REVIEW ARTICLE

Vaccines for the Control of Tilapia Lake Virus Infections in Fish: Current Status and Recent Developments – A Review

Neha Brahma[1], K. M. Ruban Chakravarthy[1], A. Deepika[1], P. Parthiban[2], S. Vimal[1]

[1]Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, [2]Department of Pathology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (Deemed to be University), Thandalam, Chennai, Tamil Nadu, India

Abstract

Aquaculture is recognized as one of the most rapidly expanding food-production sectors worldwide and has become an important source of seafood for human consumption. However, this sector is facing various problems, including infectious diseases, the biggest obstacle preventing the growth and economic output. Diseases have a significant effect on the marketability, fertility, growth, and death rates in aquaculture. Diseases have a significant effect on the marketability, fertility, growth, and death rates in aquaculture. In intensive aquaculture systems, infectious agents such as bacteria, viruses, fungi, and parasites can result in substantial losses of fish stocks. Viral diseases cause irreversible damage to the aquaculture sector. Tilapia is the second most commonly farmed aquatic species, but owing to tilapia lake virus (TiLV), there has been a major loss, with mortality rates peaking at more than 90%. It is a contagious disease that spreads via horizontal and vertical transmission. Vaccination is an effective method of disease prevention and control of infectious diseases in aquaculture. Recently developed TiLV vaccines showed promising results in reducing infections. With the ongoing global expansion of aquaculture, the demand for new vaccines will persist far into the future. This article provides a thorough insight into TiLV, especially its different types of vaccines and immune responses that have been developed so far.

Key words: Aquaculture, sustainability, tilapia lake virus, vaccines

INTRODUCTION

Aquaculture is among the most rapidly expanding food-production sectors worldwide, with seafood production reaching 181 million tonnes during 2020–2022. Aquaculture fish farming in inland and coastal areas increases the production of seafood, which benefits both the local food supply and economy (Arumugam *et al*., 2023). Asia is the biggest producer of seafood; this is attributable to an increase in fish intake per capita. To accomplish the demand for food and the economy, culturally relevant fish species, as well as production methods, were practiced. To achieve these needs, tilapia is considered the second most broadly cultured fish variety in aquaculture.[1] Tilapia fish are affordable, can tolerate high as well as low-level water temperatures, and withstand high water salinity in a culture environment. It is considered a rich source of protein and a nutrient provider among cultured fish.[2-4] Among Tilapia species, Nile Tilapia (*Oreochromis niloticus*) is the most cultivated fish, which comes under the family Cichlidae. Tilapia is a freshwater animal and plays an important role in the ecosystem by maintaining the balance of the freshwater ecosystem, and is rarely found in brackish water.[2,5] Over the ages, tilapia production has rapidly increased, and in the year 2020, more than 4.5 million tonnes of tilapia fish

Address for correspondence:
Dr. S. Vimal, Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Thandalam - 602105, Chennai, Tamil Nadu, India.
E-mail: vimalbiotechs@gmail.com

Received: 11-05-2026
Revised: 18-06-2026
Accepted: 25-06-2026

were produced worldwide.[6] Most probably, tilapia is highly cultivated over the other aquatic species because of its ability to resist viruses, bacteria, and pathogen infections.[7] Despite tilapia's origin from Africa and the Levant region, it is now mainly cultivated and produced in Asia. China is considered the largest tilapia-producing country, followed by Egypt, Indonesia, and Bangladesh.[8] Tilapia can also be vulnerable to viral and bacterial infections, such as *Vibrio, Aeromonas, Pseudomonas,* and *Streptococcus*.[2,5,9] Due to the demand for tilapia in aquaculture, the farming practice has also increased, which leads to high stress levels in the culture environment. The factors that can induce stress levels in fish can be due to an increase in density, poor water quality, standard fish farming practices, transportation, and netting, which have been well-documented.[10] Changes in the environmental conditions may increase the stress level in fish, which also results in immunosuppression. This condition weakens the innate immune system of fish against pathogens, and becomes more vulnerable to infections caused by viral and bacterial diseases.[10]

The outbreak of tilapia lake virus (TiLV) in tilapia fish caused a huge loss in aquaculture production. The virus is a novel orthomyxo-like RNA virus and represents an important threat to the sustainability of tilapia farming.[11-13] The virus was first reported in Israel,[14] and was followed by 15 countries and 4 continents that were severely affected.[15,16] This causes 80% mortality and a high morbidity rate during the early stage of the disease, and is susceptible to being affected in any life stage form.[15,17] This review is a brief study and development of the TiLV vaccine across the globe in the past 5 years.

THE ORIGIN AND EPIDEMIOLOGY

TiLV disease (TiLVD) results from infection with an enveloped, single-stranded RNA virus that contains a 10-segmented genome, in which individual segments range from 456 to 1641 nucleotides and the total genome size is 10,323 nucleotides.[17] These 10 segments encode 14 proteins, which are responsible for the primary pathogenicity in tilapia.[11,12,14,18] TiLV diameter ranges between 55 nm and 2200 nm.[11,14,17-20] In the summer of 2009, large-scale tilapia fish died due to an unknown virus in Israel that caught the attention of scientists. However, the disease was not officially diagnosed as TiLV until 2013.[14] In the same year, TiLV was reported in Ecuador.[19] Nevertheless, genomic epidemiology suggests that the TiLV likely originated between 2003 and 2009, approximately 5–10 years before its first official recognition in Israel, where it has become widely distributed across Asian countries. TiLV has been verified to be present in 16 countries, including Colombia,[21] Egypt,[22,23] Thailand,[15,18] India,[24] Malaysia,[25] Bangladesh,[26] Uganda,[27] Tanzania,[27] the USA,[28] and China.[16] TiLV is considered a significant threat in over 45 countries, spanning various regions [Figure 1].[15,18]

The risk of a catastrophic pandemic in tilapia aquaculture is considerable because the virus is widely distributed across tropical regions, including the Americas, Asia, and Africa. Moreover, the importation of tilapia seed from countries where TiLV has been reported may increase the likelihood of intercontinental spread, placing many African nations at risk.[29] Tilapia species showed mortality reaching up to 90%, and all the growth stages are susceptible to TiLV infection. The mortality rate of tilapia fish differs across geographical regions. The outbreak reported in Israel, Egypt, and Thailand occurred mainly during the hot summer time, particularly during the months of May–October.[14,22] In Ecuador, the outbreak ends up till November.[19] The water temperature, ranging from 22 to 32°C, was reported for the clinical outbreak of TiLVD because of the underlying factors. It assumes that the actual prevalence and distribution of TiLV is inaccurate due to a lack of comprehensive research and in-depth studies of mortality-related events in the tilapia industry, the absence of standard TiLV testing techniques, restrictions on certain national veterinary services, and various political issues.[29,30]

A CONCISE INTRODUCTION TO IMMUNIZATION IN FISH

In aquaculture, fish infections have been a significant concern, resulting in substantial economic losses worldwide.[31] Despite numerous trials and the development of new therapies, there has been no significant improvement seen in the health of aquatic fish. Antibiotics or chemical treatments for fish disease may be available; however, they come with clear drawbacks, including drug-resistant bacteria and consumer and environmental safety concerns.[32] A vaccine is a biological agent formulated to stimulate or strengthen adaptive immune responses by activating cytotoxic T-lymphocytes and antibody-secreting B cells. Its purpose is to confer immunity to individuals against a specific illness or a variety of illnesses. These vaccines are usually developed from the disease source, its components, or a synthetic substitute serving as an antigen without inducing the disease. As a biological agent, vaccines entail exposing the fish's immune system to either the complete pathogen or its components before encountering an infection from a highly pathogenic wild-type. At the beginning of the tradition, vaccine studies were conducted exclusively on vaccinated fish. Since the early reports published in the 1940s, the development of multiple vaccines has markedly lessened the losses linked to bacterial and viral infections in fish.[33,34] Preventing disease outbreaks remains a major challenge in aquaculture, as production systems are constantly exposed to opportunistic bacteria, viruses, and parasites. Because these pathogens may persist in the environment or be carried by apparently healthy fish, aquaculture systems are particularly prone to health-related disturbances.[35] The first fish vaccine to be reported was an oral vaccine administered to cutthroat trout, which was a killed variant of *Aeromonas salmonicida*, a

