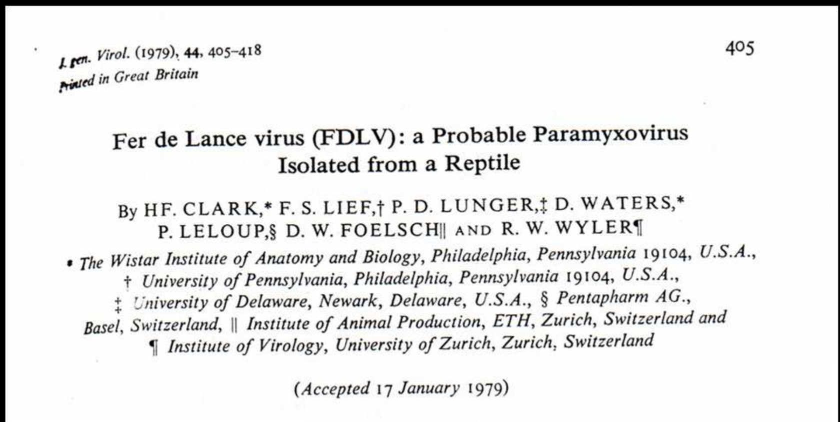
Paramyxoviral Infection of Reptiles


J. gen. Virol. (1979), 44, 405–418 405
Printed in Great Britain

Fer de Lance virus (FDLV): a Probable Paramyxovirus Isolated from a Reptile

By HF. CLARK,* F. S. LIEF,† P. D. LUNGER,‡ D. WATERS,* P. LELOUP,§ D. W. FOELSCH‖ AND R. W. WYLER¶

* The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104, U.S.A., † University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A., ‡ University of Delaware, Newark, Delaware, U.S.A., § Pentapharm AG., Basel, Switzerland, ‖ Institute of Animal Production, ETH, Zurich, Switzerland and ¶ Institute of Virology, University of Zurich, Zurich, Switzerland

(Accepted 17 January 1979)

September 1972 to February 1973
Die-off oF 123 OF 431
Bothrops moojeni
in a Serpentarium in Switzerland

Paramyxoviral Infection of Reptiles

Reprinted from the JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, Vol. 177, No. 9, Pages 796-799.

Paramyxo-like Virus Infection in a Rock Rattlesnake

Elliott Jacobson, DVM, PhD; Jack M. Gaskin, DVM, PhD; Charles F. Simpson, DVM, PhD; Timothy G. Terrell, DVM, PhD

SUMMARY

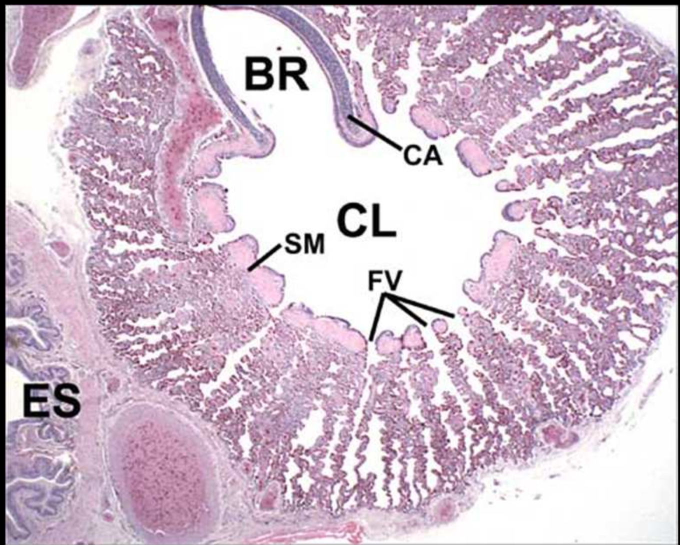
A rock rattlesnake *(Crotalus lepidus)* with a history of progressive central nervous disease was submitted for necropsy. The histopathologic findings included evidence of interstitial pneumonia, multifocal areas of gliosis in the brain, and ballooning degeneration and demyelination of brainstem and upper spinal cord axons. By electron microscopy, brainstem tissue was found to contain numerous virus particles in the extracellular spaces. A paramyxo-like virus, isolated in viper heart cells from lung tissue, was observed by electron microscopy to be similar in size and shape to the particles seen in nerve tissue.

kept in an individual cage at any one time, all snakes were intermixed during the breeding period. At this time, a new breeder male (No. 1) was introduced into the collection without being quarantined. Ultimately, this snake was in contact with all other rock rattlesnakes. On day 3 following introduction, this snake developed head tremors and loss of equilibrium, and it died on day 14. Over the next 2 months, the 3 females and 3 of the males died (No. 2–7) after manifesting similar clinical signs. Only 1 rattlesnake (No. 9) remained healthy at the time of submission of rattlesnake No. 8, and this snake has continued to be active and healthy. The rattlesnakes were the only vipers in the collection. Several species of colubrid snakes, which were maintained in the same room but not in direct contact with the rattlesnakes, remained normal.

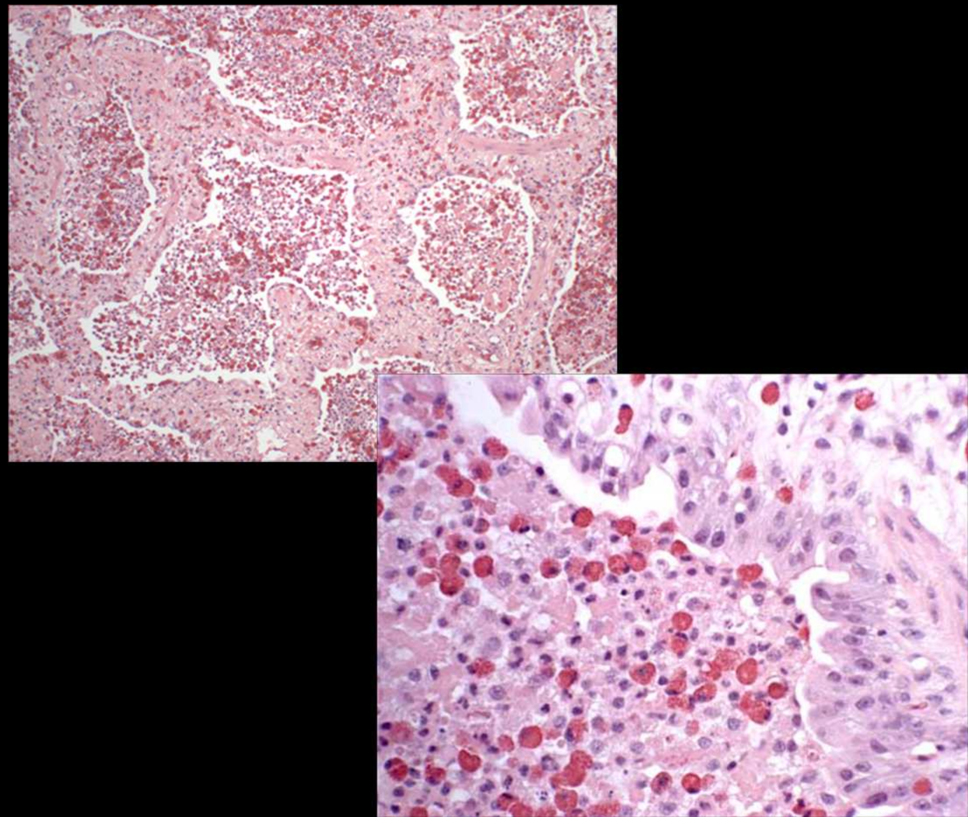
At examination, rattlesnake No. 8 had head

September to November 1979
Die-off of 8 of 9 rock rattlesnakes, *Crotalus lepidus* over a 3 month period in a private collection in Florida, USA

