Vaccinology in fish

Konstantina Bitchava

DVM, MSC, PhD, EBVS® European College of Aquatic Animal Health (dipECAAH)

Associate Professor

Department of Animal Science, School of Animal Biosciences, Agricultural University of Athens, Greece

Vaccines

- A vaccine is any biologically based preparation intended to establish or to improve immunity to a particular disease or group of diseases

- Bring the animals in contact with a harmless antigen that can elicit this immunological memory so that if an encounter with the real pathogen occurs the responses give protection against the aforementioned pathogen

- An ideal vaccine should be:

  safe – immunogenic

Benefits of vaccination

- Reduction in the use of antibiotics

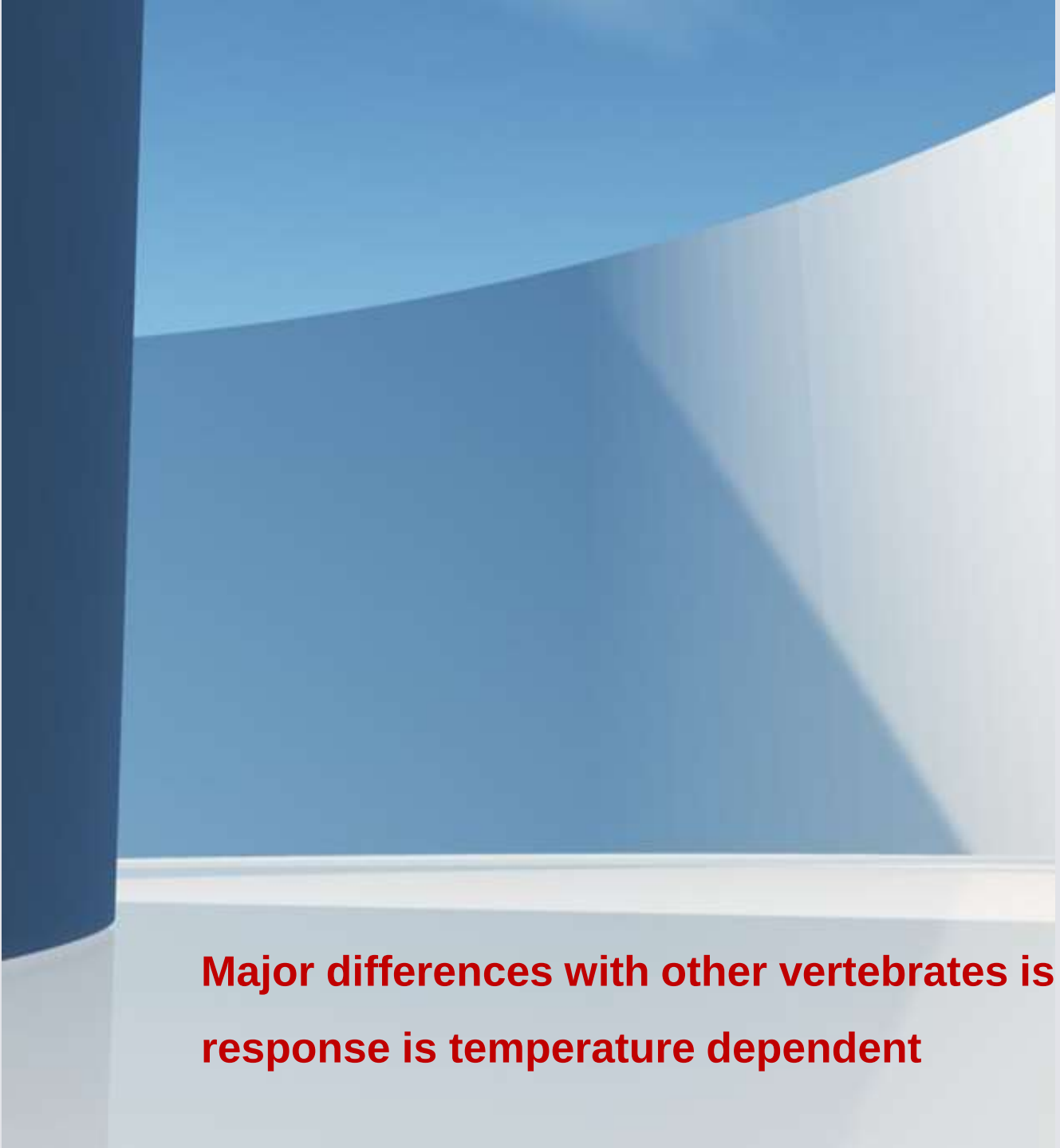
  “Dirty industry”

  Multiple resistance became commonplace

  Economic benefits

- Environmental benefits
- Animal welfare

The initial success of vaccination led to widespread adoption throughout the industry



Immunology of fish

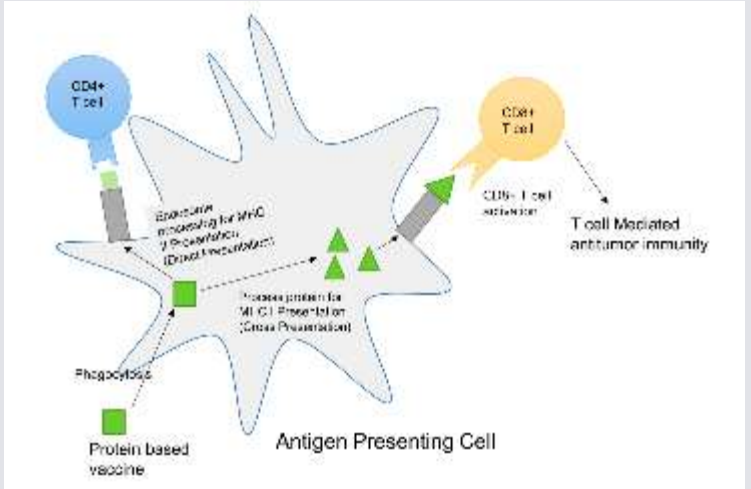
- Innate
- Adaptive immune responses

Major differences with other vertebrates is that their metabolism and immune response is temperature dependent

Adaptive immune responses

- They are responses that are mediated by lymphocytes (T cells and B cells), a particular type of white blood cell (leucocyte) that have special receptors on their surface to detect foreign molecules
- T cells are produced in the thymus and are migrating to other tissue sites to induce responses
- B cells, in teleost fish, are produced in the kidney
- The antigen receptors in T and B cells are formed from different genes and have a distinct structure
- The T-cell antigen receptor (TCR) on most T cells requires antigen presentation, in the form of processed peptides from the original protein, delivered by major histocompatibility complex (MHC) molecules
- B cells, on the other hand, can recognise soluble antigens and bind to them directly via their B-cell antigen receptor (BCR)

- Effective vaccines require efficient antigen recognition and presentation by the host immune cells
- This is performed through MHC molecules
- The MHC is a set of cell-surface proteins encoded by a family of genes that show a high degree of polymorphism between individuals
- This is one of the many genetic mechanisms that govern differential disease resistance within a species



