ARAV Infectious Diseases

Marike Visser, DVM; Daniella Inman

Moderators
Experimental Inoculation of Ball Pythons (*Python regius*) with Snake Arenaviruses

David Sanchez-Migallon Guzman, LV, MS, Dipl ECZM (Avian), Dipl ECZM (Small Mammal), Dipl ACZM, Mark D. Stenglein, PhD, Michelle Hawkins, VMD, Dipl ABVP (Avian), Valentina Garcia, M. Kevin Keel, DVM, PhD, Dipl ACVP, Tracy Drazenovich, DVM, Joseph L. DeRisi PhD

Session #291

**Affiliation:** From the Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA (Guzman, Hawkins, Drazenovich), Department of Microbiology Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University (Stenglein), Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA (Keel), Department of Biochemistry and Biophysics, School of Veterinary Medicine, University of California San Francisco, San Francisco, CA 94158, USA (Garcia, DeRisi).

Inclusion body disease (IBD) is a fatal disease of snakes. IBD is most commonly diagnosed in boas and pythons, but has been reported in several other species. Disease manifestation is variable in different species.\(^1\) Snake arenaviruses have been associated with IBD in boids,\(^2,3\) but causality has not yet been demonstrated. For this study, 4 ball pythons (*Python regius*) that tested negative on polymerase chain reaction (PCR) technique for snake arenavirus from blood and liver biopsies were used. Two ball pythons were inoculated with 2 different viral strains of snake arenavirus while 2 additional ball pythons served as a control. The viral strains used to inoculate the 2 snakes were isolated from previous boa constrictors (*Boa constrictor*) with inclusion body disease. The infected snakes developed severe neurologic clinical signs approximately 9 weeks after inoculation and had moderate encephalomyelitis, meningitis and ganglioneuritis on histopathology. The presence of viral RNA for snake arenavirus was confirmed and quantified by qRT-PCR (real-time quantitative reverse transcription PCR) in the infected snakes and immunofluorescence was performed in multiple tissues demonstrating the presence of the virus in the brain. The control snakes remained without clinical signs during the same period and no significant lesions were found on histopathology. There was no snake arenavirus RNA found in the control snakes and immunofluorescence was negative. The results of this study establish a causative relationship between snake arenavirus and IBD in ball pythons.

**Acknowledgments:** This study was supported by the Reptile Research Fund at the University of California Davis and the Howard Hughes Medical Institute.

**References**


West Nile virus (WNV) can cause mortalities in captive reared American alligators. A killed WNV vaccine is commercially available (Vetera WNV, Boehringer Ingelhein Vetmedica, Inc., St. Joseph, Missouri, 64506, USA). A case from an alligator farm in Louisiana revealed that tissues of vaccinated animals were positive for WNV by reverse transcriptase polymerase chain reaction (RT-PCR) while non-vaccinated animals were negative, suggesting the RT-PCR might detect the vaccine in the tissues. The vaccine product itself was positive for WNV via RT-PCR. This study was designed to determine if vaccination could lead to positive RT-PCR from tissues and also to evaluate the antibody response. Eighty-one, 7-day-old hatchlings were vaccinated with 0.25 ml WNV vaccine into the lateral tail musculature and 26 control alligators were injected with 0.25 ml sterile water into the lateral tail musculature on days 1 and 14. Blood was obtained daily from 3 animals in the treatment group prior to euthanasia and necropsy until all animals were euthanatized (27 days). Thirteen of the control alligators were sampled on day 14 and the remaining were sampled on day 27. WNV titers were obtained via serum neutralization. Real time RT-PCR was performed on the brain, liver, kidney, and tail musculature from each animal. Preliminary results revealed antibodies in both the control and treatment groups but viral RNA was only identified in the treatment group. The results confirm that RT-PCR can detect viral RNA from the vaccine in tissues of vaccinated alligators. The presence of antibodies in the control group without any mortalities suggests maternal transfer of antibodies.
Polymerase Chain Reaction Detection of Nidoviruses in Live Pythons and Boas

Rachel E. Marschang, PD Dr. med. vet., Dipl ECZM (Herpetology), FTÄ Mikrobiologie, ZB Reptilien, Ekaterina Kolesnik, TÄ

Session #135

Affiliation: From Laboklin GmbH & Co. KG, Steubenstr. 4, 97688 Bad Kissingen, Germany.

Viruses of the order Nidovirales have recently been detected in 2 species of pythons (ball pythons, Python regius, and Indian rock pythons, P. molurus) in the United States and Europe. They are believed to be an important cause of respiratory disease in these animals. A conventional polymerase chain reaction (PCR) was used to screen clinical samples from 201 live pythons and boas for nidoviruses (95 pythons, 84 boas, and 22 snakes of unknown species). Nidoviruses were detected in 30 of the 201 snakes examined (14.9%). This included 26 pythons (27.4% of the pythons tested), 2 boas (2.4% of the boas tested), and 2 snakes of unknown family. There was a highly significant difference in the percentage of positive pythons and the percentage of positive boas. Viruses were detected in ball pythons (16 positive), Indian rock pythons (5 positive), a Burmese python (P. bivittatus, 1 positive), green tree pythons (Morelia viridis, 2 positive), a carpet python (M. spilota, 1 positive), a python of unknown species, and boa constrictors (Boa constrictor, 2 positive). The positive samples were from Germany (16), the United Kingdom (7), Denmark (1), the Czech Republic (1), Austria (1), Switzerland (2), France (1), and Belgium (1). Sequencing of the PCR products showed that all were closely related to recently described nidoviruses from pythons with 81-97% nucleotide identity. Virus was mostly detected in oral swabs, although whole blood was positive in 2 cases.
Experimental Ferlavirus Infections in Corn Snakes: Pathologic Alterations and Impact on Lung Exchange Capacity

