Upper Respiratory Pathogens of Chelonians: A Snotty Turtle

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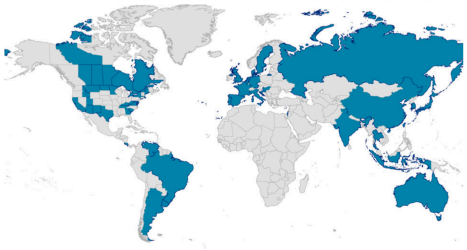
Pathogens

- Ranavirus
- Herpes
- Mycoplasma

General	Host	Type Species
Iridovirus	Insects	Tipula iridescent virus
Chloriridovirus	Insects	Mosquito iridescent virus
Lymphocystivirus	Fish	Lymphocystivirus disease virus 1
Ranavirus	Fish, Amphibians, Reptiles	Frog Virus 3
Megalocystivirus	Fish	Infectious spleen and kidney necrosis virus

Global Distribution of Ranaviruses

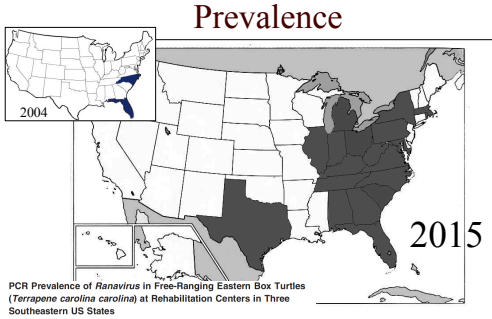


Note: This map is current as of July 2013. Since then there has been a report of ranavirus infection in Cameroon, Africa.

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



PCR Prevalence of *Ranavirus* in Free-Ranging Eastern Box Turtles (*Terrapene carolina carolina*) at Rehabilitation Centers in Three Southeastern US States

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Numerous cases

State	Species	Reference
Florida	Gopher tortoise	Westhouse et al.
	Florida Box turtle	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	Johnson et al.
	Snapping turtle	USGS
Maryland	Eastern box turtle	USGS, Mao?
	Tortoise	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	Allender et al.
Indiana	Eastern box turtle	Johnson pers. comm.
Alabama	Eastern box turtle	Allender et al.

Ranavirus: Clinical Signs

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

DIAGNOSIS

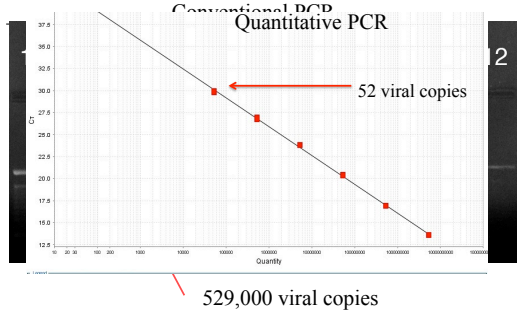
Ante-mortem Diagnostics

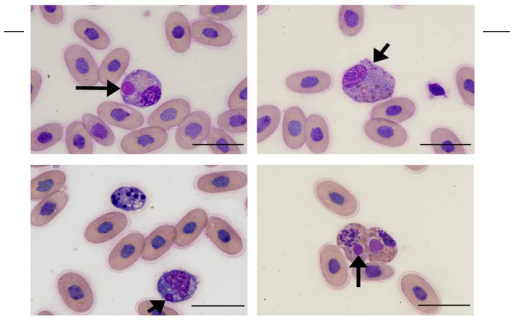
- Complete Blood count
  - Intracytoplasmic inclusions

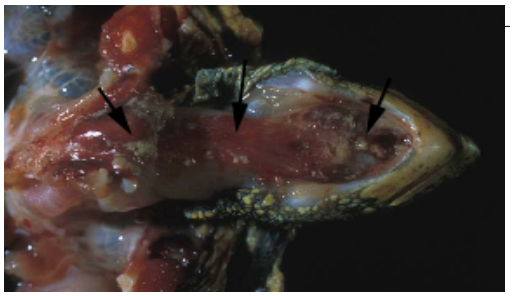
Quantitative PCR

- 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

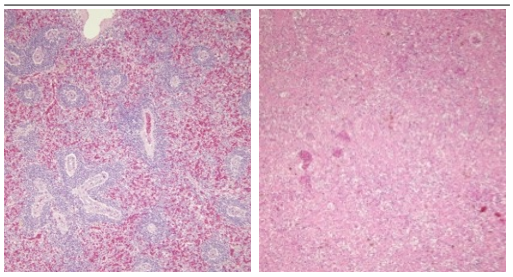
Level of Detection:







Results



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% - 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

Variable	FV3 positive	FV3 negative	Prevalence	95% CI
Female	3	168	1.8 %	0.6 – 5.0 %
Male	0	218	0.00%	0 – 1.7 %
Adult	2	398	0.5 %	0.1 – 1.8 %
Juvenile	2	55	3.5 %	0.9 – 11.9 %

