Viruses of reptiles

Rachel E. Marschang

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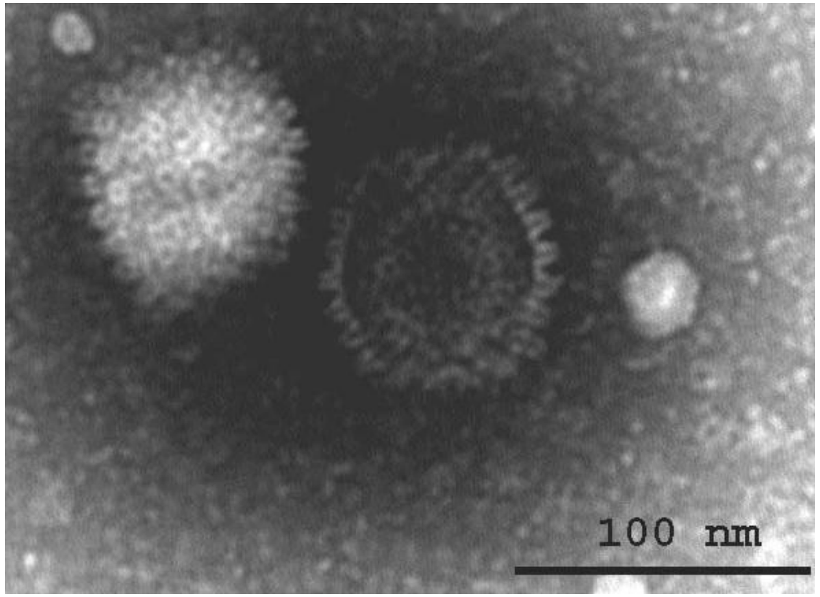
Virus of reptilesn

- DNA Viruses:
  - *Herpesviridae, Adenoviridae, Poxviridae, Iridoviridae, Hepadnaviridae, Papillomaviridae, Parvoviridae, Circoviridae, Anelloviridae, Adintoviridae, …..*
- RNA Viruses:
  - *Retroviridae, Reoviridae, Paramyxoviridae, Sunviridae, Rhabdoviridae, Bornaviridae, Peribunaviridae, Hantaviridae, Nairoviridae, Arenaviridae, Togaviridae, Flaviviridae, Caliciviridae, Picornaviridae, Tobaniviridae, Arteriviridae, Coronaviridae, Astroviridae, Kolmioviridae, …..*

Herpesviruses

- Large, enveloped, dsDNA viruses
- The family *Orthoherpesviridae* is divided into 3 subfamilies: *Alpha-*, *Beta-* and *Gammaherpesvirinae*
- Known to cause latent infections
- Genus *Scutavirus*
    - *Scutavirus chelonidalpha5* (ChHV5)
    - *Scutavirus testudinidapha3* (TeHV3)



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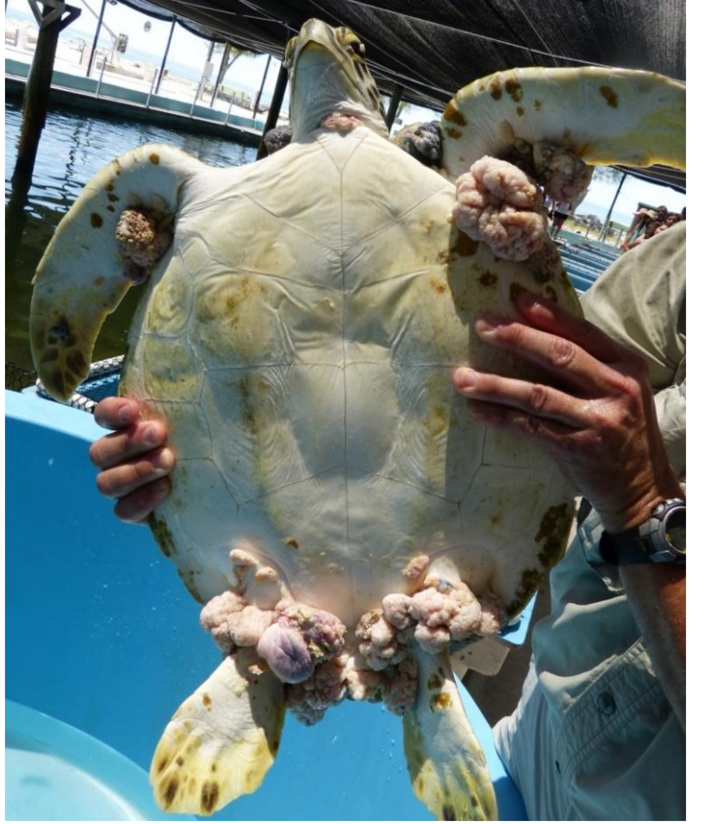
Herpesviruses: Host Range

- Chelonians:
  - Highest number of herpesvirus cases described in various chelonia species
    - Tortoises
    - Sea turtles
    - Aquatic turtles

Herpesviruses: Host Range

- Sea turtles:
  - Fibropapillomatosis
  - Lung-eye-trachea disease
  - Grey patch disease
  - Loggerhead orocutaneous HV
  - Loggerhead genital respiratory HV



Herpesviruses: Host Range

- Other turtle species (aside from tortoises):
  - Emydidae, Pelomedusidae, Chelidae (both Cryptodira and Pleurodira)
  - Associated with:
    - Hepatitis, stomatitis, skin lesions/papillomas, rhinitis, pneumonia, and in multiple species with no apparent clinical signs

Source: S. Öffner

Herpesviruses: Host Range

- Squamata:
  - Sporadic detection in lizards:
    - Iguanas (*Iguana iguana*)
    - Chuckwalla (*Sauromalus varius*)
    - Monitor (*Varanus prasinus*)
    - Agama (*Agama agama*)
    - Plated lizard (*Gerrhosaurus spp*.)
    - Gila Monster (*Heloderma suspectum*)
    - Panther chameleon (*Furcifer pardalis*)

Emerald monitor, varanid HV 1
(Source: J.F.X. Wellehan)

Panther chameleon, chamaeleonid HV1
(Source: T. Hellebuyck)

Herpesviruses: Host Range

- Herpesviruses in snakes:
  - Sporadic reports in various species
    - In venome glands of elapids, associated with reduced venome production
    - Associated with hepatic necrosis in boa constrictors
    - In mixed infections in snakes with gastro-intestinal disease
    - Disease outbreak with lethargy, anorexia, dyspnea and hepatitis in horned vipers (*Vipera ammodytes ammodytes*) (Catoi et al., 2014)
    - Associated with oropharyngeal squamous cell carcinomas in a group of wild-caught smooth green snakes (*Ophiodrys vernalis*) (Lovstad et al., 2019)

Herpesviruses: Host Range

- Detected in the cloaca in alligators (Govett et al., 2005) (possible contaminant from chelonians)
- Isolated from captive crocodiles in Australia (Hyndman et al., 2015; Shilton et al., 2016):
  - Conjunctivitis, pharyngitis, hatchling salwater crocs (*Crocodylus porosus*)
  - Poor growth, systemic lymphoid proliferation, encephalitis in young saltwater crocs
  - Multifocal lymphohistiocytic infiltration of the dermis in harvest sized saltwater crocs
  - Systemic lymphoid proliferation in captive freshwater crocs (*C. johnstoni*)

Source: C. Shilton

Herpesviruses: Clinical signs in tortoises

- Ulcerative to diphtheroid-necrotizing stomatitis and glossitis
- Lethargy and anorexia
- Rhinitis
- CNS signs

Source: S. McArthur

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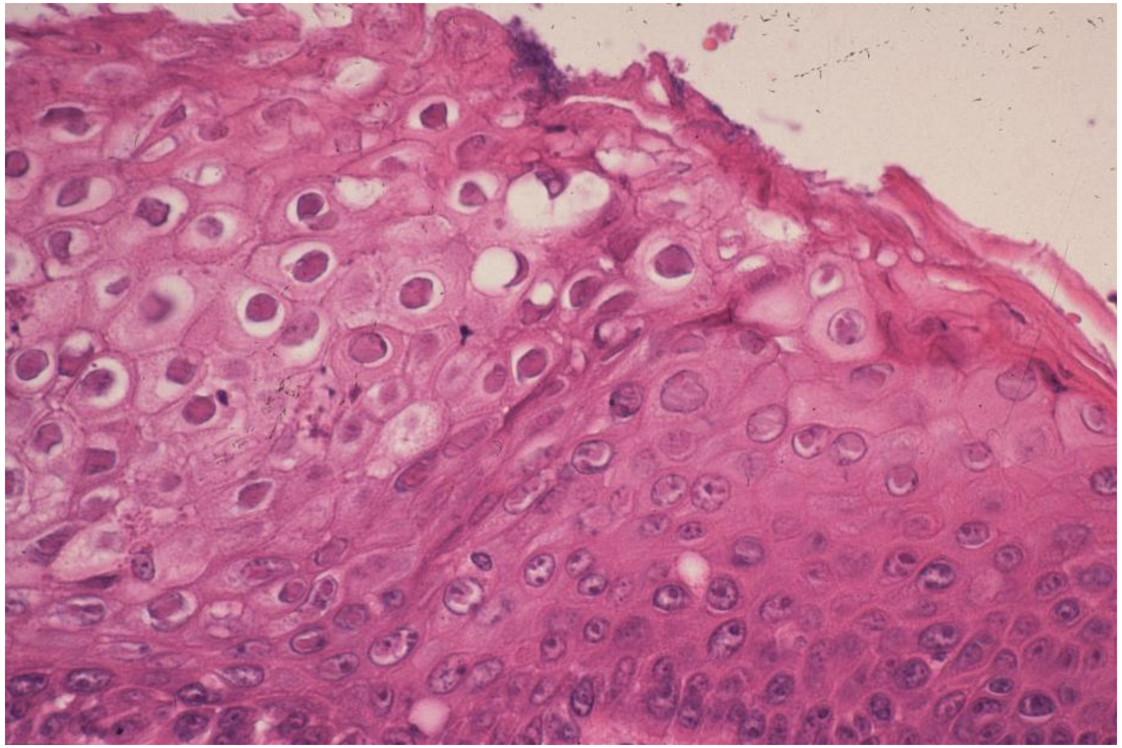
Herpesviruses: Histology

- Eosinophilic, intranuclear inclusion bodies frequently found in:
  - Epithelial cells in the tongue, trachea, and lung
  - Can also be found in: brain, stomach, large and small intestine, cloaca, liver



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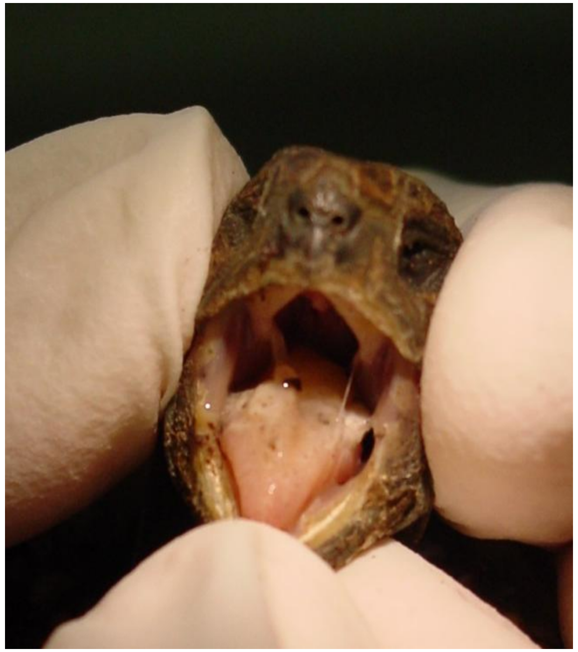
Herpesvirus types

- TeHV1
  - Found mostly in Russian tortoises
  - Incidence in veterinary practice in Europe increasing
  - Disease in sporadic cases, some outbreaks
    - Stomatitis, lethargy, anorexia
    - Relatively low morbidity in some cases
    - Mortality can be high

Herpesvirus types

- TeHV2:
  - First described in a California desert tortoise in the USA (Johnson et al., 2005)
  - Clinical signs:
    - Anorexia, lethargy, caseous plaques on the tongue and palate
  - Also found in a *Gopherus berlandieri* in Europe (Leineweber et al., 2021)

Herpesvirus types



- TeHV3:
  - Commonly found in tortoises in Europe
  - Wide host range
  - *T. graeca* and *T. marginata* relatively resistent to disease
  - Species: *Scutavirus testudinidalpha3*

JVI
Journals.ASM.org

The Genome of a Tortoise Herpesvirus (Testudinid Herpesvirus 3) Has a Novel Structure and Contains a Large Region That Is Not Required for Replication *In Vitro* or Virulence *In Vivo*

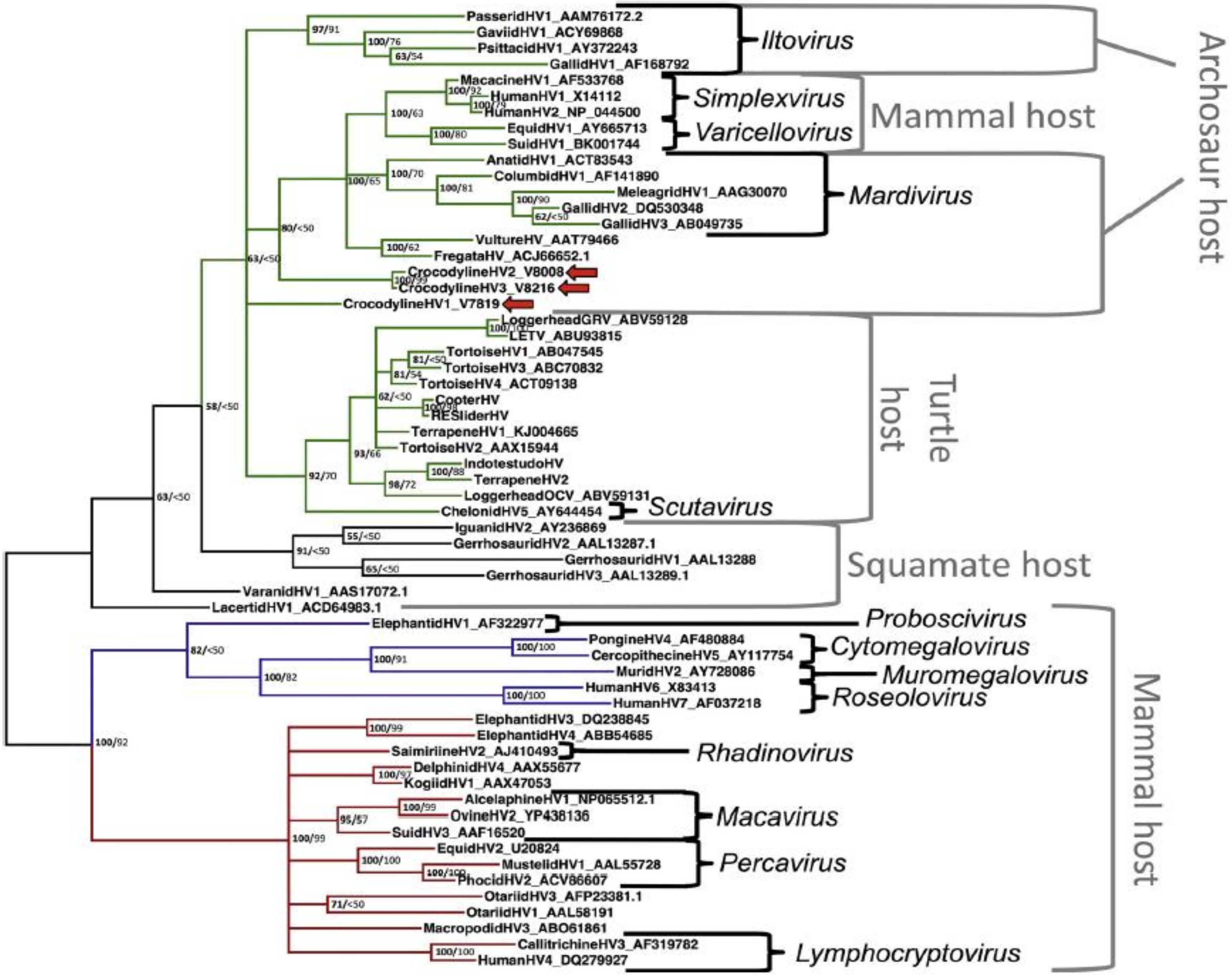
Frédéric Gandar,[a,b] Gavin S. Wilkie,[c] Derek Gatherer,[d] Karen Kerr,[c] Didier Marlier,[b] Marianne Diez,[e] Rachel E. Marschang,[f] Jan Mast,[g] Benjamin G. Dewals,[a] Andrew J. Davison,[c] Alain F. C. Vanderplasschen[a]

