Vaccination for Vibriosis in Atlantic Cod

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This study compared route of vaccination, immune response and efficacy of two *V. anguillarum* bacterin preparations in cultured Atlantic cod. Groups were either IP injected, sprayed or immersed in each bacterin. Antibody titres indicated an agglutinating response in all groups by day-47, with peak titres sustained through day-98 in the IP injected populations. Vaccine efficacy following challenge was evaluated as relative percent specific survival (RPS) through day-42 post challenge. Equally significant RPS values (87%) were only noted for the group IP inoculated with the commercial bacterin and the group immersed in the Autogenous Bacterin. Antibody titre was not consistently correlated to survival.

Introduction

Wild adult cod reared at the Sea Forest Plantation in Bay Bulls, Newfoundland, have experienced bacterial epidemics during late summer and early autumn (13-18°C) since 1988. Microbiological analysis, restriction endonuclease fingerprinting and serological evaluations confirmed the isolate was identical to *V. anguillarum* (serotype-2). Antibiotic therapy was only marginally successful, so the use of vaccination was evaluated. The findings are presented below.

Materials and Methods

Animals and Holding Conditions

Atlantic cod (*Gadus morhua*) were captured in mid-August 1991 from inshore stocks using box-type trap nets and were transported to net-pens at Sea Forest Plantation. Initial weight of the cod in the vaccination field study was 0.70-0.81 (sd0.2) kg, with a fork length of 45.09-46.59 (sd4.0) cm. Cod subsequently transferred to Wesleyville in early December 1991 for the pathogen challenge study weighed 1.64-1.81 (sd0.01) kg, with a fork length of 51.34-62.17 (sd3.8) cm.

Cod in the vaccination field study were randomly distributed between 7 adjacent seawater pens (4.7 x 5.5 x 5.5 m). Stocking density was 5.0-5.5 kg/m^3. On day-98 post-vaccination fish used for the pathogen challenge study (approx. 60 fish from the 6 vaccinated and 1 control groups) were transported to an indoor holding facility in Wesleyville. Challenge groups were randomly segregated between 7 circular fibreglass tanks (2.36 x .762 m). Stocking density during the challenge study was 29.5-32.4 kg/m^3, and water temperatures at 4-5°C.

During the vaccination study cod were fed diets of either whole thawed capelin or chopped thawed herring. For the pathogen challenge study no food was provided after day-84 post-vaccination.

Vaccine

Two formalin killed bacterin preparations were compared: the autogenous bacterin (SHAWOGEN) prepared by the Dept. Fisheries & Oceans in St. John's from a 1990 *V. anguillarum* (Serotype-2) isolate (AVC U-13220) of clinically ill cod. The bacteria was grown at 24°C in a fermenter with Trypticase Soy Broth (without dextrose) as a medium. The culture was grown for 48 h, killed with formalin (0.4%, 24 h.), and bottled and stored at 5°C until use. The commercial bacterin (VIBROGEN-2) was provided by Aqua Health Ltd. It was prepared as a formalin killed culture of three bacterial isolates; *V. anguillarum* (Serotype-1)=VA101, *V. anguillarum* (Serotype-2) New Brunswick=VA123 and *V. ordalii* = VA102.

Vaccine Delivery

Three methods were compared: a) intraperitoneal (IP) injection (0.2 mL neat bacterin) administered with adjustable multidose syringes and mild anaesthesia, b) by spraying (1:250 dilution for 30 sec) without anaesthesia on a stainless steel grading table with hand-held 11.4 L Chapin compressed air sprayers, and c) by immersion (1:500 dilution for 1 h with aeration) without anaesthesia in a 3.15 m^3 well-boat, stocked at a density of approximately 3000 lbs/m^3.

Pathogen Challenge

For the pathogen challenge study, the challenge inoculum was prepared from the same *V. anguillarum* (Serotype-2) used to prepare the autogenous bacterin. Specifically, the isolate was cultured for approx. 24 h in trypticase soy broth (described above) until reaching an optical density of 0.1, and subsequently adjusted in sterile saline for a inoculum dose of 10^6 live bacterial per 0.1 mL.

