Ecology of Ranaviruses: A State of Understanding

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UF Infectious Diseases and Pathology

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Outline

I. Ranavirus-Host Characteristics

II. Ecology: Species to Communities

III. Effects of Stressors

IV. Commercial Trade & Pathogen Pollution
Ranavirus Characteristics

- dsDNA, 150-280K bp
- 120-300 nm in diameter (3x smaller than bacteria)
- Icosahedral Shape (20)

**Family: Iridoviridae**

**Genus:** Ambystoma, Chloriridovirus, Ranavirus, Megalocytivirus, and Lymphocystivirus

**Species (6):**
- Ambystoma tigrinum virus (ATV)
- Bohle iridovirus (BIV)
- Frog virus 3 (FV3)
- Epizootic haematopoietic necrosis virus
- European catfish virus
- Santer-Cooper Ranavirus

**Paracrystalline Array**

**Species (5):**
- Ambystoma tigrinum virus (ATV)
- Bohle iridovirus (BIV)
- Frog virus 3 (FV3)
- Epizootic haematopoietic necrosis virus
- European catfish virus

**How Does Ranavirus Infect A Host?**

**Routes of Transmission**

- **Indirect Transmission:**
  - Water or Sediment
  - Skin, Gills, Intestines (epithelial cells)

- **Direct Contact:**
  - One Second Skin Contact

- **Ingestion:**
  - Incidental, Necrophagy, Cannibalism, Predation (Mortality 2X Faster)

**Ranavirus Replication Cycle**

- Viral Transcription within 3 hours of exposure
- Cell death occurs within 6 – 9 hrs PI
Gross Signs of Infected Amphibians
Edema, Erythema, Hemorrhages, Ulcerations

Signs Vary Among Species
Hyla chrysoscelis
Lithobates clamitans
Lithobates sylvaticus

Organ Destruction
3 Primary Organs: Liver, Spleen, and Kidney

Pathogenesis
Target Organ Failure
Heart Failure
Testes, Anemia

Mortality Can Be Rapid!
Quickly as 3 days!

Hoverman et al. (2011a)
Maine 2013 Die-off

6/14/13

6/15/13

What Mechanisms Lead to Outbreaks?

Gray et al. (2009)

Ecology and pathology of amphibian ranaviruses

Matthew J. Gray,*, Debra S. Miller*, James T. Hinesman*

Species Challenges
FV3-like Ranaviruses
Single-species FV3-like Challenges

Percent mortality

Phylogeny, Life History, and Ecology Contribute to Differences in Amphibian Susceptibility to Ranaviruses

Hoverman et al. (2011a): 19 Species Tested

Single-species FV3-like Challenges

Amphibians

15 Additional Species

Brenes (2013)

Life History and Phylogeny

Amphibians

Brenes (2013)

No Phylogenetic Signal

All Three Isolates

- Fast development hatching time
- Low aquatic index
- Breeding habitat (temporal)
- Breeding time (spring)

Smoky Mountains Isolate

- Distance: Population & Isolate

Storfer et al. (2007)
Single-species FV3-like Challenges
Chelonians
Terrapene carolina, T. ornata, Elseya latisternum, Emydura krefftii, Trachemys scripta

Greatest infection and morbidity with IP injection or oral inoculation.

Water bath exposure sufficient for transmission with some species.

Ariel (1997), Johnson et al. (2007), Allender (2012), Waltzek, Gray, Miller (unpubl. data)

- Soft-shelled Turtle
- Mississippi Map Turtle

Single-species FV3-like & ATV Challenges
Fishes

No Transmission:
Cyprinus carpio, Carassius auratus, Lepomis cyanellus

Low Transmission:
Amelurus melas, Esox lucius, Sander lucioperca, Micropterus salmoides

High Mortality:
Scaphirhynchus albus


- Channel catfish
- Mosquito fish

No Transmission: tilapia, bluegill and fathead minnow

Limitation: Density dependence (transmission/stressor)
Reservoirs or Amplification Hosts?
FV3-like Ranaviruses

Possible Reservoirs
- Infected, Susceptible, Recovered

- Reptiles
- Bony Fish
- Amphibians

Low Mortality (Subclinical)
- Reservoir

Low Mortality (Subclinical)
- Reservoir

Low – High Mortality (Subclinical & Clinical)
- Reservoir or Amplification

Can Interclass Transmission Occur?

