

Report on the 4th International Symposium on Ranaviruses 2017

Jolianne M. Rijks¹, PhD, DECZM (Wildlife Population Health), Bernardo Saucedo¹, MSc, Jesse L. Brunner², PhD, Paul Hick³, BVSc (Hons), PhD, David Lesbarrères⁴, PhD, Amanda Duffus⁵, BScH, MSc, PhD, Lauren V. Ash⁶, MRes, Rachel E. Marschang⁷, PhD, Dr Med Vet, DECZM (Herpetology)

¹ Dutch Wildlife Health Centre (DWHC), Utrecht University, Yalelaan 1, 3584 CL Utrecht, the Netherlands

² School of Biological Sciences, Washington State University, 283 Eastlick Hall/PO Box 644236, Pullman, WA, 99164, USA

³ OIE Reference Laboratory for Epizootic Haematopoietic Necrosis Virus and Ranavirus Infection of Amphibians, Sydney School of Veterinary Science, The University of Sydney, 425 Wecombi Road Camden, 2570, Australia

⁴ Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury, P3E 2C6, Canada

⁵ Department of Biology and Physical Sciences, Gordon State College, 419 College Drive, Barnesville, GA, 30204, USA

⁶ Department of Biology, University of Vermont, Marsh Life Science Building, 109 Carrigan Drive, Burlington, VT, 05405, USA

⁷ Laboklin GmbH & Co. KG, Laboratory for Clinical Diagnostics, Steubenstrasse 4, 97688 Bad Kissingen, Germany

Key Words: *Iridoviridae*, taxonomy, *Frog virus 3*, amphibian, global ranavirus consortium

Introduction: Ranaviruses are large, double-stranded DNA viruses in the family *Iridoviridae*. They are important pathogens in fish, amphibians, and reptiles and have caused severe disease outbreaks in captive and wild animals. Ranaviruses have been associated with population declines in amphibians in various parts of the world. The Global Ranavirus Consortium ([GRC], www.ranavirus.org) was created to help address the threat posed by these viruses by coordinating research among scientists, disseminating information, and offering training opportunities. An international symposium on ranaviruses has been organized biannually since 2011 (Lesbarrères *et al.*, 2012).

From 7 to 10 June 2017, Budapest welcomed the 4th International Symposium on Ranaviruses (4th ISR). The symposium (56 participants, 12 countries) followed the 10th International Symposium on Viruses of Lower Vertebrates ([ISVLV], 94 participants, 20 countries), with the two symposia sharing sessions on 7 June. The ranavirus symposium was sponsored by the GRC; the Association of Reptilian and Amphibian Veterinarians (ARAV); the Veterinary Faculty of the University of Budapest; the U.S. Department of Agriculture (USDA); the National Science Foundation; Laboklin GmbH & Co. KG, Germany; and Biocenter Laboratory Hungary.

During the 4th ISR, 34 scientific presentations were held on ranaviruses (29) or *Iridoviridae* in general (5), and 16

ranavirus posters were presented (https://www.ranavirus.org/wp-content/uploads/2017/02/ISR2017_Budapest_AbtractBook.pdf). Other activities included an update on the progress made by the GRC, a panel discussion on iridovirus taxonomy, regional meetings and thematic breakout groups, and a workshop familiarizing participants with the Global Ranavirus Reporting System ([GRRS], <https://mantle.io/grrs>) (Brunner, 2017). After the scientific sessions, participants could attend field trips to visit local areas of reptilian and amphibian conservation efforts (Fig. 1).

The work presented by the authors will be published elsewhere. Here, based on the studies presented, we highlight some of the progress made since the previous symposium and summarize future gaps in knowledge that the GRC intends to address.

