Isolation of Frog Virus 3 from Pallid Sturgeon (*Scaphirhynchus albus*) Suggests an Interclass Host Shift

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Topics Covered

• Pallid Sturgeon Conservation within the Missouri River Basin
  – History of the decline & subsequent restoration effort
  – Impediments to recovery
    * Iridoviral epizootics

• 2009 Blind Pony State Fish Hatchery Epizootic
  – Disease Characterization
    * Case history
    * Gross & microscopic lesions
    * Virus isolation & virion architecture
    * Genetic characterization
    * Pathogenicity trial

• Ranavirus Host Fidelity

• Significance & Future Directions
Decline of Pallid Sturgeon within the Missouri River Basin

• Damming of the MRB led to PS decline

• 1990 listed as federally endangered
  – Aging adult population estimated at 250 w/o detectable recruitment

• Breeding program in 6 hatcheries across 3 management zones
  – Upper basin
  – Middle basin
  – Lower basin
Pallid Sturgeon Restoration Effort

- Endangered adults are captured annually & transported to 1 of 6 hatcheries
- Adults spawned & then returned to the wild
- Progeny reared at 6 hatcheries for restocking 3 management zones

Blind Pony State Fish Hatchery (BPSFH)
Blind Pony State Fish Hatchery, Sweet Springs, MO

- Hatchery spawns & rears ~ 10,000 lake & 12,000 pallid sturgeon annually
- 12 raceways typically stocked at 2,000/raceway
- Raceways receive untreated H₂O by gravity from Blind Pony Lake Dam
Pallid Sturgeon Restoration Effort - Impediments

- Efforts complicated by nutritional deficiencies, water quality, & infectious etiologies
  - Significant iridovirus outbreaks since 1999…
- Family *Iridoviridae* characteristics
- Large dsDNA viruses infecting invertebrates & poikilothermic vertebrates
- Taxonomy
  - Genus *Iridovirus* (arthropod hosts)
  - Genus *Chloriridovirus* (dipteran host)
  - Genus *Lymphocystivirus* (fish hosts)
  - Genus *Megalocytivirus* (fish hosts)
  - Genus *Ranavirus* (fish, amphibian, and reptilian hosts)
  - Unassigned members
    * Pallid Sturgeon IV (PSIV), White Sturgeon IV (WSIV), Erythrocytic Necrosis Virus (ENV) et al…
Missouri River Sturgeon Iridovirus

1. Pallid (PSIV) [MRSIV]
2. Shovelnose

![Image of fish with measurement ruler]
2009 Blind Pony State Fish Hatchery Epizootic

- July – September 3/9 raceways rearing YOY PS experienced heavy losses

- Mortality in affected raceways was 80-100% over the 3 month epizootic

- Mortality obs. between 60 -78 °F with higher mortality at warmer temps (550 morts/d)

- Samples submitted to the Bozeman Fish Health Center & UCD Fish Health Lab
BPSFH PS Epizootic - Gross and Microscopic Lesions

- Dying fish displayed external and internal hemorrhagic lesions
- Histology revealed necrosis of hematopoietic (K, S) et al. internal tissues in contrast to…
  - MRSIV that typically does not generate significant internal lesions

- Renal tubular necrosis
- Cell debris within tubule
BPSFH PS Epizootic – Bacteriology, Cell Culture, SEM, PCR

- Mixed pop. of bact. cultured but mort. continued despite repeated antib. Tx
- Replicating agent observed in CHSE & sturgeon cell lines
- Negative staining SEM revealed icosahedral particles (~ 150 nm) in contrast to…
  - MRSIV that has never been isolated and possesses larger virions (~ 250 nm)
  - Samples were negative (-) against MRSIV specific PCR assay
PS Isolate Genetic Characterization

• Degenerate PCR assay targeting a portion of the viral DNA polymerase (Hanson et al. 2006)
  – 100% identical to Frog Virus 3 (680 bps, Tan et al. 2004)

• Amplification of the full length Major Capsid Protein gene
  – 100% identical to Frog Virus 3 (1392 bps, Tan et al. 2004)

• Conclusion: Over 2,000 bps from two separate loci suggests PS represent a new fish host for Frog Virus 3 (FV-3) = interclass host shift!