Paramyxoviral Infection of Reptiles



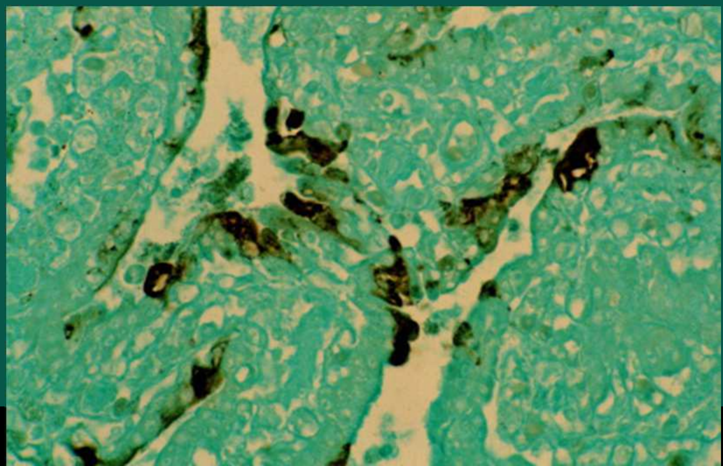
Normal Lung

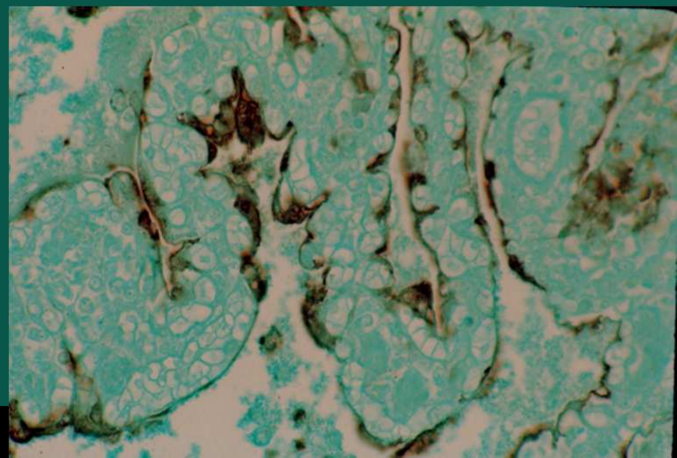


Experimentally Infected rattlesnake

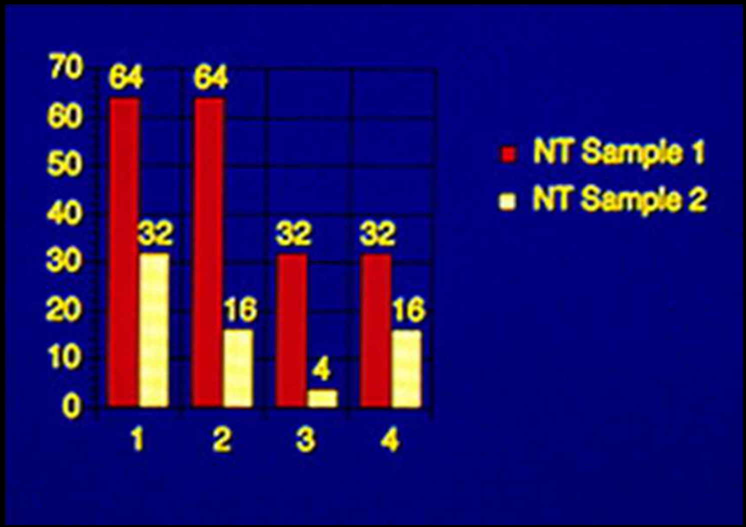
Paramyxoviral Infection of Reptiles

Demonstration of tissue antigen by the ABC immunoperoxidase kit





Hemagglutination Inhibition Test



1. Chicken red blood cells

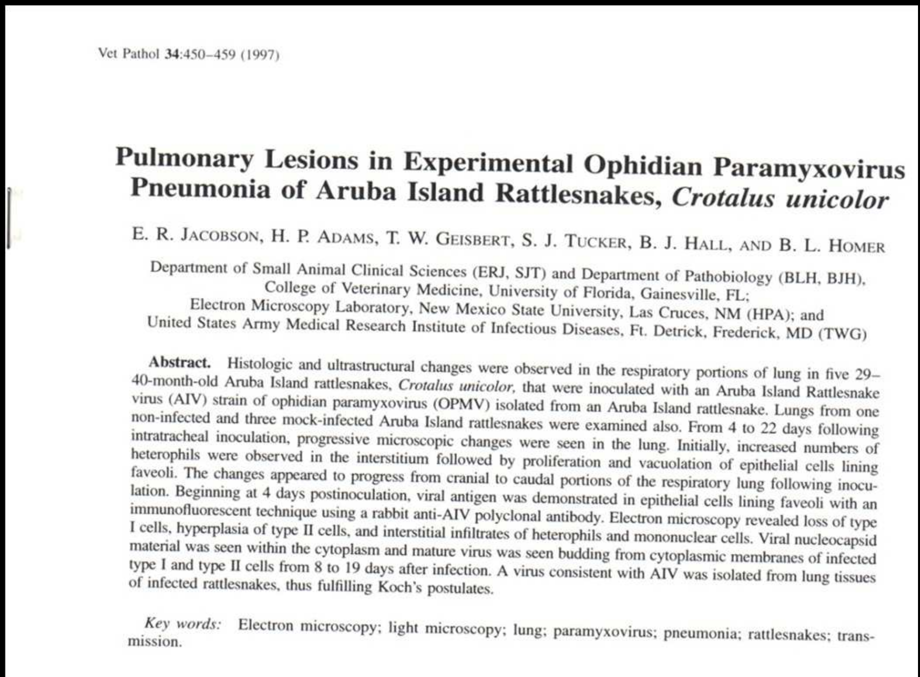
2. Guinea pig red blood cells

3. Human red blood cells

4. Sheep red blood cells

NT = Neotropical rattlesnake isolate

Experimental Infection of Paramyxovirus in Aruba Island Rattlesnakes


Vet Pathol **34**:450–459 (1997)

Pulmonary Lesions in Experimental Ophidian Paramyxovirus Pneumonia of Aruba Island Rattlesnakes, *Crotalus unicolor*

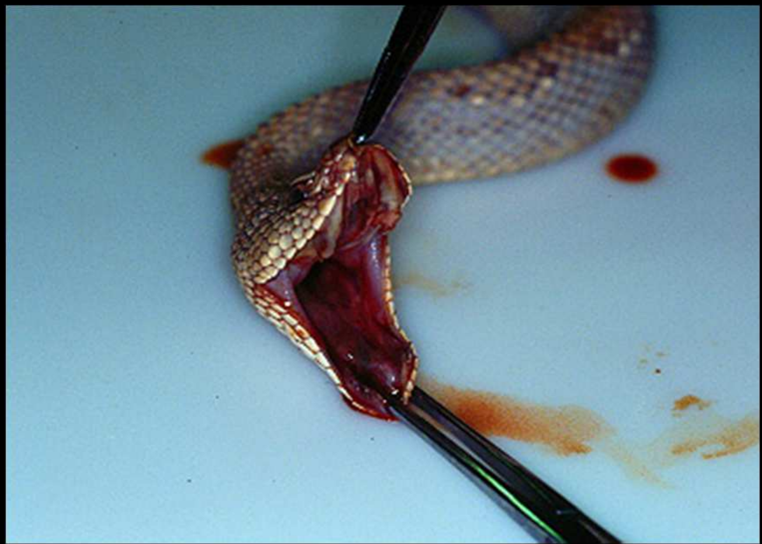
E. R. JACOBSON, H. P. ADAMS, T. W. GEISBERT, S. J. TUCKER, B. J. HALL, AND B. L. HOMER

Department of Small Animal Clinical Sciences (ERJ, SJT) and Department of Pathobiology (BLH, BJH), College of Veterinary Medicine, University of Florida, Gainesville, FL;
Electron Microscopy Laboratory, New Mexico State University, Las Cruces, NM (HPA); and
United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD (TWG)

Abstract. Histologic and ultrastructural changes were observed in the respiratory portions of lung in five 29–40-month-old Aruba Island rattlesnakes, *Crotalus unicolor,* that were inoculated with an Aruba Island Rattlesnake virus (AIV) strain of ophidian paramyxovirus (OPMV) isolated from an Aruba Island rattlesnake. Lungs from one non-infected and three mock-infected Aruba Island rattlesnakes were examined also. From 4 to 22 days following intratracheal inoculation, progressive microscopic changes were seen in the lung. Initially, increased numbers of heterophils were observed in the interstitium followed by proliferation and vacuolation of epithelial cells lining faveoli. The changes appeared to progress from cranial to caudal portions of the respiratory lung following inoculation. Beginning at 4 days postinoculation, viral antigen was demonstrated in epithelial cells lining faveoli with an immunofluorescent technique using a rabbit anti-AIV polyclonal antibody. Electron microscopy revealed loss of type I cells, hyperplasia of type II cells, and interstitial infiltrates of heterophils and mononuclear cells. Viral nucleocapsid material was seen within the cytoplasm and mature virus was seen budding from cytoplasmic membranes of infected type I and type II cells from 8 to 19 days after infection. A virus consistent with AIV was isolated from lung tissues of infected rattlesnakes, thus fulfilling Koch's postulates.

Key words: Electron microscopy; light microscopy; lung; paramyxovirus; pneumonia; rattlesnakes; transmission.

