# Adverse Effects of Fish Vaccines

Gayatri Tripathi and K. Dhamotharan

**Abstract**

Fish diseases remain to be a serious economic threat in commercial aquaculture across the world, despite several innovative approaches to novel therapy. Although antibiotics or chemotherapeutics are used to treat fish diseases, they exhibit some obvious disadvantages, such as drug resistance and safety concerns. Vaccination is regarded as an effective prophylactic measure to prevent a wide range of bacterial and viral infections in aquaculture. Fish immunization has been practiced for more than 50 years and is well recognised as an efficient means of avoiding a variety of infectious diseases of fishes. It can activate the specific and non-specific immune systems of fishes and provide long-term protection. Though vaccination is vital for preventing disease outbreaks and reducing antibiotic usage, it may also have adverse effects on the vaccinated animal, environment or the consumers of vaccinated fishes. It varies with the type of vaccine, mode of administration, environmental parameters, size, stress and immunity of fishes, etc. Inflammation, abdominal lesions, growth retardation, spinal deformities and systemic and autoimmunity development are the major adverse effects associated with injection vaccination in fishes. Apart from this, adjuvants used along with vaccines are also reported to induce granuloma in all the vital organs. Midtlyng and Speilberg scales are used as a standard method for measuring the severity of the lesions developed during fish vaccination. Vaccination is undoubtedly the most effective intervention for disease prevention and control. However, the negative consequences must be considered when creating alternative safe techniques. The present chapter deals with the major adverse effects associated with fish vaccination and their significance in vaccine development.

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M. Makesh, K. V. Rajendran (eds.), *Fish immune system and vaccines*,
https://doi.org/10.1007/978-981-19-1268-9_15

**Keywords**

Fish vaccination · Immunization · Adverse effects of vaccines · Histology · Vaccine failure · Midtlyng and Speilberg scoring

## 1 Introduction

Vaccination is the process of developing immunity to a particular disease by administering disease-specific antigens. Globally, vaccines have long been known to reduce the mortality rates in humans and animals. Vaccines are variously categorized as first, second and third generation vaccines. Conventional vaccines are grouped in the category of first generation vaccines. These require whole microorganisms for their preparation; whereas the second generation vaccines utilize the recombinant protein components. The third generation vaccines are also known as DNA vaccines, and they use the recombinant technology employing genetic engineering of bacterial plasmids.

Vaccines are vital as a preventive medicine to minimize the losses due to infectious diseases in aquaculture and to improve the welfare of farmed fishes. Intensive use of antibiotics in the aquatic ecosystem has raised concerns among fish consumers and also resulted in the development of antibiotic-resistant pathogens. An inactivated vaccine against *Yersinia ruckeri* was the first fish vaccine to be licensed in 1976 in the USA. Since then, many vaccines have been developed and licensed worldwide in order to control and combat the disease problems in aquaculture. Fish vaccines against many bacterial and viral diseases are available commercially in many countries [1]. The use of vaccines has tremendously reduced the application of antimicrobial drugs in aquaculture, particularly in the Norwegian aquaculture industry [2]. An ideal vaccine should be safe (without adverse/side effects), easy to administer, efficacious (efficient to protect the animal from diseases), stable and cheap [3]. Generally, vaccines have no hazardous risks but they do have some adverse effects. In aquaculture, injection of multivalent vaccines is a common method for preventing disease outbreaks. Many studies have found that the adverse effects of fish vaccines are mainly related to the intraperitoneal administration of oil-adjuvanted vaccines.

Vaccines may have adverse effects on the vaccinated animal, environment or the consumers of vaccinated fishes (Fig. 1). Furthermore, contaminated vaccines or unhygienic procedures adopted for injection may introduce unwanted microbes into the fishes. In aquaculture, the losses due to vaccination methods have been observed to be highest in direct immersion method, followed by injection and prolonged bathing [4].

