Norwegian University of Life Sciences

Fish Vaccination Strategies

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Content of presentation

- Definitions
- Vaccination in disease control
- Fish vaccines – current use and impact
- Type of vaccines currently used
- Vaccination modalities for fish
- Salmonids versus other farmed species
- Contrast inactivated to live vaccines
- New technologies - examples
- Conclusions – sum up

Vaccination - definition

- Vaccination is
  - the process of administering **weakened or dead** pathogens to a healthy individual
  - with the intent of conferring immunity against a targeted form of a related disease agent

The origin of vaccination

- Edward Jenner (1749-1823)
  - Discovered that vaccination of people with cowpox (virus) will protect against smallpox
  - Vaccination (Latin: vacca—cow) - the first vaccine was derived from a virus affecting cows

Milestones in the early history of vaccination

1882 — Louis Pasteur discovers vaccine against Anthrax

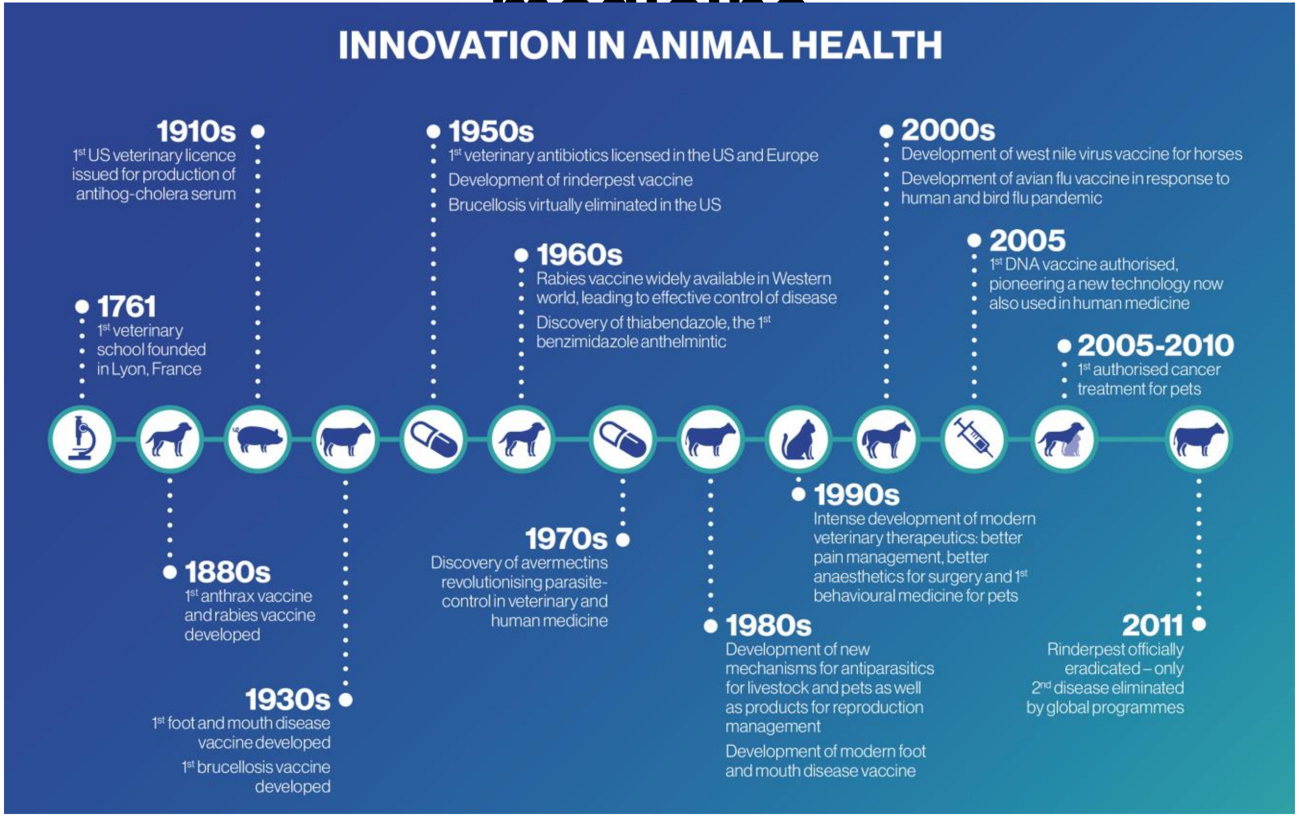
1879 — Louis Pasteur's discovery - forgotten *P. multocida* culture protected chickens against virulent challenge

1798 — Discovered that vaccination of people with cowpox will protect against smallpox. "Vacca"=cow

12th century — Variolation against small pox by the Chinese

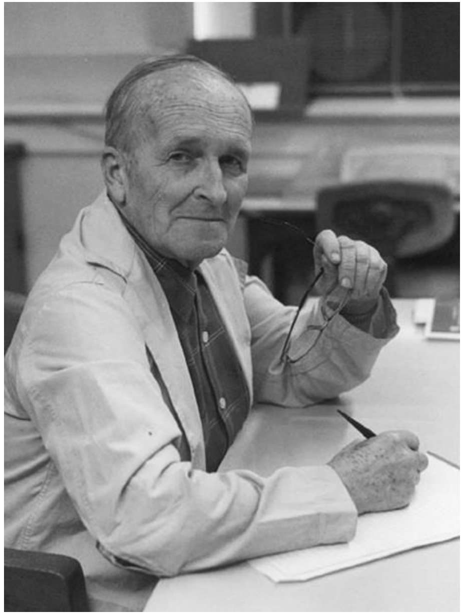
(Adapted from Tizard, 2001)

Vaccination in disease control in veterinary medicine



Fish vaccination

- The first report of disease prevention using vaccines is probably by Snieszko et al - protective immunity in carp immunized with *Aeromonas punctata* (1938)
- The first report in English - Duff in 1942
  - showed protection against *Aeromonas salmonicida* in trout immunized by parenteral inoculation and by oral administration
- The first vaccine for aquaculture (commercial)
  - prevention of yersiniosis in salmonid fish, licensed in USA in 1976 - produced by Wildlife Vaccine Inc.
- In 1988 in Norway – Atlantic salmon and rainbow trout in were vaccinated at large scale against cold-water vibriosis
  - initially via immersion, later injection
- Since 1993, all fish in Norway have been vaccinated against 3-4 bacterial diseases and since 1995 against IPN and 2007/8 also against PD



Current use of fish vaccines (globally)

