A NOVEL IGUANID HERPESVIRUS DETECTED IN ASYMPTOMATIC GREEN IGUANAS (IGUANA IGUANA) IN POLAND

Rachel E. Marschang, PD Dr med vet, Dipl ECZM (Herp), Joanna Pasterny, DVM, Jakub Sen,

Abstract: While herpesviruses are well-known pathogens in a wide variety of chelonian species, they have only sporadically been documented in squamate reptiles. Those that have been described have most often been associated with hepatic disease and oral lesions. During a study on infectious disease in pet reptiles in Poland, herpesviruses were detected in swabs from three green iguanas (Iguana iguana) from two different owners that were presented to two different veterinary clinics in Warsaw. One iguana was presented for abscesses on the head, while the other two were partner animals and remained clinically healthy throughout the course of this study. Virus was detected in oral swabs as well as combined swab samples from the oral cavity and cloaca using a panherpesvirus PCR. PCR products from all three animals were sequenced, and the detected viruses were most closely related to iguanid herpesvirus 2 from a San Esteban chuckwalla (Sauromalus varius) in the United States (GenBank accession No. AY236869.1). The single animal was retested again 1 y later and remained clinically healthy and continued to shed the same herpesvirus. This is the first description of a herpesvirus infection in pet iguanas in Europe. While the clinical relevance of the infection is not known, it is of interest that the infected animals appeared to continue to shed virus over an extended period of time.

* Herpesvirus= large dsDNA virus
* Normal chelonian, but have isolated iguanid herpesvirus 1 and IgHV2; other isolates include herpesvirus 1,2, and 3 in varanids, helodermatid herpesvirus 1, gerrhosaurid herpesvirus 1,2 and 3; Opheodrys herpesvirus 1; these animals commonly had stomatitis, proliferative oral lesions, dyspnea, sometimes oropharyngeal SCC
* Case report of 3 iguanas
	+ Iguana 1: SQ abscess over mandible, debrided abscess and did well; oral cloacal swab submitted (and then tested 1 year later)
	+ Iguana 2 and 3: housemates, routine exams and considered healthy, also had two oral cloacal swabs (one in November and then February the following year)
* Nested PCR targeting for viral DNA polymerase gene
* Herpesvirus was detected in mixed swab and oral BUT NOT the cloaca swab of Igu 1
* Herpes was detected in the mixed swab from Ig2 in November and then from both in February
* BLAST analysis showed 77% identity to IgHV2- preliminary this could be IgHV3
* Found these by chance in routine screening of animals
* Possibly iguanas kept at lower temps? Larger amount of virus released from cells incubated at lower temperatures was found in other studies

Effects of the Antivirals Lysine and Lactoferrin on Testudinid Herpesviruses and a Ranavirus in Cell Culture

Rachel E. Marschang, PD Dr med vet, DECZM (Herpetology), FTA Mikrobiologie

Abstract: Testudinid herpesviruses (TeHVs) and ranaviruses are important pathogens of tortoises, but no treatments are available. Lysine may inhibit herpesvirus (HV) replication, although its effectivity has been questioned. Lactoferrin has antiviral activity against a range of viruses by inhibiting adsorption to cells. Media were supplemented with 6.25–10 mg/ml lysine and 0.625–1 mg/ml lactoferrin, individually or combined, and the effects measured against the replication of TeHV-1, TeHV-3, or a ranavirus in Terrapene heart cells (TH-1). Cytotoxicity testing was also carried out with the same concentrations. Lysine alone inhibited cell growth after 24 and 48 h, whereas lactoferrin only reduced cell growth after 24 h. TeHV-1 titers were only reduced by the addition of a combination of 10 mg/ml lysine and 1 mg/ml lactoferrin after 48 h (43.8% reduction) and by 10 mg/ml lysine (82.2% reduction). The same concentration of the combined substances led to an average reduction in TeHV-3 titers of 43.8% after 24 h and 68.4% after 48 h, respectively, while 10 mg/ml lysine led to an average reduction of 92.5% after

48 h. Lactoferrin alone did not reduce HV titers. Both concentrations of the combined substances led to titer reductions of the ranavirus (86.7–96.8%), and testing of the individual substances led to a reduction of 97.6% after 48 h with 10 mg/ml lysine and of 82.2% after 24 h with 1 mg/ml lactoferrin.