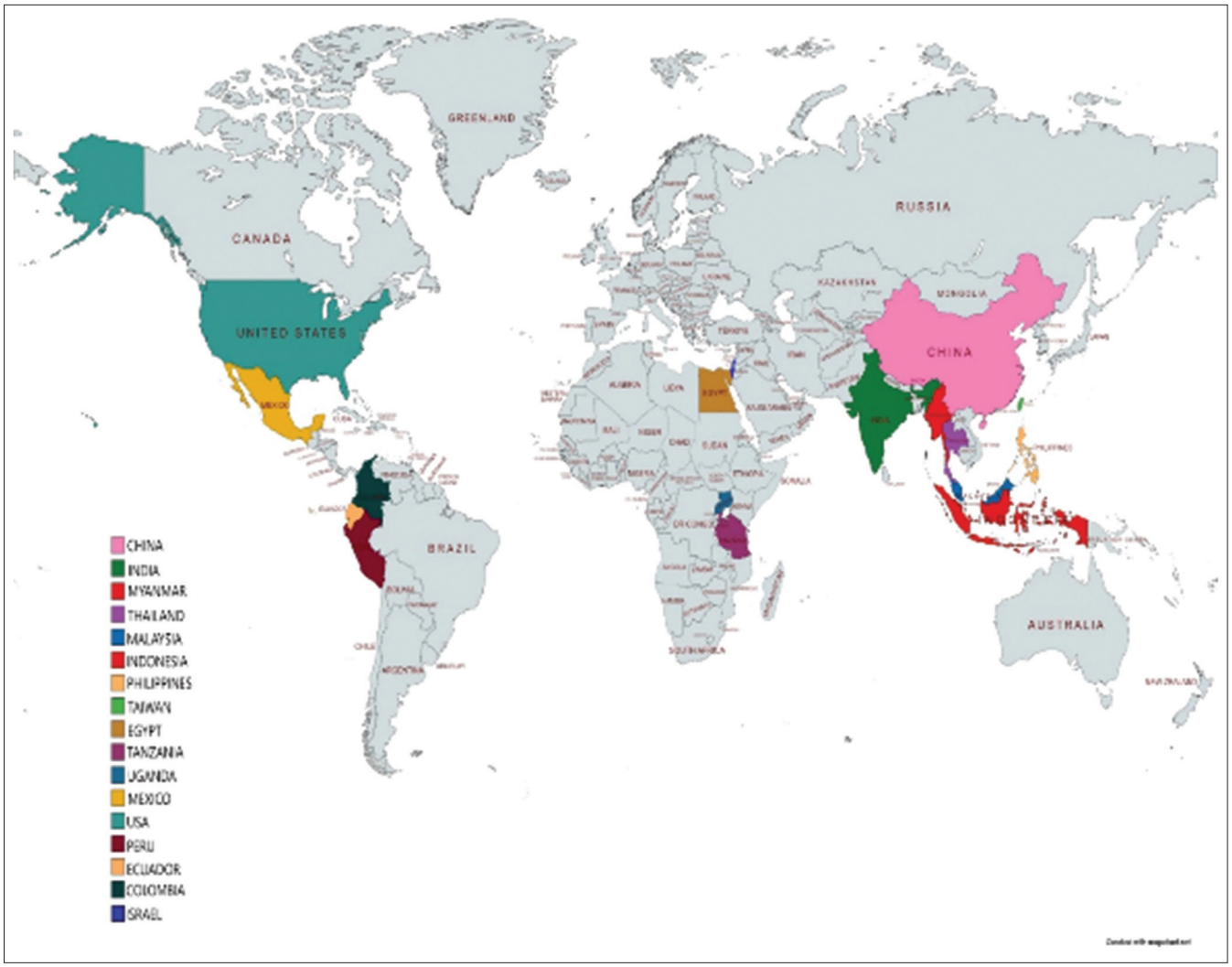

Figure 1: Representation of tilapia lake virus-affected countries worldwide

species of *Oncorhynchus clarkii*.[36,37] The first commercially available licensed fish vaccine was an immersion-administered vaccine designed to prevent the transmission of enteric redmouth disease.[38] This vaccine was inactivated and targeted *Yersinia ruckeri*. At present, 26 licensed vaccines are commercially available worldwide for application in different fish species.[32,39] Viral vaccines are commonly developed from attenuated viruses, inactivated viral particles, or specific surface proteins and other viral components. In fish, the major vaccine types include whole-pathogen vaccines, either inactivated or attenuated, together with subunit vaccines, virus-like particles (VLPs), and nucleic acid-based vaccines.[2] Since some vaccines trigger only a modest immune response on their own, adjuvants are frequently added to enhance their effectiveness.[2] Adjuvants are capable of increasing the immune responses and stimulating the cytotoxic T-lymphocytes to promote local or systemic immune activation, because they are structurally diverse compounds and can directly regulate the immunogenicity of a specific antigen.[40,41]

Disease challenge models are standardized *in vivo* models used to evaluate the effectiveness of vaccines. Both cohabitation challenge models closely resemble natural viral exposure and transmission routes, even though they present greater complexities in terms of control compared to injection challenge methods, in which every individual fish is administered an identical pathogen dosage.[2] A study has recently achieved a successful development of a vaccine for TiLV, which has been causing mortality and economic losses in the Asian aquaculture industry.[42] However, the approach of knocking out virulent genes for TiLV vaccine development faces challenges due to limited knowledge about the functions of the various genes within TiLV's 10-segment genome. To address this challenge, there is a critical need for the development of a reverse genetics approach, similar to the methodology employed for influenza A viruses. This approach will be pivotal in taking the first steps toward genetically modifying TiLV to create attenuated TiLV vaccine candidates with deleted genes. Zeng *et al*. have recently achieved success in creating a promising live-attenuated vaccine against infectious spleen and kidney necrosis virus (TiLV) by deleting specific highly protective and low-pathogenic genes. However, the development of a TiLV vaccine based on a gene-deletion strategy remains

difficult because the functions of many genes within the 10-segment TiLV genome are still not fully understood. There is a pressing need for the establishment of a reverse-genetics approach, comparable to that used for influenza A viruses, as it would represent a crucial initial step in modifying the TiLV genome to produce gene-deleted attenuated vaccine candidates.

CO-HABITATION

When sharing the same environment with tilapia in a cultivation pond, several fish species, including major Indian carp species (*Labeo rohita*, *Catla Catla*, and *Cirrhinus mrigala*), milkfish (*Chanos chanos*), pearl spot (*Etroplussuratensis*), *Mugil cephalus, Cyprinus carpio*, and *Liza ramada,* did not experience any mortality throughout the outbreak period.[22,43] In addition, a study by Jaemwimol *et al*. revealed that several tropical freshwater warm-water species, such as *Trichogaster pectoralis, Pangasianodonhy pophthalmus, Clarius macrocephalus, Channa striata, Anabas testudineus, C. carpio, Barbodes gonionotus,* and *Lates calcarifer,* exhibited resistance to TiLV infection. However, it is noteworthy that non-tilapia hosts were first identified, including the river Barb (*Barbonymus schwanenfeldii*) along with giant gourami (*Osphronemus goramy*), which were found to be infected by TiLV.[43,44]

CO-INFECTION

Reports have also documented concurrent infections involving various bacterial pathogens in fish affected by TiLV.[18,23] Various species of *Aeromonas,* including *A. veronii, A. ichthiosmia, A. enteropaelogenes, A. hydrophilia, Flavobacterium* spp., and *Streptococcus* spp., were identified in TiLV-infected fish.[23,43,44] The extent to which TiLV and co-infections contribute to clinical severity, mortality, incubation time, and other factors remains uncertain.[45]

Notably, a naturally occurring TiLV–*A. veronii* co-infection has been documented in Malaysian red hybrid tilapia.[25] Reports indicate that TiLV co-infections are observed more often with *Aeromonas* species than with other bacterial groups. This can be attributed to the presence of potent virulence factors in *Aeromonas* species and their adaptability to adverse conditions.[46] This information underscores the need for further research to verify the potential involvement of other pathogens in TiLV infections. There have been documented instances of concurrent infection involving diverse bacterial pathogens in fish afflicted by TiLV. Regarding TiLV, instances of co-infection were reported among TiLV-positive fish in Thailand. These coinfections involved various bacteria (*Flavobacterium*, *Aeromonas*, and *Streptococcus*), the monogenean parasites (*Gyrodactylus salaris* and *Dactylogyrus vastator*), and ciliated protozoa (Trichodina).[18] In both Thailand and Egypt, researchers were able to isolate multiple bacterial species from TiLV-infected fish (*Tenacibaculum maritimum*-like virus). These species included *Flavobacterium, Streptococcus*, and *Aeromonas* species have been reported in previous studies *Flavobacterium, Streptococcus*, and *Aeromonas* species, have been reported in previous studies.[18,23]. Similarly, TiLV-infected fish in Malaysia yielded concurrent isolations of *A. veronii, S. xylosus, A. sobria, A. hydrophila,* and *S. agalactiae*, as documented in research.[25,47,48]

FISH VACCINE AND CONTEMPORARY STRATEGIES FOR MANAGING TILV INFECTIONS

A vaccine is a biological product developed to enhance protection against a particular disease or group of related diseases. It works by stimulating the immune system to recognize and react to an antigen derived from a disease-causing pathogen.[23,49] Over the years, a spectrum of vaccines has emerged to combat viral infections relevant to aquatic environments. These vaccines have been developed employing various methodologies, all with the common goal of eliciting an immune response against a specific pathogen and establishing a lasting immune memory to safeguard against the target virus. Although inactivated viruses and recombinant subunit proteins remain the basis of most viral vaccines used in aquaculture, newer approaches such as attenuated vaccines and DNA-based vaccines have become increasingly important and are now commonly used in cyprinid and salmonid farming. These newer vaccine strategies are used to control diseases caused by viruses such as koi herpesvirus (KHV), infectious hematopoietic necrosis virus (IHNV), and salmonid alphavirus.[39] In Israel, the initial vaccine against T-lymphocytopenia (TiLV) was created using TiLV strains that underwent 17–20 consecutive passages of cell culture, resulting in a prototype vaccine with a relative percent survival (RPS) of more than 50% [Figure 2].[11] As of now, there are no available therapeutics or commercially produced vaccines for the control of TiLVD.