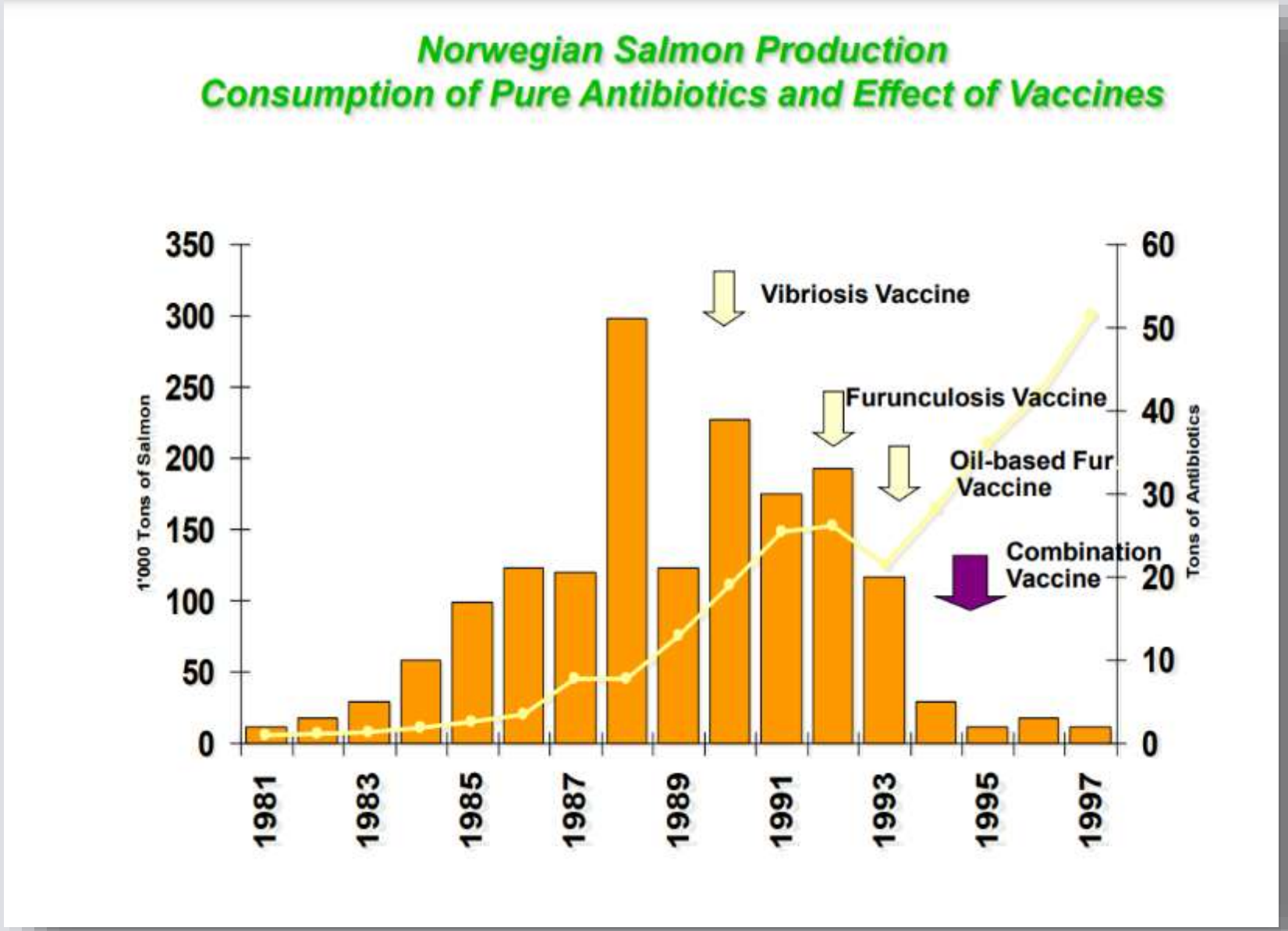
Source: PapiVax Biotech

Sustainable aquaculture

- Not possible without disease prevention
- Vaccination has become the single most important tool
- There has been a dramatic reduction in the use of antibiotics in Norwegian salmon farming since the introduction of oil-based vaccines

The situation in Norway

- In 1980, the total sales of antimicrobial agents for therapeutic use in farmed fish was very high
- Quinolones accounted for 69%
- The sales of antimicrobials declined by approximately 99% from 1987 – 1996
- Have thereafter remained low
- This reduction is mainly attributed to the introduction of effective vaccines in salmonids



Source: Tethys Aquaculture

In the salmon industry

- oil-adjuvanted, injectable vaccines
- multivalent and can contain as many as seven different antigens

e.g. Combined Vibriosis/Furunculosis/Coldwater Vibriosis/Moritella viscosa/IPNV vaccine

1982

1. **Enteric Redmouth (ERM) vaccine**
2. ***Vibrio anguillarum* vaccine**

Commercially-Available Fish Vaccines

Nowadays

1 Enteric Redmouth (ERM) vaccine
2 Vibrio anguillarum vaccine
3 Furunculosis vaccine
4 Vibrio salmonicida vaccine
5 Combined Vibriosis/Furunculosis vaccine
6 Combined Vibriosis/Furunculosis/Coldwater Vibriosis/*Moritella viscosa* vaccine
7 Combined Vibriosis/Furunculosis/Coldwater Vibriosis/*Moritella viscosa*/IPNV vaccine
8 IPN Virus vaccine
9 Pasteurella vaccine
10 Combined Pasteurella/Vibriosis vaccine
11 Vibriosis vaccine for cod
12 Shrimp Vibriosis vaccine
13 Warmwater Vibrio spp vaccine
14 SVC virus vaccine
15 *Lactococcus garvieae/Streptococcus iniae* vaccine
16 KHV vaccine
17 Aeromonas hydrophila vaccine
18 Carp Erythrodermatitis/Ulcer disease vaccine
19 *Piscirickettsia salmonis* vaccine
20 ISA virus vaccine
21 Gaffkaemia vaccine
22 *Flavobacterium psychrophilum* vaccine
23 Nodavirus vaccine
24 Pancreas disease virus vaccine
25 *Edwardsiella ictaluri* vaccine
TOTAL = 25 +

