Emerging Infectious Diseases in Amphibians: Chytrid Fungus and Ranavirus

I. Amphibian Declines and EIDs

II. Chytridiomycosis

III. Ranaviral Disease

IV. Possible Conservation Strategies to Reduce Ranavirus Emergence

Amphibian Declines and Emerging Infectious Diseases

Science 306:1783-1786
EID 5:735-748
Nature 404:752-755
Biotropica 37:463-468

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

Batrachochytrium dendrobatidis

- Western United States
- Mostly Tropical at High Elevations
- Some Species: Highly Pathogenic

Mountain yellow-legged frog (Rana muscosa)

Batrachochytrium dendrobatidis (Bd):
- Non-hyphal parasitic fungus (only chytrid spp pathogenic to vertebrates)
- Infect keratinized tissue (stratum corneum and granulosum)
- Adults: Pelvic Region
- Larvae: Mouthparts

Larvae: Mouthparts
- Adults: Pelvic Region

Life stages
- Zoospore – aquatic, flagellated (3-5μm)
- Zoosporangium – zoospores discharged (300) (4 days)

Chytridiomycosis: An Emerging Infectious Disease of Amphibians

The pathogen

Phylum: Chytridiomycota
Class: Chytridomycetes
Order: Chytridiales

- Western toad
- Wyoming toad
- Chiricahua leopard frog?
- CA red-legged frog?

>200 spp in decline

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- Chytridiomycosis
- An Emerging Infectious Disease of Amphibians
Histological Signs
Epidermis

- Discharge Tube
- Zoosporangia
- Stratum Corneum
  Normal Thickness: 2 – 5 μm
  Infected: 60 μm

Proliferation of Epidermal Cells
Epidermal Hyperplasia
Sloughing

Cause of Mortality

- Osmoregulatory Inhibition (suspected #1 cause)
  - Decreased water uptake & ion exchange; altered electrolyte/solute levels (decrease Ca, actin & myosin)
- Cutaneous Respiration
- Toxicosis
  100 Zoospores

10 – 18 days

Histological Signs
Muscular Degeneration
**Field Signs of Bd Infection**

- Infected individuals appear healthy
- Lethargic & paralysis
- Sloughing skin & lesions
- Loss of pigmentation in mouthparts of larvae

**Origins**

- **Novel Pathogen Hypothesis**
  - Out of Africa (Weldon 2004)

- **Endemic Pathogen Hypothesis**
  - Environmental changes (Pounds 2006)
Novel pathogen hypothesis

- Exotic, introduced pathogen
  - Low genetic variation globally in Bd
  - Recent global spread (Morehouse et al. 2003)
  - Broad range of host species
  - Few resistant individuals (tropics)
  - Lack of host immune response

1. *Xenopus laevis*; South Africa (1938)
2. *Xenopus gilli*; South Africa (1943)

3. *Rana clamitans*, Canada (1961) 23 years later

Rachowicz et al. (2005)
Novel pathogen hypothesis

1. *Xenopus laevis*; South Africa (1938)
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3. *Rana clamitans*, Canada (1961) 23 years later
4. 1970s North America and Australia
5. Spread around the world

Rachowicz et al. (2005)
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Endemic pathogen hypothesis

• Susceptibility of host may increase because of environmental changes
  - Immunosuppression (Carey 1993)
    • Climate (temperature & moisture, Pounds 2006)
    • UV-B radiation (Kiesecker and Blaustein 1995)
    • Downwind agriculture – Pesticide Deposition

• Species-specific Effects
  - Antimicrobial peptides (Rollins-Smith et al. 2002)
  - Life-history: habitat, basking behavior

Tropics and Subtropics: Novel Pathogen Hypothesis
Ranavirus Die-offs:
Species of Concern

Misnomer: Ranavirus only affects common species.
No evidence that Ranavirus discriminates based on USFWS ESA protection status!

