Overview

- Introduction/Background
- Diagnosis of Ranavirus
- Prevalence of Ranavirus in eastern box turtles
- Pathogenesis/Transmission
- Therapeutics
Introduction/Background

>30 cases identified since 2003

<table>
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<tr>
<th>State</th>
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<tr>
<td>Florida</td>
<td>Gopher tortoise</td>
<td>Westhouse et al.</td>
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<td>Florida Box turtle</td>
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<td>North Carolina</td>
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<td>USGS, Mao?</td>
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<td>Johnson, pers. comm.</td>
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Geographic Range

Ranavirus

- High mortality rate in diagnosed chelonia
  - All clinical cases at UT died
  - >80% in transmission study in sliders
  - Prevalence rate reflects mortality rate

- Potentially under-diagnosed
  - Illness causes turtles to thermoregulate, in high traffic/suburban areas may include on pavement, increasing chance of trauma
  - 61 of 445 animals were diagnosed with a primary infectious disease that were presented with HBC trauma and no evidence of upper respiratory signs
    - Include all causes of infectious disease
Objective 2
Diagnosis

Clinical Signs

- Present with sudden onset of severe illness or sudden death with no signs
- Clinical signs - non-specific, similar to those of mycoplasma and herpesvirus infections
- Death within hours to days of observation of clinical signs
- Variable response to supportive care
Ante-mortem Diagnostic Tests

- Complete Blood Count
  - +/- anemia
  - Intracytoplasmic inclusions

- PCR
  - Specific
  - Whole blood or oral/cloacal swabs

- ELISA
  - plasma
Polymerase Chain Reaction

- Conventional PCR has been developed and utilized in chelonians
- Real-time PCR
  - More sensitive than conventional
    - Conventional PCR – 529,000 viral copies
    - SYBR Green qPCR – 5290 viral copies
    - TaqMan qPCR – 529 viral copies
  - Detect subclinical disease states
  - Quantify virus
**Indirect ELISA**

- Substrate
- Alkaline phosphatase
- Streptavidin
- Secondary biotinylated antibody (mouse anti-desert tortoise IgY Ab)
- Primary Antibody (tortoise plasma)
- Coating antigen (iridovirus)
- Color change

**Post-mortem Diagnostics**

- Necropsy
- Virus Isolation
- PCR
- Electron microscopy
Virus Isolation
Objective 3

Prevalence
Prevalence

- No data regarding prevalence in natural populations (amphibian or reptile) in the absence of an epidemic
- Temporal and spatial patterns outside these epidemics are unclear
- Tiger salamanders
  - Screened over 4 years with PCR
  - Commonly infected in absence of clinical signs
    - Contrasts experimental data

Rehabilitation Clinics

- Populations
  - Individuals presented to wildlife clinics in the southeastern US
    - University of Tennessee (3%; 0.2-19.6%)
    - North Carolina State University (3%; 0.2-16.2%)
    - Virginia Wildlife Health Center (0%; 0-11.1%)
    - Biased population toward sick and injured animals
  - Free-ranging population in east Tennessee
    - Unbiased population in same geographic region
Free-ranging survey

- Blanding’s (58) and painted turtles (47) in Illinois
  - 0% PCR prevalence
- Gopher tortoises
  - Evaluated plasma for presence of antibodies from 5 states
  - 932 animals
  - 1.6% positive rate
    - 0-3.1% per state

Objective 4
Pathogenesis/Transmission
Transmission

- Unknown in chelonians
  - Transmission study in red-eared sliders failed to produce clinical signs in orally-inoculated turtles
  - Koch’s postulates fulfilled in injected turtles

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
Materials and Methods

- Experimental trial
  - 3 treatment groups of 5 animals each
  - Each group will be exposed to one of 3 temperatures (16, 22, 31)
  - One uninfected control in each group
  - Turtles inoculated through IM injection
  - Daily observation of clinical signs
  - Oral/cloacal swab and blood collected twice weekly
  - Euthanized when clinical signs become severe

Objective 5
Therapeutics
Treatment

• Acyclovir
  • Anti-viral drug closely related to DNA
  • Often used in treating herpesvirus infections
  • Needs to be phosphorylated by a virus thymidine kinase enzyme
  • Inserted into DNA strand during replication and stops it
  • Iridovirus TK gene is more similar to herpesvirus TK gene than that of other large DNA viruses
  • In vitro studies show that at higher dosages, ranaviral activity is slightly inhibited

References

Questions?