ALPHA DIP 2000	55242/24-08-2007	PHARMAQ AS	Sea bass	Inactivated bacterial species Listonella anguillarum (01) and Photobacterium damsela subsp. piscicida.	Designed exclusively for the protection of farmed sea bass from vibriosis that is caused by Listonella anguillarum (serotype 01) and pasteurellosis.
ALPHA DIP VIB	79093/04-08-2021	PHARMAQ AS	Sea bass	Listonella anguillarum Serotype 01 (Vibrio anguillarum) inactivated	For active immunization of sea bass in order to reduce mortality and clinical signs caused by infection by Listonella anguillarum) serotype O1 (vibriosis).
ALPHA DIP VIBRIO	86911/31-03-2020	PHARMAQ AS	Sea bass	Listonella anguillarum (Vibrio anguillarum) inactivated	For active immunization of sea bass in order to reduce mortality and clinical signs caused by infection by Listonella anguillarum) serotype O1 (vibriosis).
ALPHA JECT 2000	55244/24-08-07	PHARMAQ AS	Sea bass	Contains a non-mineral adjuvant and the inactivated bacterial species Listonella anguillarum (01) and Photobacterium damsela	Designed exclusively for the protection of farmed sea bass from vibriosis that is caused by Listonella anguillarum (serotype 01) and pasteurellosis.
ALPHA JECT MICRO 1 NODA	108576/01-12-17	PHARMAQ AS	Sea bass	Inactivated culture of: RED-SPOTTED GROUPER NERVOUS NECROSIS VIRUS (RGNNV), AL V1107	For active immunization of sea bass in order to reduce mortality caused by virus infection Red-spotted Grouper Nervous Necrosis (RGNNV)
AQUAVAC ERM.	36524/19-05-11	INTERVET HELLAS	Rainbow Trout (Oncorhynchus mykiss)	Inactivated cells Yersinia ruckeri	In Rainbow Trout of 2 grams weight or over: Active immunization against Enteric Redmouth disease (ERM) to reduce mortality caused by the Hagerman Type I strain of Yersinia ruckeri.
AQUAVAC ERM. ORAL	14042/29-02-12	INTERVET HELLAS	Rainbow Trout (Oncorhynchus mykiss)	Inactivated cells Yersinia ruckeri	Inactivated, immersion vaccine against Enteric Redmouth Disease caused by Yersinia ruckeri (Hagerman strain) in rainbow trout (Oncorhynchus mykiss).
AQUAVAC PHOTOBAC BOOST	24504/03-04-09	INTERVET HELLAS	Sea bass (Dicentrarchus labrax)	Inactivated cells of Photobacterium damsela sub-species piscicida (strain Pr85)	As an aid in the prevention of pasteurellosis caused by Photobacterium damsela sub-species piscicida.
AQUAVAC VIBRIO CS.INJ.SOL	18127/21-03-2008	INTERVET HELLAS	Rainbow Trout (Oncorhynchus mykiss)	Inactivated cells of Listonella (Vibrio) anguillarum strain 78 -SKID 75% and Inactivated cells of Listonella (Vibrio) ordalii strain MSC 275	Active immunisation to reduce mortality caused by vibriosis due to Listonella (Vibrio) anguillsrum and Vibrio ordalii in rainbow trout (Oncorhynkus mykiss)
AQUAVAC PHOTOBAC PRIME	26703/03-04-09	INTERVET HELLAS	Sea bass, sea bream	Inactivated cells of Photobacterium damsela sub-species piscicida	Inactivated, immersion vaccine against Pasteurellosis caused by Photobacterium damsella sub-species piscicida in European Sea bass (Dicentrarchus labrax) and Sea bream (Sparus aurata)
AQUAVAC VIBRIO DIP.SUSP	18127/26-01-1995	INTERVET HELLAS	Eels Trout Sea bass Sea bream	Inactivated cells of Listonella (vibrio) anguillarum (serotype I) Listonella (vibrio) anguillarum (serotype II)	For the active immunisation of fish to reduce mortality due to vibriosis caused by Listonella (Vibrio) anguillarum and Vibrio ordalii.
AQUAVAC VIBRIO ORAL	16217/13-03-08	INTERVET HELLAS	Trout and other fishes	Inactivated cells of Listonella (vibrio) anguillarum (serotype I) Listonella (vibrio) anguillarum (serotype II)	Inactivated, oral vaccine for the prevention of vibriosis caused by Vibrio anguilarum 01 and 02a (Vibrio ordalii) in rainbow trout (Oncorhynkus mykiss) and European sea bass (Dicentrarchus labrax).
AQUAVAC VIBRIO PASTEURELLA	25454/17-04-06	INTERVET HELLAS	Sea bass	Inactivated cultures of Vibrio anguillarum and Photobacterium damsela	Established bivalent vaccine used by farmers needing to protect against both pasteuerellosis and vibriosis in a single vaccination.
ICTHIOVAC LG LACTOCOCOSIS-TRUCA	58324/22-07-16	LABORATORIOS HIPRA S.A.	Trout (Oncorhynchus mykiss)	Inactivated Lactococcus garvieae	For active immunization of trouts to reduce mortality caused by infection by Lactococcus garvieae.
ICTHIOVAC - PD	24996/19/10/2020	LABORATORIOS HIPRA S.A.	Sea bream	Inactivated Photobacterium damselae subsp. piscicida DI 21, Inactivated Photobacterium damselae subsp. piscicida It1	For the active immunisation of gilthead sea bream to reduce the mortality caused by infection by Photobacterium damselae subsp. piscicida (pasteurellosis)
ICTHIOVAC STR.	88496/09/08-12-10	LABORATORIOS HIPRA S.A	Fish (scophthalmus maximus/psetta	Streptococcus parauberis inactivated, and Streptococcus parauberis inactivated.	For active turbot immunization (scophthalmus maximus / Psetta maxima), to reduce mortality associated with Streptococcus parauberis infection.
ICTHIOVAC VNN	70902/06-06-2019	LABORATORIOS HIPRA S.A	Sea Bass	Inactivated Betanodavirus	For active immunisation of sea bass to reduce the mortality due to infection by Betanodavirus Viral Necrosis.
ICTHIOVAC VR/PD	45403/16/16-08-17	LABORATORIOS HIPRA S.A	Sea bass	Listonella anguillarum, Photobacterium damsela	For the active immunisation of sea bass to reduce the mortality caused by infection by Photobacterium damselae subsp. piscicida (pasteurellosis) and by infection by Listonella anguillarum serotypes O1, O2a and O2? Onset of immunity 42 days after vaccination at 19 - 21°C.
ICTHIOVAC VR	128998/ 09-12-2020	LABORATORIOS HIPRA S.A	Sea bass (Dicentrarchus labrax) Turbot (Scopthalmu	Inactivated Vibrio (Listonella) anguillarum serotype O1 Inactivated Vibrio (Listonella) anguillarum serotype O2α Inactivated Vibrio (Listonella) anguillarum	For the active immunisation of sea bass and turbot to reduce mortality produced by Vibrio (Listonella) anguillarum (Serotypes O1, O2α and O2β).
MARIMARK N	46420/02-06-2021	BENCHMARK ANIMAL HEALTH LTD	Sea bass (Dicentrarchus labrax)	Inactivated red-spotter grouper nervous necrosis virus (RGNNV) strain K13.1	For the active immunisation of sea bass to reduce mortality caused by the Red-spotter Grouper Nervous Necrosis (RGNNV) caused by Betanodavirus
VIBRI-FISHVAX	40803/19-06-08	FATRO HELLAS	Sea bream Sea bass Trout	Inactivated cells of Listonella (vibrio) anguillarum (serotype I) Listonella (vibrio) anguillarum (serotype II)	For active immunisation of trouts to reduce the mortality due to infection caused by Listonella (vibrio) anguillarum
YERSI-FISHVAX	40796/19-06-08	FATRO HELLAS	Trout, salmonids	Inactivated cells Yersinia ruckeri	Active immunization against Red Mouth Disease caused by Yersinia ruckeri.

