Fish Vaccine Research at U of I: How can I use vaccination as a tool to reduce diseases at my facility?

Ken Cain
University of Idaho, Dept. of Fish and Wildlife Sciences and the Aquaculture Research Institute, Moscow, ID
*Correspondence: kcain@uidaho.edu

LSRCP – Annual Meeting
April 23rd, 2019, Lewiston, ID
University of Idaho – CNR and ARI Programs:

1. Fish Health/Immunology
   - Vaccine development*
   - Fish Immunology/Pathology
   - Probiotics/antimicrobial research*
   - Diagnostic improvements for pathogens*

2. Aquaculture Development
   - Burbot/freshwater cod conservation aquaculture
   - New work on commercial (foodfish) aquaculture

* Patents or licensed technology
University of Idaho – CNR and ARI Programs:

1. Fish Health/Immunology

- Disease Management
  - Coldwater disease and path to vaccine licensing and commercialization
  - Research at UI
    - Live-attenuated vaccine
    - Practical delivery methods?
    - Co-infections (IHN/CWD)?

- Summary/Conclusions
Disease Management: What are the options?

- **Drugs/chemicals** (disease treatment)
  - Antibiotics – Vet approval (VFD)
    - Antibiotic resistance?
  - Chemical therapeutants
- **Vaccines** (disease prevention)
- **Other options** (feed additives, immunostimulatnts, probiotics, etc.)

Limited (approved) disease control and prevention products available

- Need for more/improved products to prevent or minimize losses
- Alternatives to antibiotics!
Coldwater Disease

**FLAVOBACTERIUM PSYCHROPHILUM**

**PATHOLOGY**

- Causative agent of BCWD and RTFS and impacts salmonids across the globe
- Gram-negative bacteria forming yellow colonies with thin-spreading margins
- Prevalent infectivity at cold temps (up to 16°C) and can be horizontally and vertically transmitted
- Characterized by exophthalmia, erratic swimming behavior, and skin lesions and fin loss (caudal peduncle)
**F. PSYCHROPHILUM**

**ECONOMIC IMPORTANCE**

- Implications for aquaculture - both government and private hatchery systems

- Antibiotics (FFC, OTC) currently used to treat infection, following diagnostic collaboration with DVM, but resistant *F. psychrophilum* strains on the rise

- Strains may vary based on geographical location and this may impact antibiotic treatment efficacy for salmonid producers

- Aside from acute *F. psychrophilum* infection, chronic infection may render surviving fish unsuitable for stocking or market due to long-term morphological issues
Vaccine Development at UI

Goal: Develop an efficacious immersion vaccine for Coldwater disease

• UI lab – 20 years of effort
  – Early efforts:
    – Define immune response
    – Developed and tested many formulations
      – Killed cellular preparations
      – Isolated protein fractions
      – Recombinant subunit and DNA vaccines
    – Dozens of vaccine formulations tested
      – Did not work (via immersion delivery)
    – Live-attenuated bacterial strain
Isolation of rifampicin resistant *Flavobacterium psychrophilum* strains and their potential as live attenuated vaccine candidates

Benjamin R. LaFrentz\(a,1\), Scott E. LaPatra\(b\), Douglas R. Call\(c\), Kenneth D. Cain\(a,*\)

\(a\) Department of Fish and Wildlife Resources and the Aquaculture Research Institute, University of Idaho, P.O. Box 441136, Moscow, ID 83844-1136, United States

\(b\) Clear Springs Foods, Inc., Research Division, P.O. Box 712, Buell, ID 83316, United States

\(c\) Department of Veterinary Microbiology and Pathology, Washington State University, 402 Buskald Hall, Pullman, WA 99164-7040, United States
Path to commercialization

Vaccine or other product

- Marketing
- Regulatory Approval
- Bioprocess & Technology Support
- Supply Chain
- R&D Global/Regional
- Manufacturing
**F. PSYCHROPHILUM VACCINE**