Michael Pees, Prof, Dr. med. vet., Dipl ECZM (Avian), Dipl ECZM (Herpetology),
Annkatrin Neul, med. Vet.,
Volker Schmidt, Dr. med. vet., Dipl ECZM (Avian) Dipl ECZM (Herpetology),
J. Matthias Starck, Prof, Dr. rer. Nat.,
Rachel E. Marschang, PD, Dr. med. vet., Dipl ECZM (Herpetology)

Session #348

Affiliation: From the Clinic for Birds and Reptiles, University of Leipzig, An den Tierkliniken 17, 04103 Leipzig, Germany and the Department of Biology II, LMU München, Großhadener Str. 2, 82152 Planegg-Martinsried, Germany and Laboklin GmbH & Co.KG, Steubenstr. 4, 97688 Bad Kissingen, Germany.

Corn snakes (Pantherophis guttatus) were infected with defined ferlaviruses (family Paramyxoviridae) from the genogroups A, B, and C (3 groups with 12 animals each) and pathologic changes were compared to an uninfected control group. Diagnostics included clinical examination, gross pathology, histology and electron microscopy (calculation of exchange capacity of the lungs), as well as virus detection in organs. A scoring system was established to compare the different pathologic alterations among the groups, for the clinical status, lung macroscopy and microscopy, other organ microscopy changes as well as the assessment of lung morphology and the thickness of the air-blood barrier. Relevant microbiologic findings within the lung tissue also were scored. Scoring ranged from 0 (unremarkable) to 2 (severe changes) for each parameter, and a total scoring sum was calculated.

Results demonstrate significant differences in the pathogenicity depending on the virus strain used. This was consistent for all parameters evaluated, resulting in a different pathogenicity scoring between the groups. While the virus strain belonging to genogroup A only caused moderate pathologic alterations, with no snake dying within the experimental period, the isolate belonging to group B, which was originally isolated from a timber rattlesnake, caused the most severe alterations with no snake surviving past day 35 post infection. Lung tissue was most commonly affected, but changes were also noted in the liver, the spleen and the pancreas. Within the lung tissue, a massive thickening of the air-blood barrier was found, accompanied by secondary bacterial infection and inflammatory reactions.
Hemagglutination inhibition assays (HI) are regularly used for the detection of antibodies against ferlaviruses (family Paramyxoviridae) in various species including snakes, lizards, and chelonians. Some studies have shown that there may be extreme differences in HI results between different laboratories. Although some work has been done on serologic comparisons between individual isolates, no data are available on correlation between ferlavirus genogroups and serologic cross-reactivity. Four different ferlavirus genogroups have been described so far (A, B, C, and tortoise). In a previous study, corn snakes (*Pantherophis guttatus*) were inoculated intratracheally with defined ferlaviruses from the genogroups A, B, and C. The animals inoculated with the type B virus developed the most severe disease. Blood was collected from all snakes and from uninfected controls at regular intervals and tested for antibodies against representatives of all 4 known ferlavirus genogroups by HI. Increases in titers were noted 16-28 days post inoculation. The strongest cross-reactivity was noted between the genogroup A and B isolates; the lowest titers were measured against the tortoise isolate. Between the infected groups, the lowest titers against all viruses were noted in the group that received the type B virus, in which the most severe disease was noted. The results of this study show that the development of antibodies against ferlaviruses is influenced by the virus type involved in the infection and that HI results depend on the virus genotype used for testing.
Agamid Adenovirus in Clinically Healthy Bearded Dragons in Italy

Paolo Selleri, DMV, PhD, SpecPACS, Dipl ECZM (Herpetology),
Dipl ECZM (Small Mammals),
Nicola Di Girolamo, DMV, MSc (EBHC), PhD, Dipl ECZM (Herpetology),
Alessandro Montani, DMV,
Elia Zanoli, DMV,
Silvia Preziuso, DMV, PhD

Session #274

Affiliation: From Clinica per Animali Esotici, Centro Veterinario Specialistico, Via S. Giovannini 53, 00137, Roma, Italy (Selleri, Di Girolamo, Montani, Zanoli) and Department of Biosciences and Veterinary Medicine, University of Camerino, Via Circonvallazione 93/95, 62024 Matelica (MC), Italy (Preziuso).

Abstract: Adenoviruses infect various species of squamates, chelonians, and crocodiles, with the most common isolate from bearded dragons being the Agamid adenovirus 1 (AAdV-1). Bearded dragons may remain asymptomatic carriers of AAdV-1 or suffer a diverse range of issues, including neurological symptoms, immunosuppression, reduced growth and death. However, true pathogenicity is unclear and Koch’s postulates have been only fulfilled for an AdV-induced hepatic necrosis in a boa constrictor (Boa constrictor). AAdV-1 has been described in Australia, United States, and Europe. The aim of the present study was to evaluate the presence of AAdV-1 in captive bearded dragons in Italy. For this purpose, 4 private collections were sampled including a total of 39 bearded dragons of various age and sex. Oral and cloacal swabs were obtained from each bearded dragon and were tested by nested PCR to amplify a partial sequence of the adenoviral DNA polymerase gene. Ninety-five percent confidence interval for the prevalence were calculated with the Wilson procedure without a correction for continuity. Out of 39 bearded dragons sampled, 14 resulted positive (prevalence: 36%; 95% CI: 23-52%). Sequencing of the PCR products and comparison with known sequences showed the highest score with AAdV-1. Although results of this study should not be extrapolated in order to obtain the prevalence of AAdV-1 in Italy due to the limited number of samples obtained, these results confirm that AAdV-1 is well established within clinically-healthy captive bearded dragons in private collections in Italy.

References
