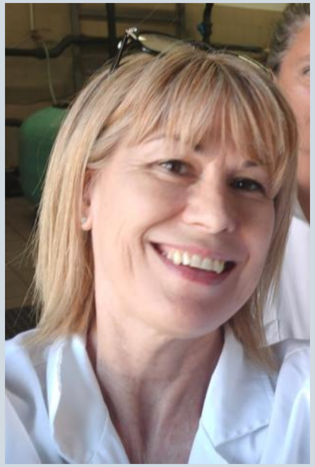
Fundamentals of vaccine immunology

DONATELLA VOLPATTI – UNIUD

SUPERTROUT WINTER SCHOOL

TORINO – NOVEMBER 21-25, 2022



Animal and Veterinary Sciences Section – Di4A - University of Udine – Italy
Didactic topics: General pathology and vet nutritional pathology

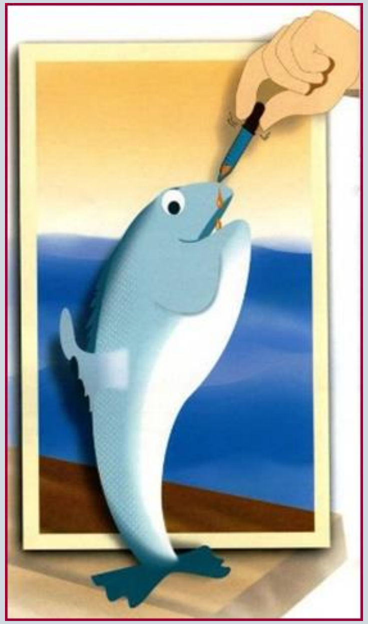
Current research activity at UNIUD - Investigations on the **pathogenesis** of infectious/un-infectious diseases in farmed fish. The main species under study are rainbow trout (*O. mykiss*) and sea bass (*D. labrax*). Current researches concerns the inflammatory and immune response against pathogens, as well as the prevention of infectious diseases by immunostimulation and/or vaccination treatments.


Reasons to study the fish immune system

Expand the knowledge on the evolution and diversification of vertebrates immune system - evolutionary and comparative immunology

Improve the knowledge on how immune system can be stimulated in order to protect fish from infectious diseases/stress – classical fish immunology and vaccinology – applicative studies

Use of fish as animal models for genetics/pathology/biology studies (e.g. zebrafish)



The history of fish immunology and vaccinology

Early studies performed between 1850 and 1940 – scientists looking at fish in terms of comparative anatomy and physiology – for example H.F. Stannius firstly described phagocytes in fish kidney

First publications on fish immune response and vaccination in 1935-1940

After 1940 numerous researchers dedicated their whole career to fish immunology/vaccinology

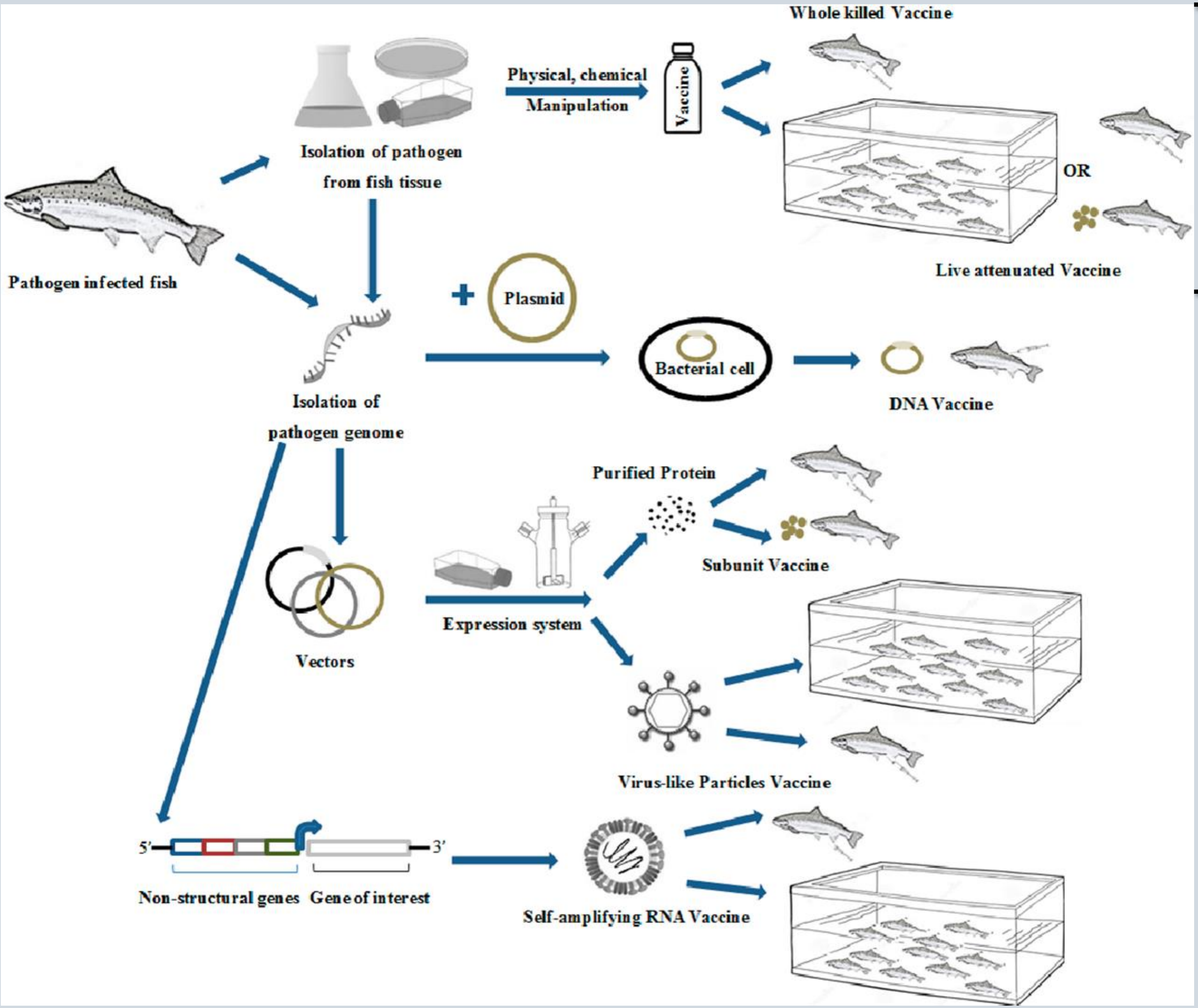
First fish-addressed vaccine approved in USA for enteric redmouth disease (1976)

Up to now 24 vaccines available (approved) worldwide, to be used in 17 fish species

Directed against 22 bacterial species and 6 virus

No approved vaccines against parasites

The majority of commercial vaccines include adjuvants and are administered by intraperitoneal injection. Some formulations are multivalent, especially those addressed to Atlantic salmon.



CLASSICAL

and

NOVEL HIGH-TECH approaches for the development and administration of fish vaccines

From innate to adaptive immune system.....passing through fish...

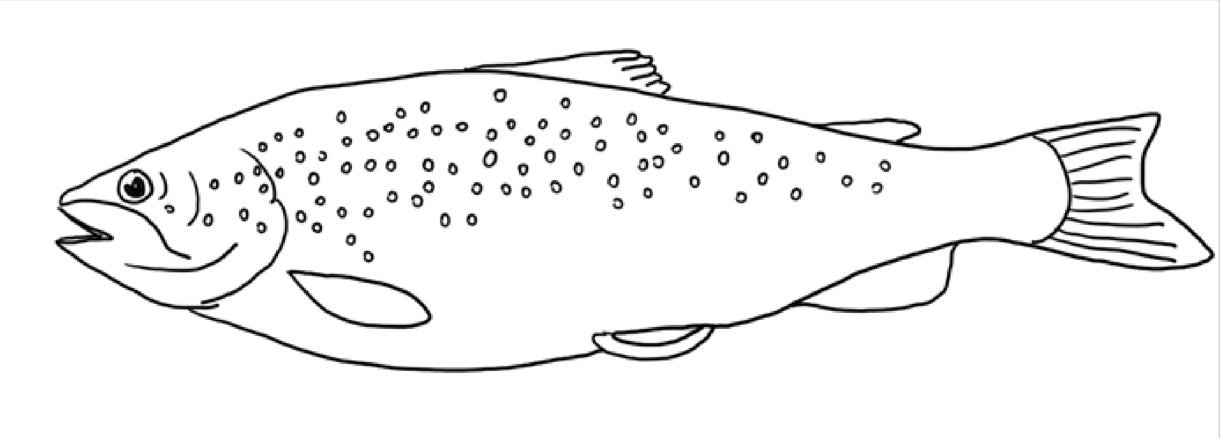




Cartilaginous and Teleost fish are the earliest vertebrates in which antibodies are present, therefore from the evolutionary point of view they represent the “key step” from INNATE IMMUNE SYSTEM to ADAPTIVE IMMUNE SYSTEM – this happened about 450 million years ago

NOTE: antibodies, T cell receptor, B cell receptor and MHC are ABSENT in invertebrates (e.g. molluscs, crustaceans) and in lower unjaved vertebrates (e.g. lampreys)

Host-pathogen interaction *versus* host-vaccine interaction



Viable virus – bacteria – parasites
enter the fish body, actively invade and colonise, actively replicate/proliferate, damage the host

Unviable virus – bacteria – parasites
(whole inactivated or fragmented vaccines) enter the fish body, are up-taken or absorbed…..without exerting pathogenicity….

In both cases the fish immune system is stimulated!