Live/attenuated viral vaccines

Live attenuated viral vaccines consist of viruses that have been weakened through genetic or chemical methods, allowing them to induce an immune response in the host for a limited period. These vaccines consist of live microorganisms, such as bacteria and viruses, which have been rendered non-pathogenic, meaning they can no longer cause the specific infection. Live vaccines may be weakened through chemical mutagenesis, serial passage in cell-culture systems under non-standard conditions, or targeted genetic modification.[39] Live vaccines are generally more immunogenic than their inactivated counterparts. This is largely because their ability to replicate or enter host tissues triggers broader cellular responses involving both innate

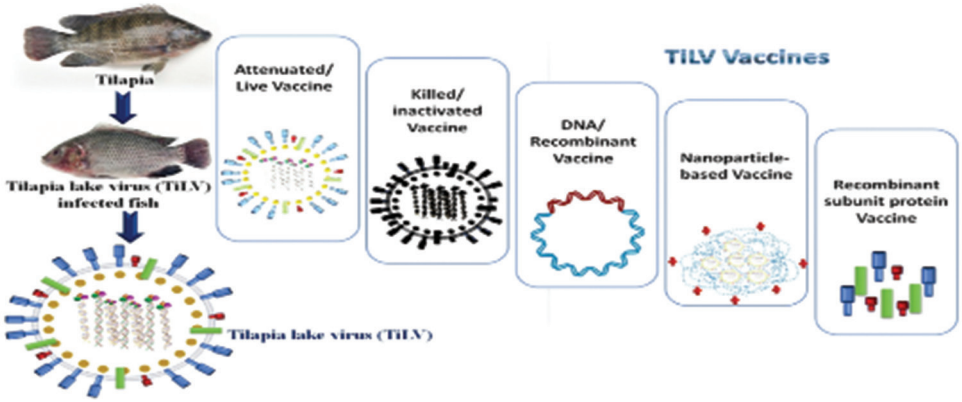

Figure 2: Representation of types of vaccines available for tilapia lake virus. (i) Attenuated/live vaccine, (ii) killed/inactivated vaccine, (iii) DNA/recombinant vaccine, (iv) nanoparticle-based vaccine, (v) recombinant subunit protein vaccine

and adaptive immunity, thereby promoting a strong and durable immune response.[50-54] Moreover, adjuvants are not necessary for attenuated viral vaccines. During vaccination, a single dose or a small number of doses will be sufficient. Traditionally, attenuation was achieved through a series of passages of the virulent strain in the presence of specific antibiotics, inducing random mutations, or in a laboratory setting, or in a controlled laboratory environment.[51-54] On the other hand, the modern attenuation strategy utilizes genetic alteration technologies, including random transposon rearrangement and allelic exchange replacement.[55,56] The modern technique has attracted considerable interest compared to the methods typically employed for killed whole cell vaccines due to its more stable and definitive attenuation [Figure 3 and Table 1].[56]

Currently, there are four licensed live viral vaccines present, which are three against bacterial disease and one against viral disease, which is KHV. This vaccine is administered via injection or the immersion route.[57] As of the present date, no alternative vaccines have been commercially released against the fish disease. Successful modification of viral genomes to generate live vaccine candidates with weakened characteristics has been demonstrated in large DNA viruses such as KHV. This achievement has led to the generation of numerous live-virus vaccine candidates for carp KHV, which are presently under consideration for use in multiple countries.[58] In Israel, a patented experimental attenuated vaccine for TiLVD has been formulated through serial passages of the TiLV virus in cell culture. Despite its development, this vaccine has not been made available in the commercial market. Bacharach *et al*. 2022 conducted an investigation to ascertain the optimal number of passages necessary for the acquisition of attenuated and avirulent TiLV vaccine candidates. The study examined passages 12, 17, and 20 of a live-attenuated TiLV strain (isolate 4/2011), which had been maintained through repeated *in vitro* passage in a permissive cell line. These virus isolates were generated through a series of *in vitro* passages in cell culture conducive to their growth. Subsequently, P12, P17, and P20 vaccine candidates were administered to Nile tilapia via intraperitoneal

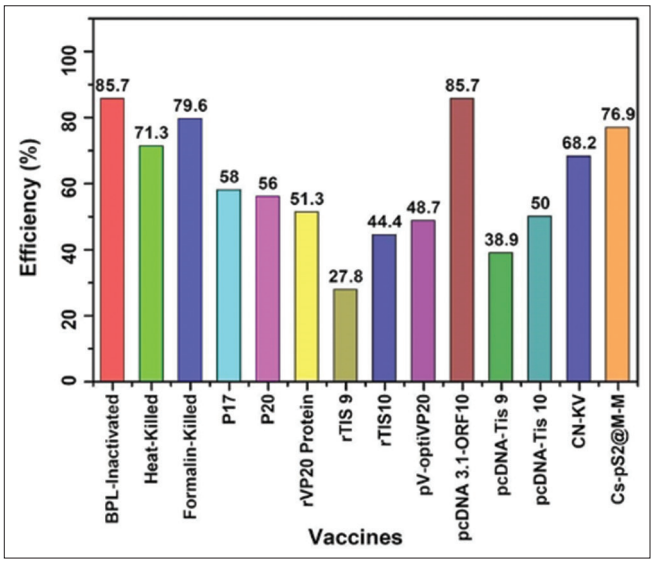

Figure 3: A visual representation of different types of tilapia lake virus vaccines and their efficiency

(IP) injection, using a phosphate-buffered saline solution containing a concentration of 1.3×10^2 $TCID_{50}$/mL of TiLV virus. After 3 weeks of vaccination, the fish were exposed through cohabitation with infected fish that had acquired infection during a 6-h exposure to fish previously placed in a tank containing wild-type TiLV that had undergone four passages to enhance virulence. In this experimental setup, mortality following challenge with the wild-type strain began between 2 and 4 days' post-challenge and continued for 5–7 days. Notably, only the attenuated TiLV strains obtained after 17 and 20 successive passages showed the desired effect in providing significant protection to the vaccinated fish, resulting in RPS values of 56–58% for P20 and P17, respectively.[11]

Despite their effectiveness in providing long-lasting protection and eliciting both cellular and humoral immunity, live vaccines face significant challenges, particularly in terms of safety, limiting their widespread adoption as commercial vaccines. Before these vaccines can be considered safe for

Table 1. List of TiLV vaccines and their mechanism of action against TiLV

Vaccine Type	Immunization Plan	Vaccine Dose	Vaccine Efficiency (%)	Survival Rate (%)	Immune Responses	Reference
Passage/Attenuated Live Vaccine						
P17	1 dose	1.3×10^2 TCID50/mL	58%	62%	Not reported	(Bacharach *et al*. 2016)
P20	1 dose	1.3×10^2 $TCID_{50}$/mL	56%	64%	Not reported	(Bacharach *et al*. 2016)
Inactivated Vaccine						
BPL- inactivated vaccine	2 doses–3 weeks apart	1.8×106 $TCID_{50}$/mL	85.7%	86.7%	Anti-TiLV IgM serum levels were identified, and the presence of neutralizing antibodies was observed.	(Zeng *et al*. 2021)
Heat- Killed vaccine	2 doses–3 weeks apart	1.8×10^6 $TCID_{50}$/mL	71.3%	81.3%	Elevation in the expression of IgM, IgT, and IgD has been documented.	(Mai *et al*. 2021)
Formalin-Killed vaccine	2 doses–3 weeks apart	1.8×10^6 $TCID_{50}$/mL	79.6%	86.3%	Increased expression of IgM, IgT, and IgD has been documented.	(Mai *et al*. 2021)
Recombinant Protein Subunit Vaccine						
rVP20 protein	2 doses–3 weeks apart	400 μg	51.3%	52.5%	Detection of anti-TiLV IgM in the serum was reported, along with the identification of neutralizing antibodies.	(Zeng *et al*. 2021)
rTIS9	1 dose	200 μg	27.8 9.6%	-	A rise in the antibody response in sera, evaluated through dot blot assay, was observed.	(Chamtim *et al*. 2022)
rTIS10	1 dose	200 μg	44.4 25.4%	-	A discernible elevation in the antibody response within the sera, evaluated through dot blot assay, was noted.	(Chamtim *et al*. 2022)
DNA Vaccine						
pV-optiVP20	2 doses–3 weeks apart	50 μg	48.7%	50%-	Elevated levels of anti-TiLV IgM antibodies in the serum and an augmentation in serum neutralizing antibodies were observed.	(Zeng *et al*. 2021)
pcDNA 3.1-ORF10	2 doses–2 weeks apart	45μg	85.7%	-	Substantial elevation in the expression of TLR2, MyD88, IL8, TNF-α, INF-β, and NF-κB was observed.	(Joaquin *et al*. 2019)
pcDNA-Tis9	1 dose	5 μg	38.9 9.6%	-	A proportional rise in the antibody response within sera, evaluated through dot blot analysis, was noted.	(Chamtim *et al*. 2022)
pcDNA-Tis10	1 dose	5 μg	50.00% 16.7	-	A relative elevation in the antibody response within sera, evaluated through dot blot analysis, was noted.	(Chamtim *et al*. 2022)
Nanoparticle-Based Vaccines						
CN-KV	1 dose	$10^3\times$ $TCID_{50}$/mL	68.2%	-	Enhanced TiLV-specific serum antibody response was observed exclusively at 14 days post-challenge (dpc), as assessed by an indirect enzyme-linked immunosorbent assay (ELISA).	(Gong *et al*. 2022)
Cs-pS2@M-M	1 dose	10 μg	76.9%	-	Elevated TiLV-specific serum antibody response, evaluated through indirect enzyme- linked immunosorbent assay (ELISA), was accompanied by a noteworthy increase in IgM expression.	(Nombela *et al*. 2019)

commercial use, several potential risks must be carefully evaluated, including reversion to a virulent form, residual virulence, and the possibility of disease occurrence in immunocompromised vaccine recipients. To assess the safety of the above-mentioned TiLV vaccine candidates, Bacharach *et al*. (2016) performed experiments in which large groups of fish were injected with doses 10 times higher than the standard vaccine dose or subjected to five serial back passages of each variant. These experiments were designed to examine remaining virulence and the likelihood of return to virulence. Importantly, neither of these approaches resulted in side effects. However, the absence of long-term follow-up should be emphasized, as important safety concerns over extended periods remain unresolved.[2] This issue is especially significant because TiLV is a segmented virus capable of reassortment, a feature that may facilitate the emergence of new viral subtypes.[26,27,59] Moreover, it is important to highlight that TiLVD has been identified in fish within their natural habitats, where monitoring reassortment events is difficult, posing a risk for the emergence of new pathogenic strains.

