Istituto Zooprofilattico Sperimentale
dell'Umbria e delle Marche "Togo Rosati"

Challenges and opportunity in the Fish Laboratories activities

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Istituto Zooprofilattico Sperimentale Umbria e Marche – IZSUM
Perugia – ITALY

Second Aquatic Animal Health Workshop for Central Asia and Transcaucasian countries
and Central Asia and Transcaucasian Aquatic Animal Health Network
Tashkent - Uzbekistan
24th – 26th April 2024

FISH FARMING

- Non-specific and not always pathognomonic symptoms;
- acute or chronic mortality;
- need to check post-therapy health status;
- need to identify healthy carriers;
- farmer's need to start early therapy.

WILD ENVIRONMENT

- Non-specific and not always pathognomonic symptoms;
- often acute mortality episodes with a strong impact on public opinion (sites of tourist interest);
- need for the competent authorities to resolve the problem as quickly as possible.

LABORATORY DIAGNOSIS

Istituti Zooprofilattici Sperimentali (IZS) are public veterinary institutes covering the whole Italian territory.

Main fields of interest of the Institutes are:

- animal health and welfare
- infectious diseases of animals
- zoonosis
- food safety
- risk analysis & communication
- epidemiology

for all animal species, including aquatic animals.

Headquarter: Perugia (Umbria Region)

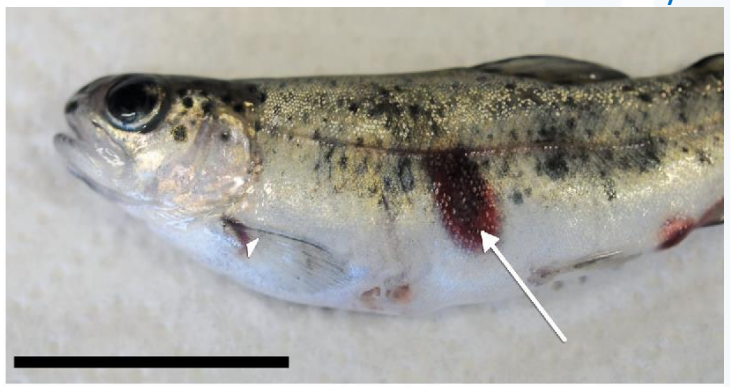
Specialistic Center for Fish Diseases: Terni (Umbria Region)

- Anatomo-pathological, virological, bacteriological, parasitological exams
- Share diagnostic protocols
- Share references materials
- Training of personnel
- Collaboration in research
- Partecipation to proficiency tests
- Partecipation in annual meeting between IIZZSS