Host range, host specificity and hypothesized host shift events among viruses of lower vertebrates
Bandin & Dopazo (2011)

Evidence from the Wild

13 February 2012

26 of 31 Box Turtles Die from Ranaviral Disease
2008 – 2011

North Branch Stream Valley State Park

Deadly virus hits turtles, tadpoles in Montgomery County

Larval anurans and salamanders dead too
Farnsworth and Seigel (2013)
Evidence of Interclass Transmission
Bayley et al. (2013)

Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts

Pike-perch Iridovirus
Common Frog Tadpoles

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Evidence of Interclass Transmission
Waltzek, Gray, and Miller

0% mortality in controls

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Evidence of Interclass Transmission
Waltzek, Gray, and Miller

Pallid Isolate Caused Mortality; Bullfrog Isolate Resulted in Infection
Can ranavirus move among host species?

**Interclass Transmission**

Sympatric Ectothermic Vertebrate Species

- Amphibian
- Fish
- Reptile

Brenes (2013)

**Experiment**

- Direct exposure
  - Exposed to $10^3$ PFU/mL
  - 3 days
- 12-L containers divided in half by a 2000 µm plastic mesh
- Different species in each side of the container

**Turtle and Fish Results**

Brenes et al. (in review; PLoS ONE)

- All classes tested can transmit the virus
- Turtles infected tadpoles
  - 50% mortality
- Fish infected tadpoles
  - 10% mortality

- 50%
- 10%
Amphibian Results

Brenes et al. (in review; PLoS ONE)

- Amphibians transmitted to turtles but not fish
- No mortality of turtles or fish exposed to infected tadpoles
- Supporting that turtles and fish may be reservoirs of ranavirus
- Amphibians may be amplifying species

Superspreaders and Amplifying Species

Paull et al. (2012)

- Persistence
- Dispersal
- Host Community
- Contact Rate

Disease Hotspots

- Superspreading Individuals
- Amplification Species
- Shedding Rate
- Contact Rate

Superspreading Individuals

From superspreaders to disease hotspots: linking transmission across hosts and space

Reilly, Gray, & Miller (unpubl. data)

- 6 hrs cohabitation
- 3-100 IPD/mL

Ranavirus Superspreaders
Community Level Transmission
Brenes, Gray, & Miller (unpubl. data)

Does Exposure Order or Composition Matter?

Exposure Order Matters
Brenes (2013)

Exposure Treatments
- Only Wood Frogs
- Only Chorus Frogs
- Only Spotted Salamanders
- Control

Exposure Order
- Appalachian: Wood frog, chorus frog, spotted salamander
- Coastal Plains: Gopher frog, chorus frog, southern toad

Community Composition Matters
Brenes (2013)

Exposure Treatments
- Only Gopher Frogs
- Only Chorus Frogs
- Only Southern Toad
- Control

Community Composition
- Appalachian: Wood frog, chorus frog, southern toad
- Coastal Plains: Gopher frog, chorus frog, southern toad

Design
- n = 5 pools/trt
- 10 larvae/spp
- 60 days
Impacts of Stressors

Gray et al. (2009)

χ² = 40.1; p < 0.001

Hatchling – 3X > Embryo
Larval – 4X > Embryo
Metamorph – 5X > Embryo

Impacts of Development
Across Seven Species

Haislip et al. (2011)

ML Estimate: Egg membrane may act as a protective barrier

Natural stressors and disease risk: does the threat of predation increase amphibian susceptibility to ranavirus?

Kerby et al. (2011)
Anax increased susceptibility to ATV (A. tigrinum)
Competing Temperature Hypotheses

• Virus Replication Hypothesis  
  Bayley et al. (2013)
  – Ranavirus replication increases with temperature up to 32 C
  High Pathogenicity at Higher Temperatures

• Temperature Induced Stress Hypothesis
  – Early Spring Breeding Species:
    • Stressed by Warm Temp
  – Summer Breeding Species:
    • Stressed by Cold Temp

Pathogenicity is species-specific and related to typical water temperature experienced during tadpole development.