Inactivated vaccine

Inactivated vaccines are usually made from harmful disease-causing pathogens. A special treatment is applied to these pathogens, which makes them unable to cause infection or multiply inside or outside a host. This treatment can involve physical methods, chemicals, or radiation, but it does not affect the germ's ability to trigger the immune response.[60] The earliest viral vaccine developed for fish was an inactivated bivalent vaccine targeting carp rhabdovirus, the causative agent of spring viremia in carp.[51] This vaccine was formulated from two inactivated isolates of spring viremia of carp virus, mixed in oil and delivered by injection. Nowadays, while they exhibit varying effectiveness, most viral vaccines employed in aquaculture are inactivated vaccines. This approach involves isolating viral particles from infected fish, propagating them in culture, and then inactivating them so that they can no longer cause infection or disease while still retaining their immunogenic properties. Virus inactivation can be achieved by chemical treatment using compounds such as formalin, formaldehyde, ethylamine, or β-propiolactone (BPL), as well as by physical approaches, including heat treatment and ultraviolet irradiation, which can alter the viral surface glycoproteins involved in binding to the cellular receptors during the infection.[61] Since inactivated vaccines often elicit weaker protective immunity, the use of immunological adjuvants and repeated booster immunizations may be required to strengthen the immune response.[60] These vaccines are most commonly administered by IP injection and are generally valued for their stability, safety, and ease of production.[39,61] A recent study[56] evaluated the effectiveness of a BPL-inactivated vaccine developed against the TiLV 2017A isolate among tilapia. This evaluation was carried out both with and without the inclusion of Montanide IMS 1312 VG as an adjuvant. Researchers reported that the most substantial RPS values, measuring 85.7% and 64.3%, respectively, were achieved after high doses of the chemically inactivated vaccine (calculated from pre-inactivation viral titers) had been administered, specifically 1×10^8 $TCID_{50}$/mL and 1×10^7 $TCID_{50}$/mL, respectively, and when the adjuvant was included in the immunization process. These findings indicate that the two factors, the dose used for the inactivated vaccine along with the inclusion of an adjuvant, play important roles in determining vaccine efficacy. In addition, the BPL-inactivated vaccine was found to induce strong serum immunoglobulin M (IgM) and neutralizing antibodies specific to TiLV responses within the 3rd week after initial immunization. This effect became more pronounced when the inactivated vaccine doses were at 1×10^8 and 1×10^7 $TCID_{50}$/mL, particularly when combined with the adjuvant. In these cases, the titer of neutralizing antibodies reached markedly elevated levels at around approximately 6 weeks post-prime immunization. The study also examined the expression of genes associated with immune responses, including interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), CD4, and MHC Class I and Class II, in the kidney and spleen of vaccinated fish. These genes showed markedly elevated expression, particularly around 6 weeks post-primary immunization (WPPI), when higher doses of the inactivated vaccine were administered with an adjuvant. The results suggest that both cellular and humoral immune mechanisms contribute to the TiLV-specific immune response detected in vaccinated tilapia. In addition, the response of cytokines (TNF-α, IL-1β, and IFN-γcytokines) plays a role within this immune reaction. After a challenge with the virulent TiLV 2017A strain, vaccinated tilapia showed lower viral loads in the liver, spleen, and kidney, with the greatest reduction observed in fish that received higher vaccine doses together with an adjuvant. These findings indicate that vaccination effectively suppressed viral replication. Notably, the researchers also reported that the BPL-inactivated vaccine provided better protection than the formaldehyde-inactivated vaccine. This finding suggests that the method of inactivation can also influence the effectiveness of chemically inactivated vaccine formulations. In a separate investigation, the study was conducted to evaluate the effectiveness of two distinct vaccine formulations against the TH-2018-K strain of TiLV.[62] These formulations included a heat-inactivated water-based vaccine (HKV) and a formalin-treated inactivated vaccine (FKV), and notably, neither of them included the use of an adjuvant. In a manner consistent with the observations made by Zeng *et al*. 2021, the onset of mortality in the groups subjected to vaccination, following exposure to the virulent TH-2018-K TiLV strain, was first observed at approximately 7 days post-challenge. Notably, the study conducted by Mai *et al*. 2021 had a more protracted vaccination trial period of 70 days, surpassing the 57-day timeline utilized.[56,62] In a subsequent research investigation, both the HKV and FKV formulation were used to assess whether IgM antibodies specific to TiLV induced in *O. niloticus* broodstock could provide passive immune protection to juvenile tilapia.[63] The study also aimed to determine whether immunizing the parental brood stock

would lead to the protection of larvae through the transfer of passive maternal antibodies. In this study, both male and female broodstock were subjected to IP immunization with the HKV and FKV vaccines. Over the course of the study, blood samples, fertilized eggs, and larvae were collected on a weekly basis, and the levels of IgM antibodies were quantified. In addition, sera obtained from female broodstock that had been immunized with either HKV or FKV were utilized for passive intramuscular immunization in juvenile tilapia. Within this field of aquaculture, several investigations have explored the efficacy of inactivated RNA virus vaccines. An intriguing finding is the heightened effectiveness, with an RPS of 81.9 %, observed in the context of viral encephalopathy and retinopathy (VER) when employing a formalin-inactivated vaccine delivered via IP injection in European sea bass.[64] Of particular interest, the same study showed that the BPL-inactivated VER vaccine generated a relatively strong VER-specific IgM response. In contrast, the heat-inactivated vaccine delivered intraperitoneally produced more variable serum IgM levels, although some individuals exhibited relatively high responses. Furthermore, formalin-treated, BPL-treated, and heat-inactivated vaccine preparations, when delivered through the immersion route, resulted in significantly diminished and irregular IgM responses across the tested samples assessed by the researchers. This differs markedly from the strong IgM responses observed in the intraperitoneally vaccinated groups, highlighting IP injection as the preferred route for administering inactivated vaccine formulations. The same study also made an observation with possible implications for future TiLV research, namely that certain RNA viruses may show resistance against both the chemical and physical inactivation approaches.[64] This underscores the necessity for meticulous verification of complete inactivation before vaccination.

Recombinant vaccine

Subunit vaccines are developed by exploiting the capacity of selected protein antigens to trigger immune responses, including the production of high-affinity, isotype-switched antibodies. Unlike whole-virus vaccines, they contain only a limited number of viral proteins or antigenic determinants, often together with purified viral components rather than the entire viral particle.[39] Because subunit vaccines cannot replicate within the host, they carry no risk of reverting to a pathogenic form. These vaccines are designed to focus the immune response on specific viral targets, particularly envelope-associated components involved in viral attachment and entry.[50] In practice, protein subunit vaccines are commonly produced by expressing antigenic proteins through recombinant systems or by isolating and purifying the relevant proteins. While this approach minimizes the likelihood of severe adverse effects, it necessitates the administration of multiple booster doses and the inclusion of an adjuvant to enhance immunization strength and longevity.[65] On the contrary, VLP vaccines consist of subunit components of a particular virus that are autonomously gathered from those viral structural proteins, forming structures that closely resemble authentic virions.[66] In contrast to real virus particles, VLPs do not contain genomic material and only display multiple copies of the same or a restricted number of antigens on their surface.[50] These vaccine varieties have the benefit of being constructed from viral structural proteins capable of eliciting robust immune responses while remaining non-infectious. Consequently, they eliminate any potential for reverting to a pathogenic infectious form, making them well-suited for immunizing individuals with weakened immune systems. Although various VLP production systems provide the flexibility to precisely customize VLPs, their development remains intricate, and assembling them poses technical challenges, particularly in the case of enveloped VLPs.[66] Recently, a novel *in vivo* cloning approach using the yeast *Saccharomyces cerevisiae* was successfully applied to express recombinant open reading frames (ORFs) of TiLV.[67] This approach has the potential to support the efficient production of large amounts of recombinant viral proteins for vaccine development. Although yeast-based vaccines have not yet received licensing for use in aquaculture, they are garnering increasing attention within this field. The approach described by Cueva *et al*. is particularly notable because it allows one or more viral fragments to be cloned into a single plasmid [Figure 4].[67] This advancement opens up possibilities for

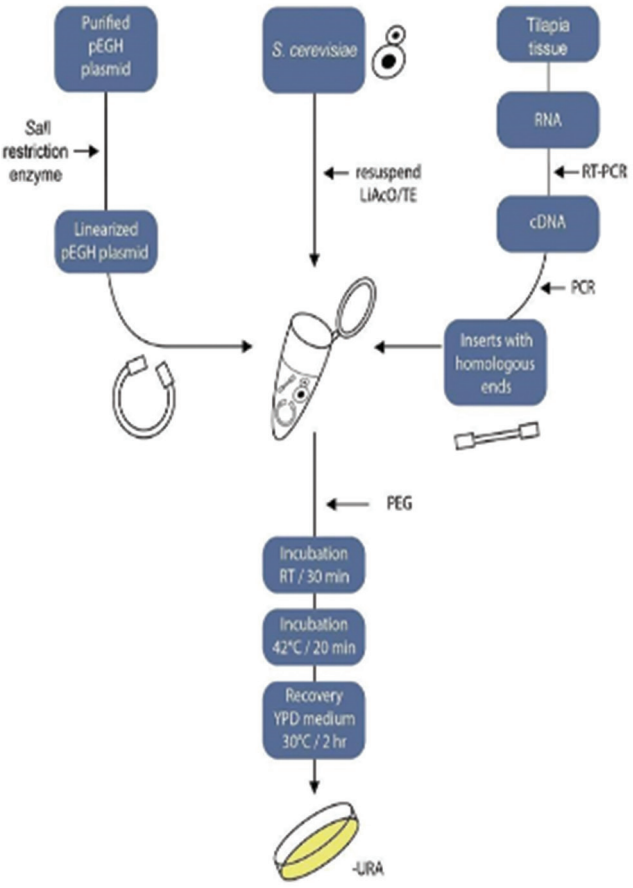

Figure 4: The figure illustrates the *in vivo* cloning method in the yeast, *Saccharomyces cerevisiae*. (Reprinted with permission from Cueva *et al*.,[67] Ulmer *et al*.[68])

the development of VLP vaccines, which have not yet been created for TiLVD prevention and infection control. Based on the advances discussed above, the development of subunit vaccines for TiLVD, particularly those based on recombinant protein expression, is already well in progress.