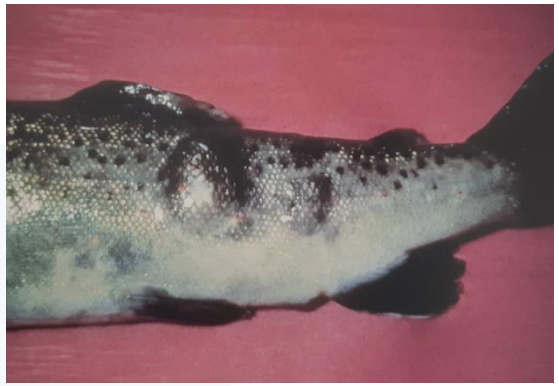
Red Mouth Disease by *Yersinia ruckeri*

Agnetti, 2022

Forunculosis by *Aeromonas salmonicida*

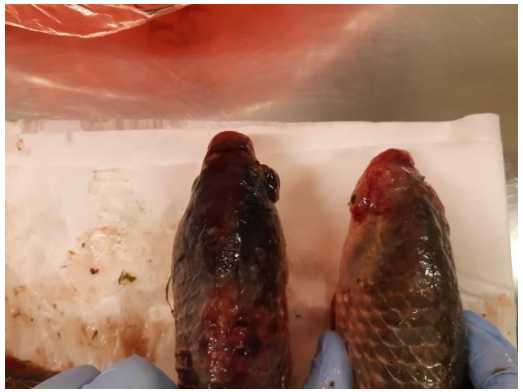


Menanteau-Ledouble, 2016



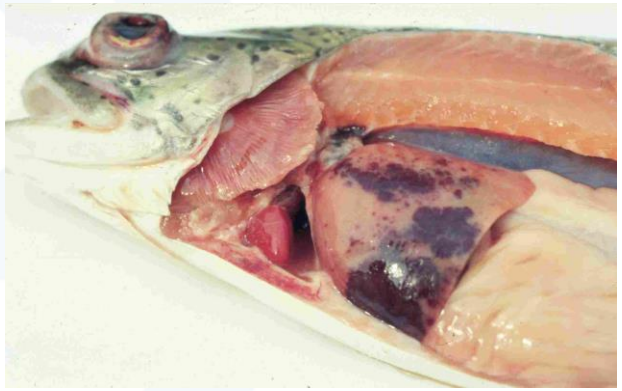
C. Ghittino, 2003

Bacterial septicemia by *Aeromonas* spp.



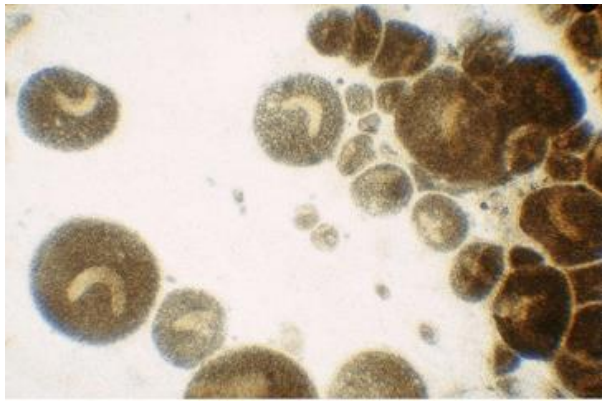
Agnetti, 2021

Lactococcosis by *Lactococcus garvieae*



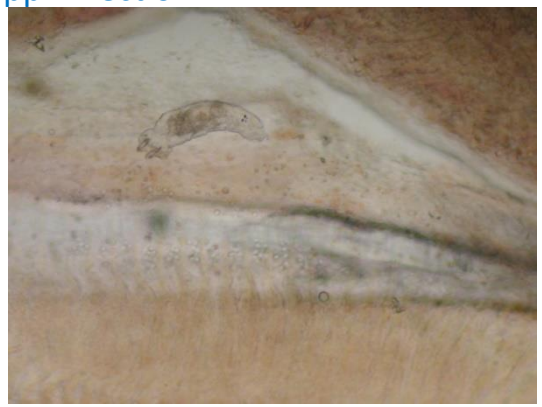
Ghittino, 2003

Ichthyophthirius spp. infection



Agnetti, 2011

Gyrodactylus spp. infection



Agnetti, 2011

Viral Haemorragic Septicemia or Infectious Haematopoietic Necrosis

Ghittino, 2003

Spring Viremia of Carp or Koi Herpesvirus or other Cyprinid viral diseases

Agnetti, 2021

Diagnostic protocols

Salmonids/Cyprinids

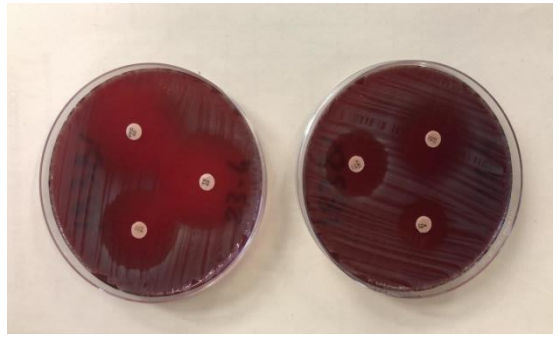
- Viral diseases:
  - isolation on cells cultures and identification through direct immunofluorescence (10-15 days)
  - End-point PCR (3-5 days)
- Bacterial diseases:
  - Isolation and identification through cultural standard methods, biochemical test, end-point PCR, mass spectrometry (Maldi-tof) (3-5 days)
- Parasitical diseases:
  - Direct microscopical examination (1 day)
  - artificial digestion of muscle samples (1-3 days)

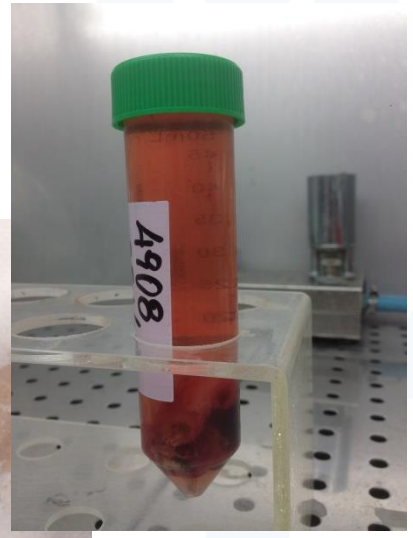
The sample should be fresh and arrive at the lab within 24 hours!

