Ranavirus in Chelonians of North America

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Overview

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

Objective 1

Introduction/Background

>30 cases identified since 2003

State	Species	Reference
Florida	Gopher tortoise Florida Box turtle	Westhouse et al. Johnson et al.
North Carolina	Eastern box turtle	DeVoe et al., Allender et al.
Tennessee	Eastern box turtle	Allender et al.
Pennsylvania	Eastern box turtle Snapping turtle	Johnson et al. USGS
Maryland	Eastern box turtle Tortoise	USGS, Mao? Mao?
Rhode Island	Painted turtle	USGS
Kentucky	Eastern box turtle	Ruder et al.
Georgia	Burmese Star tortoise	Johnson et al.
New York	Eastern Box turtle	Johnson et al.
Texas	Eastern box turtle	Johnson et al.
Massachusetts	Eastern box turtle	Allender
Virginia	Eastern box turtle	Johnson pers. comm.
Indiana	Eastern box turtle	Johnson pers. comm.

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

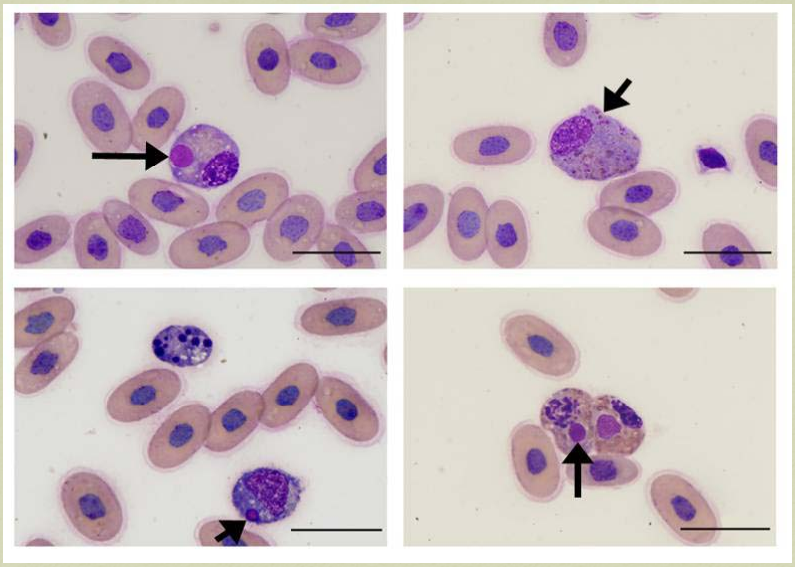
Diagnosis

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

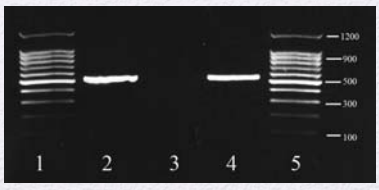
Ante-mortem Diagnostic Tests

- Complete Blood Count
  - +/- anemia
  - Intracytoplasmic inclusions
- PCR
  - Specific
  - Whole blood or oral/cloacal swabs
- ELISA
  - plasma

