

# The Potential for Vaccines in Controlling Bacterial Kidney Disease

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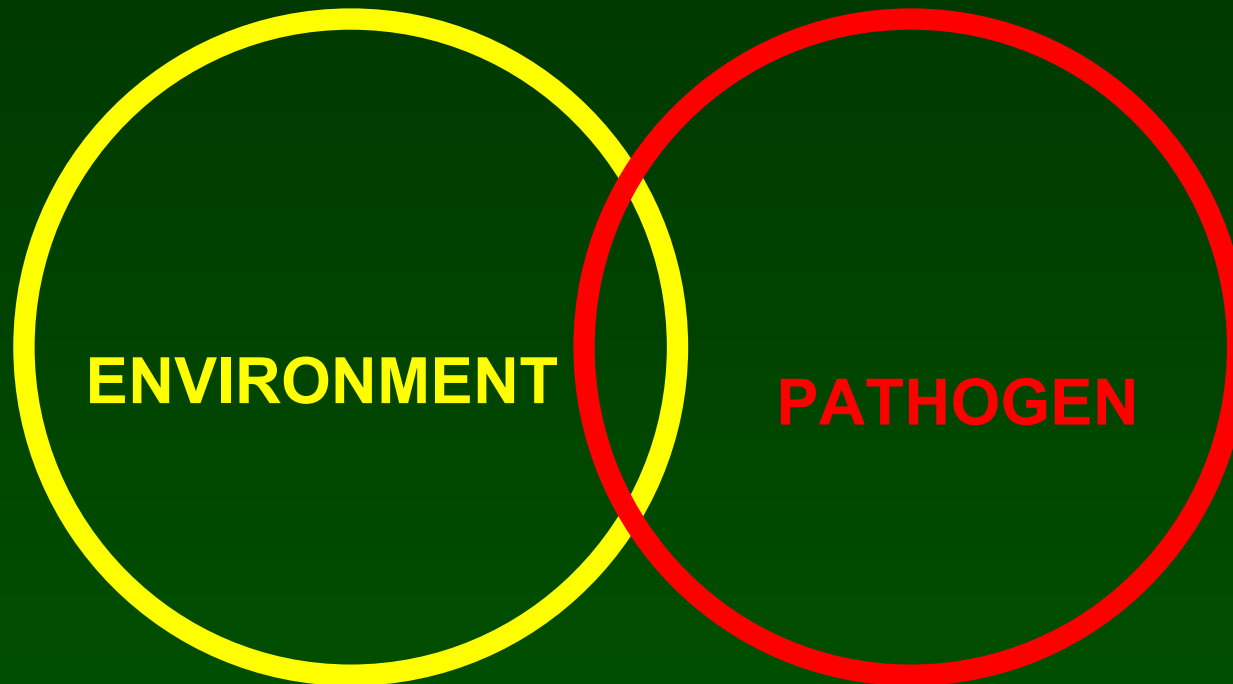
#Olympic National Park, Port Angeles, WA



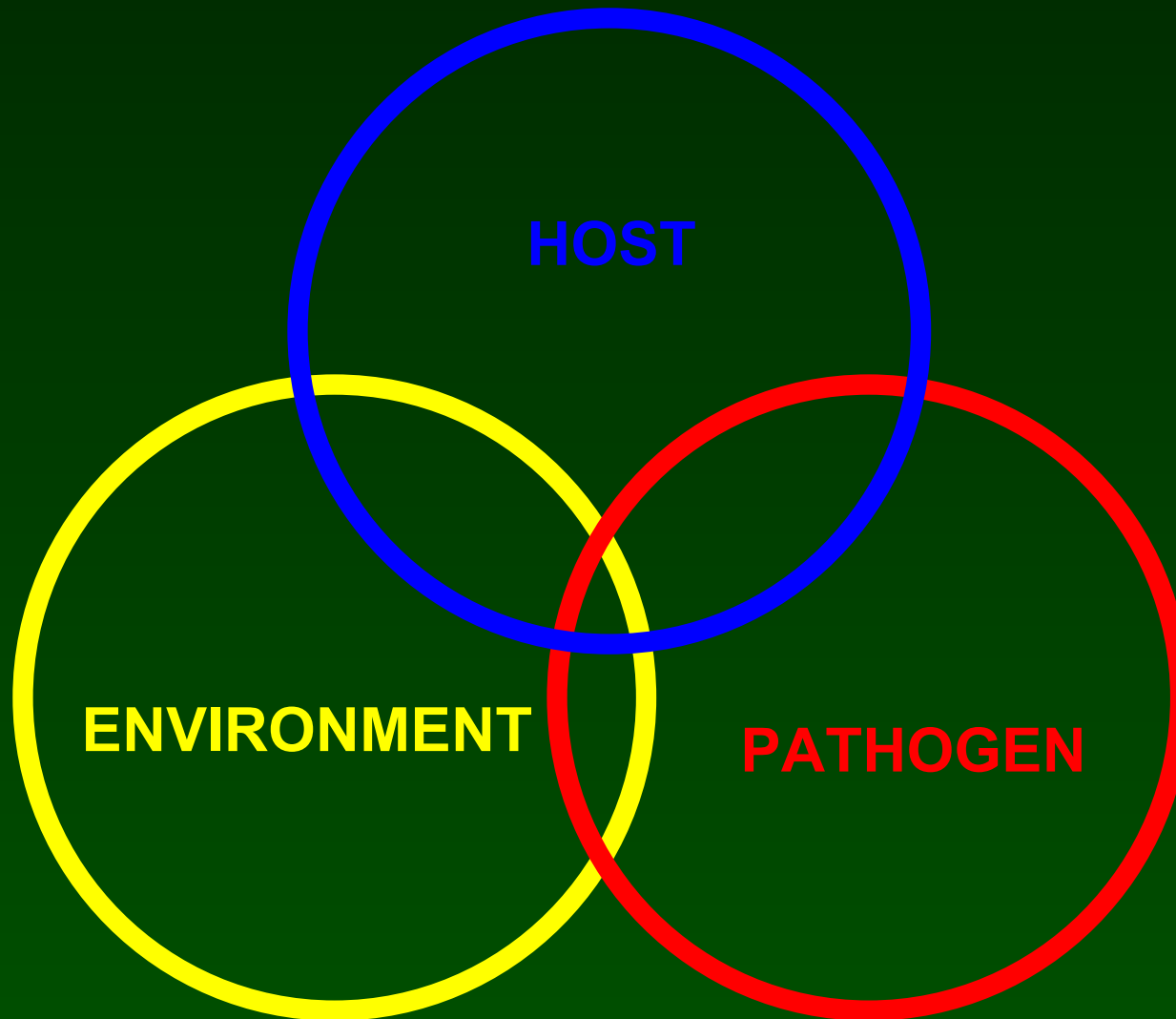
# Host-Pathogen-Environment



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# Common Microbial Diseases of Salmon and Trout

