Upper Respiratory Pathogens of Chelonians: A Snotty Turtle

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Pathogens
- Ranavirus
- Herpes
- Mycoplasma

<table>
<thead>
<tr>
<th>Genus</th>
<th>Host</th>
<th>Type Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iridovirus</td>
<td>Insects</td>
<td>Tipula iridescent virus</td>
</tr>
<tr>
<td>Chloriridovirus</td>
<td>Insects</td>
<td>Mosquito iridescent virus</td>
</tr>
<tr>
<td>Lymphocystivirus</td>
<td>Fish</td>
<td>Lymphocystivirus disease virus 1</td>
</tr>
<tr>
<td>Ranavirus</td>
<td>Fish, Amphibians, Reptiles</td>
<td>Frog Virus 3</td>
</tr>
<tr>
<td>Megalocystivirus</td>
<td>Fish</td>
<td>Infectious spleen and kidney necrosis virus</td>
</tr>
</tbody>
</table>
Ranavirus: Chelonian Significance

- Emerging disease in wild and captive chelonia around the world.
- Clinical signs include dyspnea, ocular, nasal and oral discharges, and death.
Prevalence

Numerous cases

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>Gopher tortoise</td>
<td>Westhouse et al.</td>
</tr>
<tr>
<td>North Carolina</td>
<td>Eastern box turtle</td>
<td>Johnson et al.</td>
</tr>
<tr>
<td>Tennessee</td>
<td>Eastern box turtle</td>
<td>DeVoe et al., Allender et al.</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Eastern box turtle</td>
<td>Allender et al.</td>
</tr>
<tr>
<td>Maryland</td>
<td>Eastern box turtle</td>
<td>USGS</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>Painted turtle</td>
<td>USGS</td>
</tr>
<tr>
<td>Kentucky</td>
<td>Eastern box turtle</td>
<td>Ruder et al.</td>
</tr>
<tr>
<td>Georgia</td>
<td>Burmese Star tortoise</td>
<td>Johnson et al.</td>
</tr>
<tr>
<td>New York</td>
<td>Eastern box turtle</td>
<td>Johnson et al.</td>
</tr>
<tr>
<td>Texas</td>
<td>Eastern box turtle</td>
<td>Johnson et al.</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>Eastern box turtle</td>
<td>Allender</td>
</tr>
<tr>
<td>Virginia</td>
<td>Eastern box turtle</td>
<td>Allender</td>
</tr>
<tr>
<td>Indiana</td>
<td>Eastern box turtle</td>
<td>Johnson et al.</td>
</tr>
<tr>
<td>Alabama</td>
<td>Eastern box turtle</td>
<td>Allender et al.</td>
</tr>
</tbody>
</table>

Ranavirus: Clinical Signs

- Present with sudden onset of severe illness or sudden death with no signs
- Rhinitis, conjunctivitis, oral plaques
- Death within hours to days of observation of clinical signs
- Variable response to supportive care
Ante-mortem Diagnostics

- Complete Blood count
  - Intracytoplasmic inclusions

Quantitative PCR

- TaqMan primer-probes were designed using Primer Express targeting a portion of the MCP that was contained within the 531 bp product
  - Forward: AACGCCGACCGAAAACTG
  - Reverse: GCTGCCAAGATGTCGGGTAA
  - Probe: CCGGCTTTCGGGC
- Resultant segment was a 54 bp product

Level of Detection:

- Conventional PCR: 529,000 viral copies
- Quantitative PCR: 52 viral copies
Ranavirus: Conservation

- Expanding range of Ranavirus in US
  - Human-induced spread documented in previous reports
- Proposed modes of transmission
  - Ingestion of infected material
  - Blood feeding parasites
  - Mosquitos evaluated as transmission source in Indiana
  - Mechanical transmission
  - Vertical transmission

Ranavirus: Conservation

- High mortality rate
  - >80% in transmission study in sliders
- Low prevalence rate reflects mortality rate
  - 0.3% in eastern box turtles in TN
  - 3.1% in rehab pops in SE US
  - 3.0% in Indiana
    - Occurred in ponds with anurans and salamanders