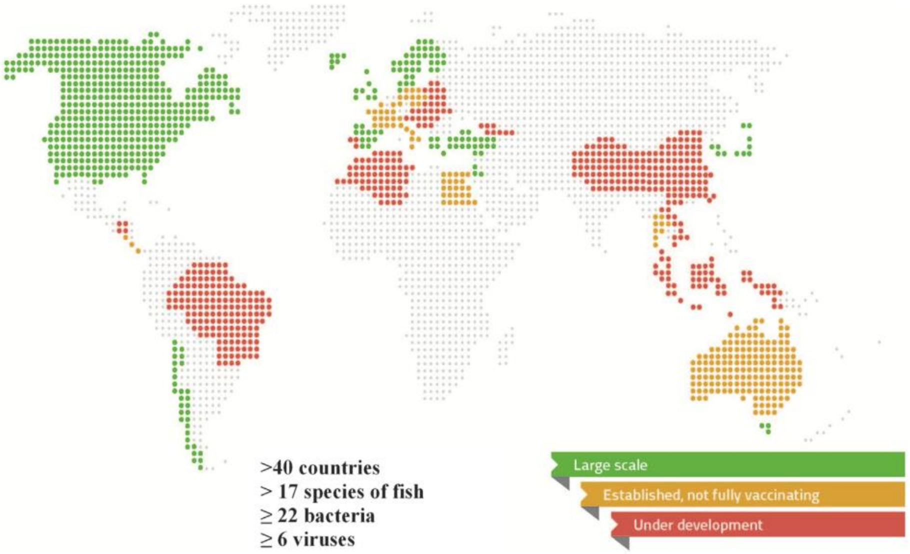


Fig. 2. A categorisation of the countries according to the use and implementation of fish vaccination. Green shows countries where vaccination is commonly used. Yellow are countries where vaccination is used, but not fully implemented. Red are countries where fish vaccination is under development. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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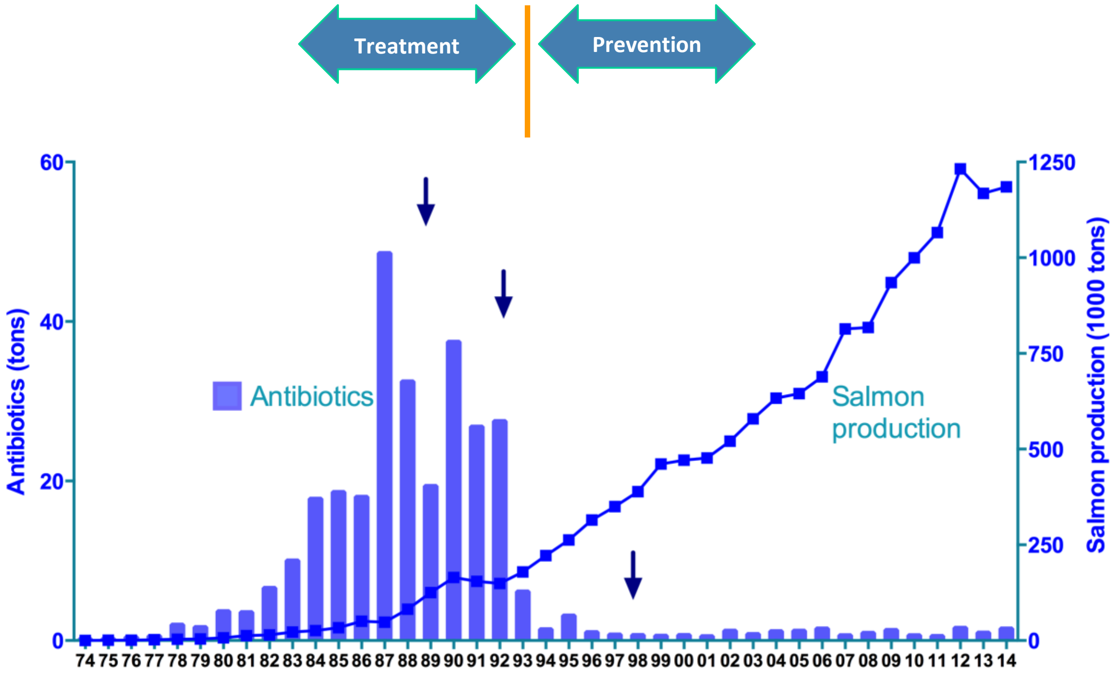
Brudeseth BE1, Wiulsrød R, Fredriksen BN, Lindmo K, Løkling KE, Bordevik M, Steine N, Klevan A, Gravningen K. Status and future perspectives of vaccines for industrialised fin-fish farming. Fish Shellfish Immunol. 2013;35(6):1759-68.

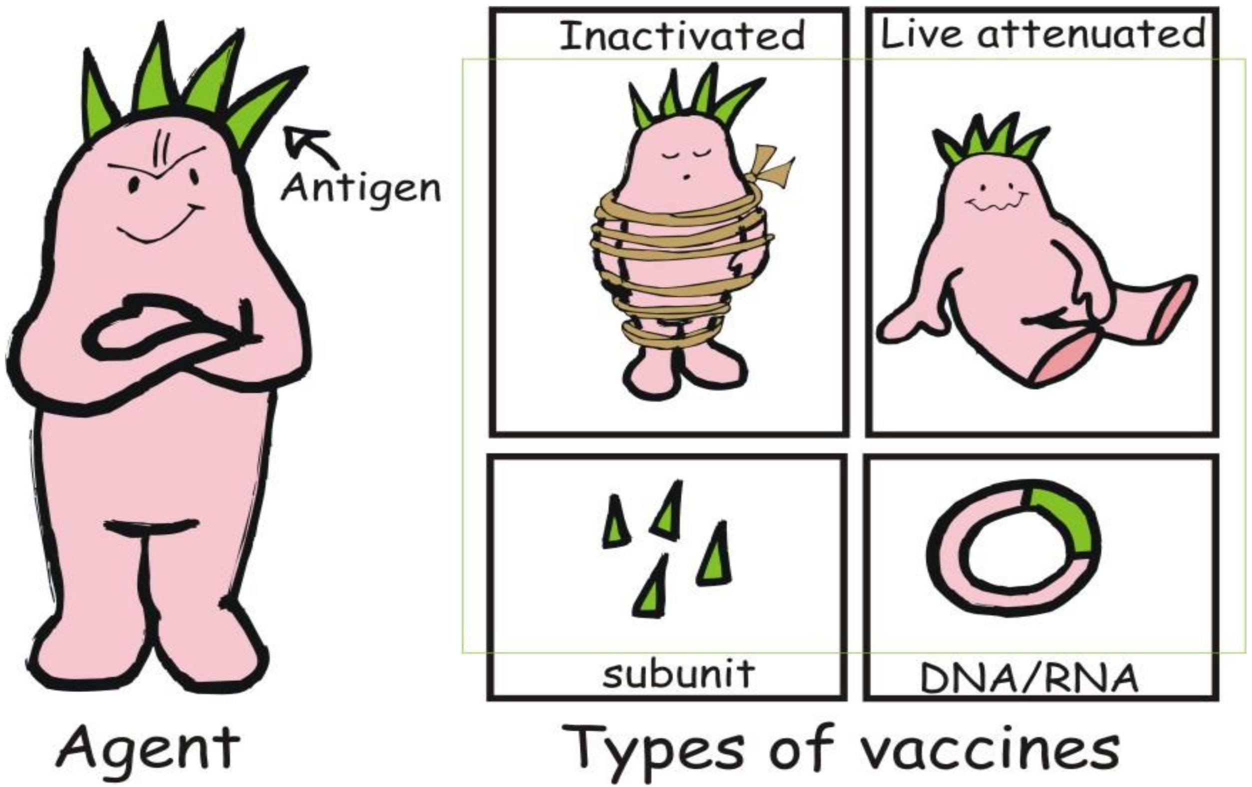
Have vaccines contributed to the control of diseases in salmon farming?

Salmon production and use of antibiotics

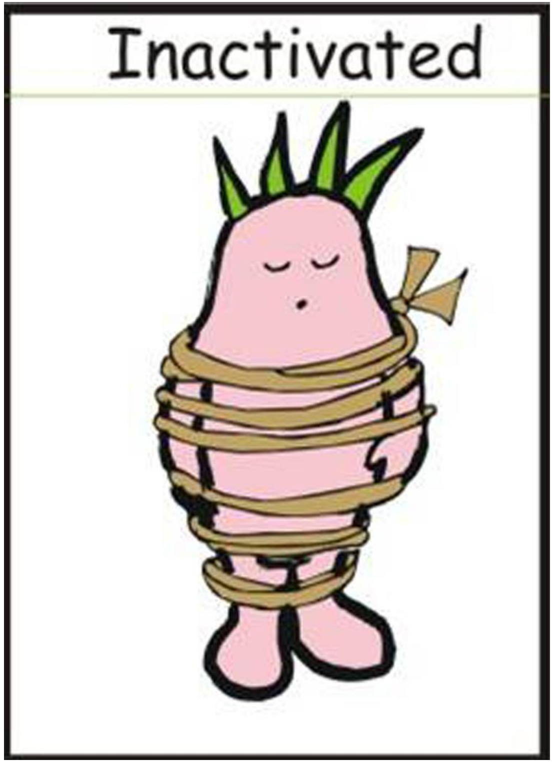


Which vaccines are being used today and how are they used?