Rana muscosa
R. aurora
Bufo boreas
Ambystoma tigrinum stebbinsi

Ranavirus in Tennessee

Previous Documentation

What do we know?
- Widespread
- Geographic Distribution
- Across all Taxa
- Across all Elevations & Latitudes

Blount County, Great Smoky Mountains National Park

1999: Pickerel Frog, Spotted Salamander
(Gray et al., unpubl. data)

2001: Wood Frog, Eastern Newt, Marbled Salamander
(Gray & Miller, unpubl. data)

2005: Cumberland Plateau
Green Frog, American bullfrog
Gray et al. (2007)

2007: GSMNP
85 of 89 Plethodontids
(10 species: adults)
(Gray & Miller, unpubl. data)

Ranavirus Characteristics

Family: Iridoviridae
Genera: Iderovirus, Chlorideovirus, Ranavirus, Megalocytivirus, and Lymphocystivirus

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

Major Capsid Protein (MCP)
Paracrystalline Array
Candidate Species: R. catesbeiana virus Z (RCV-Z)
Ranavirus Phylogeny

New Species in the Smokies?

% Identity:

- Our Species
- Previous Isolates

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D. quadramaculatus  D. monticola  G. porphyriticus  D. instaurae

Species/Strain Virulence

Are all isolates equally virulent?

- Hoverman et al. (unpubl. data)
- Schock et al. (2008): ATV and FV3 most virulent in salamanders and anurans, respectively.
- Sterler et al. (2007): ATV isolate from bait shop more virulent than wild ATV.
- Majji et al. (2006): RCV-Z more virulent than FV3 (exposure order)

Ranavirus Replication Cycle

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

- Protein synthesis within hours of infection
- Cell death occurs within 6 – 9 PI
- Doubling rate 0.7 – 1.8 days

12 – 32 C
**Ranavirus Replication Cycle**

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

- Enveloped Virion
- Non-enveloped

**Ranavirus: Gross Signs**

- Edema
- Erythema and Dermal Ulcerations

Signs can be Associated with Other Pathogen: *Aeromonas hydrophila*

- Inanition, incoordination, emaciation

**Ranavirus: Gross Signs**

- Kidney Hemorrhages
- Pale and Swollen Liver
Ranavirus: Histopathological Signs

3 Primary Organs: Kidney, Liver and Spleen


Viral Inclusions:
- Erythrocyte
- Liver
- Kidney

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

Routes of Transmission

- Oral inoculation
- Ingestion
- Water Bath Contaminated Sediment
- Horizontal vs. Vertical:
  - Only Horizontal Transmission Demonstrated
  - Duffus et al. (2008): Vertical Transmission Suspected

Reservoirs and Environmental Persistence

Vertebrate Reservoirs:

1. Amphibians
   - Intraspecific Reservoirs (Brunner et al. 2004)
   - Salamanders (Duffus et al. 2008)
   - Overwintering Tadpoles (Gray et al. 2007)
   - Xenopus laevis (Hubert et al. 2007)

2. Fish:
   - BIV & barramundi (Moody & Owens 1994)
   - SBV & TV2 viruses identical (Mao et al. 1999)

3. Turtles:
   - Eastern box turtle (Allender et al. 2006)
   - ATV:2 weeks @ 25°C water bath lost infectious capability.
     Jancovich et al. (2001)
   - ATV did not infect tadpoles or fish
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Potential Natural Stressors

1) Water Temperature
   - Negative Relationship: Immune Function & Ambient °C

2) Development
   - Immune Function: Egg, Hatchling, Larval, Metamorph, Adult

3) Population Density
   - Contact Probability
   - No relationship detected

4) Genetic Diversity
   - Genetically Isolated Populations More Susceptible

5) Predation
   - Exposure to Predators: Corticosterone Synthesis
   - Elevated Corticosterone: Increased Parasite Infection

6) Other Pathogens
   - Secondary Infection: Ranavirus, Bd, Aeromonas hydrophila, Saprolegnia