* Herpesvirus: associated with lesions in upper digestive and respiratory
* Alphaherpesivirus (turtle and tortoise herpesvirus)
* Ranavirus in chelonians: nasal discharge, oral plaques, severe systemic disease; close relation to FV3
* No treatment for either disease
* Acyclovir and Valacyclovir have been evaluated and showed promise in cell culture; however, PK studies proved that maintaining therapeutic levels problematic
* Lysine- showed promise in vitro but not considered anti-viral; antagonization of arginine (essential amino acid for some herpesviruses)
* Lactoferrin: glycoprotein- has antibacterial, antimycotic, and antiviral properties; suspect inhibition of virus adsorption to host cells by blocking cellular receptors or by binding to virus particles as well as by enhancing the production of immunoactive cytokines
* Evaluated lysine alone, lactoferrin alone, and then both together (two different concentrations 1 low of each and 1 high of each) on TeHV1, TeHV3 titers and ranavirus
* For herpes: lysine and lactoferrin together had a minor reduction after 48 hours, lysine alone had clear titer reduction after 48h
* For rana: highest average reductions in titer were after lysine alone after 48 hours of treatment and in the combined higher concentration lysine and lactoferrin after 24 and 48 hours
* Arginine levels are expected to influence the effectivity of lysine on virus replication- speculation that lysine could be a treatment for turtles and tortoises with herpesvirus
* Lysine can also be toxic to cell growth
* In FV3- late stages of the virus depended on arginine
* Lactoferrin had no inhibitory effect on replication of the herpesviruses- concentrations may have been insufficient- but higher concentrations was likely toxic to the cell lines
* Rana was inhibited by lactoferrin

Take away: Lysine may have an inhibitory effect on the growth of tortoise herpesvirus and ranavirus in cell culture. Lactoferrin may have an inhibitory effect on ranavirus in cell culture.

**PREVALENCE OF MULTIPLE REPTILIAN PATHOGENS IN THE OROPHARYNGEAL MUCOSA, CLOACAL MUCOSA, AND BLOOD OF DIAMONDBACK TERRAPIN (MALACLEMYS TERRAPIN) POPULATIONS FROM MARYLAND AND GEORGIA, USA.** JWD, 58(4), 782-790 (2022). Bryan S. Vorbach, Leigh A. Clayton, Willem M. Roosenburg, Terry M. Norton, Laura Adamovicz, Catherine A. Hadfield, Matthew C. Allender. - review by LMumm

Abstract: The diamondback terrapin (Malaclemys terrapin) is a coastal turtle with a range from Massachusetts to Texas and is the only exclusively brackish water turtle in North America. Two populations of wild terrapins from Maryland (n=55) and Georgia (n=7) were examined and tested for potential reptile pathogens. Whole blood and a mucosal (combined oropharyngeal and cloacal) swab from each animal were evaluated by quantitative PCR for 15 potential pathogens including frog virus 3, box turtle Mycoplasmopsis, Mycoplasma agassizii, Mycoplasma testudineum, Salmonella Enteritidis, Salmonella Typhimurium, Borrelia burgdorferi, Anaplasma phagocytophilum, tortoise intranuclear coccidia, testudinid alphaherpesvirus 2, terrapene herpesvirus 1, and terrapene adenovirus. Swabs were positive for a DNA segment 100% homologous to M. testudineum in both populations, with Maryland animals 87% (48 of 55) positive and Georgia animals 86% (6 of 7) positive. Although Mycoplasmopsis spp. are important respiratory pathogens for members of the order Testudines, none of the animals in the study showed any sign of upper respiratory disease. **Our data suggest that M. testudineum may survive in non-Testudinidae turtles without causing clinical signs of disease** and suggesting appropriate precautions should be taken in facilities that house multiple species of turtles simultaneously.

