

1st International Symposium on Ranaviruses

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The First International Symposium on Ranaviruses was held this summer at the annual Joint Meeting of Ichthyologists and Herpetologists (JMIH) in Minneapolis, MN. The full day symposium featured 23 speakers from nine countries to discuss the current status of knowledge among the world's experts regarding Ranavirus from a wide variety of perspectives.

Background

Ranaviruses are widespread pathogens known to infect ectothermic vertebrate species worldwide, and have been implicated as a cause of amphibian declines in many populations. The genus *Ranavirus* is one of five genera within the family *Iridoviridae*. Despite their name, ranaviruses infect not only amphibians, but also reptiles and fish. These double stranded DNA viruses are an emerging threat to cold-blooded vertebrates and are listed as a notifiable disease by the World Organization for Animal Health. Therefore, understanding their pathogenicity is of concern not only for herpetologists, but also for ichthyologists.

Despite the ability to detect ranaviruses via a number of methods (including PCR, cell culture and histology) and the relatively large amount of information known about ranavirus replication cycles, there is still relatively little known regarding the host-pathogen interactions of ranaviruses beyond the cellular level. With the current focus on chytridiomycosis, ranaviruses (and other amphibian pathogens) can easily be overlooked as a cause for mass die offs in wild populations. Ranaviruses can also be overlooked as a research priority because of perceptions that they may play only minor roles in host population ecology and persistence. This symposium sought to act as a summation of the current knowledge on the pathology, immunology, genetics, and ecology of ranaviruses. Risk assessment and conservation concerns were also discussed.

The symposium began with a historical talk from the keynote speaker, Dr. Greg Chinchar (University of Mississippi

Medical Center). Ranaviruses were first isolated in 1965 by Allan Granoff (St. Jude's Children Research Hospital) from leopard frogs. One of the early isolates was from a frog bearing a tumor and was designated Frog Virus 3 (FV3). This strain of ranavirus has served as a model for much of the early and current work regarding the biology, immunology and pathogenicity of this group of iridoviruses. In the 1980s, 'frog' viruses were identified as the cause of mortality in several fish and reptilian species indicating that this genus possessed a much larger host range than previously thought. In view of that, a greater focus was cast on understanding both the pathogenicity and method of spread among species.

Pathogenicity and Immunology

The virus infects cells directly, although interestingly the cells and tissues targeted seem to vary by both strain and host. Unfortunately, comparative pathology is only in its beginning stages, but there are contemporary methods (e.g., immunohistochemical staining) that can identify tissues targeted by ranaviruses. A diseased amphibian displays hemorrhaging, subcutaneous edema and erythema, and epidermal ulcerations. Drs. Debra Miller (University of Tennessee), D. Earl Green (U.S. Geological Survey), and Ana Balsiero (SERIDA, Spain) described the presence of intracytoplasmic inclusion bodies in organs such as the kidneys, liver, and spleen as a strong indicator of ranaviral infection. An interesting difference was noticed between affected frogs from SE Asia in the presentation of the gross lesions. Many of the large facial lesions shown in slides from frogs in SE Asia have never been recorded by researchers in North America. Studies in *Xenopus laevis* also suggest a potential role of macrophages in persistence of infection. In addition, PCR primers have been developed that amplify a conserved



sequence from the major capsid protein of the ranavirus genome. These primers can be used to determine the presence of a ranavirus in a sample but because it is such a conserved DNA sequence it is limited in its ability to determine anything regarding the type of ranavirus present.

Currently, there is no known cure or vaccine for ranavirus. Dr. Jacques Robert (University of Rochester Medical Center) has done extensive work examining the anti-viral immune defenses of amphibians using *X. laevis* as a model organism. Unfortunately, due to the limited availability of tools for typical immunological work in amphibians, this work has been challenging. The most exciting work from this is the recent generation of FV3 knock out mutants to better identify the genes involved. These discoveries will hopefully lead to the development of an attenuated viral vaccine that can be used in captive populations.

Distribution

Ranaviruses have been detected in nearly every area of the world. These pathogens have been detected on all continents, save Antarctica, and have been discovered in aquaculture facilities, zoos, and in wild populations. The symposium hosted presentations from Drs. Danna Schock (Keyano College, Canada), Amanda Duffus (Gordon College), Rolando Mazzoni (Universidade Federal de Goiás,

Brazil), Yumi Une (Azabu University, Japan), Somkiat Kanchanakhan (Aquatic Animal Health Research Institute, Thailand), Matt Allender (University of Illinois), Britt Bang Jensen (Norwegian Vet Institute, Norway) and Rachel Marschang (Hohenheim University, Germany) summarizing the state of ranavirus in several countries including: Australia, Brazil, Canada, Croatia, Denmark, Japan, Netherlands, Spain, Thailand, and the United Kingdom. While the mechanism of spread worldwide is still unclear, Dr. Angela Picco (U.S. Fish and Wildlife Service) discussed that the movement of ranavirus infected individuals both within the US and across the globe can and does occur via the pet, bait, and food trades. These routes are thought to be associated with outbreaks in the United States. Phylogenetic work by Dr. James Jancovich (California State University, San Marcos) has revealed that ranaviruses, originally fish pathogens, have jumped hosts several times to infect amphibians and reptiles. This reveals the adaptable nature of the pathogen and the importance of understanding its pathogenicity and methods for controlling it.

