Ecology and Pathology of Ranaviruses

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Outline

I. Ranavirus Die-offs and Host Effects

II. UT Research: Host-Pathogen Interactions

III. Can Ranaviruses Contribute to Declines?

IV. Anthropogenic Effects and Disinfectants

Amphibian Declines and Emerging Infectious Diseases

Science 306:1783-1786
EID 5:735-748
Nature 404:752-755
Biotropica 37:143-148

Chytrid Fungus
Adults: >95% (Europe)
Larvae: 80-100%
Ranaviruses

43% in Decline
34% in Risk of Extinction
History of Ranavirus Die-offs

First Isolated:
• Dr. Allan Granoff
• St. Jude Hospital
• *Rana pipiens* (1962)

First Large-scale Die-offs:
• Dr. Andrew Cunningham
• Institute of Zoology, ZSL
• *Rana temporaria* (1992)

First North American Die-offs:
• Dr. Jim Collins and students
• Arizona State University
• *Ambystoma tigrinum stebbinsi* (1985, 1997)

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Global Distribution of FV3-like Infections

All Latitudes, All Elevations
14 Families: Alytidae, Ranidae, Hylidae, Bufonidae, Leptodactylidae, Discoglossidae, Myobatrachidae, Rhacophoridae, Scaphiopodidae, Ambystomatidae, Salamandridae, Hynobiidae, Cryptobranchidae

>70 Species

5 Continents: 1992

Miller et al. (2011)

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Reported Amphibian Die-offs in North America: *Ranavirus*

Uncommon

>30 States & 5 Provinces; 28 Spp.
Case Example
North America
Jamie Barichivich (USGS) and Megan Todd-Thompson (UT)

May 2009
Spotted & Marbled Salamander, Wood Frog, Spring Peeper, Southeastern Chorus Frog

Ranavirus Characteristics

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

Family: Iridoviridae
Genera: Iridovirus, Chloriridovirus, Ranavirus, Megalocytivirus, and Lymphocystivirus

Paracrystalline Array
Species (6)
- Ambystoma tigrinum virus (ATV)
- Bohle iridovirus (BIV)
- Frog virus 3 (FV3)

How does Ranavirus Kill A Host?
Routes of Infection

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

Direct Contact
- One Second Skin Contact

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

**Ranavirus Replication Cycle**

Chinchar (2002), Chinchar et al. (2006)

Protein synthesis within hours of infection

Cell death occurs within 6 – 9 hrs PI

12 – 32 C

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**Gross Signs of Infected Amphibians**

Edema, Erythema, Hemorrhages, Ulcerations

N. Haislip, UT

Hyla chrysoscelis

Lithobates clamitans

Lithobates sylvaticus

A. Duffus, ZSL

B. Green, USGS

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**Signs Vary Among Species**

Haislip et al. (unpubl. data)

Lithobates clamitans

Hyla chrysoscelis

Lithobates sylvaticus
Internal Signs of Ranaviral Disease

- Kidney Hemorrhages
- Pale and Swollen Liver

Organ Destruction

3 Primary Organs: Liver, Spleen, and Kidney

- Liver Necrosis
- Spleen Necrosis
- Kidney Degeneration

Pathogenesis:
- Target Organ Failure
- Heart Failure
- Toxicosis, Anemia

Mortality Can Be Rapid!
- Quickly as 3 days!
- Die-offs: 2 weeks

Imagine if Ranaviruses could Infect Humans

- Monday: Fever
- Wednesday: Hands, Feet, Legs Swollen
- Friday: Bedridden, Body Enlarged 2X, Lesions, Hemorrhaging Internally, and from Orifices
- Sunday: Begging Dr. Death (Jack Kevorkian) for a quick end.