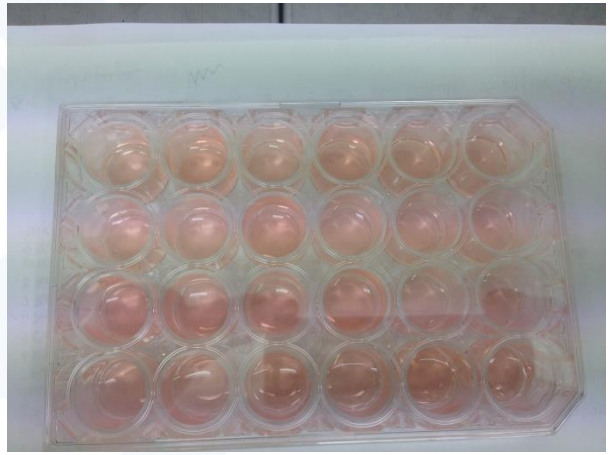
It is better to take moribund fish or fish that have been dead for a few hours.

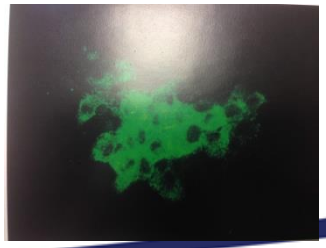
Ideal transport temperature: +4-5°C.

Pictures by F. Agnetti, 2023









Laboratory as an observation point for antimicrobial resistance (AMR)…

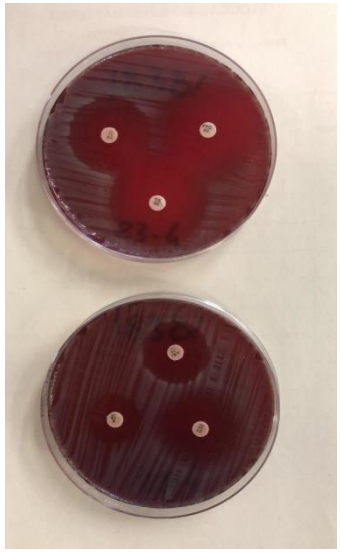


Table 6. Estimated PCU (in 1,000 tonnes) of the population of food-producing animals[1,2], by country, in 2020

Country	Cattle	Pigs	Poultry	Sheep and goats	Fish	Rabbits	Horses	Total
Austria	422	357	89	36	0	0	38	942
Belgium	484	832	285	19	0	4	122	1,745
Bulgaria	114	76	42	100	14	<0.01	23	368
Croatia	103	92	49	52	21	0.01	11	329
Cyprus	20	45	13	43	0	0.1	2	123
Czechia	288	199	131	16	20	7	39	699
Denmark	380	1,754	123	12	46	0	70	2,385
Estonia	59	43	2	5	1	0	5	116
Finland	207	146	85	12	15	0	30	494
France	3,065	1,811	1,087	644	46	39	272	6,965
Germany	2,922	3,534	1,022	132	19	23	520	8,173
Greece	77	103	144	759	129	2	2	1,217
Hungary	152	315	211	82	9	8	24	801
Iceland	19	6	6	41	41	0	23	135
Ireland	1,304	294	111	344	38	0	100	2,190
Italy	1,424	782	766	571	59	30	157	3,790

European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption, 2021.
'Sales of veterinary antimicrobial agents in 31 European countries in 2019 and 2020'. (EMA/58183/2021)

Laboratory as an observation point for antimicrobial resistance (AMR)…

- Current resarch project: Evaluation of Epidemiological Cut Offs (ECOFF) for the execution of Minimal Inhibitory Concentrations (M.I.C.) against the main bacterial pathogens of fish MIC

