

Optimizing a live attenuated *Flavobacterium psychrophilum* vaccine for coldwater disease (CWD)

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University of Idaho

Outline

1. Coldwater disease (CWD)
2. Vaccine Development
 1. Initial (B.17) vaccine
 2. “Newer” **(FP-B.17-ILM)** vaccine
3. Research
 - Study 1 (Coho salmon)
 - Study 2 (Immersion delivery duration)
 - Study 3 (Size at vaccination)
 - Study 4 (Dose, booster, and duration of protection)
4. Field trials
5. Summary/Conclusions



Coldwater Disease (CWD)/Rainbow trout fry syndrome (RTFS)



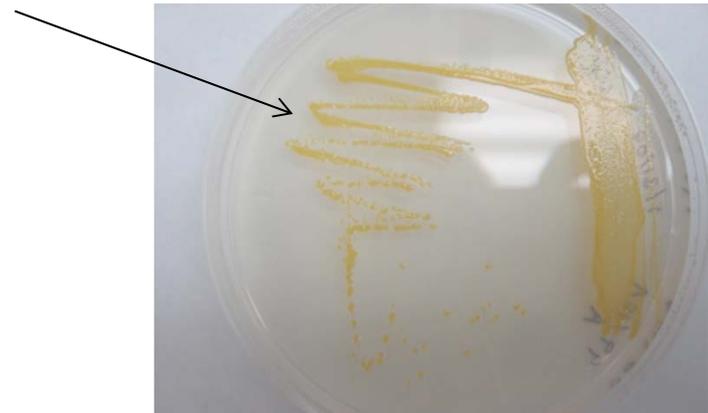
- Number 1 disease problem for US trout Industry
- Global problem in salmonid aquaculture
- Causes greater mortality for US public salmon/trout aquaculture than any other disease

Coldwater Disease (CWD)/Rainbow trout fry syndrome (RTFS)



Steelhead

Yellow pigmented bacteria



Steelhead

Vaccine Development

CWD/RTFS vaccine:

- **University of Idaho lab – over 15 years of effort**
 - Many experimental CWD vaccines
 - Limited success via **immersion** delivery
- **Shifted focus - Live attenuated vaccine**
 - Stimulate both innate and adaptive immune responses
 - Three live fish vaccines approved and commercialized
- **Initial live attenuated *F. psychrophilum* (B.17) vaccine developed** (LaFrentz et al., 2008)
- **Enhanced “new” live FP-B.17-ILM vaccine developed** (Long et al., 2013)
 - Producing in Iron Limited Media



Attenuated Vaccine

Results of the early “initial” B.17 testing:

- Vaccine is safe (field/lab)
- Some inconsistencies with field and lab immersion vaccination trials
- Reports/data submitted to USDA
 - Only outstanding requirement – serial safety testing at USDA licensed facility

Could improvements be made?

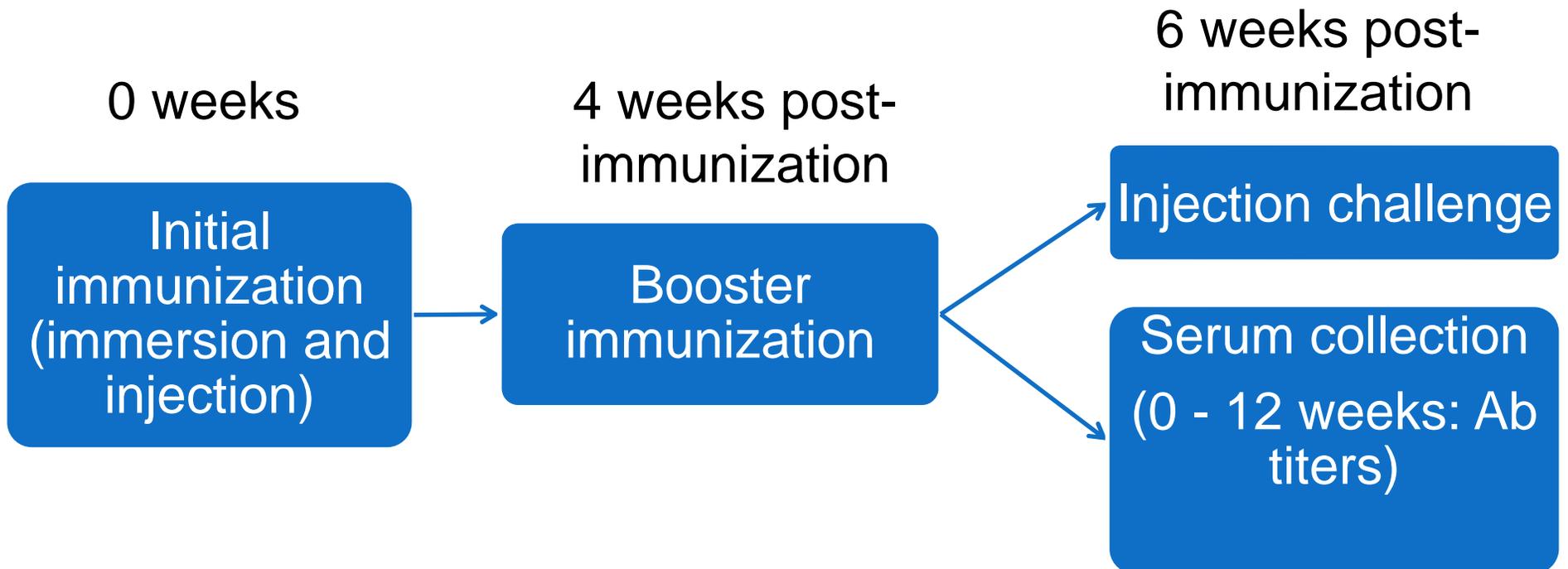
- Can B.17 vaccine be enhanced to provide greater protection/immunity when delivered by immersion?
 - **Iron-limited media (ILM)**

Study 1

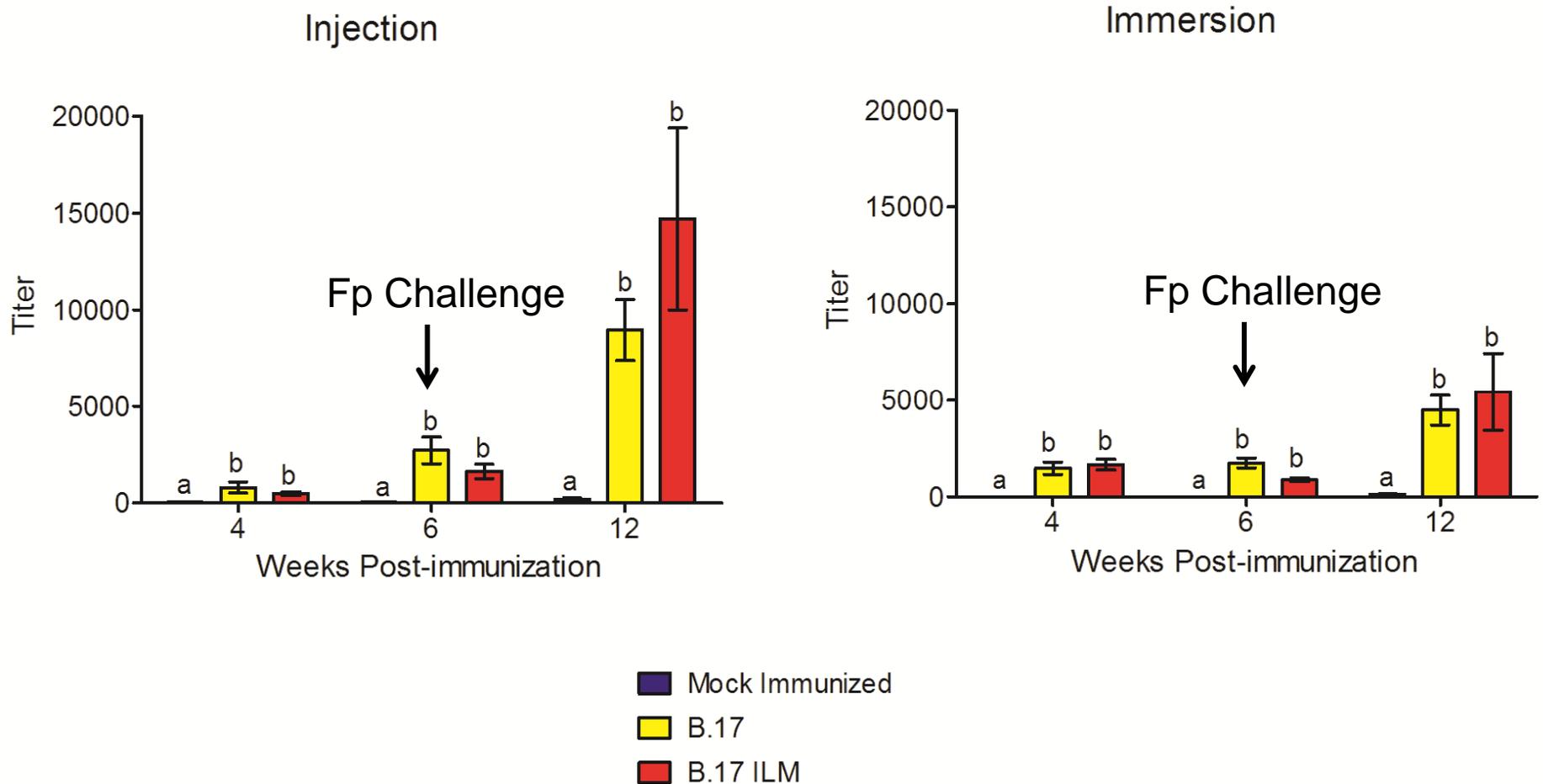
(FP-B.17-ILM) vs initial (B.17) vaccine)

