Vaccine Development for Asian Aquaculture

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ABSTRACT

In conjunction with good health management, vaccination is a powerful tool for disease control in modern day fish farming. Vaccination of fish has become a standard operating procedure in most countries in Europe and North America. In Asia, with the exception of Japan, vaccines are not commonly used for fish disease control. This is inconsistent with the large quantities of fish that are being produced in this region. There are several reasons for the lack of vaccine products in Asia. Firstly, more resources are needed to understand the basic epidemiology of diseases and the immune system of many species. Secondly, most of the farms are operated on a small scale with little technical support. Farmers focus more on treatment than prevention as antibiotics are largely available. In addition, since development and commercialization of vaccines requires a great deal of time and resources, only few companies are committed and specialized in this field. The major advantages of vaccination over therapeutic treatments are that vaccines provide long-lasting protection and leave no adverse residues in the product or the environment. A critical milestone in vaccine development is the understanding of the disease etiology and epidemiology. At present, more and more information is being generated by governmental institutes, universities and the private sector. In the foreseeable future, this knowledge will lead to successful development of vaccines specifically for the Asian aquaculture industry.

INTRODUCTION

While the intensification of aquaculture has led to remarkable improvements in productivity, it is also associated with disease epidemics, involving bacterial, fungal, viral and parasitic pathogens. Disease is undoubtedly one of the biggest constraints on production, development and expansion of the aquaculture industry. Diseases can be controlled in a number of ways, for example, introduction of specific-pathogen-free (SPF) broodstock, optimization of feed, improvement of husbandry techniques and good sanitation. In conjunction with good health management, prophylactic immunization (vaccination) is an indispensable tool for disease control in aquaculture (Evelyn, 1997, 2002; Gudding *et. al*., 1999).

Vaccination has become an increasingly important aspect of aquaculture. Several bacterial and viral vaccines, either mono- or multivalent, have been successfully developed and commercialized (Bostock, 2002; Evelyn, 2002). They have proved to be cost effective. In salmonid farming, the use of vaccines is now so widespread that basically all fish stocked in sea cages have been vaccinated. Taking Norwegian salmon farming as an example, the use of antibiotics has dropped to virtually zero and production has increased tremendously

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(Bostock, 2002, Markestad and Grave, 1996). While the success of the Norwegian salmon industry is directly associated with advances in culture methods, feeding strategies, processing technology, marketing, and legislation of disease prevention, vaccination has certainly played a significant role.

Norwegian salmon farming is often taken as an example of how things should or could progress in aquaculture. However, the production of fish in tropical and subtropical areas is quite different. Differences involve not only in the species cultured, but also (and mainly) the scientific knowledge that is available on reproduction, husbandry, feed requirements, diseases and immunology specific to the farmed species. Taking these differences into account, the knowledge that has been gathered in salmon vaccinology can be used to advance the science more efficiently in other farmed species. In this paper, an overview is given on the current situation of fish vaccination with an emphasis on fish cultured in tropical areas.

COMPARISON OF SALMON PRODUCTION WITH ASIAN AQUACULTURE

Salmon is an anadromous fish species, i.e., it spends most of its life in the marine environment but reproduces in freshwater. The larvae and fry are produced in freshwater and subsequently migrate to the seawater environment. The most economically significant diseases (e.g., furunculosis, classical vibriosis, infectious pancreas necrosis and coldwater vibriosis) occur in the marine environment. This compartmentalized development of salmon provides a convenient vaccination window to assist disease prevention. The fry are vaccinated during the freshwater phase well before their transfer to seawater so that they have time to develop protective immunity against the disease agents that they will encounter during the grow-out phase in seawater. With the available adjuvanted multivalent vaccines, a single intraperitoneal injection in juveniles can confer long-term protection in seawater stage (Evelyn, 2002). Most fish species in Asia are either cultured solely in seawater or freshwater and therefore the specific vaccination opportunity that exists for salmon is not available for these species.

Asian aquaculture is characterized by an enormous diversity of species, with over one hundred species being farmed. In other regions, the number of species cultured is far less, i.e., in Northern Europe, the only family cultured until recently was salmonids. Consequently, all resources available in Western countries were spent on the optimization of the culture for salmonids including disease control. In Asia, given the large number of species cultured, resources are spread thinly across species, resulting in sporadic and fragmented knowledge on each individual species.

