

Meeting report

Ranavirus: past, present and future

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Emerging infectious diseases are a significant threat to global biodiversity. While historically overlooked, a group of iridoviruses in the genus *Ranavirus* has been responsible for die-offs in captive and wild amphibian, reptile and fish populations around the globe over the past two decades. In order to share contemporary information on ranaviruses and identify critical research directions, the First International Symposium on Ranaviruses was held in July 2011 in Minneapolis, MN, USA. Twenty-three scientists and veterinarians from nine countries examined the ecology and evolution of ranavirus–host interactions, potential reservoirs, transmission dynamics, as well as immunological and histopathological responses to infection. In addition, speakers discussed possible mechanisms for die-offs, and conservation strategies to control outbreaks.

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1. INTRODUCTION

Viruses have a long history of impact on wildlife and ranaviruses are no different. Ranaviruses are large icosahedral viruses with double-stranded DNA genomes that infect cold-blooded vertebrates, including fish, amphibians and reptiles. Over the past 20 years, several studies have been performed on ranaviruses, yet, the scientific community had not convened specifically to discuss the current state of knowledge on ranaviruses. Thus, 23 academic researchers and government scientists from nine countries came

together at the First International Symposium on Ranaviruses (held on 8 July 2011 during the Joint Meeting of Ichthyologists and Herpetologists in Minneapolis) to highlight recent advances and identify future research directions.

In the keynote address, Greg Chinchar (University of Mississippi Medical Centre) presented the history of ranavirus research. In 1965, the first ranaviruses were isolated from northern leopard frogs (*Lithobates pipiens*) by Allan Granoff while attempting to generate cell lines that would support growth of Lucké herpesvirus. Unexpectedly, the cultures displayed cytopathic effect, from which he isolated several viruses. One of them, obtained from a tumour-bearing frog, was designated *frog virus 3* (FV3) and became the type species of *Ranavirus*. Although the early work of Granoff and others demonstrated the power of using ranaviruses as a model for examining various aspects of virus replication, little consideration was given to ranaviruses as disease agents. This view changed by the late 1980s as a series of iridoviruses, later identified as ranaviruses, were associated with mortality events in ecologically and economically important fish, amphibians and later reptiles. Ranaviruses have now been identified in North and South America, Europe, Asia and Australia in aquaculture, zoo and wild populations. Given their wide host range and the movement of ranavirus-infected animals in commerce (e.g. American bullfrogs, *Lithobates catesbeianus*), ranaviruses have become an increasing threat to captive and wild populations, especially amphibians.

2. ECOLOGY AND EVOLUTION

Although ranaviruses have been identified as the causative agent of mortality events for several ectotherms across the globe, our understanding of the host and geographical ranges of ranaviruses is hindered by a lack of virus characterization. The FV3 designation is based almost exclusively on the major capsid protein (MCP) gene. However, most ranaviruses show greater than 90 per cent sequence identity within their MCPs. Although diagnostic tests based on the MCP gene are useful for detecting ranaviruses in a sample, partial sequences of the MCP gene alone are insufficient for differentiating among ranavirus types. Therefore, designations such as ‘FV3-like’ are often used but should be interpreted cautiously, particularly in the absence of any sequence data (James Jancovich, California State University).

(a) *Differential impacts on species*

Studies involving well-characterized isolates demonstrate that similar FV3-like strains can cause mortality events in hosts as genetically diverse and geographically separated as the European common frog (*Rana temporaria*) in the UK, wood frog (*Lithobates sylvaticus*) near the Arctic Circle in Canada, various species chelonians in Europe, Australia and USA, and even the pallid sturgeon (*Scaphirhynchus albus*) in MO, USA. (Amanda Duffus, Gordon College; Danna Schock, Keyano College; Rachel Marschang, Höhenheim University; Ellen Ariel, James Cook University; Thomas Waltzek, University of Florida). Another distinct type of ranavirus, *Ambystoma tigrinum* virus (ATV), has thus far been found only in tiger

salamanders (*Ambystoma mavortium*) in western North America. In addition, phylogenetic evidence shows that several testudine reptile ranaviruses are most closely related to amphibian ranaviruses, suggestive of repeated interclass host shifts (Rachel Marschang, University of Hohenheim), and there is phylogenomic evidence that ancestral ranaviruses infected fish hosts and subsequently jumped into amphibian and reptilian hosts (James Jancovich, California State University). Such urgency for a better characterization of ranavirus isolates, their geographical distributions in the wild, and their host ranges is underscored by the fact that ranaviruses can be translocated over large distances as a result of international commerce of their vertebrate hosts (Angela Picco, US Fish and Wildlife Service). The ramifications of possible ranavirus translocations as a result of aquaculture were mirrored in reports ranging from lethal ranaviruses in goldfish (*Carassius auratus*) in Thailand (Somkiat Kanchanakhan, Aquatic Animal Health Research Institute) and American bullfrogs in Japan (Yumi Une, Azabu University) and Brazil (Rolando Mazzoni, Universidade Federal de Goiás).