Paramyxoviral Infection of Reptiles



Post-challenge Day 19

Hemorrhage in Respiratory Tract

Paramyxoviral Infection of Reptiles

ELSEVIER

Virus Research 63 (1999) 65–74

Virus Research

www.elsevier.com/locate/virusres

Comparative sequence analyses of sixteen reptilian paramyxoviruses

W. Ahne [a,*], W.N. Batts [b], G. Kurath [b], J.R. Winton [b]

[a] *Institute of Zoology, Fishery Biology and Fish Diseases, University of Munich, Kaulbachstrasse 37, D-80539 Munich, Germany*

[b] *Western Fisheries Research Center, Biological Resources Division, USGS, 6505 NE 65th Street, Seattle, WA 98115, USA*

Abstract

Viral genomic RNA of Fer-de-Lance virus (FDLV), a paramyxovirus highly pathogenic for reptiles, was reverse transcribed and cloned. Plasmids with significant sequence similarities to the hemagglutinin–neuraminidase (HN) and polymerase (L) genes of mammalian paramyxoviruses were identified by BLAST search. Partial sequences of the FDLV genes were used to design primers for amplification by nested polymerase chain reaction (PCR) and sequencing of 518-bp L gene and 352-bp HN gene fragments from a collection of 15 previously uncharacterized reptilian paramyxoviruses. Phylogenetic analyses of the partial L and HN sequences produced similar trees in which there were two distinct subgroups of isolates that were supported with maximum bootstrap values, and several intermediate isolates. Within each subgroup the nucleotide divergence values were less than 2.5%, while the divergence between the two subgroups was 20–22%. This indicated that the two subgroups represent distinct virus species containing multiple virus strains. The five intermediate isolates had nucleotide divergence values of 11–20% and may represent additional distinct species. In addition to establishing diversity among reptilian paramyxoviruses, the phylogenetic groupings showed some correlation with geographic location, and clearly demonstrated a low level of host species-specificity within these viruses.

Keywords: Fer-de-Lance virus; Paramyxovirus; Reptilian paramyxoviruses

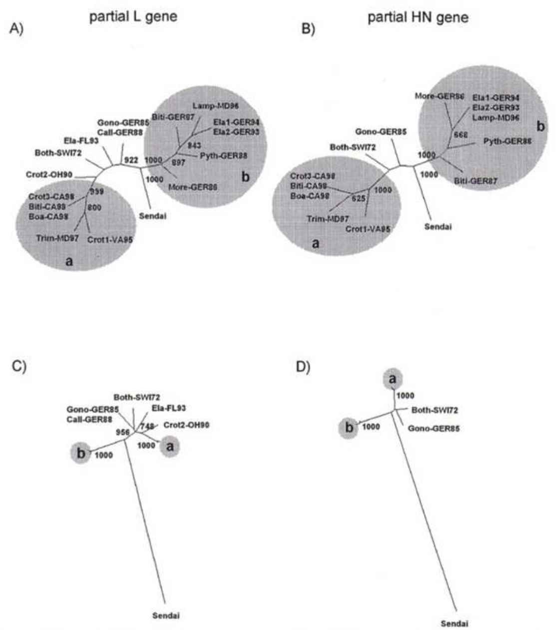
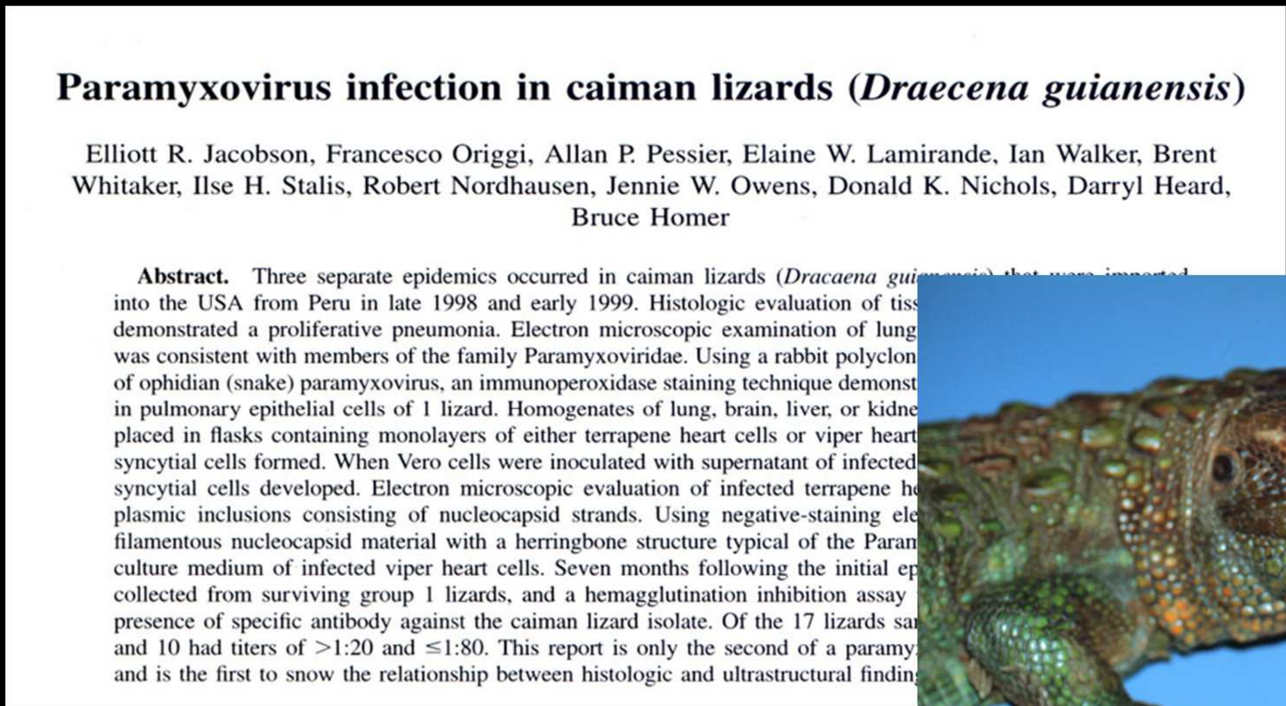


Fig. 4. Phylogenetic trees generated by maximum parsimony (A and B) and Fitch DNA distance (C and D) analyses of twelve 518-nt partial L gene sequences (A and C) and nine 352-nt partial HN gene sequences (B and D) from reptilian paramyxoviruses. In all analyses, the corresponding L and HN genomic sequences of Sendai virus (Genbank M30202) were used as an outgroup. Shading in panels A and B identifies isolates within subgroups 'a' and 'b' (see text), and shaded circles in panels C and D indicate the same subgroups, that clustered in Fitch analyses with branch lengths too short for labeling of individual isolates. Each analysis was done on 1000 bootstrapped data sets, and bootstrap values above 600 are shown on the trees. For the Fitch DNA distance method (C and D) the trees shown were generated in non-bootstrapped analyses to retain branch length information, and values from bootstrapped analyses of the same sequences were placed at the analogous branches of the trees.

Paramyxoviral Infection of Caiman Lizards



Paramyxovirus infection in caiman lizards (*Draecena guianensis*)

Elliott R. Jacobson, Francesco Origgi, Allan P. Pessier, Elaine W. Lamirande, Ian Walker, Brent Whitaker, Ilse H. Stalis, Robert Nordhausen, Jennie W. Owens, Donald K. Nichols, Darryl Heard, Bruce Homer

Abstract. Three separate epidemics occurred in caiman lizards (*Dracaena gui*
into the USA from Peru in late 1998 and early 1999. Histologic evaluation of tiss
demonstrated a proliferative pneumonia. Electron microscopic examination of lung
was consistent with members of the family Paramyxoviridae. Using a rabbit polyclon
of ophidian (snake) paramyxovirus, an immunoperoxidase staining technique demonst
in pulmonary epithelial cells of 1 lizard. Homogenates of lung, brain, liver, or kidne
placed in flasks containing monolayers of either terrapene heart cells or viper heart
syncytial cells formed. When Vero cells were inoculated with supernatant of infected
syncytial cells developed. Electron microscopic evaluation of infected terrapene h
plasmic inclusions consisting of nucleocapsid strands. Using negative-staining ele
filamentous nucleocapsid material with a herringbone structure typical of the Param
culture medium of infected viper heart cells. Seven months following the initial ep
collected from surviving group 1 lizards, and a hemagglutination inhibition assay
presence of specific antibody against the caiman lizard isolate. Of the 17 lizards sa
and 10 had titers of >1:20 and ≤1:80. This report is only the second of a paramy
and is the first to snow the relationship between histologic and ultrastructural finding