R&D AT UI

1. Optimization trials continuing at UI on this live-attenuated immersion vaccine

1. Long-term protection conferred (months)

1. Rainbow trout and Coho investigated to date
   - More salmonid species will be tested

1. Size range for vaccine efficacy

1. Wide range of cross-protection against various *F. psychrophilum* strains

1. Practical delivery options for hatcheries using tank immersion
Vaccine Optimization

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RPS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 g-ILM</td>
<td>60</td>
</tr>
<tr>
<td>1 g-ILM</td>
<td>59</td>
</tr>
<tr>
<td>0.5 g-ILM</td>
<td>55</td>
</tr>
</tbody>
</table>
## PROTECTION AGAINST MANY STRAIN BACTERIAL STRAIN SELECTION

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Host species</th>
<th>Geographic origin</th>
<th>Sequence Type / Serotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 45</td>
<td>Steelhead trout</td>
<td>MI, USA</td>
<td>ST78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Van Vliet, et al., 2016</td>
</tr>
<tr>
<td>US 54</td>
<td>Steelhead trout</td>
<td>MI, USA</td>
<td>ST267&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Van Vliet, et al., 2016</td>
</tr>
<tr>
<td>US 79</td>
<td>Rainbow trout</td>
<td>PA, USA</td>
<td>ST10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>US 149</td>
<td>Atlantic salmon</td>
<td>WA, USA</td>
<td>ST70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>03-179</td>
<td>Steelhead trout</td>
<td>WA, USA</td>
<td>ST294&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ramsrud, et al., 2007</td>
</tr>
<tr>
<td>622-97</td>
<td>Atlantic salmon</td>
<td>Chile</td>
<td>ST79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ramsrud, et al., 2007</td>
</tr>
<tr>
<td>950106-1/1</td>
<td>Rainbow trout</td>
<td>Denmark</td>
<td>ST2&lt;sup&gt;a&lt;/sup&gt;, Fd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Madsen &amp; Dalsgaard. 1999</td>
</tr>
<tr>
<td>900406-1/3</td>
<td>Rainbow trout</td>
<td>Denmark</td>
<td>ST2&lt;sup&gt;a&lt;/sup&gt;, Th&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Madsen &amp; Dalsgaard. 1999</td>
</tr>
<tr>
<td>99-10A</td>
<td>Rainbow trout</td>
<td>Denmark</td>
<td>ST10&lt;sup&gt;a&lt;/sup&gt;, Fp&lt;sup&gt;T&lt;/sup&gt;, Fd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Madsen &amp; Dalsgaard. 1999</td>
</tr>
<tr>
<td>CSF-259-93</td>
<td>Rainbow trout</td>
<td>ID, USA</td>
<td>ST10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Van Vliet, et al., 2016</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sequence Type; <sup>b</sup> Serotype


* Unpublished MLST data provided by Tom Loch and Chris Knupp (Michigan State University)
Vaccine Optimization

<table>
<thead>
<tr>
<th>Challenge strain</th>
<th>RPS_{28\text{day}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>US45-V</td>
<td>53</td>
</tr>
<tr>
<td>US54-V</td>
<td>51</td>
</tr>
<tr>
<td>US79-V</td>
<td>71</td>
</tr>
<tr>
<td>US149-V</td>
<td>58</td>
</tr>
<tr>
<td>03-179-V</td>
<td>66</td>
</tr>
<tr>
<td>622-97-V</td>
<td>60</td>
</tr>
<tr>
<td>950106-1/1-V</td>
<td>67</td>
</tr>
<tr>
<td>900406-1/3-V</td>
<td>61</td>
</tr>
<tr>
<td>99-10A-V</td>
<td>72</td>
</tr>
<tr>
<td>CSF259-93-V</td>
<td>70</td>
</tr>
</tbody>
</table>
PRACTICAL DELIVERY OPTIONS

1. Injection vaccination?
   - Not practical for most operations

2. Oral vaccination (in feed)?
   - Poor protection for most vaccines

3. Immersion vaccination?
   - 1-3 minute dip – efficacious but can take time and added labor cost
   - 30 minute bath?
Cumulative Percent Mortality (CPM)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>CPM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min-B.17-ILM</td>
<td></td>
</tr>
<tr>
<td>30 min-Control</td>
<td></td>
</tr>
<tr>
<td>6 min-B.17-ILM</td>
<td></td>
</tr>
<tr>
<td>6 min-Control</td>
<td></td>
</tr>
<tr>
<td>3 min-B.17-ILM</td>
<td></td>
</tr>
<tr>
<td>3 min-Control</td>
<td></td>
</tr>
<tr>
<td>1.5 min-B.17-ILM</td>
<td></td>
</tr>
<tr>
<td>1.5 min-Control</td>
<td></td>
</tr>
</tbody>
</table>
IMPLEMENTING AT HATCHERY