The intensification of salmon production has led to separation of fry production and on-growing sites, optimized feed and feeding strategies, good quality fingerlings that are virtually disease free and good farm management. In Asia, most farms produce different species of fish at the same site. No segregation in year classes is made, something that is obligatory for salmon in Europe, trash fish is widely used as feed, fry are often caught wild or derived from wild-caught broodstock and the culture techniques per species are not yet established. Furthermore, legislation and implementation regarding farming license and zoning policy are not in place in most Asian countries. With the gold rush mentality, this often results in too many fish and too many farms in a concentrated area that promotes the spread of diseases. The combination of all these factors together with the diversity of organisms in tropical waters leads to a truly challenging disease situation with a variety of entry points for pathogens. While the use of vaccines will make a contribution, all other aspects of farming operations must be improved for Asian aquaculture to remain sustainable.

VACCINATION VS ANTIBIOTIC TREATMENT

While under certain circumstances antibiotics can provide a useful means of helping to control some bacterial diseases, there are many problems associated with their use. An important side effect on the use of antibacterial drugs in aquaculture, apart from residue problems and increasing consumer concerns, is the development of drug resistance among bacterial pathogens (Huovinen, 1999; MacMillan, 2001; Smith *et al*., 1994, Tendencia and De La Pena, 2001). Also as sick fish do not eat, the efficiency of delivering antibiotics orally is often questionable.

The principal difference between the two approaches is that vaccination is a preventative measure, dependent on the immune system of the animal. The use of antibiotics, on the other hand, is a curative measure to treat an existing infection and does not normally rely on physiological processes.

Two side remarks should be made regarding antibiotics:

1) by nature they are active mainly against bacterial pathogens and have no direct effect against viral and other infections; and
2) antibiotics work only as long as they are present in appropriate concentration in the target organ.

In contrast, vaccines can act against bacterial, viral and, at least experimentally, parasitic infections and they will usually act specifically against only the targeted pathogens. The duration of protection obtained with vaccines normally exceeds by and large that of antibiotics. Fig. 1 shows that the introduction of vaccines has been instrumental in huge reduction in the use of antibiotics in Norwegian salmon production.

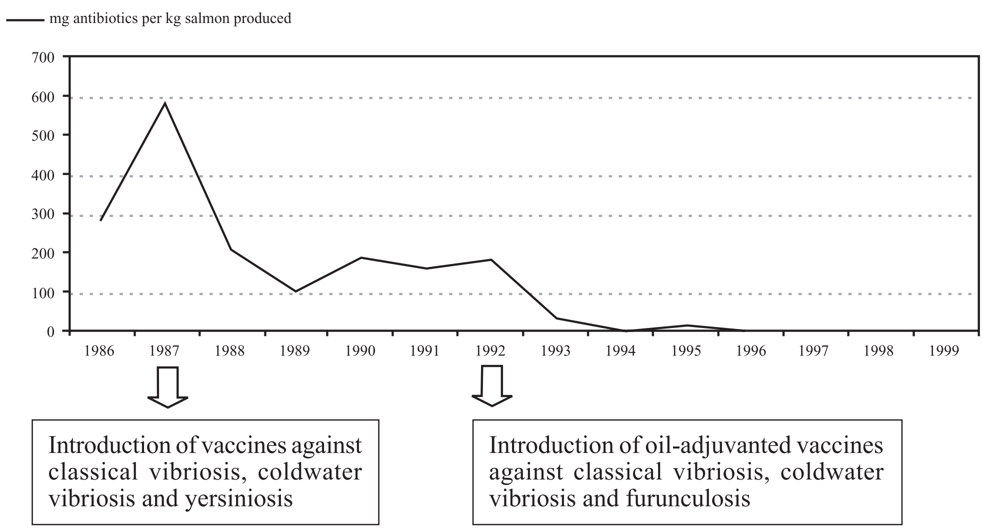

Figure 1. Consumption of antibiotics in the Norwegian salmon industry, 1986-1999.

Table 1. Registered and commercially available antigens for fish and crustaceans (from Bostock, 2002).

Antigen	Available region and target species		
	Europe/North America	Mediterranean	Asia
Aeromonas salmonicida	Salmonids		
Vibrio anguillarum	Salmonids	Seabream/Seabass	*Seriola* spp.
V. salmonicida	Salmonids		
V. viscosus (Moritella viscosa)	Salmonids		
V. ordalii	Salmonids		
Yersinia ruckerii	Salmonids		
Renibacterium salmoninarum	Salmonids		
Flexibacter columnarae	Salmonids		
Piscirickettsia salmonis	Salmonids		
Lactococcus garvieae	Salmonids	Seabream/Seabass	*Seriola* spp.
Streptococcus iniae	Salmonids	Seabream/Seabass	
Pasteurella piscicida (*Photobacterium damselae* subsp. *piscicida*)		Seabream/Seabass	
Edwardsiella ictaluri	Catfish		
V. harveyi			Shrimp
V. parahaemolyticus			Shrimp
V. alginolyticus			Shrimp
V. vulnificus			Shrimp
IPNV	Salmonids		
IHNV	Salmonids		
ISAV	Salmonids		
VHSV	Salmonids		
SPDV	Salmonids		
GCRV			Grass Carp
Iridovirus			*Seriola* spp.

