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### SECOND INTERNATIONAL SYMPOSIUM ON RANAVIRUSES: A NORTH AMERICAN HERPETOLOGICAL PERSPECTIVE

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Ranaviruses are large double stranded DNA viruses of poikilothermic vertebrates including amphibians, reptiles and fish. In North America, ranaviral disease and ranavirus-related die-off events have been documented in all three classes. Ranaviruses are found worldwide, appear to be emerging in some regions, and are increasingly recognized as a threat to many species.

In July 2013, the Second International Symposium on Ranaviruses was held in conjunction with the 62<sup>nd</sup> International Conference of the Wildlife Disease Association in Knoxville, Tennessee, USA. While the scope was international, a large number of the presentations and posters addressed North American species and issues: 15 of 27 research talks focused on ranavirus infections or disease in amphibians, reptiles and fish native to North America. The symposium was organized into five sessions with talks focusing on: Emergence and Conservation; Pathology and Physiology; Virology and Immunology; Diagnosis, Treatment and Management; and Ecology and Epidemiology. Each session started with an invited overview presentation and was followed by several research presentations.

On the first day of the Symposium, Dr. Matthew Gray, Director of the Global Ranavirus Consortium (University of Tennessee-Knoxville) welcomed the symposium participants. The keynote address was given by Prof. Richard Speare, Emeritus Professor, Anton Breinl Center for Public Health and Tropical Medicine, James Cook University and the Director of Tropical Health Solutions Pty Ltd.. Prof. Speare spoke about amphibian declines and the role of emerging infectious diseases. Specifically, he discussed the emergence of *Batrachochytrium dendrobatidis* (*Bd*) and ranaviruses, and how these two pathogens may act synergistically to cause population

declines. He emphasized the need to work in multidisciplinary research teams to fully understand the epidemiology of these pathogens, and suggested that ecologists should include both pathogens in pathogen surveillance studies.

Dr. Trenton Garner from the Institute of Zoology, Zoological Society of London, in an overview talk on Emergence and Conservation spoke about patterns of ranavirus emergence in amphibians and using those patterns to identify ranavirus-associated conservation threats. Dr. Julia Earl (University of Tennessee-Knoxville) discussed her research where, using stage-structured models, she has determined that if a ranavirus was introduced into populations of adult *Rana sevosa*, the Mississippi Gopher Frog, it could accelerate the speed at which this species would become extinct. Her models also demonstrated that survival of pre-metamorphic life stages is most important for *R. sevosa* sustainability. Scott Farnsworth (Washington State University) spoke about his work in central Maryland with Eastern Box Turtles (*Terrapene carolina carolina*). During a radio telemetry study to assess the successfulness of a relocation program, 26 of Scott's marked turtles died due to ranaviral disease. Moreover, turtle die-offs were associated amphibian die-offs from ranavirus in constructed wetlands. His work emphasizes the possibility of ranaviruses jumping between amphibians and reptiles, and that constructed wetlands may be hotspots for ranavirus infection. Dr. Kristine Smith (Eco-Health Alliance) discussed the potential role of the international trade contributing to the emergence of ranavirus. In a collaborative study with several investigators, Dr. Smith screened dozens of amphibians shipped from Hong Kong, Madagascar, and the Dominican Republic to the USA, and a large percentage from each country test-

ed positive for ranavirus. These results emphasize the importance of screening amphibians that arrive in the US for ranavirus infections according to the World Organization for Animal Health (OIE) recommend procedures. Ranaviruses that infect amphibians are considered a notifiable pathogen by the OIE. Other presentations included discussions of the distribution of amphibian ranaviruses in China, Costa Rica and Japan.

Dr. Debra Miller from the Center for Wildlife Health and the Department of Biomedical and Diagnostic Science at the University of Tennessee-Knoxville provided an overview of ranaviral disease pathology and physiology. Work that she performed shows unique and similar pathologies of ranaviral disease among amphibian, reptilian and fish species. Grossly, amphibians often display edema and systemic organ hemorrhages, while reptiles (especially chelonians) can have nasal discharges and severe necrosis in the oral cavity and esophagus, and fish may only display hemorrhages. Dr. James Wellehan (University of Florida) spoke about *frog virus 3* (FV3); the type virus of ranaviruses; infections in Eastern Box Turtles. He discussed how other pathogens (e.g., *Terrapene herpes virus 1*, *Mycoplasma* sp) have been found in ranavirus-infected animals. He hypothesized that these co-infections could affect the severity of ranaviral disease, and encouraged the group not to focus solely on one pathogen when screening for disease. Other presentations in this session discussed ranavirus infections and pathology in non-North American reptiles and the utility of the Wood Frog (*Lithobates sylvaticus*) as a model for studying ranaviral disease pathogenesis.