Herpesvirus types

- TeHV4 in a bowsprit tortoise (*Chersina angulata*):
  - Bicknese et al., 2005
  - Imported into the USA from South Africa
  - No disease observed in the infected tortoise
    - Virus detected during routine quarantine exam
- Case in a leopard tortoise (*Stigmochelys pardalis*) in Germany
  - Kolesnik et al., 2016
  - Sudden deaths in a mixed species collection
  - URTD, mycoplasma also detected
  - Detected in additional cases since then

Herpesvirus phylogenetics and co-evolution

Hyndman et al., 2015.
Vet Microbiol



Herpesvirus diagnosis

- Direct virus detection: virus or parts of virus present in clinical samples

and/or

- Indirect: Immune response
  - In reptile medicine = serology = detection of antibodies against the virus

Herpesvirus detection

- PCR
  - Can be done using oral swabs and biopsies from live animals or tissues from dead animal
    - ➢Tongue, esophagus, trachea, small intestine
  - DNA from formalin-fixed tissues
  - Pan-herpesvirus PCR often used for the detection of herpesviruses in reptiles (VanDevanter et al., 1996)

Herpesvirus: Diagnostic samples

- Diagnostic testing in our lab shows:
  - 6.5% of chelonians tested HV positive (Leineweber et al. 2021)
    - Testudinid herpesviruses:
      - 45.83% TeHV1, 0.32% TeHV2, 49.4% TeHV3, 0.96% TeHV4
    - Aquatic turtles:
      - 2.99% of sliders tested positive
      - 23.33% of box turtles tested positive

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Herpesvirus serology

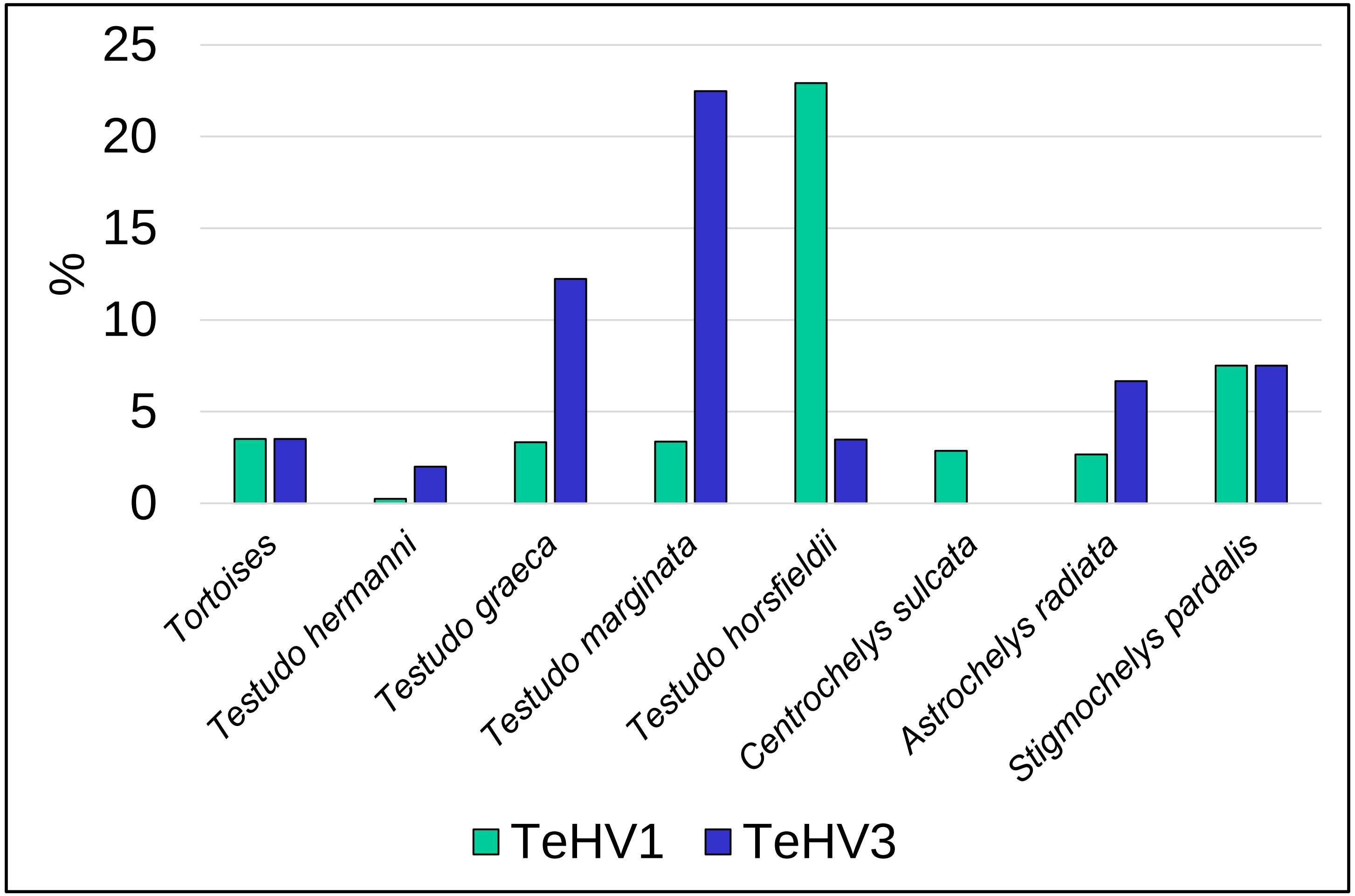
- Virus neutralization test (VNT)
  - Most commonly used
  - Slow (about 2 weeks)
  - Problems can occur with toxic sera

Herpesvirus serology

- General problems:
  - Existence of different serotypes
    - Only European tortoise herpesviruses have been used for serological testing so far (TeHV1 and TeHV3)
  - Differences in antibody titers between tortoise species:
    - *T. graeca* and *T. marginata* →measurable titers
    - *T. hermanni* and *T. horsfieldii* →low to non-existent titers
  - Antibody titers can sink below measurable levels periodically in positive animals

Herpesvirus serology

- Leineweber and Marschang, 2022. 1728 samples tested, 7.06% positive



Herpesvirus: Differential diagnoses

- Mycoplasma
- Ranaviruses
- Picornaviruses (Virus „X“)
- TINC

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Herpesviruses: Conclusions 1

- Appropriate diagnostic test depends on many factors:
  - Tortoise species involved
    - *T. graeca* and *T. marginata* different than *T. hermanni* and *T. horsfieldii*
  - Virus type involved
  - Health status of tortoises
  - Time post infection
- General rules:
  - Best to use at least 2 different methods for diagnosis
  - Repeat testing for negative animals

Herpesviruses: Conclusions s

- Quarantine procedures:
  - At least 6 months
  - Serological testing at least twice, at the beginning and at the end
  - Virological testing at least once at the beginning
- Rules for keeping a tortoise collection herpesvirus-free:
  - Keep different species separate
  - Keep animals in small groups
  - Use a strict quarantine procedure
  - Herpesviruses can cause latent infections
  - Diagnostic tests carried out on latently infected animals may be negative

Adenoviridae

- Nonenveloped dsDNA viruses
- Genera found in reptiles:
  - *Barthadenovirus* (previously known as *Atadenovirus*)
  - *Testadenovirus*
  - *Siadenovirus*
- Squamate reptiles:
  - Mostly barthadenoviruses
- Wide variety of adenoviruses found in chelonians:
  - *Testadenovirus*
  - *Siadenovirus*
  - *Barthadenovirus*
- Adenoviruses in crocodialians not yet characterized
- Co-evolution with hosts hypothesized

Adenoviruses

- Most often found in lizards and snakes, esp. bearded dragons
- Associated with hepatitis, enteritis, nephritis, pneumonia, regurgitation, anorexia, lethargy and CNS signs
- Liver and GI tract most often affected

Photo courtesy of Jutta Wiechert

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Adenoviruses in chelonians

- **Siadenoviruses** (Rivera et al. 2009; Schumacher et al. 2012)
  - Sulawesi tortoises (*Indotestudo forstenii*) and other species illegally imported into USA and contact animals
  - Anorexia, lethargy, ulcera on the oral mucosa, nasal and ocular discharge, diarrhea
  - High mortality rate
  - Similar virus recently discovered in multiple species of freshwater turtles in the USA with no apparent disease association

Adenoviruses in chelonians

- **Testadenoviruses:**
  - Detected in terrestrial tortoises and freshwater turtles
  - Wild animals and in managed care
  - High prevalences of around 30% reported in some cases (Doszpoly et al., 2013)
  - Possible co-evolution with chelonian hosts
  - Associated with disease in some cases
    - Hepatic lipidosis and hepatitis (Farkas and Gál 2009); Enteritis, gastritis, nephritis (Brown et al., 2014)
- **Barthadenoviruses**
  - Single case in a spur-thighed tortoise (*Testudo graeca*) in Europe (Garcia-Morante et al. 2016)
    - Poor body condition and weakness, died several days after presentation
    - Hyperplastic esophagitis and stomatitis
  - Found sporadically during diagnostic testing (Salzmann et al., 2021)

Adenoviruses

- Shed mostly through the cloaca
- Likely fecal-oral transmission
- Transmission study with liver suspension in a boa constrictor (Jacobson et al., 1985):
  - Intracoelomic inoculation
- Viruses very stable in the environment
- High prevalence in some countries

Adenoviruses

- Often detected together with other viruses
  - Dependoviruses
  - Invertebrate iridoviruses
  - Ferlaviruses
  - Reoviruses

  and/or with protozoa (coccidia) and/or with bacteria
- Primary pathogenic role?

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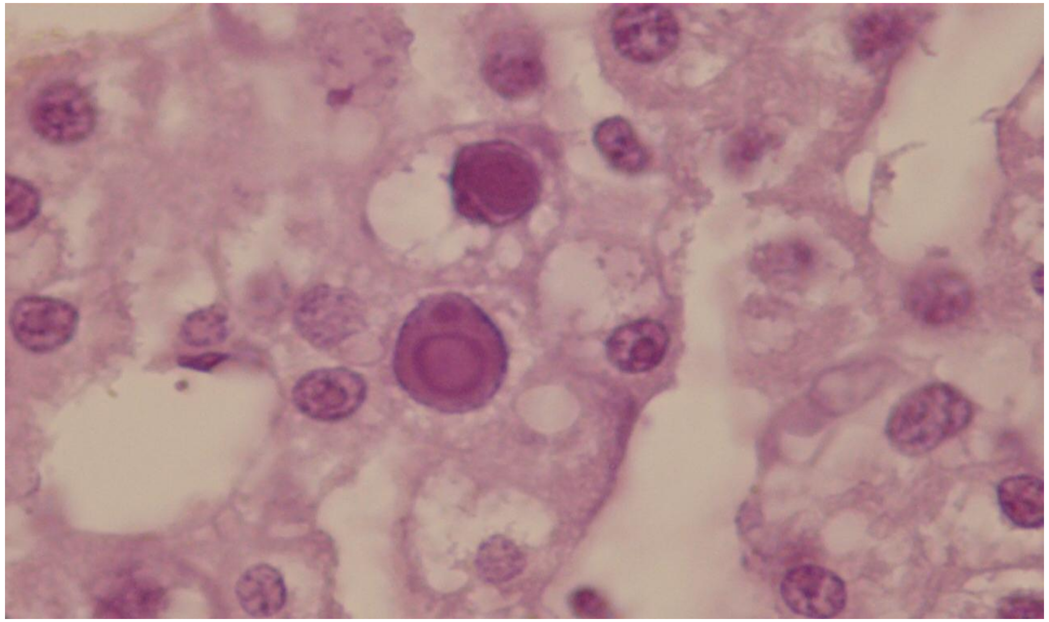
Adenoviruses: Diagnosis

- Histology
  - Large intranuclear inclusion bodies
- Virus detection:
  - Pan-adenovirus PCR (Wellehan et al., 2004)
  - Strain or species specific PCRs
- Genetically diverse adenoviruses found in reptiles
- Samples:
  - Cloacal swabs
  - Liver, intestine



juvenile bearded dragon, liver

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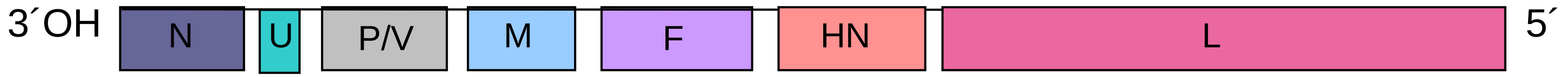
Ferlaviruses: History

- First described 1976 by Fölsch and Leloup
- Snake farm in Switzerland
- Dyspnea, lethargy and death in fer-de-lance (*Bothrops atrox*)
- Kurath et al. (2004) proposed the classification of FDLV in the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, in a new genus (*Ferlavirus*), species *Ferlavirus reptilis*

Bothrops moojeni

FDLV: 15378 nt



Ferlaviruses

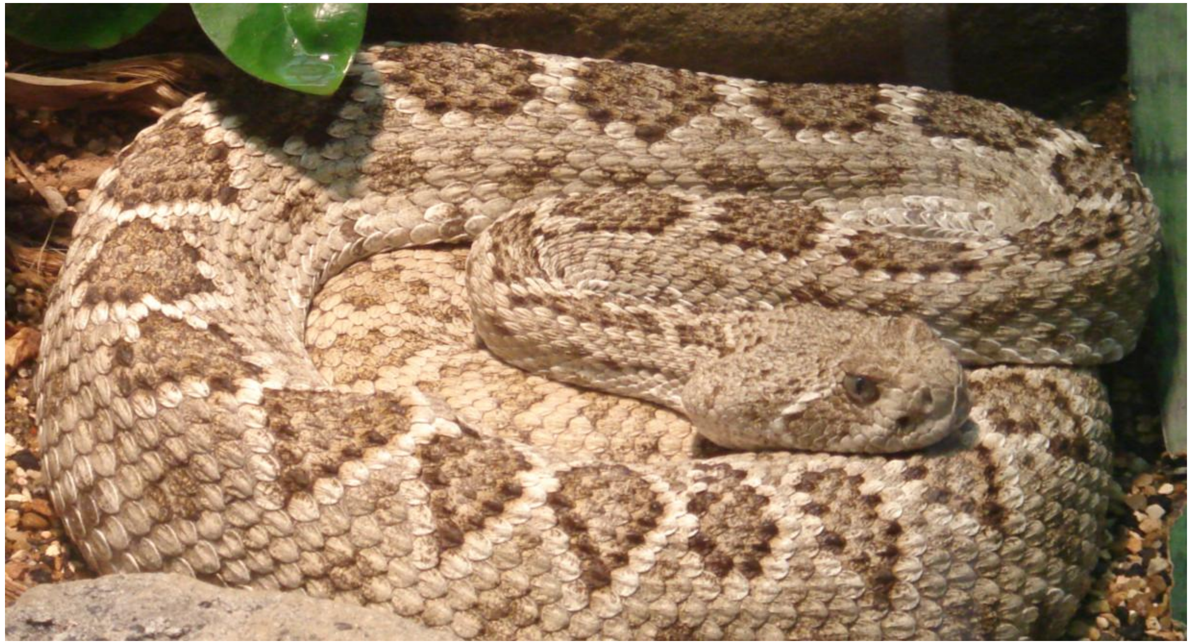
- Most commonly involve the respiratory system and CNS
  - Gaping of the mouth
  - Mucus or blood in the oral cavity
  - Gagging, regurgitation
  - Convulsions, abnormal behavior, loss of equilibrium, opisthotonus

Source: E.R. Jacobson, Univ. Florida Gainesville

Photo source: Jutta Wiechert

Host Range

- In snakes, PMV infections have most commonly been found in viperids
- Also described in colubrids, elapids, and boids
- Also found sporadically in lizards and chelonians



Host Range

- Found in captive populations
- Also detected in wild and wild-caught animals
  - A *Bothrops jararaca* from Brazil
  - Caiman lizards (*Dracaena guiaensis*) from Peru
- Serological evidence for PMV infection found in several South and Central American countries
  - Wild lizards in Honduras (Gravendyck et al., 1998):
    - *Iguana iguana*, *Ctenosaura similis*, *Ctenosaura bakeri*
  - Wild-caught lizards from Mexico (Marschang et al., 2002):
    - *Xenosaurus grandis*, *Xenosaurus platyceps*, *Abronia graminea*