## Viral

Infectious Hematopoietic Necrosis Virus

Infectious Pancreatic Necrosis Virus

Viral Hemorrhagic Sepsis Virus

Infectious Salmon Anemia Virus

## Bacterial

Enteric Redmouth Disease

*Yersinia ruckeri*

Furunculosis

*Aeromonas salmonicida*

Vibriosis

*Vibrio salmonicida*

Coldwater Disease

*Flavobacterium psychrophilum*

Bacterial Kidney Disease

*Renibacterium salmoninarum*

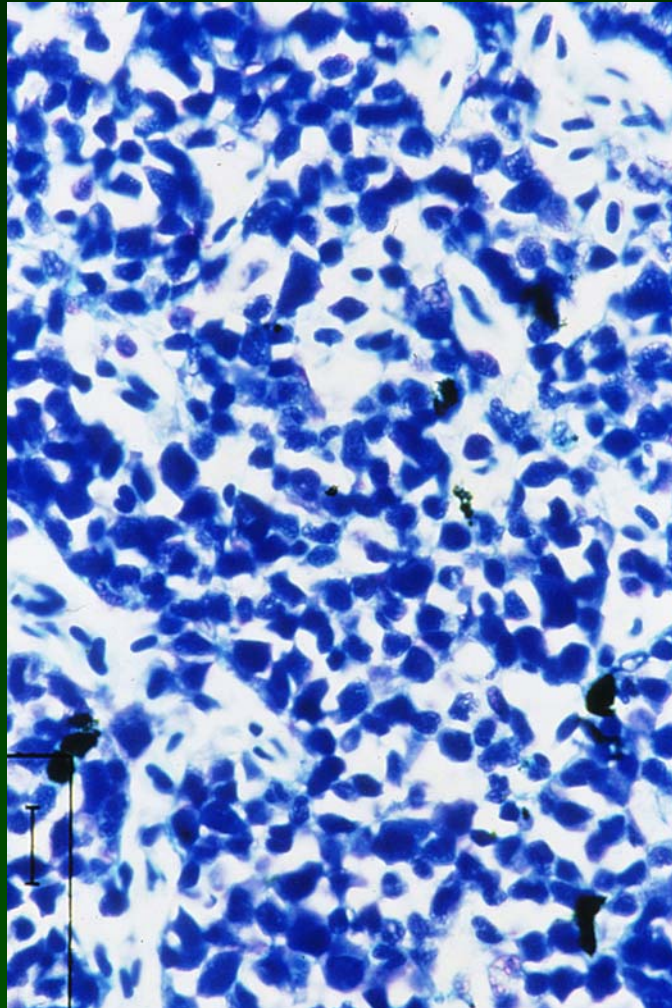


# Bacterial Kidney Disease

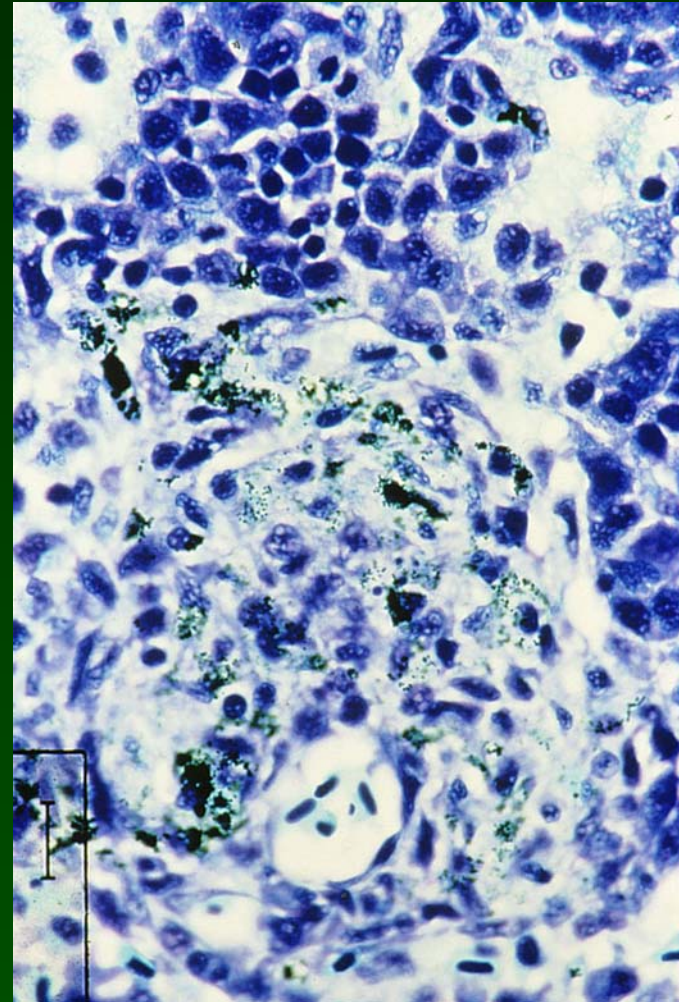
- Chronic granulomatous disease, often systemic
- Dark pigmentation, ascites, exophthalmia
- Granulomatous kidney lesions
- Asymptomatic carriers
- Horizontal transmission
- Vertical transmission
- Causative agent:  
*Renibacterium salmoninarum*



