Vol. 5: 179–183, 1988 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published December 2

Vibriosis vaccination of rainbow trout *Salmo gairdneri* at varying temperatures and seasons. I. Effects on mortality and feed conversion in four Swedish field trials

Margaret A. Thorburn[1,*], Tim E. Carpenter[1], Olle Ljungberg[2]

[1] Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616, USA
[2] National Veterinary Institute, Box 7073, S-750 07 Uppsala, Sweden

ABSTRACT: Vibriosis vaccine trials were conducted on 4 Swedish brackish water rainbow trout *Salmo gairdneri* net-pen farms. Intraperitoneal (IP) vaccination of fall- and spring-transport yearlings at 3 to 16 °C provided significant protection against mortalities from *Vibrio anguillarum*, serotype 1 (VA). Dip-vaccination of fingerlings provided only slight protection of limited duration. Non-vaccinated trout on one farm had a higher feed conversion ratio (FCR) than corresponding vaccinated trout. However, on 2 other farms, there were no significant differences in the FCRs of non-vaccinated and vaccinated trout. Any benefits of the efficiency of food utilization conferred by vaccination therefore appeared to be farm-specific, but contributing factors were not identified.

INTRODUCTION

Vibriosis is the most prevalent disease in Swedish brackish water rainbow trout *Salmo gairdneri* farming facilities (Forskningsrådnämnden 1982). In Sweden, mortalities from *Vibrio anguillarum* have only been positively confirmed during the warmer part of the growing season (approximately mid-June through mid-October). Losses occur, most commonly, in yearling trout during the first growing season following their transport, either in the spring (70 to 200 g average weight) or the previous autumn (40 to 150 g average weight), from freshwater to brackish water farms.

In initial field trials conducted to assess the efficacy of vibriosis vaccination in Sweden, rainbow trout were vaccinated in the spring by intraperitoneal (IP) injection, after water temperatures had exceeded 10 °C and 2 to 4 wk prior to transport to brackish water (Ljungberg 1983). These trials gave promising results, but the recommended vaccine protocol has presented serious difficulties for freshwater breeders, due to severe space and time shortages in the spring. The purpose of the studies reported in this paper was to investigate alternative vibriosis vaccination procedures suitable to Swedish conditions. Results published from vaccine tests conducted elsewhere were used to help design the various trials.

Based on Horne et al.'s (1982) report of long-term persistence of protection following IP-vaccination of rainbow trout at 6 °C, we chose, in trout destined for spring transport, to test the efficacy of IP-vaccination at winter-time temperatures.

Trout destined for autumn transport, and vaccinated prior to transport, require a vaccine procedure which provides protective immunity of adequate duration. Håstein et al. (1980) reported adequate protection over a 1 yr period in rainbow trout vaccinated shortly before late autumn transport. We tested this procedure in one group of fall-transport trout. We also tested the efficacy of dip-vaccination in the spring in groups subsequentally transported to brackish water in late summer and late autumn.

In one group of autumn transport trout, we waited and vaccinated (IP) trout at the brackish water site, several months after transport, but prior to the seasonal

* Present adress: National Veterinary Institute, Box 7073, S-750 07 Uppsala, Sweden

0177-5103/88/0005/0179/$ 03.00

Table 1. Summary of vibriosis vaccine trials on Swedish brackish-water commercial rainbow trout net-pen farms, 1984 to 1987

Farm	Group	No. of pens per group[a]	Vaccination				Introduction to brackish water	
			Fish size (g)	Method	Date	Water temp. (°C)	Transport date	No. of days after vacc.
A	V1	1	2.3	dip	May 84	16	Jul 84[b]	43
	C1	1	–	–	Control	–	Jul 84[b]	–
	V2	2	2.3	dip	May 84	16	Nov. 84	189
	V3	2	95	IP	Nov 84	11	Nov. 84	18
	C2	2	–	–	Control	–	Nov. 84	–
B	V1	3	85	IP	Jun 85	14	Jun 85	23
	C1	1	–	–	Control	–	Jun 85	–
C	V1	3	45	IP	May 85	3[c]	Jun 85	36
	V2	1	55	IP	May 85	10[c]	Jun 85	21
	C1	2	–	–	Control	–	Jun 85	–
D	V1	2	65	IP	May 87	8	Oct 86	–[d]
	C1	1	–	–	Control	–	Oct 86	–

[a] Number of fish per net-pen ranged from 1800 to 5600
[b] Groups A, V1 and A, C1 were held in brackish-water concrete tanks from July to November 1984. In November, they were transported to net-pens adjacent to Groups A, V2, A, V3 and A, C2
[c] Farm C was located in northern Sweden, where water temperatures remain very low until mid-summer. Group C, V2 was acclimated to 10 °C for 1 wk prior to vaccination and held at 10 °C until transport, while groups C, V1 and C, C1 were held at the natural hatchery temperatures
[d] Group D, V1 was vaccinated at the brackish water site, 7 mo after transport

rise in water temperatures associated with vibriosis outbreaks.