Types of fish vaccines

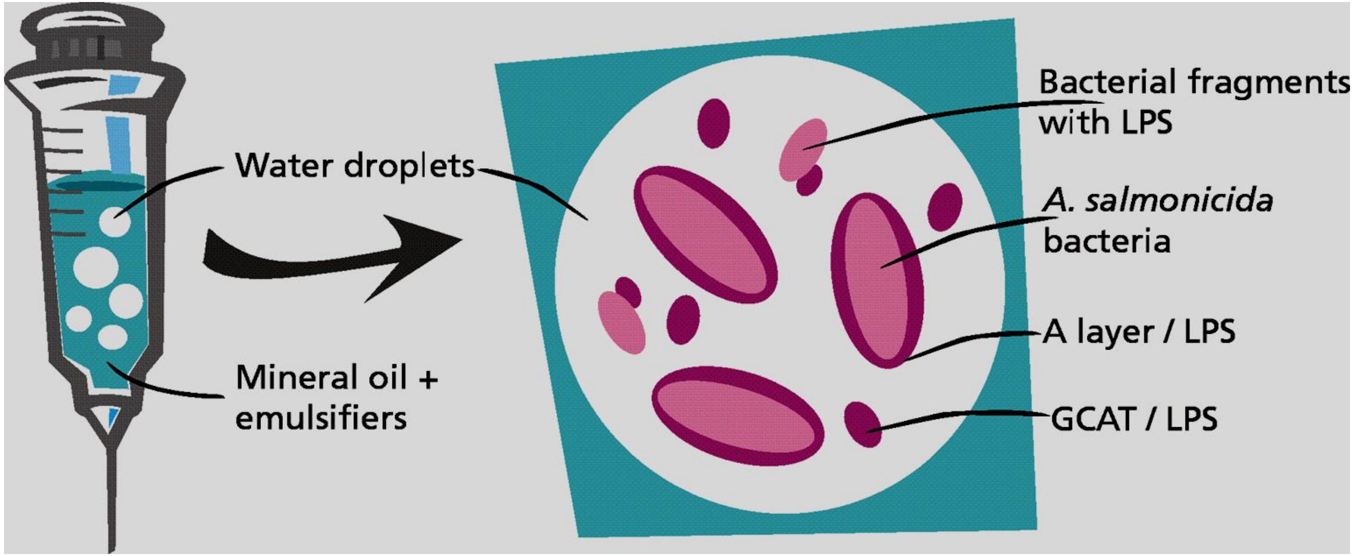


Non-replicating vaccines are poor immunogens



- Inactivated vaccines are poor immunogens
- Need adjuvant to «kick off» the inflammation and the immune response
- An adjuvant in general use in vaccines for salmon – oil adjuvanted as an water-in-oil formation

“Structure” of a w-o formulation (schematic)



This has been the solution in salmon farming particularly for vaccination against bacterial diseases

Vaccination modalities

Modalities

- Efficacy
- Stress
- Costly
- Time consuming

- Efficacy
- Stress
- Costly
- Time consuming

- Efficacy
- Stress
- Costly
- Time consuming

- Efficacy
- Stress
- Costly
- Time consuming

Injection vaccination strategies in fish

Comply with industry needs

- **But also develop new protocols and procedures together**



Injection vaccination - the “Wet Method”

Injection vaccination - “Dry Method”

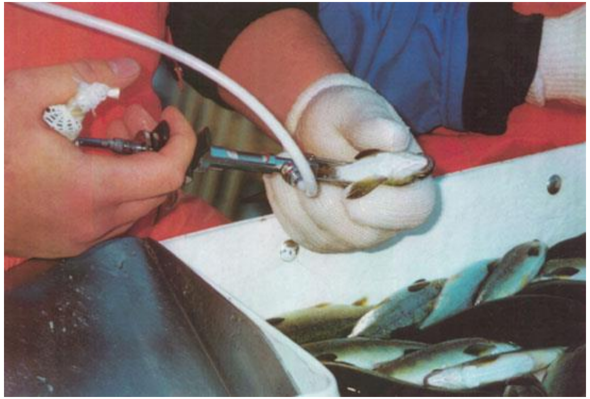
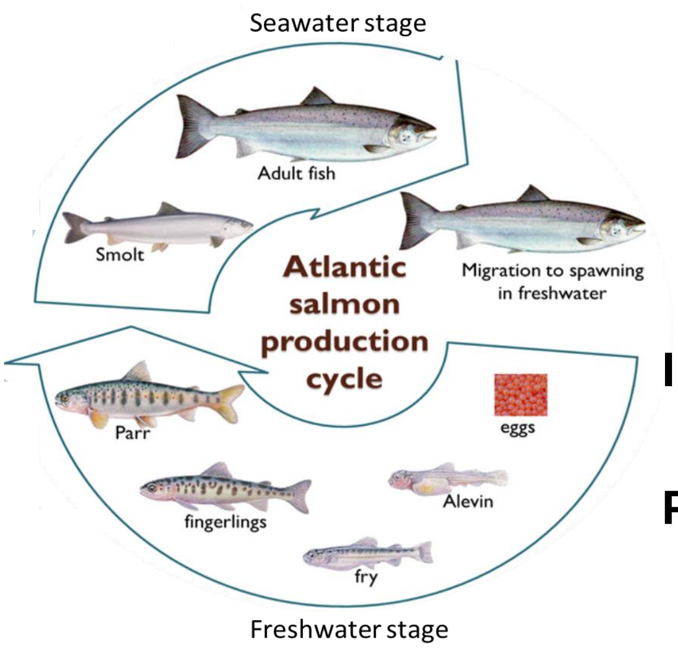


Figure 4. Manual vaccination of Atlantic salmon. Two metallic bows are attached to the injection unit to aid a correct intraperitoneal injection of the vaccine dose.

Vaccination timing
(when to vaccinate)



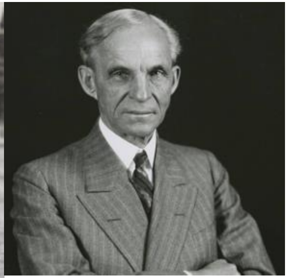
Vaccination: at parr stage/end of freshwater period

Immune induction period
- Prior to or at smoltification

Post Immune induction
- Period of protection

The salmon story – can it be applied universally?

Vaccination of pangassius - Vietnam



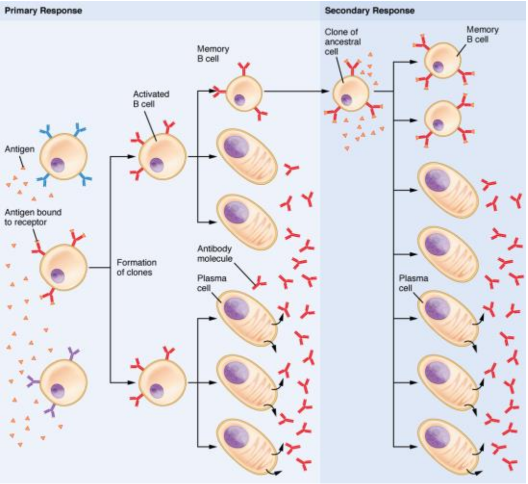
"You can have any color you like, as long as it is black"