Background: Diamondback terrapin = only exclusively brackish water turtle in N. America

* Known threats = anthropogenic = habitat destruction, drowning in crab pots, boat/vehicle strike

Methods: Tested for 15 pathogens via oropharyngeal-cloacal (OC) swabs and whole blood samples in wild terrapins under surveillance in Maryland (n=55) and HBC terrapins in rehab in Georgia (n=7)

Key Points:

* High prevalence of *Mycoplasma testudineum* → detected in 56/64 OC swabs via qPCR
	+ Clinical signs: none/asymptomatic suggests symbiosis in non-Testudinidae turtles
		- In susceptible species causes upper respiratory disease
	+ Location: MD and GA equally affected (but very different sample size…)
		- GA animals did have lower bacterial load but were receiving vet care/antibiotics
	+ Sex: no difference in sex
	+ Age: juveniles had lower infection rates than adults suggests horizontal transmission
	+ Year: higher detection in 2016 (vs. 2015)
* No other pathogens detected in OC swabs and none detected in blood at all
	+ Negative for salmonella, anaplasma, herpes, adenovirus, TIC, etc.
	+ Salmonella spp. previously reported in diamondback terrapins but not found in this study

TLDR: *Mycoplasma testudineum* has high prevalence in diamondback terrapins without clinical disease

* Care should be taken in captive settings as can be pathogenic to other species (i.e. tortoises)
* Terrapins have a low pathogen microbiome as no other pathogens were detected in this study

**Absence of Herpesvirus in a Survey of Alligator Snapping Turtles (*Macrochelys temminckii*) in Illinois and Louisiana.** JHMS, 31(1):43-47 (2021). Taylor J. Willis, Laura Adamovicz, Ethan Kessler, Peter M. DiGeronimo, Matthew C. Allender. - review by LMumm

Abstract: Herpesviruses are significant pathogens of captive-reared and free-ranging chelonians worldwide. Lesions associated with chelonian herpesvirus infection are species dependent and include stomatitis in tortoises, hepatic necrosis in pond turtles, and fibropapillomas in sea turtles, among other conditions. Herpesviruses also have been detected in several free-ranging freshwater turtle species with no clinical signs of illness at a prevalence from 2 to 56%. The alligator snapping turtle (Macrochelys temminckii) is a freshwater turtle species endemic to the United States that has experienced declines throughout its range. Reintroduction of this species is currently underway in several states. As part of a health surveillance program, in concert with reintroduction efforts, we investigated the presence of herpesvirus in captive and free-ranging alligator snapping turtles. Using conventional consensus polymerase chain reaction, we tested combined oral/cloacal swab DNA samples (n = 197) from head-started alligator snapping turtles prerelease and postrelease in southern Illinois (n = 153), prerelease samples from adult turtles confiscated from Florida (n = 18), and prerelease samples from captive-reared individuals from northern Louisiana (n = 26). Herpesvirus DNA was not detected in any sample. Possible explanations for these results include lack of exposure, latency in tissues not sampled, and viral quantities below the level of detection of the assay. Continued surveillance for this and other pathogens is helpful in characterizing potential disease risks from captive-reared reintroduction programs and will enhance future conservation efforts of this species.

