CONTROL OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN COMMERCIALLY REARED SALMONID FISHES

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PROJECT OBJECTIVES

The research in this final year of the three year IHN project falls within four objectives. These are: (1) to field test experimental vaccines for inducing specific immunity to IHNV in rainbow trout; (2) to begin research on the interaction between cold-water disease and IHN; (3) to field test means for controlling IHNV in rainbow trout using application of low levels of elemental iodine; and (4) to develop extension products to summarize the large body of research and to transfer these findings to the commercial sector.

ANTICIPATED BENEFITS

This project will develop a focused effort to translate the large body of information developed during the course of the 9 year WRAC IHN project into practical and cost-effective strategies to reduce IHN-associated losses among commercially-reared rainbow trout and to conduct field trials of these strategies. In addition, a significant extension component is included that will summarize the extensive body of research and to make the results of the work available to the trout and salmon industry. The project will conduct field trials of vaccines, examine the relationship between coldwater disease and IHN infections in commercial facilities, and conduct field trials of low levels of iodine for control of IHN. Researchers are expected to work very closely with the trout industry to coordinate the field trials and to insure that commercial concerns are included in the experimental approach. A substantial extension component will prepare an extensive summary of the scientific research to include lists of publications, synopses of the research findings, and recommendations for future work. Other, more general, extension products were to be developed to transfer the complex body of knowledge to the trout and salmon industry in a highly accessible form.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. Conduct field tests of recombinant vaccines for induction of specific resistance to IHNV

Sub-objective 1.1. Comparison of recombinant vaccines in laboratory trials

Several candidate vaccines have been developed during the course of the WRAC-funded IHN project. These include: a recombinant *E. coli* expressing most of the glycoprotein gene, a baculovirus vector expressing the entire glycoprotein gene, two ISCOM preparations containing native glycoproteins harvested from wild-type virus or baculovirus expression vectors, and a plasmid containing a cDNA copy of the glycoprotein gene under control of a CMV promoter for use as a naked DNA vaccine. These preparations and controls (saline, a killed virus vaccine, empty ISCOMs, and attenuated virus) were delivered by waterborne and/or injection routes for comparison (ten preparations in all) in a large-scale, laboratory vaccine trial at Oregon State University. The trial, the largest fish vaccine trial ever conducted, was completed in 1998 with convincing results that at least three preparations (the killed virus, the attenuated virus, and the DNA vaccine) provided substantial protection for small fish.

The most efficacious IHNV vaccine was the IHNV DNA vaccine, CMV-IHNV-G, which protected from 100% to 96% of the fish in a 10^5 PFU/ml challenge and 100% to 98% at a 10^3 PFU/ml challenge. This is in comparison to the DNA vaccine control, CMV-Luciferase, which only protected 18% to 11% of the fish at 10^5 and only 55% to 66% of the fish at 10^3. The other vaccine preparation that provided good protection was the b-propiolactone inactivated IHNV vaccine. This vaccine protected from 100% to 98% of the fish from a 10^5 PFU/ml challenge and 98% to 96% of the fish from a 10^3 PFU/ml challenge. The attenuated IHNV vaccine provided moderate protection with 60% and 50% protection at 10^5 and 82% to 78% protection at 10^3. The subunit vaccine (pXL3/pLON3), the native G, Baculovirus G, and ISCOM G, as well as the vaccine controls only afforded from 26% to 4% protection at 10^5 PFU/ml and 66% to 49% protection at 10^3 PFU/ml.

Sub-objective 1.2. Induction of specific immunity using adjuvants—ISCOMS

In a previous study of the native G ISCOMS conducted at Clear Springs, an RPS of 78% was achieved over unvaccinated controls. We are addressing the ISCOM formation problem by altering the amount and composition of the lipid used. Experiments at UC Davis have shown that the amount of membrane lipid in the starting material can affect the formation of the cage-like ISCOM structure. The original ISCOM protocols, developed by Bror Morein, called for the inclusion of cholesterol and phosphatidylcholine (PC). However, Karsten, et al. (Biochem. Biophysica Acta 1062:165-171, 1991) could not form ISCOMS using PC in the lipid mix and only had success when using phosphatidylethanolamine (PE) in place of PC. In the proposed study we plan on 1) testing PC vs PE in the lipid mix and 2) incorporating different levels of lipid during the formation of the ISCOMS. The formation of ISCOMS will be confirmed by EM. When the correct balance of lipid has been determined we will produce ISCOMS incorporating $recG_{low}$ from the rAcG897 cells. Rainbow trout will be immunized with recG ISCOMS and appropriate controls. The fish will then be challenged with virulent IHNV. Efficacy of the vaccine will be measured by RPS and serum from vaccinated controls and challenge survivors will be tested for IHNV neutralization activity.