Nucleic acid vaccines

Nucleic acid vaccines, consisting of RNA vaccines and DNA vaccines, involve the use of plasmids that contain a gene that encodes an antigen protein. These plasmids are introduced into the host so that host cells can express the antigenic protein and subsequently trigger an immune response against it.[69] Nucleic acid vaccines are relatively simple vaccine platforms based on DNA or mRNA molecules that encode the target antigen(s). Their development is generally straightforward, as it requires only the genetic sequence of the viral antigen of interest together with an appropriate delivery system.[68] These vaccines are administered safely without the risk of reverting to a pathogenic state. Their streamlined design makes them well-suited for quick and easy deployment during disease outbreaks.[70]

DNA vaccines

DNA vaccines are based on expression plasmids that carry a specific gene encoding an antigenic protein. Bacterial cells amplify plasmid production, and the gene, placed between promoter and termination elements, induces robust immune responses in the host.[71,72] The first reported application of a DNA vaccine for aquaculture purposes was to combat IHN, which was tested on rainbow trout.[71,72] Out of over 420 distinct DNA vaccine candidates extensively studied in laboratory trials during the past quarter-century.[73] Yet, only a few of these vaccines have been brought to market and made available for commercial use. The incorporation of surface glycoproteins from VHSV and IHNV as antigens in DNA vaccine expression plasmids resulted in improved protection, strong antibody responses, and successful immunization.[74-76] DNA vaccines, safer than live attenuated ones, generate specific antigenic protein segments, not the entire organism. Limited understanding exists of how these antigens interact within the host, but the genetic expression ensures prolonged immune responses.[77]

Yu *et al*. developed and evaluated a pcDNA3.1-ORF10 DNA vaccine targeting TiLV infection in Nile tilapia.[78] Intramuscular administration of this vaccine significantly upregulated several genes associated with immune responses, including IgM, TLR2, MyD88, IL-8, TNF, IFN-γ, and NF-κB across the spleen, liver, and kidney tissues of vaccinated fish. Among these, marked increases were observed in IFN-γ expression in the liver, IgM, IFN-μ, and NF-κB expression in the spleen, and IL-8 and TNF-α expression in thespleen, and IL-8 and TNF-α in the kidney. Vaccinated fish also showed a delayed onset of mortality, and the RPS increased from 60.71% to 85.72% as the vaccine dose increased. Following viral challenge, tilapia immunized with pcDNA3.1-ORF10 had significantly lower viral loads in the spleen, liver, and kidney, indicating that the vaccine was capable of inducing protective immunity.

Zeng *et al*. developed and assessed a plasmid DNA vaccine (pV-optiVP20) derived from VP20 (segment 8) against TiLV.[79] Intramuscular vaccination of tilapia using the codon-optimized pV-optiVP20 DNA vaccine led to a notable rise in antigen-specific serum IgM, further strengthened post-booster immunization at three WPPI. This vaccine additionally increased the expression of immune-associated genes, including IL-1, TNF-α, CD4, and MHC-II, suggesting the activation of both antibody-mediated and cell-mediated immune responses. In addition, splenic IgM concentrations rose significantly, and viral burden in the liver and kidney was markedly reduced after challenge with the virulent TiLV 2017A strain. Despite a 50% survival rate, fish vaccinated with PV-optiVP20 showed a 48.7% RPS value.

A recent study evaluated two DNA vaccine candidates, pcDNA-Tis9 and pcDNA-Tis10 constructs, based on TiLV segments 9 and 10, following intramuscular administration in young hybrid red tilapia.[80] Vaccination using these constructs produced RPS values of 38.89 ± 9.62% for pcDNA-Tis9 and 50.00 ± 16.67% for pcDNA-Tis10, even though only 5 μg of each vector was used. These findings suggest that the ORF encoded by TiLV segment 10 is more immunogenic than that derived from segment 9.

A related study outlined the initial steps in formulating another DNA vaccine against TiLV. Their approach involved the use of a recombinant plasmid vector carrying a genetic sequence originating from TiLV segment 4. Detection of the amplicon gene occurred as early as 8 h after two injections into juvenile tilapia. This DNA vaccine was originally developed guided by the assumption that TiLV segment 4 encoded the viral neuraminidase protein, akin to the membrane-bound surface glycoprotein in influenza viruses, making it a plausible target for vaccine development. However, studies discovered that this genomic segment actually codes for the TiLV nucleoprotein, thereby refuting the initial hypothesis.[81]

RNA-based vaccines

RNA vaccine formulations are non-invasive, hence, they are safer to use, and they are highly effective in stimulating the immune system.[82] Currently, RNA vaccines are classified into two main types based on their translational capacity: conventional non-replicating mRNA vaccines and self-replicating mRNA vaccines, commonly referred to as replicons.[83] The design of mRNA vaccine platforms has faced numerous challenges, primarily due to the inherent properties of the mRNA molecule. Naked, unprotected mRNA can be degraded by nucleases.

However, the development of an mRNA vaccine strategy targeting TiLV has yet to be realized. Significant progress

in this field could occur by utilizing the broad host cell functionality of the alphavirus replicase, particularly if the genetic sequences encoding the alphavirus structural proteins are replaced with those of TiLV.

Coxsackievirus and adenovirus receptors were discovered in response to TiLV infection in tilapia in a transcriptome investigation. After examining the effect of OnCAR on TiLV proliferation, the researchers found that the ORF of tilapia OnCAR was 516 bp in length and encoded a 171-amino-acid protein containing both a transmembrane region and an Ig-like domain. The tilapia challenge's heart, liver, and muscle tissues had the greatest levels of this OnCAR gene's broad expression across all examined tissues. Analysis of subcellular localization showed that the OnCAR protein expression was mainly detected on the membrane of tilapia brain cells. These findings provide a useful foundation for basis for designing effective RNA-based vaccines to prevent and control TiLVD. They will also help to better understand the antiviral mechanism of tilapia (TiLVD).

NANOPARTICLE VACCINES

Nanovaccines are known for their capacity to stimulate innate as well as adaptive immune responses in fish. Nanoparticle carriers carrying targeted antigens are capable of stimulating specific cellular and humoral immune responses, it plays a crucial role as efficient delivery systems, enabling the precise control of vaccine molecule administration and their gradual release, which, in turn, facilitates sustained preventive measures and serves as effective agents for therapeutic delivery.[84] Numerous investigations have delved into the incorporation of diverse nanoparticles as adjuvants in the development of vaccines, with the selection often contingent upon their suitability for the host. Widely employed nanoparticles encompass common nanocarrier materials, including alginate, chitosan, PLGA, PLA, dendrimers, and liposomal systems. However, their practical application still depends on overcoming several important limitations. However, it is crucial to highlight that the crucial step lies in connecting vaccine components with the right nanocarriers to improve vaccine qualities and enhance their delivery, ultimately leading to an effective immune response. Recent progress in the field of nanotechnology has made it possible to create a new category of vaccines. These vaccines utilize very tiny particles known as nanoparticles or nanocarriers to either encapsulate or connect with virus antigens. The resulting nanoparticle-based formulation acts as an effective system for delivering vaccines. It safeguards the enclosed antigen from degradation, enhancing its stability and overall vaccine effectiveness.[2] Chitosan nanoparticles have received considerable attention and have been broadly applied in aquaculture for creating fish vaccines. Chitosan nanoparticles have been explored as vaccine delivery systems in several fish species. For example, they have been used in a vaccine targeting infectious salmon anemia virus that combined an inactivated virus with a DNA sequence encoding alphavirus replicase, which functioned as an adjuvant. Chitosan nanoparticles have also been applied in the development of an oral DNA vaccine targeting turbot reddish body iridovirus, which is a member of the fish-associated iridoviruses.[85] More recently, a TiLV vaccine based on a chitosan nanoparticle-based vaccine has been developed for tilapia. This vaccine, administered by immersion, contains formalin-treated TiLV strain VET-KUTV08 and shows adhesive properties toward the gill mucosa. In a cohabitation challenge model, fish vaccinated with this CN-KV nanovaccine achieved significantly higher RPS values (68.17%) than fish receiving the inactivated virus alone (25.01%). In addition, CN-KV-vaccinated fish exhibited stronger TiLV-specific antibody responses at 14 days' post-challenge than untreated control groups given the inactivated virus preparation or chitosan nanoparticles alone. In field-farming settings, where natural TiLV exposure was permitted 28 days after vaccination, the CN-KV-immunized group recorded an RPS of 52.22%, whereas the control group receiving only chitosan nanoparticles showed lower protection. Comparable to oral PLGA- and liposome-derived nanovaccine systems, the CN-KV formulation remains particularly attractive because it enables mass vaccination through immersion, reducing labor demands and making it more practical for farm use.[86]

A biomimetic nanoscale delivery platform, designated Cs-pS2@M-M, was developed with fish red blood cell membranes that had been modified with DSPE-PEG-Man mannose. This system was used to deliver an anti- TiLV DNA-based vaccine. The biomimetic nanoformulated vaccine was given as an injection into the muscle. This system enabled prolonged and effective DNA expression in muscle and spleen tissues. It also induced a strong systemic antibody response and upregulated the expression of several immune-related genes, showing greater immune stimulatory activity than the same dose of non-mannosylated nanoparticles or the uncoated DNA vaccine. Fish that received the mannose-modified vaccine showed a 76.9 % protection rate, whereas the group that received the naked DNA vaccine had a 50.0 % protection rate. This new vaccine, which uses a modified fish cell membrane, was injected into the fish's muscle and turned out to be very effective. It stimulated the fish's immune system, resulting in a higher level of protection against TiLV.[87] Nevertheless, injecting this biomimetic nanovaccine into the fish's muscle is a slow and demanding process, which can be expensive and impractical for large-scale vaccination in aquaculture settings.