• PS ranavirus (PSRV) the cause of the mortality???
Virus Pathogenicity Study

- PSRV tested against YOY PS in flow-through 30 L tanks

60 Pallid Sturgeon (21 ± 3 cm)

- Control MEM-2-H

- High Titer
  1.3 x 10^5 TCID₅₀/ml

- Low Titer
  1.3 x 10^4 TCID₅₀/ml

ACCLIMATION
23°C for 14 days

EXPOSURE (bath)
Mortality recorded for 21 days

Morts processed for virus isolation, histology, and PCR
Virus Pathogenicity Study - Mortality

• 10% survival in low & high titer exposures vs. 100% in controls

• Qualitatively high titer treatment killed PS in less time
Virus Pathogenicity Study – Gross & Microscopic Lesions

- Reproduced previously observed necrosis of K, L, S, SB, Mes
Virus Pathogenicity – Cell Culture

- Virus recovered from 36/36 morts, 2/4 virus exposed survivors, 0/5 controls

- High viral titers ($3.1 \times 10^7 – 6.7 \times 10^8$ TCID$_{50}$/ml) recovered from individual pooled tissue homogenates

- Koch’s postulates fulfilled
2009 Blind Pony State Fish Hatchery Epizootic – Summary

• 2009 BPSFH PS epizootic attributed to a FV-3 like agent

• Induced gross & microscopic lesions, virion architecture, & in vitro characteristics typical of systemic IVs

• Virus isolated from hatchery PS in Sept, Oct, & Dec 2009 during & after active outbreak. PS lots destroyed.

• Oct 28 2009: 8 adult bullfrogs tested for ranavirus by PCR (all negative).

• Sept 07 2010: Given the untreated intake water 31 adult bullfrogs, 26 adult plains leopard frogs, & 4 plains leopard frog tadpoles from adjacent watersheds were cultured for virus (all negative).
Epizootic began in July with rising water temperatures
- All raceways experience 100% mortality
- Samples negative (-) for MRSIV
- Mixed bag of bact. cultured but mort. continued despite repeated antib. Tx
- External & internal hemorrhagic lesions
- Necrosis of haematopoietic tissues and mesentery
- Virus isolated on several cell lines
- Negative staining SEM revealed icosahedral particles (~ 160 nm)
- Comparison of the 2001/2009 full MCP seq. revealed they are identical!!!

PSRV continues to threaten conservation effort

What is the source of the virus???
- Sympatric amphibians contaminating hatchery intake H₂O in 01 & 09 (2x HS)
- Adult population infected & pass vertically to progeny during manual spawning
- Another hatchery reservoir?
# Host Range of Frog Virus 3 like Agents