Our major objective was to determine to what extent these vaccination schemes reduced mortalities from vibriosis, relative to traditional control methods (antibiotic treatment or no treatment). We also wished to investigate whether vaccination increased the efficiency of feed utilization.

MATERIALS AND METHODS

Four commercial rainbow trout farms (referred to in this text as Farms A, B, C and D) located along the Swedish east coast participated in the vibriosis vaccination trials. Commercially available bivalent bacterins were used in all trials. The bacterins employed on Farms A, B and C contained formalin-killed whole cell preparations of North American strains of *Vibrio anguillarum*, serotype I, and *V. ordalii*, serotype II, while that on Farm D contained formalin-killed whole cell preparations of Swedish strains of *V. anguillarum*, serotypes I and II. (The North American and Swedish serotypes I and II have been typed as 1 and 2, respectively, by Sørensen & Larsen 1986). Dip-vaccinated fish were immersed for ca 10 s in a 1:10 dilution of vaccine. Injected fish were first anaesthetized with chlorobutanolum, then injected intraperitoneally with 0.10 ml of undiluted vaccine. Feed was withheld 2 to 4 d prior to injection and 1 d prior to dip-vaccination. All vaccinated fish in each trial originated from the same lots as the non-vaccinated control fish and were first separated from controls 3 to 4 d prior to vaccination.

In these studies 'control fish' were fish that received the standard reference treatment. The treatment consisted of no vaccination, but optional antibiotic therapy during vibriosis outbreaks (75 mg oxytetracycline kg^{-1} fish d^{-1} for 7 to 12 d, orally).

Table 1 presents the various vaccination schemes employed on the different farms. The fish on Farms A, B and C were all vaccinated at the freshwater sites from which they originated, at least 18 d before transport; those on Farm D were IP-vaccinated at the brackish water site, 7 mo after transport. Both dip-vaccination (in the spring) and IP-vaccination (in the autumn) were tested on Farm A. Farm B's fish were IP-vaccinated according to the 'standard' protocol. Farm C included a group of fish IP-vaccinated at winter temperatures (3 °C).

We gave farmers on all farms sheets for daily recording of mortalities, disease signs and amount of feed fed. Other events, such as times and amounts of medication and the results of sample weighings, were also recorded. Samples of moribund fish were collected from affected net-pens, and, if available, from non-affected pens, during disease outbreaks. In addition, farmers were requested to freeze occasional trial fish found dying throughout the summer. We examined all

fresh and thawed frozen fish by bacteriological culture techniques to determine the cause of death. Kidney material was streaked on blood agar, and isolates were identified with rabbit anti-*Vibrio anguillarum* serum by the rapid slide agglutination technique.

Exact vibrio-specific mortalities (%) were not determined because not all of the dead fish were examined bacteriologically. In any given net-pen the vibrio-specific mortality was calculated by multiplying the total mortality by the proportion of the sampled dead fish yielding pure cultures of *Vibrio anguillarum*. Total mortality was calculated as follows:

$$\text{Total mortality} = \frac{\text{Number of fish dying in net-pen in period from 3 d prior to first } V.\ anguillarum \text{ isolation to 3 d after mortality decreased to pre-outbreak levels}}{\text{Number of fish in net-pen at start of outbreak}}$$

We used a 1-way analysis of variance (ANOVA), followed by the Tukey method of multiple comparisons (Neter & Wasserman 1974), and a Student's *t*-test to test for significant ($p < 0.05$) differences in mean vibriosis-specific mortality among non-vaccinated and vaccinated groups on the 2 farms (A, 1985; C) where data from replicate test and control pens were available. The normal approximation to the binomial distribution (Remington & Schork, 1970) was used to test for significant ($p < 0.05$) differences in vibrio-specific mortality on the remaining farms (A, 1984; B and D). Significance results obtained from trials where data were not available from replicate pens should, however, be interpreted with some caution, due to the possible influence of non-quantifiable 'pen-effects' on observed mortality (Michel et al. 1984).

