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VACCINE OIL ADJUVANTS

FOR THE DEVELOPMENT OF AQUACULTURE

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SUMMARY
Aquaculture is a fast growing industry, which produces today more than 30 species of fish. The growth of aquaculture in the last decades has been supported by the development of oil adjuvanted injectable vaccines that allowed a long term protection of fish and a strong reduction of the use of antibiotics. Today, injectable vaccines for fish are administered through intraperitoneal injection and are usually adjuvanted with water in oil emulsion adjuvants. Montanide™ ISA 763A VG is a non mineral oil based adjuvant which has been extensively used for vaccination of diverse fish species (salmon, trout, seabass, tilapia, ect.). Other routes of administration such as immersion and oral administration are also considered and new adjuvants and formulations are being developed for these applications.

Key words: Fish vaccines, aquaculture, adjuvants, Montanide™.

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ВАКЦИННЫЕ МАСЛЯНЫЕ АДЪЮВАНТЫ

ДЛЯ РАЗВИТИЯ АКВАКУЛЬТУРЫ

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РЕЗЮМЕ
Сельское хозяйство является быстроразвивающейся отраслью, которая на сегодняшний день производит более 30 видов рыб. Развитию аквакультурной отрасли в течение последних десятилетий способствовала разработка инъекционных вакцин на основе масляного адъюванта, что позволило обеспечить длительную защиту рыб и значительно снизить использование антибиотиков. Сегодня инъекционные вакцины для рыб вводятся внутрибрюшинно, и в них обычно добавляется масляный адъювант для эмульсионных вакцин. Montanide™ ISA 763A VG является адъювантом без минерального масла, который широко использовался для вакцинации разнообразных видов рыб (лосось, форель, сибас, тилапия и т.д.). Также рассматриваются другие способы введения вакцины, такие как погружение или пероральное введение. Для этого разрабатываются новые адъюванты и составы.

Ключевые слова: вакцины для рыб, аквакультура, адъюванты, Montanide™.

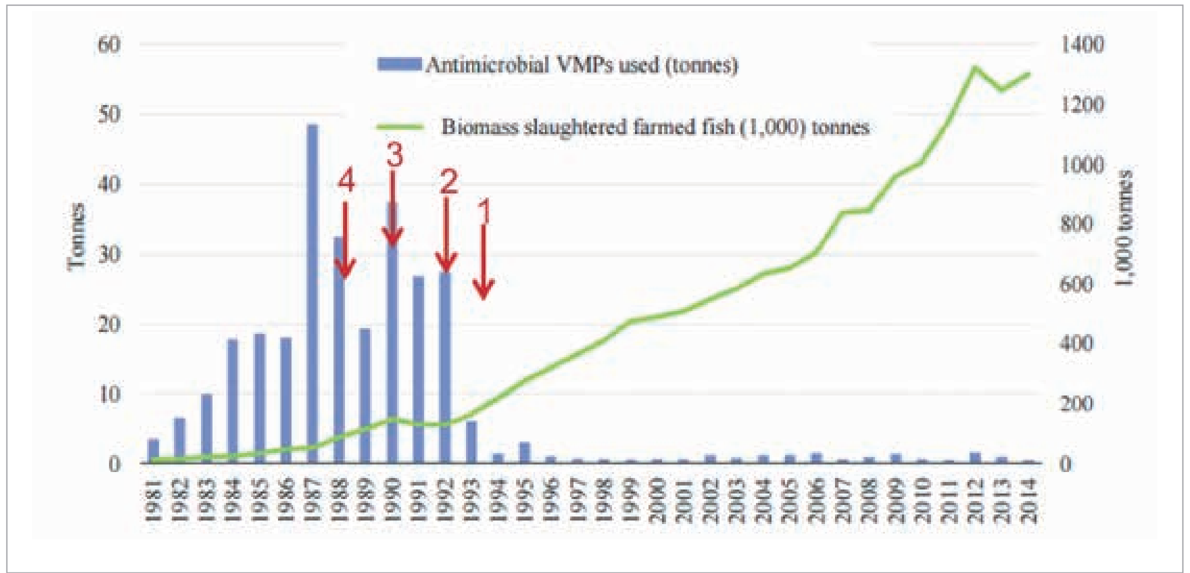


Fig. 1. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway
1 – Vibriosis vaccine, 2 – Furunculosis vaccine, 3 – Oil-based vaccine, 4 – Combination vaccine. NORM NORM-VET 2014

INTRODUCTION

The aquaculture industry produced globally 65 million tonnes in 2014, representing 150 billion of US $. Aquaculture industry is a fast growing industry (10 to 12% per year). It is expected that aquaculture should produce over 100 million tonnes of fish in 2050. Today, fish from aquaculture represent 40% of the whole fish consumption globally. It is expected that in 2025 it will be 50%. More than 30 species of fish are produced, as well as shellfish and crustaceans [4].