No Amphibian Pathogen: Diversity of Gross Signs or Kills as Quickly

There is no Cure!
University of Tennessee
Ranavirus Research
Gray, Miller, Hoverman, Haislip, Bryan, Brenes, Hilzinger and others

- Species Susceptibility
- Isolate Virulence
- Developmental Stage
- Risk of Predation
- Disinfectants
- Community Level Effects
- Interclass Transmission
- Environmental Persistence

Broad Host Range
Hoverman et al. (2011): 35 Species Tested
Expanding to 40 spp (2012)

- Frequently associated with die-offs
- Species of concern

Impacts of Development
Across Seven Species
Haislip et al. (2011)

$\chi^2 = 40.1; p < 0.001$

Egg membrane may act as a protective barrier

ML Estimate:
- Hatching: $> 3x >$ Embryo
- Larval: $> 4x >$ Embryo
- Metamorph: $> 8x >$ Embryo
Community Level Transmission
Does it Matter Who is Infected?
Species Introduce Pathogens

High Medium Low
High Transmission and Mortality?
Low Transmission and Mortality?

Can Certain Species Drive Outbreaks?

Results: Wood Frog
Mortality rates after 60 days depended on which species was initially exposed to the pathogen.

Brenes et al. (unpubl. data)

Wood frogs experienced greatest mortality in all treatments
100% Mortality: All exposed or they were exposed.
65% Mortality: Chorus frogs exposed.
15% Mortality: Spotted salamanders exposed.

Results: Spotted Salamander
Mortality rates after 60 days depended on which species was initially exposed to the pathogen.

Brenes et al. (unpubl. data)

Spotted Salamanders experienced lowest mortality among treatments
0 – 15% Mortality
Slightly higher mortality if wood frogs or all species exposed.
Results: Upland Chorus Frog

Mortality rates after 60 days depended on which species was initially exposed to the pathogen.

Brenes et al. (unpubl. data)

Chorus frogs experienced moderate mortality among treatments

20 – 50% Mortality

Similar mortality if wood frogs, all species, or only chorus frogs were exposed

Pathogen Transmission

Community Predictions Confirmed

• Wood frog tadpoles appear to function as superspreaders
• Exposure of upland chorus frog tadpoles caused an outbreak.
• Exposure of spotted salamander larvae was insufficient to cause an outbreak.

What about other Ectothermic Vertebrates?

26 of 31 Box Turtles Die from Ranaviral Disease

2008 – 2011

Farnsworth and Seigel, Towson U.
Cases of FV3-like Ranaviral Disease in Reptiles


Gopherus polyphemus, Testudo hermanni, Terrapene carolina carolina, Trionyx sinensis, Uroplatus fimbriatus, and Chondropython viridis

Over >95% homology with 1000-bp region of MCP

High homology does not imply interclass transmission is possible!

Cases of FV3-like Ranaviral Disease in Fish

Pallid Sturgeon

Waltzek et al.

Blind Pony Fish Hatchery, Missouri

Wild Case

Identical with R. aurora ranavirus

Mao et al. (1999)

Experimental Challenges

Anurans

2-L Tubs
Pallid & Turtle
3 Day WB
21 Days

Turtles

12-L Tubs
Pallid & Bullfrog
3 Day WB
Infected WF (3 d)
28 Days

$\Delta = 20 / \text{trt} + 20 \text{ controls}$
Water Change: 3d
Morbidity & Mortality
qPCR for Infection

$10^3 \text{ PFU/mL}$
Transmission to Anurans

Final Mortality

0% mortality in controls

- Pallid Isolate (35 – 70%)
- Pallid Isolate (95%)

Survival

Pallid Isolate

Resulted in faster mortality than any other previously tested isolate

- Pallid Isolate Caused Mortality
- Bullfrog Isolate Resulted in Infection

Transmission to Turtles

Final Mortality

- Pallid Isolate Caused Mortality
- Bullfrog Isolate Resulted in Infection
Transmission to Turtles
Survival Curves

**Conclusions**

- Interclass Transmission is possible
- Pallid isolate was more virulent than box turtle or bullfrog
  - 15 – 65%
  - 1 – 10 days
  (One Isolate)
- Turtle and bullfrog isolates resulted in infection in wood frogs and red-eared sliders, respectively

**Ranavirus Ecology**

Gray et al. (2009)
Are Ranaviruses Capable of Causing Local Extirpations and Species Declines?