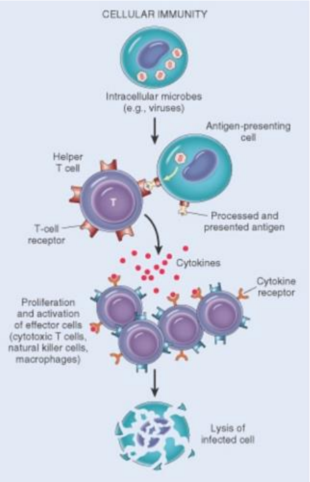
Tailor-made vaccines

- **Price (low values species)**
  - **Development, manufacturing and distribution cost**
- **Comply with production environment**
  - **Provide solutions during different stages of the production cycle**
    - **Modalities**
- **Founded on sound disease diagnostics**
  - **Etiology**
- **Training of personnel**
  - **Correct usage**
- **Develop government knowledge and confidence in disease control**
- **Regulatory authorities**
  - **Understanding disease, disease control in aquaculture and the use of vaccines**
  - **and understand how vaccines work, what you can expect**

Inactivated, oil-adjuvanted vaccines induce primarily humoral responses

- **Inactivated vaccines are B cell vaccines**
  - **give a humoral immune response**
  - **protect against pathogens in the extracellular environment**
    - **viruses (sometime or partially) because virus spread extracellularly**
    - **extracellular bacteria**
  - **poor protection against intracellular pathogens**
- **Vaccines against intracellular pathogens**
  - **Live vaccines or DNA vaccines confer immunity to intracellular pathogens**
    - **Tc – cytotoxic T cells – or by**
    - **activating macrophages that will kill cell/intracellular pathogen**





Protection against mortality
Protection against shedding and spreading of pathogens

Polio vaccine

- **There are two kinds of polio vaccine**
  - **IPV (Salk's) is given by injection (i.m.)**
  - **OPV (Sabin's) is given orally in drop form**

- **Inactivated polio vaccine (IPV)**
  - **IPV vaccine is safe for use**
  - **protects against systemic infection (CNS)**
  - **<u>does not protect against fecal shedding / spread of virus</u>**

- **Oral polio vaccine (OPV)**
  - **long-term immunity achieved with a live, attenuated virus**
  - **induces local (gut) and systemic immunity (circulating ab)**
  - **<u>prevents spread of virus (fecal-oral)</u>**

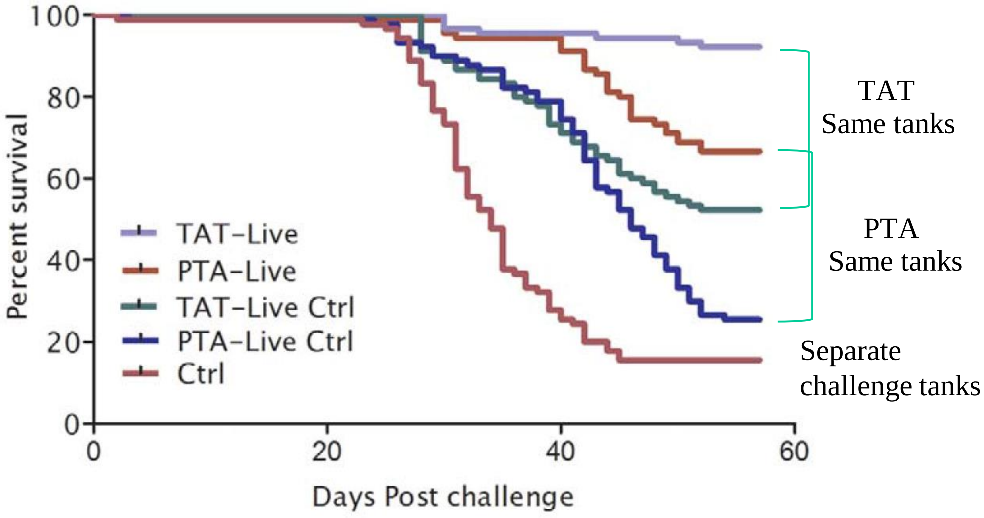
Use of live vaccines in finfish aquaculture (bacterial / viral)

- Advantages
  - High efficacy (elicit long-lived, humoral and cellular immunity)
  - Lower production cost compared to inactivated vaccines (lower antigen dose needed) – viral and bacterial
  - Easy to administer, can be delivered by dip, bath, oral route and for efficient systemic distribution
  - Humoral and cell-mediated immunity
- Challenges
  - Vaccine safety
  - Reversion to virulence
  - Attenuation by use of genetic modification (GMO issues)

Characteristics of live vaccines

Example from a virus of salmon (infectious pancreatic necrosis virus)

- **Protection in vaccinated individuals depends on immunogenicity**
- **Dynamic interaction between vaccinated and naïve individuals in the population**
  - **naïve cohabiting individuals can be immunized through spread of the vaccine virus from the vaccinated animal**
- **Vaccine with higher residual virulence/spreading potential "infects/immunizes" naïve controls and protect them (better) than more attenuated vaccine strains of IPNV**

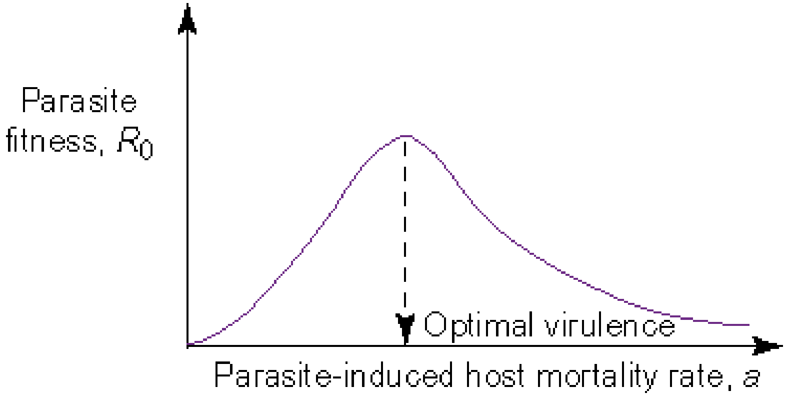



OPEN ACCESS Freely available online PLOS ONE

Immunogenicity and Cross Protective Ability of the Central VP2 Amino Acids of Infectious Pancreatic Necrosis Virus in Atlantic Salmon (*Salmo salar* L.)

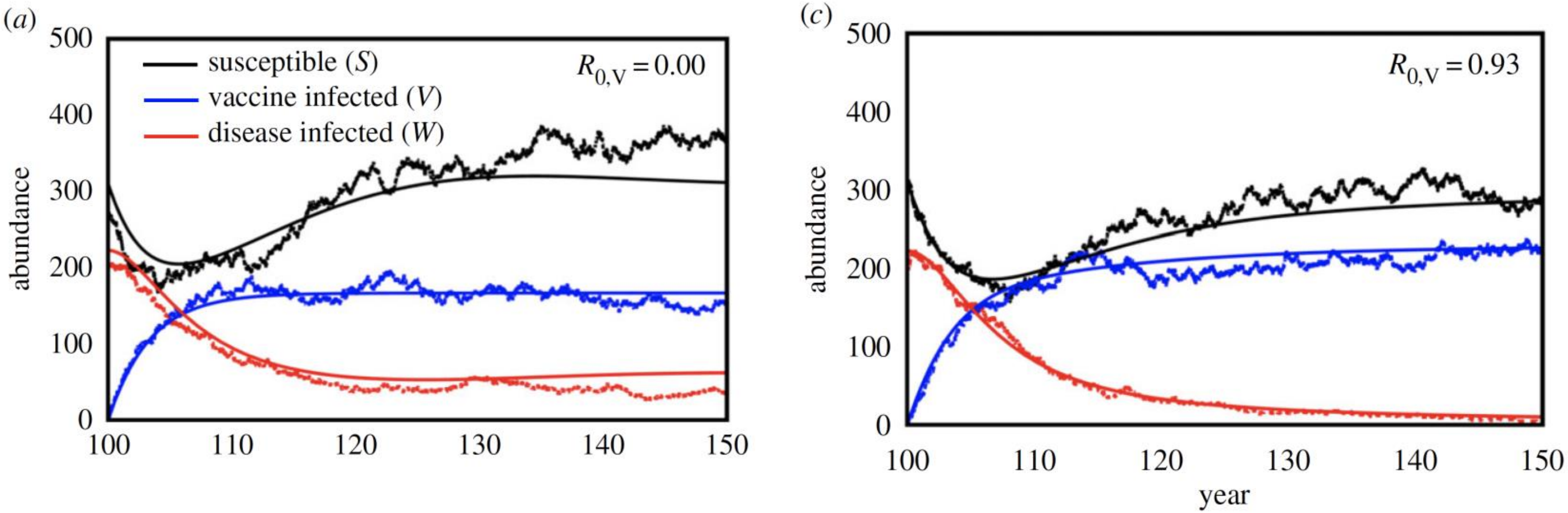
Hetron M. Munang'andu[1], Ane Sandtrø[1], Stephen Mutoloki[1], Bjørn E. Brudeseth[2], Nina Santi[3], Øystein Evensen[1*]