More fully sequenced ranavirus isolates have consequences for classification and beyond: The family *Iridoviridae* currently contains two subfamilies: the *Alphairidovirinae*, comprising three genera of viruses that infect ectothermic vertebrates, and the *Betairidovirinae*, including two genera of viruses that infect mostly invertebrates (Chinchar *et al.*, 2017a, 2017b). Viruses belonging to the genus *Ranavirus*, subfamily *Alphairidovirinae*, infect fish, amphibians, and reptiles and presently include seven member species: *Ambystoma tigrinum virus*, *Bohle iridovirus* (BIV), *Epizootic*



Figure 1. Hungarian meadow viper (*Vipera ursinii rakosiensis*). Photograph taken during the postsymposium trip to the Hungarian Meadow Viper Conservation Center near Budapest, Hungary. This facility is dedicated to protecting these endangered animals through habitat reconstruction and breeding. Little is currently known about the occurrence or effects of ranaviruses in wild squamate reptiles.

hematopoietic necrosis virus (EHNV), *European catfish virus* (ECV), *Frog virus 3* (FV3), *Santee-Cooper ranavirus*, and *Singapore grouper iridovirus* (Chinchar *et al.*, 2017a). This classification was made based on genomics, phylogenetics, phenetics, restriction fragment length polymorphism profiles, protein profiles, antigenicity, host and geographic ranges, pathology, and pathogenesis of these viruses (Waltzek, 2017), although we lack complete information on some viruses for each type of data. Updated species- and genera-level classifications will be generated by increased information, particularly complete genome sequences. One feature distinguishing the different iridovirid genera is gene order, a feature that is quite conserved within genera, with either complete colinearity or differences only in the inversion of a block of genes, or by the presence or absence of one or more genes (Chinchar *et al.*, 2017a).

Many more ranaviruses have been fully sequenced since the Third ISR, which prompted a well-organized discussion regarding *Ranavirus* genus classification during the panel discussion on taxonomy led by Tom Waltzek. One point was whether, within the *Ranavirus* genus, clades with unique genome arrangements corresponding to the inversion of a block of genes should be considered as different species, particularly when this inversion is paired with differences in host and geographic range, and in pathology. For example, all strains currently grouping with common midwife toad virus (CMTV), an important pathogen in wild amphibians in Europe, show inversion of part of the genome compared to FV3. It is expected that the commonly used addition “-like” to the names of strains will disappear with the creation of new species in this context. However, if gene colinearity is a major taxonomic feature, some viruses currently considered to belong to the

Ranavirus genus may no longer classify as such. Other examples challenging the idea of what defines a ranavirus species included the high similarity in genomic sequence and structure of BIV with FV3 despite the former being considered its own species. The similarity of ECV and EHNV was noted to be very high within 26 core iridovirus genes (Jancovich *et al.*, 2015). However, when considering the full genome, it was noted that 84.4% overall pairwise similarity highlighted considerable differences in the genome. The need to define the genomic features relevant for species demarcation was discussed. Here, virus species demarcation that considered the geographic restriction and natural host range, in addition to sequence information, supported maintaining the present classification as distinct species. It was noted in that particular case, listing of EHNV, but not ECV, by the OIE had established trade restrictions aimed at preventing commercial and ecological mishaps. When making taxonomic changes, it is important to realize that such taxonomic operations impact currently accepted preventive measures, including diagnostic criteria.

The taxonomic classification is a work in progress, subject to the new insights gained by studies. For example, at the symposium Papp and Marschang (2017) challenged the host range separation between the *Alphairidovirinae* and *Betairidovirinae* through their experiments testing the hypothesis that an invertebrate iridovirus could infect reptiles, whereas Subramaniam *et al.* (2017) provided evidence that squamate erythrocytic iridoviruses form a clade distinct from established iridoviral genera and suggested a distinct novel genus in the *Alphairidovirinae* subfamily, to be named *Hemocyttivirus*.

New findings emphasize the ranavirus threat resulting from human economic activities: Ranaviruses are considered a threat because they can infect multiple species of fish, reptiles, amphibians, or several of these, and may lead to high mortality rates. Local populations or even communities can be wiped out. This can have economic, ecological, or biodiversity consequences. As with other emerging diseases, intensive fish or amphibian farming systems and other trade activities with long-range translocations may facilitate the movement and introduction of ranaviruses. A recent example was provided by identification of ranaviruses in zoological collections that were extremely similar to BIV, which was initially isolated in Australia. Namely, ranaviruses infecting leaf-tailed gecko (*Uroplatus fimbriatus*) in Germany and boreal toads (*Anaxyrus boreas boreas*) in the United States shared an identical major capsid protein gene sequence that was >99.6% identical to BIV (Cheng *et al.*, 2014; Stöhr *et al.*, 2015; Hick *et al.*, 2016). Epizootic hematopoietic necrosis disease and ranavirus infections in amphibians are considered threats and are therefore reportable to the OIE, though reporting to OIE for these pathogens can still be substantially improved (Black *et al.*, 2017).