- Participants:
- **U.O. IMS 01** – Amedeo Manfrin CRN **IZS delle Venezie** – Lab. di batteriologia
- **U.O. IMS 02** - Monia Cocchi – **IZS delle Venezie** - Sezione di Udine
- **U.O. IMS 03** – Marica Toson – **IZS delle Venezie** - SCS4 Lab. epidemiologia applicata all'ambiente acquatico
- **U.O. IMS 04** – Giuseppe Arcangeli – **IZS delle Venezie** – Lab. molluschi
- **U.O. IMS 05** – Paolo Pastorino– IZS Piemonte Liguria Valle d'Aosta – Paolo Pastorino– **IZS Piemonte Liguria Valle d'Aosta** – Laboratorio di Acquacoltura, Ittiopatologia e Biologia degli ambienti acquatici
- **U.O. IMS 06** – Francesco Agnetti – **IZS Umbria e Marche** - Sezione di Terni
- **U.O. IMS 07** – Francesca Susini – **IZS Lazio e Toscana** – Lab. ittiopatologia Sezione di Pisa
- **U.O. IMS 08** – Teresa Bossù – **IZS Lazio e Toscana** – Roma – Centro Regionale per gli enterobatteri patogeni
- **U.O. IMS 09** – Fabio Di Nocera – **IZS del Mezzogiorno** – Portici (NA) – Lab. diagnostica speciale: ittiopatologia
- **U.O. EMS 10** – Marialetizia Fioravanti – **University of Bologna -** Dipartimento di Scienze Mediche Veterinarie

Aim

- Determine the Epidemiological Cut Offs of the most important marine bacterial pathogens (*Vibrio anguillarum, Vibrio harveyi, Photobacterium damselae subsp. piscicida*) and freshwater (*Aeromonas salmonicida, Yersinia ruckeri and Lactococcus garvieae*) in relation to the main drugs that can be used as therapy in aquaculture

Material and method

- Standardize the M.I.C. methods (hands on training)
- Test a large panel of bacterial isolates coming from the repositories of the different IIZZSS
- Data collection and analysis

- **Results:** preliminary results on *Aeromonas salmonicida*

LABORATORIO	N° ISOLATI	% RELATIVA
IZSLT-PISA	15	15%
IZSUM	15	15%
IZSVE-CSI	23	23%
IZSVE-UDINE	15	15%
UNIBO	33	33%
TOTALE	101	100%

susceptible strain

	1	2	3	4	5	6	7	8	9	10	11	12
A	ERY 32	ERY 16	ERY 8	ERY 4	ERY 2	ERY 1	ERY 0,5	ERY 0,25	ERY 0,125	ERY 0,063	GC+	GC-
B	ENR 8	ENR 4	ENR 2	ENR 1	ENR 0,5	ENR 0,25	ENR 0,125	ENR 0,063	ENR 0,031	ENR 0,016	ENR 0,008	ENR 0.004
C	FLM 32	FLM 16	FLM 8	FLM 4	FLM 2	FLM 1	FLM 0,5	FLM 0,25	FLM 0,125	FLM 0,063	FLM 0,031	FLM 0,016
D	AMX 32	AMX 16	AMX 8	AMX 4	AMX 2	AMX 1	AMX 0,5	AMX 0,25	AMX 0,125	AMX 0,063	AMX 0,031	AMX 0,016
E	FLL 64	FLL 32	FLL 16	FLL 8	FLL 4	FLL 2	FLL 1	FLL 0,5	FLL 0,25	FLL 0,125	FLL 0,063	FLL 0,031
F	OXY 16	OXY 8	OXY 4	OXY 2	OXY 1	OXY 0,5	OXY 0,25	OXY 0,125	OXY 0,063	OXY 0,031	OXY 0,016	OXY 0,008
G	DOX 16	DOX 8	DOX 4	DOX 2	DOX 1	DOX 0,5	DOX 0,25	DOX 0,125	DOX 0,063	DOX 0,031	DOX 0,016	DOX 0,008
H	T/S 8/152	T/S 4/76	T/S 2/38	T/S 1/19	T/S 0,5/9,5	T/S 0,25/ 4,75	T/S 0,125/ 2,375	T/S 0,063/ 1,188	T/S 0,031/ 0,594	T/S 0,016/ 0,297	T/S 0,008/ 0,148	T/S 0,004/ 0,074