The overview presentation for Virology and Immunology was given by Dr. Jacques Robert from the University of Rochester Medical Center. He discussed how the immune system of the host and the ranavirus interact. While the immune system tries to control or limit the ranavirus infection, it may be promoting the evolution of viral persistence. He discussed the molecular biology associated with viral immune evasion as it pertains to ranavirus infection and how ranaviruses possess well-developed immune evasion mechanisms. Dr. Jennifer Hausmann from the Maryland Zoo discussed a mortality event caused by FV3 in their captive box turtle population that caused 48% of the population to die. To determine if the surviving turtles were immune to the ranavirus, an exposure experiment was conducted. Interestingly, 6 of 7 inoculated turtles survived despite all of them shedding ranavirus virions. One exposed turtle died, but the pathological indicators of disease were less severe than those documented in the original outbreak. Thus, turtles that survive infections appear to develop immunity against ranavirus. Other research presentations addressed the molecular biology of fish ranaviruses from Asia and the transcriptional response of immune-related genes in cells exposed to ranavirus in cell culture.

Dr. Allan Pessier, from the Amphibian Disease Laboratory at San Diego Zoo provided an overview of Diagnosis, Treatment and Management of ranavirus infections. He discussed various diagnostic methodologies including histology, ranavirus isolation, quantitative PCR (qPCR), traditional PCR, electron microscopy, and their limitations. He emphasized that detection of ranavirus DNA via PCR does not imply infection nor is it an indicator of disease. Ideally, histology should be coupled with information on viral load (via qPCR) and cell culture to make assessments on disease. Dr. Matthew Allender (University of Illinois) presented a new qPCR technique

that was used to survey over 700 Eastern Box Turtles. He noted that ranavirus infection prevalence was higher in animals that were brought into rehabilitation centers. He reported that effects of generalized antiviral therapy were variable. To date, a vaccine has not been developed for FV3-like ranaviruses. Dr. Richard Sim from the Wildlife Center of Virginia (formerly of the Maryland Zoo) discussed the ranavirus outbreak that affected the captive box turtle population in the Maryland Zoo. He discussed the pathological findings in these animals and the therapy that was provided to affected animals. Therapy included heat treatment, proper nutrition and fluids, as well as the administration of antibiotics and antiviral drugs. As the survival rate in this outbreak was higher than those reported by others, it is likely that the treatment provided to the affected animals increased their likelihood of survival. Other talks in this session examined disease surveillance after ranavirus outbreaks in the Netherlands, surveillance of ranavirus infections in and around ranaculture facilities in Brazil, and how risk analyses that include ranaviruses are important for conservation planning.