Experimental design:

- Coho salmon (3.6g)
- 1×10^7 - 10^8 cfu/ml
- **adipose fin clip** incorporated for immersion treatment
- Injection vaccination or 1 hour immersion (B.17 or ILM vaccine)



Results: Antibody titers (Coho salmon)



Note – fish were injection challenged with Fp 259-93 at 6 weeks

Coho salmon vaccine trial results

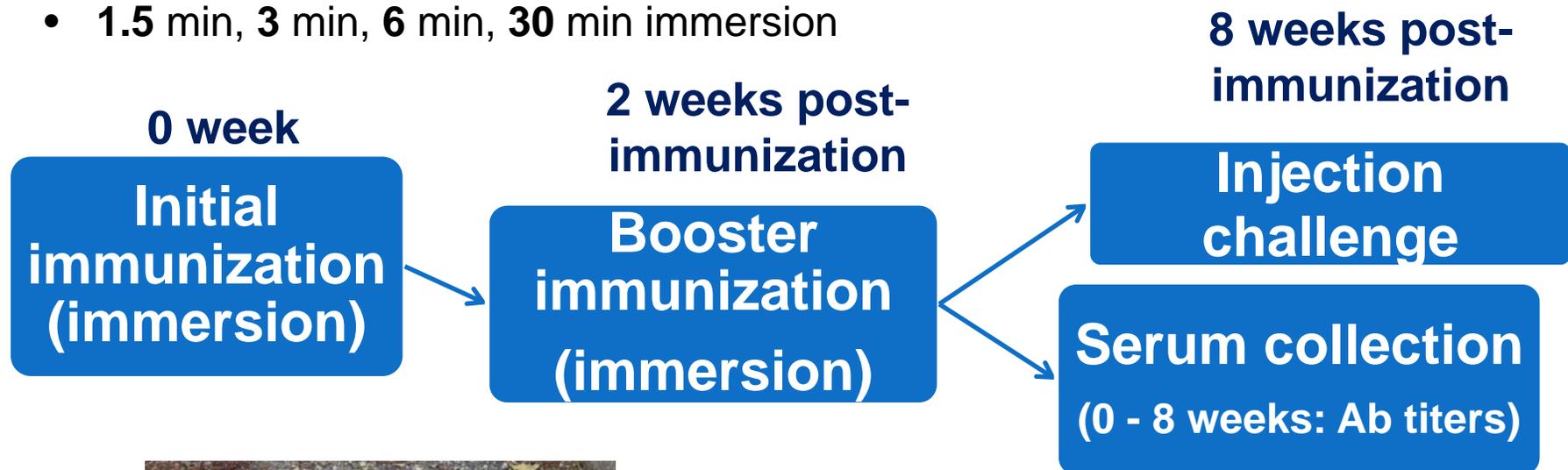
Vaccine Delivery Method	Treatment	CPM \pm SEM	Relative percent survival
Injection	Mock Immunized	65.3 \pm 10.4 ^a	
	B.17	6.7 \pm 3.5 ^b	90
	ILM vaccine	1.3 \pm 1.3 ^b	98
Immersion	Mock Immunized	54 \pm 2 ^a	
	B.17	29.3 \pm 10.7 ^{a,b}	46
	ILM vaccine	14.7 \pm 6.7 ^b	73

Note – adipose fin removed just prior to immersion delivery of vaccine

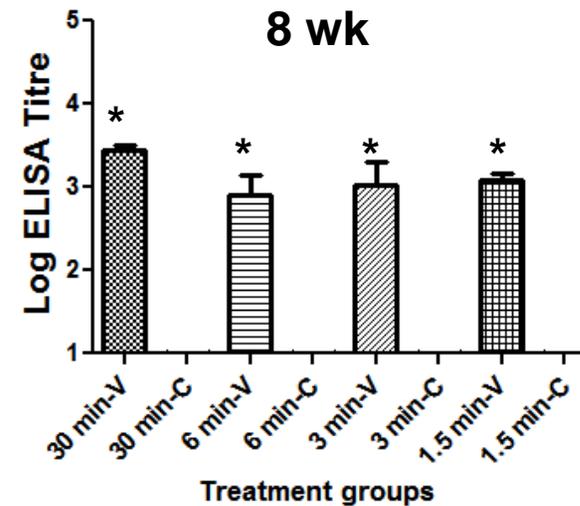
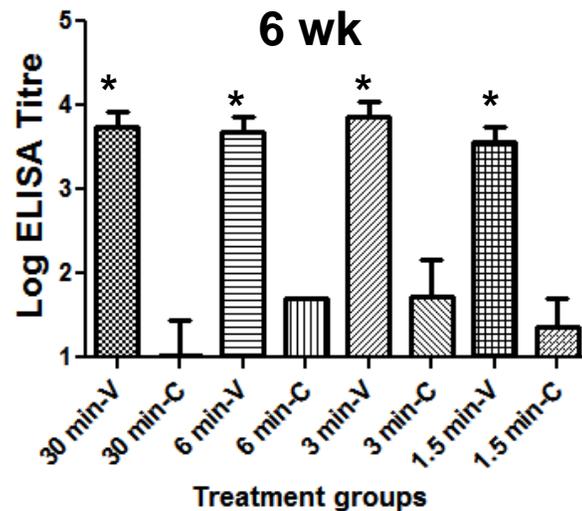
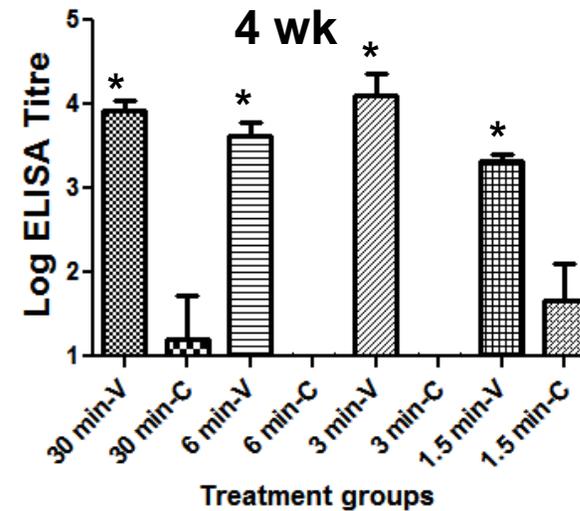
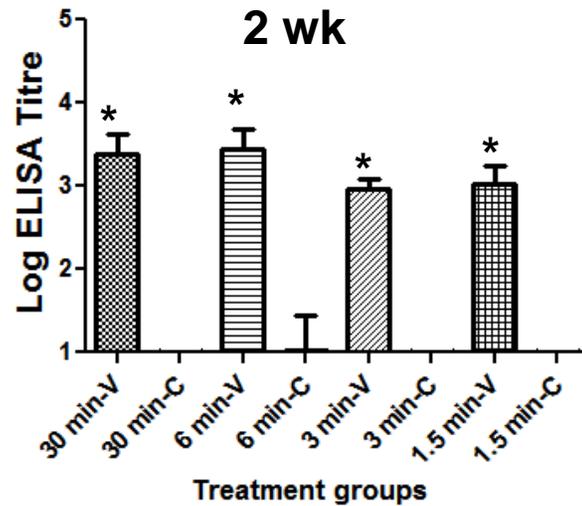
Study 2 (FP-B.17-ILM optimization): Delivery duration for immersion vaccination

Experimental design

- Rainbow trout: mean size 2.6 g
- **FP-B.17-ILM** initial vaccination dose : 5.0×10^{10} cfu ml⁻¹
- Booster dose: 4.0×10^{10} cfu ml⁻¹
- **1.5 min, 3 min, 6 min, 30 min** immersion