VACCINE ADMINISTRATION

Fish vaccines are typically given through oral administration, injectable routes such as IP or intramuscular delivery, and immersion-based methods. The choice of delivery route is influenced by the method and depends on various factors, including the type of vaccine, the life stage of the fish

(larval, fry, juvenile, adult, or spawning stages), production system, immune-memory status, pathogen type, life-cycle characteristics, and labor costs.[61,88] The chosen delivery methods can affect the elicited immune response and the level of protection against the targeted pathogen.[89]

Oral vaccines

While administering oral vaccines is straightforward, safe, and stress-free for fish across all life stages and sizes, the resulting immunological responses are frequently inconsistent and suboptimal. This is likely related to antigen degradation in the gastrointestinal tract and the limited transfer of antigens from the intestinal lumen to immune-responsive cells in fish.[90] Nevertheless, whole-pathogen antigens, subunit antigens, and nucleic acid antigens, particularly DNA vaccines, all remain potential candidates for oral delivery.[91] In fact, the majority of nanovaccine formulations documented in aquaculture are delivered through oral means.[85,92,93] As noted, oral vaccines may serve as boosters following primary vaccination, enhancing protection against specific chronic endemic diseases.[90] As highlighted, this protection appears to be mediated mainly by antibody-mediated immune responses instead of humoral immune responses, rather than by cell-mediated or innate immune responses.[94] As mentioned above, recently, *S. cerevisiae* was effectively employed for the *in vivo* cloning of viral complementary DNA derived from all 10 TiLV genome segments, presenting opportunities for enhancing the efficacy of oral vaccines in tilapia.[67]

Injectable vaccines

The optimal approach for vaccination is through injectable immunization. this the preferred vaccination method, ensuring consistent vaccine dosage for each fish and fostering a strong, lasting immune response for enduring protection.[69] In contrast to oral vaccination, injection (IP or IM) demands a smaller antigen quantity while ensuring precise and uniform vaccine dosing for each fish. Intramuscular injection is a common route for administering DNA vaccines,[95] whereas adjuvants, particularly oil adjuvants, are frequently employed with IP injections.[61] All the presently documented inactivated TiLV vaccines are administered through the IP route uniformly.[56,62] In addition, the newly disclosed nanovaccine against TiLV, featuring mannose functionalization and biomimetic properties, utilizes modified membranes from tilapia erythrocytes.[96] Automated injection, in contrast to manual injection, minimises fish stress, decreases labour intensity, shortens injection duration, and addresses certain drawbacks associated with the injection immunisation method.[69] Due to the potential mortality resulting from stress induced by injectable vaccination, the limitation of not being able to perform multiple administrations of injectable vaccines during the fish production cycle is a significant drawback.[2] The primary drawback associated with injectable vaccines is their economic impracticality for repeated administration throughout the fish production cycle. Furthermore, due to the underdeveloped nature of the immune system in early life stages of fish, injectable vaccines cannot be efficiently administered during this period.[61]

Immersion vaccine

Vaccination by immersion is a unique approach for aquatic vaccines, which involves antigens being absorbed by mucosal tissues such as gills and skin. This swift activation of the fish's mucosal immune system extends to systemic tissues, such as the peripheral blood, kidney, and spleen, prompting an effective systemic immune response.[97] In contrast to the method of injection, the utilization of an immersion-based approach for vaccination represents a straightforward and uncomplicated strategy. Immersion vaccination can be executed through both dip and bath vaccination methods. In dip vaccination, fish are immersed for a short period, usually about 30 s, in a highly concentrated vaccine solution. In contrast, bath vaccination involves exposing fish for a longer duration, typically from one to several hours, in a more diluted vaccine solution.[98] The dip immersion method is preferred for vaccination as it allows for the rapid vaccination of a larger number of fish in a more efficient manner.[61] The rationale behind the widespread application of immersion in administering live attenuated and vector vaccines, as opposed to injection, is likely attributed to its ability to mimic the natural route of infection in fish. This method has been widely applied in the context of vaccines such as the CN-KV chitosan nanovaccine designed for TiLV, indicating the versatility of immersion even for nanovaccines.[86] One limitation of the immersion method is that it generally provides protection for a shorter duration. This limitation may pose challenges, especially in the context of cultivating various fish species for an extended period.[98] However, despite the numerous advantages associated with this delivery route, it appears that the establishment of immunity takes a longer duration, ranging from 3 to 12 months.[94] In addition, the duration of immunity seems to be relatively shorter, necessitating the administration of supplementary booster doses.[98] Furthermore, the application of the immersion method becomes impractical for larger fish, primarily due to factors such as the longer time duration, associated costs, induced stress, and the inherent difficulty in utilizing multiple immune-stimulating agents and adjuvants.[98]

CONCLUSION

This review presents an assessment of the effectiveness and survival rates of various vaccines introduced in the last 5 years, revealing a positive trend in vaccine development. The TiLV poses a major challenge for aquaculture production, causing substantial losses. Developing a treatment and prevention strategy is crucial, with vaccines regarded as one of the most sustainable and economically practical

approaches for managing major diseases in aquaculture and mitigating financial losses from outbreaks of fish diseases. An effective fish vaccine should be safe for both fish and the environment, cost-effective for mass production, easily administered, capable of generating robust immunity during vulnerable periods, and exhibit minimal adverse effects. Emerging and alternative fish vaccines are leveraging advanced technologies initially designed for human or animal healthcare, demonstrating significant potential for application in aquaculture. Various methods are being explored to develop vaccines for preventing TiLV infection. While these approaches offer hope in conferring immunity against TiLV, most do not specifically target the viral envelope glycoproteins, apart from inactivated and live-attenuated vaccine candidates, partly due to the unidentified nature of these viral proteins. Despite this limitation, considering the potential for TiLV to cause large-scale mortality, it is imperative to explore alternative vaccine development approaches, including multiepitope-based methods such as multiepitope VLPs and mRNA nanoparticle-based vaccines. A better understanding of fish immune pathways is expected to support the design of improved vaccines and immunization strategies that incorporate effective, naturally derived adjuvants, such as previously identified CpG DNA fragments, which are associated with fewer side effects. As tilapia aquaculture continues to expand worldwide, additional diseases relevant to the industry may emerge, creating the need for new vaccines or for the adaptation of existing ones. Harnessing all available biotechnology to address these emerging diseases is of paramount importance, considering the ongoing global expansion of aquaculture, ensuring a continual demand for new vaccines in the future.

ETHICS

This study did not involve human participants or experimental animals directly; therefore, ethical approval was not required.

ACKNOWLEDGMENT

None.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

DATA ACCESS

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Rohani MF, Islam SM, Hossain MK, Ferdous Z, Siddik MA, Nuruzzaman M, *et al*. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. Fish Shellfish Immunol 2022;120:569-89.
2. Kembou-Ringert JE, Steinhagen D, Readman J, Daly JM, Adamek M. *Tilapia* lake virus vaccine development: A review on the recent advances. Vaccines (Basel) 2023;11:251.
3. Dong HT, Chaijarasphong T, Barnes AC, Delamare-Debouteville J, Lee PA, Senapin S, *et al*. From the basics to emerging diagnostic technologies: What is on the horizon for *Tilapia* disease diagnostics? Rev Aquac 2023;15:186-212.
4. Hounmanou YM, Mdegela RH, Dougnon TV, Achoh ME, Mhongole OJ, Agadjihouèdé H, *et al*. *Tilapia* Lake virus threatens tilapiines farming and food security: Socio-economic challenges and preventive measures in Sub-Saharan Africa. Aquaculture 2018;493:123-9.
5. Chen CY, Chao CB, Bowser PR. Infection of *Tilapia oreochromis* sp. By *Vibrio vulnificus* in freshwater and low-salinity environments. J World Aquac Soc 2006;37:82-8.
6. Food and Agriculture Organization of the United Nations. In Brief to the State of World Fisheries and Aquaculture 2022: Towards Blue Transformation. Rome: FAO; 2022.
7. Popma T, Masser M. *Tilapia* Life History and Biology. SRAC Publication No. 283. Southern Regional Aquaculture Center; 1999.
8. Prabu E, Rajagopalsamy CB, Ahilan B, Jeevagan IJ, Renuhadevi M. *Tilapia*-an excellent candidate species for world aquaculture: A review. Ann Res Rev Biol 2019;31:1-14.
9. Haenen OL, Dong HT, Hoai TD, Crumlish M, Karunasagar I, Barkham T, *et al*. Bacterial diseases of *Tilapia*, their zoonotic potential and risk of antimicrobial resistance. Rev Aquac 2023;15:154-85.
10. Wang B, Thompson KD, Wangkahart E, Yamkasem J, Bondad-Reantaso MG, Tattiyapong P, *et al*. Strategies to enhance *Tilapia* immunity to improve their health in aquaculture. Rev Aquac 2023;15:41-56.
11. Bacharach E, Mishra N, Briese T, Zody MC, Kembou Tsofack JE, Zamostiano R, *et al*. Characterization of a novel orthomyxo-like virus causing mass die-offs of *Tilapia*. mBio 2016;7:e00431-16.
12. Food and Agriculture Organization of the United Nations. Outbreaks of *Tilapia* Lake Virus (TiLV) Threaten the Livelihoods and Food Security of Millions of People Dependent on *Tilapia* Farming. Rome: FAO; 2017.
13. Aich N, Paul A, Choudhury TG, Saha H. *Tilapia* lake virus (TiLV) disease: Current status of understanding. Aquac Fish 2022;7:7-17.
14. Eyngor M, Zamostiano R, Kembou Tsofack JE, Berkowitz A, Bercovier H, Tinman S, *et al*. Identification of a novel RNA virus lethal to *Tilapia*. J Clin Microbiol 2014;52:4137-46.