Vaccines in Greece

Vaccines in Italy

ICTHIOVAC VR/PD, emulsion injectable for Sea bass	LABORATORIOS HIPRA S.A.	Sea bass (Dicentrarchus labrax)	Inactivated Photobacterium damselae subsp. piscicida DI 21; Inactivated Listonella anguillarum serotype 01; Inactivated Listonella anguillarum serotype 02α; Inactivated Listonella anguillarum serotype 02β	For the active immunisation of sea bass to reduce the mortality caused by infection by Photobacterium damselae subsp. piscicida (pasteurellosis) and by infection by Listonella anguillarum serotypes O1, O2α and O2β.
ICTHIOVAC-LG LACTOCOCOSIS TRUCHA Inactivated vaccine in injectable emulsion for trout	LABORATORIOS HIPRA S.A.	Trout (Oncorhynchus mykiss)	Lactococcus garvieae	For active immunisation of trouts to reduce mortality due to infection by Lactococcus garvieae.
ICTHIOVAC-PD PASTORELLOSI ORATA Concentrate for suspension for dipping for sea bream	LABORATORIOS HIPRA S.A.	Sea bream	Photobacterium damselae subsp. piscicida	For active immunisation of sea bream to reduce the mortality due to infection by Photobacterium damselae, subsp. piscicida (Pasteurellosis).
ICTHIOVAC VNN, emulsione iniettabile per spigole	LABORATORIOS HIPRA S.A.	Sea Bass	Inactivate Betanodavirus subsp. 1103	For active immunisation of sea bass to reduce the mortality due to infection by Betanodavirus Viral Necrosis.
VIBRI-FISHVAX Inactivated vaccine in suspension for dip or injectable for trout, salmonids, sea bass and sea bream	FATRO S.P.A.	Trout (Oncorhynchus mykiss) and salmonids, sea bass (Dicentrarchus labrax), Sea bream (Sparus aurata)	Listonella anguillarum antigen; Vibrio ordalii antigen	Vaccination against Vibriosis. To reduce mortality, clinical sign and vibriosis injuries caused by L. anguillarum and V.ordalii
YERSI-FISHVAX Inactivated vaccine suspension for dip or injectable	FATRO S.P.A.	Trout and Salmonids	Inactivated Yersinia ruckeri PRS > 70	Immunisation against Redmouth
ALPHA DIP Vib concentrate for dip suspension, vaccine for sea bass	PHARMAQ AS	Seabass (Dicentrarchus labrax L)	Inactivated Listonella anguillarum (Vibrio anguillarum) serotype O1, strain AL 112, RPS ≥ 75	For active immunisation of sea bass to reduce mortality and clinical signs caused by infection with Listonella anguillarum) serotype O1 (vibriosis).
ALPHA JECT micro 1 Noda, emulsion for injection for seabass	PHARMAQ AS	Seabass (Dicentrarchus labrax L)	Inactivated Red-spotted Grouper Nervous Necrosis Virus (RGNNV) strain ALV1107 ≥ 0.07 antigenicity units	For active immunisation of sea bass to reduce mortality caused by Red-spotted Grouper Nervous Necrosis Virus (RGNNV).
Aqua Vibrio oral	MSD ANIMAL HEALTH S.R.L.	Rainbow trout (Oncorhynchus mykiss)	Listonella (Vibrio) anguillarum inactivated cells strain 78-SKID: Vibrio ordalii inactivated cells strain	For Rainbow Trout of 2 grams weight or over: For the active immunisation of fish to reduce mortality due to vibriosis caused by Listonella (Vibrio) anguillarum and Vibrio ordalii.

AquaVac ERM Concentrate for Dip Suspension for Rainbow Trout	MSD ANIMAL HEALTH S.R.L.	Rainbow trout (Oncorhynchus mykiss)	Yersinia ruckeri inactivated cells (Hagerman type I strain)	In Rainbow Trout of 2 grams weight or over: Active immunization against Enteric Redmouth disease (ERM) to reduce mortality caused by the Hagerman Type I strain of Yersinia ruckeri. 336 degree days are required for the development of full immunity (28 days at a water temperature of 12°C). The time for development of protective immunity will depend on water temperature.
AquaVac ERM orale Oral emulsion for rainbow trouts	MSD ANIMAL HEALTH S.R.L.	Rainbow trout (Oncorhynchus mykiss)	Yersinia ruckeri inactivated cells (Hagerman type 1 strain).	Active immunization of rainbow trout, 26 g and above against Enteric Redmouth disease (ERM) to reduce mortality caused by the Hagerman Type I strain (serotype 01) of Yersinia ruckeri. The vaccine is indicated for use in fish that have been vaccinated by immersion with AquaVac ERM within the previous 4 to 6 months.
AquaVac Relera Concentrate for Dip Suspension or Suspension for Injection for Rainbow Trout	MSD ANIMAL HEALTH S.R.L.	Rainbow trout (Oncorhynchus mykiss)	Yersinia ruckeri inactivated cells (strain Hagerman type 1), Yersinia ruckeri, inactiveted cells (EX5 biotype strain)	Active immunization against Enteric Redmouth disease (ERM) to reduce mortality caused by Hagerman type 1 and EX5 biotype strains of Yersinia ruckeri
AquaVac Vibrio Immersion et Injection	MSD ANIMAL HEALTH S.R.L.	Rainbow trout (Oncorhynchus mykiss)	Listonella (Vibrio) anguillarum inactivated cells strain 78-SKID: RPS60(*) > 75%, Vibrio ordalii inactivated cells strain MSC 275: RPS60(*) > 75%	For Rainbow Trout, from 2 grams weight by immersion or 6 grams weight by injection: For the active immunisation of fish to reduce mortality due to vibriosis caused by Listonella (Vibrio) anguillarum and Vibrio ordalii.
Aquavac Vibrio vab, Immunisationsolution for dip for salmonids, eels, sea bass, sea bream and turbot	MSD ANIMAL HEALTH S.R.L.	Salmonids, Eels, sea bass, sea bream and turbot	V. anguillarum (78-SKID) inactivated ? 60% RPS*, V. anguillarum (MSC 275) inactivated ≥ 60% RPS*	Vaccination of fish against vibrosis, belonging to the following species: Salmonids, Eels (Anguilla anguilla), sea bass (Dicentrarchus labrax), sea bream (Sparus aurata), turbot (Scophtalmus maximus)
BI-FISHVAX Inactivated vaccine in suspension for dip or injectable	FATRO S.P.A.	Trout (Oncorhynchus mykiss)	Listonella anguillarum antigen, Yersinia ruckeri antigen	Active immunisation of trout and salmonids against Redmouth disease and Vibriosis.

Research prospects

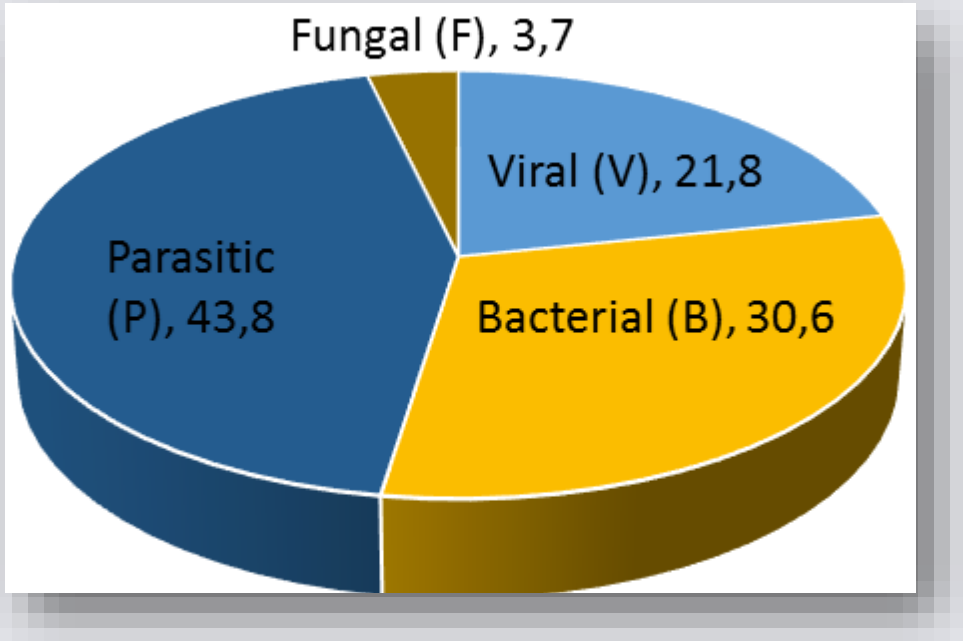
- European One Health Action Plan against Antimicrobial Resistance
- Vaccine development for bacteria
- Some commercial vaccines exist for the fish species reared in the Mediterranean area
- They do not cover the spectrum of emerging diseases that cause important losses in the area

Aquaculture:
the first export of Greece

- Fish aquaculture in marine and freshwater environment is by far the most important part of the sector (88% of the volume and 99% of the production value)
- 113.000 tonnes of seabream and sea bass and together with Turkey (125.000) cover the 66% of the world production for these species

Fish Diseases

- Among the most important threats of this fast-growing sector are the economic losses due to the presence of diseases
- They cause important mortalities to the fish population



Bacterial Diseases

- An emerging problem is the presence of myxobacteria (*Tenecibaculum maritimum*) in juvenile sea bass and sea bream
- Also, the presence of different vibrio strains compared with that in vaccines
- Only three commercial vaccines for *Vibrio anguillarum, Photobacterium damsealae* subsp*. piscicida and Lactococcosis*
- *Photobacterium damselae* subsp*. damselae*, Aeromonas spp.