Wood Frog
Survival and Infection Prevalence

<table>
<thead>
<tr>
<th></th>
<th>Warm</th>
<th>Cold</th>
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</thead>
<tbody>
<tr>
<td>No Control Mortality</td>
<td>100%</td>
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<tr>
<td>Clinical</td>
<td>152484</td>
<td>84</td>
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Spotted Salamander
Survival and Infection Prevalence

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<tr>
<th></th>
<th>Warm</th>
<th>Cold</th>
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<tr>
<td>No Control Mortality</td>
<td>45%</td>
<td>100%</td>
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<td>Clinical</td>
<td>6837</td>
<td>1700</td>
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<tr>
<td>Subclinical</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

Brand et al. (unpubl. data)
Green Frog Survival and Infection Prevalence

15% Control Mortality in Warm Chamber

Clinical

\[ \times_{FPU} = 1871 \]

\[ \times_{FPU} = 9 \]

Subclinical

Virus Replication Hypothesis

\[ \times_{FPU} = 103 \]

Reilly, Gray and Miller (unpubl. data)

25°C Chamber

Median days to mortality:

- Minnesota = 5.5 d
- Tennessee = 6 d

Tennessee = 10 d, Faster

15°C Chamber

Median days to mortality:

- Minnesota = 15.5 d
- Tennessee = 18 d

In Vitro Replication Stops at 12°C (Chinchar 2002)

Factors Contributing to Emergence

Anthropogenic Stressors:

1) Herbicide (Atrazine)
   Insecticide (Carbaryl)
   \[ \times_{A. nigrius} \]
   ATV Susceptibility

2) Cattle Land Use:
   Prevalence
   Green Frogs and Tiger Salamanders

Pathogen Pollution:

- Picco et al. (2007)
- Schloegel et al. (2009)

- Picco et al. (2007)
- Schloegel et al. (2009)

- Other Possible Stressors:
- Pesticide Mixtures, Nitrogenous Waste, Endocrine Disruptors, Acidification, Global Warming, Heavy Metals

- Pathogen Pollution:
- (Cunningham et al. 2003)

Anthropogenic introduction of novel strains to naïve populations

- Fishing Bait
- Ranaculture Facilities
- Biological Supply Companies
- International Food & Pet Trade
- Contaminated Fomites

Tennessee and Minnesota Wood Frogs
Risk of Pathogen Pollution
Majji et al. (2006), Storfer et al. (2007), Mazzoni et al. (2009), Hoverman et al. (2011a)

Per cent mortality

Ranaculture isolate 2X more lethal than FV3

Commercial Trade and Emergence
Drs. Andrew Storfer and Angela Picco
Storfer et al. (2007), Picco & Collins (2008)

- 85% bait shops had ≥1 infected salamander
- 32% prevalence (n = 2228)
- Anglers: used (26–73%) and released (26 – 67%)
- Different ATV strains are being transported
- Phylogenetic Concordance Analysis
  - Lack of co-evolution: host-pathogen phylogenies
  - Complete concordance when adjusted for human trade
- Emergence: pathogen pollution

Global Trade of Ranavirus Hosts
Kristine Smith, DVM

From 2000-2006, the U.S. imported >1.5 billion individual animals (fish & wildlife; Smith et al. 2009)

- 90% fish, 2% amphibians, 1% reptiles
- 25 million live amphibians imported to U.S./year

Ranavirus Positive
- Hong Kong = 89%
- Dominican Republic = 70%
- Madagascar = 57%

Smith et al. (unpubl. data)
World Organization for Animal Health

OIE Aquatic Code
Chytridiomycosis
Notifiable Diseases
Ranaviral disease
Certification for
2008
Shipment
Schrøgel et al. (2010)

International
Transport of
Animals

Disinfection: Johnson et al. (2003), Bryan et al. (2009);
Golds et al (2014)

• Bleach > 4%
• EtOH > 70%
• Virkon > 1%
• Nolvasan > 0.75%

$75/bottle

OIE
Notifiable Disease
Certification for
Shipment
Schrøgel et al. (2010)

International
Transport of
Animals

What do we Know?

• Ranavirus are Multi-species Pathogens
• Amphibians with fast-developing larvae most susceptible
• Interclass Transmission can occur
• Community Composition matters
• Amplification: amphibians, Reservoirs: all classes
• Transmission is efficient – Multiple Routes
• Environmental Persistence is long
• Pathogenicity might increase in Warm Temperatures
• Anthropogenic Stressors and Pathogen Pollution contribute to Ranavirus Emergence
• Enforcing OIE regulations and Biosecurity is Essential

Ranaviruses represent a significant threat to the global biodiversity of ectothermic vertebrates

Global Ranavirus Consortium
http://fwf.ag.utk.edu/mgray/ranavirus/ranavirus.htm

Symposia
Discussion Groups
Website
Reporting System
Outreach Resources
Springer eBook

The goal of the GRC is to facilitate communication and collaboration among scientists and veterinarians conducting research on ranaviruses and diagnosing cases of ranaviral disease

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