Traditional Theory
(Anderson and May 1979)

Extirpation is possible if:

Frequency Dependent

1. Multiple Host Pathogens Where Susceptibility Differs
   - Asymptomatic Carriers

   Is at least one of these conditions satisfied in the ranavirus-host system?
   - Survive Outside Host

2. Environmental Reservoir
   - Survive Outside Host

3. Clustering of Individuals
   - Sexually transmitted disease

Evidence of Alternate Hosts

1. Multiple-host Pathogen:

2. Other Ectothermic Vertebrates

Ranaviruses can infect multiple host species & some serve as asymptomatic carriers – #1 Met
Evidence of Environmental Persistence

(1) EHNV Persistence (Langdon 1989)
- Distilled Water: 97 d
- Dry Infected Tissue: 113 d
- Frozen Infected Tissue: 2 yr (permanent wetlands at colder temperatures) – #2 Met
- Soil: 13-22 d
- Soil: 30-48 d

(2) FV3, FV3-like (Nazir et al. 2012)
- PW (unsterile): 22-34 d
- PW (unsterile): 58-72 d
- 20 C = T-90 Values
- 4 C = T-90 Values

Evidence of Individual Clustering and Transmission

(1) Breeding (Brunner et al. 2004)
Frequency dependent transmission is possible in larval and adult age classes – #3 Likely.

(2) Larval Clustering
- Increase Infection Rates
- Vegetation Reduction (Greer et al. 2008)

Local Extirpations and Declines?
YES, all 3 characteristics met in the Ranavirus-Host System
Caveat: Community and Site Dependent!

Evidence of Declines

Dr. Amber Teacher
Southeastern England
1996/97 and 2008

Ranavirus (+) populations
81% Median Reduction

Ranavirus (+) populations
81% Median Reduction
Evidence of Re-occuring Die-offs

Dr. Jim Petranka
Tulula Wetland Complex, NC
1998-2006

Recruitment at most wetlands failed due to ranavirus

Persistence Possible from Source Populations

Uncommon Species?
Should we be concerned with a few uncommon species?

Commonality of Being Uncommon
Southeastern United States

Federally Listed: Rana capito sevosa, Ambystoma cingulatum, Phaeognathus hubrichti, Ambystoma bishopi

Species of Concern: 113 Species and 25 Genera Total  50% U.S.

1) Alabama = 14 species (11 genera)
2) Arkansas = 25 species (12 genera)
3) Florida = 19 species (12 genera)
4) Georgia = 22 species (15 genera)
5) Kentucky = 22 species (11 genera)
6) Louisiana = 15 species (10 genera)
7) Mississippi = 18 species (12 genera)
8) North Carolina = 41 species (15 genera)
9) South Carolina = 19 species (13 genera)
10) Tennessee = 26 species (14 genera)

If uncommon species are highly susceptible, ranaviruses could have a significant impact on amphibian communities.

Ranavirus Landscape Prevalence
Tennessee Ponds
Hoverman et al. (2012)

Ranaviruses are common hence have the potential to have landscape scale impacts.

Ranavirus Distribution: 83% of Ponds Sampled
Hotspots: ≥40% in 15 out of 40 Ponds Sampled
•85% Infection
•100% Infection
(morbid and dead)

Uncommon Species?
Should we be concerned with a few uncommon species?
Take Home Messages

Should we be Concerned?

- Ranavirus Die-offs have Global Distribution
- Ranavirus Prevalence can be High
- Ranaviruses Infect Multiple Amphibian Species with Different Susceptibilities
- Community Composition may Matter
- Interclass Transmission is Possible – Abundant Reservoirs

Epidemiological Theory Supports the Premise that Ranaviruses Could Cause Local Population Extirpations and Contribute to Species Declines.

Uncommon Species with High Susceptibility are at Greatest Risk!

Impacts of Stressors

Factors Contributing to Emergence

**Anthropogenic Stressors:**
- A. tigrinum Herbicide (Atrazine), Insecticide (Carbaryl)
- Cattle Land Use: Prevalence Green Frogs and Tiger Salamanders
- Other Possible Stressors: Pesticide Mixtures, Nitrogenous Waste, Endocrine Disruptors, Acidification, Global Warming, Heavy Metals

**Pathogen Pollution:**
- Anthropogenic introduction of novel strains to naïve populations
- Fishing Bait
- Ranaculture Facilities
- Biological Supply Companies
- International Food & Pet Trade
- Contaminated Fomites

Reference:

Questions??

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