Paramyxoviral Infection of Caiman Lizards

Thickened lungs and Exudate

in Central Chamber

INCLUSION BODY DISEASE OF BOID SNAKES

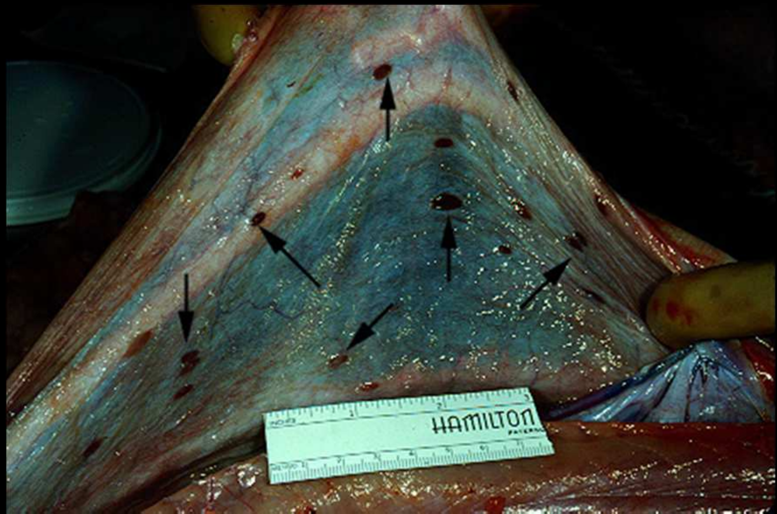
Clinical signs Seen in Snakes with IBD

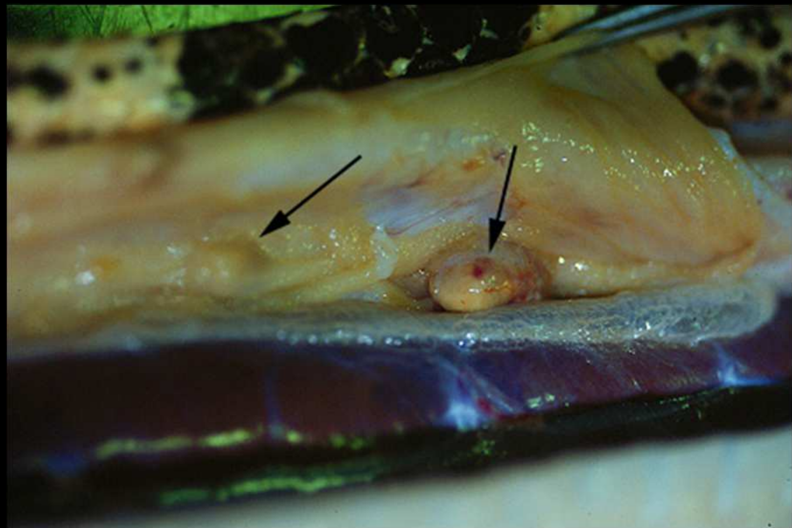
- CNS disease
- Regurgitation
- Stomatitis
- Pneumonia
- Enteritis
- Lymphoma
- Round cell tumors