Routes of vaccine administration

Intra-peritoneal (intra-coelomic) injection

Immersion

Bath

Oral

Spray

Intra-muscular injection

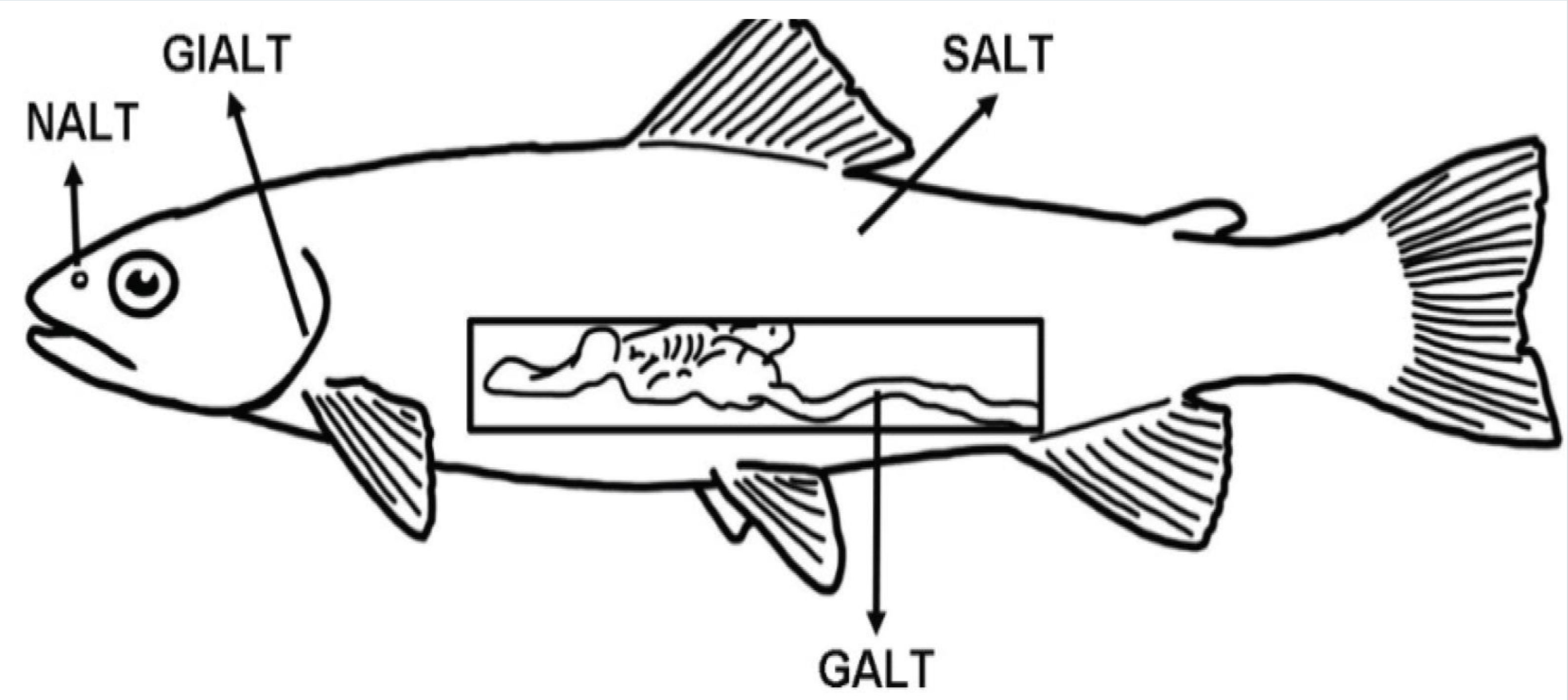
Jose Priya and Kappalli
Vaccine 40 (2022)
5873–5881

Table 1
The immunological perspective of vaccine administration methods.

Criteria	Injection delivery		Immersion delivery		Oral delivery	
	Intraperitoneal	Intramuscular	Direct immersion	Spray vaccination	Vaccine through feed	Encapsulated vaccines
Vaccination process	Time-consuming		Less time-consuming		Requires no extra time than routine farm husbandry	
Manual labour	labour intensive		Less labour		Requires no extra labour than routine farm husbandry	
Mass vaccination	Difficult to administer a large number of animals		A large number of animals can be vaccinated at the same time			
Stress to the immunised animal	Stress due to several steps, including fasting, weighing, anaesthetising, injection, and recovery		Do not cause stress	Stress due to spray pressure	Do not cause stress	
Immune response	Sub-optimal and short-term protection		Less chance of antigen uptake	Elicits both local and systemic innate and adaptive immune responses	Provoke poor and not long-lasting immunity	Elicit long-lasting and robust immunity
Antigen dosage	Low amounts of antigen are sufficient		A large dose of antigen is required for effective uptake	A large dose of antigen is required	A large dose of antigen is required for effective immunity	Low amounts of antigen are sufficient
Antigen delivery to immune responsive sites	Fast and complete absorption of antigen into the systemic circulation via capillary and lymphatic transport	Antigen find its way between muscle fibres, but slow release into the target tissue	Antigens are taken up by the skin, gills or gut and processed by the immune system		Low pH and high enzymatic activity in the foregut tend to destroy the vaccine and cause poor antigen delivery to the hindgut and other lymphoid organs	Encapsulation material helps to resist the vaccine destruction in the foregut, favouring better antigen delivery to immune responsive sites in the hindgut
Target animal	Cultured fish should be of a reasonable size. Fry cannot be vaccinated by i.p injection	Enable vaccinating fry of any size above the critical size of immune responsiveness	Cultured fish of any size can be vaccinated			
Specific target immunity	Systemic immunity	Local inflammatory responses	Mucosal immunity		Elicits both local and systemic innate and adaptive immunity	
Key sites of antigen uptake	Peritoneal cavity	Inflammatory cells	Olfactory organ, skin and gills		Hindgut and other lymphoid organs	
Need for an adjuvant	Oil-based adjuvants are required to prolong the protection					Adjuvants are not required

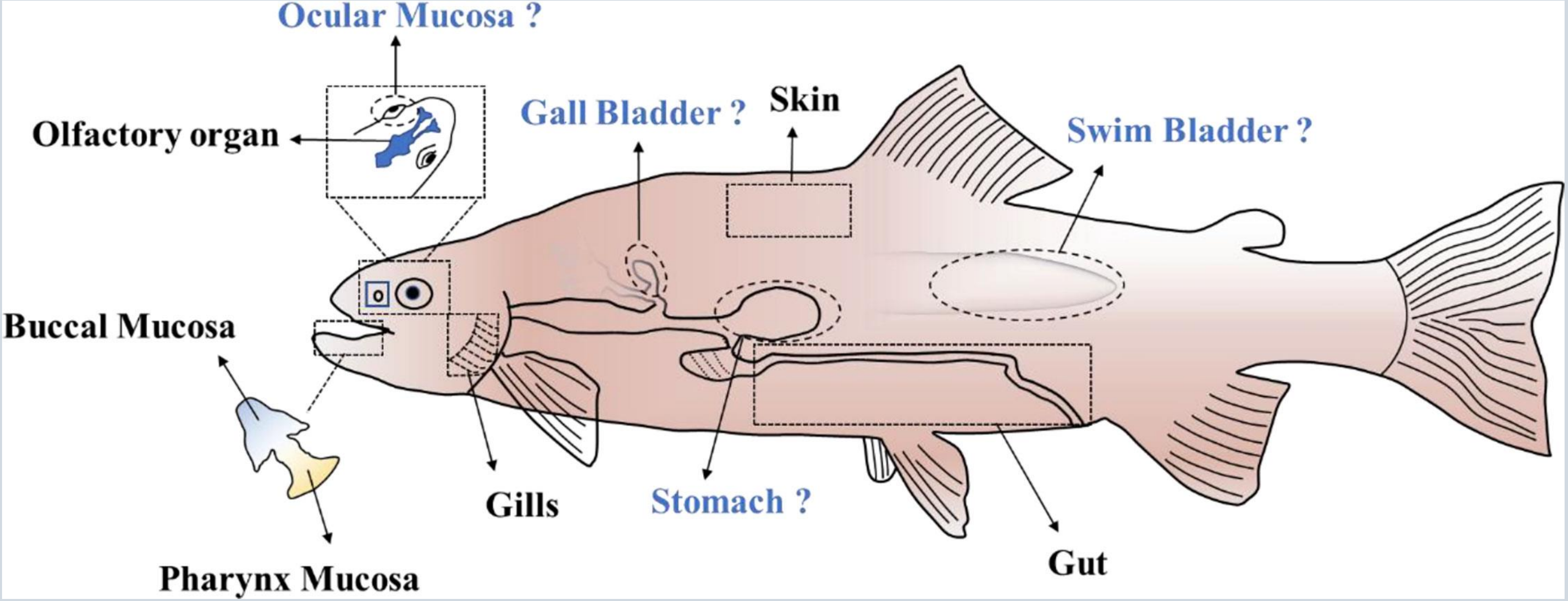
The vaccines are able to stimulate fish local (mucosal) and systemic immunity