- **R0 - the total number of secondary infections produced by a primary infection in a population of susceptible hosts**



•R_o = 1 (A primary case must attempt to generate at least one new case)
•R_o > 1 (Expansion of infected individuals)
•R_o < 1 (Shrinking pool of infected individuals).

R0,V – spread of a vaccine virus
i.e. transmissible vaccines



The densities of susceptible host individuals (black lines), vaccine infected individuals (blue lines) and disease infected individuals (red lines) predicted by the stochastic simulations (jagged line showing one of the 100 runs) and deterministic model (smooth line) over the 50 years following initiation of the vaccination programme in year 100.

Nuismer SL, Althouse BM, May R, Bull JJ, Stromberg SP, Antia R. 2016 Eradicating infectious disease using weakly transmissible vaccines. **Proc. R. Soc. B** 283: 20161903.

In a fish farming environment - would live vaccines be allowed?

Chilean fish farming taking an important innovative step to control Salmon Rickettsial Septicaemia (SRS) Syndrome

Chilean fish farming taking an important innovative step to control Salmon Rickettsial Septicaemia (SRS) Syndrome - read more

Chilean fish farming taking an important innovative step to control Salmon Rickettsial Septicaemia (SRS) Syndrome

- First attenuated live vaccine against SRS*, ALPHA JECT LiVac® SRS, has been approved by the Chilean authorities and is available for customers in Chile.
- The vaccine was developed to fight the SRS disease that causes significant costs to the Chilean fish farming industry.

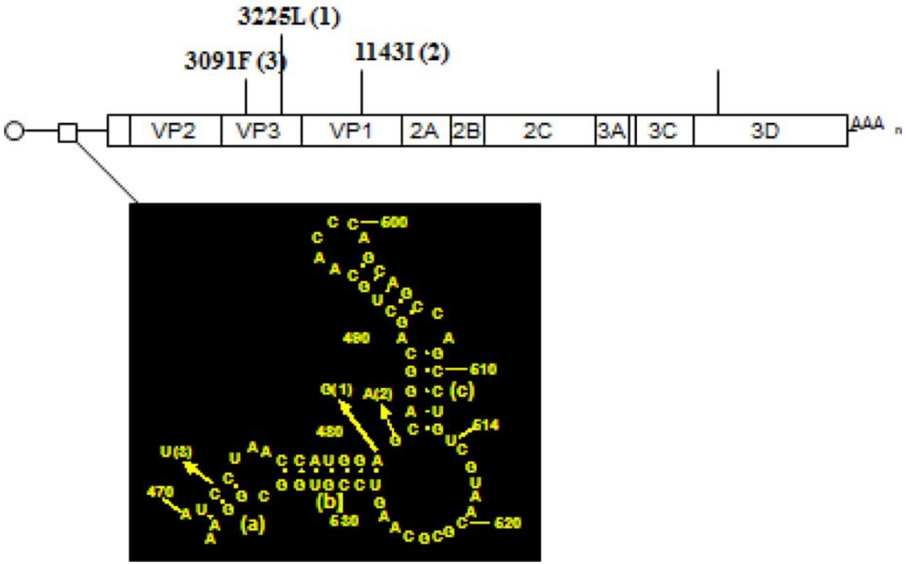
Live vaccine candidates tested experimentally

TABLE 2: Live vaccines.

Virus	Abbreviation	Fish host	Mode of attenuation	Protection	Reference
Cyprinid herpesvirus subtype 3	CyHV-3	Carp	Natural selection	High	[177]
Viral hemorrhagic septicemia	VHSV	Rainbow trout	Naturally attenuated	High	[178]
	VHSV	Rainbow trout	Naturally attenuated	High	[84]
	VHSV	Olive flounder	Recombinant (RG) modification	High	[179]
	VHSV	Rainbow trout	Recombinant (RG) modification	High	[180]
	VHSV	Zebra fish	Recombinant (RG) modification	High	[181]
Infectious hematopoietic necrosis virus	IHNV	Rainbow trout	Multiple serial passage	High	[86]
	IHNV	Rainbow trout	Naturally attenuated	High	[83]
	IHNV	Rainbow trout	Natural selection	High	[182]
	IHNV	Rainbow trout	Recombinant (RG) modification	High	[91]
	IHNV	Rainbow trout	Recombinant (RG) modification	High	[183]
Infectious pancreatic necrosis virus	IPNV	Atlantic salmon	Avirulent strain/low dose	High	[82]
Rock bream iridovirus	RSIV	Rock bream	Low temperature	High	[184]

Munang'andu & Evensen 2015

Determinants of attenuation



Mutations affecting the virulence of the three Sabin vaccine strains of poliovirus. Each has a <u>mutation in Domain V of the 50 non-coding region</u> and each at least one mutation in the capsid region. The types concerned are shown in paranthesis.

Minor, Vaccine 2015

Approach to attenuate VHS virus

 Nucleic Acids Research, 1996, Vol. 24, No. 21 ***4197–4201***

Secondary structure of the panhandle RNA of influenza virus A studied by NMR spectroscopy

Hae-Kap Cheong, Chaejoon Cheong[1] and Byong-Seok Choi*

Department of Chemistry, Korea Advanced Institute of Science and Technology, Kusung-dong 373-1, Yusung-gu, Taejon 305-701, Korea and [1]Magnetic Resonance Group, Korea Basic Science Institute, Eoun-dong 52, Yusung-gu, Taejon 305-333, Korea

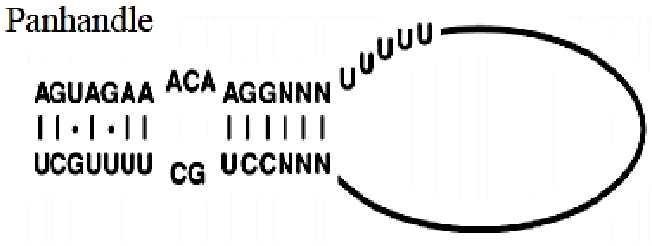
Received July 15, 1996; Revised and Accepted September 12, 1996