Background:

* Herpesviridae: enveloped, double stranded DNA virus
	+ URI and oral signs (stomatitis\*, rhinitis, glossitis, conjunctivitis) in tortoises
	+ Hepatic necrosis in pond turtles
	+ Fibropapillomas or URI signs with skin lesions in sea turtles

Methods: Tested for herpesvirus DNA via combined oral/cloacal swabs from free-ranging and captive reared ASTs (n=153) associated with reintroduction projects in southern Illinois (pre- and post-release) and northern Louisiana (pre-lease) using conventional PCR

Key Points:

* All ASTs sampled were negative for herpesvirus via PCR
	+ Potential reasons: 1) lack of prior exposure (majority of turtles were captive reared juveniles), 2) lack of viral shedding due to latency, 3) inappropriate testing site, 4) inadequate sensitivity, 5) insufficient sampling effort to detect virus at very low prevalence
* Recommend pairing PCR with 2nd test in clinically healthy chelonians
	+ Virus isolation: can get false negatives due to inactivation of virus in transport, overgrowth/inhibition of virus in cell culture d/t contaminants, or lack of viral shedding
	+ Serum neutralization: only indicates animal has been in contact with virus however for life-long infections (like herpesvirus) does indicate infection
	+ ELISA: less labor-intensive test and quicker results than serum neutralization, good for detecting potential carriers, however serologic tests in new studies can be misleading – antigenic variation in pathogen of interest, potential for cross-reacting Abs, and differences in host serologic response

**TLDR:** Herpesvirus DNA via PCR was not detected in ASTs during surveillance study in IL and LA

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[**EMYDID HERPESVIRUS 1 INFECTIONS IN WESTERN POND TURTLES (*ACTINEMYS MARMORATA*) AND A RED-EARED SLIDER (*TRACHEMYS SCRIPTA ELEGANS*) WITH FATAL AND NONFATAL OUTCOMES**](https://doi.org/10.1638/2021-0044)

Sim RR, Ossiboff RJ, Nelson J, Oddo T

**ABSTRACT:** Herpesviruses are important pathogens in zoologic chelonian collections and have been associated with fatal disease in turtles of the family Emydidae. In this report, three western pond turtles (*Actinemys marmorata*), living in a mixed-species freshwater turtle exhibit, presented with varying degrees of lethargy, pallor, generalized edema, and cloacal hemorrhage before death within a 2-wk period. Postmortem findings included necrohemorrhagic enterocolitis, necrotizing splenitis, hepatic necrosis, esophagitis, thymic necrosis, and pneumonia with epithelial necrosis and degeneration of the trachea and kidneys. Intraepithelial, intranuclear, amphophilic to eosinophilic, Cowdry type A viral inclusion bodies were identified in the intestinal tract, liver, spleen, kidney, trachea, lung, and thymus. PCR amplification and sequencing of liver tissue produced amplicons that were 100% homologous with emydid herpesvirus 1 (EmyHV-1). Molecular screening of cohoused emydid turtles revealed that a red-eared slider (*Trachemys scripta elegans*) and a western pond turtle, both asymptomatic, were PCR-positive for EmyHV-1 on combined oral-cloacal swabs. This report describes, for the first time, EmyHV-1-associated disease in western pond turtles and molecularly identifies EmyHV-1 in an asymptomatic red-eared slider.

**Background**

* Western pond turtle: range CA to WA
	+ Testudinid herpesvirus and Emydid herpesvirus - *Alphaherpesvirinae* genus *Scutavirus*

