Comparative Pathology of Ranavirus Infections in Wild Amphibians

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Lethal Ranaviral Infections, USA

Epizootiology:
- Kills larvae & larvae in metamorphosis
- Mortality rate often >95% of larvae
- Onset is sudden (explosive)
- Seldom affects adult amphibians in USA

Hosts:

<table>
<thead>
<tr>
<th>Frogs</th>
<th>Toads</th>
<th>Salamanders</th>
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</thead>
<tbody>
<tr>
<td>True frogs</td>
<td>True toads</td>
<td>Mole sal’s</td>
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<tr>
<td>Chorus frogs</td>
<td>Clawed toad?</td>
<td>Newts</td>
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<tr>
<td>Treefrogs</td>
<td>Spadefoots?</td>
<td></td>
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<tr>
<td>Unknown: Woodland salamanders, Sirens, Caecilians</td>
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Hosts of Lethal Ranaviral Infections by Genera, USA

Anurans: 4 genera, 18 spp.
Caudates: 2 genera, 6 spp.

Genera:
- Ambystoma
- Bufo
- Hyla
- Notophthalmus
- Pseudacris
- Rana

Lethal Ranaviral Infections, USA

Findings by National Wildlife Health Center:
- Cases: ~300 die-offs or disease outbreaks since 1996
- Ranavirus cases: >75
- Isolates of ranaviruses: >175 (+70 wild research frogs)

Hosts:
- 85-90% of ranaviral die-offs occur in larvae in USA
- Ranaviral infections in PM amphibians are uncommon
  (exceptions are R. luteiventris, newts & a few treefrogs)
Ranavirus Die-offs: Distribution
Frogs and Toads (tadpoles)

Revised June 2011

Puerto Rico
Alaska
Hawai’i

Repeated die-offs
One or 2 die-offs

Ranavirus Die-offs: Distribution
Salamanders (larvae & newts)

Revised June 2011

Puerto Rico
Alaska
Hawai’i

Repeated die-offs
One or 2 die-offs
Ranavirus Die-Offs

Larval Tiger
Salamanders: Yellowstone NP, WY
Photo by: Dr Sophie St Hilaire

Field (gross) Signs of Ranavirus Infection

Larvae show 4 characteristic abnormalities:
- Reddening or hemorrhages in ventral skin (near vent)
- Bloating or fluid accumulation under skin
- Skin ulcers are uncommon: pinpoint or large
- Live larvae are weak, swim poorly, may float upside down

(Hemorrhages may occur anywhere on body, including eyes and internal organs)
Field (gross) Signs

Larval Tiger Salamanders:
Caribou-Targhee NF, ID
Photo by: Devon Green, NFS

Field Signs: Skin Hemorrhages

A. maculatum  A. tigrinum  A. opacum
Field Signs: Skin Hemorrhages (tadpoles)

Field (gross) Signs: Skin Ulcers

A. tigrinum

R. catesbeiana
**Field (gross) Signs: Tadpole Skin Ulcers**

*R. catesbeiana, 22704*

*R. luteiventris, 20426*

**Field (gross) Signs: Skin Edema**

(due to excess fluid accumulation under skin in lymphatic sacs)

Bloated Bullfrog  Normal Bullfrog  Larval Bullfrog
Field (gross) Signs: Skin Edema
(due to excess fluid accumulation under skin in lymphatic sacs)

A. maculatum

A. macrodactylum

Liver:
Petechiae & Enlargement

Spleen:
Diffuse hemorrhage, enlargement or miliary necrosis

Body cavity:
Hydrocoelom

Stomach & Intestine:
Hemorrhage—petechial or diffuse

Mesonephroi: glomerular petechiae

Internal Gross Findings

Tiger salamanders (Ambystoma tigrinum)
Internal Findings

Bullfrogs (*Rana catesbeiana*) with palatal ulcer and white foci of necrosis in spleen

Histological Features of Ranavirus

**Organs affected:**
- Epidermis, dermis, blood vessels, liver, spleen, mesonephroi, stomach, intestine

**Occasionally affected organs:**
- Pancreas, lungs, gills

**Abnormalities:**
- Necrosis of endothelium, macrophages, lymphocytes, liver cells, pancreatic acini, GI tract epithelium, renal glomeruli, renal interstitial myeloid cells ("bone marrow")
- Hemorrhages: dermis, muscles, eyes, many visceral organs
**Histological Findings: Liver**

Early Changes: Necrosis of endothelium lining sinusoids; swelling of liver cells

Advanced Changes: Necrosis of liver cells and macrophages of PMAs

*Rana heckscheri*, larva, GA, 19709

**Histological Findings: Mesonephroi ("kidneys"):**

Necrosis of glomeruli and interstitial cells, rarely tubules

*Rana heckscheri*, larva, GA, 19709
**Histological Findings: Spleen**

- **Early Changes:** Multiple tiny foci of necrosis
- **Advanced Changes:** Necrosis is coalescing or diffuse
- **Viral inclusion bodies are rarely seen in spleen**

*Rana heckscheri*, larva, GA, 19709

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**Histological Findings: Epidermis (skin)**

- **Early Changes:** papules (swollen cells), necrosis of cells, spreading necrosis to form vesicles, ending with ulcers of skin

*Bufo boreas*, skin papule

*R. warszewitschii*, oral disc vesicles
Key Histological Feature:

Basophilic intra-cytoplasmic inclusion bodies (ICIB)

Most easily found in cells of:
- Epidermis (skin) and liver

Occasionally found in cells of:
- Pancreas, mesonephroi, stomach and intestine

Rarely found in cells of:
- Blood vessels, glomeruli, renal interstitium, spleen

Ranaviral ICIB in Skin

Bufo boreas, 19780-01, AK
Ranaviral ICIB in Liver

Rana heckscherii

Ambystoma tigrinum

Ranavirus: Ultrastructure

Ranavirus particles are icosohedral, c.160nm, and strictly cytoplasmic

Vesicles in toothrows

USGS
Ranaviruses In USA: Summary

1. Kills massive numbers of larvae; rarely affects adult amphibians
2. Skin hemorrhages, ulcers & bloating are key field (external) abnormalities
3. Diagnosis is made by
   - Culture / isolation of virus in lab, or
   - Histology of tissues
4. Key histological features are ICIB with widespread necrosis of liver, spleen, mesonephroi and blood vessels

END

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Ranavirus: Gross Findings
(Adult Treefrogs)

1. Sporadic outbreaks of epidermal ulceration (mostly in dorsal skin)
2. Low mortality (assumed)
3. May be reported in captive (zoo) frogs
4. Virus isolated from skin ulcers only (not viscera)
5. Somewhat resembles reported herpesviral infections in Europe

Field Signs: 22199-03 RaBla Nebr