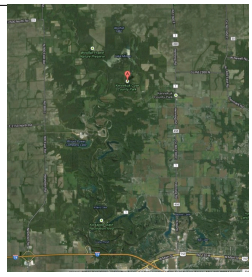
Sex: p=0.157, observed power = 0.73

Age: p=0.081, observed power = 0.91

Outbreak

Kennekuk Cove

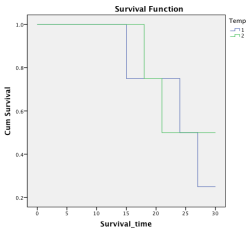
- July-August 2013
  - 21 dead box turtles (92% mortality) from one park in Illinois
  - At least three species of amphibians
    - Redback salamanders
    - Northern leopard frogs
    - Cricket frog
    - Unidentified remains
  - High levels of FV3
    - Histopathologic evidence



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



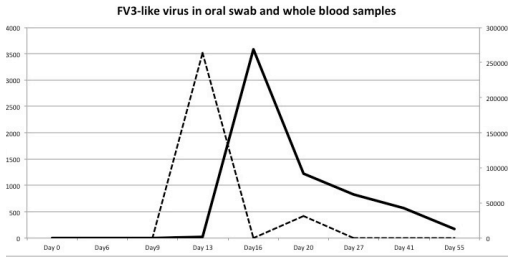
- Survival
  - 22°C – all inoculated turtles were euthanized due to severity of signs
  - 28°C – only 2 turtles were euthanized due to clinical signs
    - One uninfected control died of sepsis
- Median survival times
  - 22°C = 24 days (14 -30)
  - 28°C = 30 days (17-30)

Results

Tissue	Parameter	22C Viral Copies	28C Viral Copies
Tongue	Mean/median*	1.25 x 10^{9}*	5.94 x 10^{6}*
Skeletal Muscle	Mean/median*	3.7 x 10^{10}*	3.64 x 10^{8}*
Lung	Mean/median*	6.29 x 10^{9}*	5.01 x 10^{9}*
Heart#	Mean/median*	2.92 x 10^{10}	1.27 x 10^{9}*
Liver^	Mean/median*	2.15 x 10^{9}	1.70 x 10^{7}*
Spleen	Mean/median*	2.23 x 10^{10}*	5.44 x 10^{7}*
Ovary	Mean/median*	8.93 x 10^{9}*	9.06 x 10^{6}*
Kidney	Mean/median*	3.46 x 10^{10}*	2.54 x 10^{8}*

Significant difference between environmental temperatures, p=0.012
^ Significant difference between environmental temperatures, p=0.011

Previously infected experimental infection



Disease Translocation

Pathogen Host Switching in Commercial Trade with Management Recommendations

Angela M. Picco,[1,2] Abraham P. Karam,[1] and James P. Collins[1]

RESPONSES OF AMPHIBIANS TO RESTORATION OF A SOUTHERN APPALACHIAN WETLAND: PERTURBATIONS CONFOUND POST-RESTORATION ASSESSMENT

James W. Petranka[1], Susan S. Murray[2,3] and Caroline A. Kennedy[1]

Ranavirosis in Invasive Bullfrogs, Belgium

Ranavirus detection by PCR in cultured tadpoles (*Rana catesbeiana* Shaw, 1802) from South America

Leonardo Galli [a], Alfredo Pereira [b], Alejandro Márquez [b], Rolando Mazzoni [a,b,c,*]

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

Matthew J. Gray[1,*], Debra L. Miller[2], A. Chandler Schmutzer[1], Charles A. Baldwin[2]

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 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

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

	Number of infected turtles (number [Percentage of study population])	Survived outbreak (number [Percentage of study population])
Total study population	22	14
RV negative	3 (14)	3 (100)
HV & Myco co-infected*	2 (9)	2 (100)
RV positive (including all co-infections)	19 (86)	11 (58)
RV alone	4 (18)	2 (50)
RV & Myco	5 (23)	2 (40)
RV & HV	2 (9)	1 (50)
RV, HV & Myco	8 (36)	5 (63)

* RV, Ranavirus; HV, herpesvirus; Myco., Mycoplasma sp.

Herpesvirus: Background

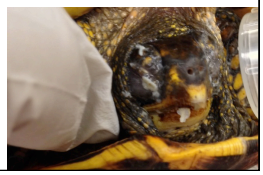
- dsDNA viruses
  - Alpha, beta, and gamma
- Variable host range
- Reported in numerous reptiles
  - Green iguana
  - Indian Cobra
  - Freshwater and sea turtles and tortoises

Herpesvirus: Background

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

Chelonian Herpesvirus

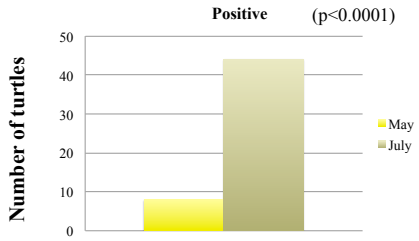
- Tortoises
  - Diphtheritic oral plaques
- Fresh water turtles
  - Hepatitis
- Eastern box turtles
  - THV1
  - THV2



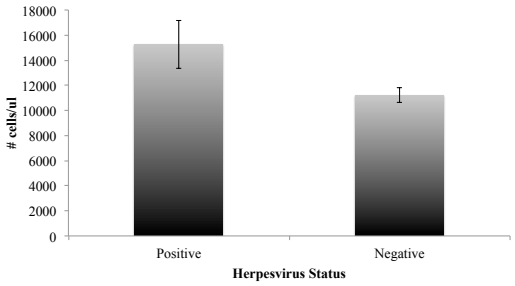
Herpesvirus: Clinical signs

- 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



Results: White Blood Cell Count



Results: Herpesvirus 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!

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