resistant strain

	1	2	3	4	5	6	7	8	9	10	11	12
A	ERY 32	ERY 16	ERY 8	ERY 4	ERY 2	ERY 1	ERY 0,5	ERY 0,25	ERY 0,125	ERY 0,063	GC+	GC-
B	ENR 8	ENR 4	ENR 2	ENR 1	ENR 0,5	ENR 0,25	ENR 0,125	ENR 0,063	ENR 0,031	ENR 0,016	ENR 0,008	ENR 0.004
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D	AMX 32	AMX 16	AMX 8	AMX 4	AMX 2	AMX 1	AMX 0,5	AMX 0,25	AMX 0,125	AMX 0,063	AMX 0,031	AMX 0,016
E	FLL 64	FLL 32	FLL 16	FLL 8	FLL 4	FLL 2	FLL 1	FLL 0,5	FLL 0,25	FLL 0,125	FLL 0,063	FLL 0,031
F	OXY 16	OXY 8	OXY 4	OXY 2	OXY 1	OXY 0,5	OXY 0,25	OXY 0,125	OXY 0,063	OXY 0,031	OXY 0,016	OXY 0,008
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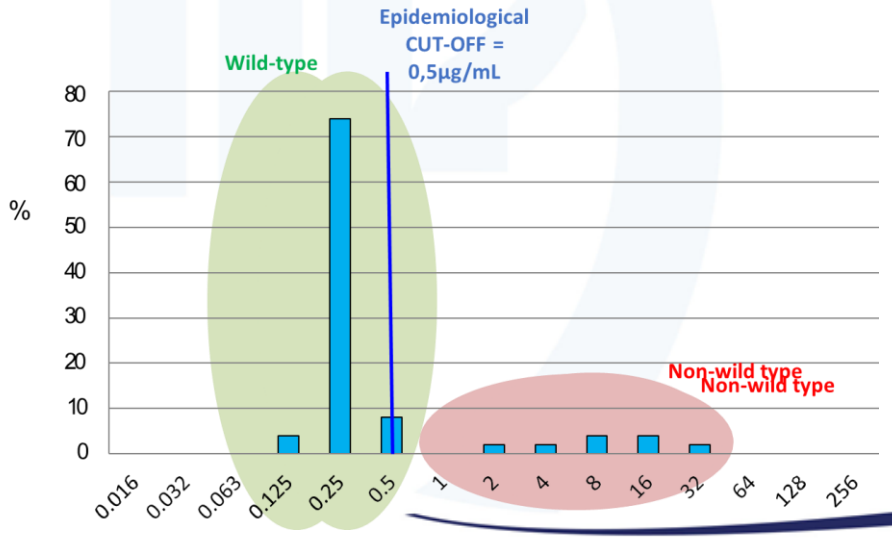
H	T/S 8/152	T/S 4/76	T/S 2/38	T/S 1/19	T/S 0,5/9,5	T/S 0,25/ 4,75	T/S 0,125/ 2,375	T/S 0,063/ 1,188	T/S 0,031/ 0,594	T/S 0,016/ 0,297	T/S 0,008/ 0,148	T/S 0,004/ 0,074

- On going analisys on *Yersinia ruckeri, Vibrio harveyi* and *Lactococcus garvieae*



