International Council for
the Exploration of the Sea

C.M. 1987/F:24
Mariculture Committee

VACCINATION AGAINST *Vibrio salmoncida* RESULTS FROM THREE LABORATORY TRIALS

by

Brit Hjeltnes, Kari Andersen, Hans-Magne Ellingsen

Directorate of Fisheries, Institute of Marine Research
Division of Aquaculture
C.Sundtsgt. 37, N-5004 Bergen, Norway

Abstract

Atlantic salmon smolts (*Salmo salar*) were vaccinated with a bacterin made from formalin-killed *Vibrio salmonicida*. In laboratory trials, fish vaccinated once showed little or no protection after challenge. Fish vaccinated twice appeared to be protected, the degree of protection depending on the administration route of the vaccine.

Introduction

Since the late seventies, the farmed Norwegian salmon have suffered from Hitra disease. In 1986-87 the mortality reached 50-90% in several fish farms in mid and northern Norway. The etiology has been discussed but part of the confusion may be due to different diseases producing same symptoms. One of these diseases is undoubtedly a vibrio infection - "Coldwater Vibriosis".

In 1981, Egidius et al. isolated a vibrio-like bacteria from fish suffering from Hitra disease. This Vibrio sp. was characterized by Holm et al (1985) and by Egidius et al (1986) who proposed a new species Vibrio salmonicida. Infection studies on rainbow trout (Egidius et al 1981) and on Atlantic salmon (Hjeltnes et al 1987) showed it to be a fish pathogen capable of producing symptoms similar to Hitra disease.

Holm and Jørgensen (1987) report on resistance after double dip vaccination with a bacteria developed from a strain of V. salmonicida. The efficiency was recorded after a natural epizootic. The purpose of the present study was to evaluate by laboratory trials the effect of vaccination against Vibrio salmoncida with special attention to revaccination and administration routes.

Materials and methods

Fish

The fish were 18-24 cm (80 g) Atlantic salmon smolts kept in UV-treated seawater with a salinity of 30 o/oo in 200 l or 400 l aquaria. They were fed Ewos dry pellets and were actively feeding before challenge.

Vaccines

Whole cell vaccines were prepared from Vibrio salmonicida by growing the cells in a liquid medium and killed by adding formalin (0.5 %). The cells were not washed before used as a bacterin. Vaccine A was prepared in our laboratorium from type strain NCMB 2262 grown to a density of $5.5 \times 10^{8.5}$ cells/ml in tryptone soy broth (TSB, Oxoid) with 15 o/oo NaCl added.
Vaccine B was prepared by A/S Apothekernes Laboratorium, Oslo, Norway in an industrial-scale fermentor (1.200 liters, Holm and Jørgensen 1987).

Vaccination

Bath vaccination was performed as described by Egidius and Andersen (1979) with a dilution of 1:200.
Dip vaccination was performed by immersing the fish one minute in a 1:5 dilution of the vaccine. Injection was performed by i.p. injection of 0.2 ml undiluted vaccine.

Challenge

Waterborne challenge was performed by exposing the fish to 5 x 10^5 bacteria/ml for 1 hr. When injection was used as a challenge, 0.2 ml of 10 bacteria/ml was injected i.p. Dead and moribund fish were collected and bacterial cultivation from the kidneys was attempted on tryptone soy (TS) agar with 5 % human blood and 15 o/oo NaCl and grown at 15 ^{o} C. 2-3 months after challenge, survivors were examined for the presence of bacteria in the kidneys.

Experiment 1

Fish were bath Vaccinated with vaccine B at 8.5 ^{o} C and kept at this temperature four weeks after vaccination. The fish were given a waterborne challenge.

Experiment 2

Fish were bath vaccinated with Vaccine A at 5 ^{o}C and kept at this temperature for four weeks. After four months, fish were revaccinated by bath or by injection at 7.5 ^{o}C. Groups of these fish were challenged by injection fifteen weeks after the first vaccination and six weeks after revaccination.

Experiment 3

Fish were vaccinated by dip and i.p. injection with Vaccine B at 12 ^{o}C. After 8 weeks, the fish were revaccinated by bath, dip and injection at 7.5 ^{o}C. Six weeks after revaccination, the fish were challenged by injection.

Results

Experiment 1

A single bath vaccination did not appear to give any significant protection although a small difference was observed (Tab. 1).

Experiment 2

Again a single bath vaccination did not appear to give any significant protection against infection by V. salmonicida although some improvement was observed during the later challenge (Table 2). Revaccination by bath gave good protection and revaccination by injection apparently gave total protection.