Fish vaccination involves greater ecological risks than in terrestrial animals due to environmental complexities. Pre-licensure and post-licensure vaccine safety, food safety and maximum residual limits of the vaccine in the animals should be evaluated for licensing of fish vaccines. Though vaccination is important to prevent

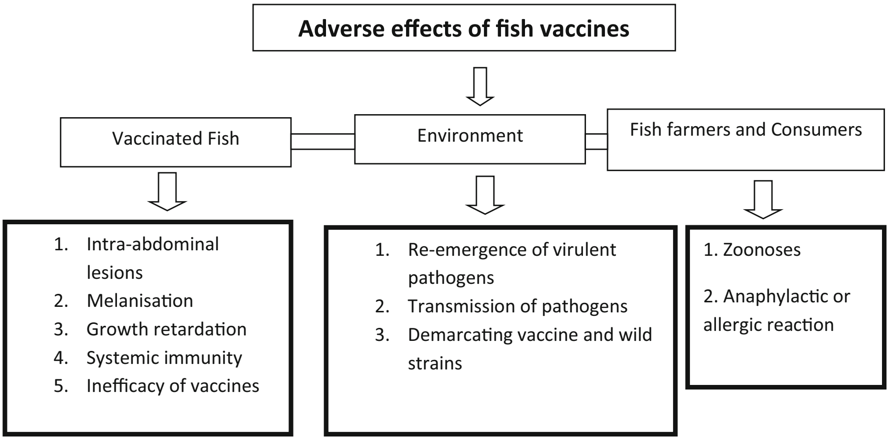


**Fig. 1** Adverse effects associated with fish vaccines

disease outbreak and to reduce the use of antibiotics, the associated adverse effects cannot be neglected for developing alternative safe methods [5].

## 2 Adverse Effects of Different Types of Fish Vaccines

### 2.1 Live-Attenuated Fish Vaccines

Live-attenuated vaccines are produced by different methods like serial passaging of pathogens through cell cultures, chemical or physical mutagenesis and genetic modifications [6]. The main advantage of live vaccines is that they are able to infect and multiply inside the host without causing clinical disease and are capable of eliciting both adaptive and innate immunity. Live-attenuated vaccines are efficient against many fish diseases as they provoke cell and antibody-mediated immunity.

Safety concerns affiliated with live-attenuated vaccines are reversion from avirulent to virulent strain, risk of transmission of pathogens, release of vaccine strain into the environment and risk of infecting other animals or humans. Live attenuated vaccines are not pathogenic to targeted species but may pose risk to other non-targeted aquatic organisms [7]. The attenuated vaccines must allow serological differentiation from infected animals, and the vaccine strain should also be traceable in the environment [8]. These safety issues restrict the wide use of live attenuated vaccines in the aquatic ecosystem. Nevertheless, attenuated vaccines are commercially available and used in aquaculture without any report of adverse effects [2].

## 2.2 Inactivated/Killed Vaccines

Inactivated vaccines consist of pathogens or products that are killed or inactivated by various chemicals (formaldehyde or β-propiolactone or binary ethylenimine or urea or tri-*N*-butyl phosphate) or by physical (ultraviolet [UV] light, heat, and sonication) methods. During inactivation, care is taken not to alter their immunogenicity; hence the inactivated pathogen is still capable of eliciting immune responses [3]. Inactivated/killed vaccines against several fish pathogens are commercially available in many countries and are the most common types of vaccines used in aquaculture [7, 9].

Generally, inactivated vaccines are injected as monovalent or multivalent vaccines and have to be evaluated for possible risk of causing clinical infections in fishes. Killed/inactivated vaccines have a possible risk of delivering viable pathogens due to inadequate killing or due to contamination of vaccines with extraneous infectious agents [3]. Improper inactivation would lead to a lack of antigen recognition by the fishes. Moreover, the presence of various chemical residues in vaccines that were used for inactivation/killing needs to be assessed for its safety and persistence. In this vaccination method, booster doses are required to increase the protective efficacy, and this requirement of repeated administration of vaccines by injection method inflicts enormous stress and adverse effects on the fishes. Inactivated vaccines are usually injected intraperitoneally as oil or water adjuvanted emulsion. Most of the reported adverse effects using inactivated vaccines are mainly due to intraperitoneal injection of various adjuvants (refer Sect. 3.3). For this reason, the adverse effects of intraperitoneal injection of oil-adjuvanted inactivated vaccines should be evaluated before authorization, marketing and field application.

