2011 International Ranavirus Symposium

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Ranaviruses are large dsDNA viruses in the family *Iridoviridae*. They were originally detected in frogs (hence the name of the genus, *Ranavirus*) but are now known to infect and cause disease in fish and reptiles as well as in amphibians. Ranaviruses have been associated with numerous die-offs in amphibian populations and have also been increasingly found in fish and reptiles in recent years. Their role in amphibian declines and as emerging pathogens has led to increased awareness of the importance of these pathogens in conservation and ectothermic vertebrate medicine. Although more research is now being carried out on these viruses, many researchers have realized a need for better communication and contact among the scientific community, including herpetologists, virologists, and veterinarians, to help fully understand these viruses.

The First International Symposium on Ranaviruses was held on 8 July 2011 in Minneapolis, MN, in conjunction with the 2011 Joint Meeting of Ichthyologists and Herpetologists. In total, 23 scientists from nine countries presented the latest information on ranaviral disease, ranavirus biology, and host-pathogen interactions. The symposium was organized by Matthew Gray (University of Tennessee, Knoxville, TN) with help from Debra Miller (University of Tennessee), Jesse Brunner (Washington State University, Pullman, WA), Jason Hoverman (University of Colorado, Boulder, CO), and Andrew Storfer (Washington State University). An overview of the program, as well as future research directions and slides and videos of most presentations are available at the symposium web site (http://fwf.ag.utk.edu/mgray/ranavirus/2011Ranavirus.htm). Many organizations and agencies sponsored the symposium, including the Association of Reptilian and Amphibian Veterinarians.

The meeting started with a presentation by keynote speaker Dr. V. Gregory Chinchar (University of Mississippi Medical Center, Jackson, MS) on "Ranaviruses past, present and future," detailing the history of the isolation of the first known ranavirus from northern leopard frogs (*Lithobates pipiens*) in cell culture by Allan Granoff in 1965. That virus was named frog virus 3 (FV3) and became the type species of *Ranavirus*. Although ranaviruses were initially considered relatively harmless viruses, they are now known to cause die-offs among various classes of ectothermic vertebrates.

The role of ranaviruses in amphibian disease and die-offs was explored by several presenters. Matt Gray discussed the potential of ranaviruses causing population extirpations and species declines based on the ability of the pathogen to infect multiple host species with different susceptibilities, long environmental persistence of virions, and the clustering nature of many amphibian tadpoles and breeding adults. Jesse Brunner presented data on ranaviral persistence in the environment and in some host species, demonstrating that ranavirus infections can persist in some species of amphibians that survive infection for months. The global distribution of ranaviral disease was highlighted not only in Dr. Gray's presentation, but also in presentations by Ana Balseiro (SERIDA, Spain), Danna Schock (Keyano College, Fort McMurray, Alberta, Canada), Amanda Duffus (Gordon College, Barnesville, GA), Rolando Mazzoni (Universidade Federal de Goiás, Brazil), Yumi Une (Azubu University, Japan), and Somkiat Kanchanakhan (Aquatic Animal Health Research Institute, Bangkok, Thailand), each presenting data on outbreaks of ranaviral disease and die-offs of amphibians in North and South America, Europe, and Asia. These presentations also highlighted the large host range of these viruses among amphibians.

A number of presentations documented the ability of some ranaviruses to infect hosts from different classes. Thomas Waltzek (University of Florida, Gainesville, FL) reported on the isolation of a FV3-like ranavirus from pallid sturgeon, indicating a potential recent host switch from amphibians to fish. Rachel Marschang (University of Hohenheim, Stuttgart, Germany) reported that some ranaviruses isolated from reptiles in Europe are more closely related to amphibian ranaviruses than to other reptilian ranaviruses. Matt Allender (University of Illinois, Champaign-Urbana, IL) presented results from Ellen Ariel (James Cook University, Australia) that demonstrated an amphibian ranavirus causing disease in Australian reptile species. A study analyzing full genome sequences from a number of ranaviruses by James Jancovich (California State, San Marcos, CA) revealed rearrangements of ranavirus genomes and suggested that ancestral ranavirus was a fish virus, with subsequent host switches to amphibians and reptiles.

A number of presentations dealt with the pathological features associated with ranaviral infections. David Earl Green (U.S. Geological Survey, Madison, WI) presented the comparative pathology of ranavirus infections in wild amphibians from a large number of ranaviral disease outbreaks in the United States since 1996. Most of these cases involved larvae, with skin hemorrhages, ulcers, and bloating being key abnormalities observed in the field. Ana Balseiro documented two main ranaviral disease syndromes in amphibians in Europe—a chronic ulcerative skin syndrome and an acute hemorrhagic systemic syndrome. Both syndromes have been observed in tadpoles and adults in various European countries. Debra Miller presented comparative pathological data on ranavirus infections in amphibians, reptiles, and fish. She highlighted similarities in lesions observed among vertebrate classes, including endothelial cell and hematopoietic necrosis.