IPNV: Infectious pancreatic necrosis virus; IHNV: infectious haematopoietic necrosis virus; ISAV: infectious salmon anemia virus; VHSV: viral haemorrhagic septicaemia virus; SPDV: salmon pancreatic disease virus; GCRV: grass carp aquareovirus

In Asia, easy access to antibiotics has led to their use not only for curative purposes but also as a form of "preventative measure", where antibiotics are administered in anticipation of an expected disease outbreak. This has resulted in a rather heavy use of antibiotics (Choo, 2000). At present, trade barriers for Asian aquaculture products, increasing public awareness and concern for residues in fish and crustacean products, and the development of multiple antibiotic resistant bacterial strains will lead to a shift from disease treatment through antibiotics to disease prevention by other means such as vaccination.

Commercially Available Antigens

A partial overview of the available bacterial antigens in commercial products is given in Table 1 (from Bostock, 2002). Although a number of vaccines are being used in specific countries that are either locally produced as autogenous vaccines or are still in an experimental phase, these antigens have been omitted from this list. Most of the antigens are developed and marketed for salmonids. In the Asia-Pacific region excluding Japan, the only antigens available are for shrimp and grass carp. Therefore, there is a discrepancy between the number and volume of species cultured in Asia and the status of disease preventative measures such as vaccination.

It seems strange that up to now international companies have not developed and commercialized any fish vaccines for the lucrative Asian market. The main reason is the lack of scientific knowledge on the diseases and animals specific to Asian aquaculture (see other sections in this paper). Also, most of the farms are operated on a small "backyard farming" scale with little technical support. As antibiotics are largely available, the focus is more on treatment than prevention; and until recently, there were few regulations on their use in aquaculture (Choo, 2000). Another reason is driven by economics. Vaccine development is a lengthy and expensive process that involves a great deal of time, usually 5-8 years from identification of disease-causing agent, and significant amounts of resources and funds are necessary for research, testing and licensing. Few companies have the know-how, resources and commitment to engage in this business.

VACCINE DEVELOPMENT

The development of a vaccine typically follows a sequence of activities that eventually leads to the availability of a product. Ideally, a vaccine should be:

1) safe for both the fish, the administrator and the consumer;
2) have a broad strain or pathogen coverage;
3) provide 100% protection;
4) give a long-lasting protection, preferable as long as the production cycle;
5) be easy to apply;
6) be applicable in various species;
7) be cost effective; and
8) be readily licensed or registered.

Of principal importance in the entire vaccine development process is the precise identification of the causative organism, including the existence and significance of serotypes, and a full understanding of the epidemiology of the disease. Clearly identification of the disease agents is needed to allow for the proper selection of appropriate strains or antigens to be included in the vaccine. Epidemiological information is required to establish the duration of protection needed and to determine the window for vaccination, i.e., when the fish should be vaccinated. A combination of both subsequently determines the application method of choice and the vaccination schedule.

Knowledge on the prevailing diseases, their economic significance and the pathogens associated are key information required to support a vaccination program. Unfortunately such information are still lacking for most species cultured in Asia and for most pathogens involved. Far too often, disease outbreaks are described based on disease signs and not on the isolation and characterization of the pathogen. A classical example is a disease referred to as vibriosis. The classical definition of vibriosis is a septicemia caused by *Vibrio anguillarum* serotypes O_1 and/or O_2. This disease typically affects a wide variety of fish species including salmonids, European seabass and seabream, and Japanese yellowtail. In Southeast Asia, the term vibriosis is used for a disease situation from which members of the genus *Vibrio* were isolated, typically *V. harveyi, V. alginolyticus* or *V. parahaemolyticus.* Given the specificity of the immune system in fish, a classical vibriosis vaccine, with *V. anguillarum* as antigen, will not provide specific protection against other *Vibrios* spp., e.g., *V. alginolyticus* (Toranzo *et al*., 1997).