## 2.3 Subunit Vaccine

Subunit vaccine consists of recombinant proteins that are part of the microbes which can induce protective immunity. Inconsistent efficacy and lack of humoral responses are reported in subunit vaccinated fishes [10]. Purifying the proteins for subunit vaccination may reduce the immunogenicity and this may demand the need for an immunogenic carrier protein or adjuvant. The use of adjuvants further increases the adverse effects.

## 2.4 DNA Vaccine

In DNA vaccination, bacterial plasmid DNA encoding antigen of a fish pathogen is injected into the animal for protecting it from diseases. APEX-IHN DNA vaccine manufactured by Vical-Aqua Health Ltd. of Canada (Novartis) is the first DNA vaccine for use in aquaculture [11].

The adverse effects of DNA vaccines include possible integration of DNA within the genome of the vaccinated animal, inducing an anti-DNA immune response, autoimmunity, immune tolerance, injection site inflammation, tissue destruction and other wild bacteria taking up the plasmid DNA [12]. In some countries, DNA-vaccinated animals are referred to as a genetically modified organism (GMO) [13]. The risk assessment for all the above aspects has to be conducted for the approval of any DNA vaccine, besides assessing the fate of plasmid DNA (stability, persistence and integration) in the vaccinated fishes and assessing the post-consumption effect (if any), when the DNA vaccinated fishes are eaten by predatory fishes or by humans.

## 3 Adverse Effects of Different Vaccination Methods

Three methods—injection, immersion and oral—are commonly used for vaccine administration in aquaculture, with each having its own advantages and disadvantages. The adverse effects associated with each of these methods are discussed below:

### 3.1 Immersion Vaccination

The different types of immersion vaccination methods used in aquaculture include hyperosmotic infiltration (HI), direct immersion (DI) (bath, dip and flush), spray, ultrasound immersion and puncture immersion. Besides its inconsistent efficacy, the immersion vaccination method is wasteful as it requires large quantities of the vaccine. This method may not be applicable to all types of vaccines. The spray method is found to be more stressful than DI methods [14].

### 3.2 Oral Vaccination

Oral vaccination is the most suitable method for aquaculture as it involves no stress to the fishes and less labour cost [15]. Noted adverse effects of oral vaccination methods include inconsistent efficacy and a considerable waste of vaccine than ingested. The duration of protection is less and fishes may develop immunosuppression in oral vaccination methods [16]. To increase the efficacy, different encapsulation methods like alginate, PLGA, chitosan, liposomes, silver or gold nanoparticles are utilized. Use of silver or other metal nanoparticles are toxic to the environment and to the fishes [17].

## 3.3 Injection Vaccination

Injection vaccination is the most efficacious method of vaccination. Fishes are vaccinated either intraperitoneally or intramuscularly as oil-adjuvanted vaccines to protect them from different bacterial or viral diseases. In this method, handling, anaesthetization and injection are stressful to the fishes and it is a labour-intensive method. Commercially available fish vaccines contain oil-based adjuvants which can protect the animal for a longer duration but severe adverse effects are reported with the use of different adjuvants and intraperitoneal injections in several fishes. The main adverse effects associated with the injection vaccination method are grouped into three categories: (a) inflammation and abdominal lesions, (b) growth retardation and spinal deformities and (c) systemic autoimmunity [18–20].

### 3.3.1 Inflammation and Abdominal Lesions

Antigen injected intraperitoneally with oil adjuvants acts as inflammation stimulant, initially attracting acute inflammatory reaction components—lymphocytes, polymorphonucleocytes (PMN), eosinophilic granulocytes/mast cells and, later, macrophages. This stimulation persists at the injection site for many months [21]. Following injection, oil-adjuvanted vaccines develop into cell vaccine mass (CVM) which is found to have a blood vessel-like connection between internal organs. Cell vaccine mass originally comprises neutrophils and macrophages but eventually develops into granulomas inside the peritoneal cavity in advanced stages [22]. Lesions were observed in many vaccinated fishes [23, 24] around the pyloric caeca and spleen, near the urinary bladder and oesophagus and intra-abdominal lesions in pancreas, pyloric caeca and the spleen [21]. Ocular inflammation and occlusion of uveal vessels are also found in vaccinated fish [25].