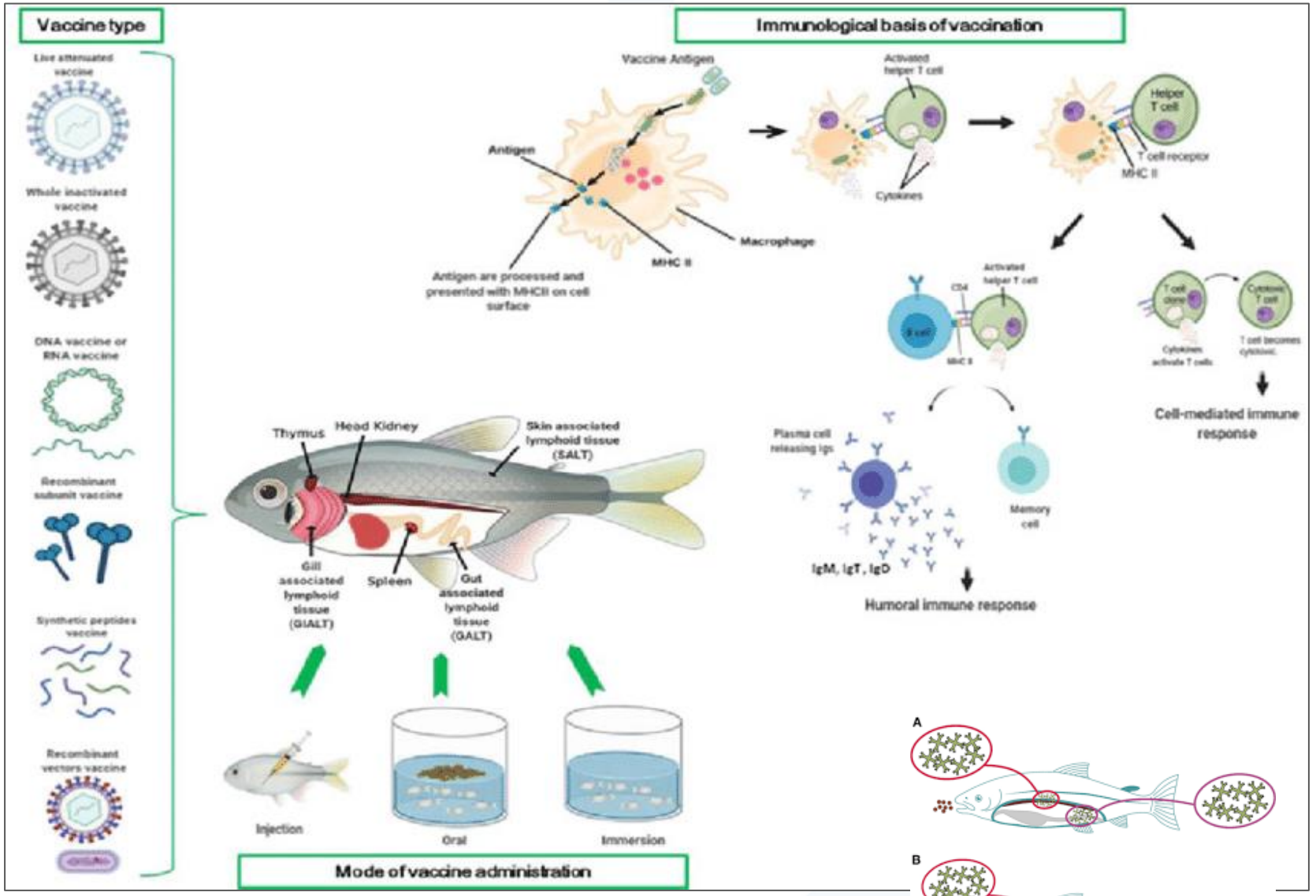
Project: **“Dietary supplementation with olive mill waste in farmed fish” - RC2021**

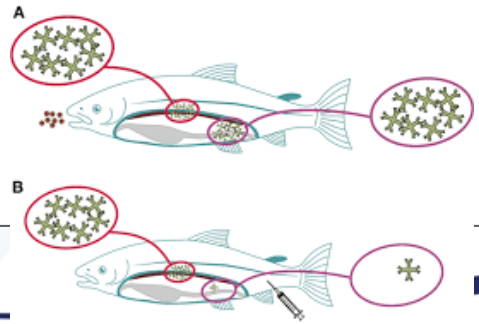
financed by Italian Ministry of Health

- evaluate the possibility of integrating the diet with polyphenolic extracts from vegetation waters and the effects on production performance, of fish (sea bream, *Sparus aurata*);
- evaluate the effects on animal welfare and **immune response**;
- estimate the presence of polyphenols in feed and in the meat of fish subjected to a controlled diet and their effects on the oxidative status.