Retrospective genotyping of ranaviruses causing outbreaks with high mortality in 1998 and 2006 at a bullfrog (*Lithobates catesbeianus*) farming facility, performed by

Claytor *et al.* (2017), showed how topical the subject is. The first outbreak was caused by a CMTV-like virus, previously detected only in wild amphibians in Europe and captive amphibians in Asia, and the second outbreak was due to a chimeric virus, with CMTV-like gene fragments integrated into a wild-type FV3 backbone. The latter virus, likely a product of recombination enhanced by the trade and farming conditions, is highly virulent. Surveillance of wild amphibian populations in the area surrounding the open ranaculture facility is ongoing.

Another example of ranavirus introduction through an infected host, this time not through farming but the ornamental trade, was given by Saucedo *et al.* (2017a). Two strawberry poison frogs (*Oophaga pumilio*), imported from Nicaragua into the Netherlands via Germany, died of FV3 infection upon arrival. To the best of our current knowledge, FV3 has not been detected in the wild in the Netherlands. Had the imported specimens only had low-level infections, detection would have been difficult (Brunner *et al.*, 2017). In this context, the possible impact of poor biosecurity during population studies in the field and the danger of scientists spreading disease on contaminated gloves or by cohousing infected and uninfected individuals, even for short periods, was quantified by Gray *et al.* (2017).

Phylogeographic patterns remain difficult to grasp but are needed to refine risk analysis: Ranavirus threat is detected principally through mortality events, specifically those with characteristics of virgin soil epidemics. Such epidemics may be initiated by introductions, either the introduction of a novel ranavirus into a susceptible host population, or the introduction of a novel host species into the “host and geographic range” of a given ranavirus. Such introductions are often human mediated through farming and other trade activities. However, epidemics displaying such characteristics can also occur within existing ecosystems, as part of the host pathogen evolution process, without direct relation to human activity. Such epidemics may occur, for example, at range limits where endemicity is not continuously maintained (Bienentreu *et al.*, 2017), or following mutations of the local ranavirus that increase its virulence, transmission, or persistence. Drivers, impact, and preventive options will differ according to context; therefore, it is important to gain a better understanding of the ranaviruses currently associated with different ecosystems.

On this note, Price *et al.* (2017) and Garner *et al.* (2017) presented data on the phylogeographic patterns among European ranaviruses, with a particular focus on the history of FV3 in the U.K. Phylogenetic analysis of fully sequenced ranaviruses gave an indication of divergence time, but interpretation was difficult and insights about the spatial origins of lineages or patterns of broad-scale emergence remained speculative, showing how challenging this enterprise is. Therefore, long-term surveillance activities with the help of citizens and international cooperation remain important pillars for such studies, as ranaviruses

probably surfaced at the time of the last ice age and have evolved along with their amphibian hosts ever since.

The utility of environmental DNA (eDNA) techniques as a quick and efficient tool for detection and characterization of ranaviruses in ecosystems was also discussed. Studies presented by Hall *et al.* (2017) on FV3 in wood frogs in the United States and by Saucedo *et al.* (2017b) on CMTV-like ranaviruses in amphibians in the Netherlands showed the increasing feasibility for detecting ranavirus infection in amphibian communities not exhibiting high mortality by surveillance for the pathogen in amphibians and water (eDNA) throughout the season. An eDNA approach to ranavirus surveillance is also probably an efficient and cost-effective screening method in a variety of captive settings (Brunner *et al.*, 2017), and one of the outcomes of the ranavirus symposium was to share eDNA protocols. Using eDNA techniques to identify the ranaviruses currently associated with open water ecosystems may be more challenging than in defined microcosms, particularly if water bodies are large or connected, thus diluting or mixing eDNA from various sources.

Ranavirus infection affects amphibian population demography: A study in the U.K. investigated five ranavirus-positive and five ranavirus-negative populations and found that ranavirus-positive populations contained a higher proportion of younger individuals than the negative populations, indicating a shift in age structure (Campbell *et al.*, 2017). There was no effect on growth rates or age of sexual maturity of individuals. As in previous meetings, there was broad agreement that we need a greater understanding of the impacts of ranaviruses on wild populations and communities in other taxa (e.g., reptiles) and other locations.

