Ranaviruses: Cold Blooded Killers!

Matthew J. Gray
University of Tennessee, Center for Wildlife Health
Department of Forestry, Wildlife and Fisheries
12:20 p.m.
17 February 2010
160 Plant Biotechnology Building

Outline

I. Emerging Infectious Diseases

II. Die-offs and TN Surveillance

III. Ranavirus Pathology and Ecology

IV. Future Research Directions

Amphibian Declines and Emerging Infectious Diseases

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

Global Distribution of Ranavirus Die-offs

5 Continents, All Latitudes, All Elevations

11 Families: Ranidae, Hylidae, Bufonidae, Leptodactylidae, Discoglossidae, Rhacophoridae, Myobatrachidae, Ambystomatidae, Salamandridae, Hynobiidae

Reported Amphibian Die-offs in North America: Ranavirus

Families
- Ranidae
- Hylidae
- Bufonidae
- Ambystomatidae
- Salamandridae
- Lithobatidae

Uncommon

>30 States & 20 Spp, 4 Provinces

Lithobates sylvaticus
Pond Surveillance
2008 Sampling

Seasons and Land use:
Green Frog, Bullfrog, Pickerel Frog, Newt, Tiger and Spotted Salamanders

Ranavirus Hotspots:
Jan: 1 Pond = 57%
Apr: 4 Ponds >30% (43%)
July: 1 Pond = 33%
Oct: 5 Ponds >50% (90%, 100%)

Southern Appalachia:
What about Plethodontids?
12 Species & 4 Genera:

Aquatic vs. Terrestrial

Another Family of Concern
2009

Hellbenders are Suitable Hosts
Ecology and Pathology of Amphibian Ranaviruses

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

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

Candidate Species: R. catesbeiana virus Z (RCV-Z)

Ranavirus Replication Cycle

Chinchar (2002), Chinchar et al. (2006)
Ranavirus Replication Cycle
Chinchar (2002), Chinchar et al. (2006)

Protein synthesis within hours of infection

Cell death occurs within 6 – 9 hrs PI

Ranavirus: Gross Signs
Edema, Erythema, Hemorrhages, Ulcerations

Ranavirus: Internal Signs
Kidney Hemorrhages  Pale and Swollen Liver
**Ranavirus: Histopathological Signs**

3 Primary Organs: Kidney, Liver and Spleen

- Kidney Degeneration
- Spleen Necrosis
- Viral Inclusions

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

Disease Can Progress Fast: 1-3 d signs, 3-7 d mortality; 2 wks die-off

**Routes of Transmission**

- Oral inoculation
- Ingestion
- Water Bath Contaminated Sediment
- Necrophagy
- Cannibalism

- Gruia-Gray & Desser (1992)
- Brunner et al. (2004), Pearman et al. (2004), Hary & Petrasko (2006)

Horizontal vs. Vertical:
- Only Horizontal Transmission Demonstrated
- Duffus et al. (2008): Vertical Transmission Suspected

**Variation in Susceptibility to Ranavirus Among Species and Tadpole Developmental Stages**

Nathan A. Haislip
M.S. Candidate
Species Comparison

Across All Development Stages

Logistic Analyses Species*Stage Interactions

ML Estimate:
- * A. americanus = 4X > P. feriarum
- ** H. chrysoscelis = 10X > P. feriarum
- L. pipiens = 13X > P. feriarum
- L. clamitans = 1.3X > L. pipiens, 16X > P. feriarum
- S. holbrooki = 65X > P. feriarum
- L. sylvaticus = 7X > L. pipiens
- 70X > P. feriarum

Ranids

Lithobates clamitans

Lithobates pipiens

Lithobates sylvaticus

Hylids

Pseudacris feriarum

Hyla chrysoscelis
Toads
*Anaxyrus americanus*

- Similar to Ranids
- *Scaphiopus holbrookii*
- Fast Developers

Potential Natural Stressors

1) Development (N. Haislip)
   - Immune Function: Egg, Hatchling, Larval, Metamorph, Adult
2) Water Temperature
   - Positive Relationship: Virus Replication; Immune Function
3) Population Density
   - Competition (L. Rucker)
   - Contact Probability
4) Genetic Diversity
   - Genetically Isolated Populations More Susceptible
5) Predation (N. Haislip)
   - Exposure to Predators: Corticosterone Synthesis
   - Elevated Corticosterone: Increased Parastic Infection
6) Other Pathogens
   - Secondary Infection: Ranavirus, *R.* *Aeомonas hydrophila*, *Saprolegia*