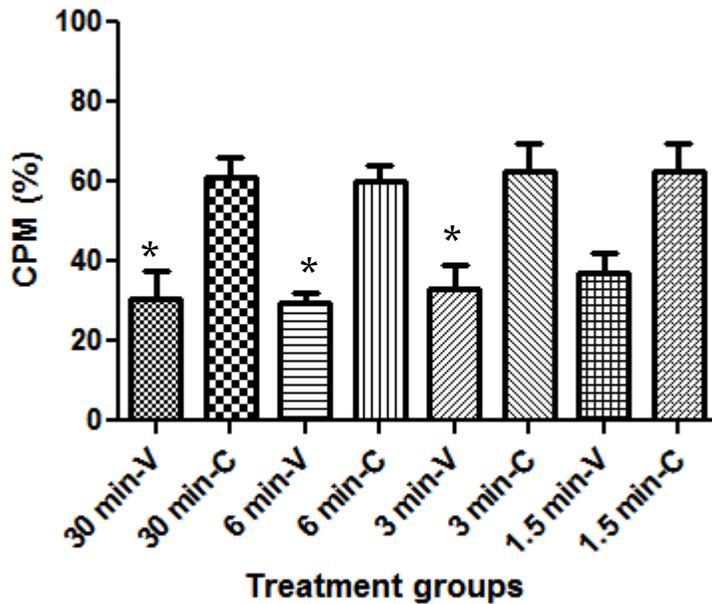


Duration of vaccine delivery - antibody titers



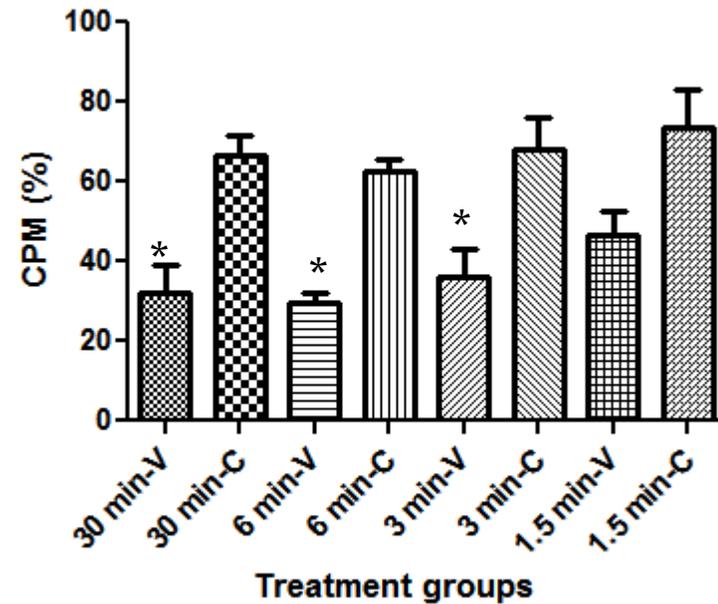
Cumulative Percent Mortality (CPM)

CPM 60%



At time point when CPM reached 60% in control groups

Final CPM 28 days



At the end of 28 days challenge

28 day relative percent survival (RPS)

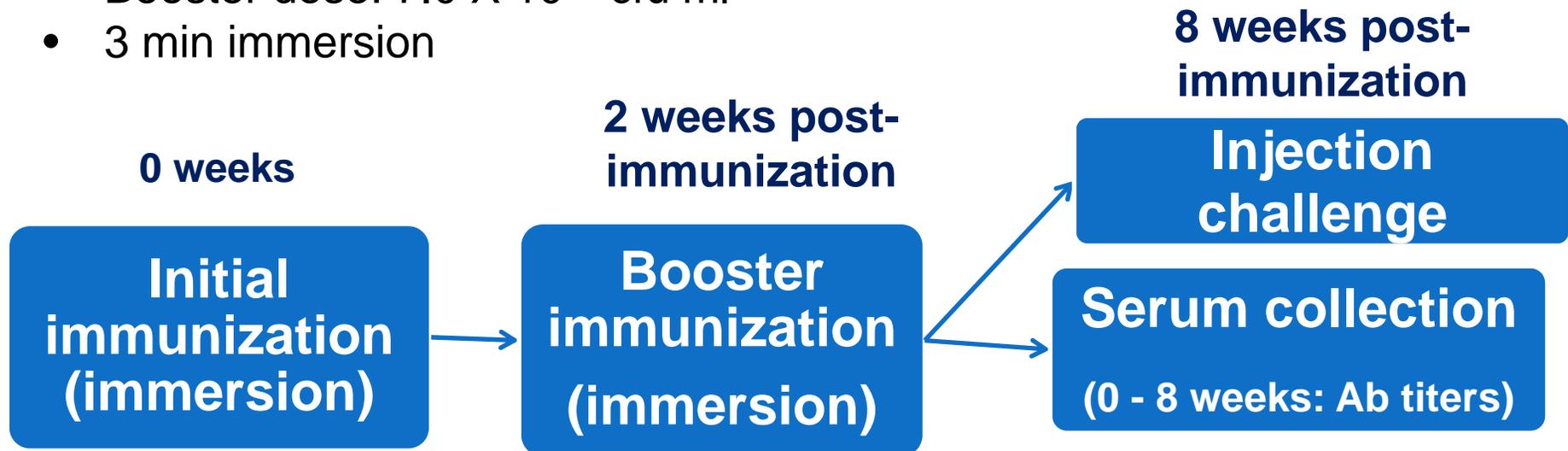
Treatments	Fish/ treatment	RPS (%)
30 min vaccinated	25 fish x 3 tanks	52
6 min vaccinated	25 fish x 3 tanks	53
3 min vaccinated	25 fish x 3 tanks	47
1.5 min vaccinated	25 fish x 3 tanks	36

Challenge dose of virulent *F. psychrophilum* (cfu fish⁻¹) = 1.46×10^7

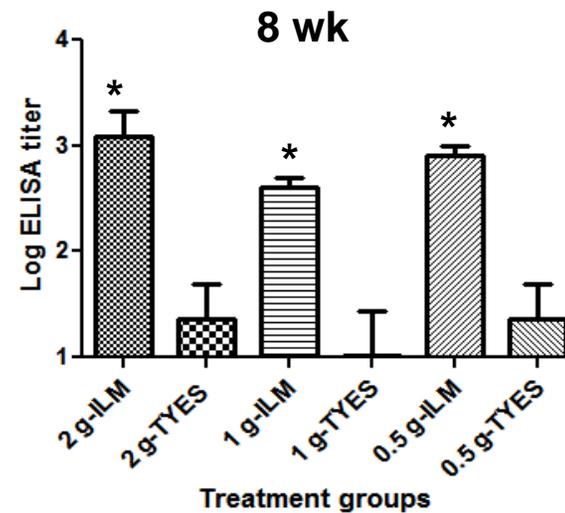
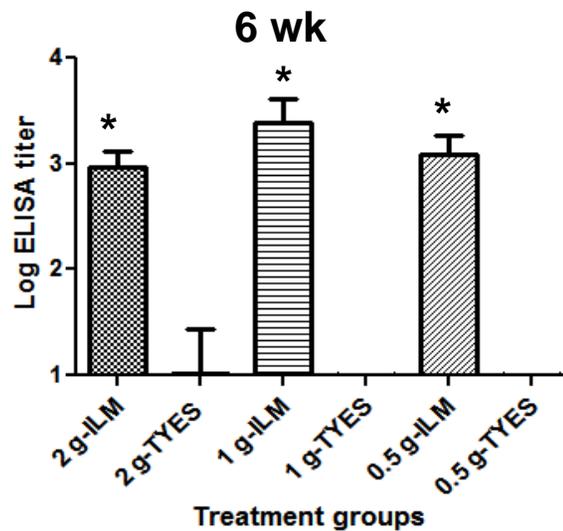
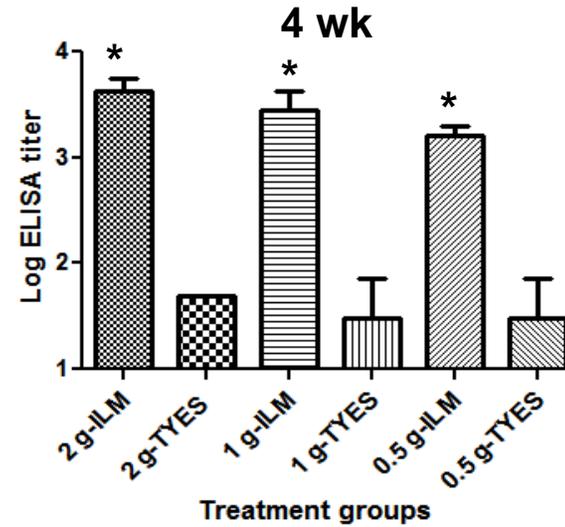
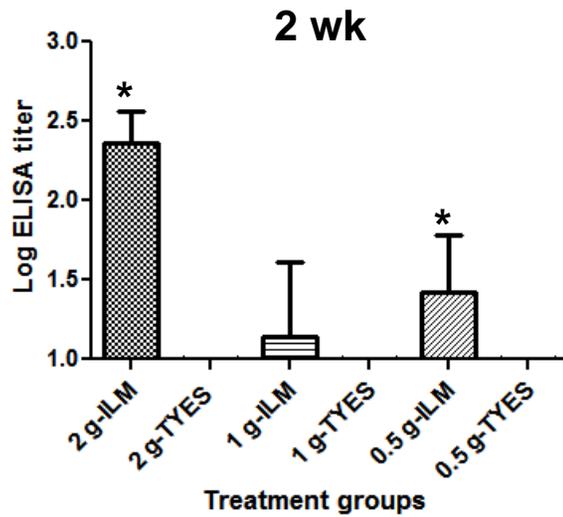
Study 3: FP-B.17-ILM testing (size at vaccination)

