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Vaccines in Mediterranean aquaculture: Practice and needs

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Abstract. Commercial conventional vaccines, which have proved their efficacy under laboratory conditions and in field trials, are available for the main bacterial diseases threatening marine finfish productions in the Mediterranean. New technologies can help develop more efficient vaccines or products for emerging diseases but regulatory aspects are still limiting their field of application. The claims of unsuccessful vaccination using a commercial vaccine should be investigated with a proper diagnosis of the resulting mortality. This approach shows in most cases that the vaccine itself is not at fault, since the vaccination strategy applied was not properly adapted to the farming conditions on site, to the disease considered and its epidemiology or the species needing protection. Vaccination programmes represent only one part of health management and should be applied parallel to other prophylactic methods, such as sanitary rules to optimize production results.

Keywords. Mediterranean – Finfish – Vaccine – Procedures – Strategies.

Les vaccins en aquaculture méditerranéenne : Pratiques et besoins

Résumé. *Des vaccins commerciaux conventionnels contre les principales maladies bactériennes affectant les élevages de poissons marins méditerranéens et ayant démontré leur efficacité lors d'essais de laboratoires et de terrain, sont maintenant disponibles. Les nouvelles technologies peuvent aider au développement de vaccins plus efficaces ou de produits contre les nouvelles maladies émergentes. Mais les aspects réglementaires limitent leur champ d'application. Les échecs de vaccination avec des vaccins commerciaux doivent être analysés avec soin, ainsi que les mortalités en résultant. Cette approche montre dans la plupart des cas que le vaccin n'est pas incriminé mais que la stratégie de vaccination mise en place ne correspondait pas aux conditions d'élevage sur le site, la maladie visée et son épidémiologie ou l'espèce à protéger. Les programmes de vaccination ne représentent qu'une partie des programmes préventifs et doivent être couplés avec d'autres méthodes prophylactiques comme l'hygiène afin d'optimiser la production.*

Mots-clés. *Méditerranée – Poissons – Vaccins – Procédures – Stratégies.*

I – Introduction

In Europe, as in others countries such as North America, the legislative framework is still being developed for the marketing of veterinary products, limiting the number of licensed products available for treatment in aquaculture. The increasing request of consumers for quality products, the implementation of quality schemes and environmental issues are pressuring the producers into reducing the use of antibiotics in their production. For these main reasons, fish vaccinology is becoming a major issue and an alternative in aquaculture health management. This is reflected by the involvement of large International pharmaceutical companies in this sector of the vaccine business. During the 1990's, three of the main animal health companies took over innovative start-up companies in order to enter this market segment, while the two remaining independent fish vaccine companies were bought out in the last two years (Table 1).

If pioneer work on fish vaccines was carried out in North America before 1964, scientific research leading to the first commercially available vaccines was initiated during the period

1977-1989 (Evelyn, 1977; De Kinkelin and Michel, 1984; Larsen *et al.*, 1989). Efficacious vaccines against *Vibrio* infections were applied on a commercial scale in Norway during the late 1980's and a furunculosis vaccine in the early 1990's. The first commercial applications of fish vaccination programmes were not fully successful due to in adapted vaccination strategies (Rodgers, 1991). Later one, studies of fish medicinal products sold in Norway underlined a strong correlation between the drastic drop in antibacterial compounds and the implementation of vaccination strategies in salmon production (Markestad and Grave, 1997). Data from Scottish aquaculture support these observations. More recently, the implementation of full vaccination programs to trout production or to marine Mediterranean production have led to similar conclusions in production units, when vaccination strategies were adapted and properly applied.

In Mediterranean countries, several bacterial vaccines are now available for both fresh water and marine production under different presentations, allowing the implementation of full vaccination programs covering all the production cycles.

While nowadays research is focusing on the development of vaccines for emerging pathologies and on recombinant vaccines, the next field of application of commercial vaccines in European aquaculture, and especially in the Mediterranean area, should be the prevention of viral diseases, such as Nodavirus infections.

The legislative framework developed in Europe for the marketing of veterinary products (Lee, 1997) includes fish vaccines and is being more constraining as regards permits and licenses for manufacturers and marketing authorization for each specific product.

Except for salmon production in more northern countries, the two other main aquaculture activities in Europe are represented by trout and marine finfish production. Present practices and future requirements in fish vaccinology in this latter sector of activity are reviewed.

II – Present status of vaccines and their use in the Mediterranean market

1. Regulatory and environmental aspects of fish vaccines

A. Regulatory aspects

Immunological products such as vaccines fall under the Veterinary Medicine Legislation in the European Union (Original Directives 81/851 and 81/852, amended by Directive 90/667/EC, including immunologicals in the EU medicines regulatory environment). Therefore, all fish vaccines have required a veterinary prescription in EU Mediterranean countries. In other Mediterranean countries, vaccine use has to comply with national legislation, which in most cases requires both importation and use authorization.