- New vibrio strains to cover the gap
- *Vibrio harveyi*
- *Vibrio vulnificus*
- *Vibrio alginolyticus*
- *Photobacterium damselae* subsp*. damselae*

Commercial and autogenous vaccines

Three trends for the development of vaccines

1. *The Mode of delivery*
2. *The Nature of the antigen* (this cover classical inactivated bacterial or viral vaccines)
3. *Recombinant technologies* (purified or designed subunit, protein-based vaccines are used)

Routes of administration

- **Injection** – **Intraperitoneal (adjuvanted or not)**
  - ***– Intramuscular (DNA)***
- **Immersion** – ***Bath method***
  - ***– Dip method***
  - ***– Spray vaccination***
- **Oral delivery** – ***Micro -encapsulation method***
  - ***– Bio -encapsulation method***

- Fish are transported in pipes from the rearing tanks to an anaesthetic bath
- Anaesthetized fish are injected by the vaccination team

Source: Vetcare.gr

Different vaccination pistols

Source: Zoetis (left), The fish site (right)

Automated systems

- Fully automatic vaccination machine is operated by a single operator
- Can vaccinate and grade up to 40 000 smolt

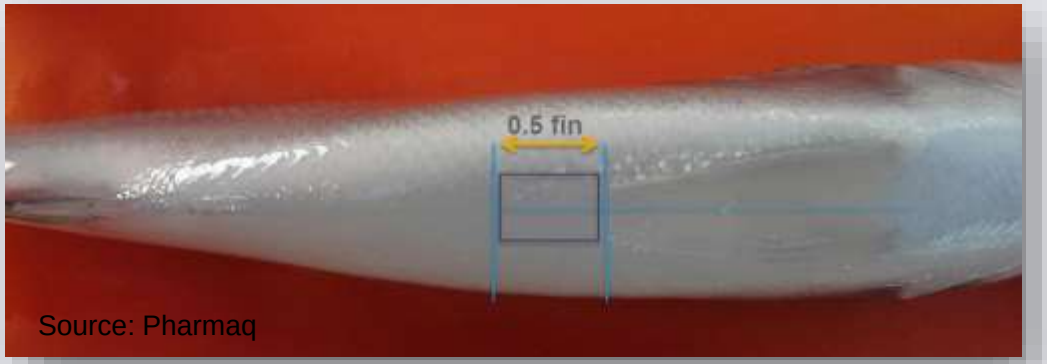
  per hour
- The machines can vaccinate single, double,

  triple and intramuscular doses

  simultaneously

Advantages & Disadvantages of Injection vaccination

- Most common method of vaccine delivery in fish
- Highly efficient in generating both humoral (antibody) and cellular cytotoxic response
- Unsuitable for small fish
- Needs automated machines or skilled teams
- Significant handling stress and risk of post vaccination infections
- Local reactions

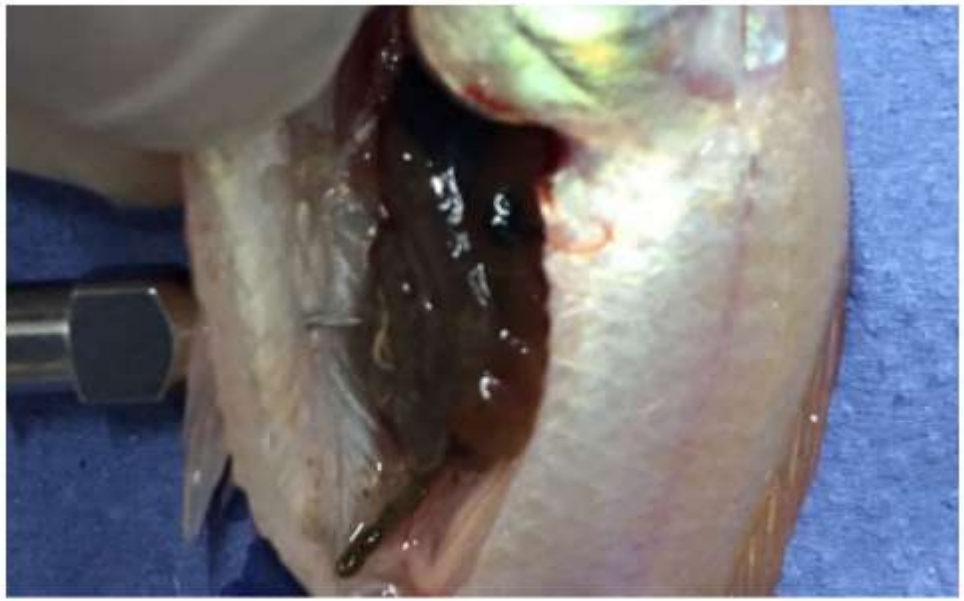


Source: Pharmaq

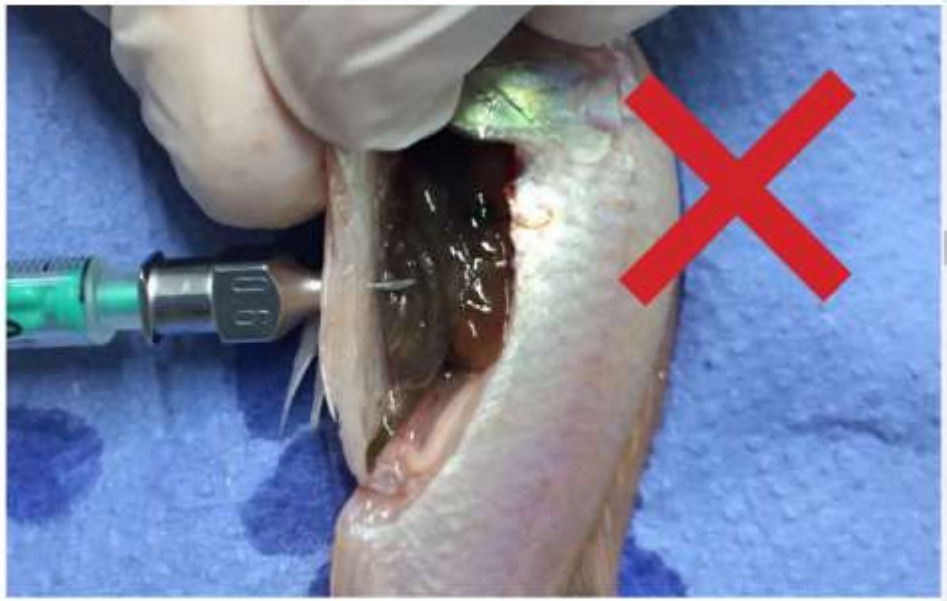
Mistakes during vaccination

- Excess pressure in the puncture sight
- Wrong timing
- wrong injection site, wound in the muscle
- wrong injection site, wound in the intestine or other internal organ
- incorrect needle use or wrong angle of vaccination

Source: Pharmaq



Picture 11: Correct needle length, with the whole bevel within the peritoneum



Picture 12: Long needle may give damage to internal organs

Source: Pharmaq

Intensity scale of side effects in European sea bass (*Dicentrarchus labrax*) post intraperitoneal injection with commercial oil-adjuvanted vaccines.