Mismatches in the local adaptation of host and pathogen can have severe impacts on a host's ability to fend off ranavirus disease. Dr. Andrew Storfer (Washington State University) reported that introduced ranavirus strains have been found in declining populations, and that there is some level of local adaptation occurring where some host populations are more resilient to more virulent strains of ranavirus. When these virulent strains are exposed to tiger salamander larvae from populations that co-exist with less virulent strains, there is a marked increase in salamander mortality. His lab is currently identifying the genes associated with this increase in virulence in the virus as well as associated genes in the salamanders. Dr. Jason Hoverman (University of Colorado) has shown differential effects of the same strain of ranavirus across 19 North American amphibian species, further validating that ranaviruses infect multiple hosts, and raising the possibility that community composition may impact the dynamics of a disease outbreak.

Ecology and Conservation

Dr. Matt Gray (University of Tennessee) discussed the threat of ranaviruses



Photo: Matt Niemiller

to wild populations, and presented an epidemiological explanation why ranaviruses can cause at least local extirpations of populations. He emphasized that the greatest threat of ranaviruses is to highly susceptible species that are uncommon (e.g., gopher frog) and coexist with species that function as ranavirus reservoirs. He emphasized the need for more extensive ranavirus surveillance and population monitoring, especially at reoccurring die-off sites.

Virions can be spread in a number of ways, but work by Dr. Jesse Brunner (Washington State University) suggests that ranaviruses most typically spread in aquatic environments especially in close contacts among individuals. Their transmission is frequency-dependent, which allows them to drive their host populations to extinction. Moreover, they can persist in chronically infected individuals, alternate host species, and to at least some degree in the environment, so they may be difficult to control and isolate.

An important need for wild populations is better monitoring, including better non-lethal diagnostic tests for use in the field. It is clear that these viruses are found worldwide, with some having dramatic impacts on populations and others simply persisting in low level infections. With the recent focus on chytrid fungus in frogs, there is a prime opportunity to monitor populations for the presence of

ranavirus as well. This is perhaps even more important given the potential for at least some ranaviruses to not only move among amphibian hosts, but to reptiles and fish as well. From the symposium, we surmise that at least 10 fish species, 40 amphibian species, and six reptile species can be infected by ranavirus. There is currently an effort underway to develop an online reporting system that can be used by researchers around the world.

A second need is for field-based studies to be sequencing ranaviruses detected during field surveys. Although most studies use the primers to amplify the highly conserved region of the genome previously mentioned, many don't take advantage of the important information that can be gleaned from that collected DNA. As a result, we are currently at very early stages of understanding the true diversity and distributions of ranaviruses in wild fish, amphibian and reptile populations.

Finally, there is a significant need to better understand the sub-lethal effects of infection. Some lab-based studies have demonstrated slowed growth rates and altered developmental rates in infected amphibian larvae. Both growth and developmental rates directly affect recruitment rates so understanding sub-lethal effects in wild populations will be key to understanding the role ranaviruses may play in host population dynamics.

Separate studies by myself and Dr. David Lesbarrères (Laurentian University, Canada) have demonstrated that the presence of both pesticide and metal pollutants can increase the susceptibilities of hosts to the pathogen. These findings emphasize the need to understand how anthropogenic stressors can significantly influence disease dynamics within a population.

Future Directions

At the close of the meeting, two roundtable discussions were held to identify the needs for future research at both the immunological and ecological levels. The organizers have established a website for the symposium that contains all of these future directions as well as copies of all the presentation files used in the symposium: <http://fwf.ag.utk.edu/mgray/ranavirus/2011Ranavirus.htm> Videos of these presentations are downloadable from

iTunes as well: <http://itunes.apple.com/us/itunes-u/2011-international-ranavirus/id452252707a>

Both websites are excellent resources for those wanting to know more about ranavirus.

Support

This effort would not have been possible without the significant sponsorship (over \$22,000) of several organizations: University of Tennessee Institute of Agriculture, Association of Reptilian and Amphibian Veterinarians, Australian Commonwealth Scientific and Industrial Research Organisation, Environment Canada, National Wildlife Research Centre, Morris Animal Foundation, Tennessee Wildlife Resources Agency, U.S. Forest Service, Pacific Northwest Research Station, American Society of Ichthyologists and Herpetologists, Missouri Department of Conservation, Partners in Amphibian

and Reptile Conservation, Tennessee Herpetological Society, USGS Amphibian Research and Monitoring Initiative, Global Ranavirus Consortium, and the UT Department of Forestry, Wildlife and Fisheries. There are plans underway for a second international symposium to be held in 2013 and continued support is needed. Information will be posted on the ranavirus website (above) as it becomes available.

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