<table>
<thead>
<tr>
<th>Frog Virus 3 like agents</th>
<th>Host Class</th>
<th>Host</th>
<th>Host common name</th>
<th>MCP % ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog Virus 3 (FV3)</td>
<td>Amphibia</td>
<td><em>Rana pipiens</em></td>
<td>Leopard frog</td>
<td>/</td>
</tr>
<tr>
<td>Redwood Park Virus (RPV)</td>
<td>Amphibia</td>
<td><em>Rana aurora</em></td>
<td>Northern red-legged frog</td>
<td>100</td>
</tr>
<tr>
<td>Tadpole Edema Virus (TEV)</td>
<td>Amphibia</td>
<td><em>Rana catesbeiana</em></td>
<td>Bullfrog</td>
<td>99.6</td>
</tr>
<tr>
<td>Rana temporiana United Kingdom Virus (RUK)</td>
<td>Amphibia</td>
<td><em>Rana temporaria</em></td>
<td>European common frog</td>
<td>99.4</td>
</tr>
<tr>
<td>Rana gyrlio virus (RGV)</td>
<td>Amphibia</td>
<td><em>Rana grylio</em></td>
<td>Pig frog</td>
<td>99.2</td>
</tr>
<tr>
<td>Rana utricularia virus</td>
<td>Amphibia</td>
<td><em>Rana utricularia</em></td>
<td>Southern leopard frog</td>
<td>100</td>
</tr>
<tr>
<td>Rana clamitans virus</td>
<td>Amphibia</td>
<td><em>Rana clamitans</em></td>
<td>Green frog</td>
<td>100</td>
</tr>
<tr>
<td>Bufo bufo United Kingdom Virus (BUK)</td>
<td>Amphibia</td>
<td><em>Bufo bufo</em></td>
<td>European toad</td>
<td>99.2</td>
</tr>
<tr>
<td>Bufo marinus Venezuelan Iridovirus 1</td>
<td>Amphibia</td>
<td><em>Bufo marinus</em></td>
<td>Cane toad</td>
<td>99.2</td>
</tr>
<tr>
<td>Desmognathus quadramaculatus Virus</td>
<td>Amphibia</td>
<td><em>D. quadramaculatus</em></td>
<td>Blackbelly salamander</td>
<td>99.1</td>
</tr>
<tr>
<td>Box turtle virus 3 (TV3)</td>
<td>Reptilia</td>
<td><em>Terrapene carolina</em></td>
<td>Eastern box turtle</td>
<td>100</td>
</tr>
<tr>
<td>Tortoise virus 5 (TV5)</td>
<td>Reptilia</td>
<td><em>Testudo horsfeldi</em></td>
<td>Russian tortoise</td>
<td>99.1</td>
</tr>
<tr>
<td>Gopher tortoise virus</td>
<td>Reptilia</td>
<td><em>Gopherus polyphemus</em></td>
<td>Gopher tortoise virus</td>
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</tr>
<tr>
<td>Burmese star tortoise virus</td>
<td>Reptilia</td>
<td><em>Geochelone platynota</em></td>
<td>Burmese star tortoise</td>
<td>100</td>
</tr>
<tr>
<td>Leopard tortoise Virus</td>
<td>Reptilia</td>
<td><em>Geochelone pardalis</em></td>
<td>Leopard tortoise</td>
<td>99.4</td>
</tr>
<tr>
<td>Softshell turtle iridovirus (STIV)</td>
<td>Reptilia</td>
<td><em>Trionyx sinensis</em></td>
<td>Chinese softshell turtle</td>
<td>99.7</td>
</tr>
<tr>
<td>Stickleback Virus (SBV)</td>
<td>Osteichthys</td>
<td><em>Gasterosteus aculeatus</em></td>
<td>Threespine stickleback</td>
<td>100</td>
</tr>
<tr>
<td>Pallid sturgeon Ranavirus (PSRV)</td>
<td>Osteichthys</td>
<td><em>Scaphirhynchus albus</em></td>
<td>Pallid sturgeon</td>
<td>100</td>
</tr>
</tbody>
</table>
Significance

• Ranaviruses are a global threat to both cultured & feral populations of poikilothermic vertebrates (fish, amphibians, & reptiles)

• RVs are especially concerning emerging pathogens given their high virulence & low host specificity (interclass host shifts/reservoirs)

• PSRV represents the first fully characterized example of a FV-3 isolate infecting a fish host

• These epizootics are especially concerning given the federally endangered status of pallid sturgeon
Future Directions

• Prevent future BPSFH PSRV epizootics
  – Install disinfection and chiller systems for incoming water
  – Sample a diversity of sympatric amphibians/life stages
  – Sample adult PS during manual spawning (reproductive products)

• Determine the pathogenicity of the PSRV isolates to other fish (sturgeon, LMB, CC, tilapia), amphibian, & reptilian spp.
  – PSRV exposed wood frog tadpoles succumbed within 7 days!
Future Directions

• Determine the pathogenicity of the PSRV isolates to reptilian spp.
  – Red-eared sliders up next…

• Functional phylogenomic analyses to compare PSRV to other RVs to determine genetic markers for pathogenicity & host fidelity
  – Sucrose pur. PSRV, WSIV, MRSIV, et al. submitted for 2nd Gen Seq
Future Directions

• Characterize other fish FV-3 like isolates
  – White sturgeon RV (1998 CA epizootic, closest relative Rana esculenta virus)
  – Et al…
Thanks for your attention!

Questions?

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