7) Adult Breeding: Transmission & Shedding

Factors Contributing to Emergence

Anthropogenic Stressors:
- Forson & Sterler (2006); Gray et al. (2007)
- 1) Herbicide (Atrazine)
  - Fertilizer (sodium nitrate)
  - ATV Susceptibility
  - Inconclusive

2) Cattle Land Use: FV3 Prevalence
   - 150 head per ha per month

Other Possible Stressors: Pesticides, Fertilizers, Heavy Metals, pH, UV-B, Thermal Pollution

Pathogen Pollution: (Cunningham et al. 2003)
- Anthropogenic introduction of novel strains to naïve populations
- Fishing Bait
- Ranaculture Facilities
- Biological Supply Companies
- Contaminated Fomites
- International Trade

Picco et al. (2007)
Daszak et al. (2006)

Results

Disease of Aquatic Organisms 77:97-103

Statistical Tests: Logistic Regression and Maximum Likelihood Estimation
Does Higher FV3 Prevalence Imply Negative Consequences to Population?

Water Quality Differences

Possible Stressor Driving Trends

Ammonia (NH₃):

Jofre and Karasov (1999)

- >0.5 mg/L
- Decrease in egg & green frog tadpole survival
- Sublethal Effects?
- Stressor: Immune Function
- Increased Susceptibility
**Results**

**Seasonal Effects**

*Disease of Aquatic Organisms* 77:97-103

![Graph showing seasonal effects with statistical tests: Logit and Logistic Regressions Maximum Likelihood Estimation](image1)

- **Trt** Season
- Did not interact,
- \( P > 0.30 \)

**Season**
- Winter
- Summer
- Fall

- More Likely!!
- 7.7X
- 4.7X More Likely!

**Statistical Tests:** Logit and Logistic Regressions

**Results**

**Developmental Stages**

*American Bullfrog*

*Disease of Aquatic Organisms* 77:97-103

![Graph showing developmental stages with statistical tests: Logistic Regression, Odds-Ratio Estimates](image2)

- 28% Decrease in the Predicted Odds of Infection with each unit increase in Gosner stage.

- Statistical Tests: Logistic Regression, Odds-Ratio Estimates

**Possible Mechanisms Driving Trends**

- **Water Temperature:**
  - T lymphocyte proliferation and serum complement activity less at low temperature in *R. pipiens*.
  - Maniero and Carey (1997)
  - Raffe et al. (2006): + WBCs and temperature
  - Rojas et al. (2005): + Survival and temperature: Ranavirus ATV

- **Developmental Stages:**
  - Tadpole immunity increases through development in *Xenopus laevis*.
  - Rollins-Smith (1998)
  - Early Development
  - Metamorphosis
  - Gantress et al. (2003)
  - Brunner et al. (2004)

- **Endogenous glucocorticoids**
  - Rojas et al. (2005): + Survival and temperature: Ranavirus ATV
Conservation Strategies
Minimize Stress on the Aquatic Environment

1) Establish Buffers
- EPA: >10 m
- TN BMPs: >15 m
- Terrestrial Environment: 100 m
- Rittenhouse & Semlitsch (2007)

2) Minimize Aerial Drift of Pesticides
A) Eliminate Flyovers
B) Aerial Application on Calm Days

3) For Cattle,
A) Reduce Density
B) Rotational Grazing

Conservation Strategies
Minimize Overland Transmission

4) Decontaminate Surfaces: Fomites
   - “Pathogen Pollution” (Cunningham et al. 2003)
   A) Boot Bath
   B) Chlorhexidine diacetate

5) Regulate Movement among Watersheds:
   - Salamander Larvae as Bait
   - Introduction of Novel Strains
     Jancovich et al. (2005), Picco et al. (2007)

Inactivating Ranavirus
Disinfectant Efficacy

- Nolvasan <0.75%
- Bleach <3%
- Potassium Permanganate

Bryan et al. (2009)
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Questions??