**uninfected**



**infected**



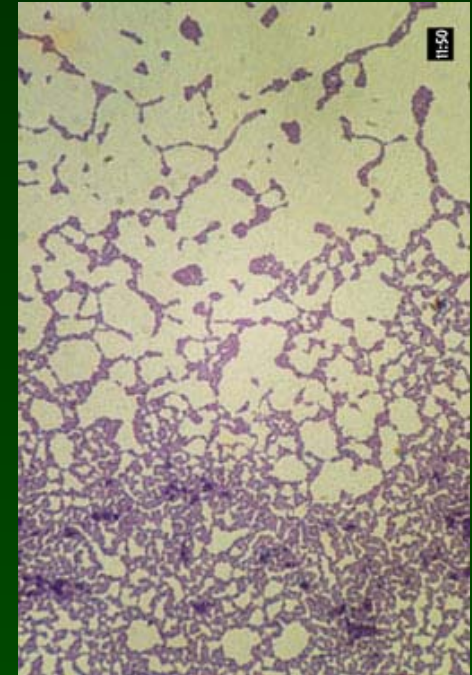
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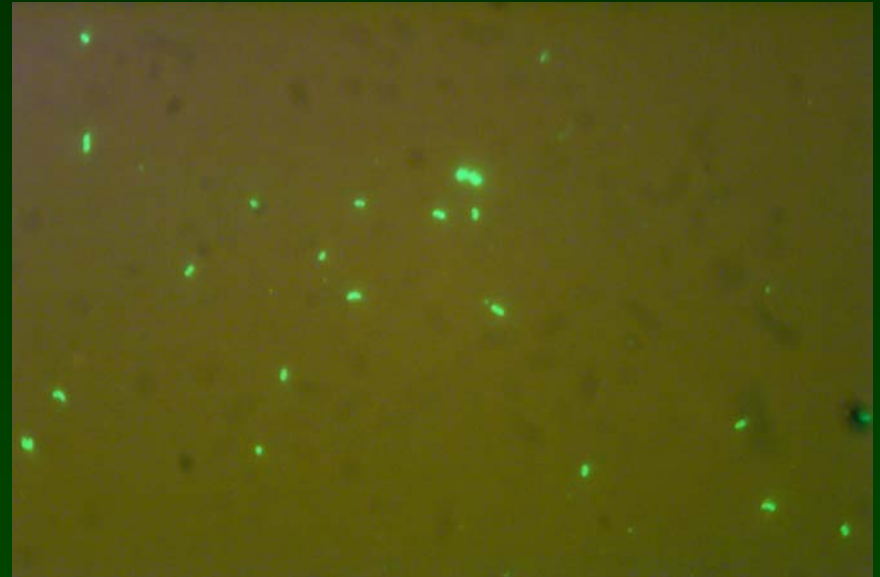
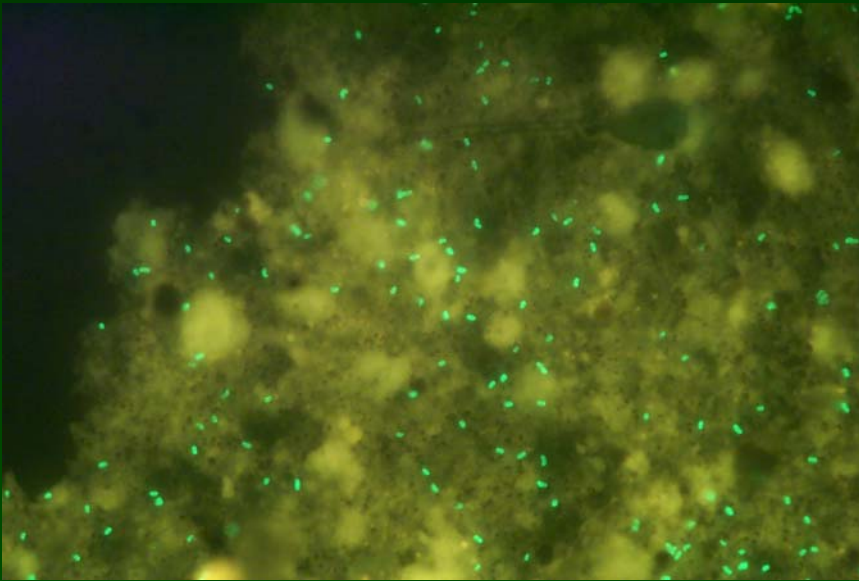


# *Renibacterium salmoninarum*

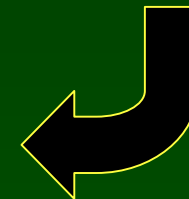
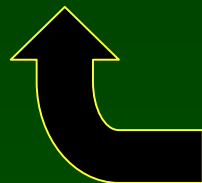
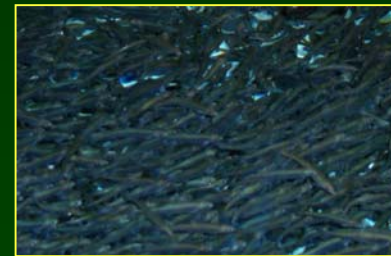
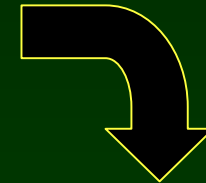
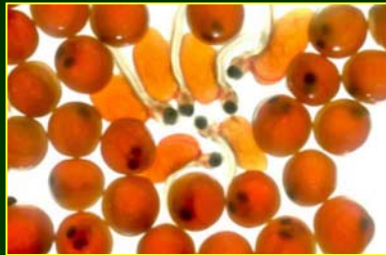
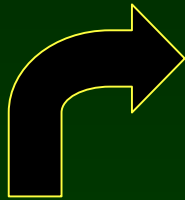
- Gram-positive diplococcobacillus
- Intracellular pathogen *in vivo*
- Environmental persistence
- Grows slowly *in vitro*  
(~24 hour doubling time)
- Immunodominant 57 kDa protein:  
major soluble antigen (MSA)



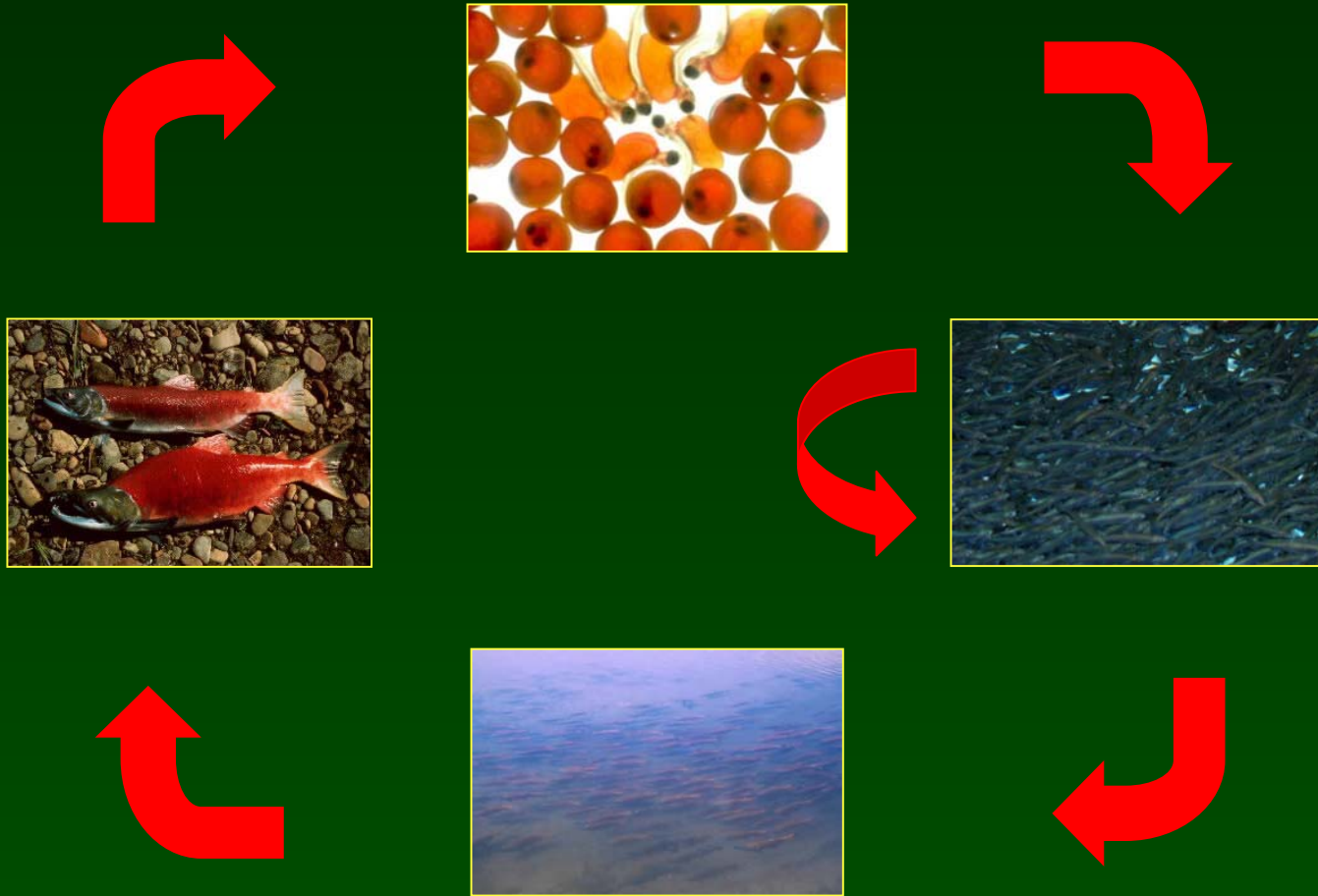
# Fluorescent Antibody Technique Microscopy