INCLUSION BODY DISEASE OF BOID SNAKES

Dysequilibrium and Ophisthotonus in Boa Constrictors

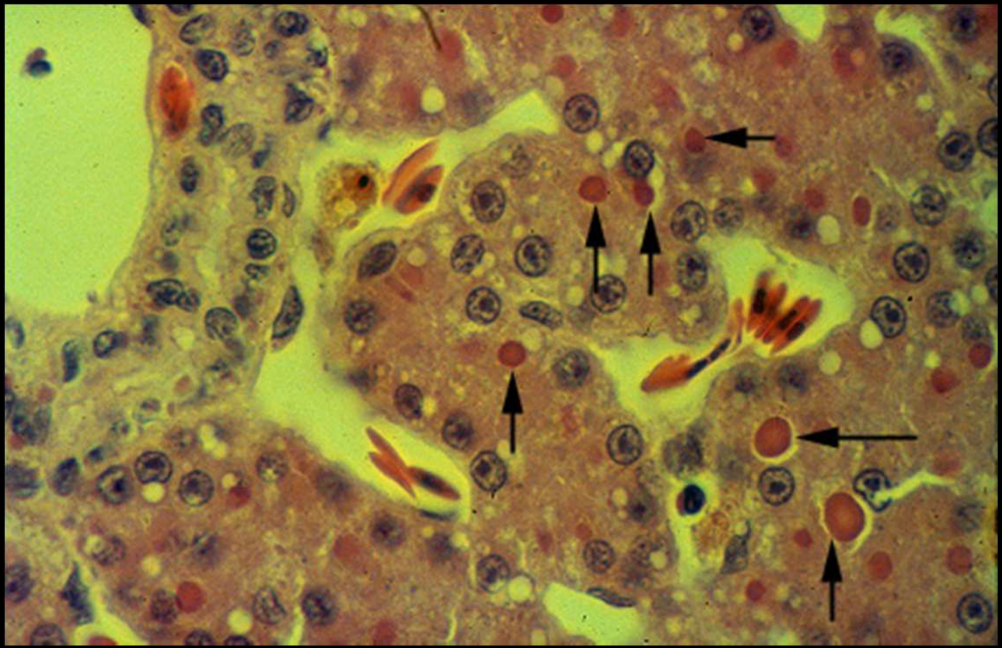
INCLUSION BODY DISEASE OF BOID SNAKES



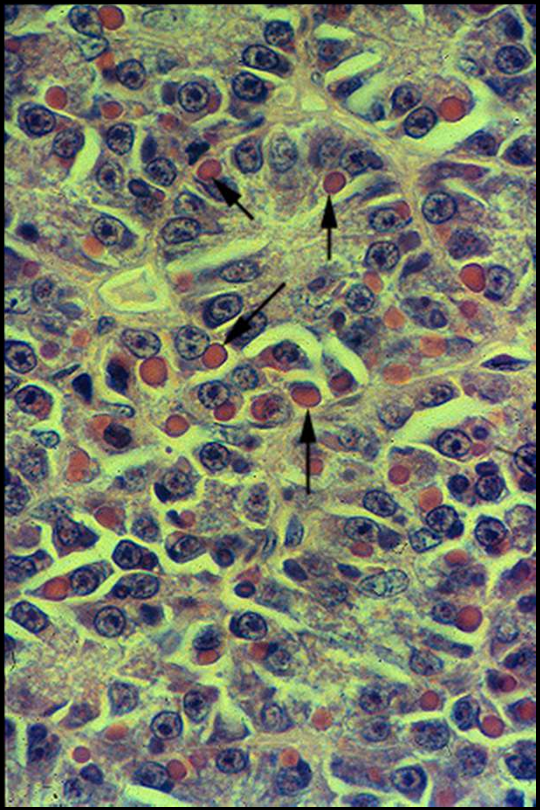


Esophageal Tonsils

INCLUSION BODY DISEASE OF BOID SNAKES

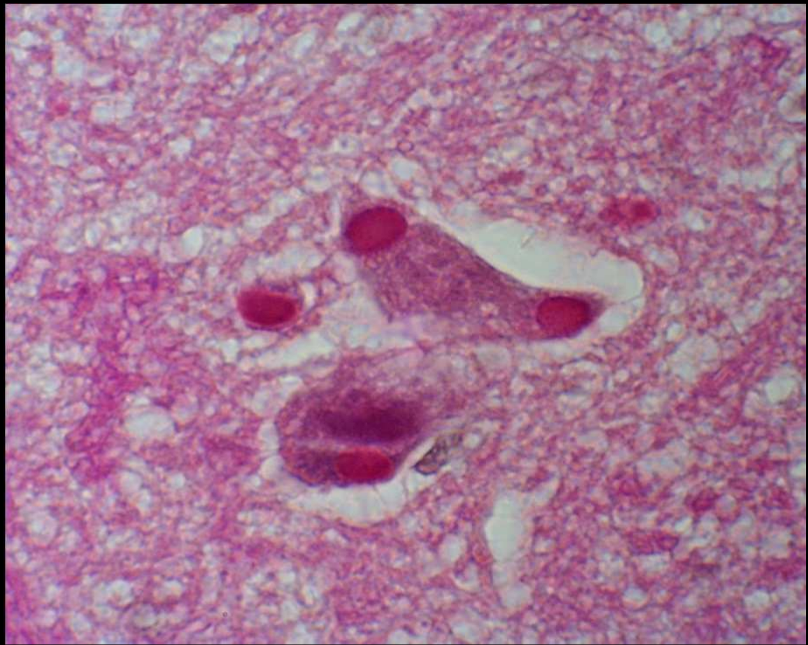


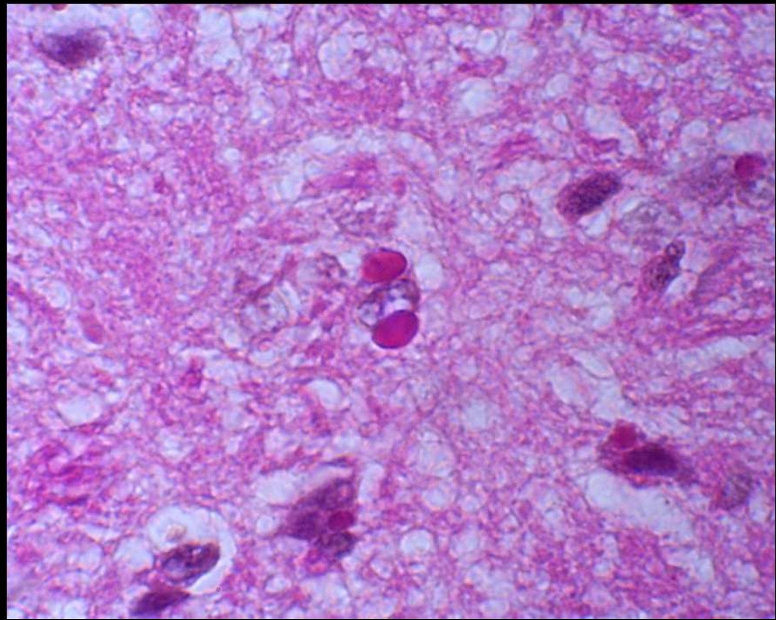
Liver - inclusions



Pancreas - inclusions

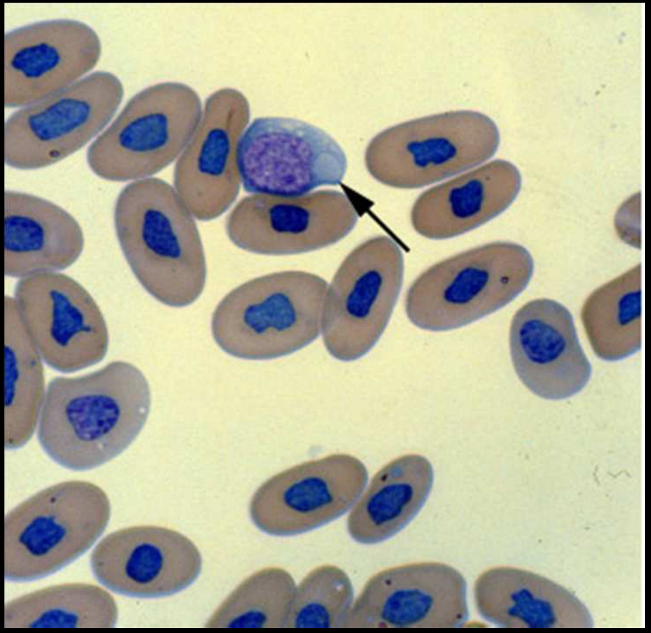
INCLUSION BODY DISEASE OF BOID SNAKES





Hindbrain - inclusions

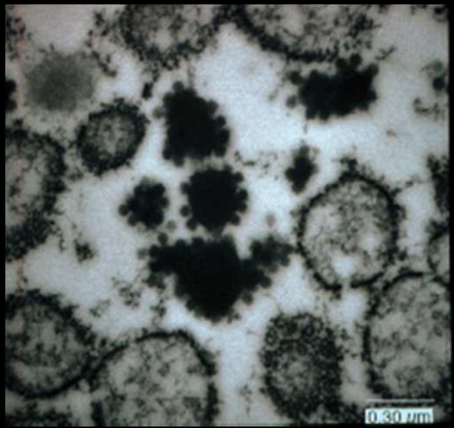
INCLUSION BODY DISEASE OF BOID SNAKES

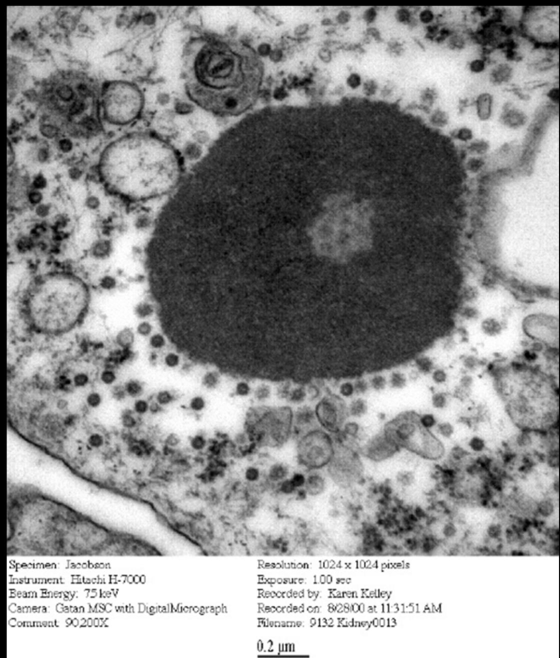


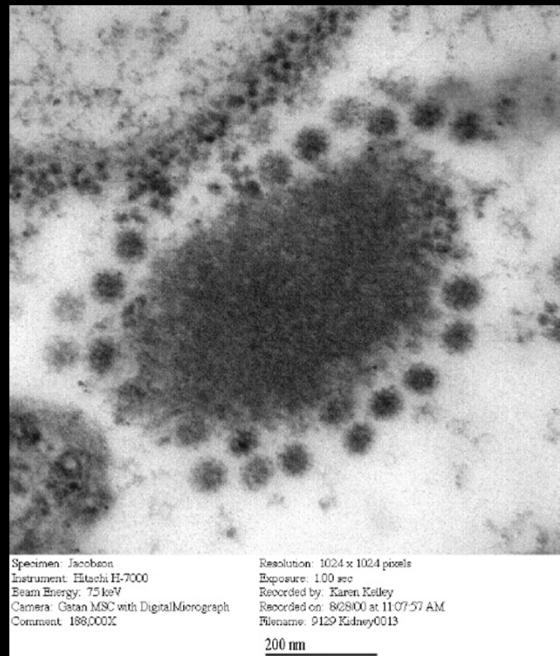
INTRACYTOPLASMIC INCLUSIONS - LYMPHOCYTES

WRIGHT-GIEMSA STAIN

INCLUSION BODY DISEASE OF BOID SNAKES







INCLUSION BODY DISEASE OF BOID SNAKES

Isolation and Characterization of an Antigenically Distinct 68-kd Protein from Nonviral Intracytoplasmic Inclusions in Boa Constrictors Chronically Infected with the Inclusion Body Disease Virus (IBDV: Retroviridae)

E. WOZNIAK, J. MCBRIDE, D. DENARDO, R. TARARA, V. WONG, AND B. OSBURN

Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA (EW,[1] JM, RT, VW, BO); and
Office of Laboratory Animal Care, Northwest Animal Facility, University of California, Berkeley, CA (DD)

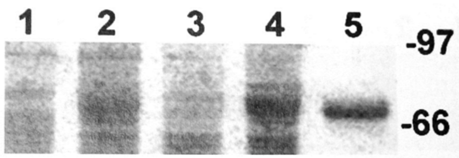


Fig. 8. Polyacrylamide gel containing electrophoretically speared proteins extracted from normal and IBDV-infected *B. constrictor* liver and kidney. Lanes 1 and 3 contain normal boa liver and kidney homogenates, respectfully. Lanes 2 and 4 contain IBDV-infected liver and kidney homogenates, respectfully. Both of the infected tissues have a high density of large inclusion bodies. A prominent 68-kd protein band is present in both IBDV-infected, inclusion-positive tissues; this band is not present in normal boa tissues. An aliquot the electrophoretically purified protein fraction is shown in lane 5. Coomassie brilliant blue R-250 total protein stain. The markers represent the molecular masses in kilodaltons.