Main mucosal sites of immunity - MALT



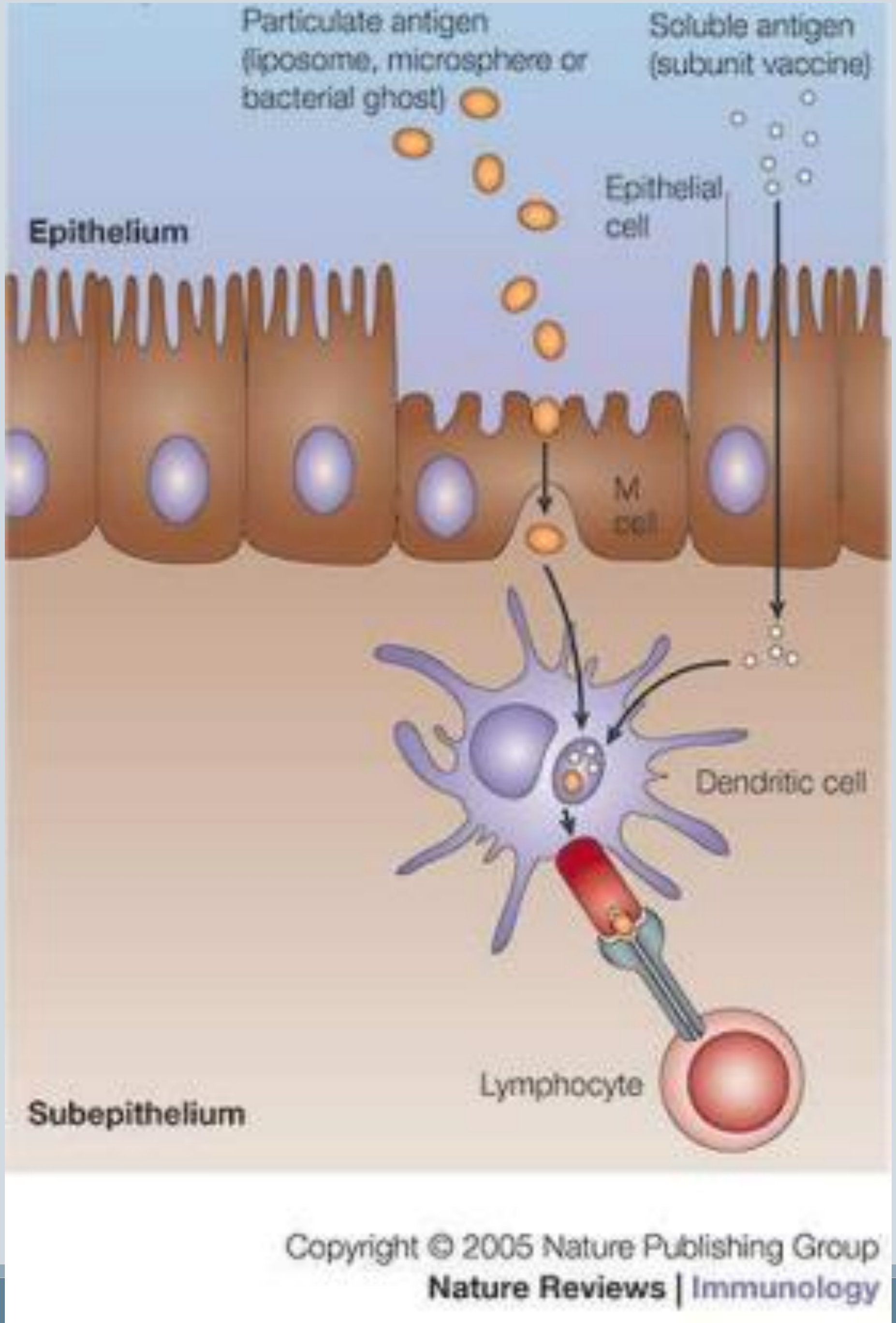
They are the "routes of entry" and antigen- processing sites for vaccines administered by immersion and bath – as well as orally

The MALTs indicate with "?" remain to be clearly delineated in teleost fish



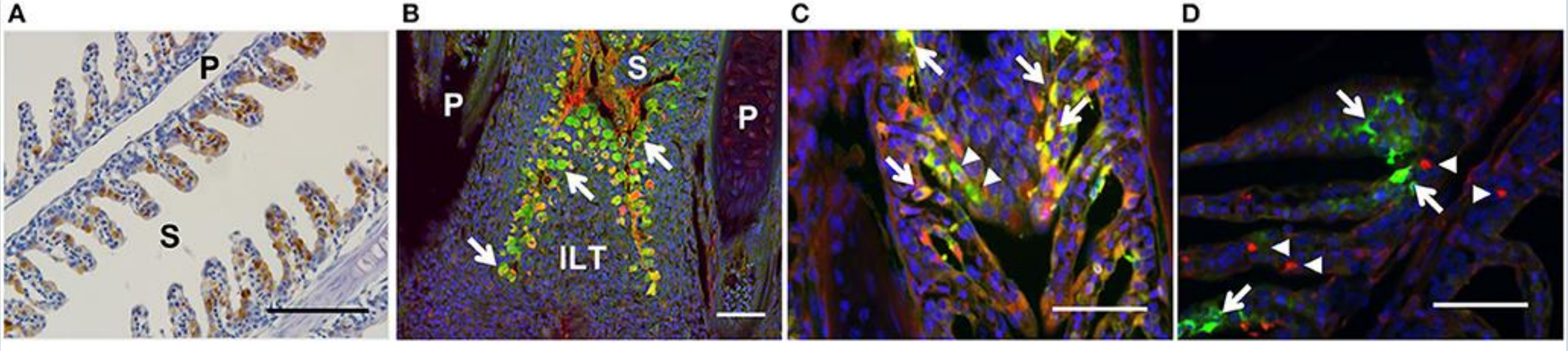
Yu Yongyao, Wang Qingchao, Huang Zhenyu, Ding Liguo, Xu Zhen
Immunoglobulins, Mucosal Immunity and Vaccination in Teleost Fish
Frontiers in Immunology
VOLUME=11 YEAR=2020

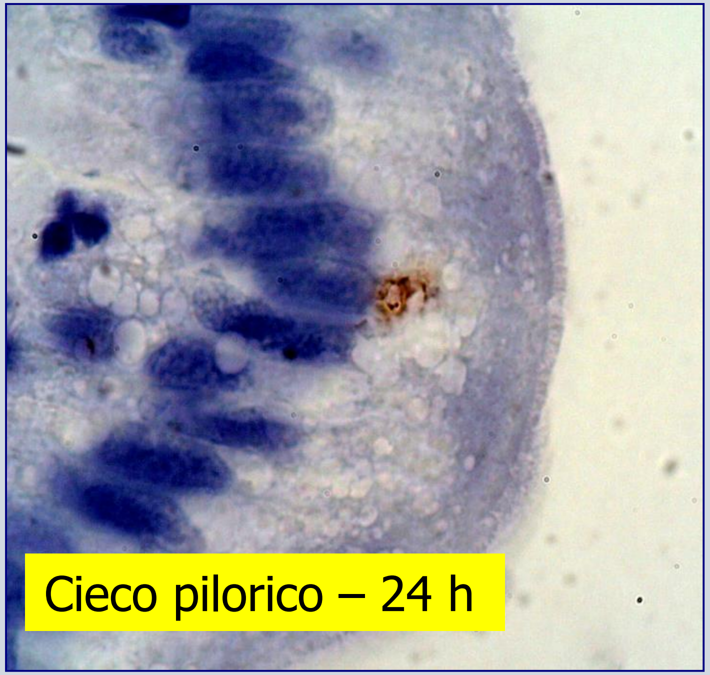
Mucosal M cells
and
antigen presenting cells

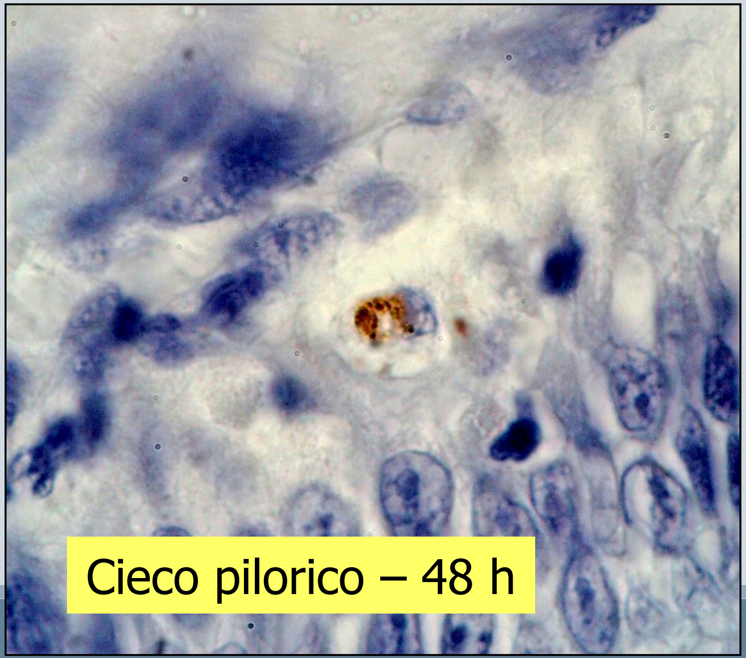


Antigen sampling cells (APCs) in the mucosal sites

To gain further insight on antigen uptake at mucosal surfaces, a key process for the optimization of mucosal vaccines, Kato et al. Front. Immunol., 20 September 2018, identified two phenotypes of ASCs able to take up antigens through rainbow trout gills. One phenotype had large vacuoles in the cytoplasm and expressed MHC-II, CD83, IL-1β. Morphologically, this subset features of monocyte, macrophage and dendritic cells







Oral vaccination of rainbow trout with *L. garvieae* Bacterin – immunohistochemical detection of the antigen in the digestive tract