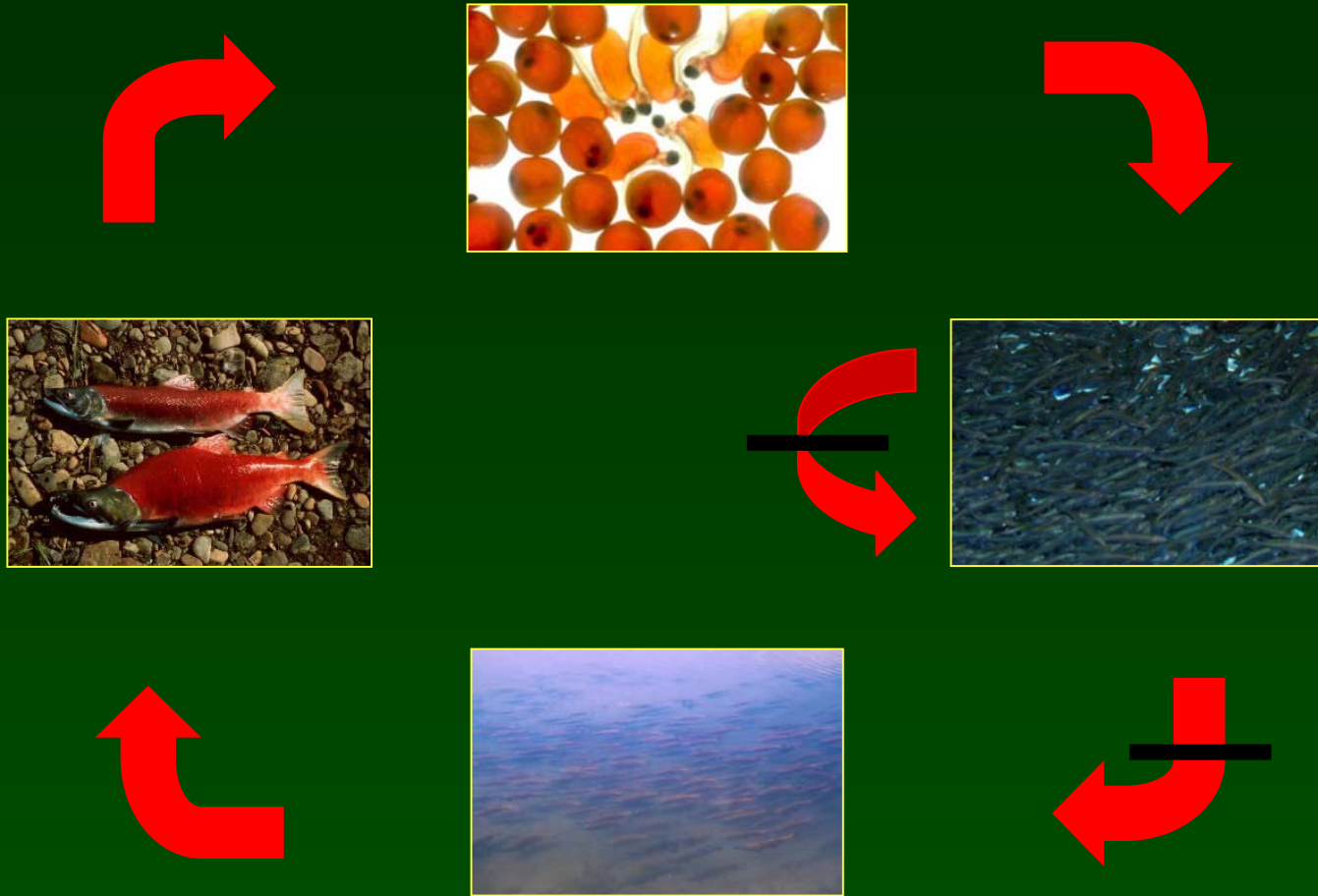
# Bacterial Kidney Disease Transmission



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# Types of Bacterial Vaccines

**live, nonpathogenic cells**

**inactivated cells (bacterins)**

**protein subunit**

**DNA**



# Trial BKD vaccines

Immunogen	Reference
whole cell; pH-lysed cells	McCarthy <i>et al</i> , 1984 (rainbow)
nutritional variants; <i>Arthrobacter</i> sp. (Renogen)	Griffiths <i>et al</i> , 1988 (Atlantic)
MSA <sup>-</sup> (p57 <sup>-</sup> ) bacterin (enzymatic digestion, heat treatment)	Wood & Kaattari, 1996 (chinook); Piganelli <i>et al</i> , 1999 (coho)
avirulent variants; MT239	Daly <i>et al</i> , 2001 (Atlantic)

