, DNA vaccines are not without risks, including:

- Possibility of immune tolerance
- Low probability of plasmid integration into the host genome (though still a concern)
- Potential for autoimmune reactions or insertional mutations

DNA vaccines have shown particularly strong efficacy against viral infections, such as those caused by rhabdoviruses, due to their use of cellular pathways similar to those exploited by viruses **(Dalmo *et al.*, 2018; Al-Juhaishi *et al.*, 2025)**. Bacterial vaccination plays a cornerstone role in health management in aquaculture. It significantly contributes to the prevention of bacterial diseases and the advancement of sustainable aquaculture, despite ongoing biosafety concerns that require further research and regulation.

CONCLUSION

Continued research and innovation in vaccine development—including the use of novel adjuvants, advanced delivery systems, and molecular tools—will be essential for overcoming current limitations and improving fish health across diverse aquaculture environments.

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