We calculated feed conversion ratios (FCRs) for the different groups of fish on Farms A, B, and C as follows:

$$\frac{\text{Total amount of feed (kg) fed in the period between first and last sample weighings}}{\text{(Average weight gain per fish [kg]) } \times \text{ (average number of fish in pen or group)}}$$

where (1) 'average weight gain per fish' was based on the difference between the results of the season's first and last sample weighings of ca 100 to 200 fish from each net-pen (Farms B and C) or group (the fish farmer at Farm A only recorded average weight for each group); and (2) the 'average number of fish in pen or group' was determined by calculating the mean number of fish in successive ca 15 d periods and taking the mean of all these periods included in the time interval between the first and last sample weighings.

On Farms A and B, sample weighings were only conducted twice, once at the start of the growing season and once towards the end of the season. Farm C, however, conducted 7 sample weighings of each net-pen at 2 to 3 wk intervals. We used the Farm C data to statistically evaluate the effect of vaccination on growth by constructing a production function based on multiple least squares linear regression analysis (Neter & Wasserman 1974). In this analysis, weight gain per fish was the dependent variable and degree-days, feed fed per fish, and vaccine status (control, cold-water vaccination or warm-water vaccination) were the independent variables. A significant contribution of vaccine status, either as an individual term (tested with the *t*-statistic, $p < 0.05$) and/or in interaction with either of the other 2 independent variables (tested with the partial *F*-test, $p < 0.05$) would indicate that vaccination had a significant effect on growth curves. In particular, significant interactions between vaccine status and feeding rate would denote differences in FCRs among the particular vaccine groups indicated.

RESULTS

Table 2 presents the estimated mortalities and FCRs for the various trial groups. In all cases of vibriosis the isolated pathogen was exclusively *Vibrio anguillarum*, serotype 1 (using the serotype classification scheme proposed by Sørensen & Larsen 1986). As shown, the non-vaccinated groups on Farms B, C and D each underwent one course of oxytetracycline treatment. No vaccinated groups were treated.

The IP-vaccination schemes tested on Farms B's and C's spring-transport trout provided significant levels of protection against vibrio-induced mortality. The trout vaccinated according to the previously recommended protocol on Farms B (V1) and C (V2) remained disease-free, while corresponding non-vaccinated fish (B, C1 and C, C1) yielded, despite medication, significantly higher vibrio-specific mortalities of 3.9 and 5.05 % (average percentage), respectively. The trout vaccinated at 3 °C on Farm C (V1) also had a 0 % vibriosis-specific mortality, which was significantly lower than the average mortality (5.05 %) for the non-vaccinated trout (C, C1).

The fall-transport fish on Farm D, which were IP-vaccinated in the spring following transport (V1), remained essentially disease-free (vibrio-specific mortality of at most 0.1 %) while corresponding non-vaccinated trout had a significantly higher vibrio-specific mortality of 6.5 % despite medication.

Intra-peritoneal vaccination in the fall, prior to transport to brackish water, protected Farm A's fish (V3) against the following summer's vibriosis outbreak. The vibrio-specific mortality of A, V3 (0.3 % or less) was significantly lower than that of the November transport control fish (C2, 7.5 %).

Dip-vaccination gave a mild protection of limited duration. Vibriosis occurred in Farm A's summer-transport dip-vaccinated (V1) and non-vaccinated (C1) trout

Table 2. Estimated vibriosis mortality (%) and feed conversion ratios (FCRs) for rainbow trout on Swedish brackish water commercial net-pen farms participating in vibriosis vaccine trials, 1984 to 1987