Free-ranging Box Turtles

<table>
<thead>
<tr>
<th>Variable</th>
<th>FV3 positive</th>
<th>FV3 negative</th>
<th>Prevalence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>3</td>
<td>168</td>
<td>1.8%</td>
<td>0.6 – 5.0%</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>218</td>
<td>0.00%</td>
<td>0 – 1.7%</td>
</tr>
<tr>
<td>Adult</td>
<td>2</td>
<td>398</td>
<td>0.5%</td>
<td>0.1 – 1.8%</td>
</tr>
<tr>
<td>Juvenile</td>
<td>2</td>
<td>55</td>
<td>3.5%</td>
<td>0.9 – 11.9%</td>
</tr>
</tbody>
</table>

Sex: p=0.157, observed power = 0.73
Age: p=0.081, observed power = 0.91
Outbreak

Kennekuk Cove

- July-August 2013
- 21 dead box turtles (92% mortality) from one park in Illinois
- At least three species of amphibians
  - Redback salamanders
  - Northern leopard frogs
  - Cricket frog
  - Unidentified remains
- High levels of FV3
  - Histopathologic evidence

Experimental Evidence

- Experimental trials – red-eared sliders
  - Groups exposed either 22°C or 28°C
  - n=8 for each trial
    - Computer randomization to make group assignments
    - Four uninfected controls in each group
    - Four turtles inoculated through IM injection
Experimental Evidence

Survival
- 22°C: all inoculated turtles were euthanized due to severity of signs
- 28°C: only 2 turtles were euthanized due to clinical signs
- One uninfected control died of sepsis

Median survival times
- 22°C = 24 days (14-30)
- 28°C = 30 days (17-30)

Results

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Parameter</th>
<th>22°C Viral Copies</th>
<th>28°C Viral Copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>Mean/median*</td>
<td>1.25 x 10^9</td>
<td>5.94 x 10^6</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>Mean/median*</td>
<td>3.7 x 10^6</td>
<td>3.64 x 10^6</td>
</tr>
<tr>
<td>Lung</td>
<td>Mean/median*</td>
<td>6.29 x 10^9</td>
<td>5.01 x 10^9</td>
</tr>
<tr>
<td>Heart</td>
<td>Mean/median*</td>
<td>2.82 x 10^8</td>
<td>1.27 x 10^9</td>
</tr>
<tr>
<td>Liver</td>
<td>Mean/median*</td>
<td>2.15 x 10^8</td>
<td>1.70 x 10^8</td>
</tr>
<tr>
<td>Spleen</td>
<td>Mean/median*</td>
<td>2.32 x 10^8</td>
<td>5.44 x 10^8</td>
</tr>
<tr>
<td>Ovary</td>
<td>Mean/median*</td>
<td>8.95 x 10^8</td>
<td>9.05 x 10^8</td>
</tr>
<tr>
<td>Kidney</td>
<td>Mean/median*</td>
<td>3.46 x 10^7</td>
<td>2.54 x 10^7</td>
</tr>
</tbody>
</table>

* Significant difference between environmental temperatures, p=0.012
^ Significant difference between environmental temperatures, p=0.011
Previously infected experimental infection

![Graph showing viral load over time in oral swab and whole blood samples.]

Disease Translocation

**Pathogen Host Switching in Commercial Trade with Management Recommendations**

![Image of disease distribution in invasive bullfrogs.]

**Ranavirus in Invasive Bullfrogs, Belgium**

- Ranavirus detection by PCR in cultured tadpoles (Rana catesbeiana Shaw, 1802) from South America
- Leopardus catesbeianus, Alfredo Perrone
- Alberto Montes
- Rolando Montes

**Frog virus 3 prevalence in tadpole populations inhabiting cattle-access and non-access wetlands in Tennessee, USA**

- Matthew J. Gray
- Beth L. Miller
- A. Chandler Schubert
- Charles A. Baldwin

Transmission

- Pond substrate
- Cannibalism
- Asymptomatic carriers
- High-density environments have shown to decrease mortality
  - May lead to more common infection
- Co-infection with myxosporidians
Transmission

- Brenes et al., 2014
  - Infected treefrog tadpoles can transmit to other tadpoles and turtles in water with no direct contact
  - Exposed fish did not become infected
  - Infected turtles and fish transmitted infection to amphibians
  - Exposed turtles and fish did not develop disease

- Goodman and Ararso, 2012
  - Evaluated concurrent chytrid and Ranavirus
  - Found no ranavirus in amphibians, but sympatric turtles were positive