Use of delivery systems

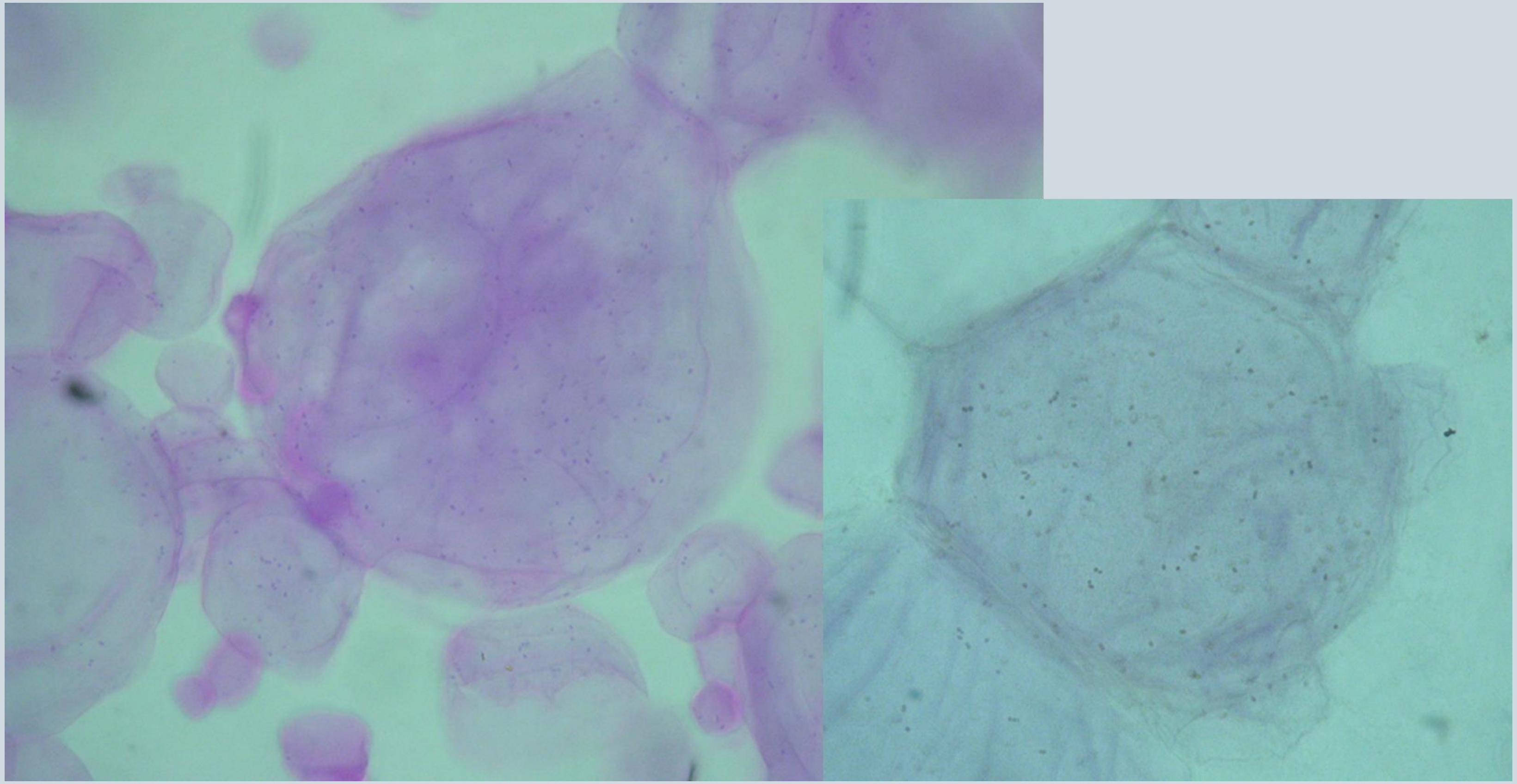
SVILUPPO E PRIMA VALIDAZIONE DI SISTEMI VACCINALI ORALI HI-TECH CONTRO *LACTOCOCCUS GARVIEAE* IN *ONCORHYNCHUS MYKISS*

Volpatti D.*, Cocchietto M., Galeotti M.*, Bulfon C.*, Zorzin L.**, Ballestrazzi R.*, Bassignana D.*, Voinovich D.***, Gallo D.**, Prearo M.****, Tesei E.*, Sava G.**/*******

**Sezione di Patologia Veterinaria, Dipartimento di Scienze degli Alimenti, Facoltà di Medicina Veterinaria, Università degli Studi di Udine;*
***Fondazione Callerio Onlus, Istituti di Ricerche Biologiche, Trieste;*
****Dipartimento di Scienze Farmaceutiche, Università degli Studi di Trieste;*
*****Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta;*
******Dipartimento di Scienze della Vita, Università degli Studi di Trieste.*

XVII Convegno Nazionale
19-21 maggio 2011
Ostuni (BR)

SOCIETA' ITALIANA PATOLOGIA ITTICA

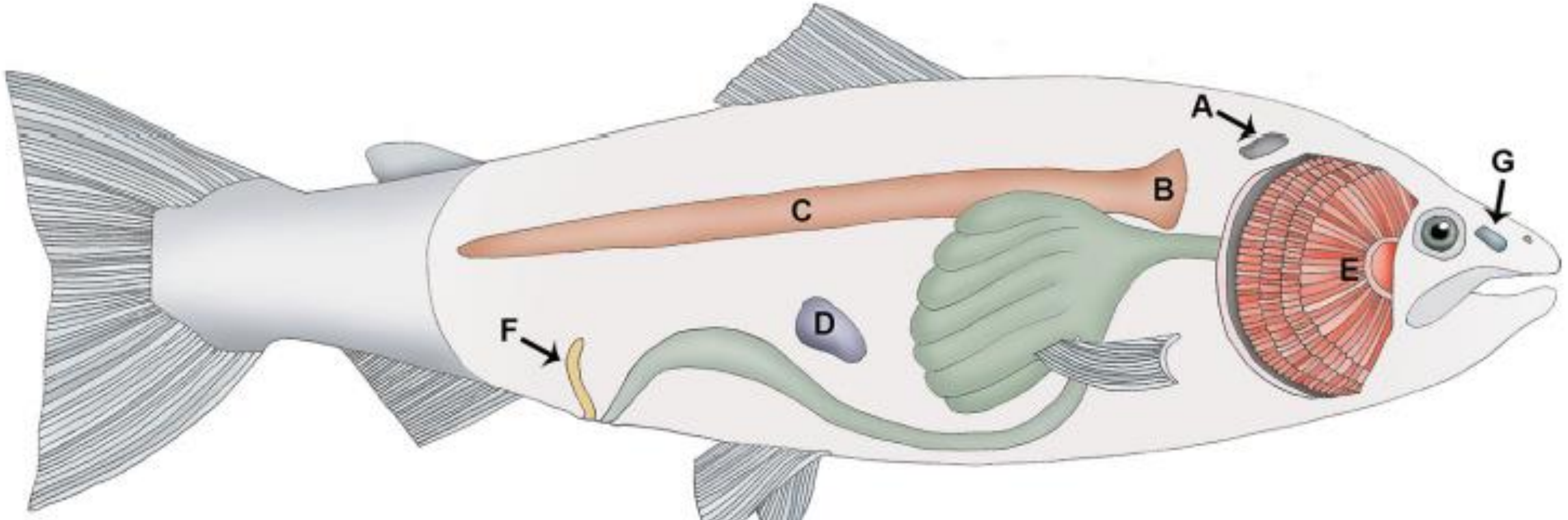


Systemic immunity

It consists in the involvement of immune cell populations colonising the central immune system – **thymus – head kidney – spleen.**

Antigens administered in the abdominal cavity (adipose tissue) are processed in this area and then can reach the central organs in order to promote a systemic response (T and B cells activation, cytokines synthesis, specific IgM synthesis)

Fig. 1 Schematic topography of immune organs in Atlantic salmon. **A** Thymus, **B** head kidney, **C** trunk kidney, **D** spleen, **E** gills with the interbranchial lymphoid tissue (ILT), **F** salmonid bursa, **G** olfactory organ with the nasopharynx-associated lymphoid tissue (NALT)



Håvard Bjørgen · Erling Olaf Koppang - Immunogenetics (2021) 73:53–63

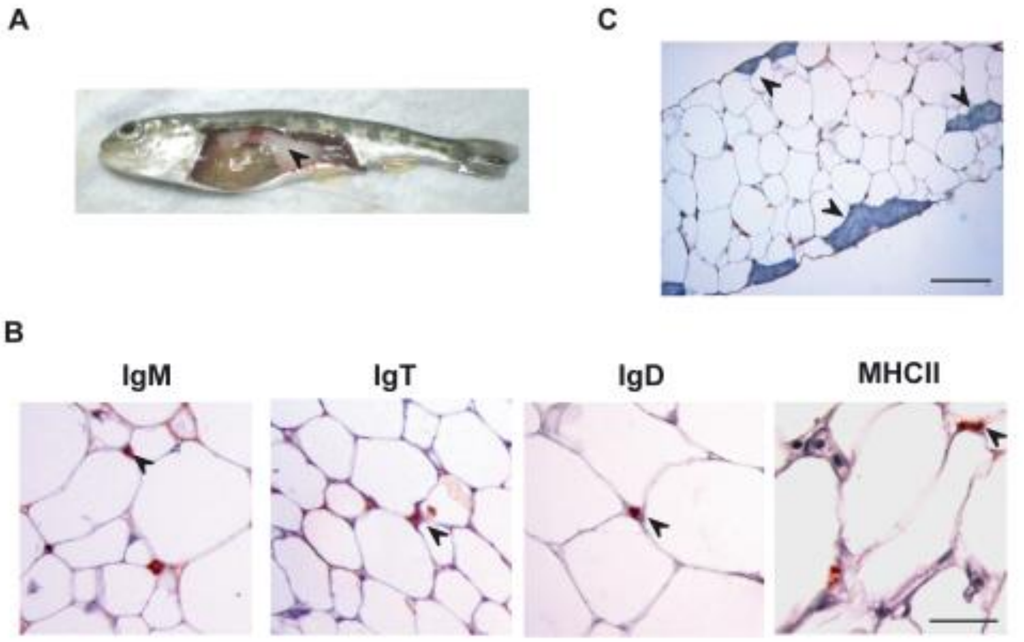
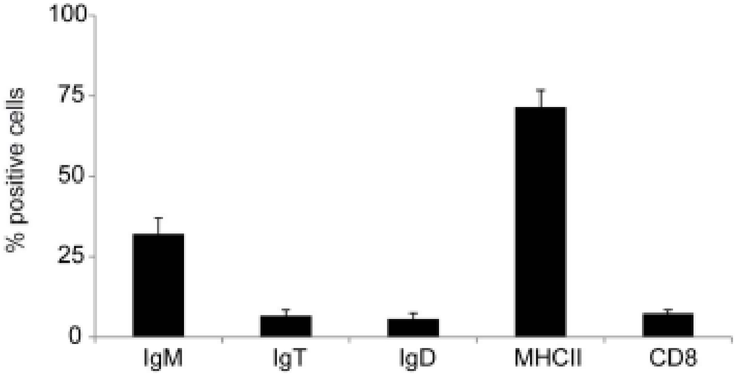


Figure 1. Immunohistological analysis of visceral rainbow trout AT. (A) The rainbow trout visceral AT (black arrow) was removed, fixed in Bouin's solution, embedded in paraffin and sectioned at 5 µm. After dewaxing and rehydration, sections were subjected to an indirect immunocytochemical method to detect trout IgM, IgT, IgD and MHC-II (B) Arrow heads point to representative positive staining. Scale bars, 50 µm. (C) Representative photomicrograph of an IgM immunostained section showing structures that resemble mammalian milky spots (arrow heads). Scale bars, 100 µm.
doi:10.1371/journal.pone.0110920.g001

Naïve fish AT found to contain APCs and T-cells which then increased in size, number and complexity following vaccination. Following peritoneal stimulation the visceral adipose mass in fish likely plays an important role in vaccine antigen uptake and presentation by APCs, as well as subsequent T-cell activation and differentiation.