Experimental Design

Vaccination occurred on successive days in late August 1991 at 10°C. All 6 experimental groups, plus a control, were randomly assigned to cages. Efficacy was evaluated by monitoring mortality, and immune response by measuring agglutinating antibody titres against *V. anguillarum* (Serotype-1 and 2), and *V. ordalii* using doubling dilutions of plasma. Specifically, 10 fish were anaesthetized, bled and necropsied from each group on days-26, 47 and 84 post-vaccination. Plasma and bacteriological samples were subsequently evaluated.

For the pathogen challenge study, on day-98 sub-samples of 60 fish/group were assigned to separate tanks. Antibody titres were assessed on days-116 and 145 post vaccination. On day-145 (47 d after transfer from net-pens) all groups were challenged by IP inoculation. Mortalities were weighed, necropsied and cultured for the challenge organism. Efficacy was evaluated by comparing the mortality rates between control and vaccinated groups. On day-42 all remaining fish were examined, and 10 random specimens from each group were weighed, bled for plasma collection, necropsied and cultured for bacterial pathogens.

Data Evaluation

To evaluate immune response, antibody titres in each experimental group were collected on days-0, 27, 44, 84, 116 and 145 post-vaccination. Antibody data were compared using a Generalized Linear Model (GLM) analysis of variance (SAS ver. 6.03), considering the effect of method of vaccination, time of sampling post-vaccination and vaccine type. Data was transformed prior to analysis to Log_2 for GLM analysis, and significance assigned at the $P \leq 0.05$ level. Statistical analysis was completed on two different antibody outcomes; 1) the mean antibody titre of the three *Vibrio spp.* isolates (VA-123, VA-101, VA-102) used in the manufacture of VIBROGEN-2; and 2) the mean antibody titre of the Newfoundland *Vibrio anguillarum* (Serotype-2) isolate (NF-13320) used to produce SHAWOGEN.

For the pathogen challenge study, vaccine efficacy was evaluated as a function of Relative Percent Specific Survival (RPS) following pathogen challenge, and Chi-square analysis of mortality data using a Bonferroni correction factor.

Results and Discussion

Vaccination Field Study

During the field study, temperatures declined from 10°C to 6°C (early December). No mortality due to natural challenge was recorded, probably because water temperatures were not ideal for a *Vibriosis* epidemic (previously occurred above 12°C). Thus, vaccine efficacy could not be assessed as was intended.

Antibody titres showed an agglutinating response in all vaccinated and unvacciniated groups by day-47, indicating that the experimental site had undergone a mild challenge. Peak titres (≥ 30) were only sustained through November (day-84) in the two IP injected populations, suggesting that the IP vaccination was more immunogenic. The most interesting finding was the maintenance of measurably elevated titres (≤ 128) in both injected groups through day-145, even though the fish were held at 4-5°C. Evidently, cod can maintain agglutinating antibody levels even on decreased water temperature.

Evaluation (GLM) of antibody levels against the autogenous vaccine component (NF-13320) indicated significant differences due to method of vaccination (P=.0001), time of sampling (P=.0001) and the method by time interaction (P=.0063). Injection groups produced significantly higher titres to NF-13220 than did the spray or immersion vaccinated groups, irrespective of type of vaccine. Likewise, all vaccinated groups had significantly higher titres to NF-13220 than the controls. By day-84, the injected groups had significantly higher antibody titres than either the sprayed, immersed or control groups (irrespective of vaccine). By day-116, however, the spray and immersion groups had significantly higher titres than the controls, and the injection group still exhibited the highest titres. By day-145 the vaccinated groups had significantly higher antibody titres than the controls. Results support injection as the best method of producing agglutinating antibody titres to NF-13220 and indicated that agglutinating antibody titres to NF-13220 can be produced and maintained regardless of vaccine type; ie, vaccination with either VIBROGEN-2 or SHAWOGENproduced similar antibody levels to the Newfoundland *Vibrio* isolate.