Interclass transmission

- Softshell turtles – no mortality
  - 10-20% infection
- Mississippi map turtles – no mortality
  - 5% infection
- Catfish and mosquitofish experienced mortality when exposed to turtle isolates

Transmission

- Role of temperature well-established in development of clinical signs from iridoviruses
  - Epizootic Hematopoietic Necrosis virus in red perch
    - 11 day incubation at 19-21°C
    - No disease below 12°C
  - EHN in white sturgeon
    - Higher cumulative mortality and longer disease course at lower temperatures
    - Higher daily mortality and secondary infections at higher temperatures
  - Tiger salamanders with ATV
    - Survived infection at 26°C
    - All or most died at 18°C or 10°C
Anthropogenic Factors

- Exposure to cattle-access farms shown to increase infection rate by 3.9 times
- Distance to industrial activity, human habitation, and degree of human influence all significantly associated with increased infection
- Bait trade

Herpesvirus: Background

- dsDNA viruses
  - Alpha, beta, and gamma
- Variable host range
- Reported in numerous reptiles
  - Green iguana
  - Indian Cobra
  - Freshwater and sea turtles and tortoises
Herpesvirus: Background

- Freshwater turtles
  - Pacific Pond turtles
  - Painted turtle
  - False Map turtle
- All captive animals

Chelonian Herpesvirus

- Tortoises
  - Diphtheritic oral plaques
- Fresh water turtles
  - Hepatitis
- Eastern box turtles
  - THV1
  - THV2

Herpesvirus: Clinical signs

- Stomatitis
  - Inflammation of oral cavity
- Rhinitis
  - Inflammation of nasal passages
- Diphtheritic plaques
- Conjunctivitis
- CNS signs
  - Circling, head tilt
- Weight loss, cachexia, difficulty breathing
Results: Herpes Prevalence

- Edge habitat (35.1%) had a non-significantly higher prevalence than forest (28.4%) or field (26.1%)
- Females (37.8%) had a higher prevalence than males (27.9%)
- Adults and juveniles had no difference
- Higher mass in positive turtles
Herpesvirus Conservation

- Unknown impact on wild populations
- Careful consideration when releasing rehabilitation turtles
  - Nosocomial infections
- Increased surveillance is needed to characterize epidemiology

Mycoplasma agassizii

- Originally isolated from free-ranging desert tortoises (Gopherus agassizii)
- Reported in at least 19 different species of free-ranging and captive species
- Recent report of closely related Mycoplasma in box turtle from Virginia

Mycoplasma: Transmission

- Primary route is direct contact, mainly through nasal secretions
- Vertical transmission not documented in experimental study
  - May occur at low rate in other species
- Environmental transmission in wild is unlikely
- Fomite transmission is possible
Mycoplasma: Clinical Signs

- Rhinitis
- Conjunctivitis
- Nasal and ocular discharge
- Periocular edema
- Conjunctival hyperemia
Mycoplasma: Course of Disease

- 1. Primary colonization of URT
- 2. Host immune response to infection resulting in reduction in organisms, but clinical sign development
- 3. Progression to chronic state

Mycoplasma: Prognosis

- Acute mortality is rare
- Some clear infection
- Most develop chronic infections
  - Persist in nasal epithelium
  - Death due to severe debilitation

Mycoplasma: Conservation

- Once diagnoses, each animal should be assumed to be persistently infected and able to shed
- No guidelines for release of infected wild tortoises
Mycoplasma: Conservation

- Disposition of animals
  - Release into exact site of origin
  - Relocation to area with high prevalence
  - Admission to captive collections
  - Adoption as pets
  - Euthanasia

Mycoplasma: Future directions

- Effects of long-term exposure
  - Nearly catastrophic declines in California in mid 80’s may have been Mycoplasma
- Role as contributing factor in tortoise declines
- Influence of Mycoplasma on population dynamics, social structure, anthropogenic influences on disease

Conservation

- URT pathogens potentially under-diagnosed
  - Illness causes turtles to thermoregulate, in high traffic/suburban areas may include on pavement, increasing chance of trauma
  - Ranavirus-positive animals presented with HBC trauma and no evidence of upper respiratory signs
  - Not investigated by scientists routinely in field
Thank You!

- Matt Allender
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