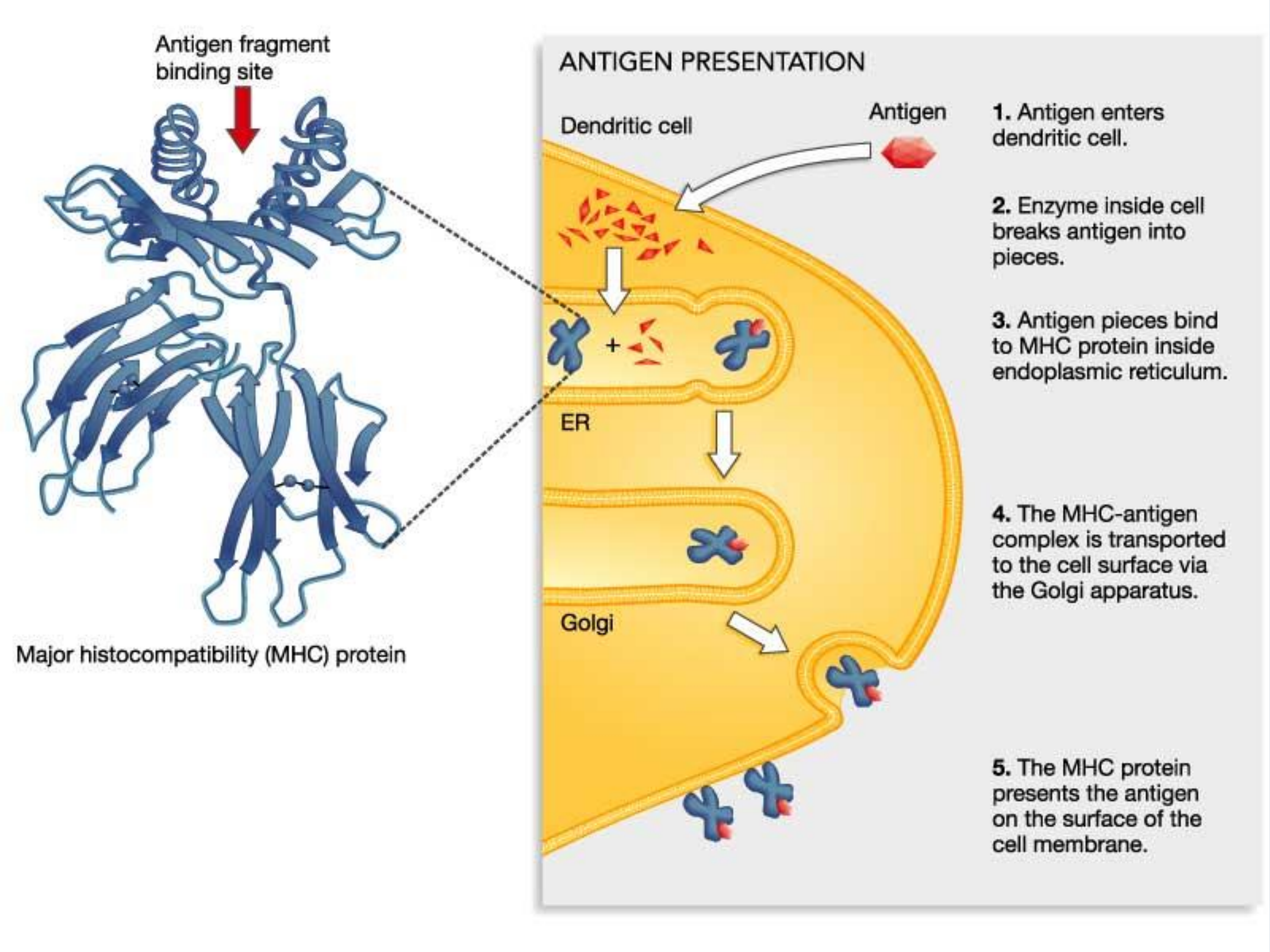


Pignatelli et al. (2014). PLoS ONE 9(10): e110920. doi:10.1371/journal.pone.0110920

Veenstra et al., 2019. Fish & Shellfish Immunology, Volume 87, https://doi.org/10.1016/j.fsi.2019.02.001.

ANTIGEN PRESENTING CELLS

THE ROLE OF MHC II



Immunoglobulin classes in fish

Teleost IgM:

tetrameric molecule - most prevalent immunoglobulin in plasma - gut and skin mucus are reported to have very low concentrations of IgM - apparent absence of intermolecular interactions mediated by immunoglobulin joining chains (J chains) - secreted mainly by plasma-like cells that are located mostly in the head kidney - after booster immunization teleost fish undergo a substantial increase in IgM titers (temperature dependent process)

Teleost IgD:

variety of secreted IgD isoforms with different molecular masses (monomers in serum) - the role of teleost IgD remains obscure

Teleost IgT:

specialized in gut mucosal immunity - present in serum as monomers, whereas in the gut mucus it forms mainly multimers similar in mass to those of IgM - most bacteria in the gut lumen of rainbow trout are coated with IgT, and IgT responses to gut parasites are measurable only in the gut, whereas IgM responses are detected only in serum - although it is suspected that IgT has a key role in other mucosal areas (such as the skin and gills) this remains to be investigated

Further relevant information on fish immunoglobulins….

First appearance of B-lymphocytes and immunoglobulins is late in marine fish compared to fresh water fish

Absence of isotype shift

Transfer of maternal antibodies to eggs and embryos demonstrated in several fish species. They may help the egg/embryo protection against vertical transfer of certain pathogens = IMPORTANT to develop protocols of vaccination addressed to broodstocks!

S. Zhang et al. / Developmental and Comparative Immunology 39 (2013) 72–78 73

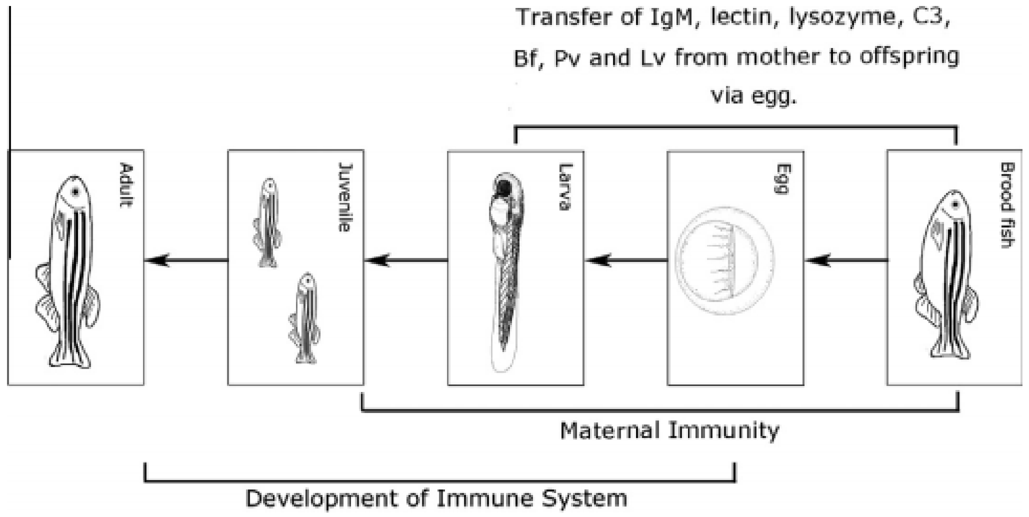
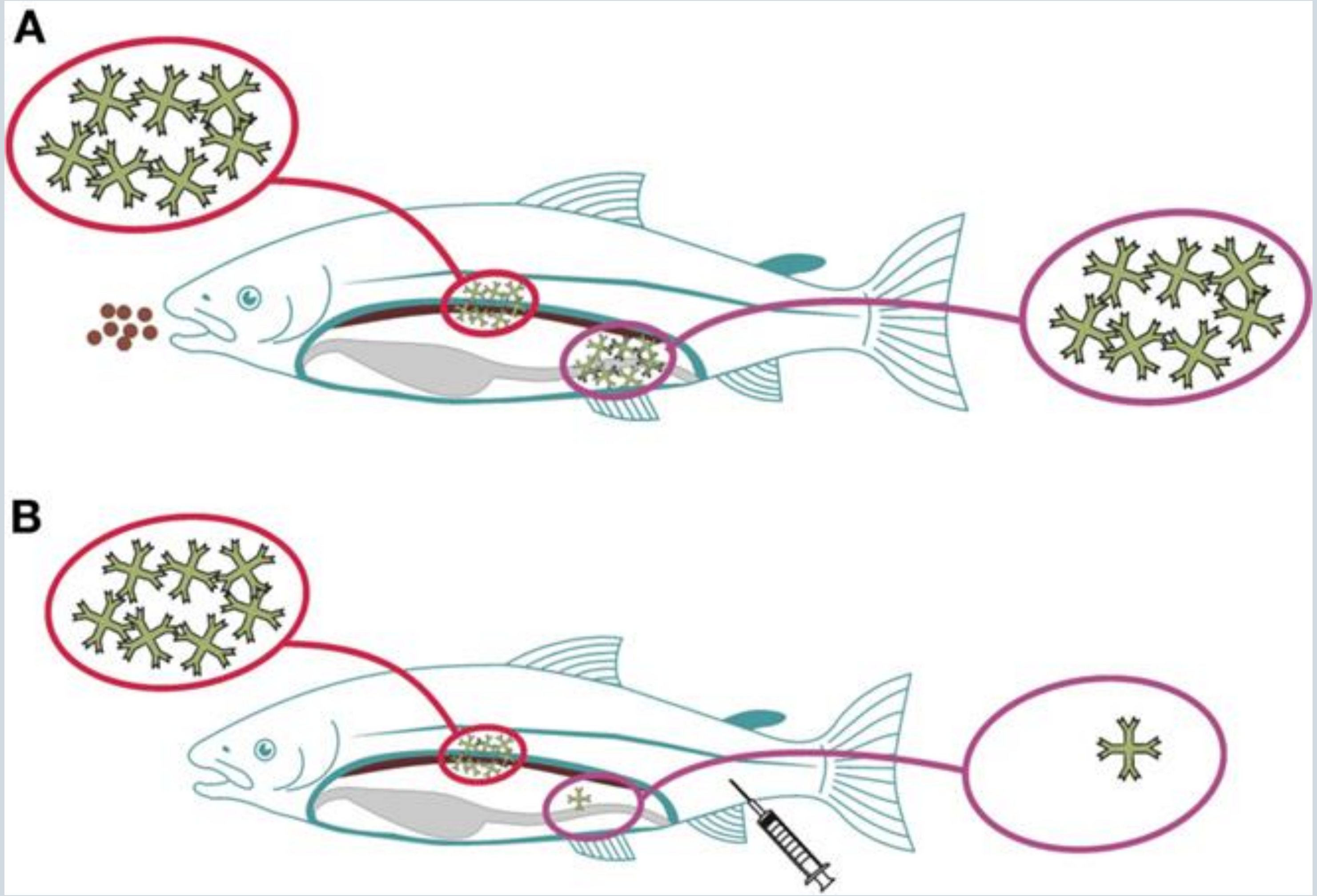