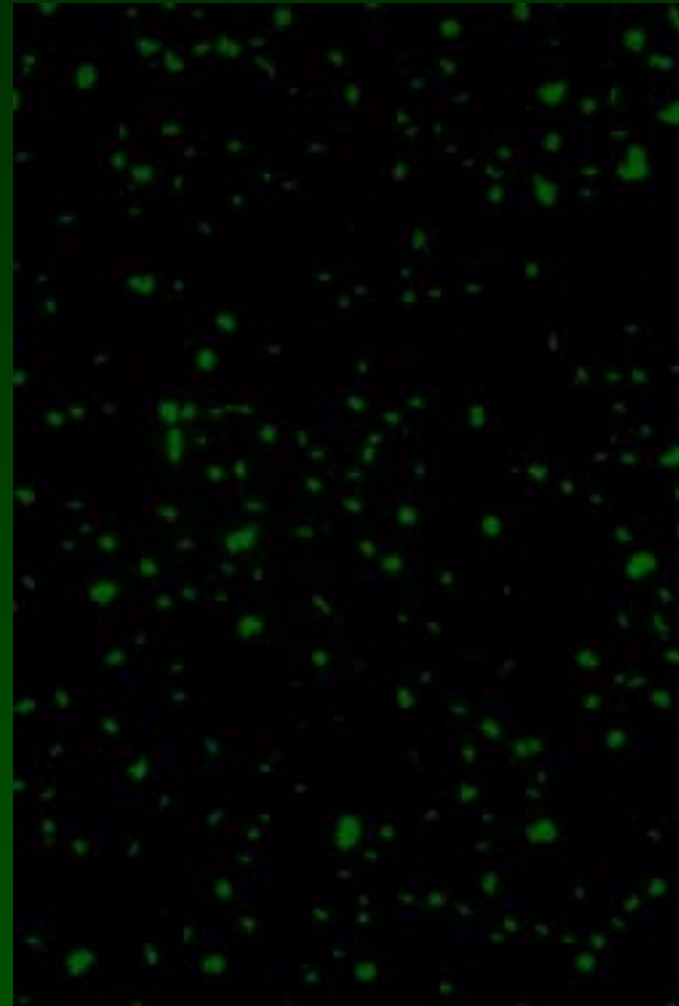
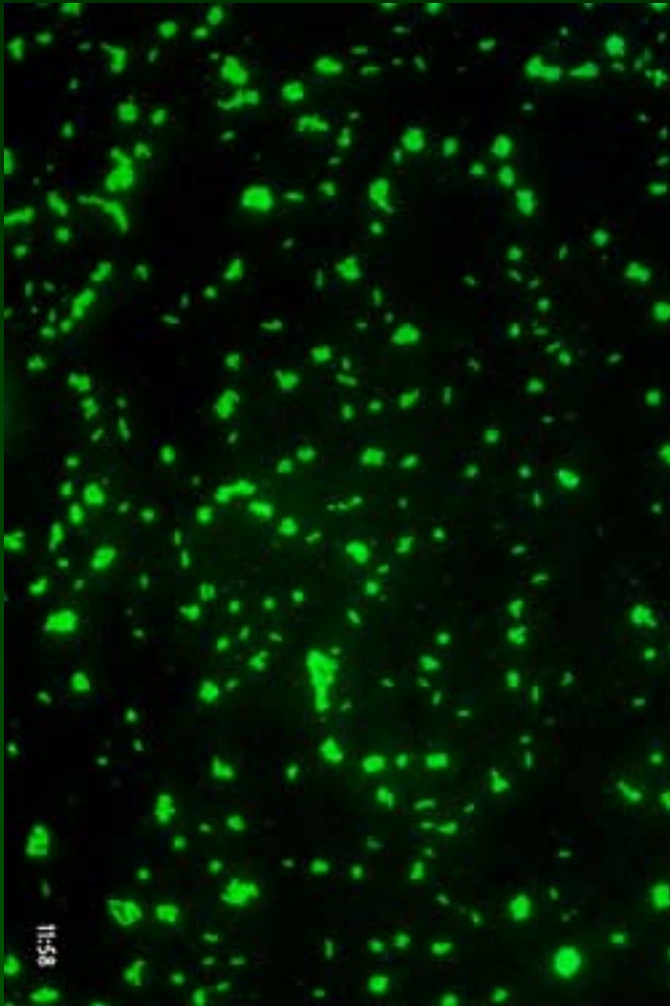


# Trial BKD vaccines

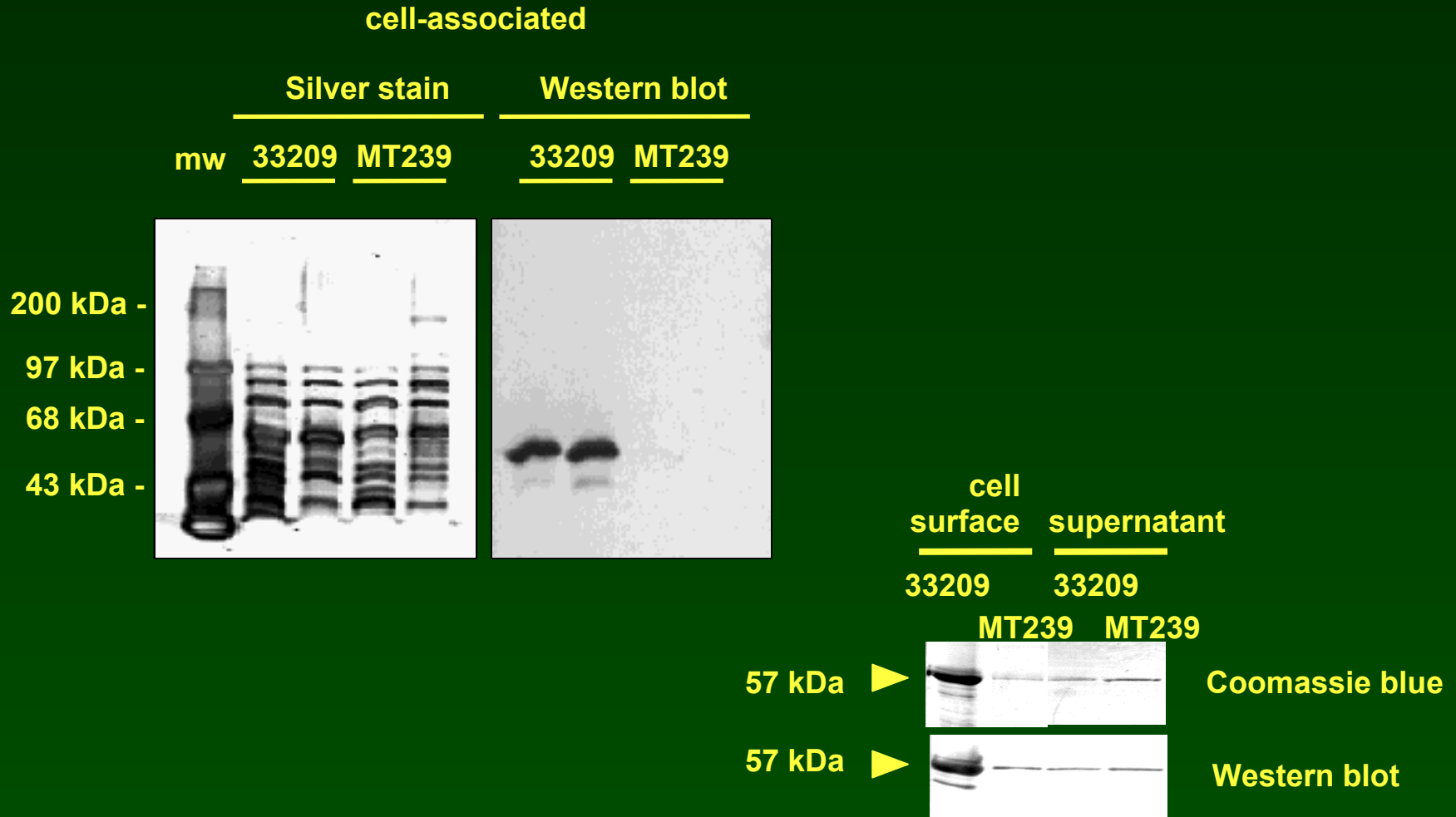
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# 33209 and MT239 differ in surface protein expression



# MT239 is defective in expressing MSA



# MT239 is an attenuated strain

Host Species	Wild Type	MT239	Reference
rainbow trout	80% (33209)	8%	Bruno, 1988
rainbow trout	92% (JD24)	7%	Senson & Stevenson, 1999
chinook salmon	73% (33209)	12%	O'Farrell <i>et al</i> , 2000



# Vaccine Reagents

## Whole Cell Vaccines

- Renogen
- MT239

## Genetic Adjuvants

- oligonucleotide
- *R. salmoninarum*  
DNA



# Whole Cell Vaccines

Cell	Type	Description	Dose
Renogen	live whole bacteria	commercial <i>Arthrobacter</i> sp. (AquaHealth)	$1 \times 10^5$ CFU / fish
MT239	killed, whole bacteria	attenuated isolate of <i>R. salmoninarum</i> (MSA <sup>low</sup> )	$5 \times 10^6$ cells / fish