Statistical evaluation (GLM) of the mean antibody levels against the commercial vaccine components (VA-123, VA-101 and VA-102) indicated significant effects due to method of vaccination (P=.0001), time of sampling (P=.0001) and vaccine type (P=.003). All methods produced significantly higher antibody responses to the commercial vaccine (VIBROGEN-2), than the controls irrespective of vaccine type; injection vaccinated groups produced higher titres than spray vaccinated groups, which were higher than immersion vaccinated groups. With regard to time of sampling, the highest antibody levels were attained between day-47 and 116, being significantly higher than those at days-26 or 145, irrespective of vaccination method or vaccine type. The antibody response to vaccines was significantly higher than in the controls, regardless of application method or sampling time. Interestingly, unlike the response noted with the Newfoundland isolate (NF-13220), the VIBROGEN-2 bacterin produced significantly higher antibody titres than the SHAWOGEN bacterin, suggesting that vaccination with the commercial bacterin produces less cross-agglutination to the Newfoundland isolate than the reverse; i.e., vaccination with SHAWOGEN produces lower antibody levels to VA-123, VA-101 and VA-102 than VIBROGEN-2.

Efficacy Evaluation

Pathogen challenge studies demonstrated equally high protection for the injection and immersion groups. By day-42 these groups had

Table 1. Mortality which occurred in vaccinated groups of cod following IP injection with 10^6 live *Vibrio anguillarum* (serotype-2) bacteria.

Treatment Group	Number Treated	Total Mortality	Confirmed Mortality	PSM[1]
Control	60	41	39	65.0
Vibrogen-2 Spray[2]	56	24	22	39.2
Vibrogen-2 Immersion	61	32	31	50.8
Vibrogen-2 Injection	62	10	5	8.1
Shawogen Spray[3]	58	26	26	44.8
Shawogen Immersion	57	5	5	8.8
Shawogen Injection	63	17	17	27.0

[1] Percent specific (confirmed) mortality
[2] Commercial bacterin supplied by AquacHealth Ltd., Charlottetown
[3] Autogenous bacterin supplied by Dept. Fisheries & Oceans, St. John's, NF

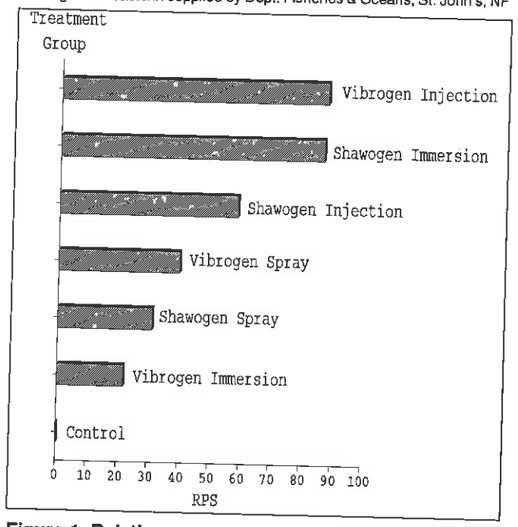

Figure 1. Relative percent specific survival (RPS) of vaccinated cod following IP challenge with 10^6 live *Vibrio anguillarum* (serotype-2) bacteria.

mortalities of only 8.1 and 8.8% compared to 65% in the controls (Table 1). Chi-square analysis indicated better survival over the controls for groups vaccinated by injection (P=0.006) and spray (P< 0.0001) with VIBROGEN-2, as well as groups immersed (P<0.0001) and injected (P=0.0002) with SHAWOGEN. The VIBROGEN-2 IP injected (P=0.0001) and SHAWOGEN immersion groups (P≤ 0.01) had significantly higher survival than the other methods, but were not significantly different from each other. An RPS value of about 87% was calculated for both groups (Fig. 1).

This finding was surprising, in that the immersion vaccinated group demonstrated relatively low antibody titres to the challenge organism (NF-13220) prior to challenge (i.e., 4.0-12.0). Whereas, the injection vaccinates maintained relatively high titres to the challenge organism throughout this period (i.e., 9.6-48.0, suggesting that agglutinating antibody titre may not consistently show a positive correlation with protection in cod (at least not during low temperature challenge). Evidently alternative immunological mechanisms (likely cell mediated) were induced by the bath vaccination with the autogenous bacterin (SHAWOGEN), and may have contributed to protection. Since the pathogen challenge study was done at 5°C, the results may not be indicative of the condition typical of *Vibriosis* epidemics in Newfoundland. Additional field studies should be undertaken to evaluate both the SHAWOGEN Immersion and VIBROGEN-2 Injection techniques at more appropriate temperatures of 10-15°C.