Fig. 1. A diagram showing the transfer and persistence of maternal immunity with respect to acquisition of immunocompetence at the different stages of development. Both innate and adaptive immune-relevant factors are transferred from mother to offspring, which play a critical role to protect the vulnerable offspring against pathogenic attacks before full development and maturation of immune system in fish.

PROPOSED ASYMMETRY FOR IMMUNE RESPONSES INDUCED VIA MUCOSAL (GUT) VERSUS PARENTERAL ROUTES IN FISH. When antigens are delivered via the gut, local and systemic immune responses will be elicited, symbolized by high amounts of circulating IgM (A). When the antigens are delivered parenterally, systemic responses will be strong, while local (gut) responses will be almost absent (B).

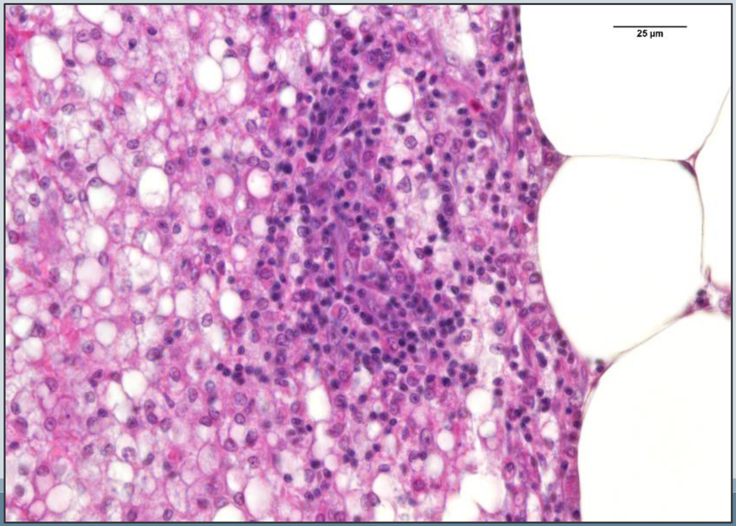
Mutoloki Stephen, Munang'andu Hetron Mweemba, Evensen Øystein. Oral Vaccination of Fish – Antigen Preparations, Uptake, and Immune Induction. Frontiers in Immunology
VOLUME=6 YEAR=2015

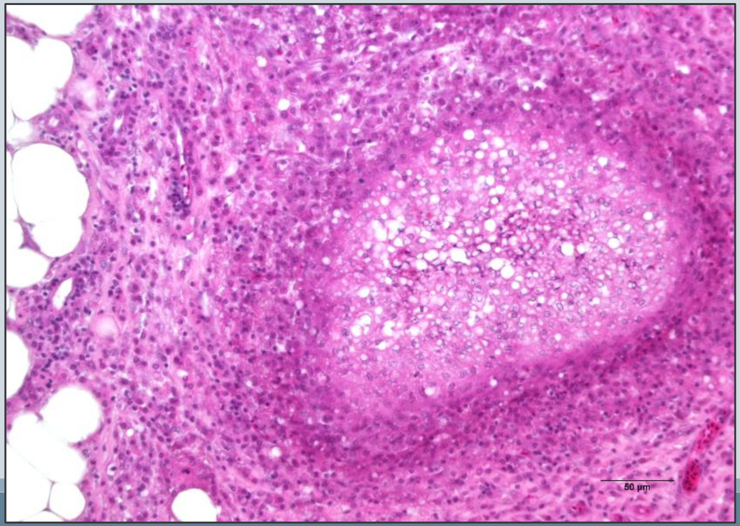


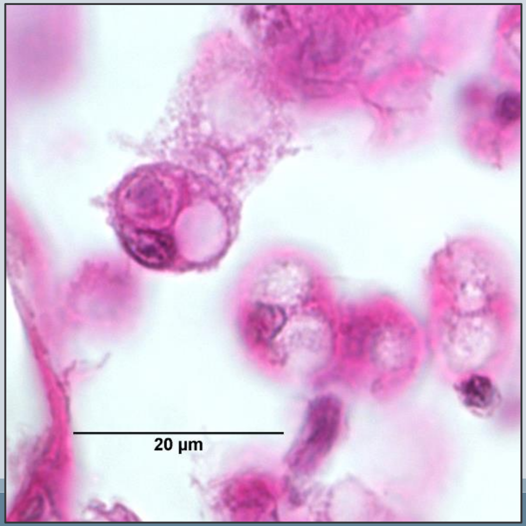
Adjuvants

To date, commercial vaccines mostly use mineral oils as adjuvants. These mineral oils help induce a more robust immune response than the antigen alone by increasing the immunogenicity of weak antigens, prolonging the duration of antigen release at the injection site, and also stimulating and modulating adaptive immune responses.

Study of vaccination side-effects



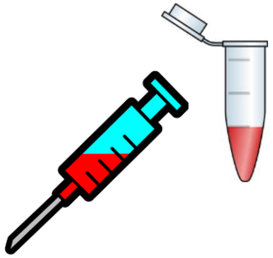




How can we evaluate the immune response of rainbow trout submitted to vaccination?

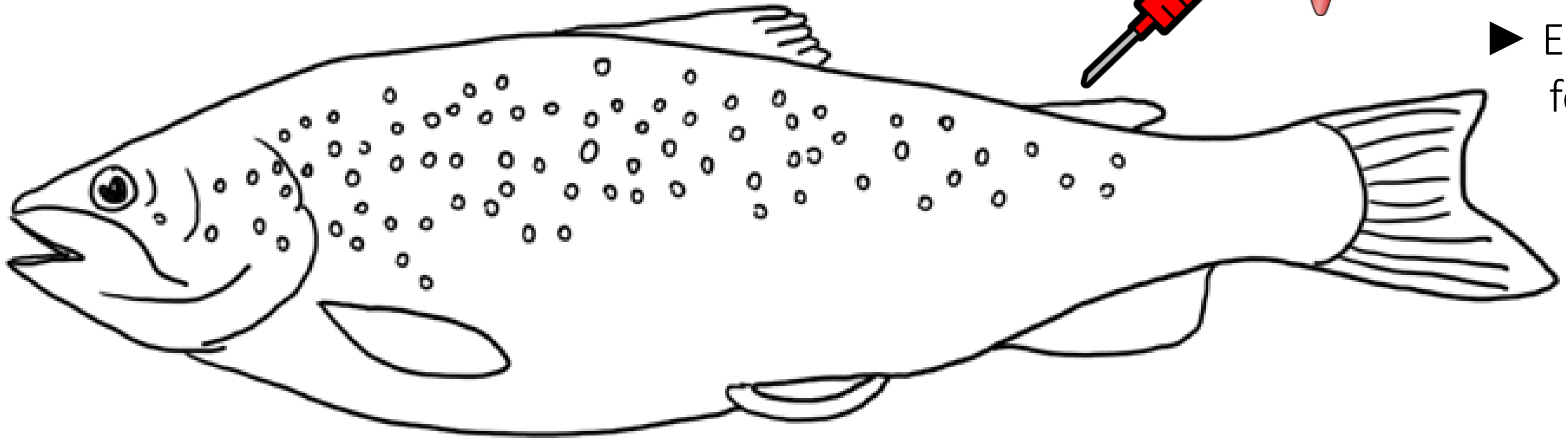
A proper sampling to study the post-vaccination response