Until 1980, very few fish vaccines were used in aquaculture. In 1982, vaccines only existed for 2 diseases (Enteric Redmouth disease (ERM) and *Vibrio anguillarum*). Today, vaccines have been developed for more than 25 diseases of fish. Mass vaccination started in the 1990s in the salmonid industry, especially in Norway. Before the generalization of the use of fish vaccines, antibiotics were used extensively to prevent diseases in fish production. The use of new vaccines for salmons allowed a strong reduction of antibiotic use and a fast development of the industry. It is considered that introduction of mass vaccination in the salmonid industry based on water-in-oil emulsions is one of the major success stories in the growth of the global salmon farming industry [6]. It allowed the salmonid production to grow from a few hundred thousand tonnes during the early 1990s to more than 1.3 million metric tonnes in 2012 (Fig. 1).

The role of the oil adjuvant in the success of vaccination of salmonids is important. It is indeed the stability and slow release of the adjuvanted antigen that allows single intra-peritoneal injection to protect through the 2 to 4 years grow-out period of salmons. This property made vaccination an economical option for preventing disease and led to almost universal adoption by salmon farmers within a few years [6].

Today, the practice of vaccination by intraperitoneal injection has been slowly transferring to non-salmonid species. This is an important transition as the major growth in finfish aquaculture is now occurring in warm-water species such as tilapia.

Three routes of administration can be considered for fish vaccination: injection, immersion and oral administration. Intraperitoneal (IP) injection (Fig. 2) of 0.1 to 0.2 ml of water in oil vaccine is highly efficient and induces high and long term protection. Specific devices are available and injectable fish vaccines are extensively used in farming. However, this route of administration is labour intensive, requires trained vaccination teams, and cannot be performed when very small or very large specimen are concerned.

To avoid these technical issues, immersion and oral vaccination are being considered. Immersion consists in dipping the fish in a bath containing a vaccine for a few minutes. Oral administration consists in mixing the vaccine with the fish feed. Both methods are easier to implement than injection, but their efficacy has been until now limited. They are usually used as a complement to boost injectable vaccines [8], or for vaccination of juveniles when injection is not yet possible. The development of immersion and oral vaccines for fish will require dedicated adjuvants or formulations to improve their efficacy.

Commonly used vaccines are based on inactivated bacterial or viral antigens. New generation vaccines comprise

Fig. 2. Intraperitoneal injection in trout

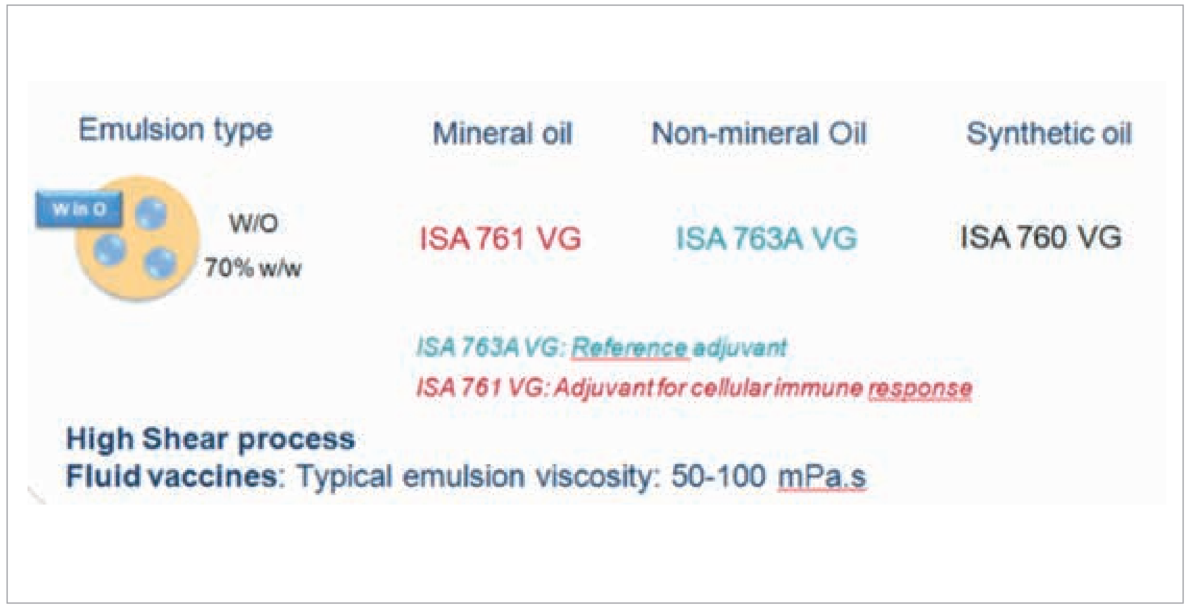


Fig. 3. Montanide™ range for injectable fish vaccines

attenuated or DNA antigens, but still represent a very small market share. Injectable inactivated vaccines are usually formulated with water in oil emulsion adjuvants to induce long term efficacy. As fish are sensitive to IP injection, oil adjuvants must be selected carefully to avoid viscosity and injectability issues (especially in cold water fish) and local reactions at the site of injection, such as melanisation and adhesions in the peritoneal cavity [7]. Such reactions should be avoided as they induce a loss of economic value. The type of oil (mineral, metabolizable, synthetic) and the quality of the oil are critical to ensure the safety and efficacy of vaccines. Metabolizable oils are usually safer than mineral oil for fish vaccines, but mineral oils can induce higher antibody titers and can be used to induce stronger cell mediated immunity.