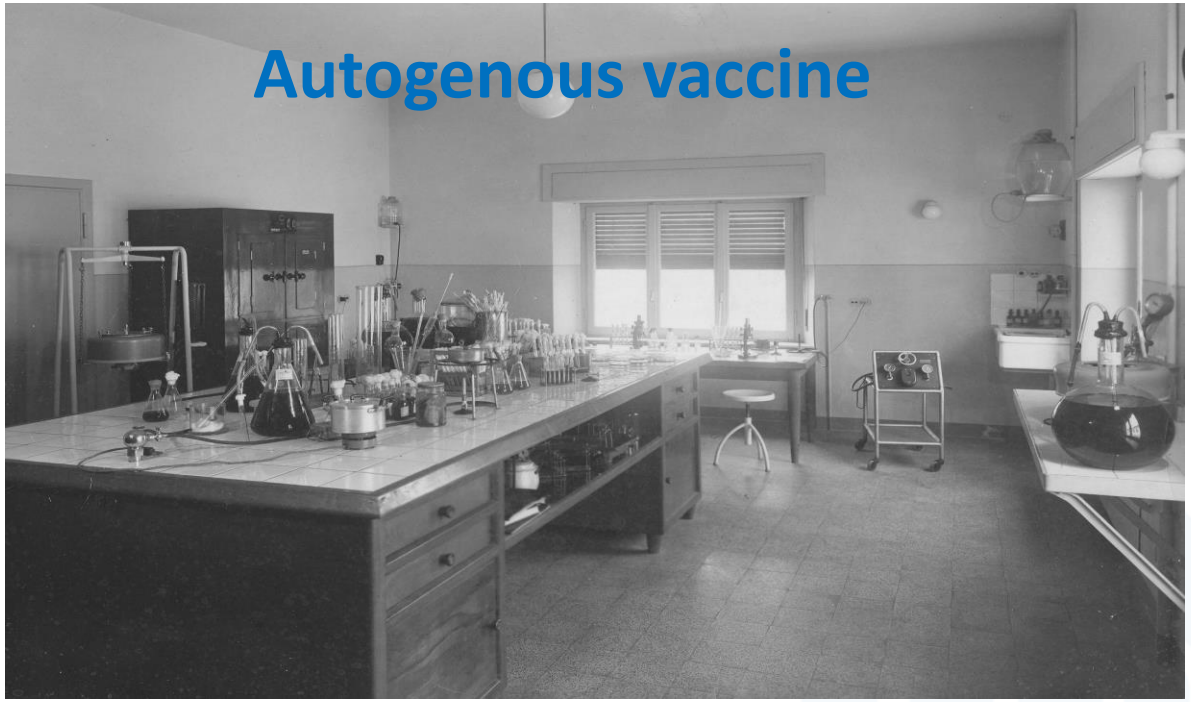
possibility of reducing drugs in aquaculture

Laboratory as a starting point for the formulation of a vaccine…





Autogenous vaccine



The autogenous vaccine is a veterinary immunological drug prepared with pathogens and / or antigens isolated from subjects affected by an outbreak of an infectious disease in a particular farm and used for the treatment of the same animals of the farm or animals of the same country, if the DVM believe it appropriate for evident epidemiological reasons (**DIRECTIVE 2001/82/EC** . Article 3. 2: Inactivated immunological veterinary medical products …)

Italian II.ZZ.SS. authorized to produce autogenous vaccines by the Ministry of Health

Lactococcosis by *Lactococcus garvieae*

IZSPLV - Turin

IZSVe - Verona

Red mouth disease by *Yersinia ruckeri* biotype II
Lactococcosis by *Lactococcus garvieae*

Vibriosis by *Vibrio harveyi*
Vibriosis by *Vibrio alginolyticus*

IZSLT - Siena

IZSUM - Perugia

Red mouth disease by *Yersinia ruckeri* biotype II
Foruncolosis by *Aeromonas salmonicida*
Lactococcosis by *Lactococcus garvieae*

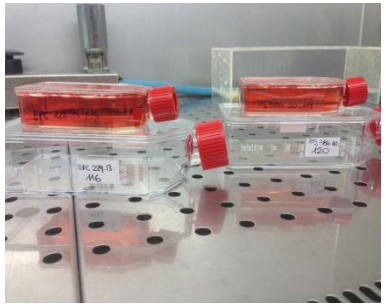
Laboratory must give a response to…

LABORATORY

- **Pharmaceutical companies**
- **Scientific community**
- **Fish farmers**
- **Competent Authorities**
- **Private citizens**
- **Other laboratories**