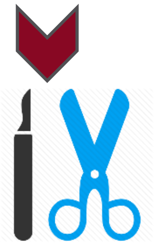
Skin mucus ► Lysozyme/Immunoglobulins



PBL ► leucogram

serum ► aspecific parameters
► E.L.I.S.A./agglutination for specific IgM

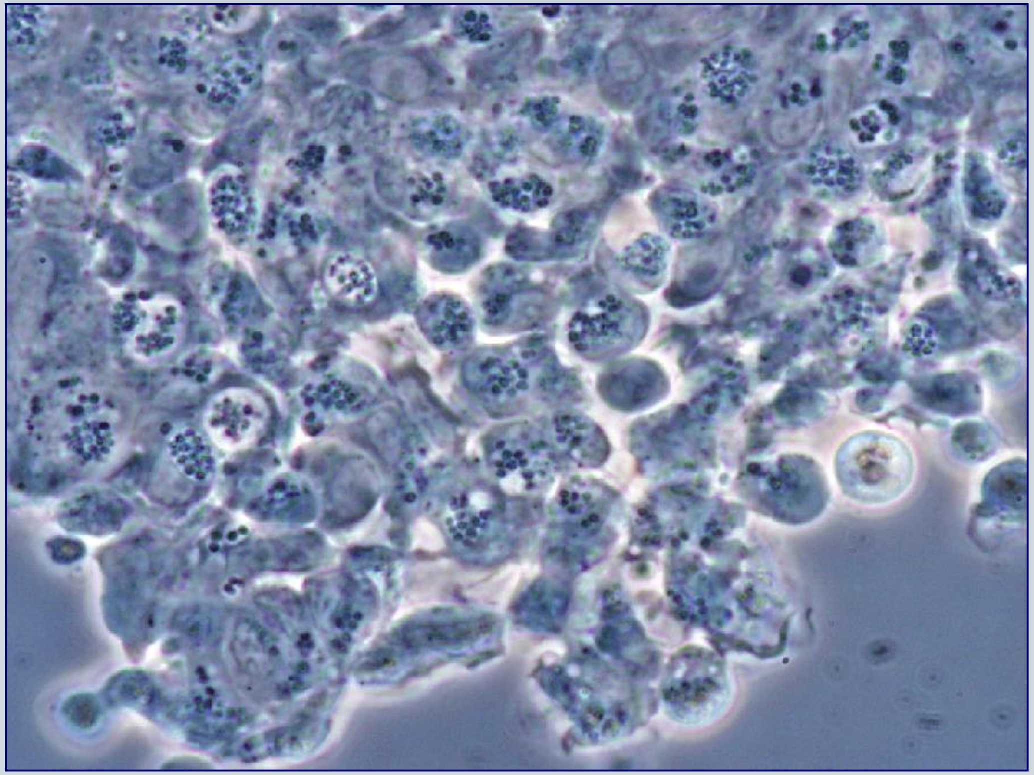




HK/PBL for leukocytes respiratory burst/proliferation
HK/spleen/gut/gills ► qPCR for immune genes expression

Pooled samples *versus* individual samples, suitable for serum/plasma, but also for leukocytes

In vitro evaluation of leukocytes ability to perform phagocytosis and respiratory burst



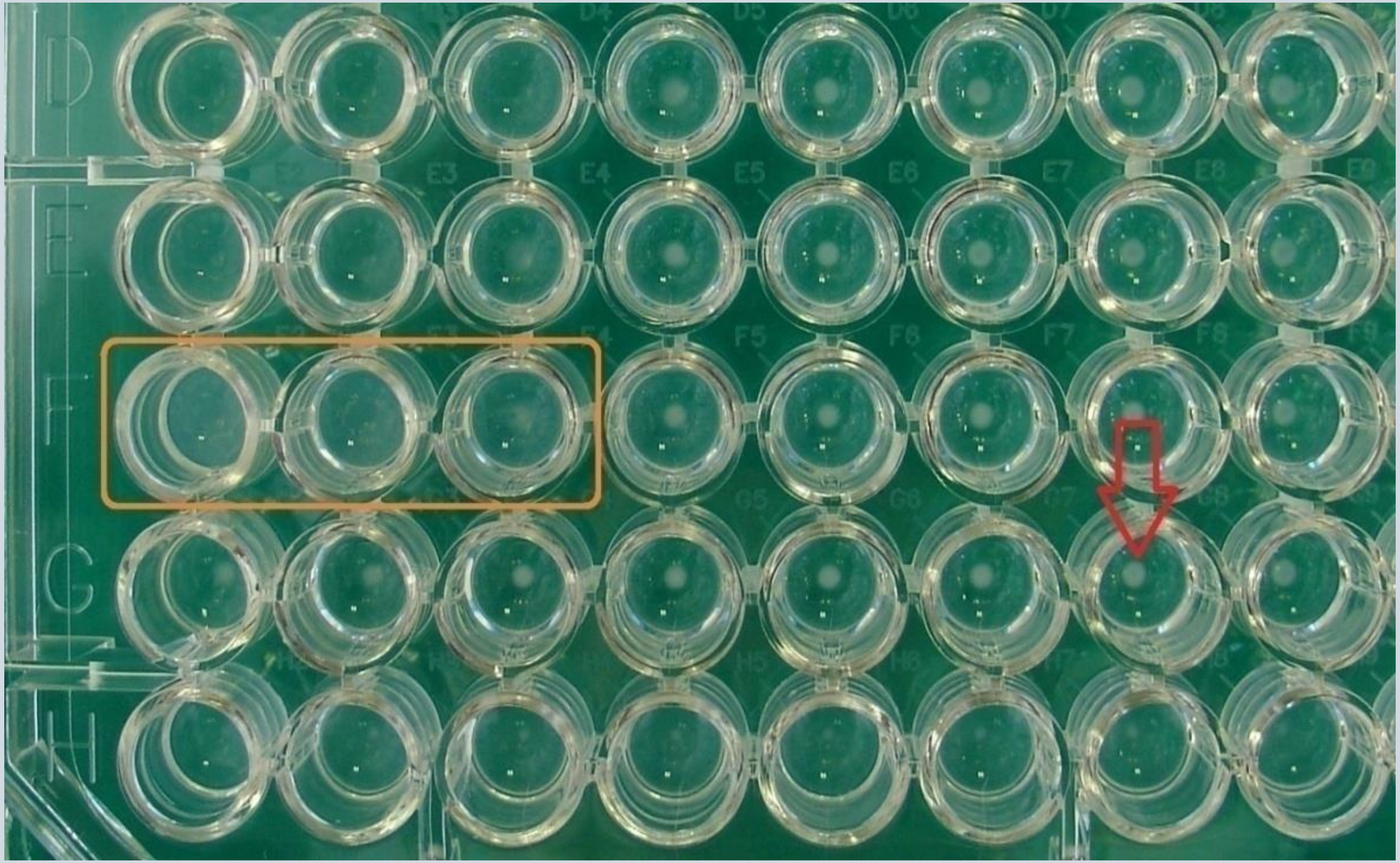
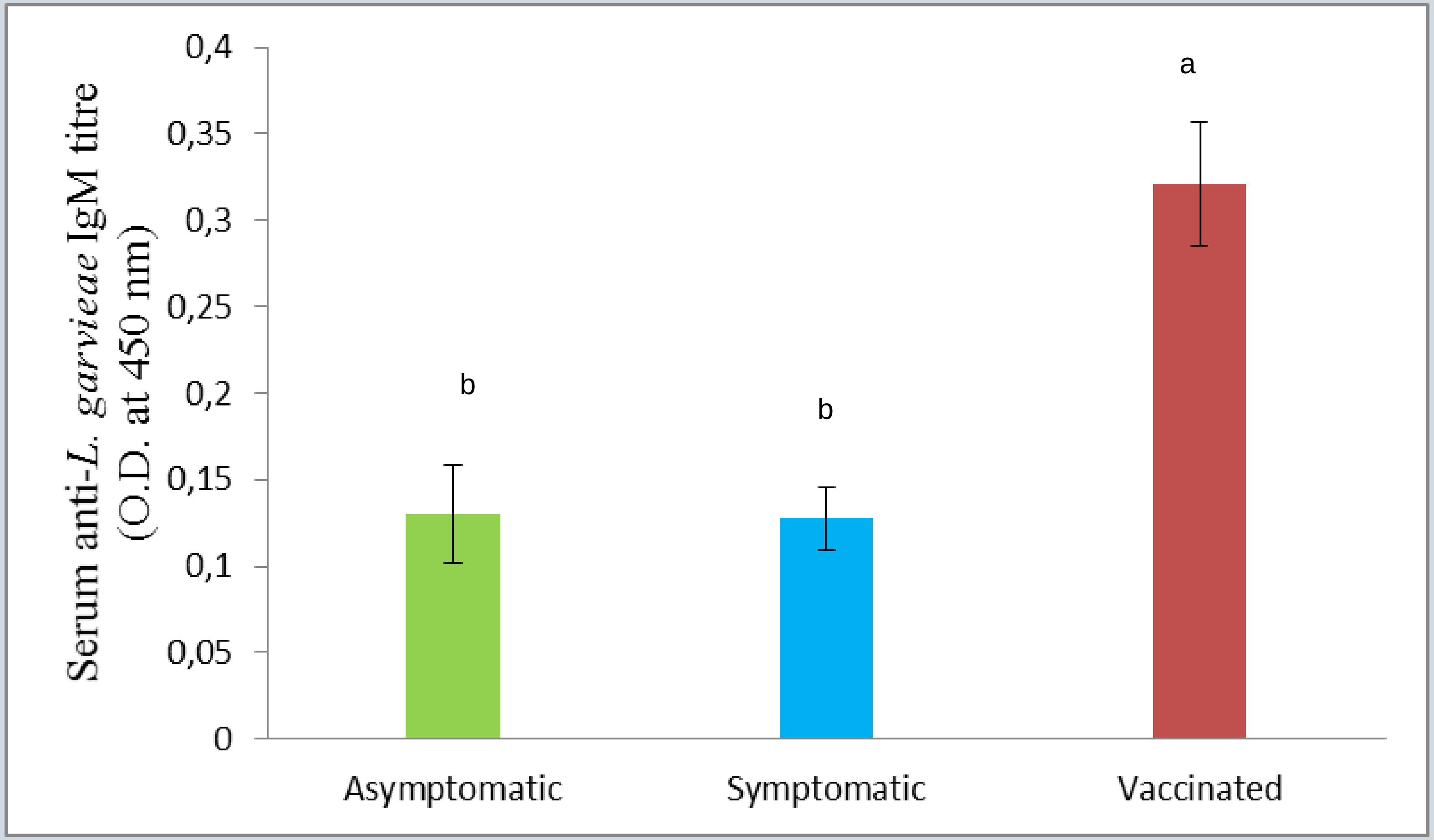