Farm	Group[a]	Vibriosis outbreak				FCR[b]
		1st VA isolation (day/mo)	Total mort.[b] (%)	Vibriospecific mort.[b] (%)	Anitibiotics fed (day/mo.)	
A	V1	1/10	8.3	7.1	No	ND
1984	C1	25/9	13.8	10.7	30/9–6/10	ND
A	V1	ND	1.1	≤ 1.1	No	ND
1985	C1	ND	1.9	≤ 1.9	No	ND
	V2	16/7	8.2	8.2	No	1.28
	V2	16/7	5.9	5.9	No	
	V3	ND	0.3	≤ 0.3	No	1.43
	V3	ND	0.3	≤ 0.3	No	
	C2	16/7	8.0	8.0	No	1.48
	C2	16/7	7.0	7.0	No	
B	V1	–	< 0.1	0.0	No	1.68
	V1	–	< 0.1	0.0	No	ND
	V1	–	0.4	0.0	No	1.49
	C1	28/8	3.9	3.9	30/8–7/9	2.65
C	V1	–	< 0.1	0.0	No	1.23
	V1	–	< 0.1	0.0	No	1.11
	V1	–	< 0.1	0.0	No	1.37
	V2	–	< 0.1	0.0	No	1.40
	C1	26/8	3.3	1.9	30/8–9/10	1.31
	C1	26/8	8.2	8.2	30/8–9/10	1.50
D	V1	ND	0.1	≤ 0.1	No	ND
	V1	ND	0.1	≤ 0.1	No	ND
	C1	19/8	6.5	6.5	1/9–7/9	ND

VA: *Vibrio anguillarum;* ND: not done; –: all culture results negative
[a] Groups are described in Table 1
[b] These terms are described in 'Materials and Methods' Weighing intervals for the FCR determinations were 64 d (Farm A), 82 d (Farm B) and 119 d (Farm C)

ca 4 mo after the dip-vaccination. The vibrio-specific mortality of V1 (7.1 %) was significantly lower than that of C1 (10.7 % despite antibiotic therapy). The dip-vaccinated fish (V2) transported to Farm A in November (after the 1984 outbreak) experienced, the following summer (1985), an average vibrio-specific mortality of 7.05 %, which did not differ significantly from that of the corresponding control fish (C2, 7.5 %), but was significantly higher than that of the IP-vaccinates (V3, 0.3 %).

The survivors of Farm A's 1984 vibriosis outbreak, Groups A, C1 and A, V1, appeared quite resistant to vibriosis the second summer. The 1985 vibrio-specific mortalities for these groups (at most, 1.9 % and 1.1 %, respectively) were substantially lower than the average mortalities of their non-exposed cohorts (A, C2: 7.5 % and A, V2: 7.05 %).

Vaccination was obviously associated with increased feed conversion efficiency on Farm B only, where non-vaccinated fish had a much higher FCR (2.65) than did corresponding vaccinated fish (average FCR = 1.59). Feed conversion ratios among cold-water vaccinates (V1) on Farm C were, on the average, slightly lower than those of the other (warm-water vaccinated, V2, and control, C1) fish. The selected production function (adjusted $R^2 = 0.969$) did not, however, include vaccine status, indicating that there were no significant differences among the FCRs or among the growth curves, given constant feeding rates, of the 3 groups.

DISCUSSION

All of the IP-vaccination schemes tested in these trials protected fish against vibriosis during their first 'high-risk' growing season in brackish water It is particularly noteworthy that vaccine injected at very low temperatures (3 °C, Farm C) provided good protection. Since vaccination at lower temperatures has been associated with delays in the immune response of salmonids (Groberg 1982), the cold-water trial trout were vaccinated more than 5 wk before transport (and, as it turned out, 16 wk before the vibriosis outbreak in

controls). These fish were apparently allowed sufficient time to mount an effective immune resonse.

Dip-vaccination provided some protection against vibriosis, but the protection was mild and of limited duration. Protection against vibriosis is stronger in fingerlings vaccinated at larger sizes (Thorburn & Jansson 1988). Furthermore, trout dip-vaccinated at 4.5 g or larger remain protected longer than those vaccinated at 2.3 g (Johnson et al. 1982). The 2.3 g vaccinated Farm C trout were not able to mount an immune response which in strength and/or duration very effectively resisted natural challenge. It would be worthwhile, however, to test whether dip-vaccination of larger fingerlings would provide adequate protection for autumn-transport yearling trout in Sweden.

The Farm A trout which survived the 1984 vibriosis outbreak (C1 and V1) apparently gained some immunity from natural 'outbreak-level' exposure, as shown, the following summer, by their low mortaility rates relative to non-outbreak-exposed cohorts (C2 and V2). This is consistent with the findings of Evelyn & Ketcheson (1980) for pen-reared sockeye salmon *Oncorhyncus nerka*. The immunity elicited by exposure, alone (C1) or following dip-vaccination (V1), appeared, however, to provide somewhat less protection than did that elicited by IP-vaccination (V3).