Experimental design:

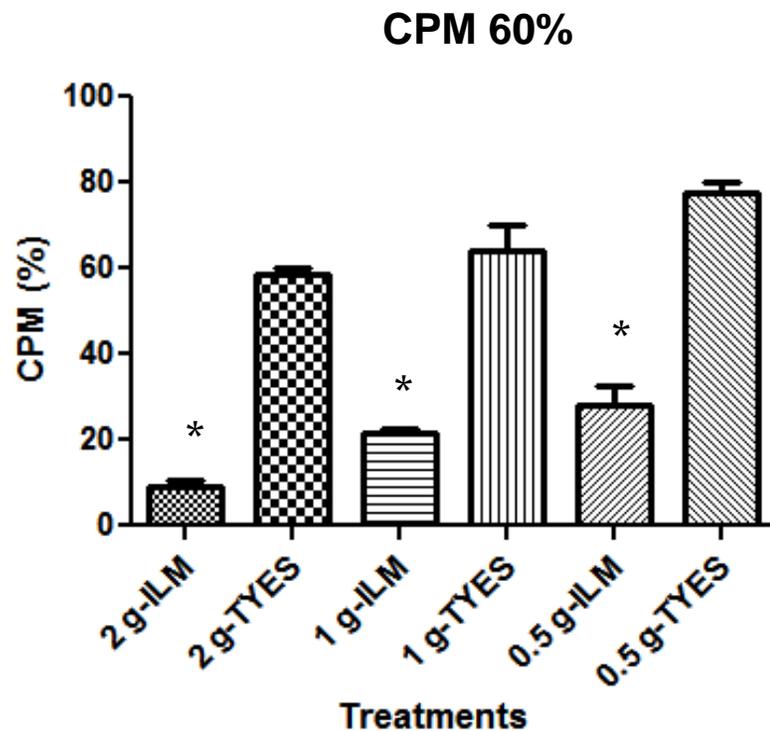
- Rainbow trout: **0.5 g, 1.0 g, 2.0 g**: 150 fish/ group
- **FP-B.17-ILM** initial vaccination dose: 9.0×10^{10} cfu ml⁻¹
- Booster dose: 7.0×10^{10} cfu ml⁻¹
- 3 min immersion



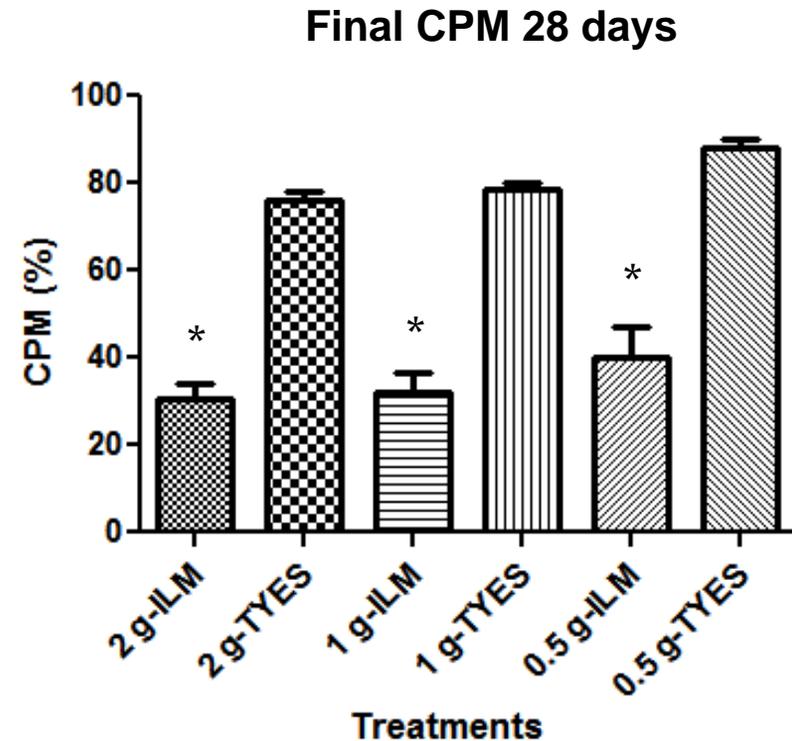
Size at vaccination - antibody titers



Cumulative Percent Mortality (CPM)



At time point when CPM reached 60% in control groups



At the end of 28 days challenge

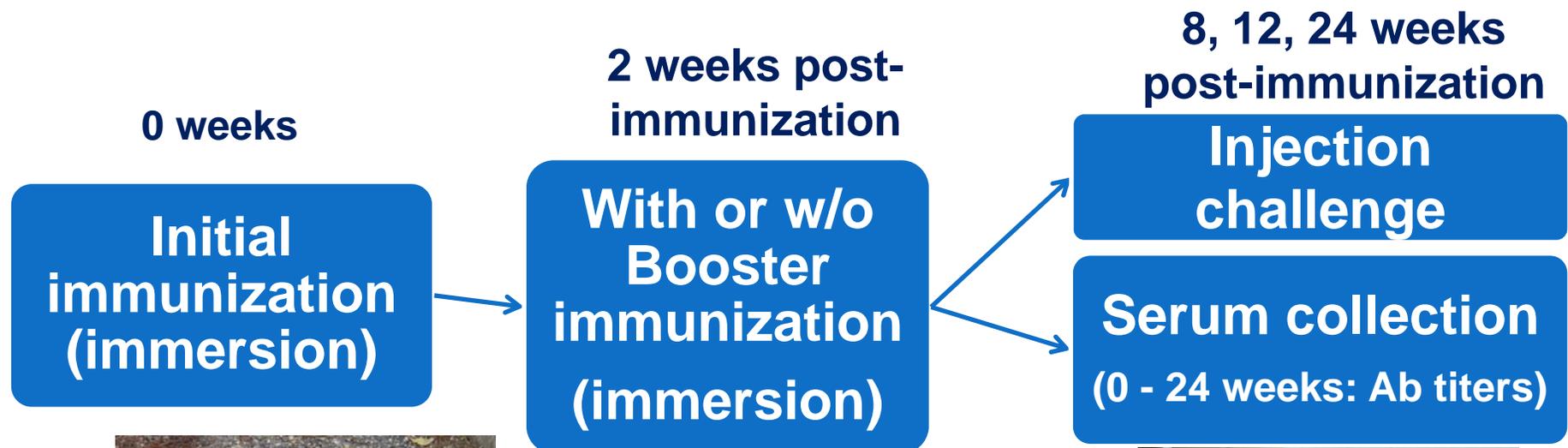
28 day relative percent survival (RPS)

Treatments	Challenge dose (cfu fish ⁻¹)	Fish/ treatment	RPS (%)
2 g-ILM	5.18 x 10 ⁷	25 fish x 3 tanks	60
1 g-ILM	3.45 x 10 ⁷	25 fish x 3 tanks	59
0.5 g-ILM	1.73 x 10 ⁷	25 fish x 3 tanks	55