Advances in detection of virulence determinants and understanding of host immunity: A thorough review of host immunology and ranavirus infection was provided by Robert (2017), with special emphasis on T-cell-mediated immunity in *Xenopus* tadpoles. The poor response to pathogens in larval stages correlates with a great immunological disadvantage in comparison to adults, for example, the inefficiency of an antibody switch from immunoglobulin (Ig)M to the more antigen-specific IgY, and a lack of natural killer cell populations and major histocompatibility complex I class expression. The role of CD8 T-cells as memory cells when battling viral infection, as well as that of macrophage-derived antiviral cytokines such as tumor necrosis factor- α , interleukin 1, and interferons, was also discussed to be slightly delayed in tadpoles (Robert, 2017; Robert *et al.*, 2017). The group also addressed the role of distinct macrophage phenotypes in response to infection (Yaparla and Grayfer, 2017), and their ability to act as carriers of the virus in a quiescent state, by isolating and labelling of peritoneal leukocytes. The group led by Qiwei Qin discussed the discovery of VP51 protein in Singapore grouper iridovirus, which is a homolog of tumor necrosis factor receptor and can modulate the immune response of

the host during viral infection by enhancing cell proliferation and inhibiting virus-induced apoptosis (Qin *et al.*, 2017).

Advances in the study of ranavirus pathogenesis: Several presenters discussed the need to use different species of ectothermic vertebrates as models to understand the pathogenesis of ranavirus infection, because the outcome of disease has been shown to differ not only in relation to virulence properties inherent to the pathogen but also in relation to host species. For example, several Australian PhD students from Ellen Ariel's group discussed the pathogenesis of ranavirus in chelonians and lizards, and the group from the Gray and Miller lab addressed the pathogenesis and immunohistochemical patterns of ranaviruses in endangered eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*). Maria Forzán presented RNA-scope *in situ* hybridization as a diagnostic tool that allows for ranaviral DNA labeling on paraffin-embedded tissues (Forzán and Sloma, 2017; Maclaine *et al.*, 2017; Miller *et al.*, 2017; Wirth *et al.*, 2017).

Another novel aspect of ranavirus pathogenicity discussed by Robert *et al.* (2017) was the discovery that ranavirus can pass the blood–brain barrier in *Xenopus laevis* tadpoles, which was studied by means of immunofluorescent labeling of the virus in infected macrophages.

Knowledge gaps identified by the thematic breakout groups:

As in previous years, the symposium included time for breakout discussion groups for important ranavirus-related issues. The topic of the first discussion group was techniques for identification and classification of isolates, which was moderated by Paul Hick. This topic has been controversially discussed among scientists and will need to be addressed proactively in response to developments in taxonomy of these viruses. These issues were considered together with diagnosis, treatment, and management.

For diagnostic testing, it was agreed that continued work and sharing of data are necessary on the development and validation of assays. The ability of various assays to detect and differentiate a range of ranaviruses and the development and availability of pan-ranavirus assays was discussed. The requirements for defining the characteristics of each test, use of quality control samples, and minimum essential information necessary to define a positive result were identified. A priority remains the need to determine the dynamic range of the tests when applied to subclinical infection and a broad range of host species, tissue specimen types, and even environmental samples. Furthermore, it was unclear how to categorize ranaviruses identified by polymerase chain reaction without partial or possibly full genome sequencing. An action point strongly supported by the group was the organization of a ring test for research and diagnostic laboratories. The GRC was also encouraged to develop white papers or position statements on minimum diagnostic standards, guidelines for diagnostic test interpretation, and biosecurity guidelines to the applicable target groups.

This discussion group also addressed disease management and therapy. Vaccines were considered a research priority, and further studies on basic immunology of amphibians, fishes, and reptiles was identified as a necessity. Another important aspect is a better understanding of pathogenesis of ranaviral disease, as it relates to methods for disease control, such as modification of environmental temperature. Additional suggestions for future development of the GRC included the facilitation or support of specific research priorities. This could include efforts such as reviewing papers, publishing research priorities, and exploring and supporting fundraising efforts.