B. Environmental aspects

Environmental bodies and organizations are putting more and more emphasis on pollution resulting from aquaculture activities. It is likely that restrictive regulations against indiscriminate use of antibiotics will be enforced. Vaccination therefore becomes an alternative, requested by an increasing number of customers and already included in the list of criteria of different quality schemes and labels: (i) hyper market quality chart in Spain; and (ii) red label and organic label in France.

2. Available products and vaccination methods

Most of the commercial fish vaccines available on the market today are produced by five pharmaceutical companies mainly focusing on the salmon industry (Table 1). First extensive field trials on marine species were initiated in Greece during the early 1990's by two of the main vaccine suppliers. However, it is only recently that interest in the vaccination of marine species has grown in the Mediterranean area and that licensed or temporary authorized products for the vaccination of these species have become available.

Table 1. Pharmaceutical companies involved in the international fish vaccine market in 2002 (modified from P.J. Midtlyng, 1997)

Fish vaccine manufacturers	Country	Pharmaceutical industry alignment
Alpharma Aquatic Animal Health	Norway / USA	Alpharma Inc.
Intervet / Shering-Plough	USA	Intervet / Shering-Plough
Aqua Health Ltd	Canada	Novartis
Microtech Inc.	Canada	Bayer AG

The first vaccines developed in the 1970's and 1980's were water based formalin-killed vaccines for dip immersion, using strong antigens such as *Yersinia ruckeri* and *Vibrio anguillarum*, which were applied by intra-peritoneal injection to larger fish. To improve the efficacy of such vaccines, especially when using weaker antigens such as *Photobacterium damsela* subsp. *piscicida*, ultrason were tested with immersion vaccination (Navot *et al.,* 2005) and mineral or non mineral oil adjuvants were applied with injectable vaccines. However, side effects rapidly became an issue, which was partly solved by using non-mineral oil adjuvant. The development of effective vaccines for oral delivery represents the last step in the development of commercial vaccines. The licensing of an oral enteric redmouth (ERM) vaccine was the first full license ever to be granted for an oral fish vaccine, but it not only has an effect on ERM vaccination strategies but establishes the credibility of the method as well. Proving the technology will have a profound effect on the vaccination strategies for other vaccines. Other antigens, such as *Vibrio anguillarum* serotypes I and II in Greece or *Lactococcus garviae* in Japan are already available for oral delivery in marine species.

Therefore, three methods of vaccination can now be applied in aquacultured marine species to protect the fish during their full production cycle. Each of them has advantages and disadvantages (Table 2), which will guide their choice, in correlation with different production factors:

(i) The economic factor represents the bottom line. Vaccination will be applied if the cost of the disease is expected to be higher than the cost of the vaccination. Different cost benefit analysis schemes have been designed to estimate this parameter. If mortality and chemotherapeutic costs have to be taken into account, then other costs need to be considered as well, such as extra work, and growth penalties.

(ii) The size of the fish will largely define if immersion vaccination is suitable or not compared to injection or oral delivery. The quantity of vaccine used for immersion is related to the biomass, while the quantity of vaccine required for the two other vaccination methods depends on the number of fish.

(iii) The production structure and production management of the site considered will be a factor mostly for booster vaccination and the choice between injection or oral delivery of the vaccine.

(iv) The period of the year and the species to be immunized will also influence the choice of the booster vaccination method. It is obvious that intra-peritoneal injection is not the method of choice when water temperatures are increasing and the fish are more sensitive to stress.

The availability of different vaccines (Table 3) for immersion, injection or oral delivery will allow the implementation of full vaccination programs covering the whole production cycles of the fish and lead to a better flexibility to adapt these vaccination strategies to the constraints specific to each production and each site.

Table 2. Vaccination methods: advantages and disadvantages

Vaccination method	Advantages	Disadvantages
Immersion vaccination: (1) Dip immersion (2) Long term bath (3) Spraying	Suitable for large quantities of small fish (<5 gr) Cost effective for small fish (1 and 3) Protective immunity for 3-5 months depending on the antigens	Difficult to apply on ongrowing units Expensive for large fish Costly (2)
Injection vaccination I.P. or I.M.	Good protective immunity, lasting up to 1 year Suitable for large fish (broodstocks) Possibility of automatization (tables) Good for weak antigens, for vaccination at low temperature	Stressful method Labor and time consuming Use of anesthetic required Side lesions
Oral vaccination	No stress, not time consuming Easy to apply on all production facilities For all sizes of fish (>10 g) and large batches	Monovalent vaccines only Protection slightly shorter than njection (8 months) Needs to be planned and requires ood feeding practice

Table 3. Licensed products in at least one of the EU States

Disease	Antigen	Water based			Oral formulation	Licensed†	
		Immersion	Injection				
			Aqueous	Adjuvated			
Vibriosis	*Vibrio anguillarum* serotypes I and II	X	X		X	L	L
Pasteurellosis	*Photobacterium damsela* subsp. *piscicida*	X	X		X	L	L
Vibrio + Pasteurellosis	*Vibrio anguillarum + Photobacterium*	X	X	X		L	L
Furunculosis	*Aeromonas salmonicida* subsp. *salmonicida*	X	X	X	X	L	L
Vibriosis + Furunculosis	*Vibrio anguillarum + A. salm. Salm.*			X		L	

†Licensed in at least one of the EU countries (L).