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Notes

1. Atlantic Veterinary College, UPEI, Charlottetown, PE.
2. Institute of Fisheries and Marine Technology, St. John's, NF.
3. Dept. Fisheries Oceans, Science Branch, St. John's, NF.
4. Sea Forest Plantation, St. John's, NF.
5. Canadian Centre for Fisheries Innovation, St. John's, NF.

Calendar

April 1993

9th International **Pectinid Workshop**, 22-27 April, Nanaimo BC. Biennial meeting of people working with scallops; international participation encouraged. Symposia and contributed papers on fisheries biology, physiology, aquaculture, genetics, diseases, processing and marketing. Abstract deadline: 1 January 1993. Contact: Dr. Neil Bourne, Pacific Biological Station, Nanaimo, BC V9R 5K6 (fax 604 756-7053).

Microalgae and Health—1st European Symposium on biotechnological and metabolic aspects of microalgae, 21–22 April. Congress Secretariat: BIOCIM/IMIM, Mini Parc, Bât 2, Rue de la Croix Verte, 34090 Montpellier, France (fax 33 67 61 15 10).

May 1993

World Aquaculture 93 Conference and Trade Show, Torremolinos, Spain 26-28 May. Torremolinos is a well-known Mediterranean seaside resort on the Costa del Sol only 7 km from Malaga. A number of optimal excursions are planned to visit sea bass, sea bream, turbot,, clam, shrimp and mussel farms. Conference information: European Aquaculture Society, Coupure Rechts 168, B-9000 Gent, Belgium. Exhibition organizer: Peter Landless, 45 Ashcroft Road, Cirencester, Glos United Kingdom GLZ 1QZ.

June 1993

Floating Structures Design Conference, 16-19 June, Seattle. Coastal and ocean engineering principles; breakwater, wave attenuator, mooring design; structural and hydrostatic design, design and construction of floating structures, pens, housing, bridging and docks; case studies and engineering economics. Fee $800. Information: 800-462-0786 (fax 608-263-3160). Contact: Prof. C. Allen Wortley, Dept. Engineering, University of Wisconsin–Madison, 432 N. Lake Street, Madison, WI 53706.

Aquaculture Engineering: An International Conference of Engineering Techniques for Modern Aquaculture, 21-23 June, Spokane, Washington. Sponsored by the American Society of Agricultural Engineers. Information: American Society of Agricultural Engineers, 2950 Niles Road, St. Joseph, Michigan 49085-9659.

Sixth Annual Atlantic Aquaculture Fair, 24-27 June, St. Andrews, NB. Trade show, industry sessions, children's program, water events, and sea farmer's market. For information: Sue Corbyn, Box 89, St. Andrews, NB EOG 2XO (tel 506 529-4578; fax 506 529-8095).

August 1993

Aquaculture Canada 93—10th Annual Meeting of the Aquaculture Association of Canada, Charlottetown, PEI, 24-28 August. "Industry and Research—Development through Cooperation", conference with special sessions, contributed papers, industry workshops, informal trade show and social events. Program: introductions and transfers, shellfish enhancement, mussel workshop, aquaculture and the environment, marketing and industry sessions. For information contact Irwin Judson, Chairman, PEI Dept. Fisheries and Aquaculture, PO Box 2000, Charlottetown, PEI C1A 7N8. For submission of papers contact: Dr. David Groman, Atlantic Veterinary College, 550 University Avenue, Charlottetown, PEI C1A 4P3.

Larviculture and Artemia Training Course—8 Aug–17 Sep, Ghent/Leuven, Belgium. Topics: biology, production, quality control and use in aquaculture of the brine shrimp *Artemia*; role of *Artemia* in solar salt production; larviculture of marine and freshwater fish, shrimp and prawn; selection and manipulation of live feeds and their supplements/substitution products; reproduction in fish; bacterial infections of fish larvae/interaction with water quality. Registration fee is 40,000 BEF (about US $1250). Contact: the Lab. of Aquaculture & ARC, c/o P. Bogaert, Rozier 44, B-9000 Gent.

January 1994

World Aquaculture 94 and Aquaculture Expo VII—the 25th Annual Meeting of the World Aquaculture Society, January 1994, Marriott Hotel, New Orleans, Louisiana. Information: Carroll Trosclair, 4640 S. Carrollton Ave., Suite 2E, New Orleans, LA 70119.