Experiment 3

In this experiment the mortality in the control group was not very high (34 %) but some differences among the groups were observed (Tab. 3).

A single dip vaccination did not give good protection. A single injection gave some protection but not as good as the revaccinated groups. Injection followed by a dip vaccination gave the best resistance.

Vibrio salmonicida was cultivated from the kidneys of all dead and moribund challenged fish. No bacteria were cultivated from the survivors.

Table 1. Experiment 1. Mortality in unvaccinated and single bath vaccinated after waterborne challenge (NCMB 2262)

Treatment (Vacc. B)		No of fish	Mortality	%
I	Unvaccinated	30	29	96
	Single bath	30	26	80
II	Unvaccinated	30	28	93
	Single bath	29	25	86

Table 2. Experiment 2. Mortality in unvaccinated and vaccinated fish after challenge by i.p. injection.
* Challenged 15 weeks after 1st vaccination (NCMB 2262)
** Challenged 6 weeks after 2nd vaccination (NCMB 2262)

Treatment (Vacc. A)

	1.		2.	No of fish	Mortality	%
*	I	Unvaccinated		46	24	52.1
		Bath		43	18	41.8
		Unvaccinated		45	43	95.5
		Bath		29	20	68.9
**	II	Bath	Bath	37	10	27.0
		Bath	Injection	41	0	0

Table 3. Experiment 3. Mortality in unvaccinated and vaccinated fish after challenge by i.p. injection (LFI 001).

Treatment (Vacc. B)

1.	2.	No of fish	Mortality	%
Unvaccinated		44	15	34.1
Dip		45	10	22.3
Dip	Bath	45	6	13.4
Injection		36	3	8.3
Dip	Dip	47	3	6.4
Dip	Injection	44	2	4.6
Injection	Dip	34	0	0

Discussion

The Hitra disease is economically the most important disease in Norwegian fish farming. Because prompt therapy is essential, medical treatment has been difficult. Since 1985 multiple-resistant strains of Vibrio salmonicida have been reported (Hjeltnes et al. 1987). The incidence of multiple-resistant strains seems to be increasing and this emphasizes the need for a vaccine.

Under laboratory conditions, Atlantic salmon can be effectively vaccinated against Vibrio salmonicida, although a single bath or dip vaccination gives little or no protection. As regards vaccination by single injection, the data are too limited for any firm conclusion before further investigation. Revaccinated fish are protected against Vibrio salmonicida. The degree of protection depending on the administration route of the vaccine. This supports the observation of Holm and Jørgensen (1987) who found in a field test that double dip vaccinated Atlantic salmon were protected against "Cold-Water Vibriosis".

The increased protection observed from booster vaccination indicates a secondary immune response in Atlantic salmon. The best protection was observed from combinations of bath and injection or dip and injection. Different ways of stimulating the immune system may be responsible for this increased protection.

Acknowledgement

This work was supported by the Norwegian Research Council of Fisheries (NFFR).

References

Egidius, E. and Andersen, K. (1978). Host-specific pathogenicity of strains of Vibrio anguillarum isolated from rainbow trout Salmo gairdneri Richardson and saithe Pollachius virens (L). Journal of Fish Diseases 1, 45-50.

Egidius, E., Andersen, K., Clausen, E. and Raa, J. (1981). Cold-water vibriosis or "Hitra-disease" in Norwegian salmonid farming. Journal of Fish Diseases 4, 353-354.

Egidius, E., Wiik, R., Andersen, K., Hoff, K.A. and Hjeltnes, B. (1986). Vibrio salmonicida sp. nov., a new fish pathogen. International Journal of Systematic Bacteriology 36, 518-520.

Hjeltnes, B., Andersen, K., Ellingsen, H.M., and Egidius, E. (1987). Experimental studies on the pathogenicity of a Vibrio sp. isolated from Atlantic salmon, Salmo salar L., suffering from Hitra Disease. Journal of Fish Diseases 10, 21-27.

Holm, K.O., Strøm, E., Stensvåg, K., Raa, J. and Jørgensen, T. (1985). Characteristics of a Vibrio sp. associated with the "Hitra-disease" of Atlantic salmon in Norwegian fish farms. Fish Pathology 20, 125-129.

Holm, K.O. and Jørgensen, T. (1987). A successful vaccination of Atlantic salmon, Salmo salar L., against "Hitra-disease" or coldwater vibriosis. Journal of Fish Diseases 1987 10, 85-90.