The next crucial step is the development of a challenge model that allows for reproduction of the disease and the disease signs. This challenge model is needed not only to fulfill Koch's postulates, thereby confirming that the disease in question is indeed caused by the pathogen, but also to allow for the evaluation of prototype vaccines. Vaccine efficacy is normally evaluated by comparing the survival of vaccinated fish with that of non-vaccinated control fish after challenge. The efficacy is then expressed as relative percentage survival or RPS. RPS is defined as: {1-(% mortality in vaccinated fish / % mortality in control fish)} x 100. In efficacy evaluations, a mortality level of more than 60% is aimed for in the control groups in order to obtain reliable results. In general, RPS values exceeding 60% are considered good.

After the development of a challenge model, prototype vaccines can be developed and tested. Different types of vaccines are possible, i.e., inactivated vaccines either adjuvanted or not, live attenuated vaccines, sub-unit vaccines, recombinant vaccines, synthetic vaccines (peptide vaccines) or DNA vaccines. The choice of vaccine type in a particular case will depend on the degree of protection that can be obtained, the duration of the protection obtained versus the required duration, the final cost of the vaccine in relation to the benefit to the farmer, and the registration limitations imposed by governments in the countries where the vaccine would be marketed. For instance, governments might object to the use of live vaccines or DNA vaccines, although often on non-scientific grounds.

VACCINE APPLICATION METHODS

Different application methods or routes of administration exist, namely:

(a) oral vaccination;
(b) immersion vaccination; or
(c) injection vaccination.

Oral vaccination

In oral vaccination, the vaccine is either mixed with the feed, coated on top of the feed (top-dressed) or bio-encapsulated (Quentel and Vigneulle, 1997). When antigens are to be incorporated in feed, heat sensitivity of the antigen needs special attention. When vaccines are to be top-dressed on the feed, a coating agent is often applied either to prevent leaching of the antigen from the pellets or to prevent breakdown of the antigen in the acidic environment of the fish stomach. For sensitive antigens, various micro-encapsulation methods are being evaluated and tested. Bio-encapsulation is used where fish or shrimp fry are to be vaccinated. In this case, live feed such as *Artemia* nauplii, copepods or rotifers are incubated in a vaccine suspension and then fed to the fry. Since these live organisms are non-selective filter feeders, they will accumulate the antigen in their digestive tract and, as such, transform themselves into living microcapsules (Campbell *et al*., 1993).

Oral vaccination has the advantage in that it is easy to administer and causes no stress to the fish. However, in most cases, only limited protection can be obtained and the duration of protection is rather short. Thus, although oral vaccination is the preferred method from a fish farmer's perspective, at present, there are few examples of effective oral vaccines.

Immersion vaccination

For immersion vaccination, two application methods exist: (a) dip vaccination; and (b) bath vaccination (Nakanishi and Ototake, 1997). In dip vaccination, fish are immersed for a short duration, usually 30 sec, in a highly concentrated vaccine solution, usually 1:9 diluted product. In bath vaccination, fish are exposed for a longer time, usually one to several hrs, in a lower concentration of antigen. Of the two alternatives, dip vaccination is more widely used since it allows for rapid vaccination of a large numbers of fish (up to 100 kg of fish for 1 L of vaccine). Immersion vaccination is widely used for fry with weights between 0.5 to 5 g. It is an effective method that results in relatively good protection for a significant period of time. The limitations of immersion vaccination are that the duration of immunity is not very long and booster vaccination is required when the disease prevails over longer periods. In addition, the method is impractical for larger size fish due to cost-effectiveness and stress issues. A few bacterial combination vaccines exist for immersion application but, to our knowledge, no inactivated viral vaccine is presently available for immersion application.

Injection vaccination

Injection vaccines are initially perceived by fish farmers as unfavorable, mainly because they fear that the stress resulting from the manipulation and injection of the fish will cause mortality and the process is time consuming. However, injection vaccines have a number of advantages that make them the preferred method. Injection vaccination provides long duration of protection, i.e., for over a year, and it allows for multiple antigens to be combined in a single vaccine and therefore in a single administration (Evelyn, 2002). At present, the most complex products provide protection against 6 agents (5 bacterial and 1 viral) and, in the near future, heptavalent vaccines will become available. The injection volumes per fish are usually 0.1 or 0.2 ml, with resulting protection throughout the production cycle. Injection vaccines can be adjuvanted with oils to increase their effectiveness as well as the duration of protection obtained. Injection is in general superior to any other vaccine application method; however, injectable vaccines can only be applied practically in fish of 15 g or greater.

Injection vaccines can be administered by intramuscular or intraperitoneal (in the abdominal cavity) injection. Given the possibility of inflammatory reactions at the injection site, most available injection vaccines are developed for intraperitoneal injection. Any vaccine remnants or inflammatory reactions can then be removed by eviscerating the fish before consumption. Injection vaccination can be performed by a specialized machine or by hand (Fig. 2). Indeed, an experienced person can inject over 1,500 fish per hour.