# Genetic Adjuvants

Adjuvant	Type	Description	Dose
CpG (CpG 1826)	synthetic oligonucleotide	5'- TCCATGAC <u>CG</u> TTCCTG AC <u>G</u> TT-3'	15 µg / fish
non-CpG (GpC 1745)	synthetic oligonucleotide	5'- TCCAATGAG <u>G</u> CTTCCT GAGTCT-3'	15 µg / fish
<i>R.salmoninaru</i> <i>m</i> DNA	chromosomal (genomic) DNA	purified from ATCC culture type 33209	10 µg / fish



# Vaccination Design

## Inocula

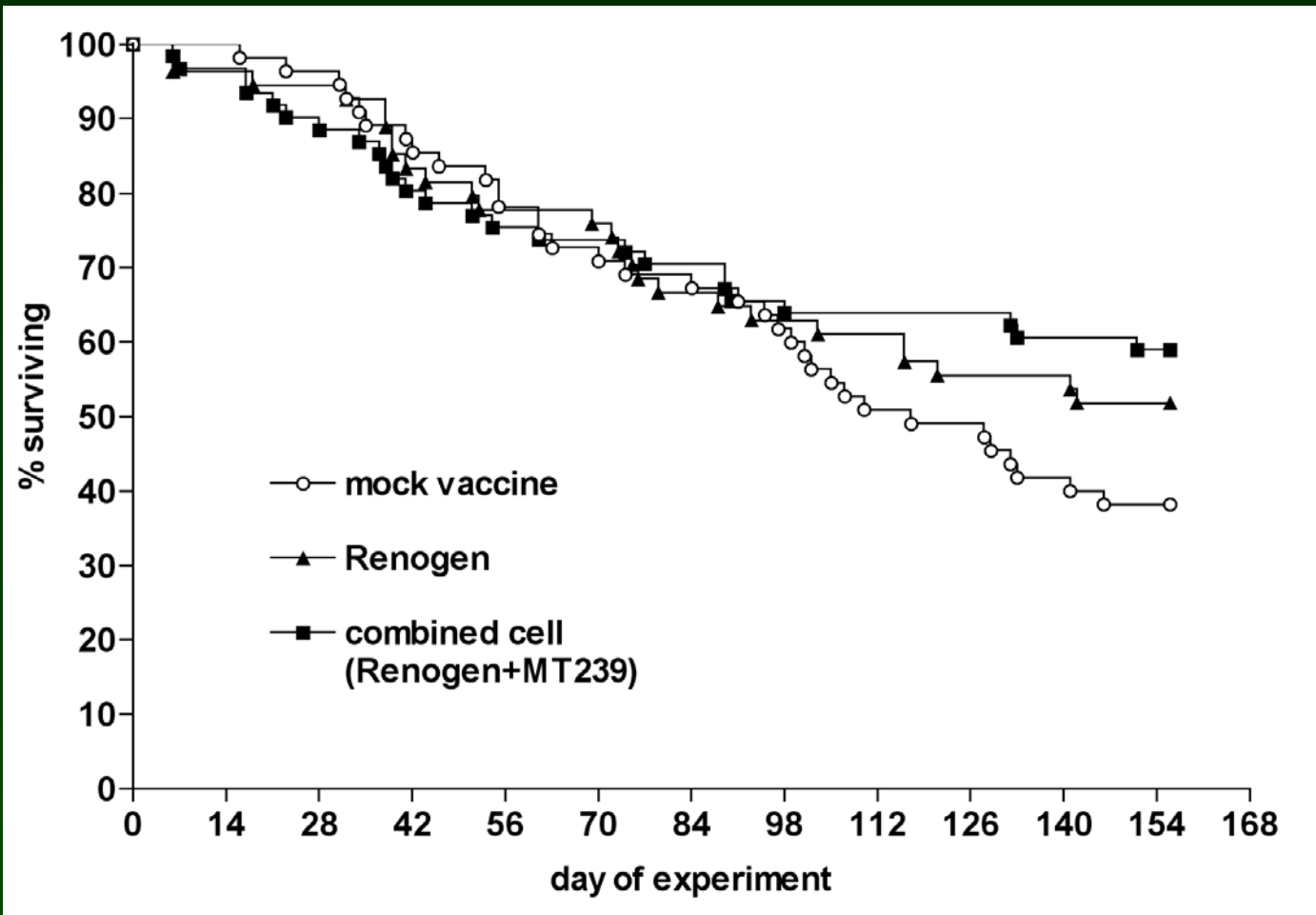
- Vaccine diluent (PBS)
- Adjuvants only (CpG, non-CpG, or *Renibacterium* DNA)
- Whole cells only (Renogen or Renogen+MT239)
- Adjuvants (CpG, non-CpG, or *Renibacterium* DNA) with Renogen+MT239

## Fish

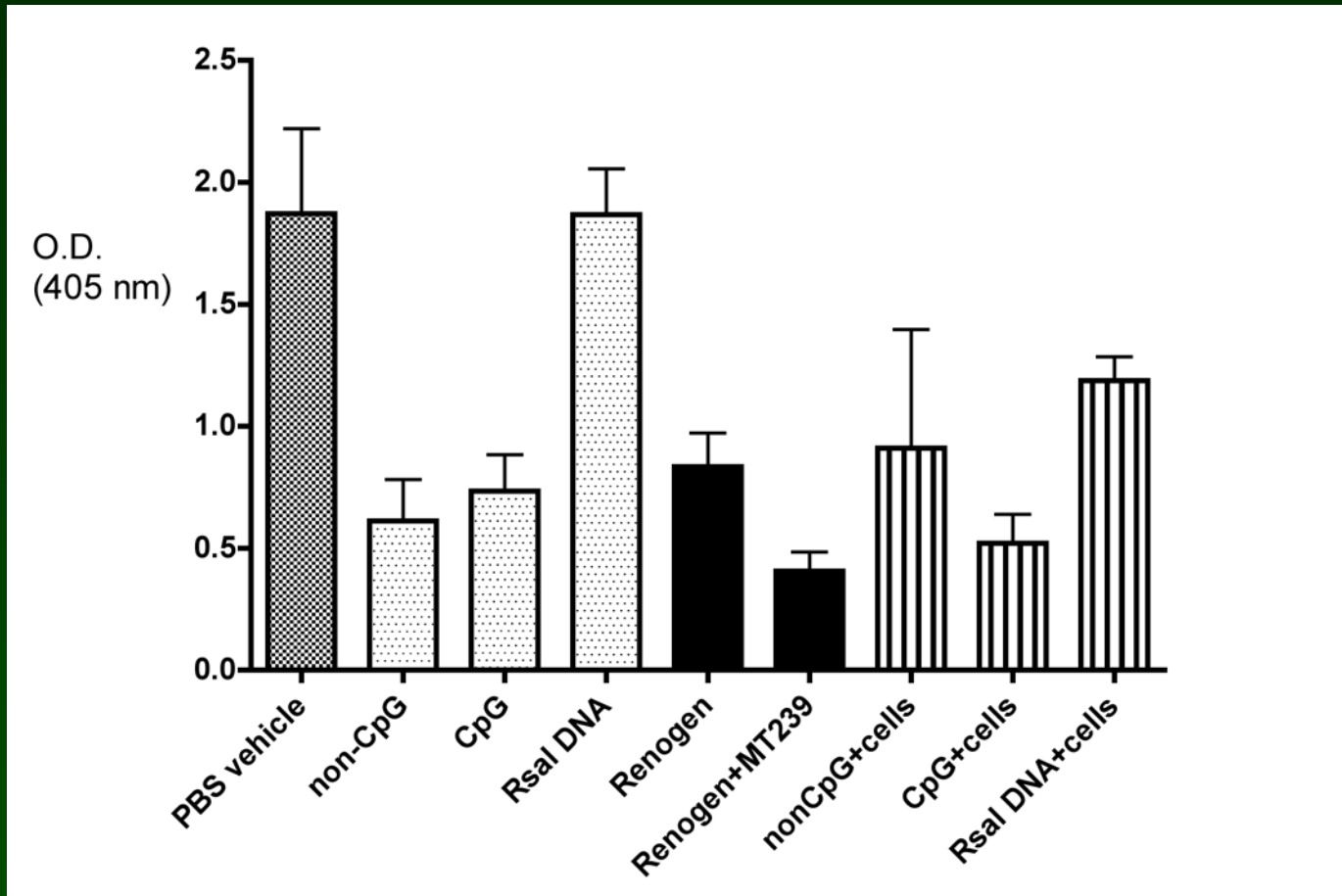
- Fish: Chinook salmon (*Oncorhynchus tshawytscha*)
- George Adams stock
- Salt water-adapted yearlings
- Kidney ELISA at start of experiment:  
mean=0.7155  
range=0.1965 to 3.2390



