

RNAVIRUS RESEARCH DIRECTIONS

Pathology, Immunology and Genetics

Gene Function and Molecular Characterization (Chinchar)

- Understand the role of viral genes in virulence
 - Immune evasion, host range
- Identify host and reservoir species
 - Is FV3 really a “frog virus” ? Is LMBV an isolate of DFV ?
- Understand what host, viral, and environmental factors trigger disease/persistence/recrudescence
 - FV1 and FV2 were isolated from “healthy” frogs; what makes LMBV pathogenic?
- Determine if susceptible species can be protected by vaccination with KO mutants?
- Does the genus *Ranavirus* consist of 6+ unique species, or are there fewer species but multiple isolates displaying various host preferences and degrees of pathology?
 - A Regulatory/Taxonomic issue?

Ranavirus Evolution and Phylogenetics (Jancovich)

- When, in evolutionary history, have the observed species jumps occurred?
 - genomic sequencing of ranavirus isolates
 - molecular clock of ranavirus evolution
 - comparative genomics
 - phylogenetics
- What molecular events facilitate species jumps?
 - host range/pathogenesis genes
 - changes in host biology

Ranavirus Immunology and Gene Function (Robert)

1. Characterize the complex interactions of RVs with larval and adult macrophages in *Xenopus*
 - a. Characterize permissive macrophages subsets using available and newly generated antibodies, follow them *in vivo* using fluorescent tracers
 - b. Determine role of macrophages as innate immune effectors and antigen presenting cells
2. Screen and identify viral genes involved in virulence and immune evasion by reverse genetics (knockout)
 - a. Investigate the role of these genes *in vitro* and *in vivo* on viral fitness (viral replication, etc)
 - b. Investigate the role of these genes on *Xenopus* host immune responses
3. Study antiviral T and B cell responses in *Xenopus* larvae and adults
 - a. Identify dominant antigens (proteins [Abs] and peptides [T cells])
 - b. Determine the respective role of classical and nonclassical MHC class I molecules in anti-viral cytotoxic T and NK cells responses

Ranavirus Hosts, Strain Variation, Serology and Pathology (Green)

1. Reservoirs or Carrier Animals
 - Host species?
 - Time (persistence) of carrier state after metamorphosis
 - Organ(s) or tissue(s) that shed infectious virus?
 - Do ranaviruses persist in any aquatic invertebrates?
2. Identification of ranaviruses by sequencing
 - How many strains? How many species?
 - Identification by sequencing should include fish & reptile viruses
 - Can geographic spread or genetic drift be determined?

3. Serology

Can sero-positive ranaviral survivors be detected?

If carriers exist, does their titer also persist? If so, how long?

4. Pathogenesis

What is route of infection? (oral, gills, skin, intestine?)

Where does virus first replicate (oral mucosa, skin, gut cells, blood cells)?

European Ranavirus Surveillance and Pathology (Balseiro)

- Study of the prevalence of Ranavirus disease in amphibians in different National Parks
- Search of reservoirs and risk factors
- Pathology and molecular characterization of possible isolated viruses

Ranavirus Pathology and Vaccine Development (Miller)

1. Expand our capabilities to identify (and visualize) the location of ranaviruses within tissue sections at various intervals post-infection.
2. Identify the cellular changes (e.g., cell signaling) that take place after infection by ranavirus.
3. Test the pathogenesis of 2 or more pathogens invading an individual and then a community.
4. Develop a vaccine that can be used in captive facilities and conservation programs.

Ecology and Conservation

Ranavirus Ecology and Declines (Gray)

- Long-term ranavirus surveillance at reoccurring die-off sites and with uncommon species
- Continued testing of amphibian species differences in susceptibility to various ranavirus isolates
- Role of community composition and super-spreaders on the likelihood of emergence
- Occurrence of interclass transmission among relevant ectothermic vertebrate species, and
- Environmental persistence of ranavirus virions.

Ranavirus Ecology (Hoverman)

- Immunological mechanisms underlying susceptibility
- Integration of ranavirus research with other amphibian disease systems
- Role of interspecific transmission within amphibian communities

Ranavirus Transmission, Genetics and Stressors (Lesbarreres)

- Transmission
 - Vertical vs. Horizontal
- Landscape genetics of an infection
 - Gene flow vs. Disease dynamics
- Metal-Induced Immunosuppression
 - Health-related trade-offs

Ranavirus and Pesticides (Kerby)

- How much impact does agricultural and/or urban pollutants have on Ranavirus dynamics?
- What is the mechanism causing increased Ranavirus susceptibility to pesticides?
- How important is land use change in understanding emerging infectious disease?