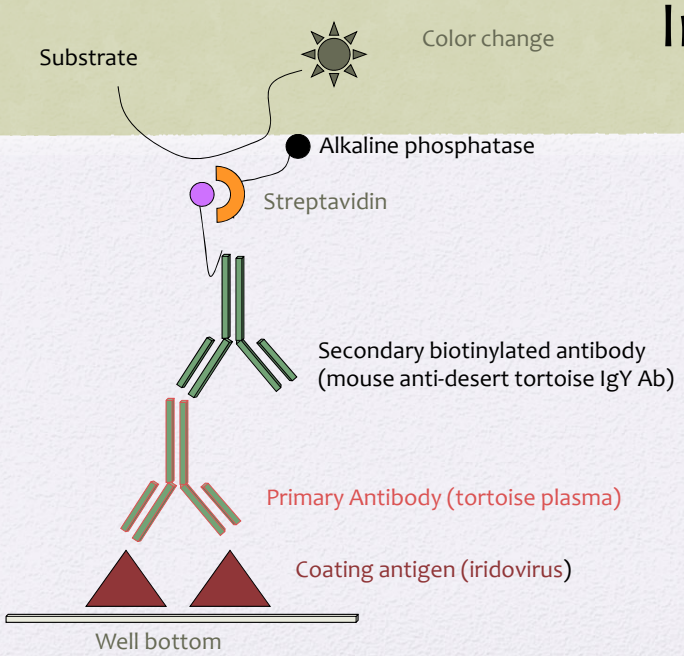


Polymerase Chain Reaction

- 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



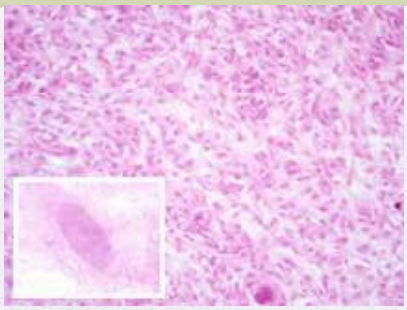
Indirect ELISA



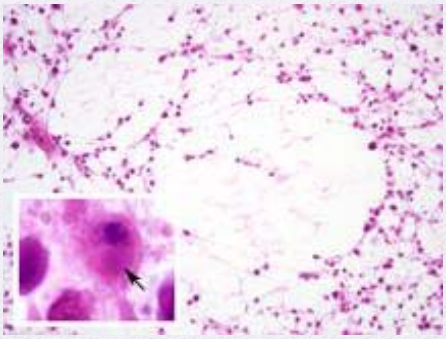
Post-mortem Diagnostics

- Necropsy
- Virus Isolation
- PCR
- Electron microscopy

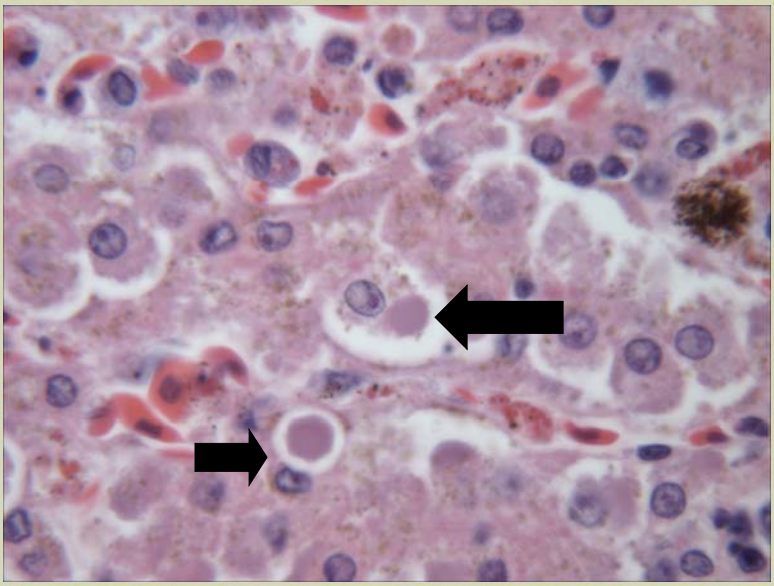
Virus Isolation

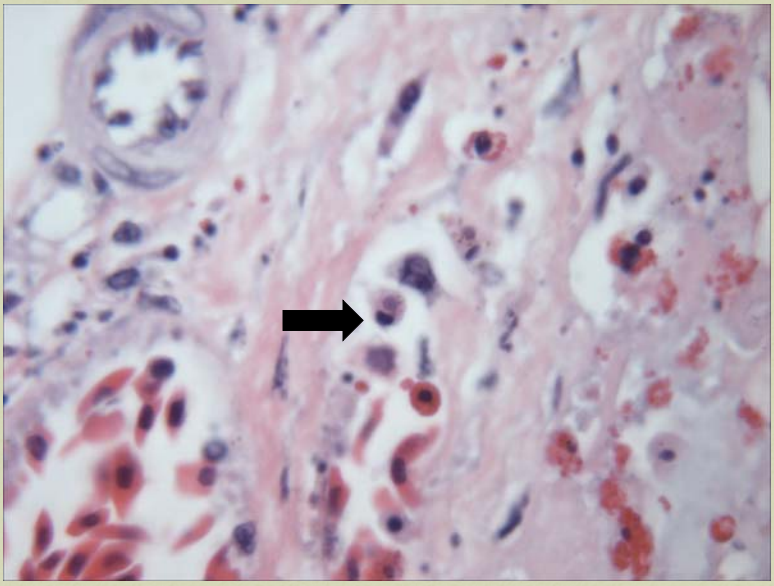


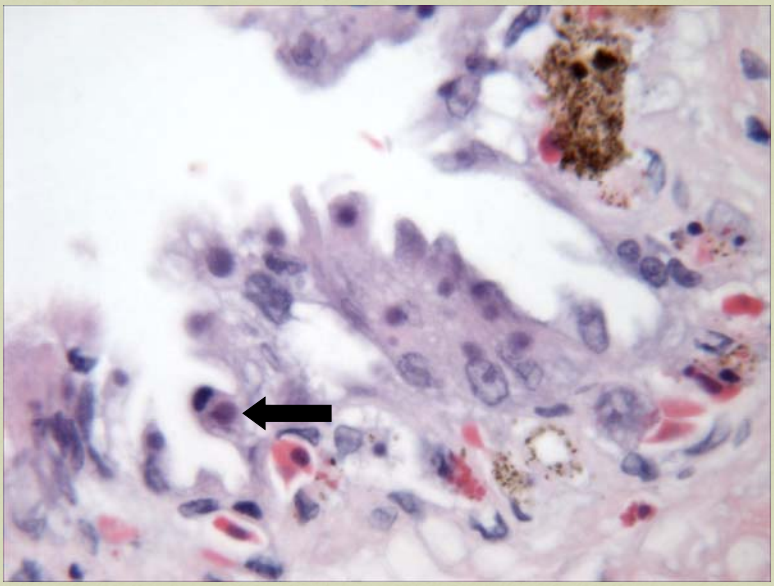
Control

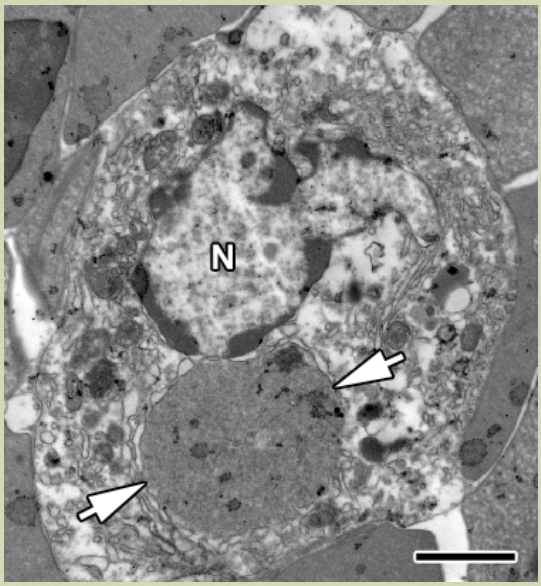