The adjuvants aid in the formation of depots of antigens at the injection site and slow release of these antigens [26]. After the acute injection site inflammatory responses, these develop into chronic inflammatory reactions characterized by the presence of visible adhesion between the organs and between organs and the abdominal wall. Granulomas, the hallmark of chronic inflammatory reaction, develop at later stages that are characterized by the presence of large numbers of macrophages, apoptotic neutrophils [22, 27, 28], lymphocytes and multinucleate cells [29, 30].

Two most commonly observed adverse effects associated with injection vaccination methods are adhesions and melanin formation. Adhesions are caused by the production of fibrinous strands [31] which surround the pancreas and pyloric caeca [28]. In addition, the formation of cell vaccine mass (CVM), viscosity and adherence of oil itself contribute to the development of internal adhesions [22]. Melanin pigmentation is a routinely observed adverse effect of the oil-adjuvanted vaccine in many fishes [31–33]. Initially, the adverse reactions increase for a period of time and then the reactions subside until the antigen is fully removed by competent cells [33]. Among different types of vaccines, the highest adverse reactions were observed in oil-adjuvanted multivalent vaccines by many researchers [27, 29].

### 3.3.2 Histological Changes in Organs

The oil-adjuvanted vaccines induce granulomatous peritonitis, oedema in splenic mesoderm [27], white military spots on liver [28], petechial haemorrhages in liver, abscess near lateral line, thickening of splenic ellipsoids, mild disruption of splenic structure, fibrin in liver, recruitment of melanomacrophage centres in anterior kidney [34], granulomas in liver, spleen, heart and kidney and hyperplastic white pulp of spleen [28].

### 3.3.3 Scoring of Adverse Effects of Vaccines

In fish vaccination, different scoring methods are used to evaluate the vaccine-induced adverse effects. Midtlyng and Speilberg score (0–6) based on visual appearance of the abdominal cavity is used as a standard method for measuring the local reactions due to intraperitoneal injections of oil-adjuvanted vaccines (Table 1) [23], and this scoring is modified into Visual Analogue Scales (VAS) for continuous scoring [35]. Vaccination-induced melanisation is evaluated using visual observation on a 0–3 scale: 0—no melanin; 1—pinpoints or small spots; 2—considerable

**Table 1** Midtlyng and Speilberg scoring of adverse effects [23]

| Score | Visual appearance of abdominal cavity | Severity of lesions |
|---|---|---|
| 0 | No visible lesions | None |
| 1 | Very slight adhesions, most frequently localised—close to the injection site. Unlikely to be noticed during evisceration | No or minor opacity of peritoneum after evisceration |
| 2 | Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall may be noticed during evisceration | Only opacity of peritoneum remaining after manually disconnecting the adhesions |
| 3 | Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall may be noticed during evisceration | Minor visible lesions after evisceration, which may be removed manually |
| 4 | Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit may be noticed during evisceration | Moderate lesions which may be hard to remove manually |
| 5 | Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas | Leaving visible damage to the carcass after evisceration and removal of lesions |
| 6 | Even more pronounced (than the lesions described above), often with considerable amounts of melanin. Viscera unremovable without damage to fillet integrity | Leaving major damage to the carcass |

amount of melanin; 3—melanin covering large areas of the abdominal wall/abdominal organs [36].

The inflammation and granuloma are assessed on a 0–2 scale where 0—none, 1—few, and 2—a large number of granulomas [33]. The spinal deformity is scored by macroscopic observation and palpation on a 0–3 scale, in which 0—no deformity, 1—palpable deformity recognizable by fish health professionals, 2—medium deformity recognizable by laymen and 3—high degree of deformity [35].

### 3.3.4 Growth Retardation and Spinal Deformities

Growth reduction due to spine deformities [35], visible vertebral changes in 10% fishes [28], reduced interest in feed, behavioural changes like decreased activity [31], weight reduction up to 20% [37] and growth reduction up to 8% [32] are observed as vaccine-associated adverse effects. Vaccine-induced abdominal lesions and adhesions also affect the normal functioning and metabolism of different organs, and may reduce appetite and damage the digestive function [38].