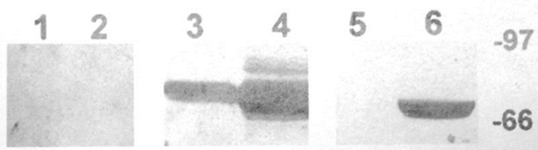
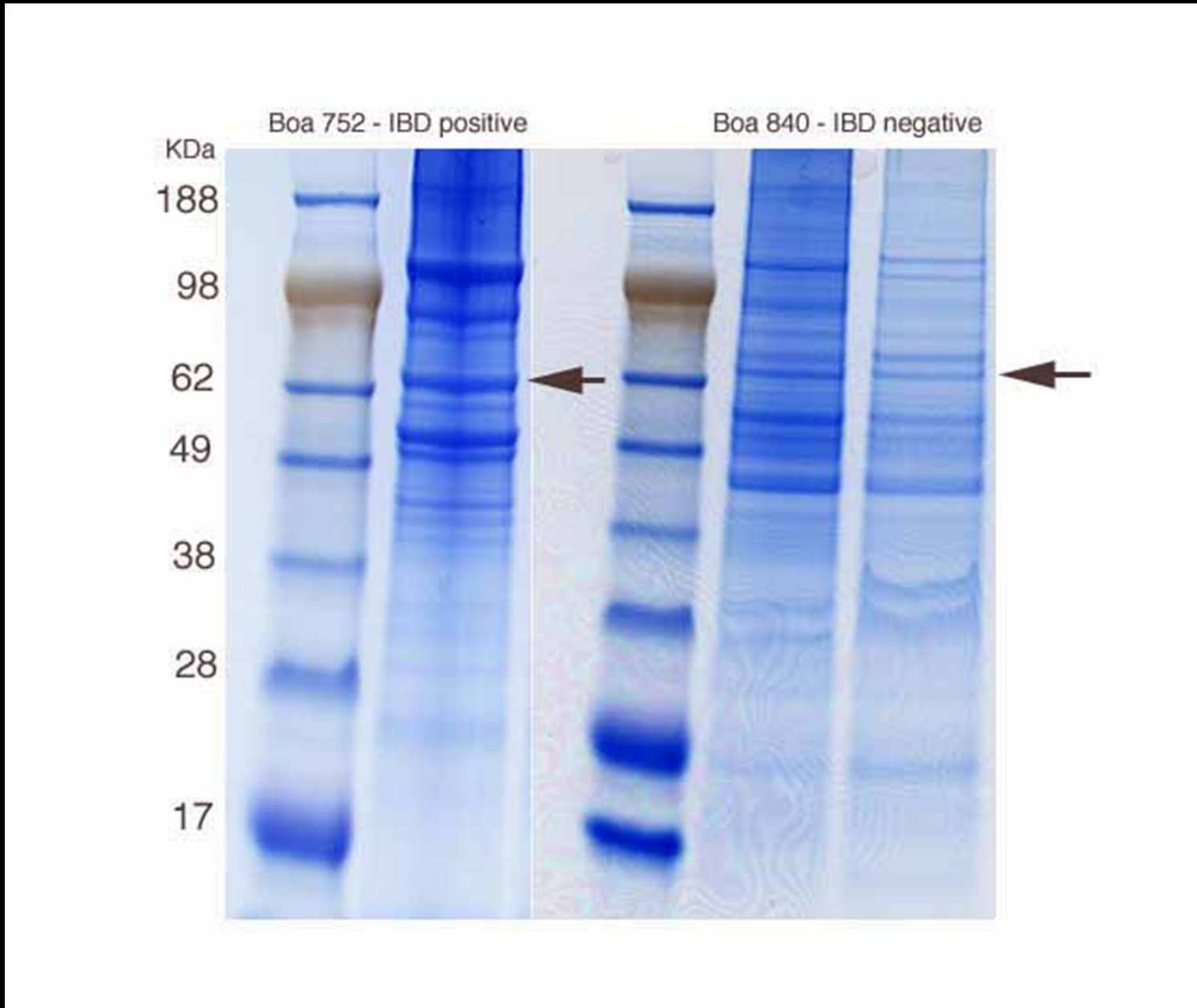
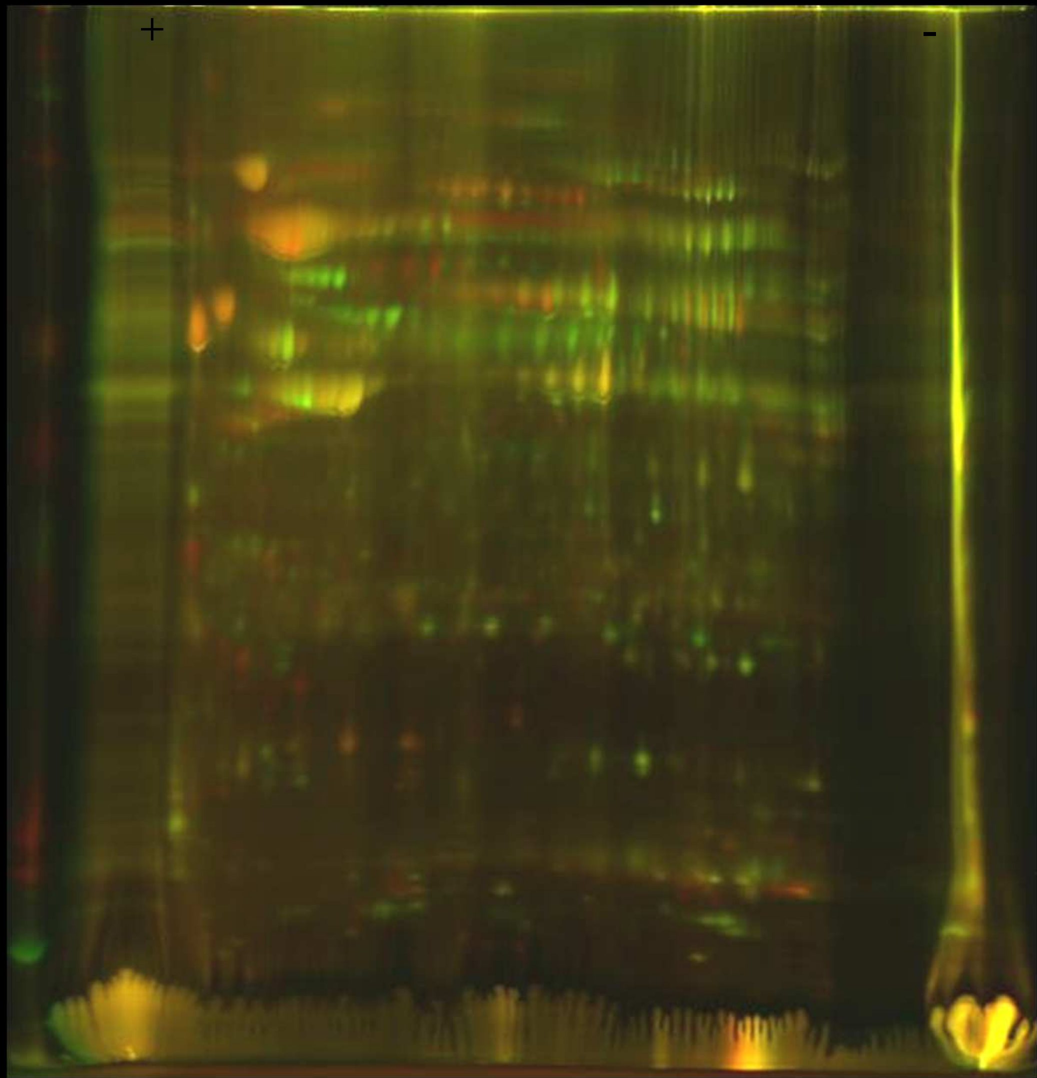


Fig. 9. Western blot of normal and IBDV-infected, inclusion-positive *B. constrictor* liver proteins. Lanes 1, 3, and 5 contain normal boa liver. Lanes 2, 4, and 6 contain IBDV-infected boa constrictor liver with a high density of large inclusion bodies. Lanes 1 and 2 were stained with baseline mouse serum. Lanes 3 and 4 were stained with polyclonal mouse antisera raised against the 68-kd IBD protein. Lanes 5 and 6 were stained with monoclonal antibody 2H2, which demonstrates specific affinity for the 68-kd IBD protein band. The markers represent the molecular masses in kilodaltons.

Gel Electrophoresis of Inclusion Proteins



6/10/04   2D DIGE of Boa liver protein.   45 ug Cy5 labeled IBD #752 protein (red) and 45 ug Cy3 labeled control #840 protein (green) mixed with 400 ug of unlabeled #752 was focused in 18 cm pH 3 to 11 IPG strip for 80 kVhr before separated in 8 to 16% Tris Glycie SDS PAGE.



INCLUSION BODY DISEASE OF BOID SNAKES

INCLUSION BODY DISEASE OF BOID SNAKES

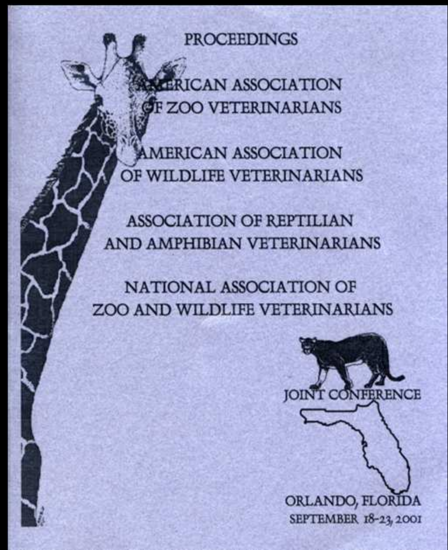
ISOLATION OF VIRUSES FROM BOA CONSTRICTORS (*Boa constrictor* spp.) WITH INCLUSION BODY DISEASE IN EUROPE

Rachel E. Marschang, DMV,[1] Udo Hetzel, DMV, Dr. Biol.,[2] Dirk Schwartz,[2] Ralf Michling,[3] and Karina Matthes[3]*

[1]Institute for Avian Medicine, Jusut Liebig University Giessen, Frankfurter Street 91, 35392 Giessen, Germany; [2]Department of Veterinary Pathology, Jusut Liebig University Giessen, Frankfurter Street, 35392 Giessen, Germany; [3]Clinic of Small Animals, School of Veterinary Medicine, Hannover, Germany

Abstract

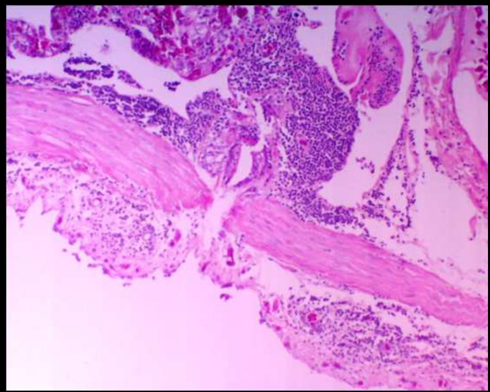
Inclusion body disease (IBD) is characterized by the formation of intracytoplasmatic inclusions in neurons and in epithelial cells of various organs. It generally affects boid snakes. Clinically, it is commonly associated with central nervous system (CNS) disorders as well as regurgitation, stomatitis, and pneumonia. The disease is believed to be of viral etiology, and retroviruses have been implicated as a possible factor.[8,16] However, boa constrictors (*Boa constrictor* spp.) have also been shown to harbor endogenous retroviruses,[13] making a definitive connection between retroviruses found in IBD positive snakes, and disease difficult.

