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## **Efficacy of autogenous vaccine administered by three routes against furunculosis by *Aeromonas salmonicida* in large trout (*Oncorhynchus mykiss*)**

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## CONTEXT

### Increased risk of furunculosis in rainbow trout farms for several reasons

- Furunculosis due to *Aeromonas salmonicida* sub. *Salmonicida* (Ass) affects salmonids of all sizes and is most prevalent when water temperatures > 18°C.
- The rearing time of rainbow trout is longer to produce fish > 2 kg for smoked products and eggs, which leads to keep fish during summer with higher water temperatures
- The global warming induces an increase of water temperatures in fish farms

### Vaccination against furunculosis in rainbow trout (RT) in France

- The efficacy is controversial
- Vaccination protocols are highly heterogeneous between farms (routes of administration, number of doses, size of fish vaccinated, season etc.)

## OBJECTIVES OF THIS STUDY

### To advise breeders for an optimal protocol, we have :

- Compared the efficacy and safety of an autogenous vaccine administered by different routes (intraperitoneal, immersion, oral) to large trout (> 150g)
- Evaluated the duration of protection to study whether booster administrations are necessary

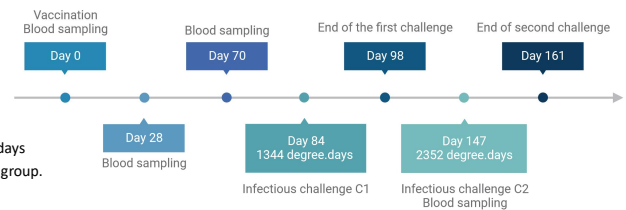
## METHODOLOGY

APAFIS #35027-2022012811456492 v1 - Oniris experimental facilities (D 44 272)

Vaccines have been provided by Biovac/CEVA (Beaucouzé, France) with the Ass strain CAE11414 isolated from RT with furunculosis

Fish: Rainbow trout were divided into 4 groups :

- NV group : 135 RT are Not Vaccinated
  - IP group : 90 RT are vaccinated by IntraPeritoneal route : injected with 100 µL of vaccine
  - Baln group : 90 RT are vaccinated by Balneation route: fish was immersed in 1L of vaccine solution during 1 min
  - VO group : 90 RT are vaccinated by Orale route : 100 µL of vaccine per RT are given with food during 2 x 5 days, spaced by 5 days
- Fish weighing around 150 g were randomly distributed between the NV, IP and VO groups. Fish weighing around 100 g for Baln group.  
Rearing temperature: 16 °C



Infectious challenge : 84 or 147 days after vaccination (1344 or 2352 degree.days respectively)

For challenges, 45 trouts of each group have been immersed in a solution of Ass (10<sup>6</sup> CFU/mL) during 24h at 16°C

Clinical signs were monitored twice a day.

Blood samples were taken at 0, 28, 70 and 147 days after vaccination to study anti-Ass antibody production by ELISA.

Trouts were weighted at 0, 70 and 147 days after vaccination.

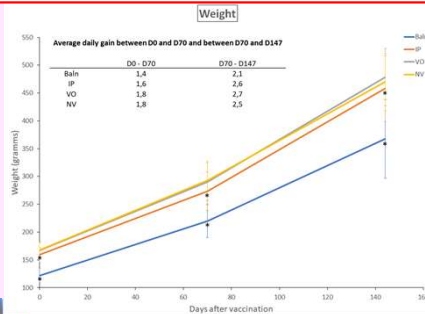
During infectious challenges, dead or dying fish were necropsied for evaluation of internal lesions and presence of Ass.

## RESULTS

### Vaccine Safety

Apart from infectious challenge period, few fish died as a result of anaesthesia or hypoxia accidents. But no difference was observed between vaccinated and non vaccinated group.

In the IP group, 82.3% of trout showed intraperitoneal adhesions at the vaccine injection site. In 53.7% of cases, these adhesions were severe.



About fish weight, smaller fish in the Baln group have been chosen (approx. 100g) due to the immersion vaccination method. Significant difference between the Baln group and the other groups persisted throughout the experiment.

At D0, a significant difference of approx. 8 g was observed between IP and NV groups despite the randomly distribution. This difference increased after vaccination to reach over 19 g at D70. Then, it decreased between D70 and D147. Between D0 and D70, the Average Daily Gain is lower in the IP group than in the NV and VO groups (p<0.05).

### Anti-Ass antibodies production

No production of anti-Ass antibodies was observed in the NV and VO groups. Oral vaccination did not induce antibody production detectable by ELISA.

A single administration of vaccine by the IP route induced a significant anti-Ass antibody production compared with NV group as early as 4 weeks after vaccination at 16°C, which lasted until the end of the study (2352 degrees. day).

Vaccine administration by immersion induces a little antibody production at 4 weeks after vaccination, compared with the NV group (p < 0.05). This increase did not last over time.

### Vaccine Efficiency

During the 2 infectious challenge, no mortality due to Ass was observed in the non infected NV group (negative control).

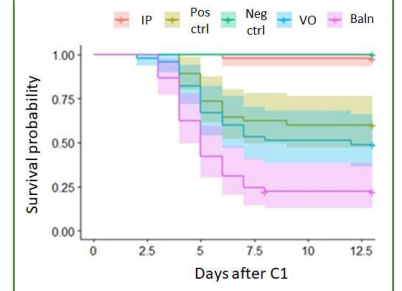
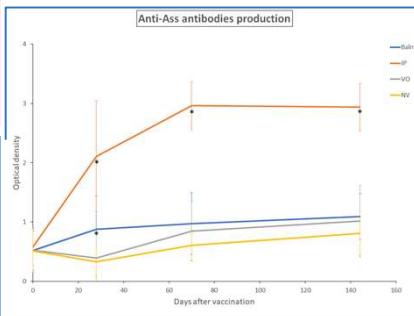
A mortality rate of 40% and 100% was observed in the infected NV group (positive control) during the first and the second infectious challenge respectively.

Only the vaccine administered by IP route showed a high efficacy, with only 1 and 4 fish dead after C1 and C2 respectively. A single IP injection protected the animals for at least 2352 degrees.day.

A single administration of the vaccine by oral or immersion route showed no efficacy. Each infectious challenge resulted in the death of more than half the fish vaccinated by these 2 routes

	1st challenge – efficiency at 1344 degrees.days				2nd challenge – efficiency at 2352 degrees.days			
	Number of fish per group	Number of deaths after challenge C1	% mortality	RPS (%)	Number of fish per group*	Number of deaths after challenge C2	% mortality	RPS (%)
NV without challenge (negative control)	42	0	0		36	0	0	
NV with challenge (positive control)	45	18	40		28	28	100	
Baln	45	35	77.8	-94.5	36	21	58.3	42
IP	43	1	2.3	94.2	21	4	19.0	81
VO	45	23	51.1	-27.8	12	8	66.7	33

\*During the infectious challenge C2 by immersion, some fish were died due to hypoxia. These fish were eliminated of the analysis, explaining that the number of fish per group were not 45 for the second challenge RPS : relative percent of survival.



## FIRST CONCLUSIONS AND PERSPECTIVES

Administration route	Safety	Efficacy	Practical feasibility	Duration of protection	Antibodies production
Intraperitoneal	No	Yes	No	>2350 degrees.day	Yes
Immersion	Yes	No	Yes		A little in the beginning !
Oral	Yes	No	Yes		No

⇒ Several administration of vaccine by immersion or orale route to induce immune responses and protection against furunculosis ?

⇒ Booster administration to maintain a long-lasting protection ?

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Further discussion? Partnership?

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