A separate group, moderated by David Lesbarrères, discussed epidemiology and surveillance. On surveillance, the group reiterated the need to continue work on eDNA and the possibility of screening eDNA collected for other purposes to increase the spatial and temporal breadth of surveillance data available. The need to validate epidemiological models was also discussed. For instance, are we certain of the relative importance of different routes of transmission (e.g., via water or vectors)? Are we confident that stressors (e.g., chemical contaminants) affect the outcome of epidemics? There was agreement that mesocosm experiments, which can evaluate the effects of key mechanisms or changes, are well suited to advance this area of research.

There was also discussion of the need to better understand how ranaviruses can persist within an ecological system. Current epidemiological models are built at the pond level, but it is necessary to study how well these models fit at the landscape level (Bientreu *et al.*, 2017; Brenes *et al.*, 2017). Another important aspect is the ability of infected metamorphs and adults to remain infectious, as well as how far these infected adults disperse and what role they then play in spreading infection. Vertical transmission, environmental persistence, and the overall impact of infection on populations and ecosystems are additional areas where more research is needed.

Additional subjects were the future publication of best practices, white papers, guidelines for surveillance, the harnessing of collaboration and storage capacities, and options for finding financial support for joint research efforts. The GRC is also considering ways to improve and optimize the GRRS, as well as how to better address die-offs in amphibians beyond just focusing on the presence of ranavirus. In addition, the need to expand research to be inclusive of all of the animal classes affected by ranaviruses was reiterated.

Conclusion statement: The 4th ISR was an opportunity for scientists from a variety of backgrounds to get together to study these important pathogens. The presentations confirmed the importance of ranaviruses in wild and captive fish, amphibians, and reptiles, highlighting new outbreaks, global dispersion, and changes in the viral genome over time. The Fifth ISR is planned to take place in Cairns, Australia, in 2019 and will be organized by Ellen

Ariel and colleagues. More information will be forthcoming on the GRC website (www.ranavirus.org).