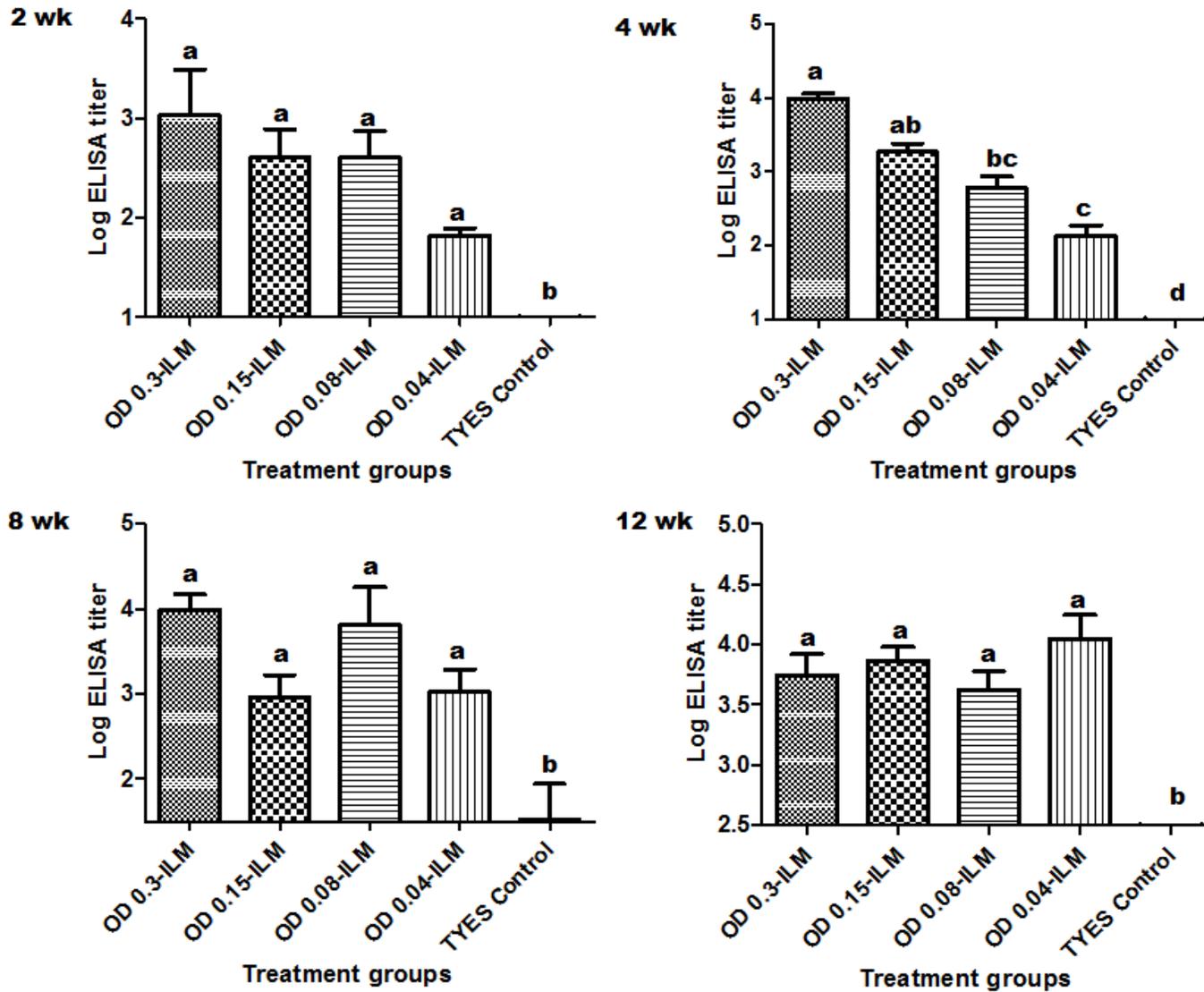
Study 4: FP-B.17-ILM dose optimization and duration of protection

Experimental design:

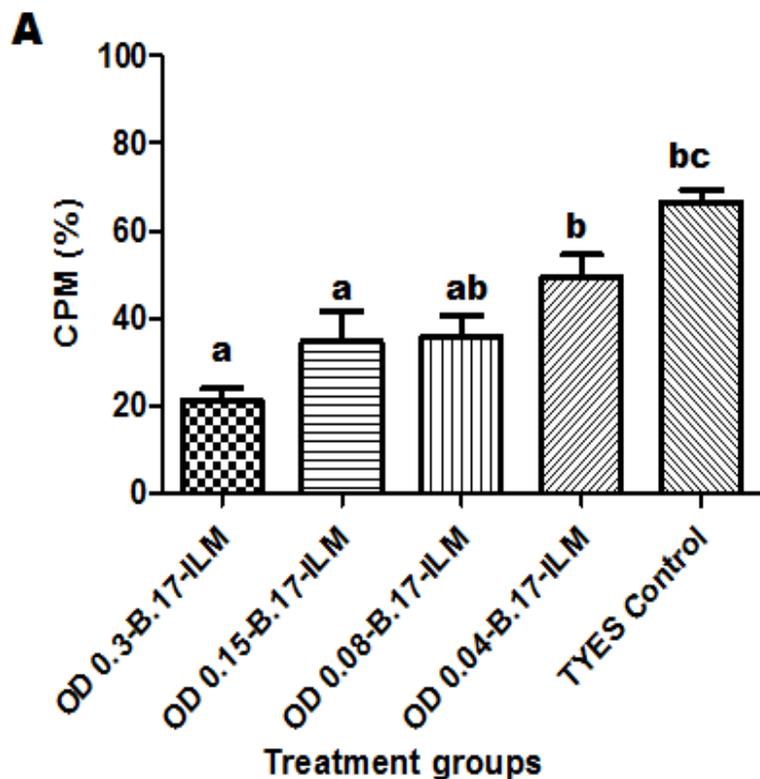
- Rainbow trout: **2.6 g**: 3 min immersion
- **FP-B.17-ILM** initial vaccination dose : ODs: 0.04, 0.08, 0.15, and 0.3:
 - (2.50×10^5 cfu ml⁻¹ to 1.58×10^{10} cfu ml⁻¹): challenge @ 8,12,24 wks)
- **One, two, or no booster** vaccination (challenge at 8 and 12 weeks)



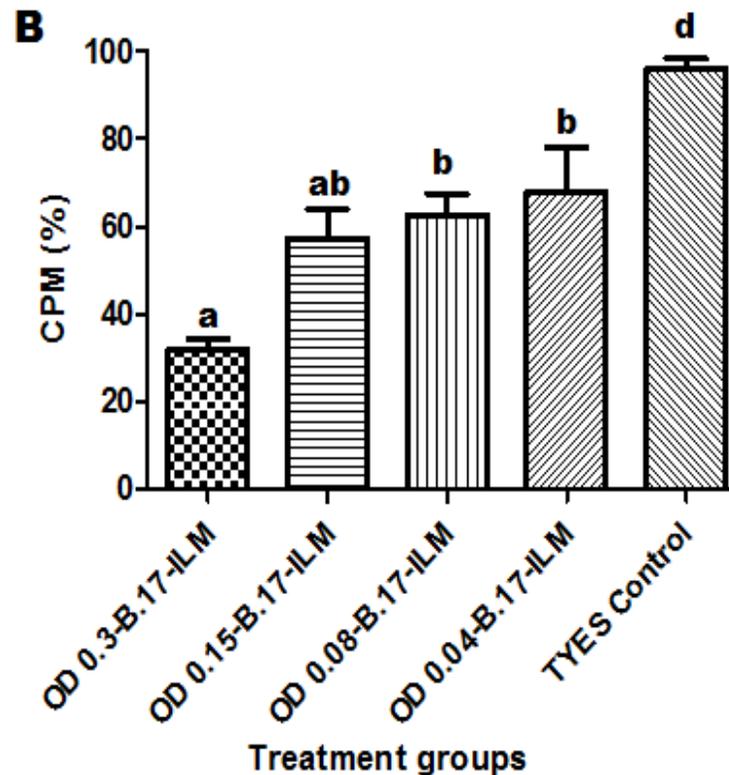
Dose and duration of protection (antibody titers)



Cumulative Percent Mortality (CPM @ 8 wks)