3. Factors influencing the vaccination strategy

To be successful, a vaccination strategy has to be adapted to the site where it will be implemented, depending on different factors:

A. The epidemiology of the diseases on the site

A proper identification of the pathogen(s) threatening the production on site is an essential primary step. Sea bream *Sparus aurata* can be affected during the early stages of their life cycle production by some serotypes of *Vibrio anguillarum.* However, *Vibrio anguillarum* serotype I and II have not been described as pathogens of economic importance for the production of this species, especially during the grow out period. Therefore, vaccination with the commercially available *Vibrio* vaccines of sea bream is not justified. The risk periods for the occurrence of the diseases to be prevented will define the vaccination calendar throughout the production cycle and the type of vaccine most suitable: monovalent/bivalent. Vibriosis occurs mainly in spring and autumn when the water temperature fluctuates, whereas Pasteurellosis occurs mainly during periods of high water temperature in summer and early autumn.

The pressure of the pathogen on site will give an indication of the method of vaccination of choice and the need for booster vaccination. IP injection will be more suitable on a heavily infected site, during the first years of the establishment of a vaccination program. In the long term, when the pressure of the pathogen will be reduced, oral booster vaccination will be easier to apply, especially in production cage structures at sea.

B. The farm production strategy and management

A minimum period, depending on the water temperature, is required for the development of the specific immune response of the fish. Any stress applied during this period might lower the immune response. Fish transferred to a contaminated area at this time will not have acquired full protection and it is essential to respect this post-vaccination period. In addition, oral vaccination requires a fifteen day period before implementation. These parameters have to be considered in conjunction with the farm production plan.

Most fingerlings are now vaccinated by immersion in hatcheries. To protect fish during their full production cycle and provide total protection, booster vaccination is often required. The implementation schedule will depend on the date of introduction of the different batches on site and the risk period for the disease considered.

C. The species

Each fish species is sensitive to a specific range of pathogens and this sensitivity can change during the different stages of their life cycle. These parameters will guide the choice of the vaccine, while the method of vaccination to apply will depend on the stress sensitivity of the species to vaccinate.

D. The hygiene on site and the health status of the fish

The health status of the fish is fundamental for successful vaccination. Gill parasitic infestation or bacterial gill disease will not allow a good uptake of antigen through this organ during immersion vaccination. For the same reason, anesthesia of the fish slows down the blood flow through the gill filaments and represents a contra-indication for vaccination of the fish by immersion (Navot, 2003).

Asymptomatic carriage will trigger the development of the immune response of the fish. This point is of prime importance in the case of bacterial pathogens such as *Photobacterium damsela* subsp. *piscicida*, which can survive in macrophages after phagocytosis. This can lead to the occurrence of some mortalities following vaccination, due to the stress of the vaccination procedure caused by: (i) manipulation of the fish; and (ii) physiological stress.

4. Actual practice in the Mediterranean marine finfish sector

Most species of cultured marine finfish are very sensitive during their early larval stages to viral and bacterial diseases. Attempts to protect them through distribution of bioencapsulated antigen in *Artemia nauplii* have not been successful, as fish do not increase their ontogenic maturity (Chair *et al*., 1994; Joosten *et al*., 1995). Immunostimulation and good hygiene procedures help to control larval pathologies.

Standard vaccination procedures of marine species include an immersion vaccination between 1-2 g, before the transfer of the juveniles from the hatcheries and booster vaccination either by injection or orally at the pregrowing or ongrowing facilities. Booster vaccination by immersion on larger juveniles ranging from 5 to 10 g average weight has been recommended for some occasions but represents quite an expensive procedure that is not easy to apply when fish are already transferred to sea in floating cage structures.

Commercial mono- or bivalent vaccines are available against vibriosis and pasteurellosis for booster vaccination. The former ones are water based vaccines while the latter are water based or adjuvated vaccines. Monovalent vaccines for oral delivery are now available for both diseases and can be coated on the dry feed either on site or at the feed mills. These oral vaccines are usually applied in conjunction with immunostimulation in order to increase the specific immune response of the fish both in intensity and duration.