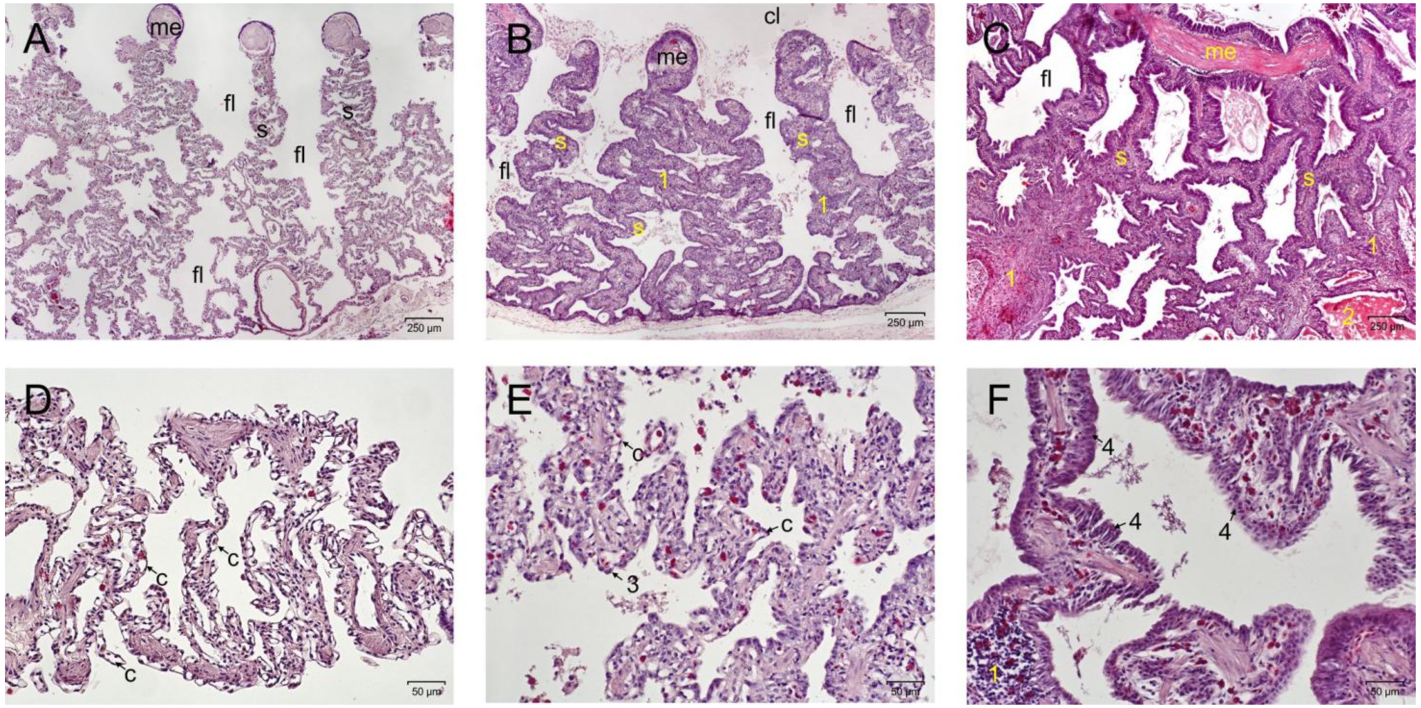
Pathology

- Congested, thickened and oedematous lungs
- Serous exudate to caseous necrotic cellular debris within the lumen of the lungs and air sacs
- Changes in liver and pancreas also possible
  - Hepatomegaly
  - White nodules in the hepatic parenchyma and enlargement of the pancreas

Histology

- Proliferative interstitial pulmonary disease
- Pulmonary epithelial cells may contain eosinophilic intracytoplasmic inclusions
- Cellular debris and exudate
- Alveolar type II cells lining the primitive alveoli undergo hypertrophy, hyperplasia and metaplasia

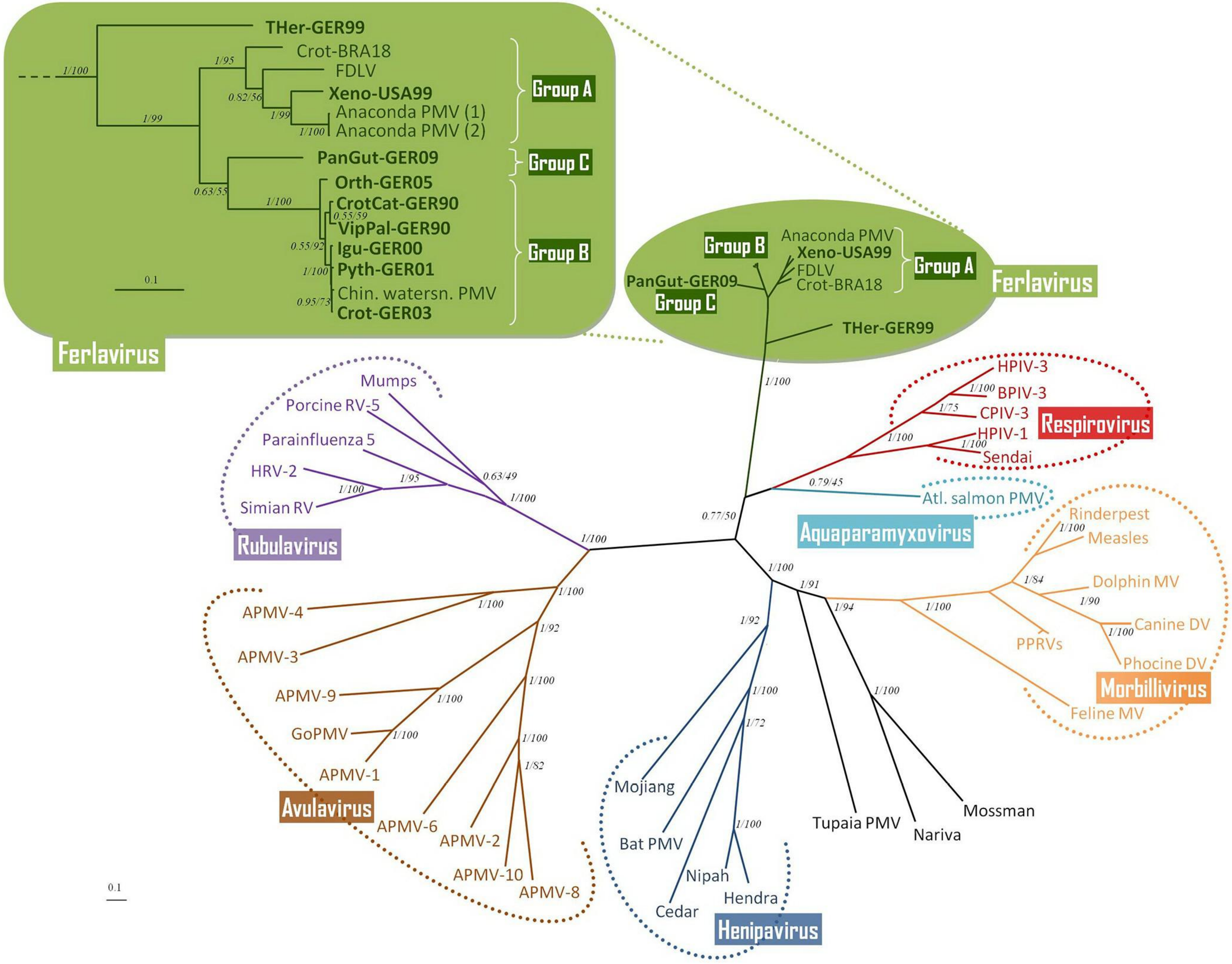
Control animal | Group A | Group B



Source: Pees et al., 2019. PLoS One

Genogroups

- A, B, C, and „tortoise“
  - A and B found in snakes, lizards, and chelonians
  - C found in snakes and lizards
  - „tortoise“ found in one tortoise with pneumonia
- All species *Ferlavirus reptilis*



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Transmission studies

- Koch‘s postulates fulfilled for 1 isolate in rattlesnakes (Jacobson et al., 1997)
- Transmission studies with corn snakes
  - Genotype A, B, and C isolates, intratracheal infection
  - Pathogenesis, virus detection, immunological reaction, immunization

Trial group	Isolate	Geno-group	Host species common name	Host species scientific name	Reference
1	Xeno-USA99	A	Flathead knob-scaled lizard	*Xenosaurus platyceps*	Marschang et al., 2009
2	Crot-GER03	B	Timber rattlesnake	*Crotalus horridus*	Marschang et al., 2009
3	PanGut-GER09	C	Corn snake	*Pantherophis guttatus*	Abbas et al., 2011

PLOS | ONE

RESEARCH ARTICLE

Three genetically distinct ferlaviruses have varying effects on infected corn snakes (*Pantherophis guttatus*)

Michael Pees[1]*, Volker Schmidt[1], Tibor Papp[2], Ákos Gellért[2], Maha Abbas[3¤], J. Matthias Starck[4], Annkatrin Neul[1], Rachel E. Marschang[5]

Immunologic responses in corn snakes (*Pantherophis guttatus*) after experimentally induced infection with ferlaviruses

Annkatrin Neul Med Vet
Wieland Schrödl PD, Dr Med Vet
Rachel E. Marschang PD, Dr Med Vet
Tina Bjick Med Vet
Uwe Truyen Prof Dr Med Vet
Heiner von Buttlar Dr Med Vet
Michael Pees Prof Dr Med Vet

OBJECTIVE
To measure immunologic responses of snakes after experimentally induc
infection with ferlaviruses.

ANIMALS
42 adult corn snakes (*Pantherophis guttatus*) of both sexes.

PROCEDURES
Snakes were inoculated intratracheally with genogroup A (n = 12), B (12
or C (12) ferlavirus (infected groups) or cell-culture supernatant (6; co
trol group) on day 0. Three snakes from each infected group were euth

Transmission study: ball pythons

- Same experimental set up
- Inoculated with 1ml of the same genogroup B isolate – same passage, same titer
- Comparison of clinical signs, pathological changes and immunological reaction

animals

MDPI

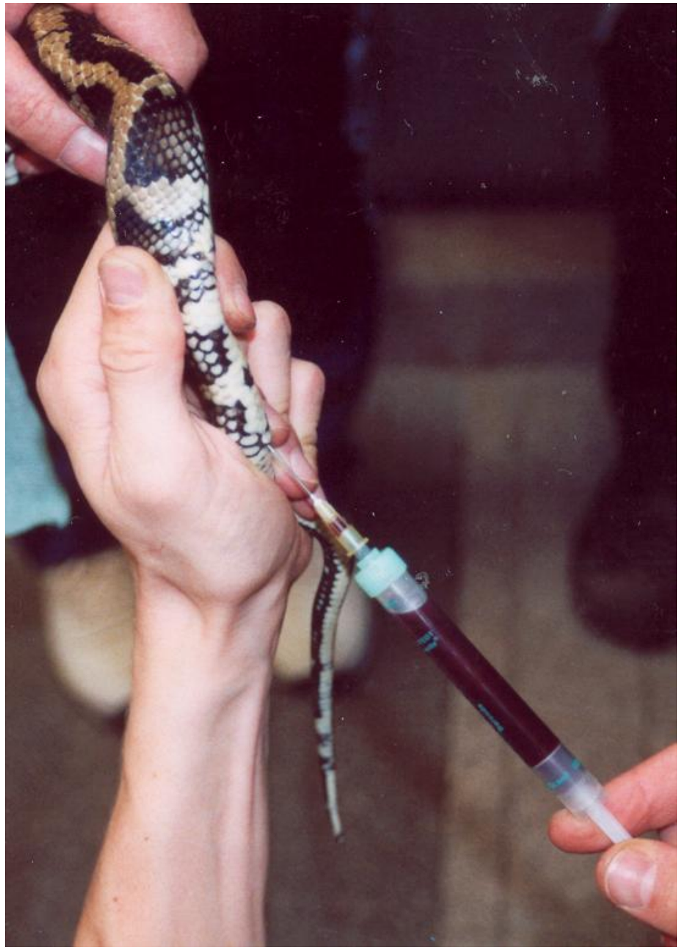
Article

The Role of Host Species in Experimental Ferlavirus Infection: Comparison of a Single Strain in Ball Pythons (*Python regius*) and Corn Snakes (*Pantherophis guttatus*)

Michael Pees [1,*], Annkatrin Möller [2], Volker Schmidt [3], Wieland Schroedl [4] and Rachel E. Marschang [5]

Diagnosis

- Virus detection
  - Immunohistochemistry
  - in situ hybridization
  - Virus isolation
  - RT-PCR
- Serological tests:
  - HI, virus neutralization



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Virus detection

- RT-PCR most commonly used, virus isolation also described occasionally
- Samples:
  - In live animals:
    - oral and cloacal swabs, tracheal wash
  - In dead animals:
    - lung, kidney, intestine and liver

Kolesnik *et al. BMC Veterinary Research* (2019) 15:281
https://doi.org/10.1186/s12917-019-2028-0
BMC Veterinary Research

RESEARCH ARTICLE Open Access

Check for updates

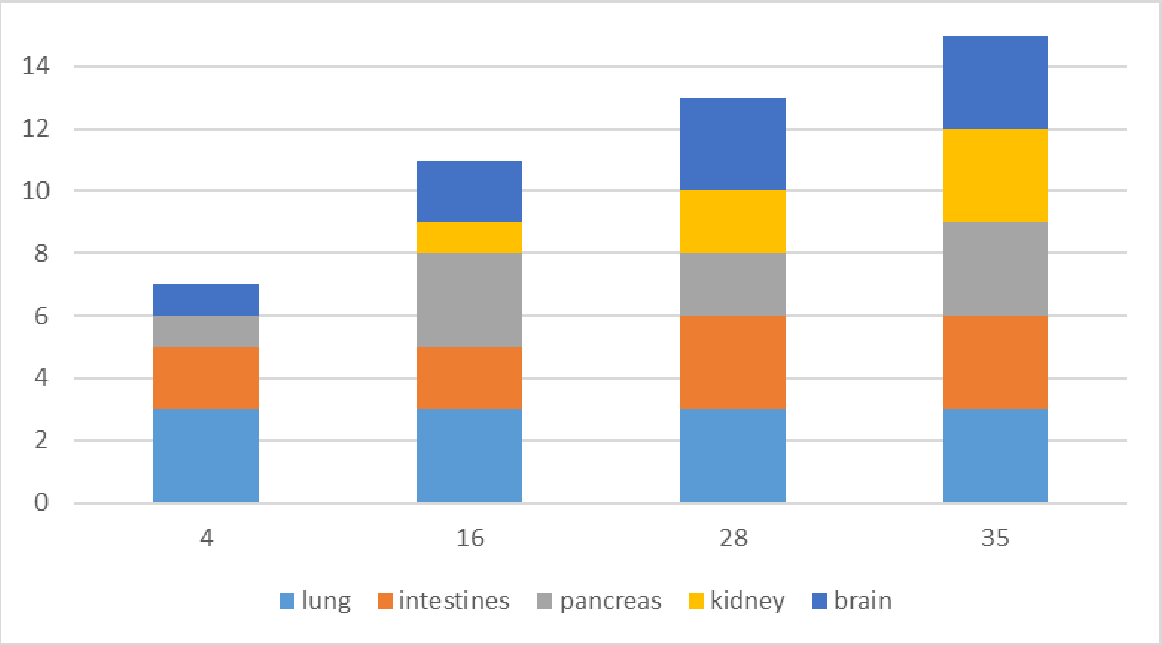
Comparison of three different PCR protocols for the detection of ferlaviruses

Ekaterina Kolesnik[1], Timothy H. Hyndman[2], Elisabeth Müller[1], Michael Pees[3] and Rachel E. Marschang[1*]

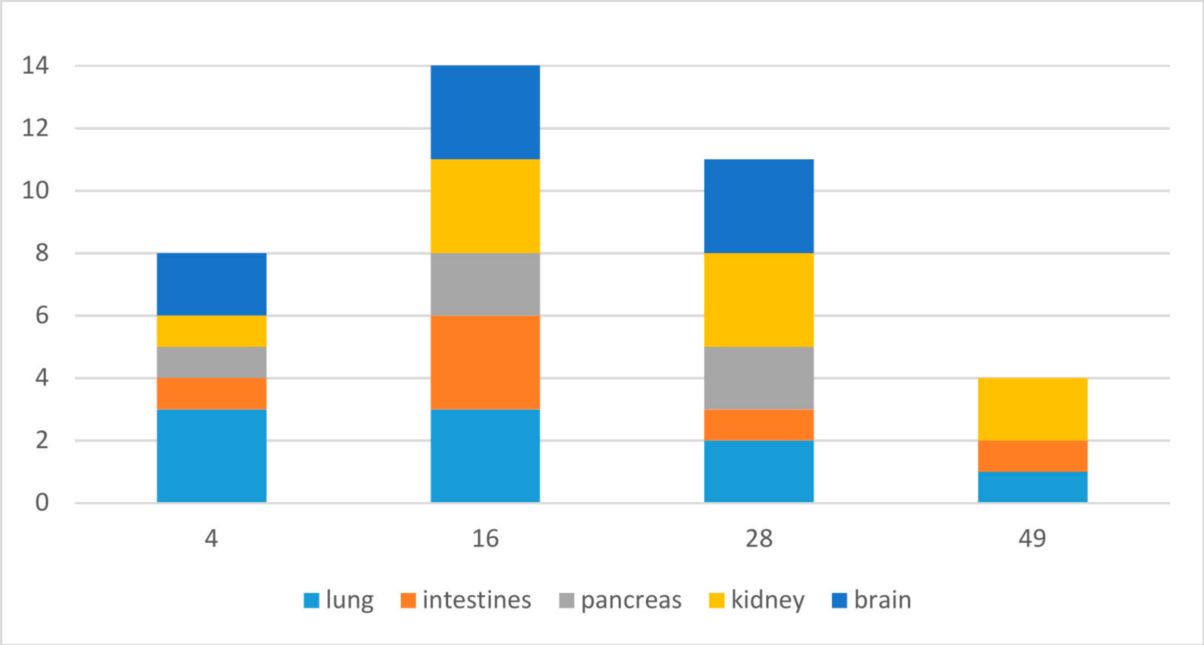
Ball pythons vs. corn snakes

- Virus detection

Corn snakes



Ball pythons



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Virus detection

- RT-PCR detection in live and necropsied animals:
  - Indication that tracheal washes are not always sufficient for virus detection