Source: Tziouvas, H., & Varvarigos, P. (2021)

Score	Description of intra-abdominal lesions	
0	No adhesions, no peritoneal lesions noted. Since some adhesions are always expected post-injection vaccination, fish with 0 score should be considered as non-injected by mistake.	
1	Soft, localised peritoneal adhesions of the visceral peritoneum hymens wrapped around the organs, affecting mostly the intestine and occasionally other organs, but not affecting the swim bladder and the parietal peritoneum lining the inner surface of the abdominal wall. Organs separate easily with minor force. Peritoneal nodules or granulomatous lesions are not visible. Usually, score 1 sea bass are found soon after injection.	
2	Soft, localised peritoneal adhesions of the visceral peritoneum. May affect the stomach, liver, mesentery, intestine and occasionally the spleen, but do not affect the swim bladder and the parietal peritoneum. Organs separate easily with minor force. Presence of few small, pin point, mostly non-pigmented (creamy coloured) peritoneal granulomatous lesions in the form of tiny nodules.	
3	Soft but widespread adhesions of the visceral peritoneum including the stomach, liver, mesentery, intestine and spleen that, however, do not affect the swim bladder and the parietal peritoneum. Organs separate relatively easy. Presence of many small, pin point, peritoneal nodules, rarely >1 mm in diameter, mostly non-pigmented (creamy coloured).	
4	Soft to moderately hard widespread adhesions of the visceral peritoneum including the stomach, liver, mesentery, intestine and spleen, but also affecting the swim bladder and the parietal peritoneum on the abdominal wall, appearing as fibrous connective tissue threads. Organs separate relatively easy with moderate force, without danger of rupture. Presence of many peritoneal nodules of variable sizes, mostly small pin point, but also larger >2 mm in diameter, in the majority non-pigmented (creamy coloured), but some pigmented (orange, grey, brownish to black).	
5	Moderately hard widespread adhesions of the visceral peritoneum including the stomach, liver, mesentery, intestine and spleen, also affecting the swim bladder and the parietal peritoneum. Moderate force is needed to separate the organs with possibility of organ rupture. Presence of many peritoneal nodules of variable sizes, ranging from pin point to >4 mm in diameter, non-pigmented (creamy coloured), but also pigmented (orange, grey, brownish to black).	
6	As for score 5 but lesions are even more pronounced with strong adhesions attaching the visceral and the parietal peritoneum, granulomatous peritoneal lesions ranging from numerous small but also many large >8mm pigmented or not nodules. Organs may rupture when their separation is attempted. No lesions or pigmentation on the fillet. Adverse effects on growth is deemed possible.	 

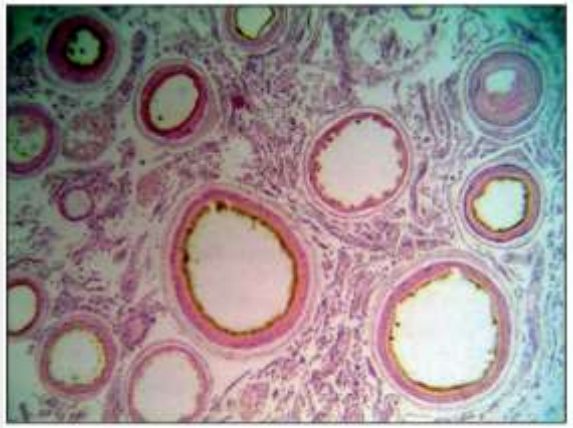


Figure 4. Histology (HE stain, 100X magnification) of peritoneal nodules 2800 DD post i.p. injection with a commercial mineral oil adjuvanted divalent vaccine against *Vibrio anguillarum* O1 and *Photobacterium damselae* subsp. *piscicida*. The nodule contents, stained brown, comprise the remnants of mineral oil adjuvanted vaccine, which has been mostly removed during the process of fixation and staining. The inner and external layers of connective tissue forming the nodules differ in thickness, presumably in part depending on the section cut.

Source: Tziouvas & Varvarigos

Consequences of local reactions

- Reduced growth
- Increased feed conversion ratio
- Problems at processing
- Welfare issue

Depending on adjuvant

- On temperature
- On dose volume
- On size of fish

Source: Pharmaq (left), The fish site (right)

Advantages & Disadvantages of immersion

- Suitable for mass vaccination of all sizes of fish
- Reduced stress for fish
- Lower labour costs
- Less risk to vaccination team
- Major **disadvantages** are the large amount of vaccine required and lower level of protection and duration of immunity

Advantages & Disadvantages of Oral vaccination

- Vaccine mixed with feed
- Easiest method for mass vaccination of all fish sizes
- Saves labour and avoids stress
- Large quantities of antigen required
- Requires all fish to be fed
- Week protection and of short duration

Which method to use?

SIZE OF FISH

>1GR

DURATION OF PROTECTION REQUIRED

TYPE OF PATHOGEN

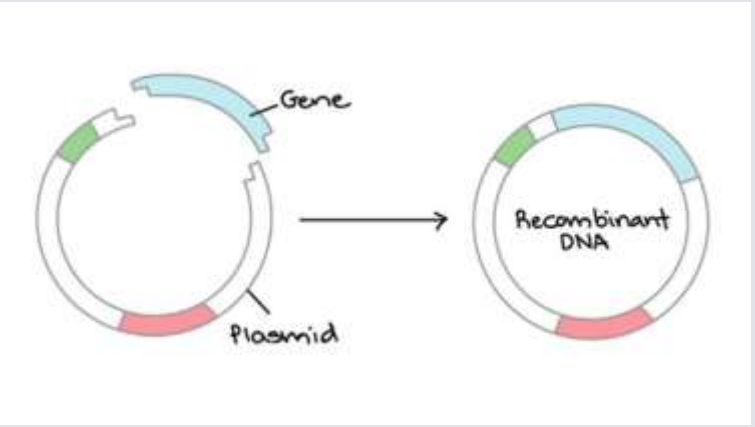
COST OF VACCINE

The nature of the antigens

- The majority of vaccines currently available are prepared by conventional methods (developed by Louis Pasteur 100 years ago)
- A suspension-based fermentation of bacteria or virus harvested from cell culture
- Inactivation methods typically include the use of formalin or alkylating compounds
- Then they are followed by either filtration (“washing”), concentration of antigens or purification of antigen preparations

Recombinant- DNA based vaccine

- Recombinant DNA: any artificially created DNA molecule which brings together DNA sequences from different sources
- Molecular cloning: the propagation of recombinant DNA inside a particular host cell so that many copies of the same sequence are produced
- Allows the genetic modification (genetic engineering) of any host organism and the expression of recombinant gene products (proteins)



Video show
Steps in gene cloning
https://www.youtube.com/watch?v=cVKsVnE3rYU

- *Escherichia coli* strains are used as competent cells for production of antigen at the end of the fermentation cycle.
- A classical example in fish vaccinology is the *E. coli* -based subunit vaccine against infectious pancreatic necrosis in Atlantic salmon.
- Recombinant vaccines have also been produced in *Saccharomyces cerevisiae*.
- Other vectors may also be used for production of recombinant vaccines, like silkworms, cabbageworms, plants and insect cells.
- Plant-based vaccines are referred to as molecular farming where whole plants or plant cells/tissues are cultured in vitro for the production of recombinant proteins.
- There are currently no commercial vaccines in the market.