ABSTRACT

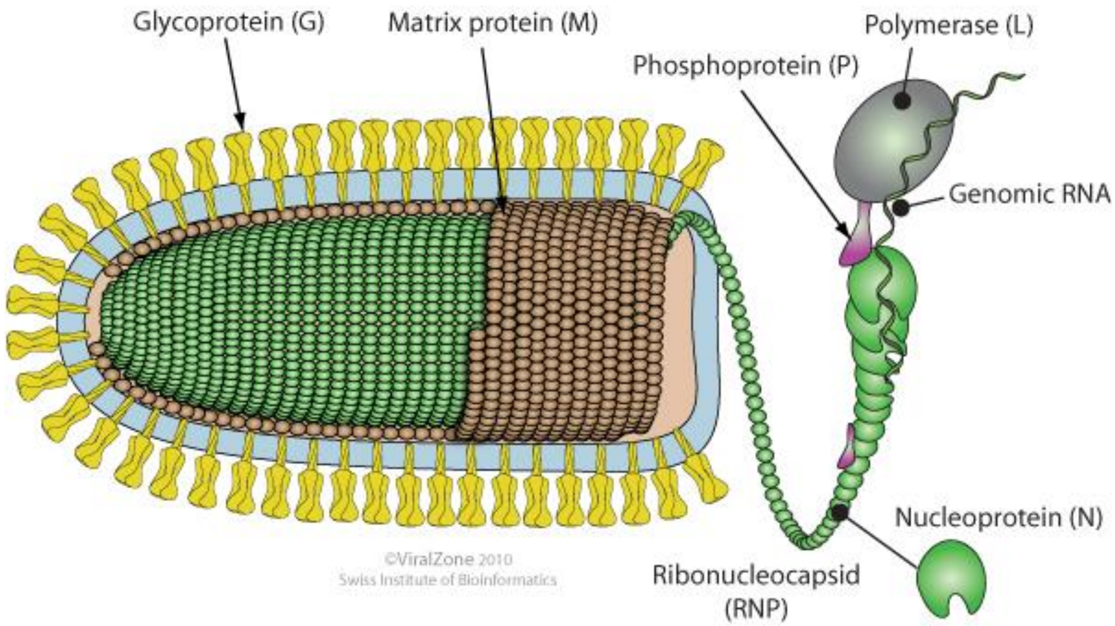
The double-stranded panhandle structure of the influenza virus RNA is important for replication, transcription and packaging into the virion of the virion RNA. The solution structure of a 34 nt RNA which contains the conserved panhandle sequences has been investigated by one- and two-dimensional NMR spectroscopy. The partially complementary 5′- and 3′-ends of the RNA form a double helical structure which is, on average, close to A-form. The stem contains bulges at nucleotides A10, A12 and C26. In between these bulges, C11 and G25 form a Watson–Crick base pair. The structural features of the panhandle provide a framework for the explanation of mutational analysis and for a better understanding of RNA–polymerase interactions.

required for polyadenylation and for transcription termination (9,10). *In vitro* studies using recombinant influenza virus polymerase have demonstrated that both viral 5′- and 3′-ends are required for polymerase activity and that the polymerase binds sequence-specifically to the partially double-stranded panhandle structure (11,12). Both an RNA polymerase binding assay and an *in vitro* transcription assay have shown that the panhandle structure is involved in initiation of transcription (13). Nuclease S1 mapping of the influenza virus RNA has indicated that the termini of the RNA segments form a 15 bp panhandle structure (8; Fig. 1A). Another alignment, which contains an internal loop, has been proposed as a model structure of the panhandle of the influenza virus RNA (14; Fig. 1B). Recently, however, by using different nucleases and chemical probes, Baudin *et al.* (15) have determined a somewhat different panhandle structure, in which the panhandle contains an internal loop and a bulge (Fig. 1C).

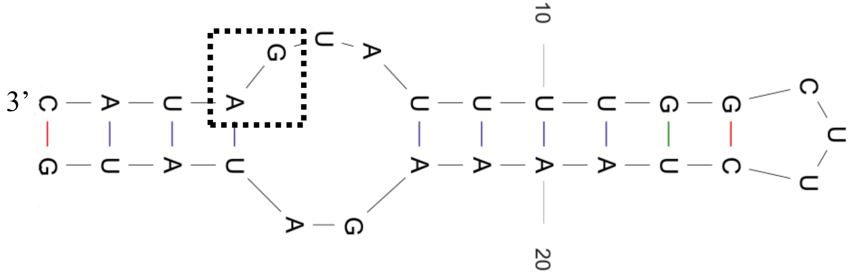
In this paper, we report the structure of this panhandle RNA

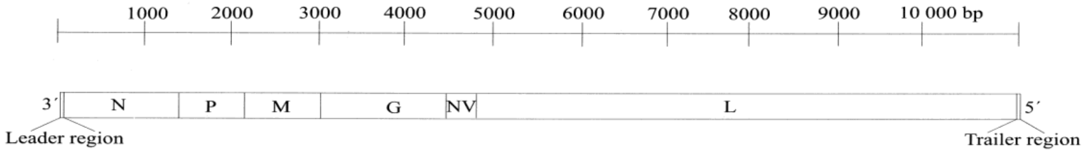


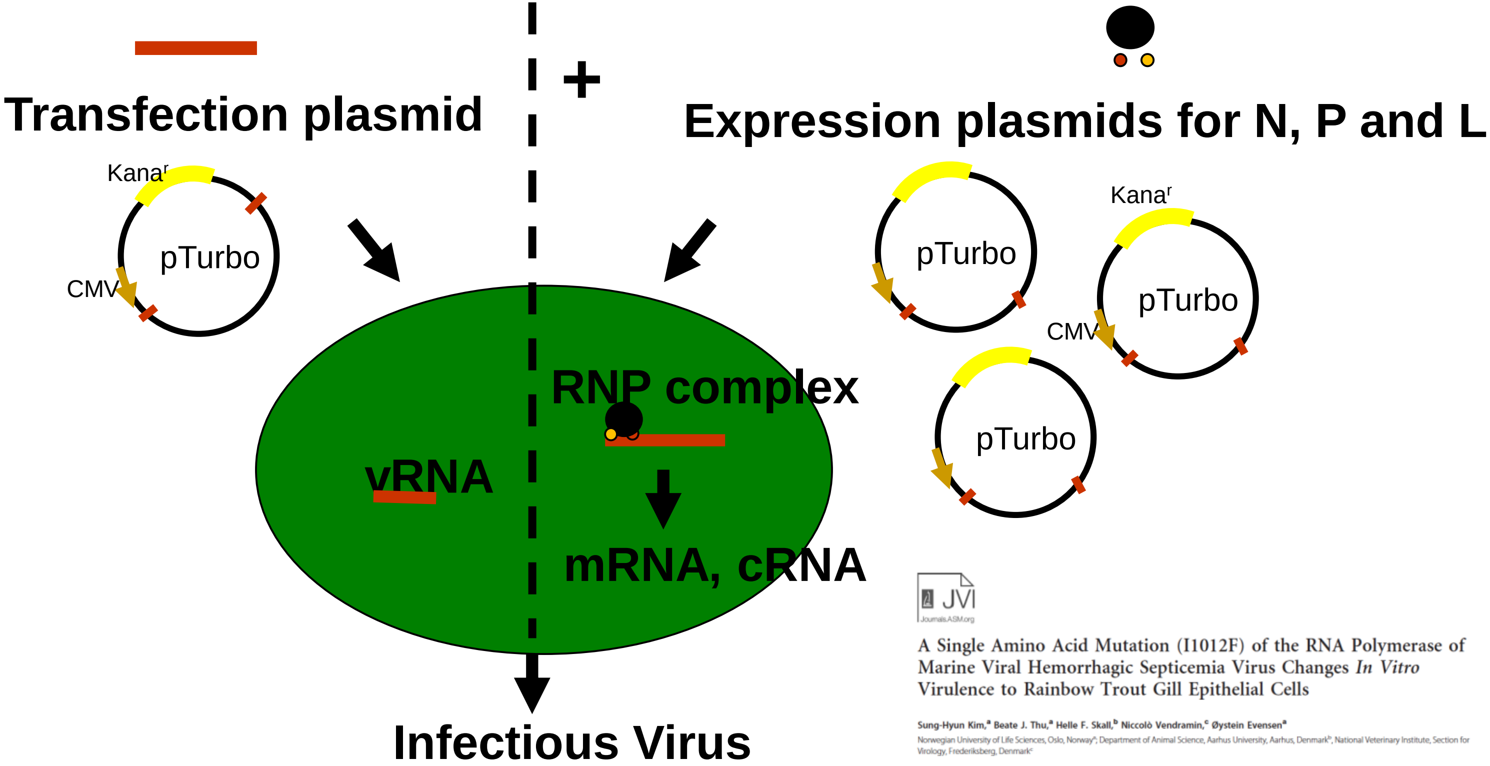
: Terminal UTR RNA (3’)-RNA (5’) interaction forms a secondary structure.