Fig. 6. Esempio di piastre usate per il test ELISA. Nel riquadro rosso è evidente la lettura ottenuta dai soggetti non vaccinati prelevati in avannotteria (controllo negativo). Nel riquadro azzurro è evidente la lettura ottenuta con un siero di trota disponibile in laboratorio (controllo positivo). Nel resto dei pozzetti sono contenuti i sieri oggetto di valutazione, con vari livelli di positività.



Serum IgM titres against *L. garvieae* detected by ELISA (OD at 450 nm) in different groups of rainbow trout. Data are expressed as mean ± SE (n = 10). Different letters indicate significant differences among groups ($P \le 0.05$).

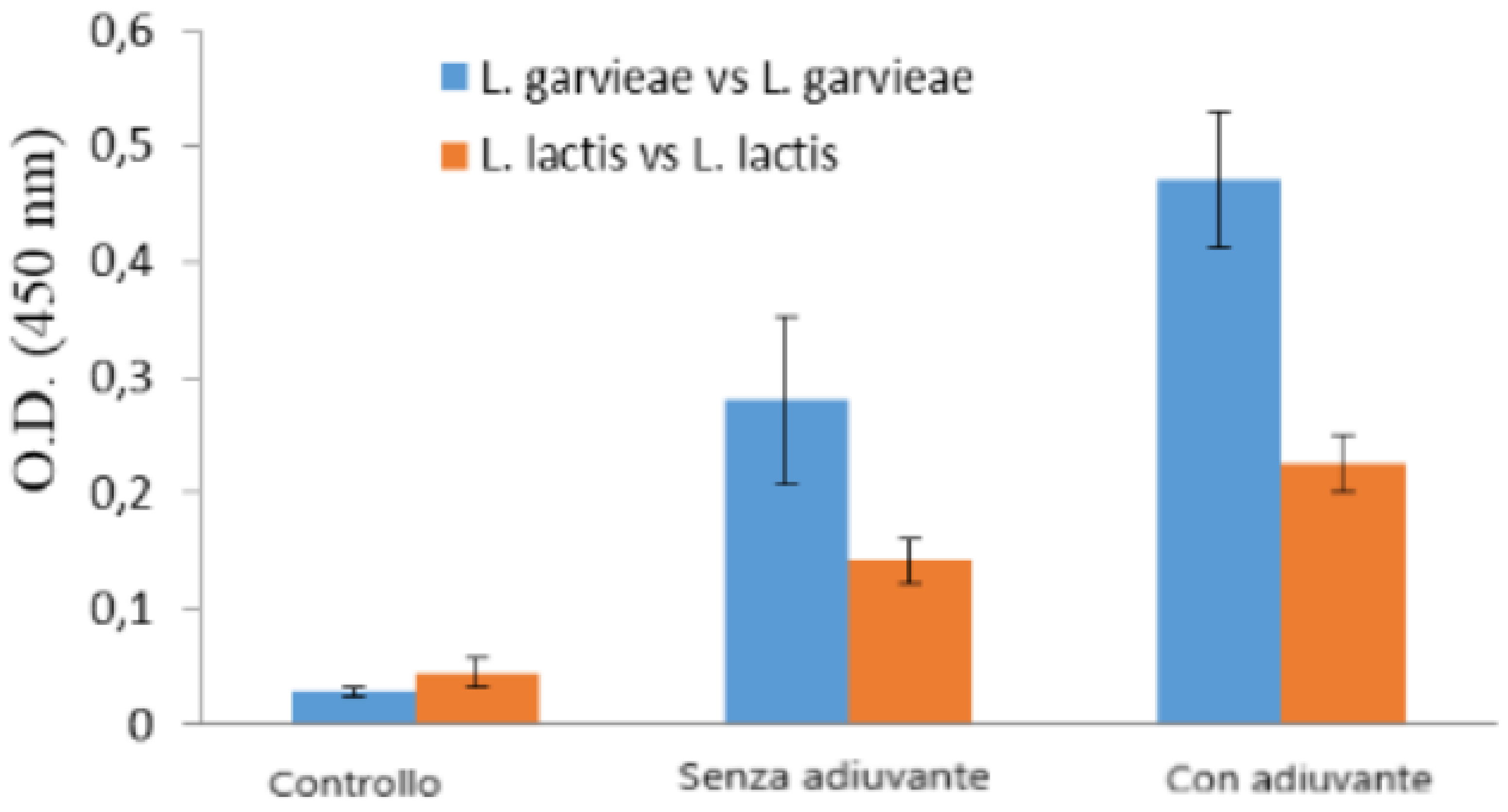


Grafico 1 - Risposta anticorpale specifica (IgM) dei soggetti vaccinati con bacterin *L. garvieae* o *L. lactis*, con e senza aggiunta di adiuvante. Nel grafico sono riportati anche i risultati ottenuti per i soggetti di controllo. Il coating è stato effettuato con il medesimo antigene usato per immunizzare i pesci. I sieri sono stati analizzati previa inattivazione al calore. Ogni gruppo sperimentale era composto da un numero di soggetti variabile da 3 a 11.

Commercially available antibodies useful to study rainbow trout immune response upon vaccination

target	details/company
Immunoglobulins	IgM IgT IgD https://vertebrateantibodies.com/products/fish/salmonids/ https://bocascientific.com/ IgM https://aquaticdiagnostics.com/ https://www.bio-rad.com/
Leukocyte populations	Linfociti T – CD3 https://vertebrateantibodies.com/products/fish/salmonids/ https://bocascientific.com/
Immunity mediators	TNF-alfa IFN-gamma IL-22 https://vertebrateantibodies.com/products/fish/salmonids/ https://bocascientific.com/

Fish cells secrete cytokines after stimulation/activation

Several cytokines (signal molecules of cellular communication) indientified and studied in fish, either through gene expression or functional activity

Target organs for expression studies… spleen, head kidney, gut mucosae

Valuable approach to study the fish response after infection/vaccination/immunostimulation

IL-1 beta (detected in 13 fish species)
TNF alfa and beta (rainbow trout, sea bass, sea bream, goldfish, catfish)
IFNs (salmonids)
……about 15 cytokines detected in fish up to now

Sebastián Reyes-Cerpa et al., 2012. Fish Cytokines and Immune Response. In: New Advances and Contributions to Fish Biology. DOI: 10.5772/53504

Reference primers available in GenBank for immune gene expression studies on rainbow trout

Gene	Forward primer	Gene	Reverse primer	GenBank accession number
T_IL-1β_F	ACATTGCCAACCTCATCATCG	T_IL-1β_R	TTGAGCAGGTCCTTGTCCTTG	AJ223954
T_IL-10_F	CGACTTTAAATCTCCCATCGAC	T_IL-10_R	GCATTGGACGATCTCTTTCTTC	AB118099
T_TNF-α_F	GGGGACAAACTGTGGACTGA	T_TNF-α_R	GAAGTTCTTGCCCTGCTCTG	AJ277604
T_IL-8_F	AGAATGTCAGCCAGCCTTGT	T_IL-8_R	TCTCAGACTCATCCCCTCAGT	AJ279069
T_IgT_F	AGCACCAGGGTGAAACCA	T_IgT_R	GCGGTGGGTTCAGAGTCA	AY870265
T_Tlr5_F	GGCATCAGCCTGTTGAATTT	T_Tlr5_R	ATGAAGAGCGAGAGCCTCAG	AB091105
T_IL-6_F	ACTCCCCTCTGTCACACACC	T_IL-6_R	GGCAGACAGGTCCTCCACTA	DQ866150
T_IgM_F	CTTGGCTTGTTGACGATGAG	T_IgM_R	GGCTAGTGGTGTTGAATTGG	S63348
T_MHC-I_F	TCCCTCCCTCAGTGTCT	T_MHC-I_R	GGGTAGAAACCTGTAGCGTG	AY523661
T_MHC-II_F	TGCCATGCTGATGTGCAG	T_MHC-II_R	GTCCCTCAGCCAGGTCACT	AF115533
T_TCR-β_F	CTCCGCTAAGGAGTGTGAAGATAG	T_TCR-β_R	CAGGCCATAGAAGGTACTCTTAGC	AF329700
T_β-actin_F	ACAGACTGTACCCATCCCAAAC	T_β-actin_R	AAAAAGCGCCAAAATAACAGAA	AJ438158

Veterinary Immunology and Immunopathology 213 (2019) 109885

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Research paper

Protec™ improves innate immune response and specific antibody response against *Lactococcus garvieae* in rainbow trout (*Oncorhynchus mykiss*)

Chiara Bulfon[a,*], Valentina Pacorig[a], Massimo Sarti[b], Umberto Luzzana[b], Marco Galeotti[a], Donatella Volpatti[a]

Thanks for your attention ☺