Sub-objective 1.3. Field tests of most effective recombinant vaccine

The three vaccines determined to be most efficacious in sub-objective 1.1 (killed, attenuated and DNA vaccines) are planned to be used in a field trial at a commercial facility in Idaho. In the proposed study, fish at rearing densities and feeding regimens used in production will be vaccinated and challenged with contaminated water from ponds with an active IHNV epizootic at the facility. Mr. Jim Parsons at Blue Lakes Trout Farm originally offered to assist in conducting the trials; however, the project may need to use other facilities in the Hagerman Valley. One possibility is Clear Springs Foods, Inc. where extensive laboratory trials with the DNA vaccine have already been conducted. In addition, Mr. Leo Ray has expressed a willingness to have the trials run at his facility, but only with the consensus of the Idaho aquaculture community. A proposal to proceed with the vaccine trials will

be brought to the Idaho Aquaculture Association in December 1999. If favorable, trials will be run in February or March of 2000.

Objective 2. Synergistic effects of Flexibacter psychrophilus and IHNV During Mixed Epizootics

Sub-objective 2.1. Determine effects of IHNV and F. psychropuilum on the immune response of rainbow trout

The enzyme-linked immunosorbent assay (ELISA) developed in the previous year was improved. The assay was used to determine the effect of exposure to IHN virus on the humoral immune response to *F. psychrophilum.* Rainbow trout (12 g) received one of three treatments: (1) intramuscular injection of 10^5 live *F. psychrophilum,* followed after 5 d by an immersion challenge in 10^3 PFU/mL of IHN virus, strain 220-90 [CWD/IHNV groups]; (2) intramuscular injection of 10^5 live *F. psychrophilum,* followed after 5 d by a mock immersion challenge [CWD groups], or (3) intramuscular injection of saline, followed after 5 d by a mock immersion challenge [mock challenged groups].Replicated CWD/IHNV and CWD challenges (n=25 fish for each replication)were performed with fish from each of four tanks, and one group offish (n=25) from each of the tanks served as the mock-challenged control. Mortalities were recorded for 28 days. At the end of the observation period, blood samples were taken from survivors for determination of anti-*F. psychrophilum* antibody titers against by the ELISA method developed in the previous year of the project.

Antibody titers against *F. psychrophilum* were identical (9.5 units) for the CWD and CWD/IHNV groups. Both challenged groups had much higher titers($P<0.001$) than controls (2.6 units). Mortalities were significantly higher ($P=0.02$,Mann-Whitney U test) in the CWD/IHNV groups than in the CWD groups (63.8 vs 37.2% mortality overall). In this experiment, the humoral immune response to *F. psychrophilum* was not affected by co-infection with IHN virus. The antigen processing and presentation stages of the immune response presumably would have been completed, however, prior to the time of IHNV challenge (5 d after *F. psychrophilum* injection); additional trials are needed to determine if IHNV infection during the earliest stages of the immune response affects the development of the humoral response to *F. psychrophilum.*

Anti-*F. psychrophilum* antibody titers were determined for 87 trout that survived *F. psychrophilum* infection. Titers were higher in fish that subsequently developed deformities (13.8 units) than in those that did not (4.1 units).

Sub-objective 2.2. Determine histopathological changes in fish expsoed to mixed IHNV/F. psychrophilum infections.

Infected tissues collected during the challenge trials described above and during natural epizootics will be examined. In this manner, pathological changes in various tissues, including liver, spleen, anterior kidney and posterior kidney, and vertebrae can be followed during the entire course of infection by both pathogens. *F. psychrophilum*-specific monoclonal antibodies and IHNV specific monoclonal antibodies will be used to label the bacterial and viral antigens in infected tissues processed for histopathology to help indicate the relationship of each pathogen to pathological changes observed.

Sub-objective 2.3. Determine histopathological changes in tissues from fish exposed to mixed IHNV/ F. psychrophilus infections

Infected tissues will be collected during the challenge trials described above and during natural epizootics. In this manner, pathological changes in various tissues, including liver, spleen, anterior kidney and posterior kidney, and vertebrae can be followed during the entire course of infection by both pathogens. *F. psychrophilus*-specific monoclonal antibodies and IHNV-specific monoclonal antibodies will be used to label the bacterial and viral antigens in infected tissues processed for histopathology to help indicate the relationship of each pathogen to pathological changes observed. This work will occur during year 2.

