Amphibian ranavirus transmission and persistence

With an emphasis on ecological relevance

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Amphibian ranaviruses

1) Transmission
   - Routes of transmission — *How* does ranavirus get around?
   - Function form of transmission — How does transmission scale with density?

2) Persistence
   - Environmental persistence
   - Persistent infections
   - Biotic reservoirs

3) Future directions
Essentially every dose-response study with ranavirus

- **BIV** Cullen et al. 1995, Cullen & Owens 2002
- **ATV** Brunner et al. 2005
- **FV3** Pearman et al. 2004, Hoverman et al. 2010, Warne et al. 2011
- **RUK** Cunningham et al. 2007
- **LMBV** Grant et al. 2003

Small particles (filtered water) and chunky bits (filtrate) are both very infectious (Brunner et al. 2007)
Harp & Petranka (2006) added water (~2L) and pond substrate (~0.3kg) from ponds undergoing die-offs to kiddie pools with wood frog tadpoles

Routes of transmission: via water

Table 1. Results of polymerase chain reaction assays for ranavirus detection in surviving wood frog tadpoles from Experiment 3.

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>Sediment</th>
<th>Water</th>
<th>No. of samples (% positive)</th>
<th>Pools (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>Unexposed</td>
<td></td>
<td>11 (45%)</td>
<td>83%</td>
</tr>
<tr>
<td>Exposed</td>
<td>Exposed</td>
<td></td>
<td>9 (44%)</td>
<td>67%</td>
</tr>
<tr>
<td>Unexposed</td>
<td>Unexposed</td>
<td></td>
<td>6 (0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Unexposed</td>
<td>Exposed</td>
<td></td>
<td>6 (0%)</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Treatments reflect all combinations of exposed or unexposed sediment and water. Exposed samples were collected during an active ranavirus outbreak and unexposed samples from a pond without ranavirus. Data represent the number of samples analyzed, the percentage of samples that were positive (in parentheses), and the percentage of pools (n = 6 per treatment) that were positive for ranavirus. Each sample reflects a pooled analysis of five tadpoles, and from one to three samples (5–15 tadpoles) were analyzed per pool.*
**Routes of transmission: via water**

Are infectious levels of virus in water reached in nature?

- Measured ATV concentration in exudate, scrapes, and water (~500mL) a week after IP injection
- In a well-mixed, 10m diameter pond 1m deep, would need 157,000 sick larvae shedding to reach $10^2$ pfu/ml!

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**Graphs:**

- **Proportion Infected** vs. **Log$_{10}$(ATV) / ml**.
- **Log$_{10}$(ATV) / ml** vs. **Proportion Infected**.

**Graph Data:**

- Brunner et al. 2005
- Brunner, unpublished data
Routes of transmission: *direct contact*

- **ATV**: one second, belly-to-belly contact caused infection in 18/21 *Ambystoma tigrinum* larvae (Brunner et al. 2007)

- **BIV**: 5/8 *Limnodynastes terraereginae* metamorphs co-housed with IP-injected frogs were infected (Cullen et al. 1995)

*but not *L. caerulea* or *Cophixalus ornatus* adults (Cullen & Owen 2002)
Routes of transmission: *direct contact*

- Infected *A. tigrinum* larvae become more infectious through time.
- Carcasses are very infectious.

Brunner et al. 2007
Routes of transmission: consumption

- Bits & pieces (nipping, biting)
  - Fed tail clips form ATV-exposed larvae (Brunner et al. 2005)
  - Orally inoculated 3 anuran spp with FV3 (Hoverman et al. 2010)

- Cannibalism

- Necrophagy/scavenging
  - Tadpoles with access to FV3-infected carcasses get sick and die faster (Harp & Petranka 2006, Pearman et al. 2004)
Routes of transmission: *vertical*

- 60% of wood frog tadpoles raised from eggs in lab “weakly positive” for FV3-like virus (Greer et al. 2005)

- FV3-contaminated wood frog eggs: 4/5 field-collected & 1/3 laid in captivity (Duffus et al. 2008)
  - Only 1/59 tadpoles tested from these four clutches was positive by PCR

Contamination or true vertical transmission?