The final session covered the ecology and epidemiology of ranaviruses, with the overview provided by Dr. Matthew Gray. Dr. Gray discussed the mechanisms that are involved in ranavirus outbreaks, such as the susceptibility of individuals to the pathogen, the presence of reservoir hosts, sublethal infections, and environmental factors (e.g. pollutants, environmental disturbances). He also discussed the relationship between ranavirus pathogenicity, the genotype of the virus, and the signs of disease displayed in affected animals. Dr. Gray provided evidence based on simulations in his lab that ranaviruses could cause extirpation of local populations of common and uncommon amphibian species. Dr. David Lesbarres (Laurentian University) presented findings from an experiment that examined the effect of temperature (an environmental stressor) in Wood Frog (*L. sylvaticus*) and Northern Leopard Frog (*L. pipiens*) tadpoles exposed to FV3. The results of the study suggest that temperature is an important environmental factor that interacts with both the genotype of the host and virus to affect pathogenicity. In a study performed by Mabre Brand (University of Tennessee-Knoxville), she found that ranavirus was more pathogenic in 25°C water compared to 10°C water for three amphibian species, which might explain seasonal trends in outbreaks. Dr. Jesse Brunner (University of Washington) addressed the within-pond epidemiology of an amphibian ranavirus. Using computer models, he demonstrated that water-borne transmission of ranaviral infections may not be as important as direct contact of individuals, and suggested that habitat structure at a breeding site could affect contact rates. Dr. Danna Schock (Keyano College) presented results on the emergence of ranaviral disease in contaminated environments in Alberta and the Northwest Territories. She documented ranavirus infections in *L. sylvaticus* in locations where they share breeding sites with other species. She observed that recurrent mortality events in *L. sylvaticus* did not appear to have a negative impact on population size at some of the study sites, while at other sites recruitment and age structure of the population was affected. Dr. Roberto Brenes (University of Tennessee-Knoxville) discussed the results of a multispecies exposure experiment. He found that a highly virulent ranavirus strain was easily transmitted between Cope's Gray Treefrog (*Hyla chrysoscelis*) larvae, Pond Slider (*Trachemys script-*

ta) juveniles and Western Mosquitofish (*Gambusia affinis*). His results demonstrate that interclass transmission of ranavirus is possible. Dr. Robin Warne (Southern Illinois University) presented on how the stress response in individual *L. sylvaticus* tadpoles affected susceptibility to ranavirus infection. The other research presentation in this session examined ranavirus disease dynamics in the European Common Frog (*Rana temporaria*).

In addition to the large number of presentations on amphibians, there were talks that addressed ranaviruses in fish. One notable talk given by Dr. Tom Waltzek (University of Florida) addressed the repeated detection of FV3 during health surveys of fish from US aquaculture facilities. He noted that FV3 was the cause of mass mortality in farmed pallid sturgeon and also presented data that showed FV3 to have loose host specificity, being able to infect three different species of fish. This underscores the potential role that interclass transmission of FV3 and FV3-like viruses may have in naturally occurring ranaviral disease and ranavirus-associated mortality events within wetland communities. Other presentations addressed the molecular biology of the Singapore Grouper Iridovirus (SGIV), which is a pathogen in economically important fish species in Asia.

Videos and slides of most presentations are available at: <http://fwf.ag.utk.edu/mgray/ranavirus/2013SymposiumPresentations.htm>. There also was a poster session in the evening after the first day of presentations that included 35 posters from ten countries. Posters addressed experimental research, but also examined other initiatives such as the Global Ranavirus Reporting System, methods for identifying ranaviruses in tissues through immunohistochemistry and ranavirus genomics. Most of the posters can be found at the website above.

Each day, breakout discussions were held for each session where participants identified areas of future research and outreach activities that could be performed by the Global Ranavirus Consortium (GRC). The meet-

ing was closed by Dr. Matthew Gray who discussed the mission of the GRC and future initiatives, which included drafting bylaws and developing a new website. Dr. Greg Chinchar (University of Mississippi Medical Center) provided an excellent overview of the symposium at the 62<sup>nd</sup> International Conference of the Wildlife Disease Association, which followed the symposium.

There were two field trips that were focused specifically on North American herpetofauna. Dr. Matthew Allender led two expeditions to look for Eastern Box Turtles with turtle dogs and took participants through how Eastern Box Turtles have not only their health assessed, but how they are sampled non-invasively for ranavirus testing. Dr. Matthew Gray led two groups of scientists, veterinarians, and students into the Great Smoky Mountains where his research group has been doing ranavirus monitoring for nearly a decade. Participants caught, identified and sampled animals non-invasively for both ranavirus and *Bd*.

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In 2015, the Third International Symposium on Ranaviruses is planned for late April following the Florida Marine Mammal Conference in Gainesville, Florida. Information will be distributed via the GRC listserv (you can join the listserv at <http://listserv.utk.edu/cgi-bin/wa?A0=GRC>) and website (<http://fwf.ag.utk.edu/mgray/ranavirus/ranavirus.htm>). If you are interested in serving on the planning committee, please contact Dr. Thomas Waltzek ([tbwaltzek@ufl.edu](mailto:tbwaltzek@ufl.edu)) at the University of Florida.