Literature Cited

- Bienentreu J-F, Grant SA, Kyle CJ, Brunetti CR, Schock DM, Lesbarrères D. 2017. Ranavirus in northern Canada: a low-diversity ecosystem for a better comprehension of disease dynamics? Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Black Y, Meredith A, Price SJ. 2017. Detection and reporting of ranavirus in amphibians: evaluation of the roles of the World Organisation for Animal Health and the published literature. Poster at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Brenes R, Hoverman JT, Miller DL, Gray MJ. 2017. Presence of amplification hosts increases mortality of syntopic amphibians by ranaviral disease. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Brunner JL. 2017. WORKSHOP: The Global Ranavirus Reporting System (GRRS). Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Brunner JL, Olson A, Rice JG, Le Sage MJ, Cundiff JA, Goldberg CS, Pessier AP. 2017. Performance of nonlethal methods of detecting ranavirus infections in captivity and trade. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Campbell LC, Garner TWJ, Tessa G, Scheele BC, Griffiths AGF, Bayer-Wilfert L, Harrison XA. 2017. Ranavirus infection potentially alters the age structure of infected populations. Poster at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Cheng K, Jones MEB, Jancovich JK, Burchell J, Schrenzel MD, Reavill DR, Imai DM, Urban A, Kirkendall M, Woods LW, Chinchar VG, Pessier AP. 2014. Isolation of a Bohle-like iridovirus from boreal toads housed within a cosmopolitan aquarium collection. *Dis Aquat Org*, 111(2):139–152.
- Clayton SC, Subramaniam K, Chinchar VG, Gray M, Mavian C, Salemi M, Wisely S, Waltzek T. 2017. Ranavirus phylogenomics: signatures of recombination and inversions among ranaculture Isolates. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Chinchar VG, Hick P, Ince IA, Jancovich JK, Marschang R, Qin Q, Subramaniam K, Waltzek TB, Whittington R, Williams T, Zhang Q, ICTV Report Consortium. 2017a. ICTV virus taxonomy profile: *Iridoviridae*. *J Gen Virol*, 98(5):890–891.
- Chinchar VG, Waltzek TB, Subramaniam K. 2017b. Ranaviruses and other members of the family Iridoviridae: their place in the virosphere. *Virology*, 511:259–271.
- Forzán MJ, Sloma E. 2017. Increased detection of ranavirus in tissue using novel in situ hybridization technique. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Garner TWJ, Price SJ, Ariel E. 2017. The European ranaviruses. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Gray MJ, Spatz JA, Carter ED, Miller DL. 2017. Poor biosecurity could lead to ranavirus outbreaks in amphibian populations. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Hall EM, Goldberg CS, Crespi EJ, Brunner JL. 2017. Seasonal dynamics of ranavirus epidemics in wood frog populations. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Hick PM, Subramaniam K, Thompson P, Whittington RJ, Waltzek TB. 2016. Complete genome sequence of a Bohle iridovirus isolate from ornate burrowing frogs (*Limnodynastes ornatus*) in Australia. *Genome Announc*, 4(4):e00632–16.
- Jancovich JK, Steckler NK, Waltzek TB. 2015. Ranavirus taxonomy and phylogeny. In Gray MJ, Chinchar VG (eds): *Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates*. Springer, New York:59–70.
- Lesbarrères D, Balseiro A, Brunner J, Chinchar VG, Duffus A, Kerby J, Miller DL, Robert J, Schock DM, Waltzek T, Gray MJ. 2012. Ranavirus: past, present and future. *Biol Lett*, 8(4):481–483. Available from: <http://doi.org/10.1098/rsbl.2011.0951>.
- Maclaine A, Scott J, Wirth W, Mashkour N, Ariel E. 2017. Pathogenesis of Bohle iridovirus in juvenile eastern water dragons, *Intellagama lesueurii lesueurii*. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Miller DL, Balseiro A, Casais R, Gray MJ. 2017. Immunohistochemistry of hellbenders exposed to an FV3-like ranavirus, alone or with one stressor. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Papp T, Marschang RE. 2017. A member of the genus *Iridovirus* in reptiles and amphibians? Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Price SJ, Wadia A, Wright O, Leung W, Cunningham AA, Lawson B. 2017. How ranavirus brought Britain closer to the EU – molecular screening of a long-term tissue archive from herpetofauna. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Qin Q, Yu Y, Huang Y, Huang X. 2017. Singapore grouper iridovirus (SGIV) TNFR homolog VP51 functions as a virulence factor by modulating cellular apoptosis and the host inflammatory response. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Robert J. 2017. The interplay between ranavirus and the amphibian immune system. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Robert J, Edholm E-S, Banach M, Kwan T, Sanchez J, Yi L, De Jesús Andino F. 2017. Different antiviral T cell type immunity in tadpole and adult *Xenopus*. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Saucedo B, Hughes J, Suárez N, Haenen O, Voorbergen-Laarman M, Gröne A, Kik MJL, van Beurden S. 2017a. Complete genome sequence of a Frog Virus 3-like ranavirus from an *Oophaga pumilio* frog imported from Nicaragua into the Netherlands. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.

- Saucedo B, Hughes J, Suarez N, Haenen O, Voorbergen-Laarman M, Kruithof N, Rijks J, Schils M, Spitzen A, Gilbert M, Gröne A, van Beurden S. 2017b. Comparative genomics and spatiotemporal characterization of two strains of common midwife toad virus in the Netherlands. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Stöhr AC, Lopez-Bueno A, Blahak S, Caeiro MF, Rosa GM, Alves de Matos AP, Martel A, Alejo A, Marschang RE. 2015. Phylogeny and differentiation of reptilian and amphibian ranaviruses detected in Europe. PLoS ONE, 10(2):e0118633.
- Subramaniam K, Waltzek TB, Stac NI, Grosset C, Wellehan JFX Jr. 2017. Phylogenomic characterization of squamate erythrocytic iridoviruses. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Waltzek TB. 2017. Overview of the nucleo-cytoplasmic large DNA viruses (NCLDV). Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Wirth W, Scott J, Ariel E. 2017. Dose-dependent morbidity of freshwater turtle hatchlings, *Emydura macquarii krefftii*, inoculated with *Bohle iridovirus* (*Ranavirus* sp., *Iridoviridae*). Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.
- Yaparla A, Grayfer L. 2017. Differentiation-dependent antiviral capacities of amphibian (*Xenopus laevis*) macrophages. Presentation at the 4th International Symposium on Ranaviruses, Budapest 7–10 June.