Experimental studies provide evidence that cross-class transmission is of critical importance in understanding spread. Within each class, hosts can be differentially impacted by infection. Among and within species, infection may range from no observable lesions or behavioural changes to severe lesions and rapid death (David Green, US Geological Survey; Anna Balseiro, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA); Debra Miller, University of Tennessee). In fact, Jason Hoverman (University of Colorado) tested the relative susceptibility of 19 North American amphibian species to two ranavirus isolates and suggested that more tolerant host species might serve as reservoirs; the most susceptible species he tested were the wood frog and gopher frog (*Lithobates capito*). Thus, species composition of communities may be a key factor in the dynamics of a disease outbreak.

(b) *Environmental dependency of disease dynamics*

Because ranaviruses infect ectothermic vertebrates, the surrounding environment should alter ranavirus–host interactions. David Lesbarrères (Laurentian University) described the role of potential abiotic and biotic mechanisms, such as temperature, larval developmental stages, density and competition for resources on the prevalence and virulence of the virus. Additionally, natural stressors (e.g. exposure to a predator) may interact with anthropogenic stressors (e.g. pesticides) to increase susceptibility to ranavirus (Jake Kerby, University of South Dakota). Finally, Andrew Storfer (Washington State University) demonstrated that anthropogenic movement of tiger salamanders have disrupted the natural co-evolutionary dynamics between this host species and endemic ATV strains leading to the evolution of viral virulence and disease emergence. Thus, to better understand the epidemiology of ranaviral diseases and forecast disease outbreaks, more investigation of host–pathogen genotypic interactions and their environmental dependence is required.

(c) *Transmission and persistence*

In many ways, ranaviruses are well suited to cause mass die-offs and even local extinctions. In addition to often being highly virulent, they are transmitted through several routes: water, brief direct contact and ingestion. Jesse Brunner (Washington State University) demonstrated that transmission among wood frog tadpoles is not density dependent but rather frequency dependent, presumably because tadpoles tend to aggregate. Pathogens transmitted in a frequency-dependent manner can, in theory, cause host extinctions, as Matt Gray (University of Tennessee) noted. However, this likely depends on site characteristics and the composition of the aquatic community. By contrast, transmission in chelonians is much less well understood as Matthew Allender (University of Illinois) explained how reared sliders were fairly resistant to ranavirus, and only became infected by intramuscular injection. Allender hypothesized that transmission between chelonians might require a vector, which would be a first for a ranavirus.

Ranavirus persistence in the environment remains poorly understood. It is increasingly clear that ranaviruses can infect a suite of hosts, and so reservoir species are probably common, and infections do, on occasion, develop into a sublethal, persistent and apparently infectious form. It is worth noting that all of these means of persisting could also contribute to the spread of ranaviruses across the landscape and between distant locations. Moreover, reservoir species, environmental persistence and frequency-dependent transmission all facilitate disease-induced extinctions (Matt Gray, University of Tennessee).

3. PATHOLOGY

The increasing prevalence, wide host range and shift capacity of ranaviruses suggest that they can rapidly adapt to hosts and overcome immune defenses. Thus, investigation of host immune responses is important for understanding the etiology of ranaviral diseases and finding ways to control them. The African clawed frog (*Xenopus laevis*) serves as an important experimental model to study amphibian host defenses against ranaviruses such as FV3 (Jacques Robert, University of Rochester). Adults have been shown to develop rapid innate and potent adaptive immune responses (including CD8 T cells and anti-FV3 antibodies) that clear FV3 within two to three weeks. By contrast, *X. laevis* tadpoles are unable to clear FV3 and most die within a few weeks after infection. Recent data suggest that in addition to the known weakness of larval antibody and T cell responses, larval susceptibility to FV3 can in part be due to an inefficient innate immune response. Another area of research opened using the *X. laevis* model is related to viral persistence (i.e. within the host) and the potential role of resistant (carrier) species in the dissemination of ranaviral infections. Convergent evidence suggests that, in *X. laevis*, some macrophages are permissive for FV3 infection and harbour quiescent virus. Subclinical infections found in various amphibian species are consistent with a possible transient quiescent phase of ranaviral infections, although the mechanisms involved and the

relevance of this phenomenon remain to be determined. Finally, the implementation of an improved method to generate FV3 knock-out mutants will provide a powerful way to identify viral genes involved in virulence and immune evasion and to develop an attenuated viral vaccine.