MATERIALS AND METHODS

Montanide™ range of water-in-oil adjuvants (Fig. 3) has been used for fish vaccination worldwide. In particular, Montanide™ ISA 763A VG is a metabolizable oil based water-in-oil adjuvant that has been shown to be safe and highly efficient for injection of diverse fish species, such as salmons, trouts, tilapia, seabass, turbot, catfish, ect. [1-3]. This adjuvant has been used for commercial vaccines formulation in the last decades.

Montanide™ ISA 763A VG is a safe adjuvant that induces only minor reactions after injection. In a safety study for sutchi catfish *Edwardsiella ictaluri* vaccine, Montanide™ ISA 763A VG was formulated with inactivated antigen (10^9 CFU/dose) and 0.1 ml of vaccine was injected to 2x30 catfish of 15 to 30 g. The fish were slaughtered at D21 post injection and local reactions were assessed following Spielberg scoring scale (score 0 (no reaction to score 6 (global adhesion to the organs)). 75% of the fish had score 0 reaction, and no fish showed adhesion above score 1 (Fig. 4).

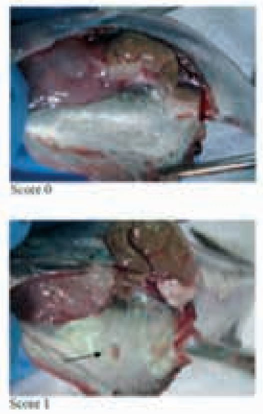
In another study, the use of Montanide™ ISA 763A VG in a turbot vaccine against *Edwardsiella tarda* increased strongly the duration of immune response compared to non adjuvanted vaccine [2]. At 1 month post injection, 100% of fish vaccinated with the adjuvanted vaccine were protected, compared to 80% in the non adjuvanted group. At 6 months post injection, still 90% of fish vaccinated with the adjuvanted vaccine were still protected, compared to 20% only in the non adjuvanted group [2]. These results and others show that the use of adapted water in oil adjuvant is necessary to protect fish on the long term with only one injection.

RESULTS AND DISCUSSION

In order to improve the efficacy of immersion and oral vaccines, dedicated adjuvant formulations have been developed and tested. Adjuvants for immersion vaccines should be aqueous adjuvants that can be added to the immersion bath. Montanide™ IMS adjuvants are aqueous adjuvants composed of a micro-emulsion and containing an immunostimulating compound. It was shown that immersion vaccination against *Yersinia ruckeri* in rainbow trout was improved by the addition of the micro-emulsion adjuvant Montanide™ IMS 1312 VG [5]. This study showed that the vaccine against yersiniosis formulated with Montanide™ IMS 1312 VG induced a strong and long term humoral and cellular immunity, and that the addition of adjuvant allowed reaching above 90% of protection against the disease after challenge, over 10 weeks after vaccination.

Developing efficient oral vaccination for fish would allow mass vaccination of the fish and a strong reduction of the workload necessary for fish vaccination. It would also limit considerably the risks of reactions after vaccination. However, as of today the efficacy of oral vaccination is not sufficient to replace vaccination by injection.

An option to enhance the efficacy of oral inactivated or subunit vaccines would be to improve the formulation of these vaccines. Oral vaccines must be mixed with feed to be administered to the fish. Vaccine can be lost in water and antigen may also be destroyed in the gastrointestinal tract of the fish. Formulations for oral vaccines should thus contain a gastro-protective matrix for



Treatments (volume injected)	Type of local reaction (%)	
	Score 0 (no lesion)	Score 1
Antigen alone 10^9 CFU/ml	98 ± 2	2 ± 2
ISA 763 A VG with 10^9 CFU/ml	77 ± 8	23 ± 8
ISA 760 VG with 10^9 CFU/ml	77 ± 5	23 ± 5
Control (saline buffer)	98 ± 2	2 ± 2

Fig. 4. No lesions above score 1 were observed in adjuvanted groups. Montanide ISA 763A VG is safe for fish vaccination

the antigen, able to stick to fish pellets in water until it has been swallowed by the fish and able to protect the antigen in the acidic part of the fish gastrointestinal tract. Such formulations are being developed and tested to improve oral vaccines.

CONCLUSION

The development of new efficient and safe fish vaccines is necessary to ensure an on-going growth of the aquaculture industry and a reduction of the use of antibiotics and anti-parasitic drugs used in fish farming.

The use of appropriate adjuvants allows the formulation of safe and protective one-shot injectable fish vaccines. The development of more efficient immersion and oral vaccines should allow an efficient mass vaccination of fish in the coming years.

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