Previous investigations into possible growth effects of vibriosis vaccination have reported conflicting results. Antipa & Amend (1977) found no significant differences in the growth rates of vaccinated and non-vaccinated coho salmon in a vibriosis vaccine trial. Sawyer & Strout (1977), on the other hand, reported improved growth of coho salmon vaccinated against vibriosis, as compared to medicated and non-medicated controls, in one field trial. These 2 reports did not present feed conversion ratios.

In the present study, vaccination was associated with markedly increased efficiency in feed utilization on only 1 of 3 farms (Farm B) surveyed. Because there was no replication of the control pen on the farm (B) where the FCR effect was noted, this result should be interpreted cautiously. The magnitude of the difference was large enough, however, to cause us to wonder whether there might not be farm-specific practices or conditions that interact with vaccination to bring about such results. If so, it would be worthwhile identifying these factors.

Acknowledgements. This work was supported by funds provided by the USDA Animal Health Act of 1977 (P. L. 95–113) and administered through the LDRL of the School of Veterinary Medicine, University of California, Davis; and by the Swedish Council for Forestry and Agricultural Research (981–84; V18:2). The support is gratefully acknowledged.

LITERATURE CITED

Antipa, R., Amend, D. F. (1977). Immunization of Pacific salmon: comparison of intraperitoneal injection and hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* bacterins. J. Fish. Res. Bd Can. 34: 203–208

Evelyn, T. P. T., Ketcheson, J. E. (1980). Laboratory and field observations on antivibriosis vaccines. In: Ahne, W. (ed.) Fish diseases. Third COPRAQ session. Springer-Verlag, Berlin, p. 46–52

Forskningsrådsnämnden (1982). Pigg som en mort: vattenbrukets hälso och sjukdomsfragor ('Aquaculture's health and disease questions'). Rapport 82:8, Stockholm, p. 32–33

Groberg, W J. (1982). Infection and the immune response induced by *Vibrio anguillarum* in juvenile coho salmon *(Oncorhyncus kisutch).* Ph. D. thesis, Oregon State University, Corvallis

Håstein, T., Hallingstad, F., Refstie, T., Roald, S. O. (1980). Recent experience of field vaccination trials against vibriosis in rainbow trout *(Salmo gairdneri).* In: Ahne, W. (ed.) Fish diseases. Third COPRAQ-session. Springer-Verlag, Berlin, p. 53–59

Horne, M. T., Tatner, M., McDermont, S., Agius, C., Ward, P. (1982). Vaccination of rainbow trout, *Salmo gairdneri* Richardson, at low temperatures and the long-term persistence of protection. J. Fish Dis. 5: 343–345

Johnson, K. A., Flynn, J. K., Amend, D. F. (1982). Duration of immunity in salmonids vaccinated by direct immersion with *Yersinia ruckeri* and *Vibrio anguillarum* bacterins. J. Fish. Dis. 5: 207–213

Ljungberg, O. (1983). Vaccination mot vibrios ('Vaccination against vibriosis'). Vattenbruk 1: 7

Michel, C., Tixier, G., Mevel, M. (1984). An assessment of the potency and economic value of fish vaccines. In: de Kinkelin, P. (ed.) Symposium on Fish Vaccination, Paris, Feb 20–22, 1984. Office International des Epizooties, p. 75–96

Neter, J., Wasserman, W. (1974). Applied linear statistical models. Richard D. Irwin, Inc., Homewood, Illinois

Remington, R. D., Schork, M. A. (1970). Statistics with applications to the biological and health sciences. Prentice-Hall, Inc., Englewood Cliffs

Sawyer, E. S., Strout, R. G. (1977). Survival and growth of vaccinated, medicated and untreated coho salmon *(Oncorhyncus kisutch)* exposed to *Vibrio anguillarum.* Aquaculture 10: 311–315

Sørensen, U. B. S., Larsen, J. L. (1986). Serotyping of *Vibrio anguillarum.* Appl. environ. Micriobiol. 51: 593–597

Thorburn, M. A., Jansson, E. (1988). The effects of booster vaccination and fish size on survival and antibody production following *Vibrio* infection of bath-vaccinated rainbow trout, *Salmo gairdneri.* Aquaculture 71: 285–291

Responsible Subject Editor: Dr T Evelyn; accepted for printing on August 18, 1988