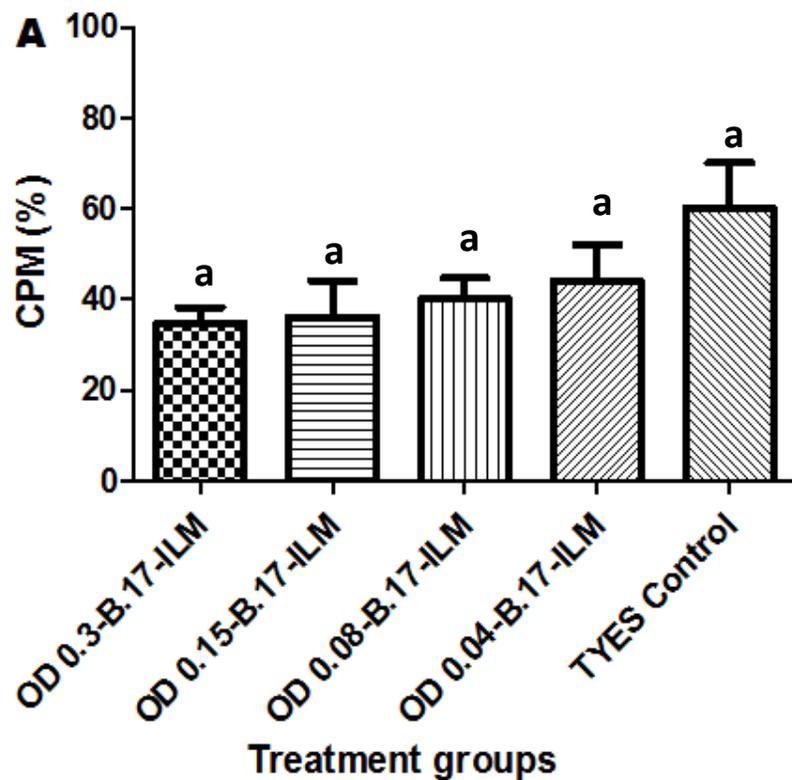


At time point when CPM reached 60% in control groups

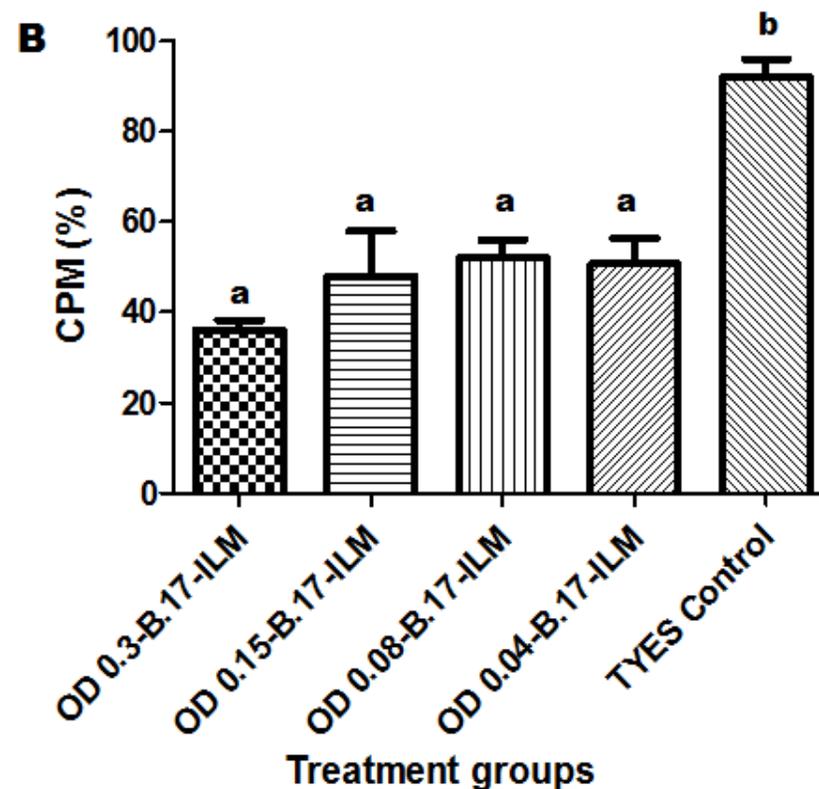


At the end of 28 days challenge

Cumulative Percent Mortality (CPM @ 12 wks)



At time point when CPM reached 60% in control groups



At the end of 28 days challenge

28 day relative percent survival (RPS)

Treatment groups	Initial vaccination dose (cfu ml ⁻¹)	RPS (%)		
		Week 8	Week 12	Week 24
OD 0.3-B.17-ILM	1.58 X 10 ¹⁰	67	61	70
OD 0.15-B.17-ILM	3.00 X 10 ⁸	40	48	55
OD 0.08-B.17-ILM	1.00 X 10 ⁶	35	43	36
OD 0.04-B.17-ILM	2.50 X 10 ⁵	29	45	34

28 day relative percent survival (RPS)

Treatments	Initial vaccination dose (cfu ml ⁻¹)	RPS (%)	
		Week 8	Week 12
One booster- 0.3-B.17-ILM	1.58 X 10 ¹⁰	67	61
Two booster- 0.3-B.17-ILM	1.58 X 10 ¹⁰	56	48
No booster- 0.3-B.17-ILM	1.58 X 10 ¹⁰	17	13

Field trial – ILM vaccine at Magic Springs hatchery

Experimental design:

- Rainbow trout (~ 1.5g): ~ 50,000 controls/50,000 vaccinates
- Primary - 3 L (bottles) vaccine (8 passes/bottle @ 10lbs/pass)
- Boosted @ 2 weeks (6 bottles vaccine)
- 3 min immersion

0 weeks

2 weeks post-immunization

Monitor for diseases/mortality

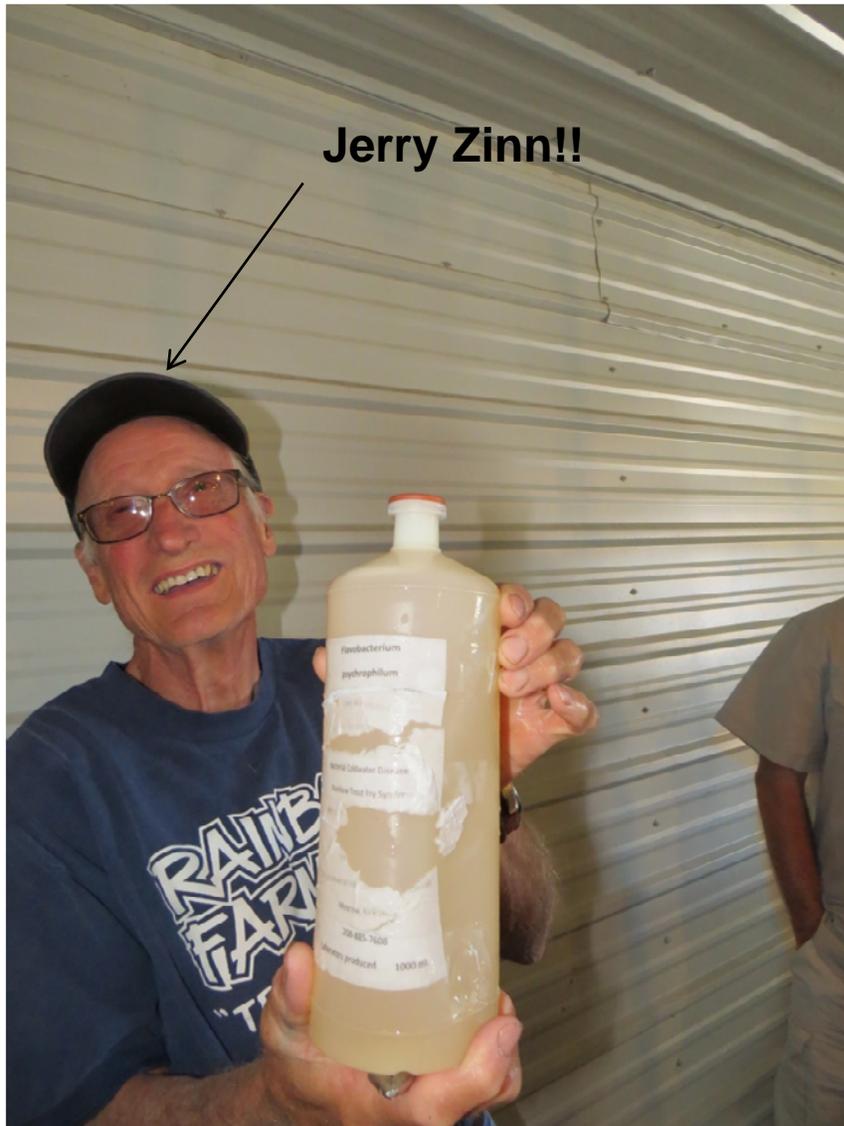
Initial immunization (immersion)

Booster immunization

Outside (duplicate) raceways



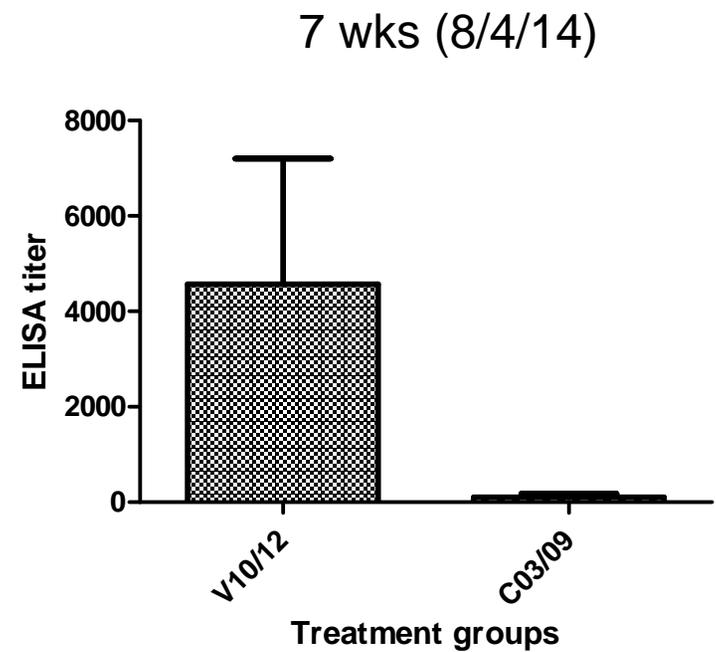
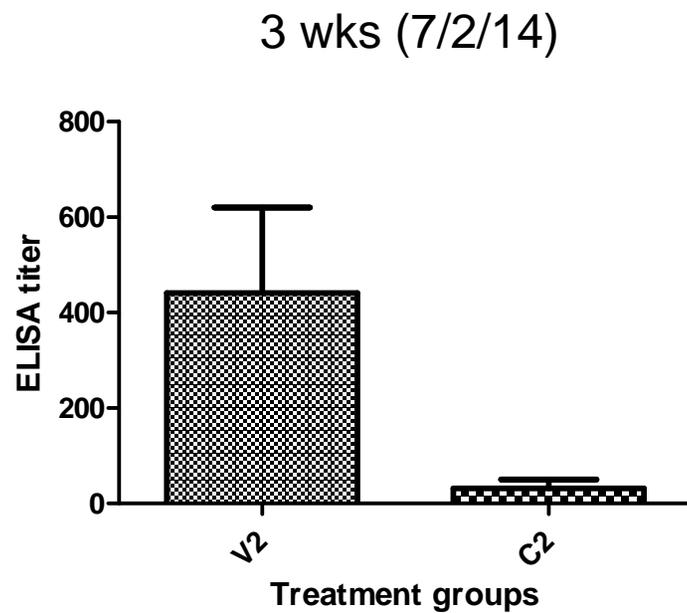
Field trial – ILM vaccine at Magic Springs hatchery