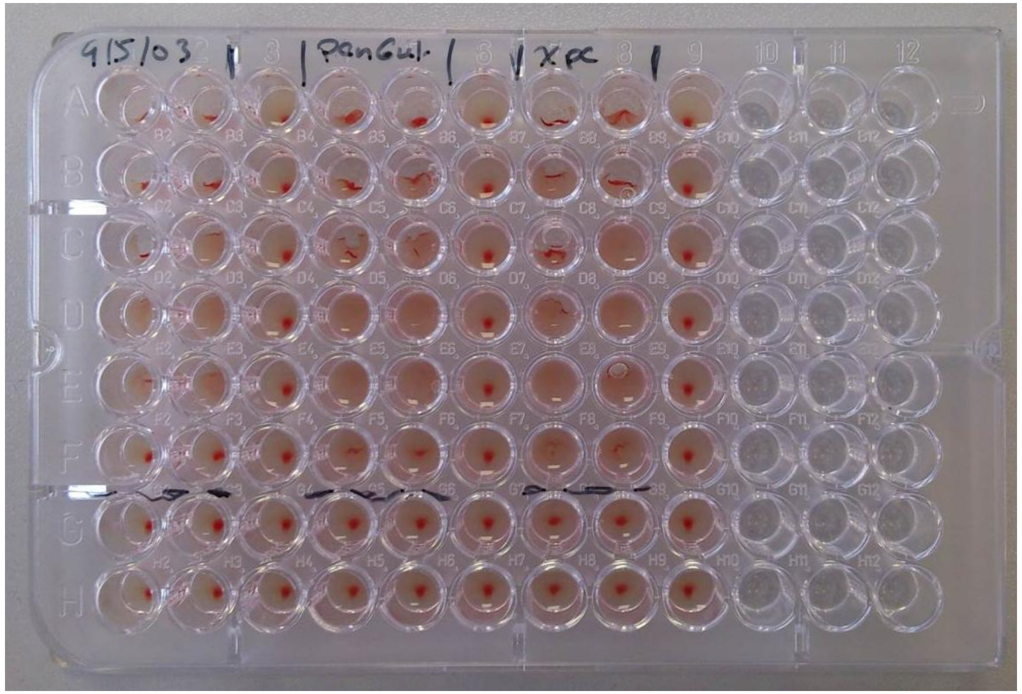
Journal of Zoo and Wildlife Medicine 49(4): 983–995, 2018

FERLAVIRUS-RELATED DEATHS IN A COLLECTION OF VIPERID SNAKES

Edmund J. Flach, M.A., Vet.M.B., Dipl.Zoo Med. (Mammals), Dipl.E.C.Z.M. (Zoo Health Management), Mark P. Dagleish, B.V.M. & S., Ph.D., F.R.C.Path., Yedra Feltrer, D.M.V., M.Sc., Dipl.E.C.Z.M. (Zoo Health Management), Iri S. Gill, H.E.Diploma Zool., Rachel E. Marschang, P.D. Dr.Med.Vet., FTÄ Mikrobiologie, Dipl.E.C.Z.M. (Herpetology), Nic Masters, M.A., Vet.M.B., Dipl.E.C.Z.M. (Zoo Health Management), Jorge Orós, D.V.M., Ph.D., Dipl.E.C.Z.M. (Wildlife Population Health, Herpetology), Ann Pocknell, D.V.M., M.Vet.Sci., Dipl.R.C.Path., Dipl.A.C.V.P., Matthew E. Rendle, Dipl. Vet. Nursing, Taina Strike, B.V.Sc., M.Sc., Dipl.E.C.Z.M. (Zoo Health Management), Benjamin Tapley, B.Sc., M.Sc., and Nick M. Wheelhouse, B.Sc., Ph.D.

Serological tests

- Seroconversion takes about 4-8 weeks
- HI:
  - Uses the hemagglutinating properties of the virus



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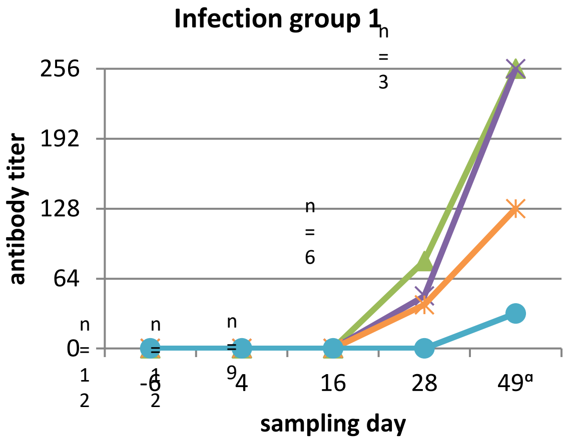
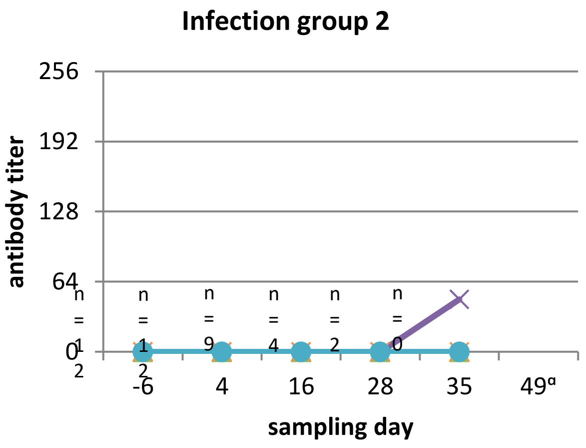
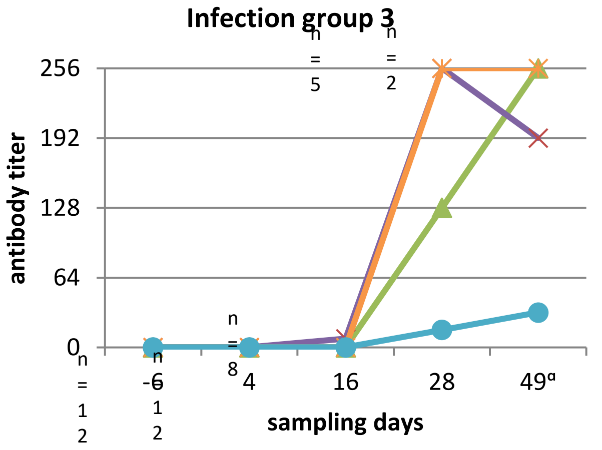
Ball pythons vs. corn snakes

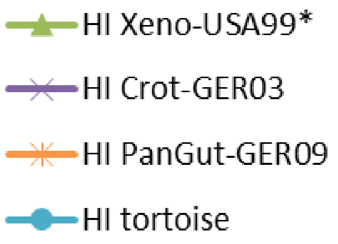
HIs:

Corn snakes

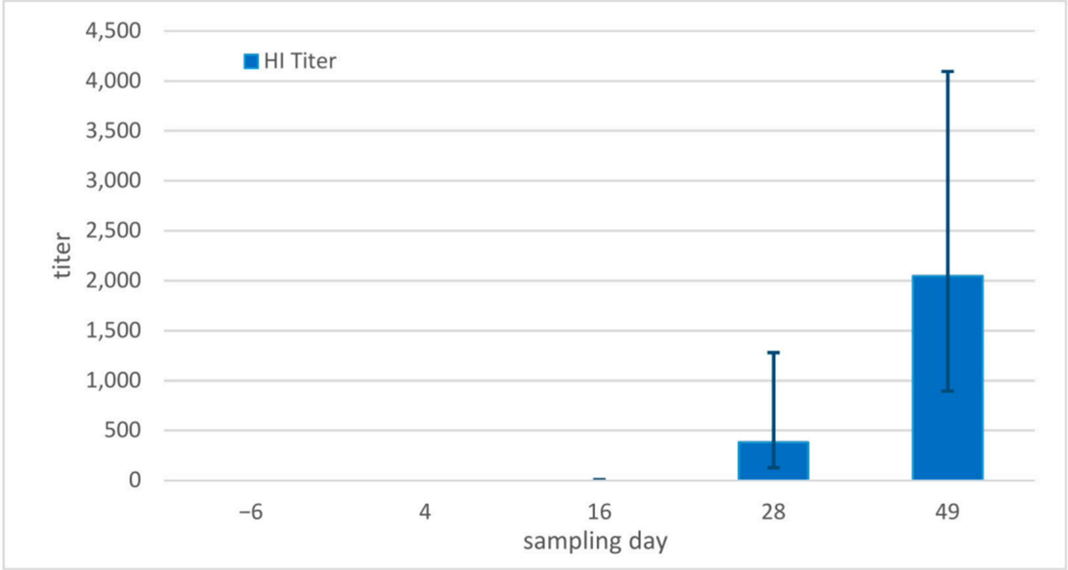








Ball pythons



Ferlavirus Serology

- Studies in the USA have shown that different laboratories can provide different results (Allender et al., 2008)
  - Different isolates?
  - Different cut-offs?
  - Repeatability and reproducibility of the results?

Journal of Zoo and Wildlife Medicine 39(3): 358–361, 2008

MEASURING AGREEMENT AND DISCORD AMONG HEMAGGLUTINATION INHIBITION ASSAYS AGAINST DIFFERENT OPHIDIAN PARAMYXOVIRUS STRAINS IN THE EASTERN MASSASAUGA (*SISTRURUS CATENATUS CATENATUS*)

Matthew C. Allender, D.V.M., M.S., Mark A. Mitchell, D.V.M., M.S., Ph.D., Michael J. Dreslik, M.S., Ph.D., Christopher A. Phillips, M.S., Ph.D., and Val R. Beasley, D.V.M., Ph.D., Dipl. A.B.V.T.

Ferlavirus Serology

- Interpretation:
  - Development of antibodies takes several weeks
  - Results dependent on virus, host, environment
  - Not all virus positive animals will develop measurable antibodies

- Carrier status

Infection, Genetics and Evolution 51 (2017) 239–244

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

ELSEVIER

Research paper

Anaconda paramyxovirus infection in an adult green anaconda after prolonged incubation: Pathological characterization and whole genome sequence analysis

CrossMark

Patrick C.Y. Woo [a,b,c,d,*,1], Paolo Martelli [e,1], Suk-Wai Hui [e], Candy C.Y. Lau [b], Joseph M. Groff [f], Rachel Y.Y. Fan [b], Susanna K.P. Lau [a,b,c,d,*], Kwok-Yung Yuen [a,b,c,d]

Treatment and prevention

- No treatment for PMV known
- Antibiotic therapy may be helpful in reducing secondary bacterial infections
- In an outbreak
  - Reduce contact between snakes
  - Divide large collections into groups
  - Disinfection of cages of dead snakes with virucidal disinfectant (e.g. 0.15% sodium hypochlorite)
  - Allow at least 2 months following last death before adding new snakes to a collection
- Quarantine: Some sources recommend at least 90 days

Differential diagnoses

- Many different factors can lead to pulmonary disease in snakes
  - Bacteria:
    - Gram-negative organisms (*Pseudomonas* spp., *Providencia* spp., *Proteus* spp., *Salmonella* spp., *Aeromonas hydrophila* and *E. coli*) most commonly isolated bacteria from the respiratory tract of reptiles with clinical signs of pneumonia
    - *Mycoplasma* are discussed as a cause or contributor to respiratory disease in various snake species
  - Various fungi
  - Paraisties
    - e.g. *Rhabdias*, pentastomids, and others
  - Viruses:
    - Reoviruses have been associated with respiratory disease in some snakes
    - Adenoviruses
    - Sunshinevirus in pythons (mostly in Australia)
    - Serpentoviruses (order Nidovirales) in pythons (and others)
    - Reptarenaviruses (mostly in boas)

Nidovirales

RESEARCH ARTICLE

CrossMark
click for updates

Ball Python Nidovirus: a Candidate Etiologic Agent for Severe Respiratory Disease in *Python regius*

Mark D. Stenglein,[a] Elliott R. Jacobson,[b] Edward J. Wozniak,[c] James F. X. Wellehan,[d] Anne Kincaid,[e] Marcus Gordon,[f] Brian F. Porter,[g] Wes Baumgartner,[h] Scott Stahl,[i] Karen Kelley,[j] Jonathan S. Towner,[k] Joseph L. DeRisi[a,l]

Photos source: Laura Hoon-Hanks, Colorado State University, USA

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Serpentoviruses and disease

- Primary pathogens:
  - Outbreaks of respiratory disease
- Chronic, sublinical infections also described
- Possible seasonal variation in prevalence

Contents lists available at ScienceDirect

Virology

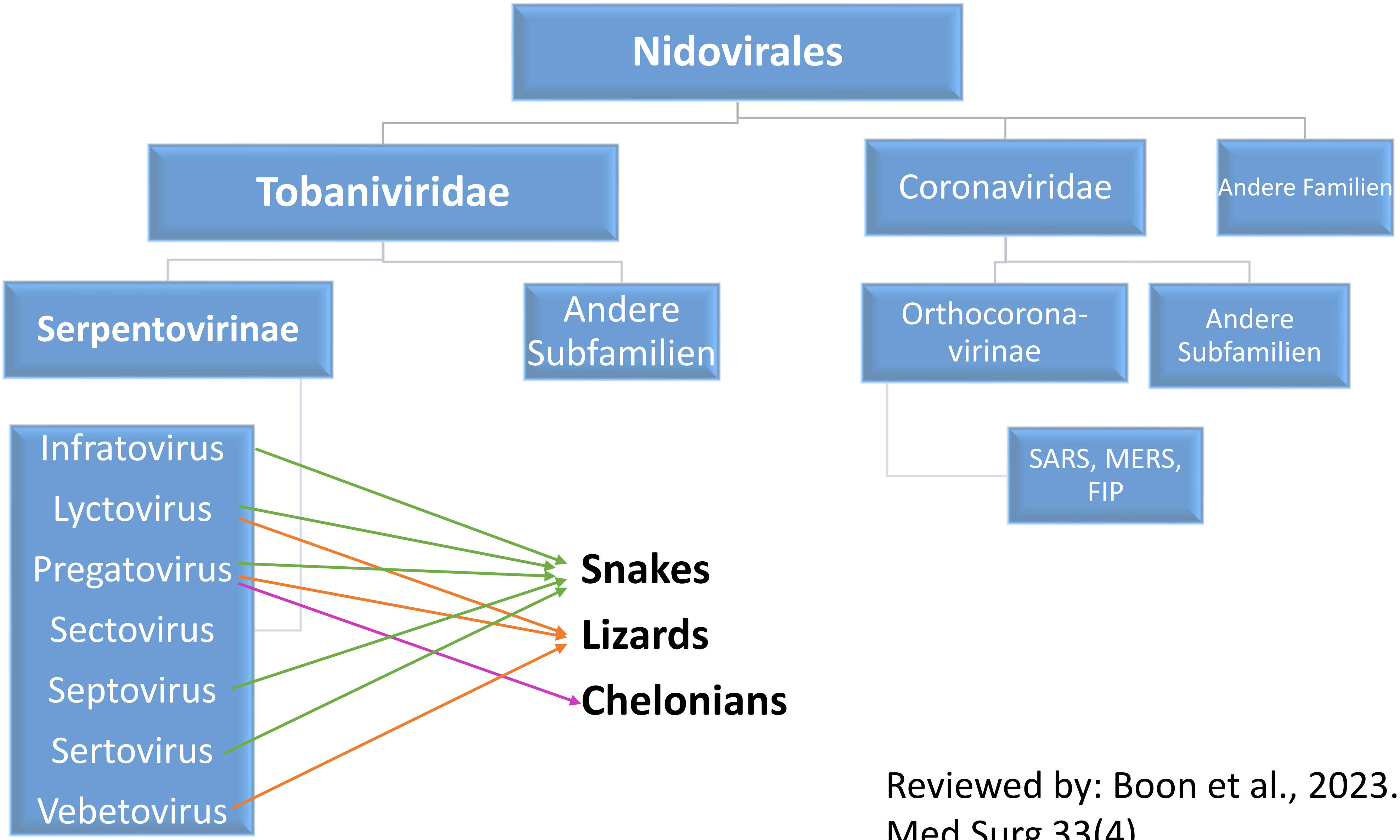
journal homepage: www.elsevier.com/locate/virology

Respiratory disease in ball pythons (*Python regius*) experimentally infected with ball python nidovirus

Laura L. Hoon-Hanks[a,*], Marylee L. Layton[a], Robert J. Ossiboff[b,1], John S.L. Parker[c], Edward J. Dubovi[b], Mark D. Stenglein[a,**]

[a] Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA
[b] Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
[c] Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Nidovirales Taxonomy – highly simplified!