- **The polymerase (L) together with P and N will attach to the 3’ end (UTR) and start transcription**







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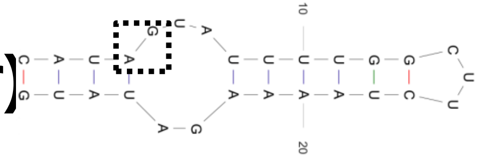
A Single Amino Acid Mutation (I1012F) of the RNA Polymerase of Marine Viral Hemorrhagic Septicemia Virus Changes *In Vitro* Virulence to Rainbow Trout Gill Epithelial Cells

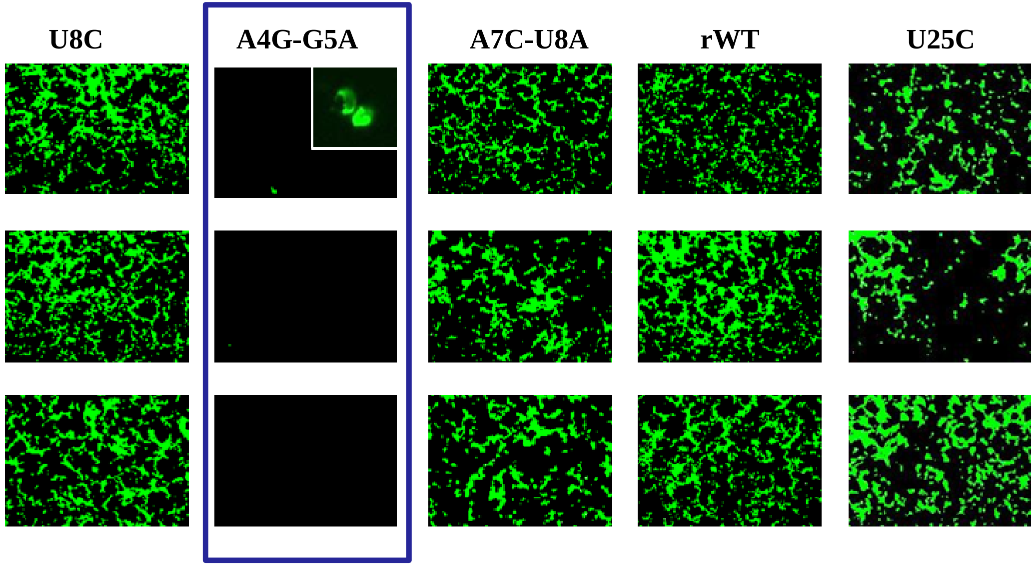
Sung-Hyun Kim,[a] Beate J. Thu,[a] Helle F. Skall,[b] Niccolò Vendramin,[c] Øystein Evensen[a]

Norwegian University of Life Sciences, Oslo, Norway[a]; Department of Animal Science, Aarhus University, Aarhus, Denmark[b]; National Veterinary Institute, Section for Virology, Frederiksberg, Denmark[c]

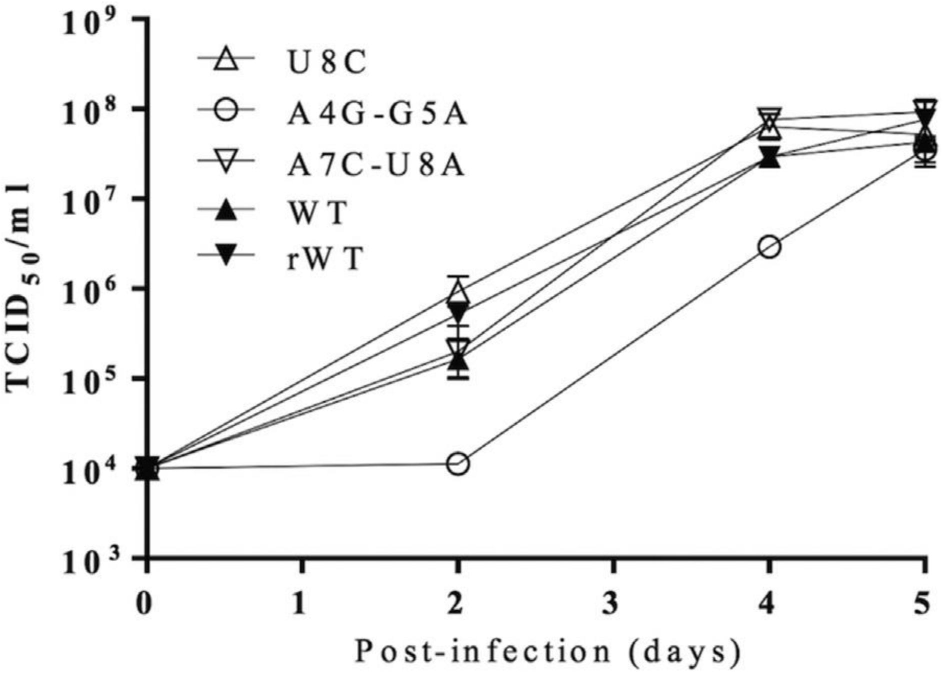
A4G-G5A

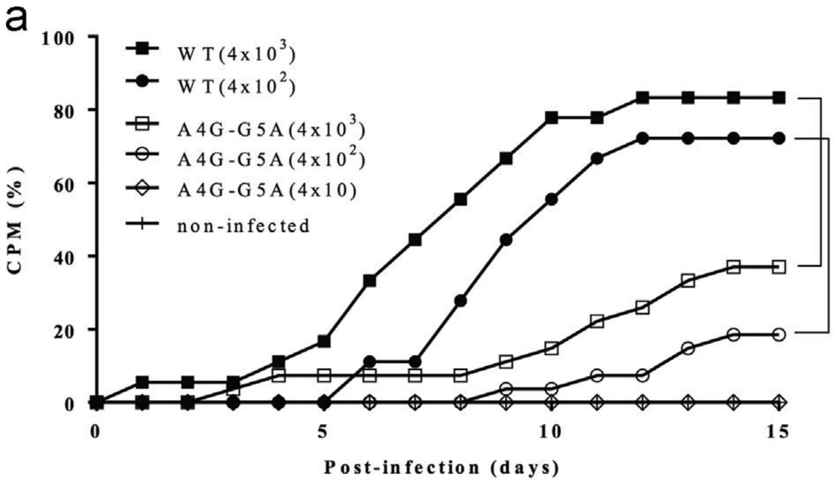
3 log lower (rescued virus titer)





Recombinant A4G-G5A VHSV strain





Contents lists available at ScienceDirect

Virology

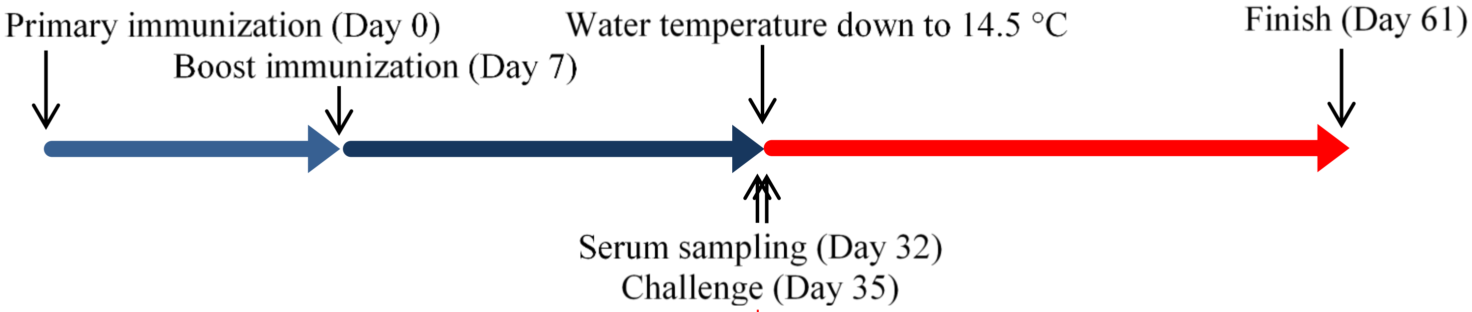
journal homepage: www.elsevier.com/locate/yviro

