Immersion vaccination of rainbow trout (*Oncorhynchus mykiss*) against *Flavobacterium psychrophilum*

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Abstract

Flavobacterium psychrophilum is the causal agent of rainbow trout fry syndrome (RTFS), which seriously affects rainbow trout aquaculture. The disease affects fish at early life stage and can cause high mortality. Within trout aquaculture several procedures have been implemented in order to reduce the disease problem. Use of ”Flavo free” broodstock and disinfection of eggs can reduce probability of introducing the disease to the farm, however, the bacterium has shown to be resistant against several of the disinfection procedures performed today. Other factors such as minimum handling of fish (less skin abrasions) and keeping an optimal fish density and water flow are prophylactic measures that can prevent development of disease [1]. If disease outbreak occurs antibiotic treatment is the most effective measure to stop infections in the early phase. Vaccination has shown to be vital for controlling disease and reducing use of antibiotics in Norwegian salmon and trout farming. Dip and bath vaccination of fry can be a method for reducing outbreaks of RTFS in trout aquaculture.

A study was performed to evaluate the efficacy of an inactivated whole-cell based immersion vaccine. Rainbow trout fry with a mean initial weight of 3 g were dip vaccinated for one minute with a 1/10 dilution of a suspension of inactivated *F. psychrophilum*. At 6 weeks (~5.0 g) post-immunization the fish were challenged by intraperitoneal injection with 5.2×10^7 CFU/fish of a highly virulent strain of *F. psychrophilum* (Serotype Th isolate, isolated in Finland). The relative percent survival (RPS) was calculated by relating the number of diseased fish in the vaccinated groups with the corresponding number in the control group. To reduce tank effects, the trial was performed in duplicates (identical groups in two tanks). The results from the challenge showed a difference in mortality between the vaccinated groups and the mock-vaccinated (PBS dip) group in both tanks, with an RPS of 50.0% and 56.3% (Tab. 1).

Table 1. Relative percent survival of vaccinated trout fry challenged 6 weeks post vaccination by intraperitoneal injection challenge with highly virulent live *F. psychrophilum*.

Tank	Vaccination	No. of fish	Dead fish	% Mortality	RPS
1	**Dip vaccinated**	40	7	17.5	56.3
	Mock vaccinated	40	16	40.0	0
2	**Dip vaccinated**	40	8	20.0	50.0
	Mock vaccinated	40	16	40.0	0

Although the results in this experiment showed good protection of the vaccine, earlier trials using the same vaccine have resulted in lower RPS levels. However, it should be emphasized that inactivated whole-cell vaccines do induce protection by injection vaccination [2].

Development of a challenge model by infection through natural pathways such as bath or cohabitant (disease carriers) is important for a better interpretation of vaccine efficacy. There are few published studies on successful bath challenge models. A trial was initiated to investigate the significance of exposure time on mortality of naïve rainbow trout. Fry 3–5g (n=50) were challenged by bath using a highly virulent Norwegian *F. psychrophilum* strain with an exposure time of 2, 4, 7 hours or 7 hours on two repeated days. The study showed that exposure time did not influence the level of mortality and a two-hour challenge was enough for inducing mortality above 50% (Fig. 2). The results showed that it is possible, under experimental conditions, to induce mortality in rainbow trout by immersion exposure to *F. psychrophilum*. However, further studies should be performed to verify the reproducibility of the bath challenge method.

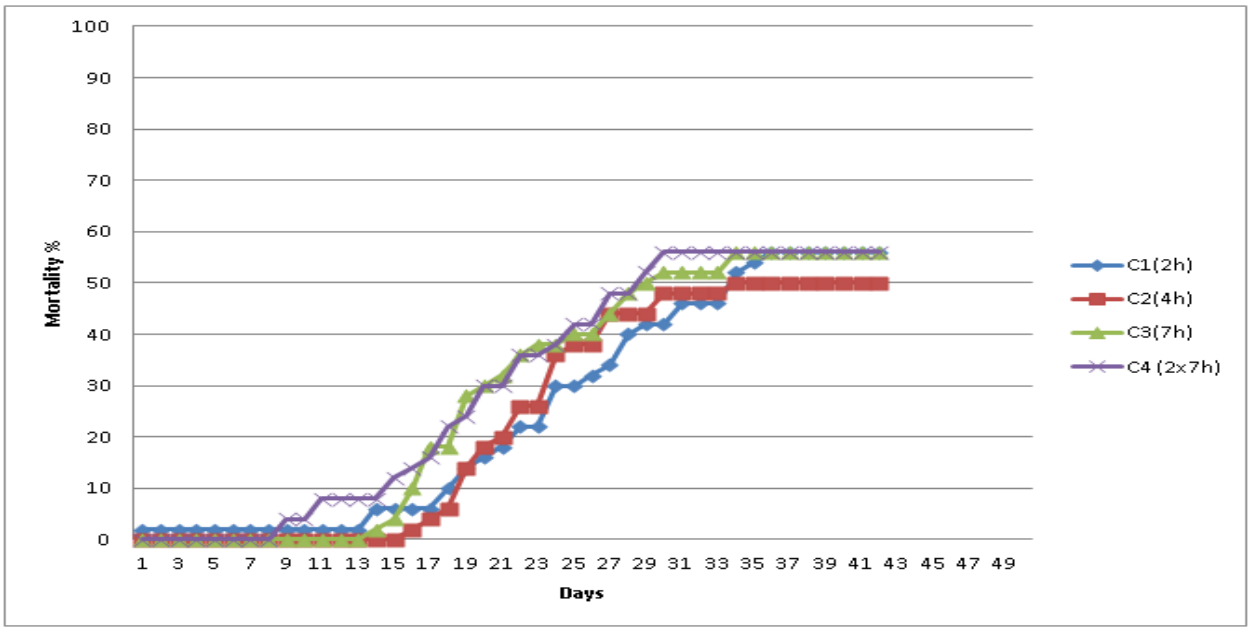

Figure 1. Cumulative per cent mortality in rainbow trout (3–5 g) challenged by bath using a Norwegian *F. psychrophilum* strain. The fish were exposed for 2, 4, 7 and 2 × 7 hours.

One of the major challenges for the development of a protective vaccine regime is the fact that the disease affects fry at early life stages prior to reaching an immunocompetent size. Studies indicate that fry should reach a size of at least 1–2 g to achieve an immune response after dip vaccination and that the onset of immunity is usually obtained after 450 day degrees (Non published PHARMAQ studies). Only healthy fish should be vaccinated to avoid that handling and stress involved in vaccination trigger latent infections causing outbreaks shortly after vaccination.

Studies to evaluate the duration of protection are essential for developing an optimal vaccine regime securing a long lasting protection until harvest. Dip vaccination trials (not published) performed by PHARMAQ using inactivated *Yersinia ruckeri* dip vaccine indicated that a double vaccination procedure should be performed in order to obtain long term protection (15

weeks). Further studies will be initiated to investigate the optimal vaccination regime for long term protection against *F. psychrophilum* infections

The Finnish authorities (Fimea) decided to make a monovalent dip vaccine with inactivated *F. psychrophilum* antigens from PHARMAQ against RTFS available in Finland on a special license due to the severe situation. A field trial experiment from dip vaccination with inactivated *F. psychrophilum* indicated that the vaccine reduces mortality after double vaccination. However, mortality also occurred in vaccinated fish groups. Further information will be collected from fish farms for evaluation of the vaccine efficacy.

References

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