Immunology and ranaviral disease in amphibians was explored by a number of presentations. Immune evasion in ranaviral infection was one of the topics discussed by Greg Chinchar in his keynote address regarding the future of ranavirus research. Jacques Robert (University of Rochester, Rochester, NY) presented on the role of the innate immune system in susceptibility to FV3. Using *Xenopus laevis* as a model system, his team has been able to study the immune response to ranaviral infection in tadpoles of this species. This system will also allow a more in-depth study of the interactions between virus and host via manipulation of the host immune system. Dr. Robert's team also studied the role of macrophages in FV3 infection of *Xenopus*, finding that macrophages are involved in the anti-FV3 immune response and may harbor quiescent FV3 DNA. Their research also led to an improved method for the generation of FV3 knock-out mutants, an important step in understanding viral genes involved in virulence and immune evasion.

Interactions between the environment, stressors, and viral infection were explored by several presentations. Andrew Storfer discussed evidence supporting host-pathogen coevolution using the tiger salamander and *Ambystoma tigrinum* ranavirus (ATV) as an example. He emphasized that, when humans introduce infected animals into naïve systems (e.g., when salamanders are used as fish bait), coevolutionary patterns are disrupted. Jacob Kerby (University of South Dakota, Vermillion, SD) presented studies monitoring the interactive effects of pesticides, viral infection, and other stressors on mortality in salamanders. He found that sublethal amounts of pesticides significantly increased the mortality rate following infection with ATV. David Lesbarrères (Laurentian University, Ontario, Canada) explored the interactions between ranavirus infection in amphibians and various environmental and host factors including population density, life stage, and host genotype and virus genotype. He concluded that environmental factors, including host density and temperature, play a role in ranavirus infection associated mortality rates. He also noted that the timing of the infection may play a key role and that susceptibility may differ among amphibian life stages.

The dangers of international commercial trade of amphibians and fish infected with ranaviruses were explored by Britt Bang Jensen (Norwegian Veterinary Institute, Oslo, Norway) and Angela Picco (U.S. Fish and Wildlife Service, Sacramento, CA). Dr. Jensen presented some of the results and the risk assessment model for the EU RANA-project. Dr. Picco presented a study on ranavirus infections in amphibians and reptiles in the international trade (e.g., food, research, and pet trade), demonstrating that such animals have repeatedly been documented to be infected with ranaviruses and that all of these trade routes can be sources of new, potentially dangerous ranaviruses for fish, amphibians, and reptiles in wild and captive populations.

The need for international monitoring and cooperation in ranavirus detection, research, and control is clear. Ranavirus infections of amphibians are now a notifiable disease according to the OIE (http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.01.02_RANAVIRUS.pdf).

At the end of the symposium, roundtable discussions were held on ranavirus pathology, immunology, genetics, ecology, and conservation. These roundtables each identified a number of urgent research directions for future ranavirus research. For ranavirus pathology, immunology, and genetics, these included the need for studies that identify mechanisms of ranavirus persistence in the host (i.e., covert infections); better characterization of immune responses against ranaviruses among different host species and amphibian developmental stages; identification of tissues targeted by ranaviruses and whether these patterns differ among amphibian species; identification of virus reservoirs; examination of the historical occurrence of ranaviruses on each continent and approximate times of introduction or emergence; determining the role of bullfrogs and ranaculture facilities in the introduction of ranaviruses into naïve amphibian populations; and development of vaccines for the treatment of individuals in captive populations and repatriation projects. In the roundtable discussion on ecology and conservation, the following research directions were identified: further work on diagnostic testing, with a need to test the sensitivity and specificity of diagnostic tests and the reliability of tissue (tail, toe, organ type) versus swab samples; and the establishment of one or more model species for ranavirus experiments so that results from different experiments are more comparable. Two model species were suggested: wood frogs (*Lithobates sylvaticus*, a highly susceptible species) and the American bullfrog (*Lithobates catesbeianus*, a low susceptible species). The group also agreed that there is a strong need to sequence the entire genome of several isolates and identify regions that are important for differentiating ranavirus strains and species. Another aspect of this research is the identification of portions of the genome that consistently contribute to virulence, leading to the development of markers for identifying highly pathogenic strains.

Another important development from the symposium was the establishment of the Global Ranavirus Consortium (GRC, http://fwf.ag.utk.edu/mgray/ranavirus/Ranavirus.htm), which is composed of scientists from around the world who are working on ranaviruses. Members of the GRC can be found at the above web site, and new members are welcomed to join. The GRC also is soliciting endorsement by conservation organizations. Future activities include planning for the 2013 International Symposium on Ranaviruses and initiating regional discussion groups around the globe. Additionally, the Southeast Partners in Amphibian and Reptilian Conservation (SE PARC) with the assistance of national PARC is developing a web site for reporting occurrences of ranavirus infection and die-offs. Amanda Duffus and Dede Olson (U.S. Forest Service, Corvallis, OR) are chairing this endeavor. This reporting web site will be a resource for sharing surveillance data.

The symposium was considered a great success by all attending, contributed greatly to the understanding of ranaviruses worldwide, identified future research and conservation needs, and facilitated cooperation among scientists and veterinarians working on ranaviruses. It was agreed that it would be helpful for the international ranavirus symposium to be a regular event to facilitate further understanding in this field. The next ranavirus symposium is being planned for 2013 in conjunction with the International Conference of the Wildlife Disease Association in Knoxville, TN.