A. Vibriosis

First attempts to vaccinate sea bass against *Vibrio anguillarum* serotype I and II were undertaken in the early 1990's and were successful. Immersion vaccination of the fry at 1.5 to 2 g rapidly became a standard procedure in the production of this species. Booster vaccination by injection has proved its efficacy, prolonging the immune protection for a year. However, since *Dicentrarchus labrax* is a stressful and difficult species to handle, major progress in the booster vaccination strategies for a full production cycle protection only came with the oral delivery of the vaccine.

B. Pasteurellosis

Development of vaccines against pasteurellosis has been the subject of numerous research studies for the last twenty years (Kitao *et al*., 1981; Kusuda and Hamaguchi, 1987; Magariños *et al*., 1994; Thune *et al.*, 1999). Different methods were investigated to improve the immunogenic properties of the bacterin and several vaccines have been tested (Kusuda and Hamaguchi, 1987, 1988; Kusuda *et al*., 1988). Specific bacterial techniques today allow the preparation of good quality antigen for the production of a conventional vaccine. However, the immune protection achieved in fish remains short and does not last for more than three months. Generally, the disease occurs during the warm water period in first year class fish, but has a chronic course in larger fish in the Mediterranean area. Therefore, it is important to consider the epidemiology of the disease on-site, in order to set up adapted and efficient vaccination strategies.

Immersion of the fingerlings in the hatchery at 1-2 g, followed by an oral or an injection booster vaccination at the ongrowing site should allow the fish to be protected during the critical period of first year production. Bivalent vaccines, including *Vibrio anguillarum*, may be used during the spring period for species sensitive to both diseases, such as sea bass.

C. Furunculosis

Furunculosis has been the great success story in fish vaccinology. In the 1990's, different authors (Hastings, 1988; Larsen and Pedersen, 1997) described the demand for an effective vaccine as imperative for the salmon industry, but no decisive success was achieved. The breakthrough came with the use of mineral oil and then non-mineral oil adjuvants. A point has now been reached where nearly 100% of farmed salmon are vaccinated against furunculosis. Data collected over this period have not only shown high levels of long-term protection but have also demonstrated dramatic reductions in the use of antibiotics.

Furunculosis vaccines are now being used with success or are being tested in other production facilities, such as brown trout, rainbow trout, cyprinids, ornamental fish and other marine species, such as turbot. Both sea bass, when reared in brackish water, and turbot are sensitive to the disease and can be effectively protected with an appropriate vaccination program.

III – Future needs in Mediterranean aquaculture

1. Ongoing research and development in fish vaccines

A. Bacterial diseases

Flexibacter infections represent one of the main bacterial diseases threatening the larval and juvenile production of both sea bass and sea bream in Mediterranean production. Despite much work in this area, including a three year research program, it still appears that the development of an effective vaccine is not close. The main issues remain the production of the antigen and the fact that the small size of the fish requiring vaccination are unable to develop a satisfactory immune response, since they have not reached ontogenic maturity.

B. Parasitic diseases

A successful recombinant vaccine against *Ichthyophthirius* has been tested in North America and represents the first antiparasitic vaccine for fish so far. However, it is currently only applicable by injection, which does not allow it to be used for mass administration. This step provides some hopes for the development of an effective vaccine against *Enteromyxum leei*, which is the major parasitic disease threatening the production of Sparidae.

The collection of antigen represents one of the issues in the development of vaccines against parasites. The knowledge of their life cycles, the identification of their intermediate host, such as freshwater bryozoans in the case of proliferative kidney disease, should offer possibilities of harvesting large numbers of parasites, which might be used in vaccine development studies.

C. Viral diseases

Attempts to develop conventional inactivated vaccines against viral disease have not met with success, except for an IHN vaccine commercialized in Canada and an Iridovirus vaccine in Japan. Recombinant vaccines have been more promising, especially in the case of IPN. Given that the salmon farming industry is suffering from increasing losses due to this disease, IPN vaccines are likely to be the first recombinant viral vaccines to be licensed for use in fish. Applying a similar method of expression to Nodaviruses should allow efficient vaccines to be developed against these viral infections in the near future. While DNA vaccines have produced good protection in trials aiming to immunize fish against VHS, there will be significant regulatory hurdles to overcome before such vaccines can reach the market.

IV– Conclusion

Commercial vaccines, which have proved their efficacy under laboratory conditions and in field trials, are available for the main bacterial diseases threatening aquaculture production facilities in the Mediterranean area. Therefore, the claims of unsuccessful vaccination using a commercial vaccine should be investigated with correct diagnosis of the resulting mortality. This approach shows in most cases that the vaccine itself is not at fault, since the vaccination strategy applied was not properly adapted to the farming conditions on site, to the disease considered and its epidemiology or the species needing protection. Vaccination programs represent only one part of health management and should be applied in parallel with other prophylactic methods, such as sanitary rules to optimize production results.

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