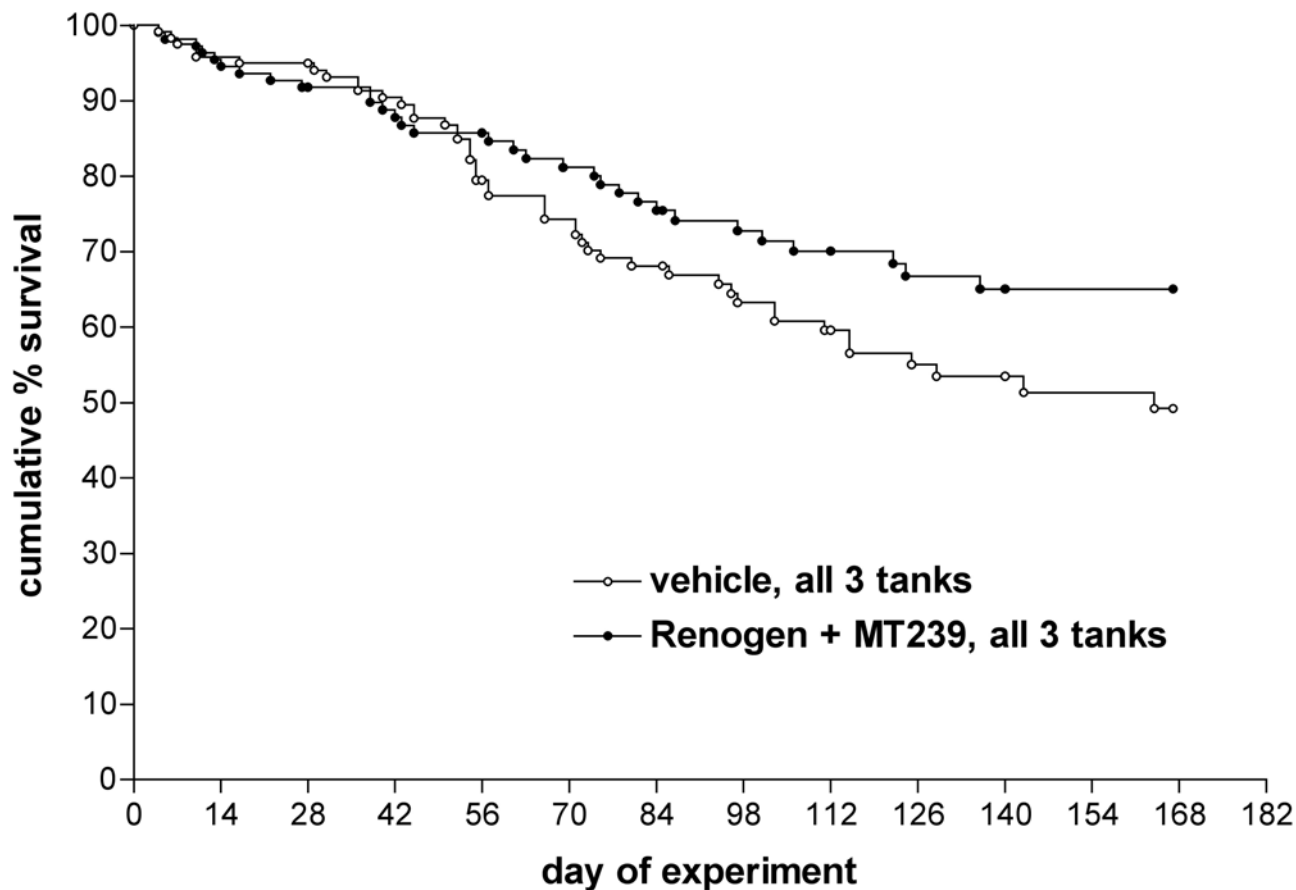
# Survival in Naturally Infected Fish



# Kidney ELISA Values in Surviving Fish



# Survival in Vaccinated Chinook (experiment 2)



# Conclusions

- **A combined whole cell vaccine of Renogen and MT239 can increase survival in infected post-smolt juvenile chinook salmon.**



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- A combined whole cell vaccine of Renogen and MT239 can increase survival in infected post-smolt juvenile chinook salmon.
- Either the oligonucleotide adjuvants or whole cell vaccines may stimulate increased clearance of *R. salmoninarum*.
- This is the first demonstration of a therapeutic vaccine against BKD.