Vertical transmission is rare

unimportant for epidemic dynamics

potentially important for year-to-year persistence
The functional form of the transmission term

\[ \text{contacts} \times \left( \frac{I}{N} \right) \times P(\text{inf} \mid \text{contact}) \times S \]

Will I, a poor susceptible tadpole, become infected?
The functional form of the transmission term

\[ \text{contacts} \times \left( \frac{I}{N} \right) \times P(\text{inf} \mid \text{contact}) \times S \]

- Contact rate increases with density \( \beta IS \)
- Contact rate is constant (density-independent) \( \beta \left( \frac{I}{N} \right) S \)

- Disease fades out before host goes extinct
- Culling is an effective control measure
- Transmission continues as host goes extinct
- Culling will not control disease
Add infected and susceptible animals to pools
Wait 24h and see how many were infected
Fit transmission terms to data
Form of the transmission term

<table>
<thead>
<tr>
<th>Transmission model</th>
<th>Central pile</th>
<th>Dispersed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>ΔAICc</td>
</tr>
<tr>
<td>Constant $\beta S$</td>
<td>1</td>
<td>17.3</td>
</tr>
<tr>
<td>Density $\beta IS$</td>
<td>1</td>
<td>35.8</td>
</tr>
<tr>
<td>Frequency $\beta (I/N)S$</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Power $\beta I^q S$</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

Brunner et al. *In prep.*, see also Greer et al. 2008
Transmission summary

**Routes of transmission**

1) Most transmission occurs by “close contact”

2) Build up of virus in the environment, particularly substrate, may increase transmission

3) Cannibalism & Necrophagy/Scavenging are probably very important

**Form of transmission**

1) Frequency-dependent (over most host densities)

2) Dose-dependent transmission from the environment is like density-dependent transmission

3) Transmission via scavenging is an added term (keep track of carcasses) and should lead to accelerating epidemics
EHNV can survive ≥ 97d in distilled water & ≥113 d at 15°C in dried culture media (Langdon 1989, reviewed in Whittington et al. 2010)

**Persistence in the environment**

**Water**

- Pond water
- Autoclaved water

**Sediment**

Sediment collected during an epidemic infected wood frog tadpoles in 9/12 pools (Harp & Petranka 2006)

*But*… when ATV-spiked pond sediment was dried & rehydrated it was not infectious to salamander larvae (Brunner et al. 2007)

Brunner, unpublished data

Log<sub>10</sub> (ATV) / ml

Days after water spiked

0 5 10 15

0 1 2 3 4
Persistence in frozen carcasses

EHNV can persist in frozen fish for $\geq 2$ years (Langdon 1989)

ATV has been detected in frozen carcasses (D. Schock, pers. comm.)
Persistence in carrier state

- *Notophthalmus viridescens* developed persistent (≥81 days) infections with T6-T20 (FV3-like) (Clark et al. 1969)
- FV3 persists and replicates in peritoneal leukocytes for ≥12 days in *Xenopus laevis* (Robert et al. 2007)
- *Ambystoma tigrinum* larvae maintained persistent, transmissible ATV infections for ≥5 months (Brunner et al. 2004)
- Evidence of carrier state in EHNV infections is mixed, but likely in redfin perch (reviewed in Whittington et al. 2010)
Persistence in alternate hosts / biotic reservoirs

No shortage of potential hosts

- ATV infects a range of salamanders (Jancovich et al. 2001) as well as frogs (Schock et al. 2008)
- FV3 infects anurans and caudates, (Duffus et al. 2008, Schock et al. 2008), and apparently fish (Mao et al. 1999) and chelonians, too (Johnson et al. 2008)
- BIV infect frogs and fish (Moody & Owens 1994)

Interspecific transmission not assured or well understood

E.g., FV3 and ATV viruses co-circulating in wood frogs and tiger salamanders, respectively (Schock et al. 2008)
Persistence summary

1) Need to think about persistence at different time scales, with varying relevance for:
   - transmission within epidemics
   - persistence between epidemics (re-current epidemics)
   - movement between populations or regions

2) Multiple means of persistence between epidemics:
   - reservoirs
   - Carriers
   - frozen carcasses

3) Environmental persistence of ranavirus least well documented or understood


Future directions / research needs

1) Establish the *ecologically relevant* environmental limits of ranavirus
   - In pond water (with algae, bacteria, etc.) & substrates
   - On potential fomites & in or on potential carriers (e.g., birds)

2) Study persistent/chronic infections
   - Commonness, duration, and cause(s) of

3) Determine the relative importance of different routes of transmission
   - From environmental sources, carcasses, and live hosts
   - Between alternate host species in the community