Field trial – ILM vaccine at Magic Springs hatchery

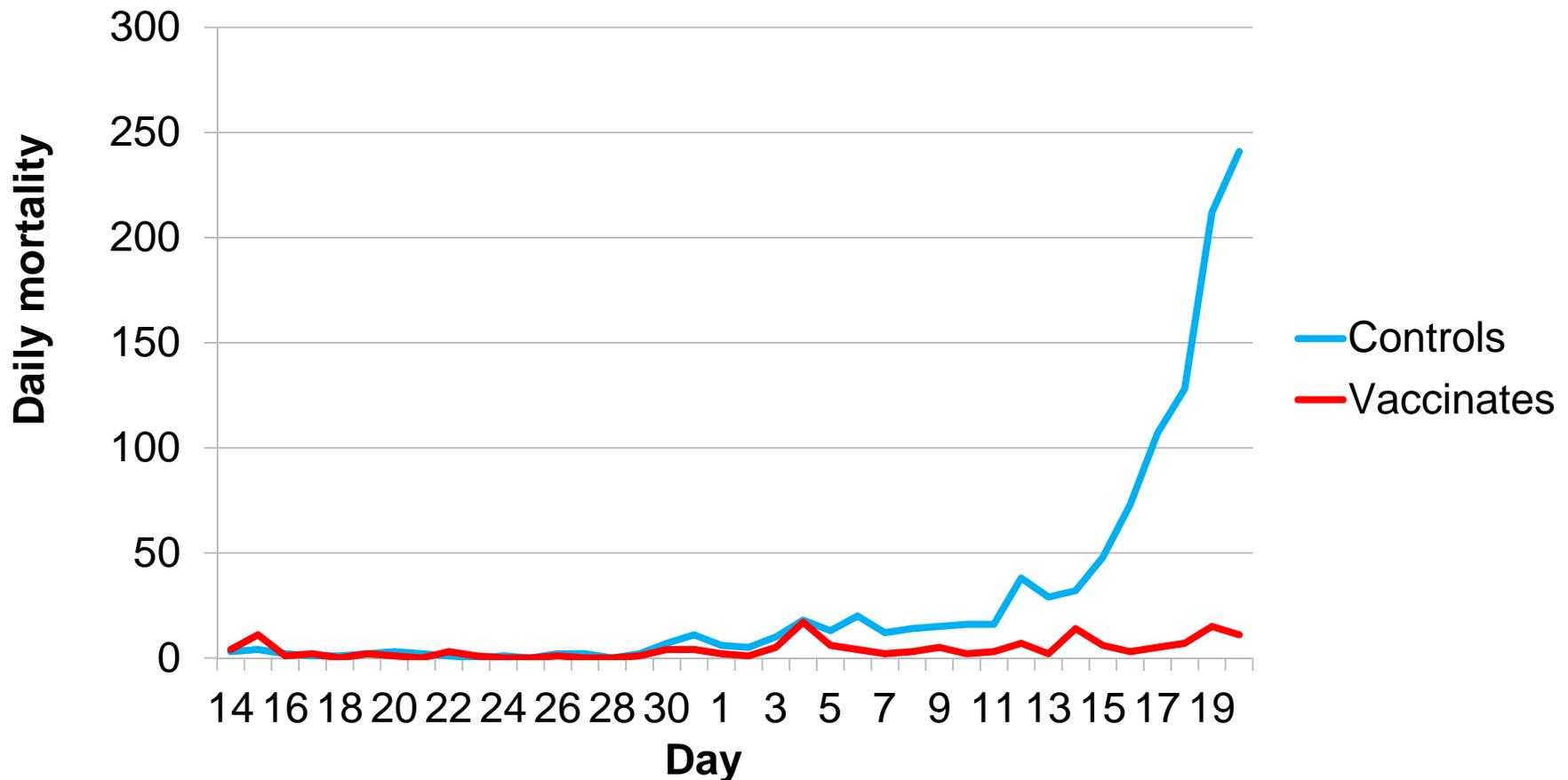


Field trial – Antibody response following vaccination with ILM vaccine



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)

- **7/31/14**
 - Slight increase in mortality in controls
 - 6 control; 6 vaccinates sampled (TYES plates for bacterial isolation)
 - Confirmed *F. psychrophilum* (2/6 controls)
- **8/13/14**
 - Increasing daily mortality in controls (significant lesions present)
 - 12 controls; 12 vaccinates sampled

CWD vaccine field trial – Magic Springs (Clinical exam of fish)

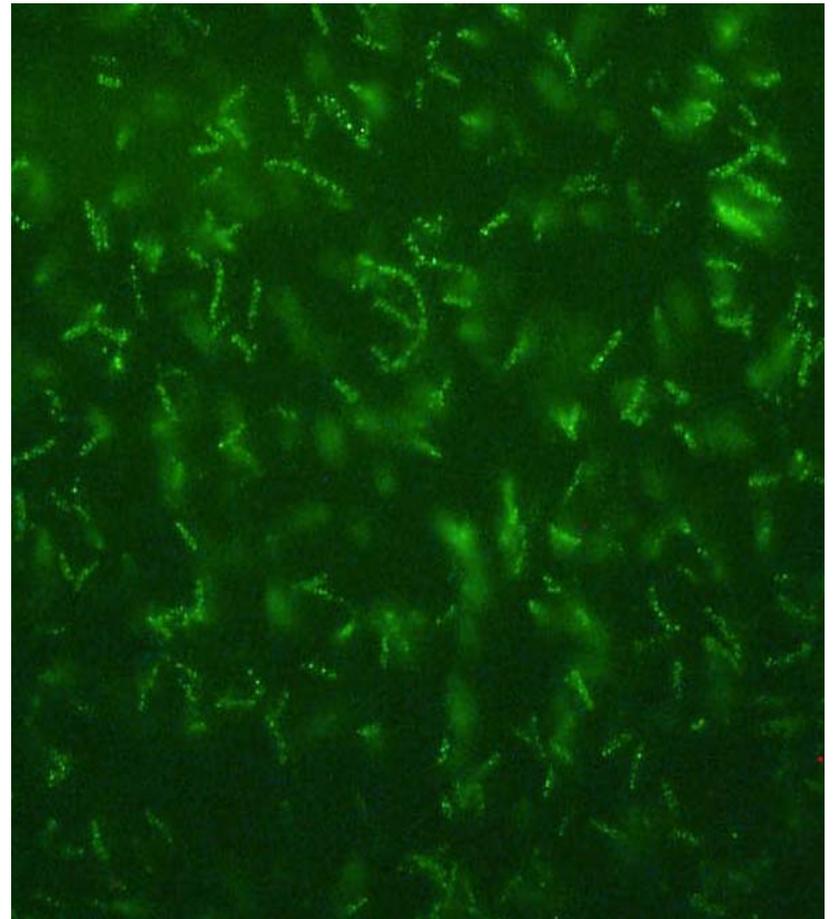
• Clinical exam results (8/13/14)

Controls

- EBI/BGD
- 9/12 fish positive for IHNV
- *F. psychrophilum* confirmed
 - (FAT and PCR) - only 1 fish