Large investments in…

Laboratory instruments



Laboratory reagents/materials

Quality certification according to current legislation

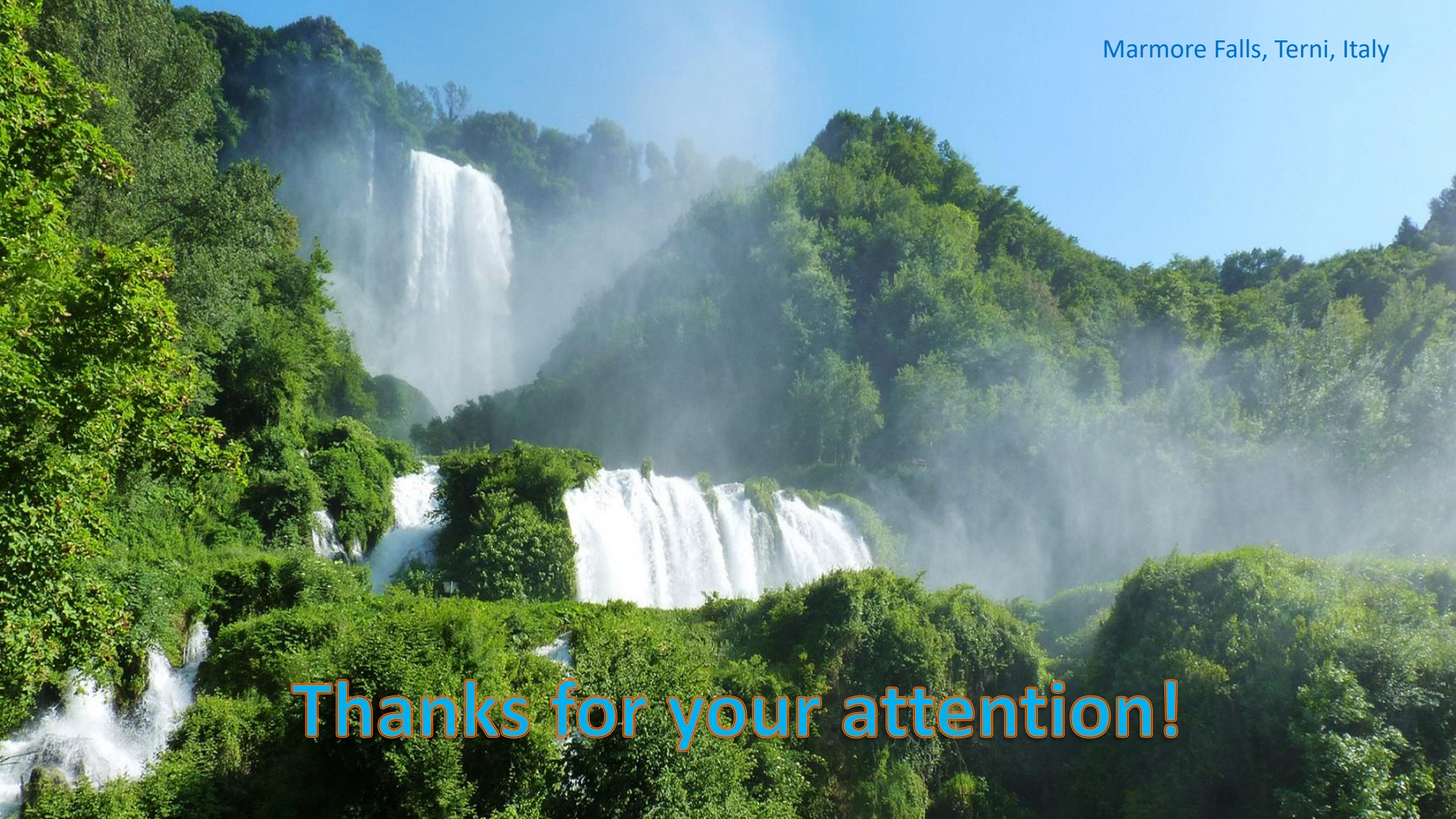
Large investments in staff training

Participation to proficiency tests (PTs)

- Test laboratory methods
- Test laboratory reagents and reference materials (controls)
- Test the operator's performance

Participation to annual laboratory meeting

- Update scientific knowledge on aquatic animal diseases
- Update on application of EU/National Regulation
- Discuss interesting clinical cases
- Discuss results of PTs


Marmore Falls, Terni, Italy
Thanks for your attention!