Infected





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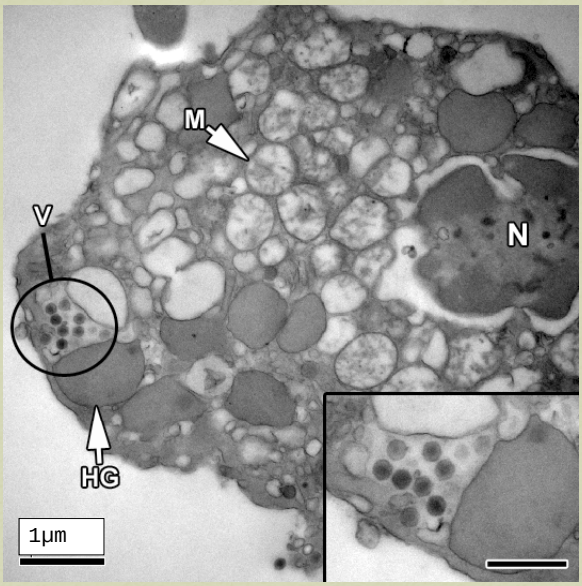

Objective 3

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 Hematopioetic Necrosis virus in red perch
    - 11 day incubation at 19-21C
    - No disease below 12C
  - 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 26C
    - All or most died at 18C or 10C

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

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Questions?