Vaccinates

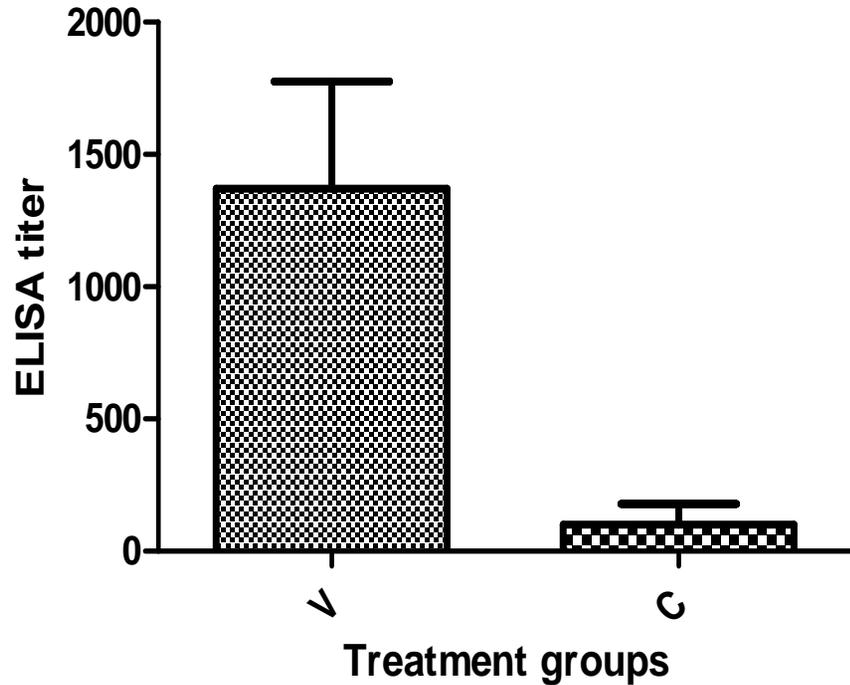
- Minor BGD
 - (limited signs or bacterial growth)
- No virus
- *F. psychrophilum* confirmed
 - Only 1 fish



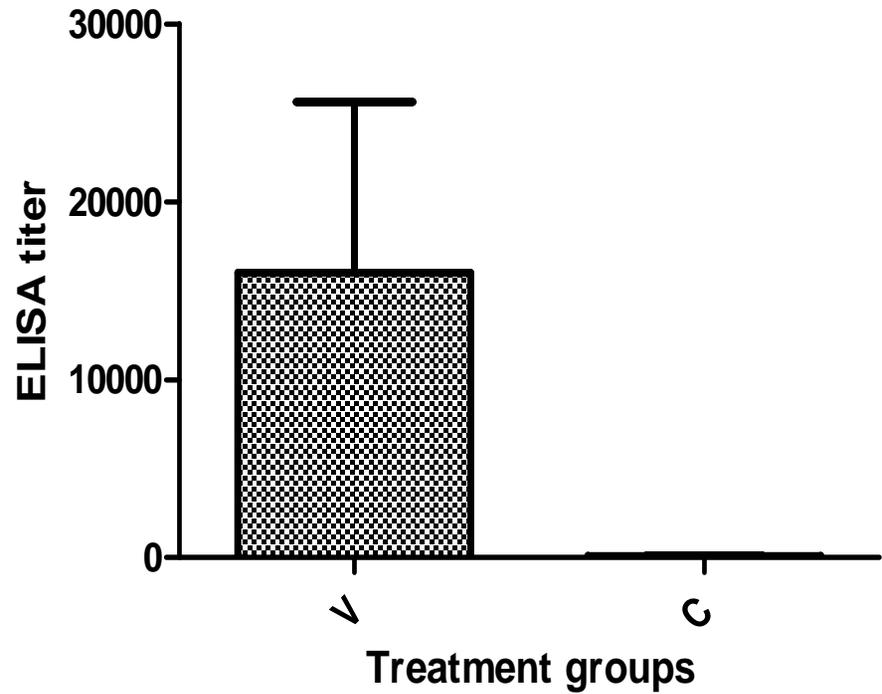
Second Magic Springs trial

Antibody responses

Wk- 4, 11/13/2014



Wk-9, 12/18/2014

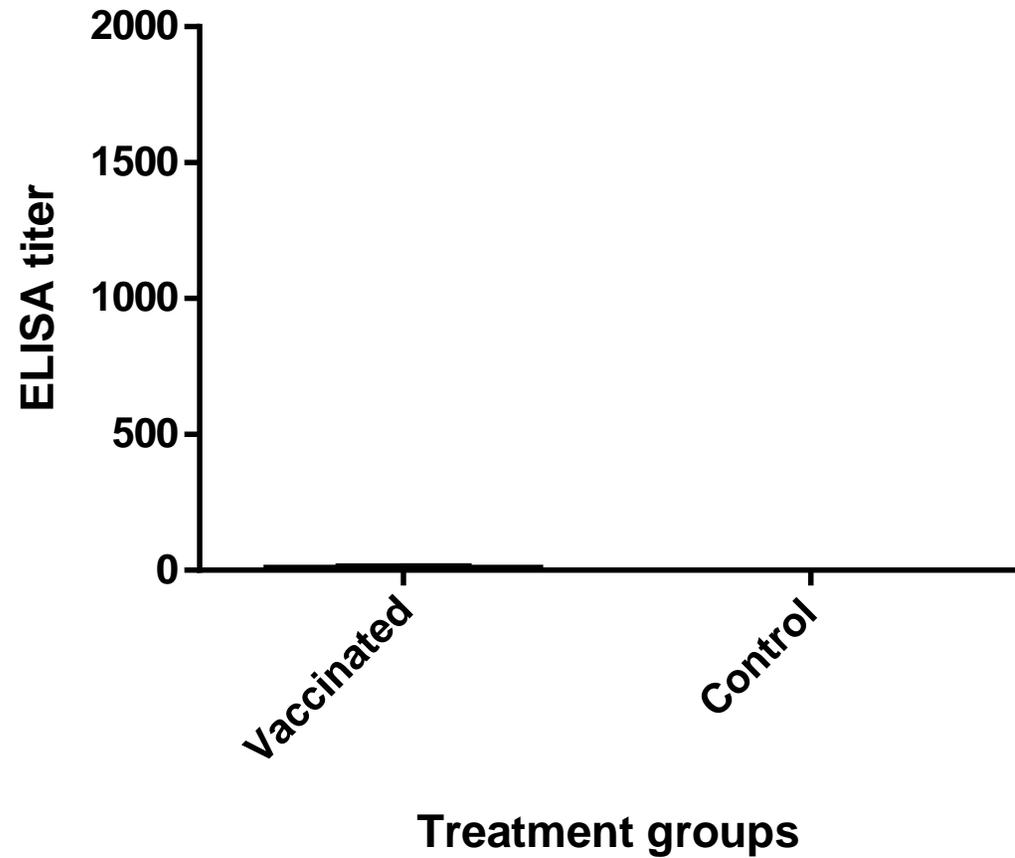


American Falls (IDFG) trial

Antibody responses

12/22/2014

Sample	Titer	Sample	Titer
V1	0	C1	0
V2	0	C2	0
V3	0	C3	0
V4	0	C4	0
V5	0	C5	0
V6	0	C6	0
V7	0	C7	0
V8	0	C8	0
V9	0	C9	0
V10	50	C10	0
V11	0	C11	0
V12	50	C12	0
V13	0	C13	0
V14	0	C14	0
V15	0	C15	0



Second Magic Springs and American Falls (IDFG) trial

Inconclusive field trial results

- **Magic Springs** – Fish responded to vaccine and produced strong antibody response
 - No CWD outbreak
- **American Falls** – Fish did not respond to vaccine (no antibodies) – something went wrong
 - Primary and booster vaccination?
 - Consistency between all vaccinations (lab and all field trials)?
 - Was there an issue with viability of attenuated strain?
 - Time of vaccination following thawing?
 - Inconsistent production at UI (not under strict quality control)?
 - All vaccination bottles checked prior to storage.

Summary

FP-B.17-ILM vaccine optimization work (lab):

- Vaccine protects Coho salmon and rainbow trout via immersion delivery
- Immersion delivery of 3.0 min or greater provides solid protection
- Vaccine protects fish as small as 0.5 g
- Level of protection is dose dependent
- One booster immunization is required
- Significant protection (high RPS) lasts for at least 24 weeks post vaccination

Summary

Field trials:

- Initial field trial (**Magic Springs**) demonstrated safety and efficacy – even in presence of mixed infection
- Second field trial (**Magic Springs**) demonstrated safety and strong immune response – no CWD outbreak so - no protection data
- American Falls trial – Fish never developed an immune response
 - Some aspect of vaccination did not work
 - Need to determine factor(s) caused this and optimize protocols for vaccine administration and/or production and storage

What's left to do?

Live attenuated FP-B.17-ILM vaccine:

- **Vaccine production** - practical large scale growth, manufacturing and storage characteristics
- **Complete optimization work**
- **Safety/efficacy trials** (USDA approval)
 - Laboratory and field safety trials on manufactured product
 - Conditional/Full licensing
 - Marketing and sales

This vaccine shows strong efficacy via immersion delivery and once optimized will be an important tool for managing CWD at salmonid hatcheries.



Acknowledgments

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Questions?