Density testing and recommendations for vaccination

- Showed that fish could be held for 30 minutes with oxygen at density of 2.0lbs/gal (.24Kg/L) with minimal observed stress.
- Method is simple to apply to hatchery tanks/troughs by lowering water and adding aeration
  - Utah Division of Wildlife Resources
- Estimate of biomass and fish numbers for vaccination:
  - At suggested vaccine dose and fish size:
    - In 1000L (264 gal) – one vial of vaccine
      - 160,000 fish (2.0g)
      - 120,000 fish (1.5g)
      - 240,000 fish (1.0g)

Practical vaccination method with minimal time/labor commitment
CWD vaccine field trial – Magic Springs
(60 days post vaccination)

Initial control population at ponding = 46,948 (raceways 3,9)
Initial vaccinate population at ponding = 50,529 (raceways 10,12)
CWD vaccine field trial – Magic Springs
CWD vaccine field trial – Magic Springs
CWD vaccine field trial – Magic Springs
CWD vaccine field trial – Magic Springs
(Clinical exam of fish)

• July 31
  • Slight increase in mortality in controls
  • 6 control; 6 vaccinates sampled (TYES plates for bacterial isolation)
  • Confirmed *F. psychrophilum* (2/6 controls)

• Aug. 13
  • Increasing daily mortality in controls (significant lesions present)
  • 12 controls; 12 vaccinates sampled
CWD vaccine field trial – Magic Springs
(Clinical exam of fish)

• Clinical exam results (Aug. 13)
  • Controls
    • BGD
    • *F. psychrophilum* confirmed
      • (FAT and PCR)
    • 9/12 fish positive for IHNV
  • Vaccinates
    • Minor BGD
      • (limited signs of bacterial growth)
    • IHNV not detected
    • *F. psychrophilum* confirmed

*Mixed Fp/IHNV infection in controls*
ADDITIONAL RESEARCH WITH IMPLICATIONS FOR NORTHWEST HATCHERIES

Flavobacterium psychrophilum (Fp)

Infectious hematopoietic necrosis virus (IHNV)
Co-infections can be common in nature and occur when a host is infected with two or more pathogens at the same time.

### Co-infection research in salmonids

<table>
<thead>
<tr>
<th>Pathogen A</th>
<th>Pathogen B</th>
<th>References</th>
</tr>
</thead>
</table>

Co-infection with *F. psychrophilum* and *IHNV* occurs in Pacific Northwest hatcheries.

These Fp/IHNV co-infections are not well documented or characterized.
RESEARCH AIMS

HYPOTHESES AND OBJECTIVES

Hypothesis: A combined infection with *F. psychrophilum* (CSF-259-93) and IHNV (220-90) would result in greater mortality than infection with each pathogen alone

Objective 1: Characterize mortality/pathology following *in vivo* challenge of rainbow trout with *F. psychrophilum* and IHNV

- Determine primary target organs of pathogen localization during single and co-infection

Objective 2: Characterize *F. psychrophilum* and IHNV viral load following single pathogen infection or co-infection

Objective 3: Determine if *F. psychrophilum* and IHNV have a synergistic (or antagonistic) interaction
TRIAL DESIGN