Objective 3. Field testing application of low levels of iodine for control of IHN

Earlier we showed that addition of low levels of elemental iodine will rapidly inactivate IHNV leading to

a potential application of the compound for disinfection of water supplies. Dick Polley of Hydrodyne Co provided a quantity of iodine at cost in exchange for access to the information for possible future commercialization of the concept. Leo Ray of Buhl, Idaho was contacted and was willing to donate a portion of his facility where IHN challenges are highly predictable. He also offered to provide rearing troughs, food, and fish for the experiments. To date, two field trials have been conducted and a third is being initiated in late September. These trials have been extremely encouraging and further work is planned. In the initial trial, an iodine diluter was fabricated from PVC pipe and installed to deliver elemental iodine to replicate commercial fiberglass troughs at 0.1 and 0.05 ppm with a pair of untreated troughs. Rainbow trout were placed in the troughs and reared according to standard procedures at the facility. After a few days exposure to iodine, the first dose appeared slightly too high and fish were observed to suffer modest mortality. The concentration was reduced for the remainder of the trial. After one week, typical IHN losses began in the control fish. By the end of the experiment, losses in the treated groups were considerable lower than controls although problems with the iodine delivery system precluded the experiment from running the intended length. Encouraged by this pilot experiment, eight stainless steel rearing troughs were purchased from Rangen's Inc. which made them available at a considerable discount from original cost. A new diluter system was fabricated and installed to deliver 0.08, 0.05, and 0.025 ppm to replicate troughs with a pair of control troughs as before. Trout were added to the system and reared using standard methods. In this trial, Mr. Ray was encouraged enough to make technical help available to us at no charge. This experiment ran for 40 days and the results were highly encouraging. Although the highest concentration again appeared to be marginally toxic to the fish, the middle and lowest concentrations provided substantial protection with losses in the middle group being about one third of the controls. The replicates were generally in good agreement. Mr. Ray is eager to pursue this work further. A third field trial will test the middle concentration (0.05 ppm) in 4 replicates with 4 control troughs. After 30 days, the iodine will be discontinued to two of the treated replicates and the fish monitored for an additional 30 days to determine if the fish developed immunity to IHNV during the treatment period.

Objective 4. Development of extension products and project synopsis

Work on this objective was delayed due to budget constraints and is now being conducted in the current year (year 3). It will include an extensive summary of the scientific research to include lists of publications, synopses of the research findings, and recommendations for future work. Other, extension products were to be developed to transfer the complex body of knowledge to the trout and salmon industry in a highly accessible form. These include workshops to present findings, a diagnostic manual and synopses of the field tests of vaccines and iodine.

USEFULNESS OF FINDINGS

Infectious hematopoietic necrosis (IHN) is the most important viral disease of fish in the United States. Causing losses estimated at several millions of dollars annually among commercially-reared rainbow trout, IHN also affects salmon and steelhead trout reared by federal, state and tribal agencies in at least five western states. Our project on development of methods for control of IHN was among the original set of proposals selected for funding by the Western Regional Aquaculture Center. During the initial 5-year project, the IHN work group established a high level of productivity publishing 27 papers in peer-reviewed journals and developing two extension products. Based upon the results from the initial project, the IHN work group was funded for an additional 4-year period. During the renewal, the work group published an additional 43 papers in peer-reviewed journals and hosted two highly successful workshops for the commercial trout growers of the Hagerman Valley of Idaho. Over the course of the project, significant accomplishments included development and testing of a subunit vaccine against IHNV that was commercially licensed; creation of new diagnostic reagents (monoclonal antibodies, DNA probe, PCR primers) that allow more rapid or more sensitive tests (these have also received commercial interest);

new information about the role of non-specific mechanisms of resistance to IHNV including resistance conferred by avirulent viruses; further understanding about the IHNV life cycle, and the susceptibility of IHNV to low levels of iodine that has generated substantial interest in the commercial industry.

WORK PLANNED FOR NEXT YEAR

This project was originally scheduled to end after 2 years on 3/31/1999 with accomplishment of all four objectives. However, at the 1997 WRAC IAC/TC meeting, funding for Objective IV was removed from the current (second) year and the project was promised a one-year extension until 3/31/2000 and funding of $30,000 to complete Objective IV. At the 1998 WRAC IAC/TC meeting, funding for the extension component was cut further to $9000 and the project scheduled to end on 3/31/2000. Thus, in the current (final) year, we will complete any unfinished portions of the work, prepare manuscripts and initiate work on a reduced level of effort in Objective IV which will be accomplished during this final year of the project. Extension products generated from the IHN project will consist of a workshop, workshop proceedings, IHN diagnostic manual and a project synopsis. The one-to-two day workshop will be divided into two parts. Part one, directed at salmonid producers, will cover the virus and its biology, specific immunity, non-specific immunity, management strategies, and results of the planned field trials. Part two, directed at both private and public fish pathologists, will cover improved diagnostics. From part one a proceedings will be published and made available to WRAC. A diagnostic manual will come from part two. The proceedings will serve as a guide for salmonid producers in managing IHN virus, while the diagnostic manual will standardize diagnostic techniques for IHN virus, thereby, facilitating the movement of salmonids within and across state lines. A large body of research has been produced from this project and there is a need to develop a project synopsis. The synopsis will summarize the work, explain the reasoning behind the research and allow easy access to the body of work. At this time next year, we will prepare a final completion report for the entire project.

SUPPORT

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT				TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	TOTAL	
98	127,400	19,975	136,500	283,299	439,774	567,174

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

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