ELSEVIER

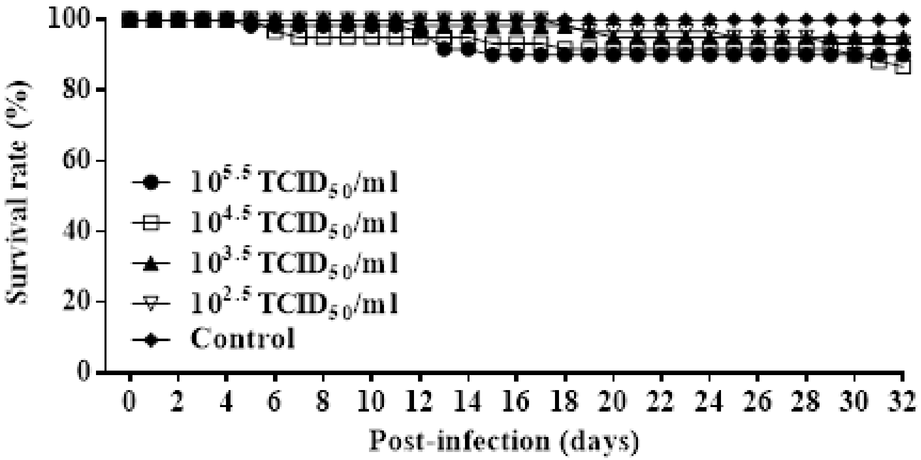
Specific nucleotides at the 3′-terminal promoter of viral hemorrhagic septicemia virus are important for virulence in vitro and in vivo

Sung-Hyun Kim [a], Tz-Chun Guo [a], Vikram N. Vakharia [b], Øystein Evensen [a,*]

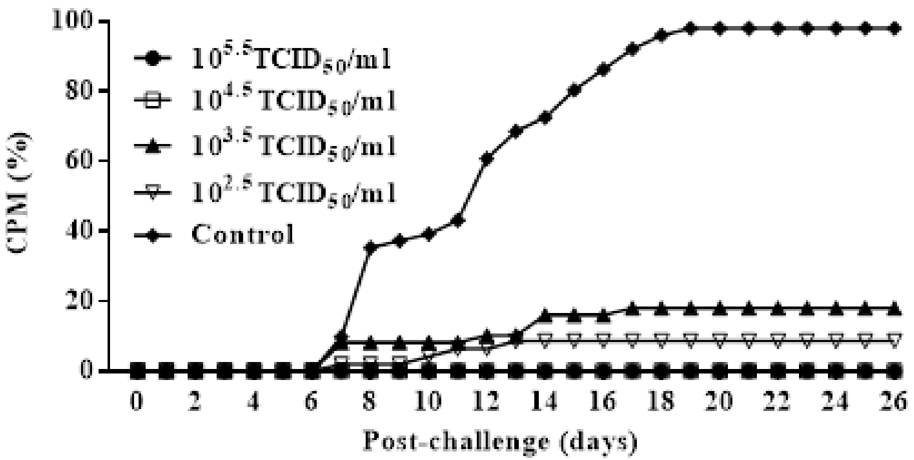
Good protection by immersion immunization – tested in Olive flounder



Immunization



Challenge



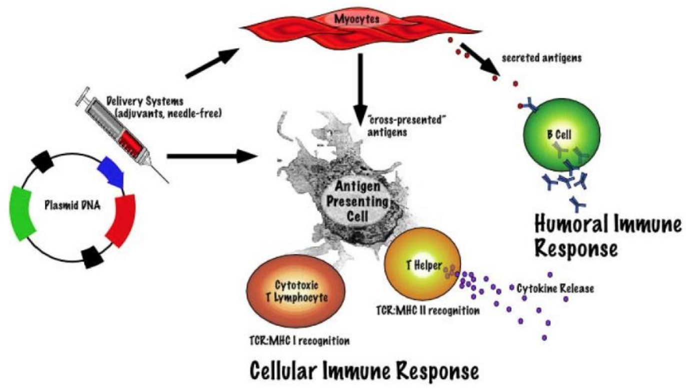
Kim et al. Vaccine 2016

Other technologies

Table 3: DNA vaccines explored in fish.

Classification	Virus family	Pathogen	Abbreviation	Antigen	Protection*	Reference
DNA viruses	Iridovirus	Red sea bream iridovirus	RSIV	Major capsid	Moderate	[185]
	Herpesviridae	Channel catfish virus (CCV)	CCV	ORF 6&59	Low	[186]
RNA virus	Rhabdoviridae	Viral hemorrhagic septicemia virus	VHSV	G	High	[95]
	Rhabdoviridae	Infectious hematopoietic necrosis virus	IHNV	G	High	[98, 187, 188]
	Rhabdoviridae	Spring viremia of carp virus	SVCV	G	High	[189, 190]
	Rhabdoviridae	*Hirame rhabdovirus*	HRV	G	High	[191]
	Birnaviridae	Infectious pancreatic necrosis virus	IPNV	SegA/VP2	Moderate	[103, 140]
	Orthomyxoviridae	Infectious salmon anemia virus	ISAV	HE	Moderate	[192]
	Togaviridae	Salmon alphavirus subtype 3	SAV-3	E2	Moderate	[157]
	Nodaviridae	Atlantic halibut nodavirus	ANHV	Capsid	Low	[193]

*Protection was determined by postchallenge relative percent survival (RPS).



DNA vaccine against PD was approved by EMA 2017

EU Commission authorises first DNA vaccine

Clynav will protect salmon against serious infectious disease

Clynav will protect Atlantic salmon against Salmon Pancreas Disease.

The first veterinary DNA vaccine to protect Atlantic salmon against Salmon Pancreas Disease (SPD) had been authorised across the European Union.

SPD is a serious infectious disease which causes damage to the heart, pancreas and skeletal muscle, leading to death in some cases. The disease has become established in some Member States and outbreaks cause significant loses in salmon farms.

DNA vaccines consist of a genetic sequence that triggers the production of proteins in the cells of the vaccinated animal. These proteins stimulate a protective immune response, thereby preventing or reducing the impact of the disease should the animal be exposed to the virus.

Clynav, marketed by Elanco Europe Ltd, is the first DNA vaccine to be recommended for marketing authorisation in the EU.

Fish vaccinated with DNA not genetically modified

TOPICS: Clynav DNA Fish Genetically Modified GMO Norway Today Vaccine

Whole salmon on ice. Pixabay.com

Summary

- Vaccination – a tool in disease control
- Fish vaccine modalities and technologies must comply with the production environment/production cycles
  - Price structure of the fish species

- Duration of immunity will depend on mode of delivery – injection vaccines provides longer immunity than immersion vaccines/oral vaccines

- Inactivated vaccines have low immunogenicity in fish – as in other animals – and strong adjuvants are needed to induce long-lasting immunity (‘antigen depot’ type of memory)

- Live vaccines, DNA vaccines or other technologies will make their way into high-price segments
  - Better disease control
  - Better at identifying determinants of attenuation

Thank you for your attention

or