Factors Contributing to Emergence

**Anthropogenic Stressors:** Forsen & Sterner (2006); Gray et al. (2007)

- 1) Herbicide (Atrazine)
- 2) Fertilizer (sodium nitrate)
- 3) ATV Susceptibility
- 4) Inconclusive

- 1) Salamander Bait Trade
- 2) Ranaculture Facilities
- More Virulent Strains

**Novel Strain Introduction:** “Pathogen Pollution”

- 1) Introduction of Novel Strains
- 2) More Virulent Strains

_Majji et al. (2006), Hoverman et al., unpubl. data_
Pathogenicity of a Ranaculture Ranavirus Isolate

Jason T. Hoverman
Post-doctoral Research Associate

---

Wood Frog

Pearson’s chi-square test

---

Southern Leopard Frog
Pickerel Frog

Green Frog

Cope’s Gray Treefrog

Percent survival over days for Pickerel Frog, Green Frog, and Cope’s Gray Treefrog.
World Organization for Animal Health

OIE Aquatic Code
Chytridiomycosis
Ranaviral disease
2008

International Transport of Animals

Notifiable Diseases Certification for Shipment
Schenkel et al. (2009)

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

- Bleach > 4%
- EtOH > 70%
- Virkon > 2.5%
- Nolvasan > 0.75%

$5/ bottle

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

Other Ectothermic Hosts
Reptiles and Fish

Are Amphibian Ranaviruses Lethal to other Ectothermic Vertebrates?

Reptiles: Gopherus polyphemus, Testudo hermanni, Terrapene carolina carolina, Triops xenis, Uroplatus fimbriatus, and Chondropython viridis
(Marschang et al. 1999, 2005; Hyatt et al. 2002; Allender et al. 2006)

Fish: BIV & barramundi: Experimental Challenge
FV3 & pallid sturgeon: 2009 Die-off

J. Briggler, MO Dept of Conservation

Blind Pony Hatchery

Important Research Directions
Pressing Research Directions
Southeast Species

1) Surveillance & Monitoring

2) Experimental Challenges

Tennessee
- Hyla gratiosa
- Acris gryllus
- Hyla versicolor
- Rana areolata
- Siren intermedia
- Ambystoma barbouri
- Ambystoma talpoideum

Pressing Research Directions
Mechanisms Driving Outbreaks?

1) Cattle Use: Nitrogenous Waste

2) Pesticides: Atrazine, Carbaryl, Malathion, Endosulfan, Glyphosate

3) Strain Virulence

Do Ranaviruses from One Region Represent Novel Pathogens in Another Region?
Hoverman et al.: Captive Facilities

Pressing Research Directions
A Mechanism Driving Future Outbreaks
Temperature-induced Stress

Ectothermic Vertebrates

Ranavirus Replication Increases with Temperature
Pressing Research Directions
Reservoirs and Persistence

1) Fish and Reptiles

2) Persistence

Pathogen Ecology
Spatially Structured Breeding Sites

\[ \text{Host-Pathogen Community} \]

\[ EM(t)_{ijkl} \]
\[ I(t)_{ijkl} \]
\[ S(t)_{ijkl} \]
\[ IM(t)_{ijkl} \]
\[ P(N_t)_{il} > 0 \]

Collaborators

University of Georgia

Dr. Debra Miller
Dr. Sandy Baldwin
Dr. Jason Hoverman
Nathan Haislip
Kevin Hamed

University of Tennessee

Funding:
- UGA Veterinary Diagnostic & Investigational Laboratory (Tifton)
- UT Institute of Agriculture
- Tennessee Wildlife Resources Agency
- Assoc. Reptile & Amphibian Veterinarians