# Research Relationships

**Applied Research:** Integrated Disease Control  
Husbandry  
Antibiotics  
Vaccination

**Basic Research:** Epidemiology  
Isolate differentiation  
Infection prevalences

**Basic Research:** Molecular Genetics  
Reverse genetics techniques  
Virulence determinant - MSA



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Vaccination

## **Basic Research:** Epidemiology

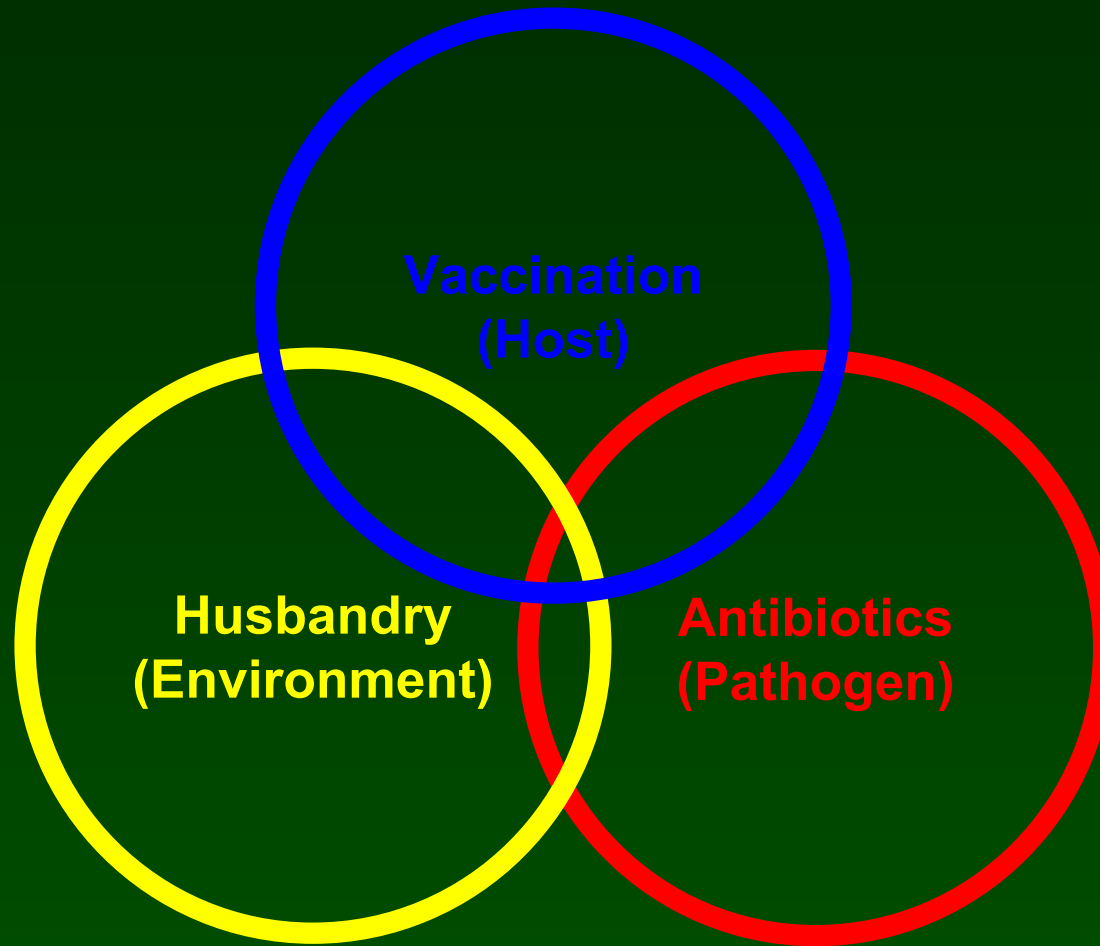
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## **Basic Research:** Molecular Genetics

Reverse genetics techniques  
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# Applied Research: Integrated Disease Control

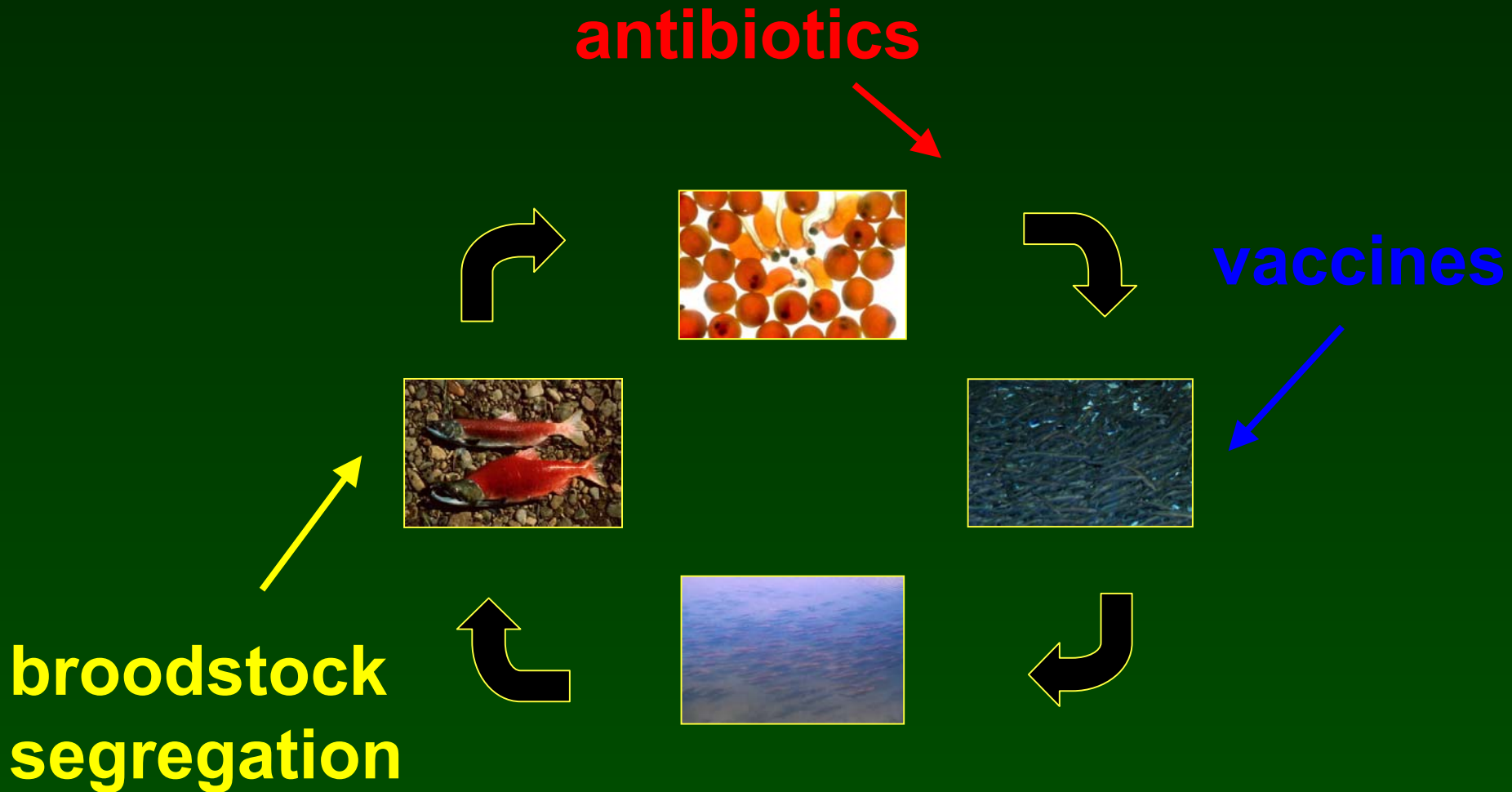


# Concerns & Barriers

Husbandry Hygiene Broodstock segregation	Cost Genetics
Antibiotics	Cost Drug resistance
Vaccines	Availability Cost



# Integrated Disease Management



# Acknowledgments

Mark Peterson (NWFSC)  
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