### 3.3.5 Systemic Autoimmunity

Autoimmunity refers to a system of the immune response against its own cells or tissues. The systemic autoimmunity following vaccination was first reported in fishes by Koppang et al. [39]. Presence of autoantibodies, thrombosis and chronic granulomatous inflammation in liver and immune-complex glomerulonephritis were observed in vaccinated fish [39]. Further, the presence of rheumatoid factor, self-reacting anti-nuclear and cytoplasmic antibodies and immune-complex-mediated glomerulonephritis, after the intraperitoneal injection of the killed multivalent mineral oil-adjuvanted vaccine confirmed this condition as vaccine-induced systemic autoimmunity [28].

## 4 Risks to Fish Farmers and Consumers

Fish farmers or technicians involved in vaccinating the fishes may be exposed to fish vaccines by accidental self-injection. During fish vaccination, vaccinators reported one to more than 50 stabs or self-injection. Even though self-injection of fish vaccines has low health effects, two cases of hospitalisation were reported because of anaphylactic/allergic reaction after accidental self-vaccination [40]. Hence, the studies on adverse reactions in humans during accidental injection also need to be taken into consideration prior to vaccine approval.

## 5 Vaccine Failures

There are many reasons for the failure of vaccines such as poor storage condition, immunizing unhealthy and immune-compromised fishes, loss of immunogenicity of antigens in a killed vaccine and use of antimicrobials along with live-attenuated vaccines [41]. Overexposure of fishes to various stressors, vaccinating unhealthy fishes [2], not following the recommended vaccination protocol in dilution, species, life stage and administration methods, etc. may result in vaccine failures [42].

## 6 Measures to Reduce the Adverse Effects

The adverse effects (internal adhesions and melanisation) caused by the parenteral vaccination of oil-adjuvanted vaccines on breeding and seed production, fish welfare and marketing of fishes can be overcome by the use of novel methods of vaccination and adjuvants. Development of adverse reactions can be reduced by increasing the fish size at vaccination [38], selective breeding method [43], use of alternate adjuvants like water-based adjuvants, liposomes, etc. In safety testing, the following parameters are assessed: the presence of viable pathogens in inactivated vaccines, contamination of vaccines by extraneous pathogens or chemicals and residual level of formaldehyde in formalin-inactivated bacterial vaccines [44–46]. Novel strategies are required to develop the most effective and efficacious oral vaccines for aquaculture. The hurdles in the development and licensing of DNA vaccines can be overcome by addressing all the research gaps in development and application.

## 7 Future Perspective

The world fish farming sector has transformed from an extensive to intensive industrial-scale production system. Global food fish production has reached an all-time high of 178.5 mmt in 2018 with an average annual growth rate of 5%. Global per capita fish consumption has increased from 9.0 kg in the 1960s to 20.5 kg in 2018, mainly due to the increase in aquaculture production [47]. Diseases are the primary constraint hindering further growth and sustainability of the sector. In recent decades, the enormous expansion of aquaculture practices including intensive stocking and transboundary movement of aquatic animals has paved the way for the emergence of many new pathogens [2]. The outbreak of certain diseases such as white spot syndrome virus (WSSV), early mortality syndrome, hepatopancreatic microsporidiosis (EHP), infectious salmon anaemia (ISA), tilapia lake virus (TiLV) and spring viremia of carp (SVC) has caused catastrophic losses in aquaculture [7]. ISA caused an economic loss of approximately US $1.0 billion from 2007 to 2011 [48], which could have been saved by the use of appropriate vaccines.

The emergence of new pathogens and detrimental use of antimicrobial drugs have to be addressed to sustain aquaculture production. Fish vaccines play a major role in maintaining the farmed fish welfare and reducing the economic losses by preventing disease outbreak in aquaculture. Vaccination is undoubtedly the most effective intervention for disease prevention and control. However, there is still a need for improving hazardless vaccine delivery methods in aquaculture.

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