The pathology associated with ranaviral disease was also discussed during the symposium. Although sub-clinical ranavirus infection results in no obvious pathological changes, clinical cases may result in mass morbidity and mortality, behavioural changes and striking lesions. D. Earl Green (US Geological Survey) described the lesions observed in amphibians during morbidity and mortality events, and stressed that in the USA, larvae appear to be the life stage most affected, with erratic swimming, haemorrhaging and swellings most often reported. Ana Balseiro (SERIDA) pointed out that adults are often most affected by ranaviruses in Europe, with disease presenting as: (i) a chronic disease characterized by skin ulceration with no obvious internal gross lesions and (ii) a peracute disease characterized by systemic haemorrhages. Debra Miller (University of Tennessee) pointed out that haemorrhages, fluid accumulation and skin ulceration may also be noted in fish and reptiles, and necrosis of the oral cavity can be especially severe in chelonians. She also noted a common histological finding across classes (amphibians, reptiles and fish) is necrosis of endothelial cells that results in destruction of many organs (e.g. mesonephroi, liver, spleen and skin) and necrosis of haematopoietic tissue, both with severity varying by host species and ranavirus isolate. Intracytoplasmic inclusion bodies (discrete stainable aggregates composed of viral particles) are seen variably in multiple cell types but can be difficult to verify by light microscopy in areas of cellular necrosis.

4. FUTURE DIRECTIONS

As the symposium unfolded, urgent research needs became evident in two major areas: (i) pathology, immunology and phylogenetics of ranaviruses and (ii) ranavirus ecology and conservation. Eleven critical directions were identified. First, there is a need to understand how ranaviruses persist within a system, which includes three focus areas: environmental persistence outside of the host, identification of host reservoirs and latent persistence within hosts via covert infections. We also need a better understanding about host immune responses to ranavirus infection, and how responses may vary among host species and developmental stages. Previous studies have shown that some ranaviruses have greater pathogenicity than other isolates; however, information on what genes are contributing to enhanced virulence is needed. Genome sequencing and use of knock-down and knock-out techniques can provide insight into gene function. Other needs in molecular research include development of ranavirus vaccines for use in captive

populations and reintroduction projects. Pathologists that participated in the symposium emphasized the need for a better understanding of what tissues are initially targeted by ranaviruses, which could be accomplished through immunohistochemical staining, and whether the target tissues change over infection time and among host species. Ecologists emphasized the need to identify model species for virus challenge experiments so that results are more comparable among studies. The wood frog (highly susceptible species) and American bullfrog (low susceptible species) are good candidates for North American studies, because of their widespread distribution and the use of the latter in international trade. Additionally, to develop an overview of ranavirus pandemics for use in epidemiological research, there is an ongoing effort to map the distribution of ranaviruses on a global scale (led by Dede Olson, U.S. Forest Service, Amanda Duffus and colleagues from Imperial College), which will help to identify species and areas that have been especially hard hit by ranaviruses as well as inform the study of ranavirus epidemiology more generally. Participants also agreed that future directions must include widespread surveillance for ranavirus and investigations into cross-class transmission within community systems. We also need a better understanding of the role of American bullfrog farming and trade in the spread of ranaviruses, and whether captive facilities contribute to evolution of highly virulent strains. Given that ranaviral disease in amphibians has been listed as notifiable by the World Organization for Animal Health, testing amphibians that are traded internationally will become standard procedure.

Finally, to facilitate greater communication and collaboration, the Global Ranavirus Consortium was formed after the symposium with the following objectives: (i) increase communication among scientists, veterinarians and natural resource practitioners conducting ranavirus research or surveillance and (ii) foster collaborations among these groups. As a result, a second international symposium on ranaviruses will take place in 2013, and will be held concurrently with the International Conference of the Wildlife Disease Association. Resources from the 2011 symposium, including the presentation slides and videos, are available at <http://fwf.ag.utk.edu/mgray/ranavirus/2011Ranavirus.htm>.

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