Recombinant Fish Vaccines

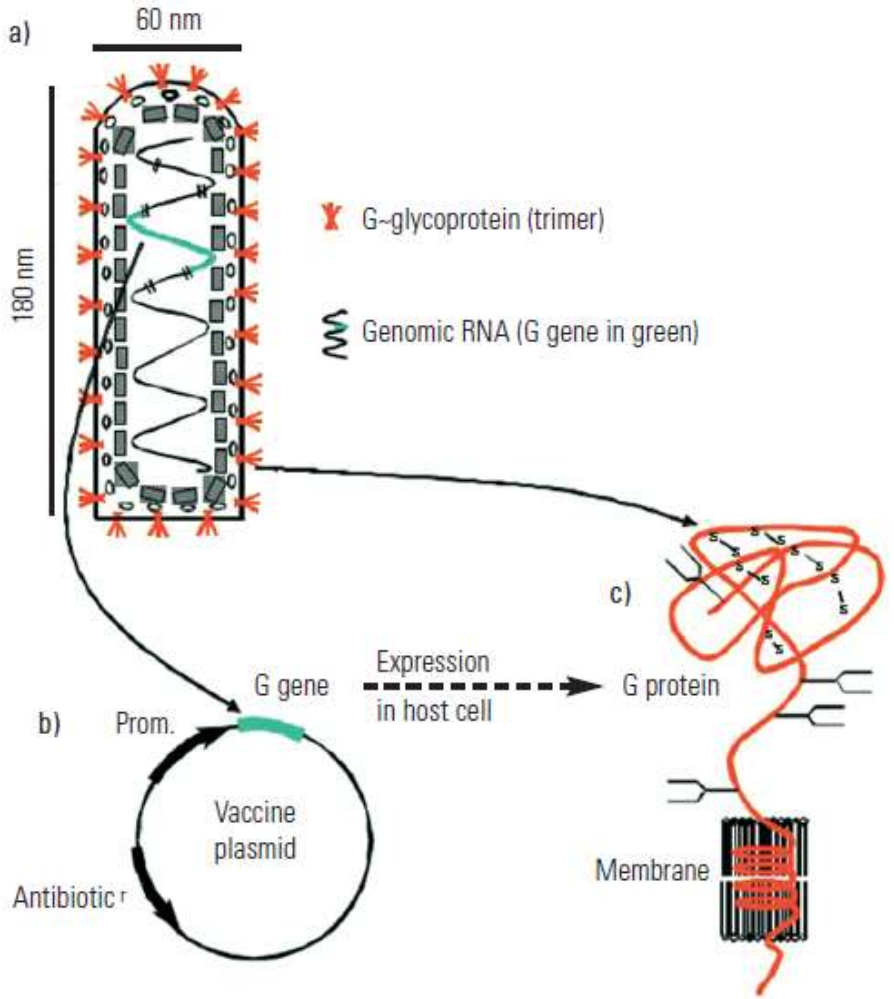
Viral: Channel Catfish Virus (CCV)

- Infectious Haematopoietic Necrosis (IHN) Virus
- Infectious Pancreatic Necrosis (IPN) Virus
- Spring Viraemia of Carp (SVC) Virus
- Viral Haemorrhagic Septicaemia (VHS)
- Infectious Salmon Anaemia (ISA)
- Whitespot Virus of Shrimp (WSV)

Bacterial: Bacterial Kidney Disease (BKD)

- Piscirickettsia salmonis (SRS)

Parasitic: *Ichthyophthirius multifiliis* (“Ich”) and *Lepeophthirius salmonis* (Salmon louse)



In the vaccine plasmid, the eukaryotic promoter (Prom.), antibiotic resistance selection marker (Antibiotic) and the inserted fish virus glycoprotein gene (G gene) are indicated (b). The G protein is a transmembrane molecule with oligosaccharide side chains ⊃— and stabilised by disulphide bonds (s—s) (c). The G protein appears on the surface of virus infected cells as well as on the surface of virus particles. Once the vaccine plasmid has reached the nucleus of a cell in the vaccinated fish, expression of G protein will be initiated and G protein molecules will appear inside the cell and on the cell's surface, as if the cell had been naturally infected with virus (52)

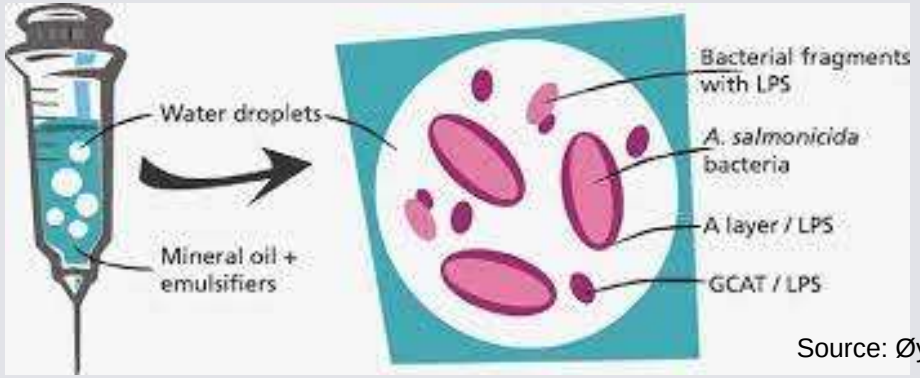
Source: Lorenzen & LaPatra

DNA vaccines

- Use the animal to "produce" the antigen via injection of plasmids encoding defined antigenic parts of the pathogen.
- Utilize the machinery of the cell to produce the G protein.
- Simple IM injection of purified plasmid DNA in a neutral buffer has proven to be more efficient in fish than in any other type of animal tested to date.

Adjuvants and how they work

- The mechanisms of adjuvanticity are complex and not fully understood.
- Adjuvants facilitate delivery of antigen (to the secondary lymphoid organs)
- Increase the immune response to a given antigen
- Prolong the immune responses, the latter being the depot effect
- It is conceived as particularly important for fish for long-term immune protection
- Fish vaccines for parenteral delivery formulated with an adjuvant are typically a water-in-oil formulation



Source: Øystein Evensen

Picture 1: Always store the vaccine in a cooler between 2-8˚C

Picture 2

Picture 3

Picture 4

Picture 2 and 4: Normal appearance of vaccine. Picture 2: Homogeneous. Picture 4: Oily layer on top, must be shaken before use. Picture 3: Separated vaccine with clear water droplets; DO NOT USE

Source: Pharmaq

- Oils used are either of vegetable or mineral origin.
- Montanide: Mineral oil adjuvants registered under the trademark of Montanide by Seppic have been optimised in order to improve efficacy and stability of vaccine formulations and to reduce side effects.
- These adjuvants are based on either mineral oil, nonmineral oil or a mixture of both.

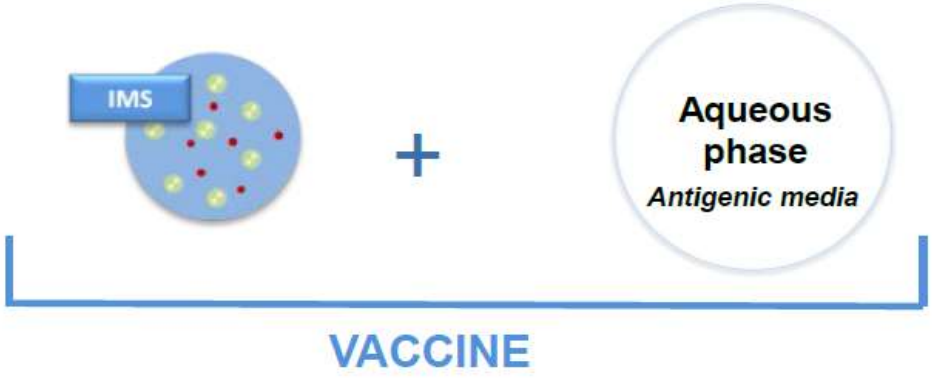
To manufacture 100 g of vaccine: Adjuvant 70 g - Aqueous antigenic medium: 30 g