**Key Points:**

* 3 cohoused western pond turtles in central Oregon presented with lethargy (limp tone to head and limbs), pallor (grey-pink skin), generalized edema/coelomic effusion, ocular discharge, and perimortem cloacal hemorrhage – all died within 2 weeks of presentation
* Intraepithelial, intranuclear, amphophilic to eosinophilic, Cowdry type A viral inclusion bodies were identified in the liver (3/3, Fig. 1B), intestinal tract (3/3), spleen (2/2, Fig. 1C), lung (2/2, Fig. 1D), kidney (1/2), thymus (1/2), and trachea (1/2).
* Postmortem: hepatocellular degeneration and necrosis, necrotizing splenitis, and interstitial pneumonia.
	+ Novel lesions in this case: necrohemorrhagic enterocolitis, esophagitis, epithelial degeneration of the trachea, and thymic necrosis.
* Oral-cloacal swabs collected of all other tankmates – red eared slider and western pond turtle (both clinically normal) tested positive
* Necrohemorrhagic enterocolitis with perimortem cloacal hemorrhage may be important clinical features of EmyHV-1 infection in western pond turtle.
* EmyHV-1 has an expanded tissue tropism in WPT compared with prior reports
* EmyHV-1 can cause significant disease in some WPT, but some are asymptomatic.
* One WPT did not develop disease, so onset of disease associated with EmyHV-1 in WPT may be conditional on other factors, like stress
* Be careful with multi-species chelonian exhibits to limit cross-species infection
	+ Alphaherpesvirus infection more severe when occurs in nondefinitive host species.

Racz, Katharina, et al. "Detection of mycoplasma and chlamydia in pythons with and without serpentovirus infection." Journal of Zoo and Wildlife Medicine 52.4 (2021): 1167-1174.

**Abstract:**

Serpentoviruses (order *Nidovirales*) are an important cause of respiratory disease in snakes. Although transmission studies have shown that serpentoviruses can cause respiratory disease in pythons, the possible role of additional potential pathogens is not yet understood. Very little information is available on the role of mycoplasma and chlamydia infections in disease in pythons. Diagnostic samples **from 271 pythons** of different genera submitted to a laboratory for **detection of serpentoviruses were also screened for mycoplasma and chlamydia infections by PCR.** Most of the samples were **oral swabs**. **Almost 30% of the samples were positive for serpentoviruses**, and **mycoplasmas were detected in more than 60% of the pythons**. The occurrence of these two pathogens correlated significantly (*P* < 0.001). Additionally, about **3% of the snakes tested positive for *Chlamydia***. This study found a **high prevalence of mycoplasmas in the tested pythons and a correlation between infections with these bacteria and serpentoviruses in python** samples submitted for diagnostic testing. Because the role mycoplasmas play in respiratory diseases of snakes is still largely unknown, further investigations are necessary to evaluate the role of mixed infections in disease.

**Key Points:**

* Snake-associated nidoviruses (single-strand RNA viruses) are members of the subfamily *Serpentovirinae*. A transmission study in ball pythons confirmed the association between serpentovirus infection and upper and lower respiratory disease.
* Mycoplasma has been described in individual snake cases. Little is known.
* *Chlamydia* is a gram-negative, obligate, intracellular bacteria with a complex life cycle (cell infectious elementary bodies and metabolically active reticulate bodies). *Chlamydia pneumoniae* is most commonly detected in snakes*.* Many infected snakes have been clinically healthy. Regurgitation, chronic wasting, stomatitis,pneumonia, anorexia, neurological signs, granulomatous disease, and respiratory signs have been described.
* Most of the samples originated from pythons of the genera *Python* (*n* = 134) and *Morelia* (*n* = 105). General health status information was not available.
* 180 of 271 samples (66.4%) tested positive for at least one of the pathogens examined.
* Serpentoviruses were detected in 79 (29.2%) of the pythons tested.
* *Mycoplasma* spp. were detected in 163 (60.2%) of the pythons tested.
* Chlamydia infections were detected in 8 (3.0%) of 271 pythons, all ball pythons (7.1% positive). Mycoplasmas was also detected in all eight chlamydia-positive samples.
* Both serpentoviruses and mycoplasmas were detected in 62 (22.9%) pythons, so that 37.4% of the mycoplasma-positive snakes were also serpentovirus-positive, and 78.5% of the serpentovirus-positive animals were also mycoplasma-positive.
* The detection of mycoplasmas and serpentoviruses correlated significantly. The probability of co-infection with mycoplasma and serpentovirus was 3x higher than infection with only one pathogen.