Reviewed by: Boon et al., 2023. J Herpetol Med Surg 33(4)

Serpentoviruses in snakes

- Found in the following families (Hoon-Hanks et al., 2018; Boon et al., 2023; Tillis et al., 2024):
    - Pythonidae
    - Boidae
    - Colubridae
    - Homalopsidae
    - Viperidae
    - Elapidae
- Most commonly found in:
    - Ball pythons
    - *Morelia* spp.

➢ Esp. genus *Pregatovirus*, subgenus *Roypretovirus*

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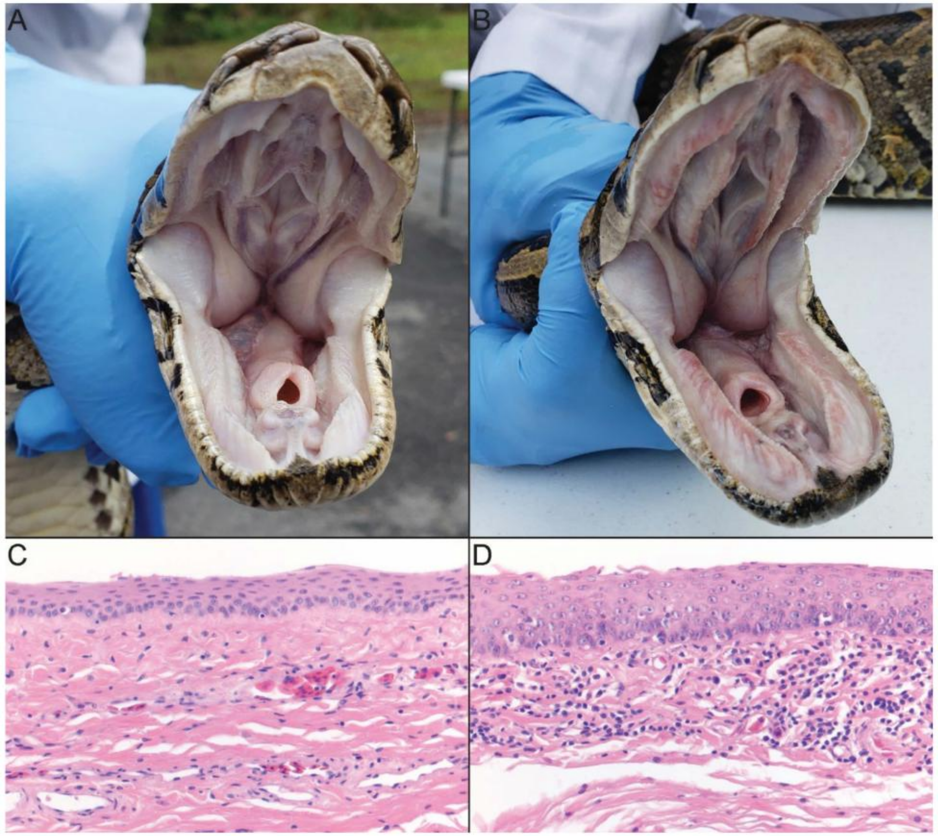
Serpentoviruses: Host species

- Genetically diverse serpentoviruses found in snakes in Florida (Tillis et al., 2022)
  - 27.8% of 318 free-ranging invasive Burmese pythons tested
  - No python serpentoviruses found in native snakes tested (n = 219)
  - Divergent serpentoviruses found in 2.3% of the native snakes tested



viruses

MDPI

Article

Divergent Serpentoviruses in Free-Ranging Invasive Pythons and Native Colubrids in Southern Florida, United States

Steven B. Tillis [1], Jillian M. Josimovich [2,†], Melissa A. Miller [3,‡], Laura L. Hoon-Hanks [4,§], Arik M. Hartmann [5], Natalie M. Claunch [6,7], Marley E. Iredale [1], Tracey D. Logan [1], Amy A. Yackel Adams [8], Ian A. Bartoszek [9], John S. Humphrey [10], Bryan M. Kluever [10], Mark D. Stenglein [4], Robert N. Reed [8,‖], Christina M. Romagosa [6], James F. X. Wellehan, Jr. [1] and Robert J. Ossiboff [1,*]

Serpentoviruses: Host species

- Related virus described in skinks in Australia with „bobtail flu“ (O‘Dea et al., 2016)
- Low prevalence (1-3%) reported in free-ranging shingleback skinks (O‘Reilly et al, 2024)
- Also found in shinglebacks in Europe

Source: Karina Mathes, Hannover

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Serpentoviruses: Host species

- Novel serpentoviruses and an orthoreovirus found in juvenile vieled chameleons (*Chamaeleo calyptratus*) with rhinitis and pneumonia (Hoon-Hanks et al., 2020)
- Many adult chameleons positive with no clinical signs – possible age-associated predisposition to morbidity and mortality

Serpentoviruses: Host species

- Disease outbreak in Bellinger River turtles (*Myuchelys georgesi*) → Bellinger River virus
  - Poor body condition, swollen eyelids, skin lesions, splenic and nephric inflammation and necrosis
  - Severe disease outbreak, very high mortality rate

https://www.flickr.com/photos/48646673@N07/16586164465/in/album-72157648599063903/

PLOS | ONE

RESEARCH ARTICLE

Identification of a novel nidovirus as a potential cause of large scale mortalities in the endangered Bellinger River snapping turtle (*Myuchelys georgesi*)

Jing Zhang[1☯], Deborah S. Finlaison[1], Melinda J. Frost[1☯], Sarah Gestier[1☯], Xingnian Gu[1], Jane Hall[2], Cheryl Jenkins[3], Kate Parrish[1], Andrew J. Read[1☯], Mukesh Srivastava[1], Karrie Rose[2], Peter D. Kirkland[1]*

1 Virology Laboratory, Elizabeth Macarthur Agriculture Institute, Menangle, New South Wales, Australia, 2 Australian Registry of Wildlife Health, Taronga Conservation Society Australia, Bradleys Head Road, Mosman, New South Wales, Australia, 3 Microbiology and Parasitology, Elizabeth Macarthur Agriculture Institute, Menangle, New South Wales, Australia

Serpentoviruses: Host species

- Bellinger River virus
  - Led to near-total loss of mature turtles in 2015
  - Monitoring of the remaining population between 2015 and 2020:
  - Prevalence 10.8% in Bellinger River turtles, and 1.0% in sympatric Murray River turtles
  - Conjunctival swabs most sensitive samples type
  - Higher prevalence in larger turtles
  - Decreasing prevalence over time (no positives detected after 2018)
  - No clinical signs observed after outbreak

viruses

MDPI

Article

Delving into the Aftermath of a Disease-Associated Near-Extinction Event: A Five-Year Study of a Serpentovirus (Nidovirus) in a Critically Endangered Turtle Population

Kate Parrish [1,2,*], Peter Kirkland [1,2], Paul Horwood [2], Bruce Chessman [3], Shane Ruming [4], Gerry McGilvray [4], Karrie Rose [5], Jane Hall [5] and Lee Skerratt [6]

Serpentoviruses: Pythons

- Long-term study in infected collections
  - Virus detectable over extended period of time in surviving infected snakes (28 months)
  - Some virus positive animals remained clinically healthy

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 03 October 2019
doi: 10.3389/fvets.2019.00338

Check for updates

OPEN ACCESS

Edited by:
Kathryn Christine Gamble, Lincoln Park Zoo, United States

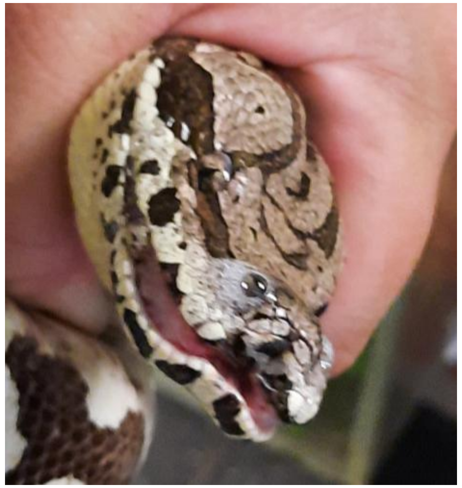
Reviewed by:

Longitudinal and Cross-Sectional Sampling of Serpentovirus (Nidovirus) Infection in Captive Snakes Reveals High Prevalence, Persistent Infection, and Increased Mortality in Pythons and Divergent Serpentovirus Infection in Boas and Colubrids

Laura L. Hoon-Hanks[1], Robert J. Ossiboff[2], Pia Bartolini[3], Susan B. Fogelson[4], Sean M. Perry[5], Anke C. Stöhr[5], Shaun T. Cross[1], James F. X. Wellehan[2], Elliott R. Jacobson[2], Edward J. Dubovi[6] and Mark D. Stenglein[1*]

Serpentoviruses: Diagnosis

- Genetically diverse group of viruses
  - Methods:
    - PCR
      - Several discribed
      - May be genus or species specific
    - NGS
      - Able to detect diverse viruses
      - Often somewhat less sensitive than PCR
    - Virus isolation



Serpentoviruses: Diagnosis

- Samples
  - In live animals:
    - Oral swabs
  - In dead animals
    - Highest viral loads found in the lungs
    - Also found in the trachea, esophagus, liver, spleen, heart, and brain

Serpentoviruses: Diagnosis

- Overview diagnostic testing of snakes in our lab

Received: 1 November 2022 | Revised: 14 December 2022 | Accepted: 23 December 2022

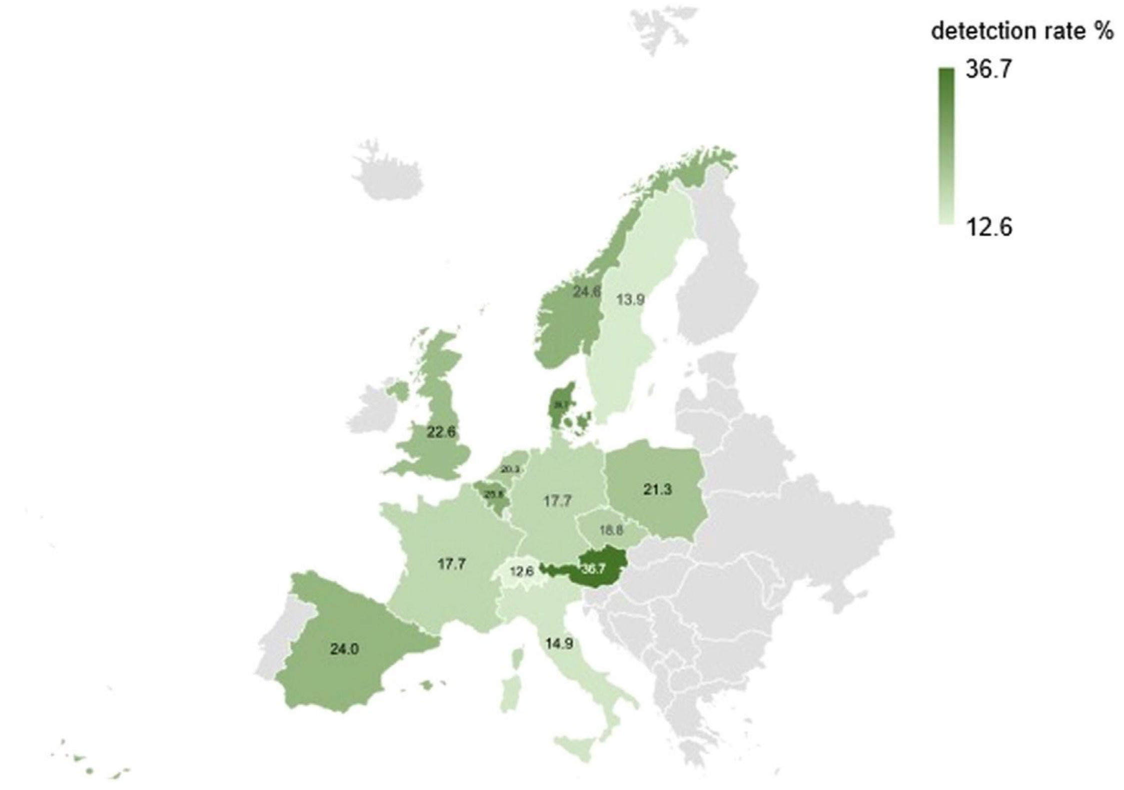
DOI: 10.1002/vetr.2588

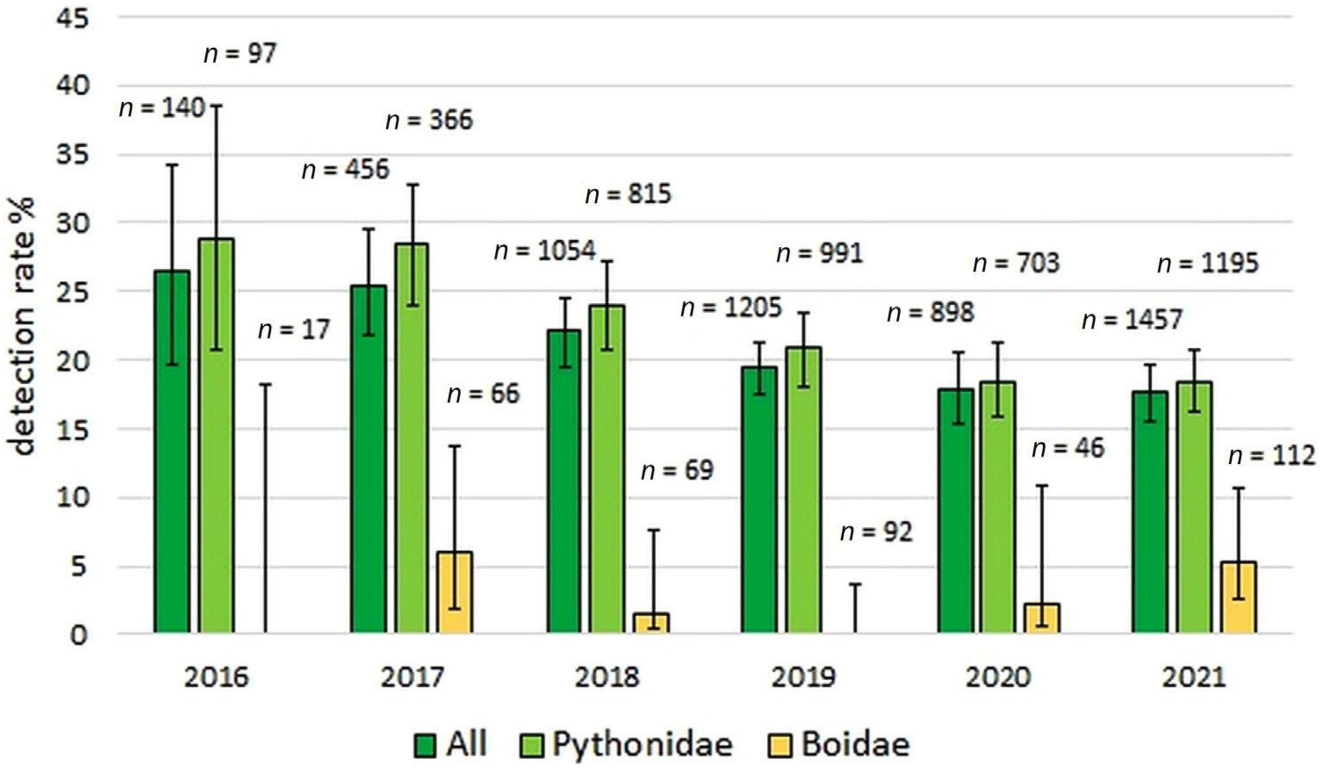
VetRecord

ORIGINAL RESEARCH

Detection of nidoviruses in samples collected from captive snakes in Europe between 2016 and 2021