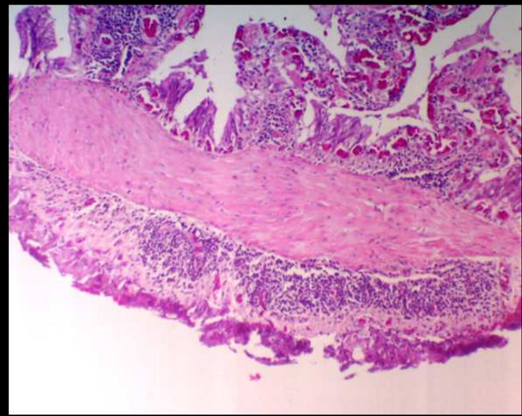


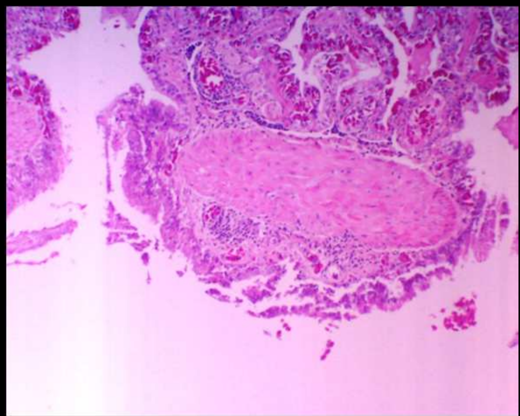
Reoviral infection

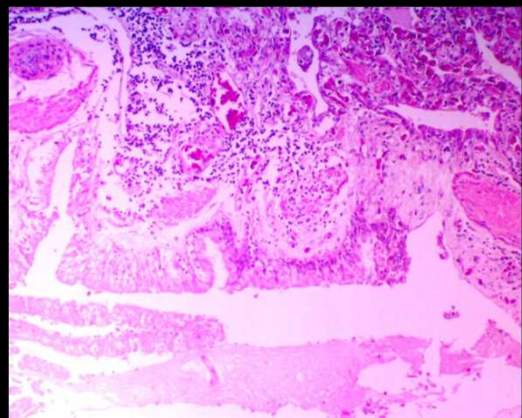
- Rattlesnakes - CNS disease and pneumonia
- Rat Snakes - pneumonia
- Boas and Pythons - associated with IBD
- Green Iguana - isolated from dead lizards

Reovirus -Moelendorf’s Rat Snake
Interstitial and proliferative Pneumonia









Reoviral Transmission Study

ELSEVIER Virus Research 63 (1999) 135–141

Virus Research

www.elsevier.com/locate/virusres

Short communication

Isolation and experimental transmission of a reovirus pathogenic in ratsnakes (*Elaphe* species)

Elaine W. Lamirande [a,*], Donald K. Nichols [a], Jennie W. Owens [b], Jack M. Gaskin [c], Elliott R. Jacobson [d]

[a] *Department of Pathology, National Zoological Park, Smithsonian Institution, Washington, DC 20008, USA*
[b] *Veterinary Resources Program, Office of Research Services, National Institutes of Health, Bethesda, MD 20892, USA*
[c] *Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA*
[d] *Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA*

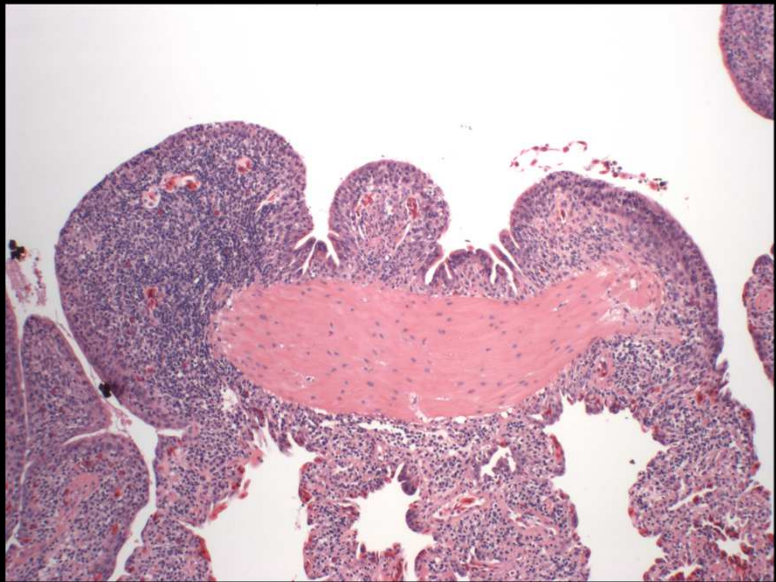
Abstract

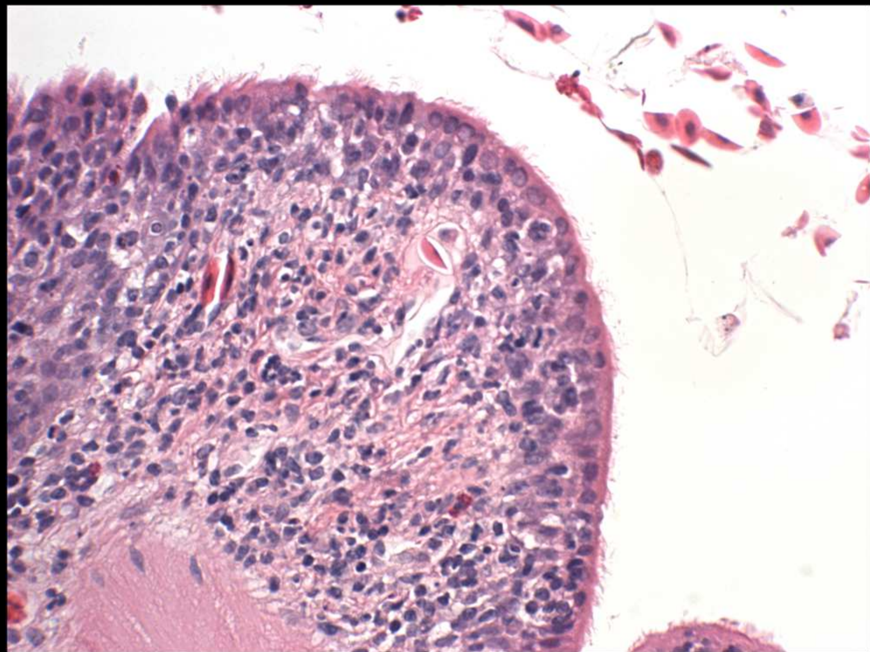
A reovirus was isolated from juvenile Moellendorff's ratsnakes (*Elaphe moellendorffi*) and beauty snakes (*Elaphe taenuris*) that died soon after importation into the USA. Viper heart (VH2) cells inoculated with tissue homogenates showed cytopathic effects consisting of large syncytia formation followed by cell detachment from the monolayer. Tissue culture supernatants failed to hemagglutinate guinea pig and chicken erythrocytes at room temperature. Electron microscopy of purified virions revealed spherical to icosahedral particles measuring 70–85 nm in diameter with a double capsid layer. Preparations of the viral genome contained ten segments of dsRNA when analyzed by polyacrylamide gel electrophoresis. A juvenile black ratsnake (*Elaphe obsoleta obsoleta*) was experimentally inoculated with the isolate and was found dead 26 days post inoculation. Necropsy revealed diffuse subacute interstitial pneumonia with respiratory epithelial cell hyperplasia and syncytia. Reovirus isolated from this snake was used to inoculate another juvenile black ratsnake which was euthanized 40 days post inoculation. Pneumonia and multifocal subacute proliferative tracheitis were found on necropsy. Reovirus was isolated from the lung of this snake and was demonstrated by transmission electron microscopy. This is the first documentation of a pathogenic reptile reovirus and the first report of experimental transmission of a reovirus in snakes.