**Take-Home Message:**

* >50% of the pythons tested were positive for mycoplasmas. However, overall clinical relevance and role in respiratory diseases in snakes is unknown. All *Chlamydia*-positive and nearly 80% of the serpentovirus-positive snakes were also infected with mycoplasmas, which suggests that mycoplasma could increase susceptibility to respiratory infections. Detection of mycoplasmas should be considered in diagnostic testing of pythons in future.

Salzmann, Ekaterina, Elisabeth Müller, and Rachel E. Marschang. "Detection of testadenoviruses and atadenoviruses in tortoises and turtles in Europe." Journal of Zoo and Wildlife Medicine 52.1 (2021): 223-231.

**Abstract:**

**Adenoviruses** have been **regularly detected in squamate reptiles**; evidence of infection in **chelonians** is described much **less frequently**. The adenoviruses found in turtles and tortoises have been genetically diverse, and have included members of the genus ***Siadenovirus*,** a proposed **testadenovirus genus**, and, in a single case, an ***Atadenovirus***. In this study, samples from **949 chelonians** submitted to a diagnostic laboratory were screened for the presence of adenoviruses by polymerase chain reaction (**PCR**) targeting a portion of the DNA polymerase gene. **Adenoviruses were detected in 22 (2.3%) chelonians** of different species. Adenovirus-positive species included Hermann's tortoises (*Testudo hermann*i), spur-thighed tortoises (*T. graeca*), Horsfield's tortoises (*T. horsfieldii*), sliders (*Trachemys* spp.), box turtles (*Terrapene* spp.) and a black pond turtle (*Geochlemys hamiltonii*). Sequencing and phylogenetic analyses of the obtained PCR products revealed that the majority of the detected adenoviruses **(72.7%) cluster with members of the proposed testadenovirus genus, while the rest (27.3%) cluster with the atadenoviruses**. This study significantly expands the known host range of both the proposed testadenoviruses and the atadenoviruses in different chelonian species and families.

**Key Points:**

* Adenoviruses are nonenveloped, double-stranded DNA viruses. Adenovirus infections are well known in squamate reptiles and are most commonly associated with gastrointestinal and neurological signs.
* Adenoviruses distinct from known genera have been detected in chelonian species, leading to the suggestion of a new genus, testadenovirus.
* Previous studies associated siadenovirus infections with high mortality rates, anorexia, lethargy, mucosal ulcerations, palatine erosions, nasal and ocular discharge, and diarrhea in Sulawesi, impressed, and Burmese star tortoises. Testadenoviruses have been associated with unspecific clinical signs and death but have also been detected in several species without any associated clinical signs. The only atadenovirus described in a tortoise was associated with stomatitis and esophagitis.
* Samples from 949 chelonians were tested. The samples included mainly oral swabs (*n* = 902), some nasal lavages (*n* = 75) and a few tissues (*n* = 18). No clinical information was provided.
* Adenoviruses were detected in a total of 22 of the 949 chelonians tested (2.3%). 16 of the adenoviruses (72.7%) were most closely related to viruses in the suggested testadenovirus genus, while 6 adenoviruses (27.3%) were most closely related to members of the genus *Atadenovirus*.
* Coinfections with mycoplasma (54.6%) or herpesviruses (13.6%) were detected in 68.2% of adenovirus-positive chelonians. No other pathogens were detected. There was no significant correlation between adenovirus and myocoplasma or between adenovirus and herpesvirus infections.
* The phylogenetic analysis of the testadenoviruses showed that the viruses clustered according to their host species. In contrast, the detected atadenoviruses did not cluster according to host species, and the closest identities for each were found in squamate reptiles.
* Base composition has been used to evaluate possible host switches among atadenoviruses, with high AT contents considered an indicator for a previous host switch.

 **Take-Home Message:**

* This study extends the known host range of testadenoviruses and atadenoviruses to different chelonian species and families. Although adenovirus infections in chelonians do not appear to be common, they should be considered as potential pathogens in these animals.