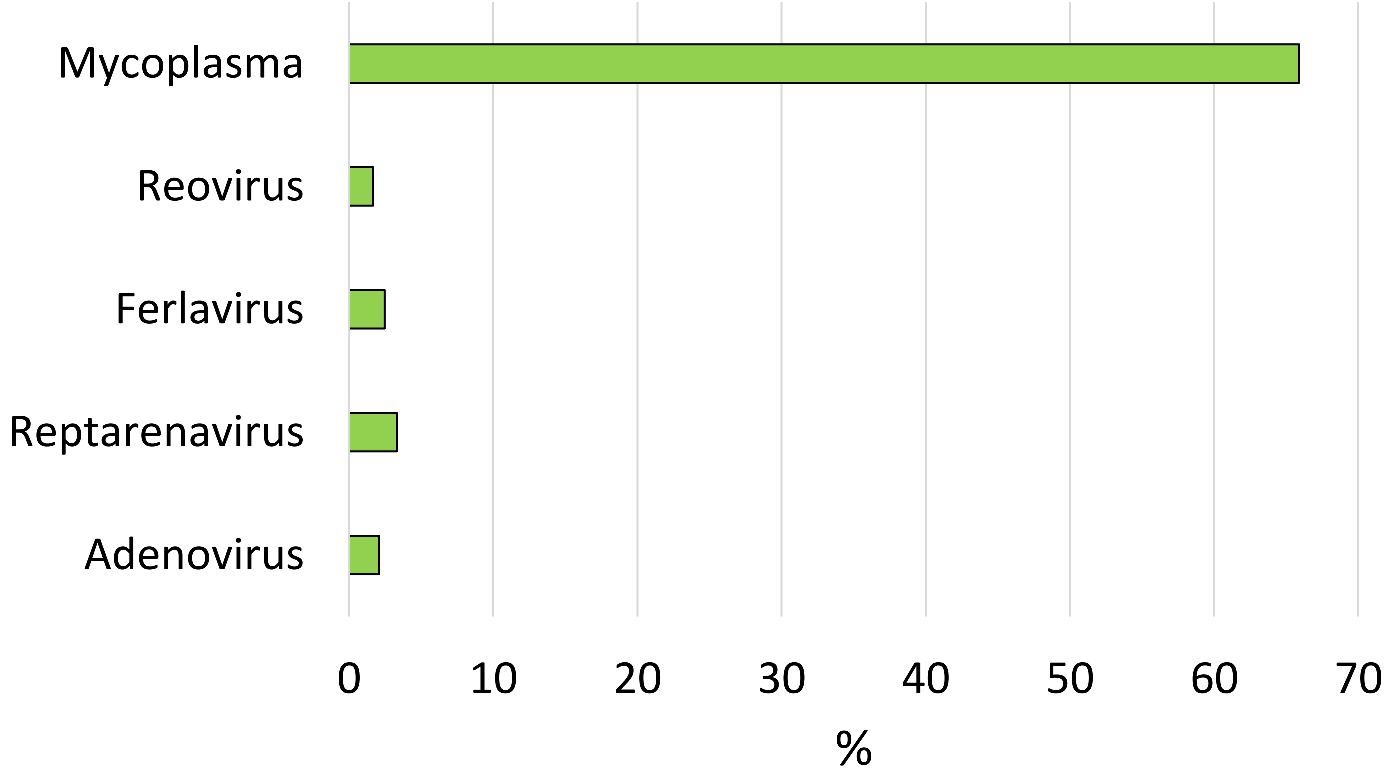
Christoph Leineweber | Rachel E. Marschang





Serpentoviruses and Other Pathogens

- Serpentoviruses also found in animals together with other potential pathogens
  - Reoviruses
  - Adenoviruses
  - Mycoplasma
  - Paramyxoviruses
  - Arenaviruses



Significant for paramyxoviruses ($p = 0.0046$) and mycoplasma ($p < 0.0001$)

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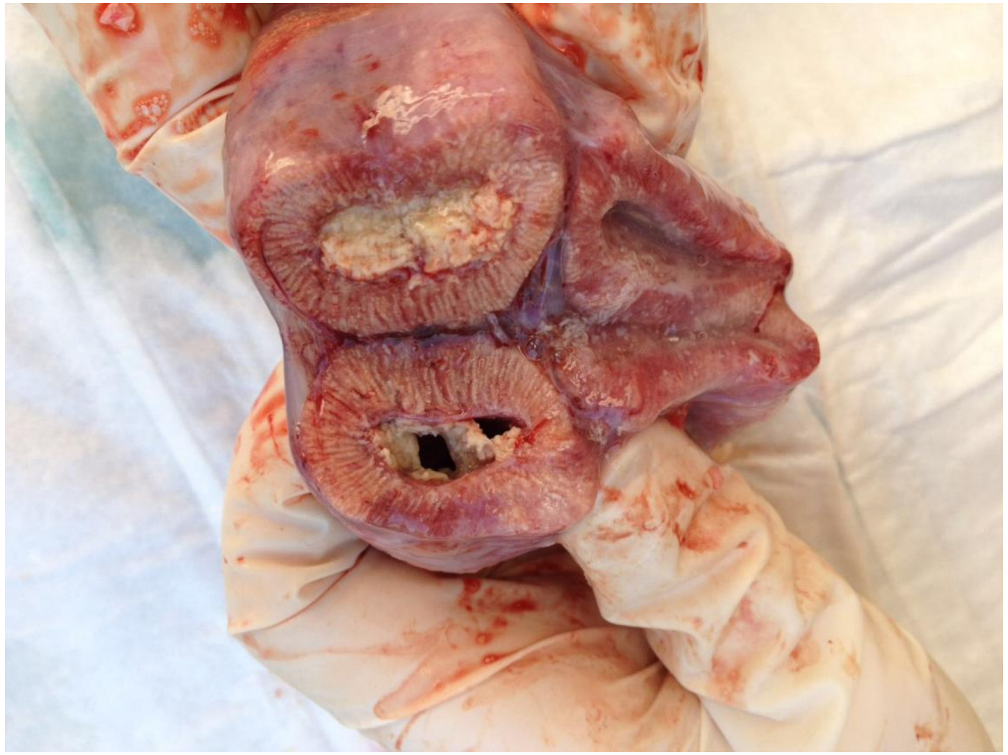
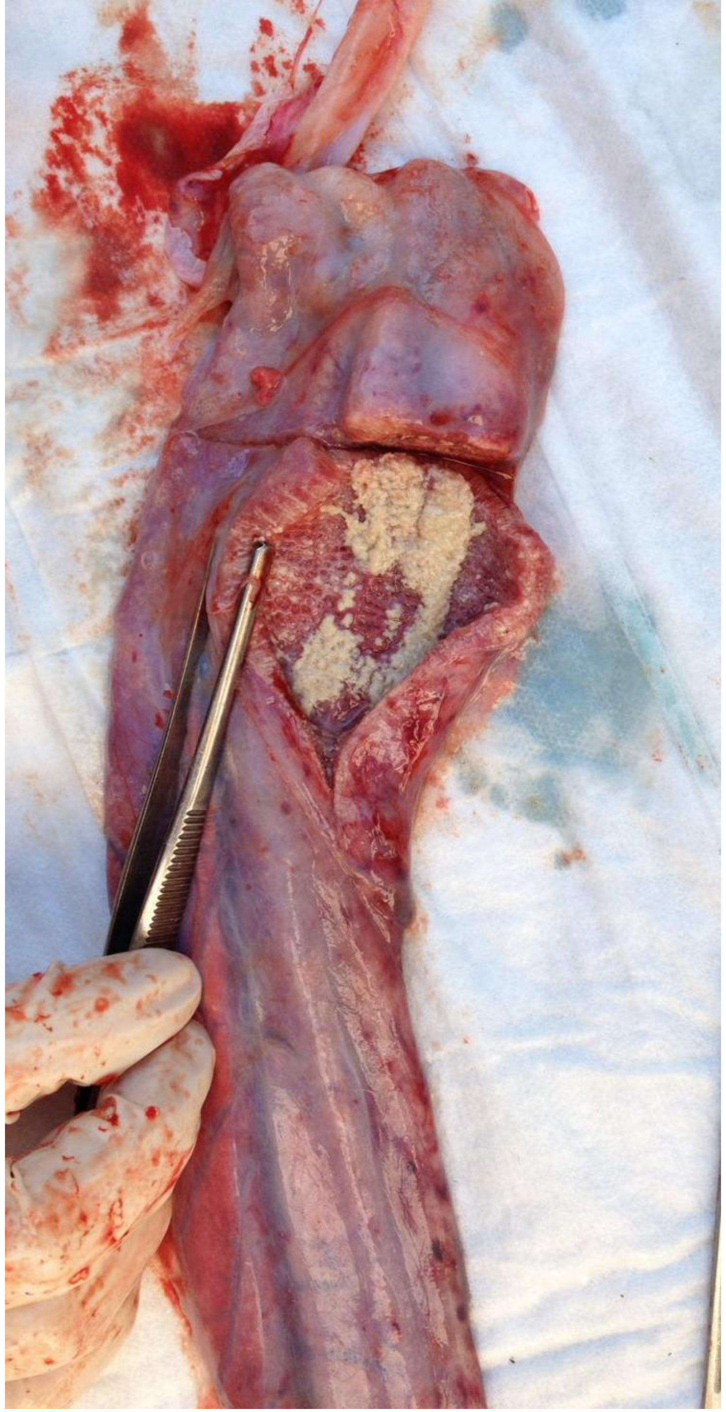




Source: Sabine Öfner, Reptile Rescue Center, Munich

Prophylaxis and treatment

- Quarantine
  - Long-term carriers possible
  - Intermittent shedding possible
- Testing
  - Genetically diverse group of viruses
  - Currently no serological tests available
- Disinfection
  - Relatively easy to inactivate (Tillis et al., 2023):
    - 10% bleach, 2% chlorhexidine, 70% ethanol: inactivation within 1 minute
    - 7% peroxide, quaternary ammonium compound (F10): inactivation within 3 minutes

Prophylaxis and treatment

- Treatment:
  - Supportive care
  - Treatment of co-infections
  - Temperature:
    - Viruses do not grow at temperatures >35° C
    - Antivirals:
      - Little data
      - Temdesivir, rebaviris and NITD-008 have potential antiviral activity against several serpentovirus isolates in vitro (Tillis et al., 2023)

microorganisms

MDPI

Article

In Vitro Characterization and Antiviral Susceptibility of Ophidian Serpentoviruses

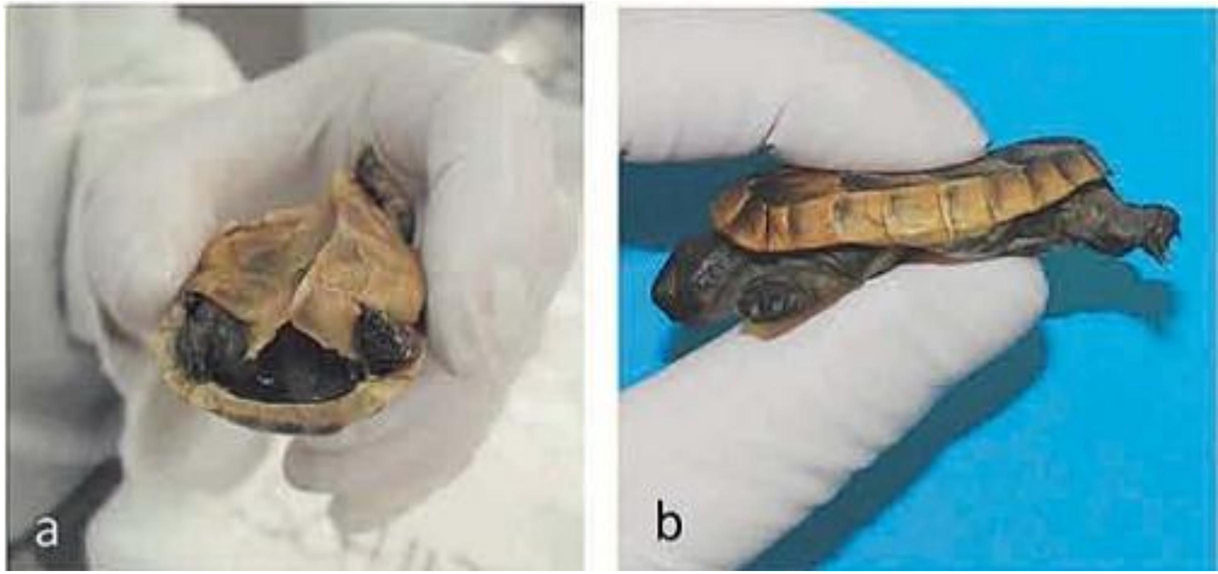
Steven B. Tillis [1], Camille Holt [2], Spencer Havens [2], Tracey D. Logan [1], Justin G. Julander [2] and Robert J. Ossiboff [1,*]

Picornaviruses:

- Known as:
  - Virus „x“
  - Genus *Torchivirus*
- Are detected regularly in tortoise collections in Europe
- Most commonly in Greek tortoises, but also in all other *Testudo* spp. and others

Virus „x“: Clinical signs

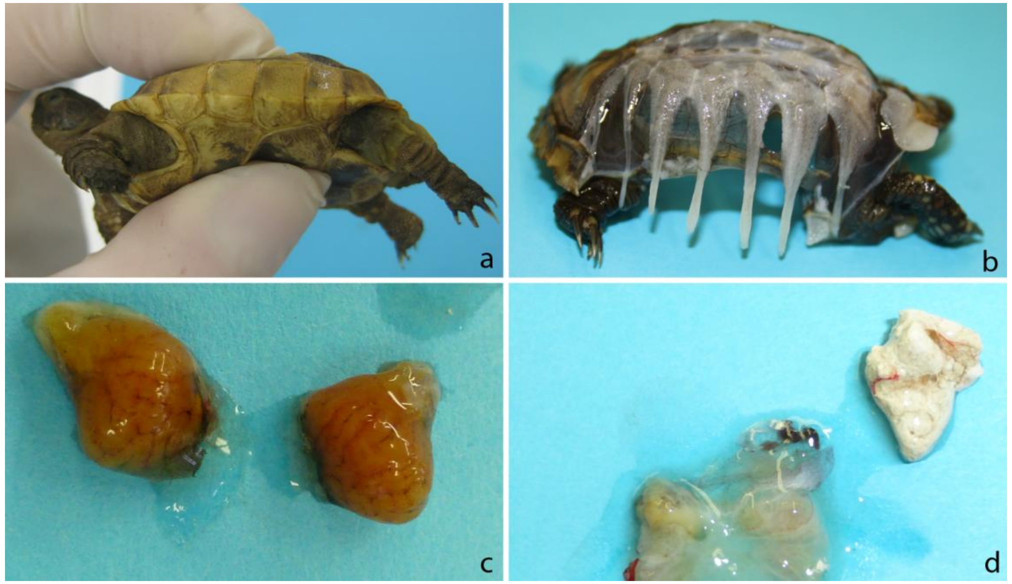
- Softening of the carapace in young animals
- Rhinitis
- Cachexia, often a sudden loss of weight
- Stomatitis
- Conjunctivitis
- Ascites
- Pneumonia
- Enteritis
- Has also been found in clinically healthy tortoises



Heuser et al., Tierärztliche Praxis, 2014

Clinical Signs

- Koch‘s postulates fulfilled for kidney disease and shell weakness in Hermann‘s and spur-thighed tortoises (Paries et al., 2019)



PLOS | ONE

RESEARCH ARTICLE

The role of Virus "X" (*Tortoise Picornavirus*) in kidney disease and shell weakness syndrome in European tortoise species determined by experimental infection

S. Paries[1]*, S. Funcke[1], O. Kershaw[2], K. Failing[3], M. Lierz[1]

Virus "x": Prevalence

- Diagnostic testing in our lab showed between 2 and 3% prevalence in samples from tortoises
  - Highest prevalence in spur-thighed tortoises (ca. 5%)
  - Higher prevalences have been reported
    - 13.8 % (RNA) and 13.5% (Ab) in a smaller study in Germany (Paries et al., 2018)
    - 42.4% by virus isolation in an earlier study (Marschang et al., 2001)
  - Antibodies have been detected in individual cases in animals from a variety of African countries

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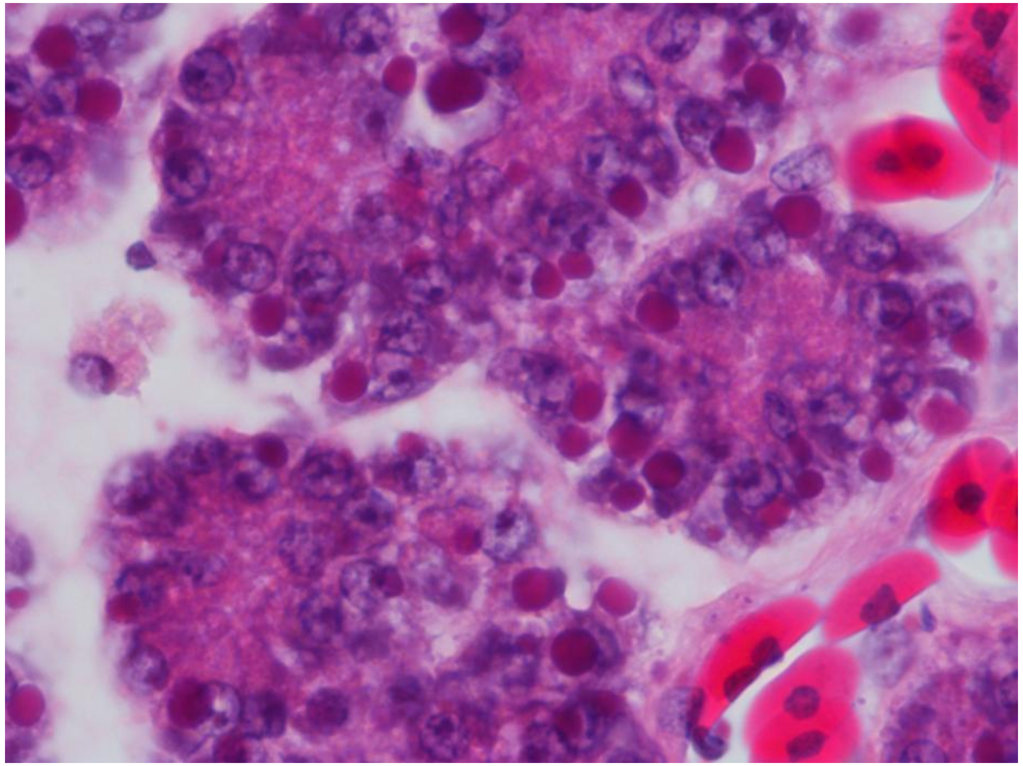
Virus “x”: Diagnosis

- Virus detection:
  - Virus isolation
  - PCR
  - Samples:
    - Oral, cloacal, conjunctival swabs
    - Intestine, kidney, multiple other tissues
- Serology
  - Neutralizations tests with virus isolates

Inclusion body disease

- IBD is a slow, progressive disease of boas and pythons
- Defined by the presence of intracytoplasmic inclusion bodies in cells of various tissues



Etiology

- Family *Arenaviridae*:
    - Enveloped ssRNA viruses with a segmented genome
    - Genus *Reptarenavirus* with multiple species

mBio

Identification, Characterization, and *In Vitro* Culture of Highly Divergent Arenaviruses from Boa Constrictors and Annulated Tree Boas: Candidate Etiological Agents for Snake Inclusion Body Disease

Mark D. Stenglein, Chris Sanders, Amy L. Kistler, et al. 2012. Identification, Characterization, and *In Vitro* Culture of Highly Divergent Arenaviruses from Boa Constrictors and Annulated Tree Boas: Candidate Etiological Agents for Snake Inclusion Body Disease . mBio 3(4): . doi:10.1128/mBio.00180-12.