Source: Seppic

Adjuvants for immersion fish vaccines

MICROEMULSIONS:
Montanide™ IMS 1312 VG



VACCINE FORMULATION:

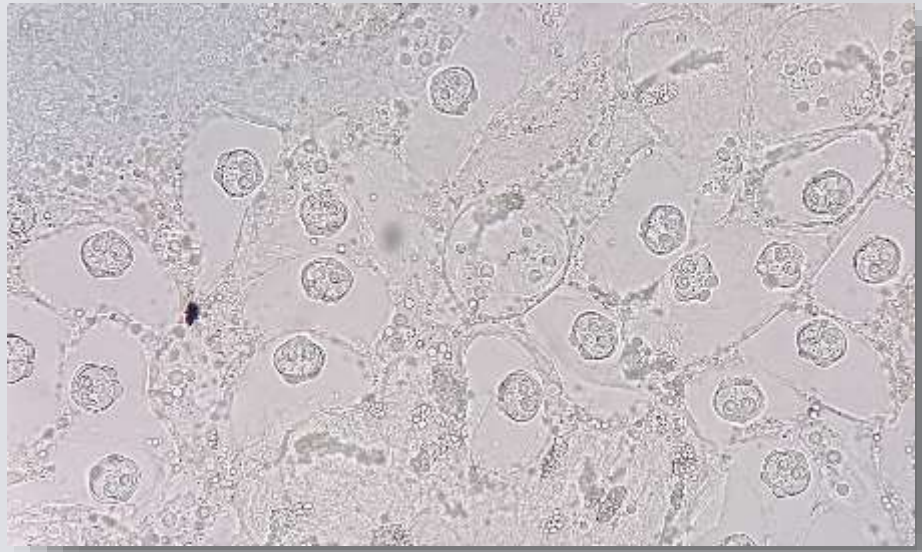
- Mix of Montanide IMS / Antigenic media (50%v / 50%v) at a low shear rate, at room temperature
- Immersion protocol: adjuvant used at 5-10% in final bath (5 to 10 times dilution of the vaccine in the immersion bath)

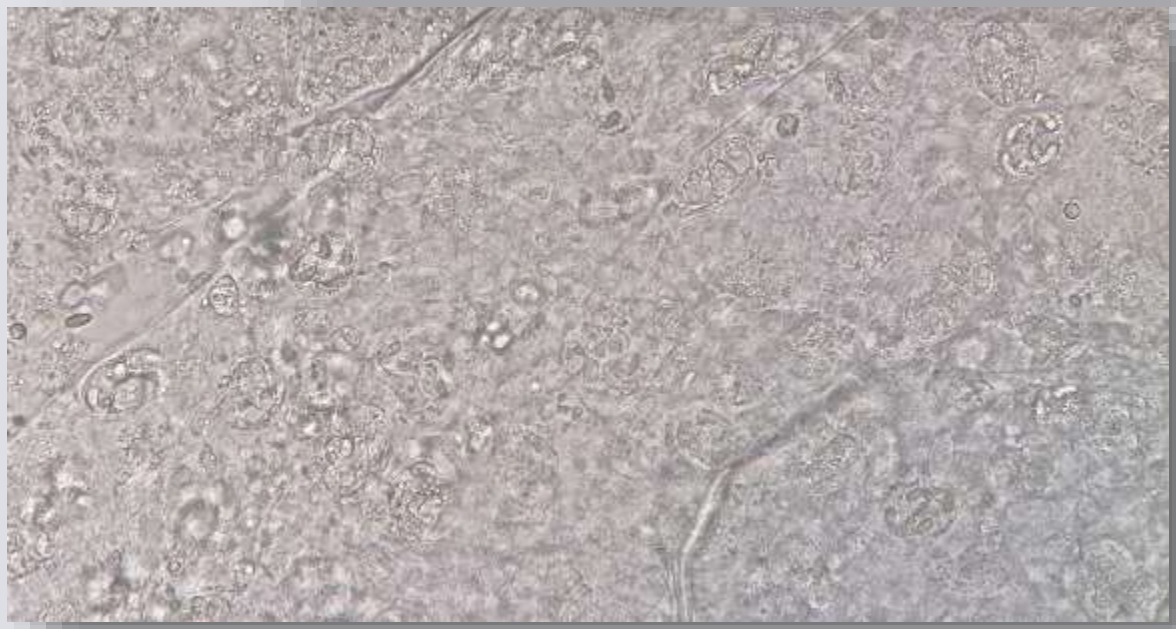
SEPPIC

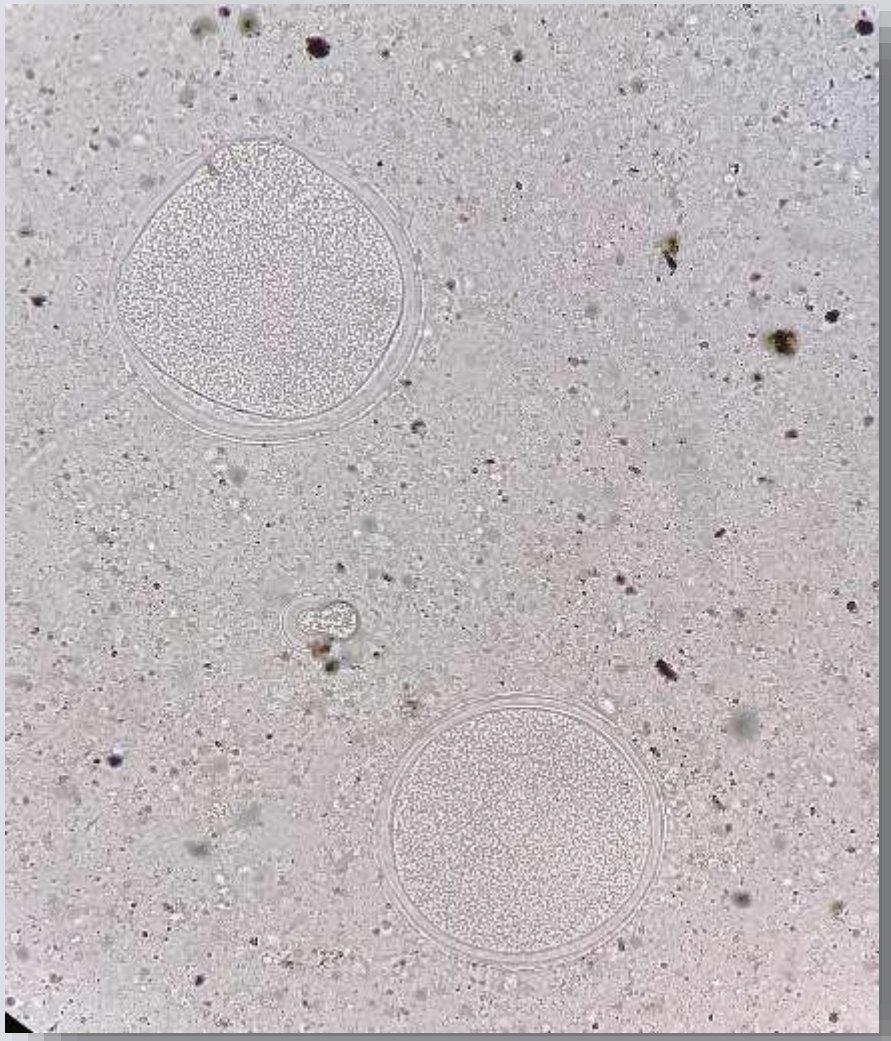
Adjuvanted vaccine reached above 90% of protection against the disease after challenge, over 10 weeks after vaccination.

Parasitic in Sea bass

Parasitic in Sea bream







Fungus

Proposed References

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SUPER TROUT

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