Chelonian Research (Allender)

- Investigate mortality events in chelonians
 - Determine cause and spatially map trends
- Determine prevalence across range of species
- Identify route of transmission in free-ranging setting
- Identify viral source/vector
- Identify early monitoring/diagnostic assays
 - Immunologic assays
- Determine appropriate therapy for endangered/at-risk populations
 - Vaccine
 - Anti-viral

Interclass Transmission (Waltzek)

- Ranaviruses are among the most pathogenic & promiscuous of viruses to infect poikilothermic vertebrates.
 - What are the genetic determinants of pathogenicity & host fidelity?
 - Given ranaviral low host specificity, is the OIE regulation to report only amphibian epizootics prudent?
- What factors most significantly influence the impact ranaviral diseases have on lower vertebrate populations?
 - Biotic factors: coinfection with Bd, pathogen pollution, low host genetic diversity, host density or life stage, resource limitation...
 - Abiotic factors: global warming, habitat destruction, pollution...
 - Pathogens that induce persistent infections are among the most difficult to detect/eradicate.
 - Discuss evidence for persistent ranaviral infections & strategies for detection/eradication

Ranavirus Surveillance in South America (Mazzoni)

- Surveillance on the presence of ranavirus in:
 - Introduced farmed American bullfrogs
 - Areas where American bullfrogs were introduced
 - Other areas
 - Compare isolates
- Characterization of South American ranaviruses
- Challenge studies in native aquatic organisms

European Ranavirus Surveillance and Ecology (Duffus)

- Increased disease surveillance with statistically valid sample sizes
- Better investigations into morbidity and mortality events that include biologists AND veterinary professionals
- We need to start performing statistically relevant experiments to:
 - Understand species and stage specificity of infection
 - Strain-species interactions
 - Understanding disease progression
- Testing multiple species and life history stages whenever possible

Ranavirus Epidemiology in Europe (Bang Jensen)

- Investigation into which factors determine host susceptibility to ranaviruses
 - Virulence factors
 - Transmission studies

- Epidemiologic investigations should be carried out when new outbreaks are detected, in order to identify sources
- Investigations into
 - Possibility for carrier status
 - Survival of virus in animals and commodities
 - Prevalence of ranaviruses in exporting and importing countries
- Best measures for control, including revision of current OIE (and EU) legislation according to new knowledge

Ranaviruses in Japan (Une)

1. Clarify:

- Distribution of ranaviruses in nature
- Source of ranavirus (native or exotic ?)
- Risk for native species.
- Evaluate pathogenicity of each ranavirus (RCV-JP, HNV, TFV) for native species
- Disease carriers (reservoirs)
- Pathogenesis of mass die-offs

2. Draw up:

- Measures to fight ranavirus in the field.

Ranaviruses in Canada and Additional Research Needs (Schock)

- At least some of the positives detected in surveys of wild amphibian populations should be sequenced.
- Guidance on which segments of the genome to use in surveys?
- Long-term studies that can speak to the role of ranaviruses in amphibian population stability, persistence, demographics
- Validated diagnostic tools necessary to conduct those long-term studies
- Ecological correlates with outbreaks / long-term effects
- Studies of wild amphibian populations (e.g., monitoring) that explicitly recognize the multi-host nature of ranaviruses
- Causes and consequences of intraspecific variation of hosts and viruses
- Sublethal effects of infection
- Interactions between FV3 strains (species?) within hosts and within populations
- Interactions between FV3 and ATV
 - within hosts? Populations? Amphibian communities?
- Interactions between ranaviruses and other pathogens (e.g. *Bd*, *Saprolegnia*)
- Interactions with other environmental stressors?

Conservation and Amphibian Trade (Picco)

- Are trade RVs more virulent than wild RVs?
- Are RV strains mixing in trade? Consequences?
- What is the likelihood that ranaviruses are transmitted from trade to amphibians in the wild?
- How is trade responsible for the spread of diseases into new areas?
- What effects do released pathogens have on native populations?
- How does pathogen pollution contribute to amphibian declines and extinctions around the world?
- What are the most effective approaches for curbing the spread of ranaviral disease into new areas?