IBD: Clinical signs

- Clinical signs of disease can be very variable
  - Immunosuppression
  - CNS disease
  - Regurgitation
  - Anorexia
  - Lethargy
  - Pneumonia

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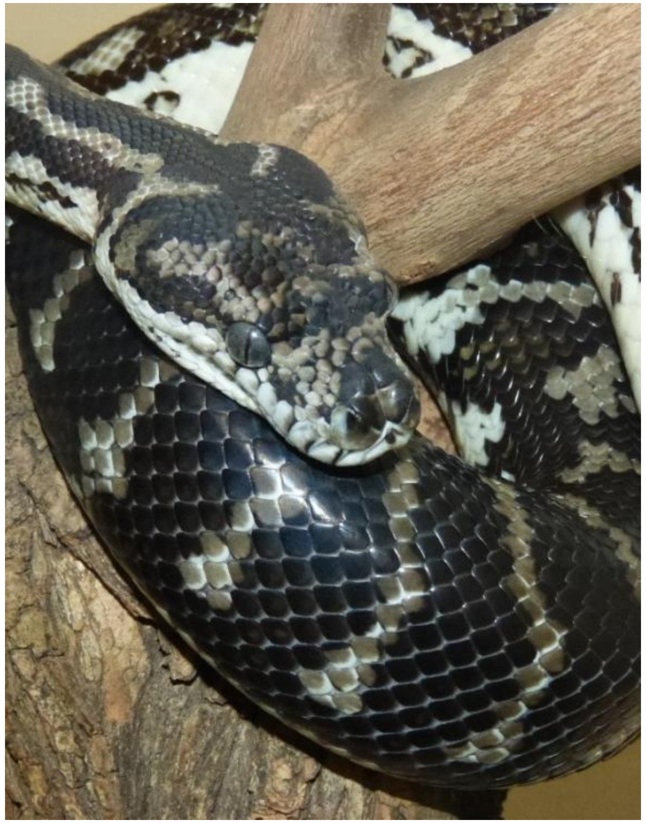
Host species

- Found (almost) only in boas and pythons
- World-wide (Europe, USA, Australia, Africa)
  - *Boa constrictor*
  - *Python bivittatus* (Burmese python)
  - *P. molurus molurus* (Indian python)
  - *P. regius* (ball python)
  - *Malayopython (Python) reticulatus* (reticulated p.)
  - *Morelia spilota variegata* (carpet python)
  - *M. spilota spilota* (diamond python)
  - *Eunectes murinus* (green anaconda)
  - *Eunectes notaeus* (yellow anaconda)
  - *Epicrates cenchria* (rainbow boa)
  - *Chilabothrus (Epicrates) striatus* (Haitian boa)
  - *Acrantophis madagascariensis* (Madagascar ground boa)
- Also found in native boa constrictors in Brazil (Argenta et al., 2020. J Virol)

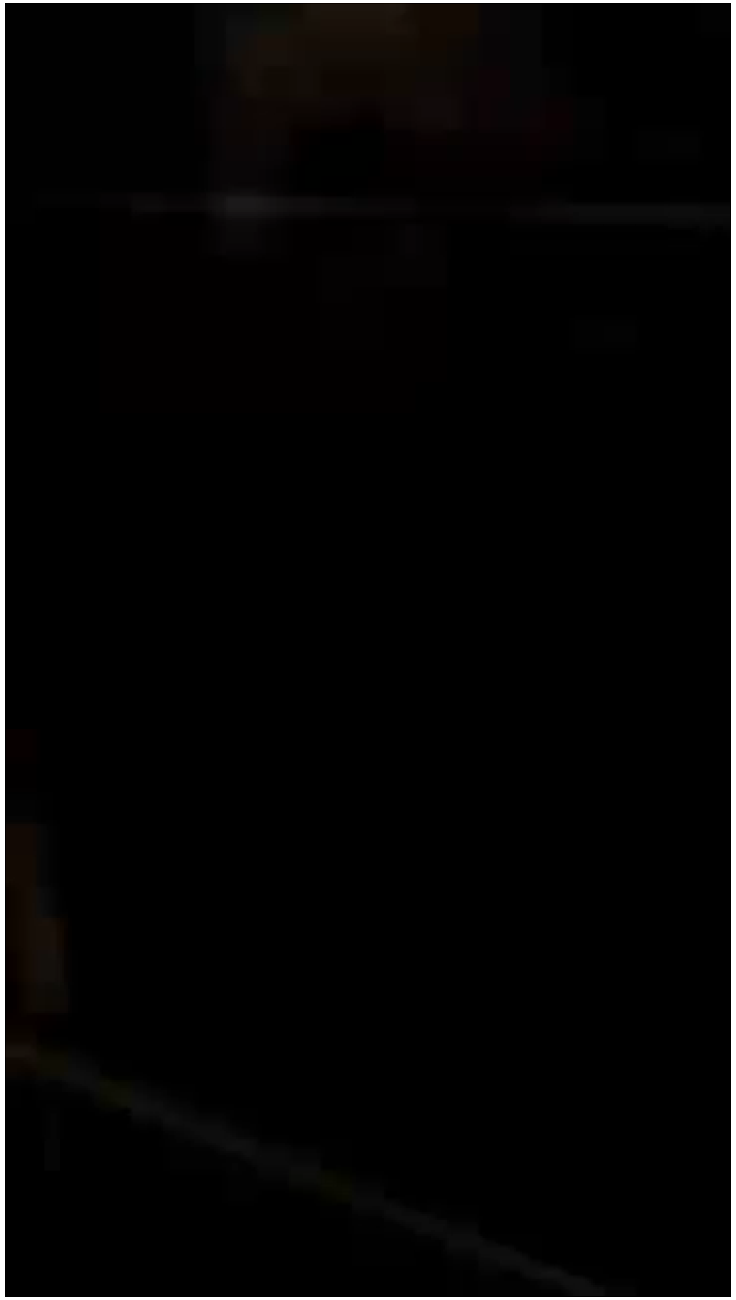


Host species

- Inclusion bodies found in colubrids and viperids in individual cases:
  - A single *Lampropeltis getula* (kingsnake)
  - Group of *Bothriechis marchi* (palm pit viper)
- Reptarenaviruses also found in a colubrid and viperids:
  - A *Pantherophis guttatus* (cornsnake)
  - Two *Tropidolaemus wagleri* (Wagler's pit vipers)

Source: J. Dietz

IBD: Clinical signs



Video source: Mark Krabbe

IBD: Clinical signs

IBD: Clinical signs

- Clinical signs may differ depending on host species
  - Pythons reported to develop disease more quickly than boas
  - Many arenavirus positive boas may remain clinically inapparently infected for extended periods of time

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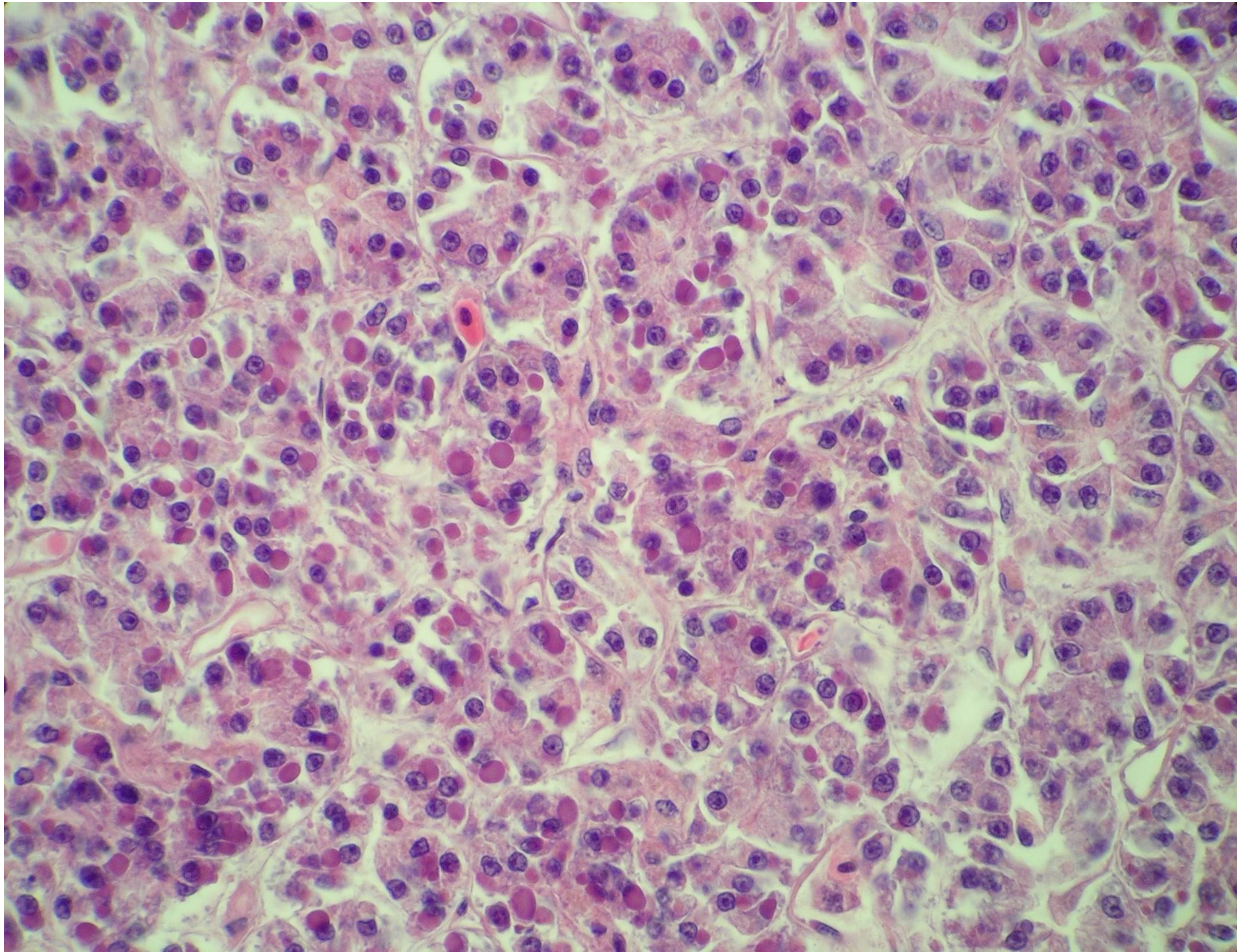
Histology

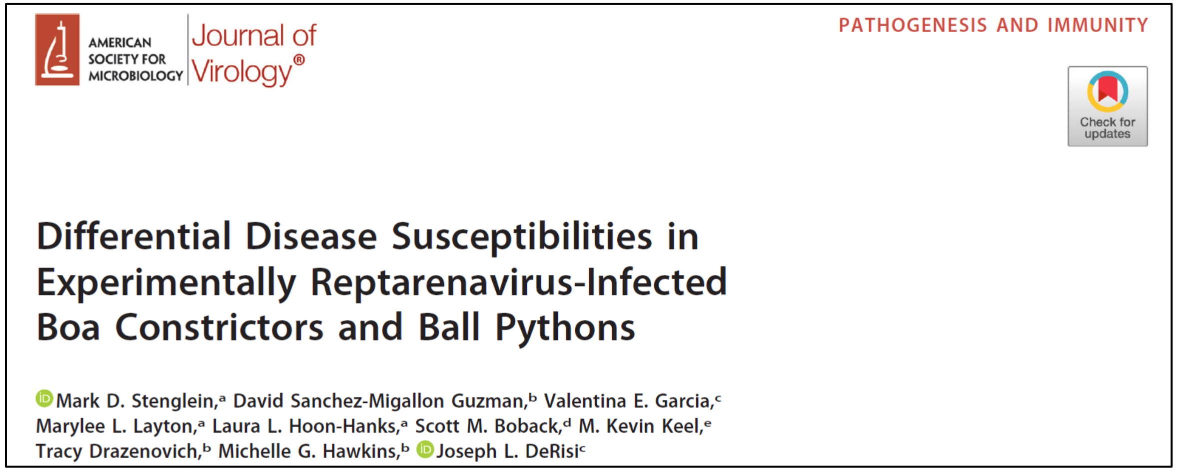
- Intracytoplasmic eosinophilic to amphophilic inclusions:
  - In epithelial cells of the respiratory, gastrointestinal and urogential tracts, cells of the central and peripheral nerve system and in hematopoeitic and lymphatic tissues
  - Made up of fine granular electron dense material
  - Inclusion body disease protein (IBDP) = arenavirus nucleoprotein

Pancreas of a *Boa constrictor* with IBD



Transmission studies

- Successful production of inclusion bodies following transmission in boas and pythons (Stenglein et al., 2017)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PATHOGENESIS AND IMMUNITY

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Differential Disease Susceptibilities in Experimentally Reptarenavirus-Infected Boa Constrictors and Ball Pythons

Mark D. Stenglein,[a] David Sanchez-Migallon Guzman,[b] Valentina E. Garcia,[c] Marylee L. Layton,[a] Laura L. Hoon-Hanks,[a] Scott M. Boback,[d] M. Kevin Keel,[e] Tracy Drazenovich,[b] Michelle G. Hawkins,[b] Joseph L. DeRisi[c]