15. Dong HT, Ataguba GA, Khunrae P, Rattanarojpong T, Senapin S. Evidence of TiLV infection in *Tilapia* hatcheries from 2012 to 2017 reveals probable global spread of the disease. Aquaculture 2017;479:579-83.
16. He T, Zhang YZ, Gao LH, Miao B, Zheng JS, Pu DC, *et al*. Identification and pathogenetic study of *Tilapia* lake virus (TiLV) isolated from naturally diseased *tilapia*. Aquaculture 2023;565:739166.
17. Tattiyapong P, Dachavichitlead W, Surachetpong W. Experimental infection of *Tilapia* lake virus (TiLV) in Nile *tilapia* (*Oreochromis niloticus*) and red *tilapia* (*Oreochromis* spp.). Vet Microbiol 2017;207:170-7.
18. Surachetpong W, Janetanakit T, Nonthabenjawan N, Tattiyapong P, Sirikanchana K, Amonsin A. Outbreaks of *Tilapia* lake virus infection, Thailand, 2015-2016. Emerg Infect Dis 2017;23:1031-3.
19. Allahbagash B, Gani T, Seepoo AM, Mohamed Jaffer AW, Abdul NA, Sivaraj M, *et al*. Immune response and protective efficacy of quercetin in *Tilapia*, *Oreochromis niloticus* against *Tilapia* parvovirus. J Fish Dis 2025;48:e70001.
20. Del-Pozo J, Mishra N, Kabuusu R, Cheetham S, Eldar A, Bacharach E, *et al*. Syncytial hepatitis of *Tilapia* (*Oreochromis niloticus* L.) is associated with orthomyxovirus-like virions in hepatocytes. Vet Pathol 2017;54:164-70.
21. Kembou Tsofack JE, Zamostiano R, Watted S, Berkowitz A, Rosenbluth E, Mishra N, *et al*. Detection of *Tilapia* lake virus in clinical samples by culturing and nested reverse transcription-PCR. J Clin Microbiol 2017;55:759-67.
22. Fathi M, Dickson C, Dickson M, Leschen W, Baily J, Muir F, *et al*. Identification of *Tilapia* Lake virus in Egypt in Nile *Tilapia* affected by 'summer mortality' syndrome. Aquaculture 2017;473:430-2.
23. Nicholson P, Fathi MA, Fischer A, Mohan C, Schieck E, Mishra N, *et al*. Detection of *Tilapia* Lake virus in Egyptian fish farms experiencing high mortalities in 2015. J Fish Dis 2017;40:1925-8.
24. Behera BK, Pradhan PK, Swaminathan TR, Sood N, Paria P, Das A, *et al*. Emergence of *Tilapia* Lake virus associated with mortalities of farmed Nile *tilapia Oreochromis niloticus* (Linnaeus 1758) in India. Aquaculture 2018;484:168-74.
25. Amal MN, Koh CB, Nurliyana M, Suhaiba M, Nor-Amalina Z, Santha S, *et al*. A case of natural co-infection of *Tilapia* Lake virus and *Aeromonas veronii* in a Malaysian red hybrid *tilapia* (*Oreochromis niloticus* × O. *mossambicus*) farm experiencing high mortality. Aquaculture 2018;485:12-6.
26. Chaput DL, Bass D, Alam MM, Hasan NA, Stentiford GD, Aerle RV, *et al*. The segment matters: Probable reassortment of *Tilapia* Lake virus (TiLV) complicates phylogenetic analysis and inference of geographical origin of new isolate from Bangladesh. Viruses 2020;12:258.
27. Mugimba KK, Chengula AA, Wamala S, Mwega ED, Kasanga CJ, Byarugaba DK, *et al*. Detection of *tilapia* lake virus (TiLV) infection by PCR in farmed and wild Nile *tilapia* (*Oreochromis niloticus*) from Lake Victoria. J Fish Dis 2018;41:1187-92.
28. Ahasan MS, Keleher W, Giray C, Perry B, Surachetpong W, Nicholson P, *et al*. Genomic characterization of *Tilapia* Lake virus isolates recovered from moribund Nile *Tilapia* (*Oreochromis niloticus*) on a farm in the United States. Microbiol Resour Announc 2020;9:e01368-19.
29. Dong HT, Siriroob S, Meemetta W, Santimanawong W, Gangnonngiw W, Pirarat N, *et al*. Emergence of *tilapia* lake virus in Thailand and an alternative semi-nested RT-PCR for detection. Aquaculture 2017;476:111-8.
30. Surachetpong W, Roy SR, Nicholson P. *Tilapia* lake virus: The story so far. J Fish Dis 2020;43:1115-32.
31. Rodger HD. Fish disease causing economic impact in global aquaculture. In: Adams A, editor. Fish Vaccines. Basel: Springer; 2016. p. 1-34.
32. Irshath AA, Rajan AP, Vimal S, Prabhakaran VS, Ganesan R. Bacterial pathogenesis in various fish diseases: Recent advances and specific challenges in vaccine development. Vaccines (Basel) 2023;11:470.
33. Duff DB. The oral immunization of trout against *Bacterium salmonicida*. J Immunol 1942;44:87-94.
34. See MS, Ching XL, Khoo SC, Abidin SZ, Sonne C, Ma NL. Aquatic microbiomes under stress: The role of gut microbiota in detoxification and adaptation to environmental exposures. J Hazard Mater Adv 2025;17:100612.
35. Jørgensen LV. Zebrafish as a model for fish diseases in aquaculture. Pathogens 2020;9:609.
36. Menanteau-Ledouble S, Kumar G, Saleh M, El-Matbouli M. *Aeromonas salmonicida*: Updates on an old acquaintance. Dis Aquat Organ 2016;120:49-68.
37. Gudding R, Goodrich T. The history of fish vaccination. In: Gudding R, Lillehaug A, Evensen O, editors. Fish Vaccination. Chichester: John Wiley and Sons Ltd.; 2014. p. 1-11.
38. Fajardo C, Santos P, Passos R, Vaz M, Azeredo R, Machado M, *et al*. Functional and molecular immune response of rainbow trout (*Oncorhynchus mykiss*) following challenge with *Yersinia ruckeri*. Int J Mol Sci 2022;23:3096.
39. Ma J, Bruce TJ, Jones EM, Cain KD. A review of fish vaccine development strategies: Conventional methods and modern biotechnological approaches. Microorganisms 2019;7:569.
40. Guy B. The perfect mix: Recent progress in adjuvant research. Nat Rev Microbiol 2007;5:505-17.
41. Sivakumar SM, Safhi MM, Kannadasan M, Sukumaran N. Vaccine adjuvants - current status and prospects on controlled release adjuvancity. Saudi Pharm J 2011;19:197-206.
42. Zeng R, Pan W, Lin Y, He J, Luo Z, Li Z, *et al*. Development of a gene-deleted live attenuated candidate vaccine against fish virus (ISKNV) with low pathogenicity and