BACTERIAL AND VIRUS

**Fp**: *F. psychrophilum* CSF 259-93 strain (LaFrentz et al., 2002)

**IHNV**: EPC cell line; IHNV (CSF 220-90 strain; LaPatra et al., 1991)
### TRIAL DESIGN

#### FISH CHALLENGE AND SAMPLE COLLECTION

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pathogen/Placebo</th>
<th>Challenge dose/Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fp</td>
<td><em>F. psychrophilum</em></td>
<td>1.0 x 10^6 CFU/fish - 2 days - MEM</td>
</tr>
<tr>
<td>IHNV</td>
<td>IHNV</td>
<td>100 PFU/fish - 2 days - TYES</td>
</tr>
<tr>
<td>Fp/IHNV</td>
<td><em>F. psychrophilum</em> and</td>
<td>1.0 x 10^6 CFU/fish - 2 days - 100 PFU/fish</td>
</tr>
<tr>
<td></td>
<td>IHNV</td>
<td></td>
</tr>
<tr>
<td>IHNV/Fp</td>
<td>IHNV and <em>F. psychrophilum</em></td>
<td>100 PFU/fish - 2 days - 1.0 x 10^6 CFU/fish</td>
</tr>
<tr>
<td>Mock-1</td>
<td>TYES</td>
<td>-</td>
</tr>
<tr>
<td>Mock-2</td>
<td>MEM</td>
<td>-</td>
</tr>
<tr>
<td>Mock-3</td>
<td>TYES and MEM</td>
<td>-</td>
</tr>
</tbody>
</table>

*3.5g Rainbow trout: low dose challenge (i.p. injection) – CPM (28 d)*
Co-infected fish exhibited clinical signs characteristic of both CWD and IHN disease.
RESULTS

CUMULATIVE PERCENT MORTALITY (CPM)

Co-infected groups had significantly higher mortality (76.2-100%) and earlier onset of disease (mortalities).
RESULTS

INDIRECT IMMUNOFLUORESCENCE ASSAY

Fp and IHNV were localized in and on the same cell.
SUMMARY/CONCLUSION

- Low dose co-infection of IHNV and Fp resulted in a synergistic interaction with significantly higher mortality (76.2-100%) compared to challenge with each respective pathogen alone (5-20%).

- Fish with an initial underlying IHNV infection (IHNV/Fp groups) exhibited greater infection severity (mortality, viral/bacterial load, and pathology) than Fp/IHNV groups.

- Co-infection with IHNV and Fp can lead to substantial mortality.

Future Research:

- Can control measures (e.g. vaccination) that target one pathogen lessen the impacts of a co-infection?
Summary/Conclusion

- **Current efforts:** Continuing work with industry partner to gain full USDA license approval for this vaccine

<table>
<thead>
<tr>
<th>Optimization criteria</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size range for vaccination</td>
<td>Complete (0.5 – 2 g)</td>
</tr>
<tr>
<td>Immersion delivery (density 2lb/gal)</td>
<td>Complete (1.5 – 30 min)</td>
</tr>
<tr>
<td>Duration of immunity (w or w/o booster)</td>
<td>Complete (&gt; 24 weeks)</td>
</tr>
<tr>
<td>Production feasibility (large scale)</td>
<td>Complete</td>
</tr>
<tr>
<td>Immunization dose determination</td>
<td>Complete</td>
</tr>
<tr>
<td>USDA regulatory approval</td>
<td>Ongoing</td>
</tr>
<tr>
<td>• Safety (R2V and shed/spread)</td>
<td></td>
</tr>
<tr>
<td>• Efficacy (multi-species)</td>
<td></td>
</tr>
<tr>
<td>• Field Safety (multiple sites)</td>
<td></td>
</tr>
</tbody>
</table>

- **Confident that this vaccine will become an important management tool for CWD at salmonid hatcheries in US and globally!**
Acknowledgments

Funding:
- Western Regional Aquaculture Center (USDA)
- Idaho Department of Commerce - IGEM
- Idaho State Board of Education – HERC
- Private Industry Partners
- WSU /UI Aquaculture Initiative (USDA)
- USDA/SBIR I&II

Collaborators:
- SeaPac/Magic Springs (Tom VanTassel)
- Utah Division of Wildlife Resources: Christine Swan, Wade Cavender, and Chris Wilson
- WSU, Doug Call/Devendra Shah,

Graduate students:
- Ben LaFrentz, Amy Long, Tarah Johnson, Nicole Lindstrom, David Burbank, Tyson Fehringer, Mark Polinski

Postdocs/Research Scientists:
- Sudheesh Ponnerasary, Jessie Ma, Tim Bruce
Questions?