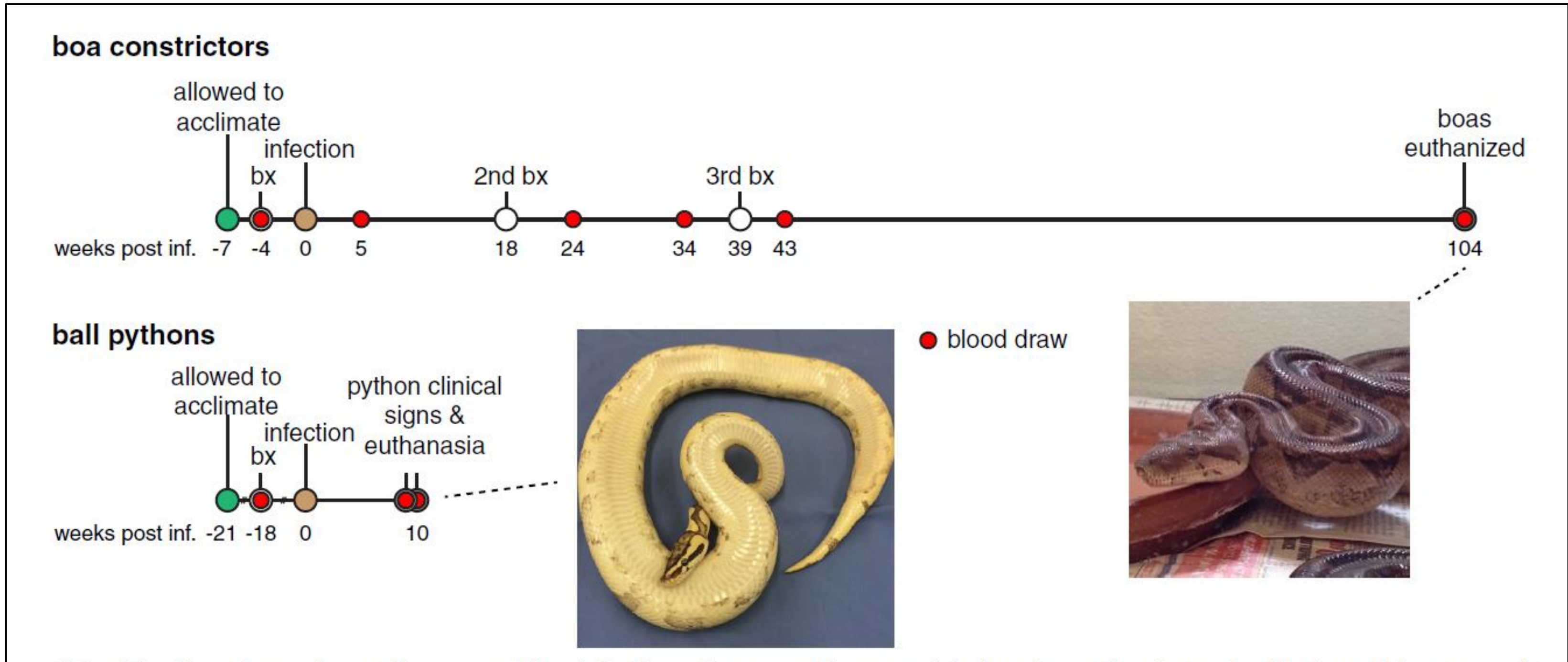

FIG 1 Timeline of experimental reptarenavirus infection of boa constrictors and ball pythons. The times of collection of the pre- and

Transmission studies

- BUT: Hetzel et al. 2021 were not able to detect inclusion bodies in inoculated pythons or co-housed boas

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PATHOGENESIS AND IMMUNITY

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Experimental Reptarenavirus Infection of *Boa constrictor* and *Python regius*

U. Hetzel,[a,c] Y. Korzyukov,[b] S. Keller,[a] L. Szirovicza,[b] T. Pesch,[a] O. Vapalahti,[b,c,d] A. Kipar,[a,c] J. Hepojoki[a,b]

IBD: Immunology

PLOS | ONE

RESEARCH ARTICLE

Antibody response in snakes with boid inclusion body disease

Katharina Windbichler[1], Eleni Michalopoulou[2], Pia Palamides[1¤a], Theresa Pesch[1], Christine Jelinek[1¤b], Olli Vapalahti[3,4], Anja Kipar[1,3], Udo Hetzel[1,3], Jussi Hepojoki[1,4]*

- Inclusion body development associated with:
  - Presence of Giessen reptarenavirus
  - Negatively correlated with the presence of anti-Giessen reptarenavirus IgY antibodies

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PATHOGENESIS AND IMMUNITY

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Experimental Reptarenavirus Infection of *Boa constrictor* and *Python regius*

U. Hetzel,[a,c] Y. Korzyukov,[b] S. Keller,[a] L. Szirovicza,[b] T. Pesch,[a] O. Vapalahti,[b,c,d] A. Kipar,[a,c] J. Hepojoki[a,b]

IBD: transmission

- Transmission route unknown, possibly via:
  - Horizontal transmission
    - Direct contact, mites
  - Vertical transmission

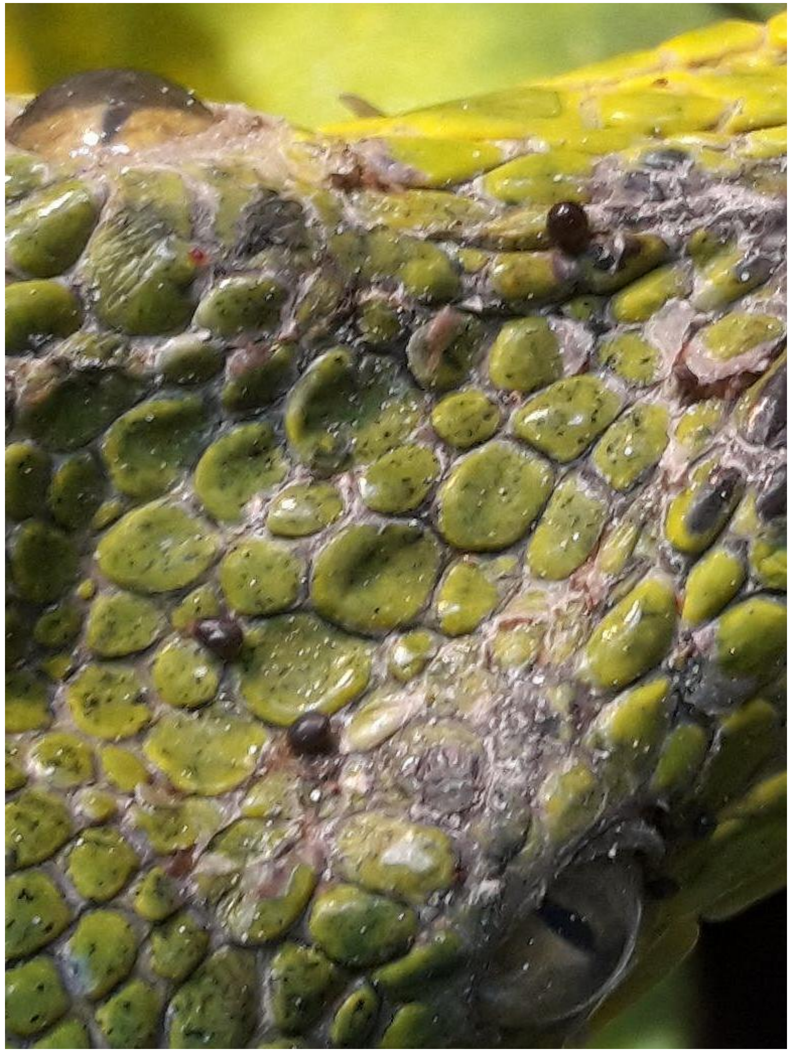


Image courtesy of Karina Mathes

Diagnosis in live animals

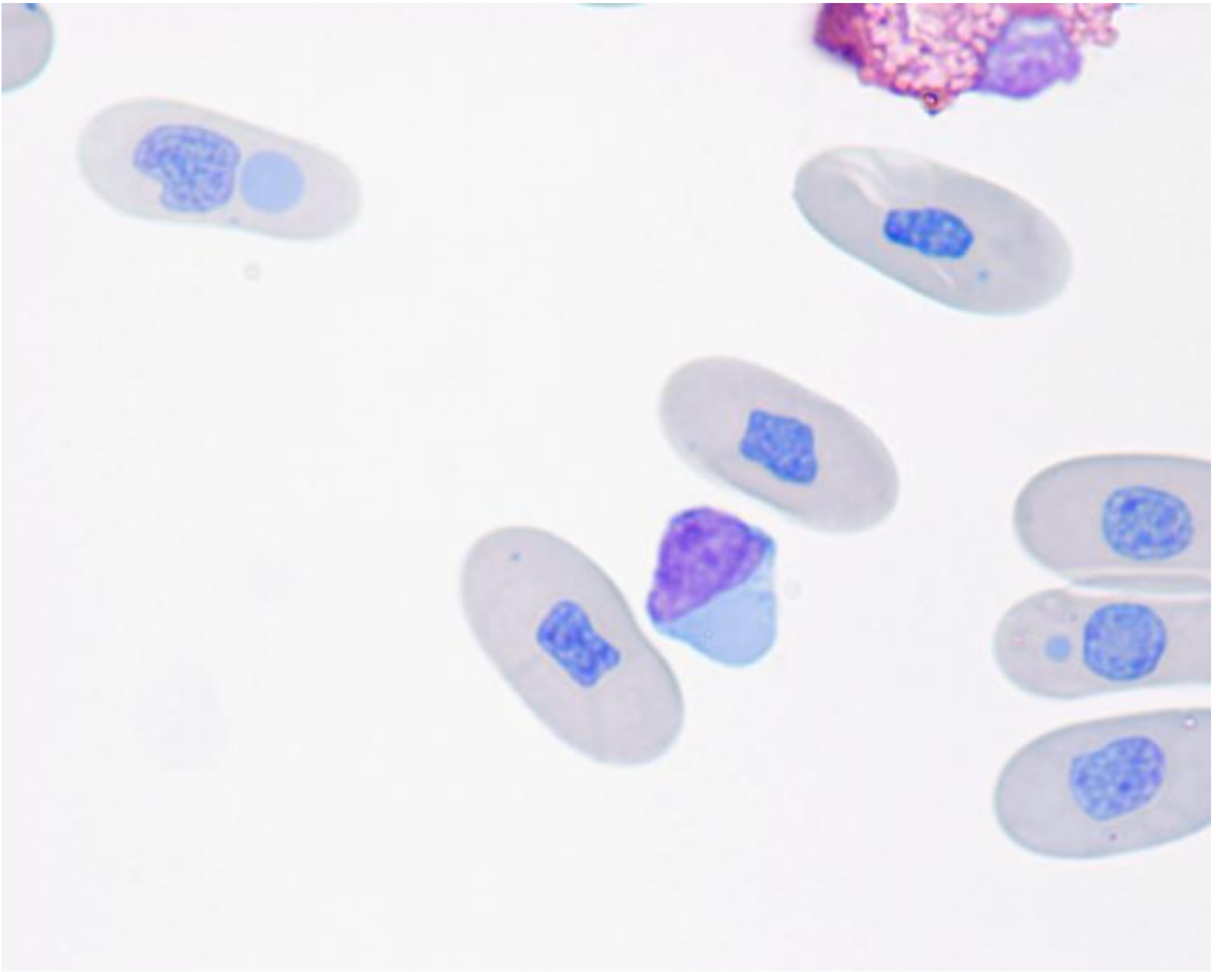
- Inclusions in:

Biopsies from the liver, kidney, and esophageal tonsils

Blood cells:

erythrocytes

lymphocytes



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Reptarenaviruses

- Highly diverse viruses found in infected snakes

PLOS | PATHOGENS

RESEARCH ARTICLE

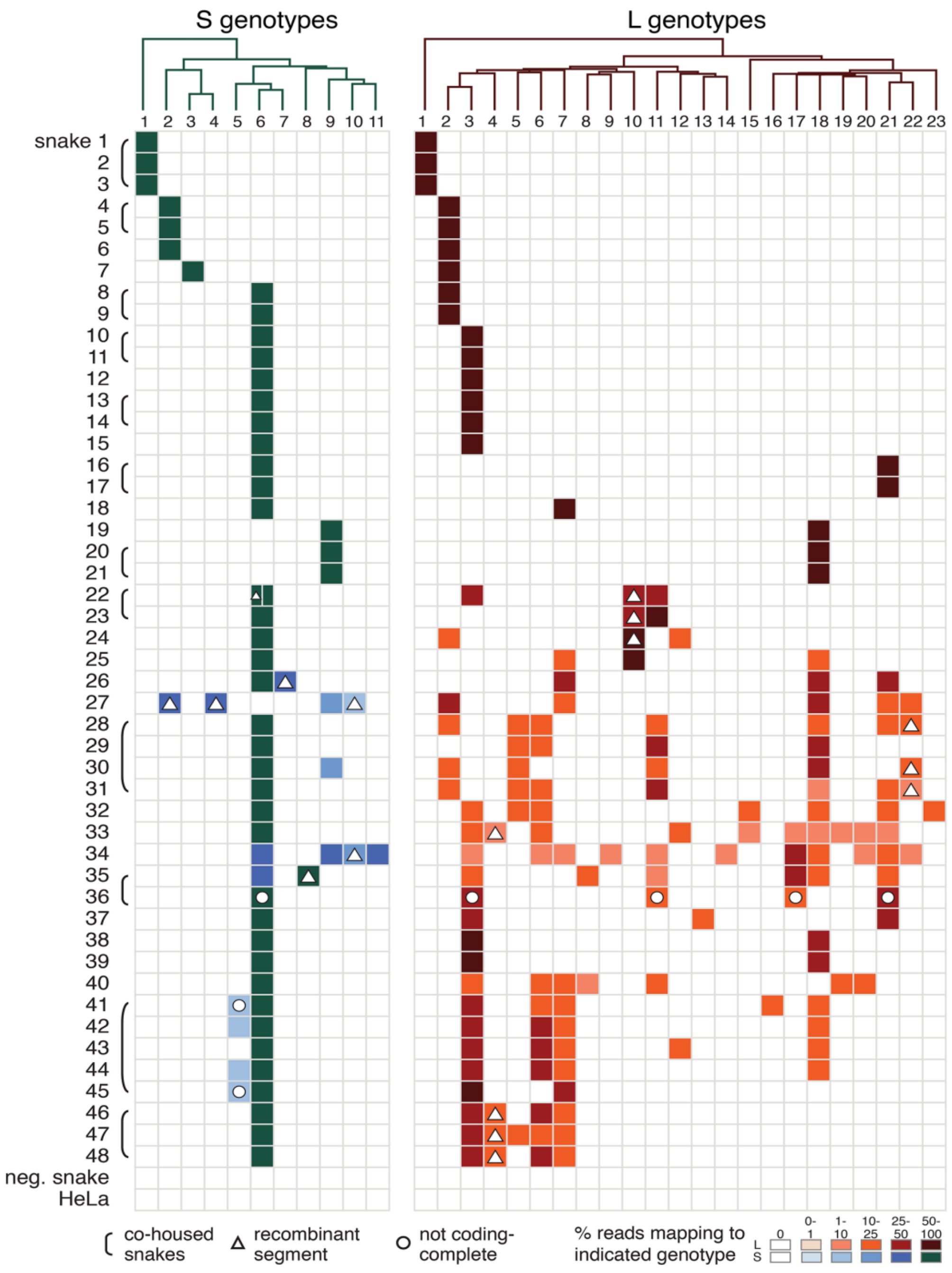
Widespread Recombination, Reassortment, and Transmission of Unbalanced Compound Viral Genotypes in Natural Arenavirus Infections

Mark D. Stenglein[1¤]*, Elliott R. Jacobson[2], Li-Wen Chang[2], Chris Sanders[3], Michelle G. Hawkins[4], David S-M. Guzman[4], Tracy Drazenovich[4], Freeland Dunker[5], Elizabeth K. Kamaka[6], Debbie Fisher[7], Drury R. Reavill[8], Linda F. Meola[9], Gregory Levens[10], Joseph

JVI Journals.ASM.org

Arenavirus Coinfections Are Common in Snakes with Boid Inclusion Body Disease

J. Hepojoki,[a] P. Salmenperä,[b] T. Sironen,[a] U. Hetzel,[c,d] Y. Korzyukov,[a] A. Kipar,[c,d] O. Vapalahti[a,d,e]



Stenglein et al. 2015

Detection

- Can be challenging
  - Inclusions:
    - Live animals: blood smears, biopsies of liver, kidney, and esophageal tonsils
    - Dead animals: liver, pancreas, brain
  - Arenavirus:
    - PCR for the detection of snake arenaviruses
    - Live animals: blood, esophageal swabs, biopsies
    - Dead animals: see inclusions (pythons)

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

RESEARCH ARTICLE

Check for updates

Reptarenavirus S Segment RNA Levels Correlate with the Presence of Inclusion Bodies and the Number of L Segments in Snakes with Reptarenavirus Infection—Lessons Learned from a Large Breeding Colony

Tanja Thiele,[a,b] Francesca Baggio,[a] Barbara Prähauser,[a] Andres Ruiz Subira,[a] Eleni Michalopoulou,[a] Anja Kipar,[a,c] Udo Hetzel,[a,c] Jussi Hepojoki[a,d]

Boas/pythons

- Arenaviruses much more commonly detected in boas than in pythons
  - Reported prevalence in boas: 25-40% (Chang et al., 2016; Simard et al., 2020)
  - Reported prevalence in pythons: 0-5.3%

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Prevention

- Quarantine
  - -Information on the source of the snakes
  - -Clinical examination
  - -Test for inclusions
  - -Test for arenaviruses
  - -Duration?
- Treat parasites
  - -Mites may play a role in transmission
- Remove positive animals from the collection
  - -Not all IBD or arenavirus positive snakes will develop clinical disease

Additional clinically relevant viruses of reptiles

- Reoviruses
- Arteriviruses
- Pox viruses
- Papillomaviruses
- Sunviruses
- Bornaviruses
- Iridoviruses

Video courtesy of Tim Hyndman

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Thank you for your attention!

European College of Veterinary Microbiology

ARAV ASSOCIATION OF REPTILIAN AND AMPHIBIAN VETERINARIANS

ECZM EUROPEAN COLLEGE OF ZOOLOGICAL MEDICINE