Figure 2. Injection vaccination of salmon by hand.

THE FUTURE

Asian aquaculture will continue to grow at a relatively fast pace both in terms of area expansion and production intensification. Under these conditions, the prevalence and spread of infectious diseases will unavoidably increase as a result of higher infection pressure and deterioration of environmental conditions. Accordingly, the effective control of infectious diseases has become more and more important in the cultivation of aquatic organisms. Good husbandry practices and health management must be emphasized. As part of health management measures, vaccination can be effective for disease control.

The development and manufacture of vaccines for aquatic species is a complex process. Important elements which have to be considered when developing vaccines and vaccination strategies aimed for Asian aquaculture, include:

1) fish farming technology (intensive production of a particular species with good management);
2) etiology and epidemiology of the diseases (identification and characterization of the disease-causing agent);
3) the ontogeny of the immune system (identification of the earliest time to vaccinate and available windows for vaccination);
4) efficacy and safety of the product, preferably applicable to multiple fish species; and
5) a good return on investment for the fish farmer. Until now, the first three elements have not yet been established for most of the Asian species.

Fish vaccination is no longer a new technology. In fact, current efforts to develop new fish vaccines have turned to recombinant DNA technologies (Husga *et. al*., 2001). Especially for viral pathogens, as well as the more complex bacterial/rickettsial pathogens, these technologies may be the method of choice to produce sufficient quantities of the antigens on an economical basis (Rödseth, 2000). There has been a great deal of interest recently in the use of plasmid DNA encoding antigens for immunization (Corbeil *et al*., 2000; Lorenzen *et al*., 2000). DNA vaccines have been shown to elicit both cellular and humoral immunity in other animals. Research on DNA vaccines in fish is ongoing in several laboratories.

Probably the most imminent and urgent task towards vaccine development for Asian aquaculture is the understanding of the etiology and epidemiology of diseases for the large variety of fish species farmed within the region. Some disease-causing agents have been described but comparative studies between isolates from different geographical locations and different fish species are generally not available. Epidemiological data are scarce as are basic data on the immune systems of Asian fish species. Nevertheless, during the last few years, an increased focus on disease diagnosis is noticeable. Furthermore, several government-owned high-tech hatcheries are being established in order to provide better quality fingerlings for stocking. The production methods developed in other regions can serve as a starting point for the development of local farming methods to further optimize the production and profitability of fish farming. The same applies for vaccination technology. Once a better understanding of the disease agents and their significance in Asia is obtained, the development of effective vaccines should be quite possible. Collaboration among governmental institutes, universities and the private sector are important to speed up the process.

CONCLUSION

The amount of aquaculture production in Asia greatly exceeds that of the rest of the world. However, in comparison, almost no specific disease preventative products, i.e., vaccines, are available in the region. There are several reasons, which explains this discrepancy. The wide variety of species cultured in Asia results in the spread of available resources to optimize the culture of any given species. In Northern Europe, salmon farming has basically been the only focus for decades and therefore the production process has been optimized in a relative short time period. In Asia, proper disease diagnosis and systematic collection of pathogen strains are limited. Farmers often use antibiotics without knowing the disease agent due to the lack of diagnostic support and alternatives for disease control. The large variety in culture methods in the region, the use of wild fingerlings, over stocking practices, and the ubiquitous use of trash fish as the principal source of feed further complicate the issue.

As Asian aquaculture continues to expand, disease problems will increase. Therefore, disease research and the implementation of new disease control concepts are critical to maintain sustainability. Vaccination is the active process of inducing protective immunological responses against specific pathogens. The development of an effective vaccine is a complex process. One of the prerequisites is understanding the basic epidemiology of diseases and the immune system of the target species. At present, knowledge in these areas are lacking. However, the importance of disease control is increasingly recognized by both farmers and governments due to the significant economic losses caused by diseases and international pressure on the use of chemicals and antibiotics. Thus, increased resources have been allocated for disease research. In turn, improved information on diagnostic techniques, infectious diseases and standardization of the culture practices will assist the development of vaccines in the future.

Over the last decade, vaccines and vaccination strategies have been successfully developed for several bacterial and viral diseases. Proven by the success of Norwegian salmon production, vaccination will be one strategy of choice, in conjunction with good health management, for effective disease control in commercial fish farming in Asia. With the continuous advancement in technology and standardization of production, it is expected that, in the near future, tailor-made vaccines for Asian aquaculture will become available.

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