high protection. iScience 2021;24:102750.
43. Pradhan PK, Paria A, Yadav MK, Verma DK, Gupta S, Swaminathan TR, *et al*. Susceptibility of Indian major carp *Labeo rohita* to *Tilapia* lake virus. Aquaculture 2020;515:734567.
44. Jaemwimol P, Rawiwan P, Tattiyapong P, Saengnual P, Kamlangdee A, Surachetpong W. Susceptibility of important warm water fish species to *Tilapia* lake virus (TiLV) infection. Aquaculture 2018;497:462-8.
45. Jansen MD, Dong HT, Mohan CV. *Tilapia* lake virus: A threat to the global *tilapia* industry? Rev Aquac 2019;11:725-39.
46. Nicholson P, Mon-On N, Jaemwimol P, Tattiyapong P, Surachetpong W. Coinfection of *tilapia* lake virus and *Aeromonas hydrophila* synergistically increased mortality and worsened the disease severity in *tilapia* (*Oreochromis* spp.). Aquaculture 2020;520:734746.
47. Abdullah A, Ramly R, Mohammad Ridzwan MS, Sudirwan F, Abas A, Ahmad K, *et al*. First detection of *tilapia* lake virus (TiLV) in wild river carp (*Barbonymus schwanenfeldii*) at Timah Tasoh Lake, Malaysia. J Fish Dis 2018;41:1459-62.
48. Basri L, Nor RM, Salleh A, Md Yasin IS, Saad MZ, Abd Rahaman NY, *et al*. Co-infections of *Tilapia* Lake Virus, *Aeromonas hydrophila* and *Streptococcus agalactiae* in farmed red hybrid *Tilapia*. Animals (Basel) 2020;10:2141.
49. Czochor J, Turchick A. Introduction. Vaccines. Yale J Biol Med 2014;87:401-2.
50. Wan Mahari WA, Waiho K, Fazhan H, Azwar E, Shu-Chien AC, Hersi MA, *et al*. Emerging paradigms in sustainable shellfish aquaculture: Microalgae and biofloc technologies for wastewater treatment. Aquaculture 2024;587:740835.
51. Sommerset I, Krossøy B, Biering E, Frost P. Vaccines for fish in aquaculture. Expert Rev Vaccines 2005;4:89-101.
52. Norqvist A, Hagström A, Wolf-Watz H. Protection of rainbow trout against vibriosis and furunculosis by the use of attenuated strains of *Vibrio anguillarum*. Appl Environ Microbiol 1989;55:1400-5.
53. Håstein T, Gudding R, Evensen O. Bacterial vaccines for fish--an update of the current situation worldwide. Dev Biol (Basel) 2005;121:55-74.
54. Li J, Mo Z, Li G, Xiao P, Huang J. Generation and evaluation of virulence attenuated mutants of *Edwardsiella tarda* as vaccine candidates to combat edwardsiellosis in flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol 2015;43:175-80.
55. Wang J, Zou LL, Li AX. Construction of a *Streptococcus iniae* sortase a mutant and evaluation of its potential as an attenuated modified live vaccine in Nile *tilapia* (*Oreochromis niloticus*). Fish Shellfish Immunol 2014;40:392-8.
56. Zeng W, Wang Y, Hu H, Wang Q, Bergmann SM, Wang Y, *et al*. Cell culture-derived *Tilapia* lake virus-inactivated vaccine containing montanide adjuvant provides high protection against viral challenge for *Tilapia*. Vaccines (Basel) 2021;9:86.
57. Dhar AK, Manna SK, Thomas Allnutt FC. Viral vaccines for farmed finfish. Virusdisease 2014;25:1-17.
58. Fuchs W, Fichtner D, Bergmann SM, Mettenleiter TC. Generation and characterization of koi herpesvirus recombinants lacking viral enzymes of nucleotide metabolism. Arch Virol 2011;156:1059-63.
59. Verma DK, Sood N, Paria A, Swaminathan TR, Mohan CV, Rajendran KV, *et al*. Reassortment and evolutionary dynamics of *Tilapia* lake virus genomic segments. Virus Res 2022;308:198625.
60. Tlaxca JL, Ellis S, Remmele RL Jr. Live attenuated and inactivated viral vaccine formulation and nasal delivery: Potential and challenges. Adv Drug Deliv Rev 2015;93:56-78.
61. Mondal H, Thomas J. A review on the recent advances and application of vaccines against fish pathogens in aquaculture. Aquac Int 2022;30:1971-2000.
62. Mai TT, Kayansamruaj P, Taengphu S, Senapin S, Costa JZ, Del-Pozo J, *et al*. Efficacy of heat-killed and formalin-killed vaccines against *Tilapia* tilapinevirus in juvenile Nile *tilapia* (*Oreochromis niloticus*). J Fish Dis 2021;44:2097-109.
63. Mai TT, Kayansamruaj P, Soontara C, Kerddee P, Nguyen DH, Senapin S, *et al*. Immunization of Nile *Tilapia* (*Oreochromis niloticus*) broodstock with *Tilapia* Lake virus (TiLV) inactivated vaccines elicits protective antibody and passive maternal antibody transfer. Vaccines (Basel) 2022;10:167.
64. Nuñez-Ortiz N, Pascoli F, Picchietti S, Buonocore F, Bernini C, Toson M, *et al*. A formalin-inactivated immunogen against viral encephalopathy and retinopathy (VER) disease in European sea bass (*Dicentrarchus labrax*): Immunological and protection effects. Vet Res 2016;47:89.
65. Holten-Andersen L, Doherty TM, Korsholm KS, Andersen P. Combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and synthetic mycobacterial cord factor as an efficient adjuvant for tuberculosis subunit vaccines. Infect Immun 2004;72:1608-17.
66. Dai S, Wang H, Deng F. Advances and challenges in enveloped virus-like particle (VLP)-based vaccines. J Immunol Sci 2018;2:36-41.
67. Cueva MD, Villena GK, Kitazono AA. Efficient cloning of *Tilapia* lake virus complementary DNAs using an *in vivo* strategy in baker's yeast. J World Aquac Soc 2021;52:1209-20.
68. Ulmer JB, Mason PW, Geall A, Mandl CW. RNA-based vaccines. Vaccine 2012;30:4414-8.
69. Du Y, Hu X, Miao L, Chen J. Current status and development prospects of aquatic vaccines. Front Immunol 2022;13:1040336.
70. Kyriakidis NC, López-Cortés A, González EV, Grimaldos AB, Prado EO. SARS-CoV-2 vaccines strategies: A comprehensive review of phase 3 candidates. NPJ Vaccines 2021;6:28.
71. Kurath G. Biotechnology and DNA vaccines for aquatic animals. Rev Sci Tech 2008;27:175-96.

72. Lorenzen N, LaPatra SE. DNA vaccines for aquacultured fish. Rev Sci Tech 2005;24:201-13.
73. Dalmo RA. DNA vaccines for fish: Review and perspectives on correlates of protection. J Fish Dis 2018;41:1-9.
74. Hølvold LB, Myhr AI, Dalmo RA. Strategies and hurdles using DNA vaccines to fish. Vet Res 2014;45:21.
75. Purcell MK, Nichols KM, Winton JR, Kurath G, Thorgaard GH, Wheeler P, *et al*. Comprehensive gene expression profiling following DNA vaccination of rainbow trout against infectious hematopoietic necrosis virus. Mol Immunol 2006;43:2089-106.
76. Utke K, Kock H, Schuetze H, Bergmann SM, Lorenzen N, Einer-Jensen K, *et al*. Cell-mediated immune responses in rainbow trout after DNA immunization against the viral hemorrhagic septicemia virus. Dev Comp Immunol 2008;32:239-252.
77. Weiner DB. DNA vaccines: Crossing a line in the sand. Introduction to special issue. Vaccine 2008;26:5073-4.
78. Yu NT, Zeng WW, Xiong Z, Liu ZX. A high efficacy DNA vaccine against *Tilapia* lake virus in Nile *tilapia* (*Oreochromis niloticus*). Aquac Rep 2022;24:101166.
79. Zeng W, Wang Y, Chen X, Wang Q, Bergmann SM, Yang Y, *et al*. Potency and efficacy of VP20-based vaccine against *Tilapia* lake virus using different prime-boost vaccination regimens in *tilapia*. Aquaculture 2021;539:736654.
80. Chamtim P, Suwan E, Dong HT, Sirisuay S, Areechon N, Wangkahart E, *et al*. Combining segments 9 and 10 in DNA and recombinant protein vaccines conferred superior protection against *Tilapia* lake virus in hybrid red *tilapia* (*Oreochromis* sp.) compared to single segment vaccines. Front Immunol 2022;13:935480.
81. Abu Rass R, Kustin T, Zamostiano R, Smorodinsky N, Ben Meir D, Feder D, *et al*. Inferring protein function in an emerging virus: Detection of the nucleoprotein in *Tilapia* Lake virus. J Virol 2022;96:e0175721.
82. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov 2018;17:261-79.
83. Cho SY, Kim HJ, Lan NT, Han HJ, Lee DC, Hwang JY, *et al*. Oral vaccination through voluntary consumption of the convict grouper *Epinephelus septemfasciatus* with yeast producing the capsid protein of red-spotted grouper nervous necrosis virus. Vet Microbiol 2017;204:159-64.
84. Harshitha M, Nayak A, Disha S, Akshath US, Dubey S, Munang'andu HM, *et al*. Nanovaccines to combat *Aeromonas hydrophila* infections in warm-water aquaculture: Opportunities and challenges. Vaccines (Basel) 2023;11:1555.
85. Zheng F, Liu H, Sun X, Zhang Y, Zhang B, Teng Z, *et al*. Development of oral DNA vaccine based on chitosan nanoparticles for the immunization against reddish body iridovirus in turbots (*Scophthalmus maximus*). Aquaculture 2016;452:263-71.
86. Tattiyapong P, Kitiyodom S, Yata T, Jantharadej K, Adamek M, Surachetpong W. Chitosan nanoparticle immersion vaccine offers protection against *Tilapia* lake virus in laboratory and field studies. Fish Shellfish Immunol 2022;131:972-9.
87. Gong YM, Wei XF, Zhou GQ, Liu MZ, Li PF, Zhu B. Mannose functionalized biomimetic nanovaccine enhances immune responses against *Tilapia* lake virus. Aquaculture 2022;560:738535.
88. Assefa A, Abunna F. Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish. Vet Med Int 2018;2018:5432497.
89. Palm RC Jr., Landolt ML, Busch RA. Route of vaccine administration: Effects on the specific humoral response in rainbow trout *Oncorhynchus mykiss*. Dis Aquat Organ 1998;33:157-66.
90. Brudeseth BE, Wiulsrød R, Fredriksen BN, Lindmo K, Løkling KE, Bordevik M, *et al*. Status and future perspectives of vaccines for industrialised fin-fish farming. Fish Shellfish Immunol 2013;35:1759-68.
91. Roberts RA, Eitas TK, Byrne JD, Johnson BM, Short PJ, McKinnon KP, *et al*. Towards programming immune tolerance through geometric manipulation of phosphatidylserine. Biomaterials 2015;72:1-10.
92. Tian J, Yu J. Poly(lactic-co-glycolic acid) nanoparticles as candidate DNA vaccine carrier for oral immunization of Japanese flounder (*Paralichthys olivaceus*) against lymphocystis disease virus. Fish Shellfish Immunol 2011;30:109-17.
93. Phanse Y, Puttamreddy S, Loy D, Ramirez JV, Ross KA, Alvarez-Castro I, *et al*. RNA nanovaccine protects against white spot syndrome virus in shrimp. Vaccines (Basel) 2022;10:1428.
94. Newaj-Fyzul A, Austin B. Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. J Fish Dis 2015;38:937-55.
95. Evensen Ø, Leong JA. DNA vaccines against viral diseases of farmed fish. Fish Shellfish Immunol 2013;35:1751-8.
96. Nombela I, Lopez-Lorigados M, Salvador-Mira ME, Puente-Marin S, Chico V, Ciordia S, *et al*. Integrated transcriptomic and proteomic analysis of red blood cells from rainbow trout challenged with VHSV points towards novel immunomodulant targets. Vaccines (Basel) 2019;7:63.
97. Du Y, Tang X, Sheng X, Xing J, Zhan W. The influence of concentration of inactivated *Edwardsiella tarda* bacterin and immersion time on antigen uptake and expression of immune-related genes in Japanese flounder (*Paralichthys olivaceus*). MicrobPathog 2017;103:19-28.
98. Mohamed LA, Soliman WS. Development and efficacy of fish vaccine used against some bacterial diseases in farmed *Tilapia*. Nat Sci 2013;11:120-8.

Source of Support: Nil. **Conflicts of Interest:** None declared.