Keywords: *Elaphe* sp.; Pneumonia; Reovirus; Snake; Tracheitis

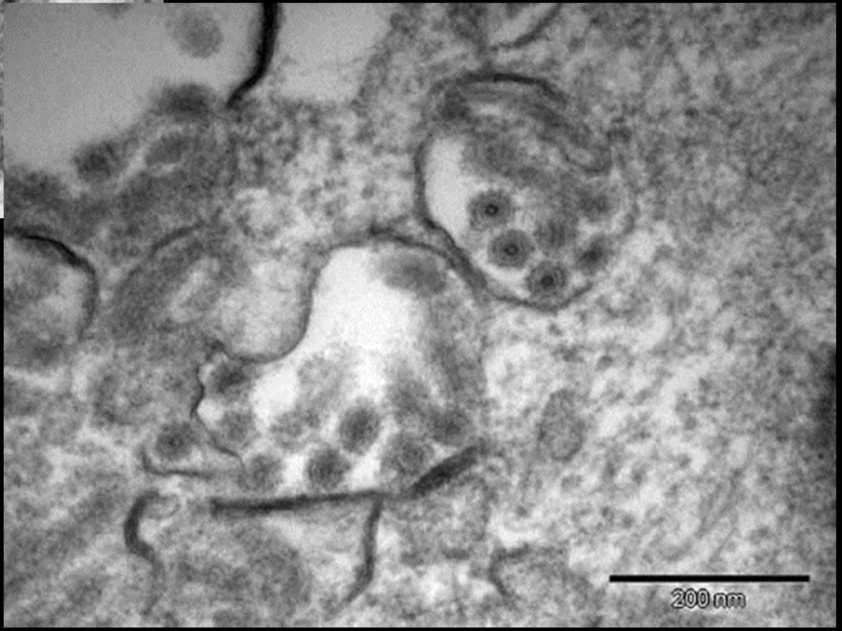
Pulmonary Disease of Ball Pythons, *Python regius*

Pulmonary Disease of Ball Pythons

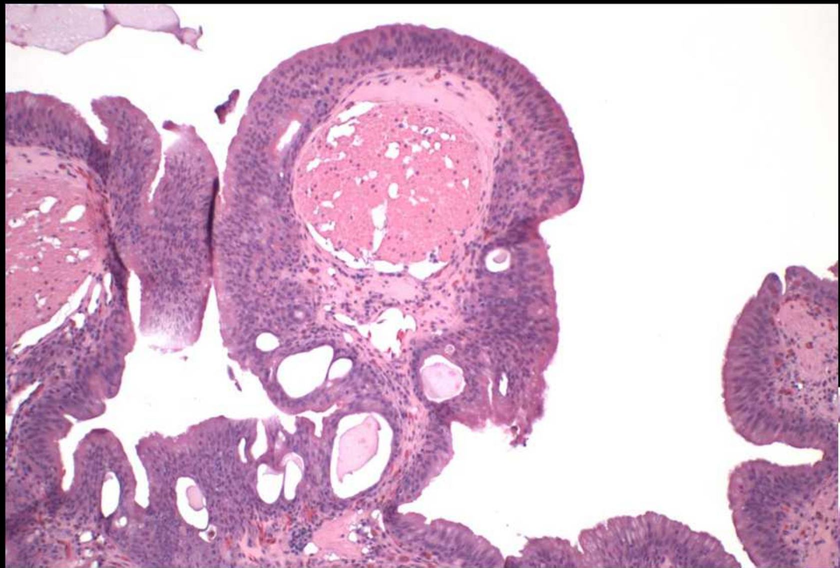


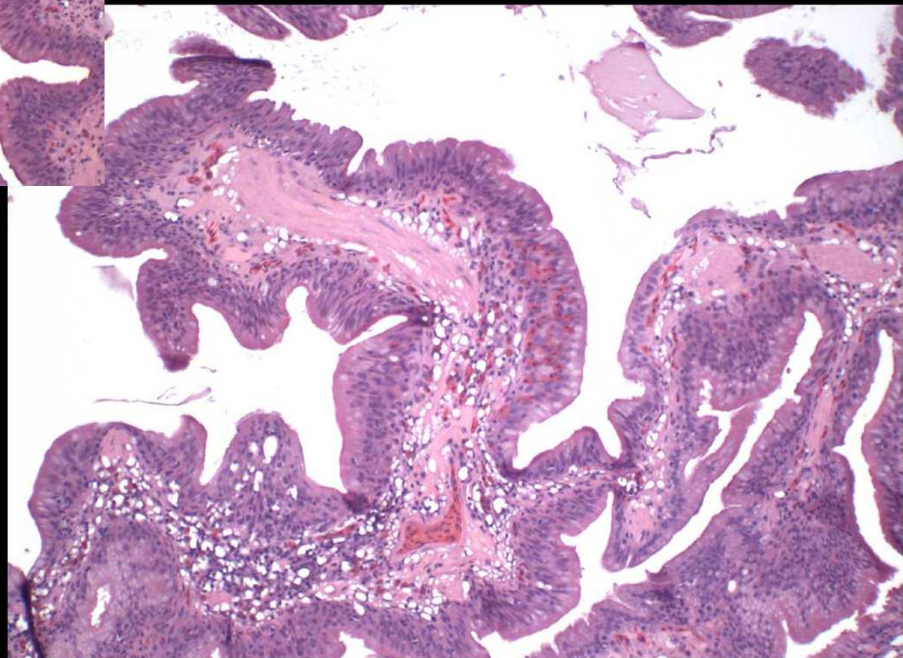


Pulmonary Disease of Ball Pythons

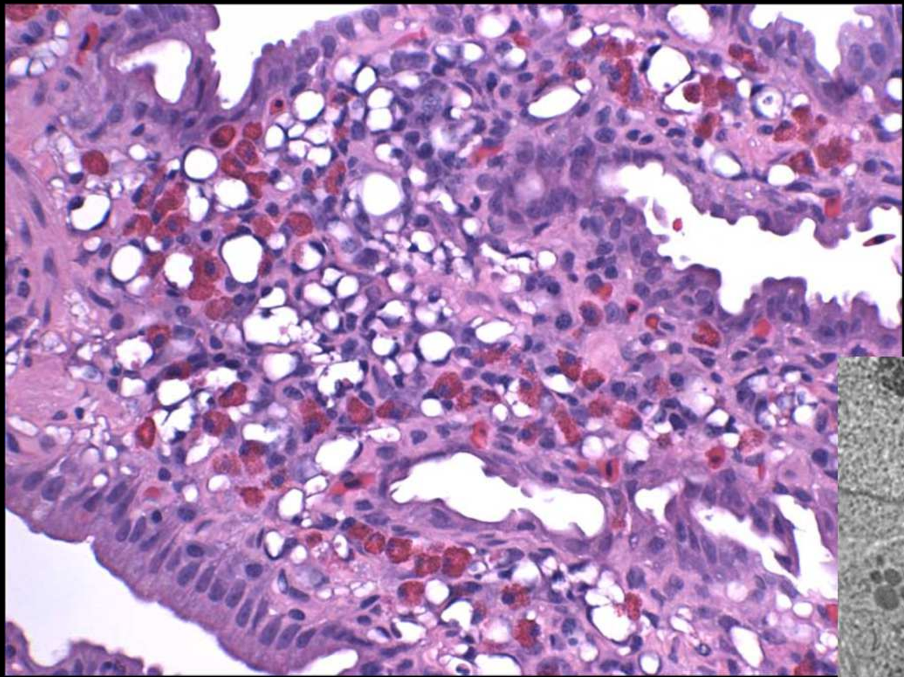


Pulmonary Disease of Ball Pythons

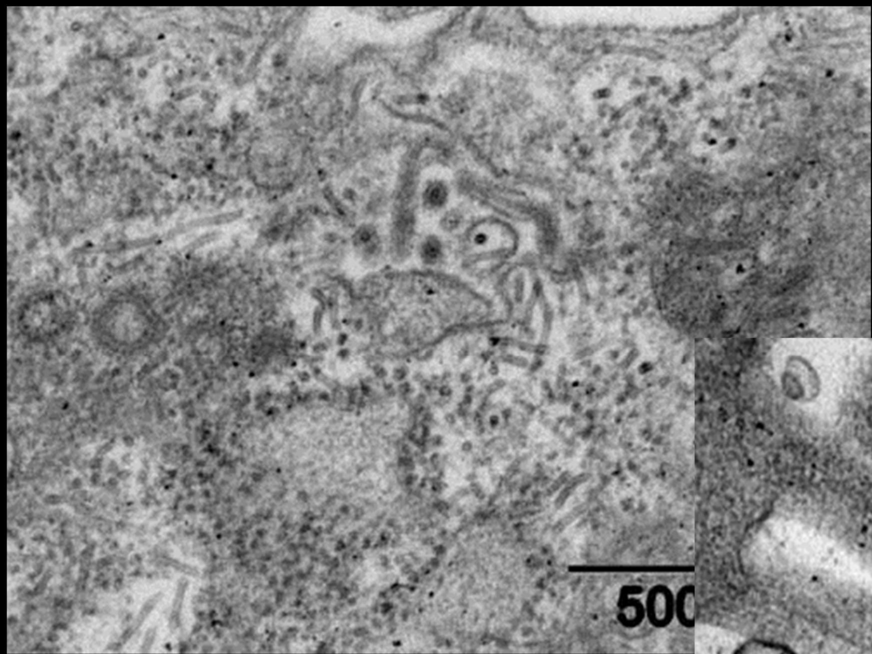


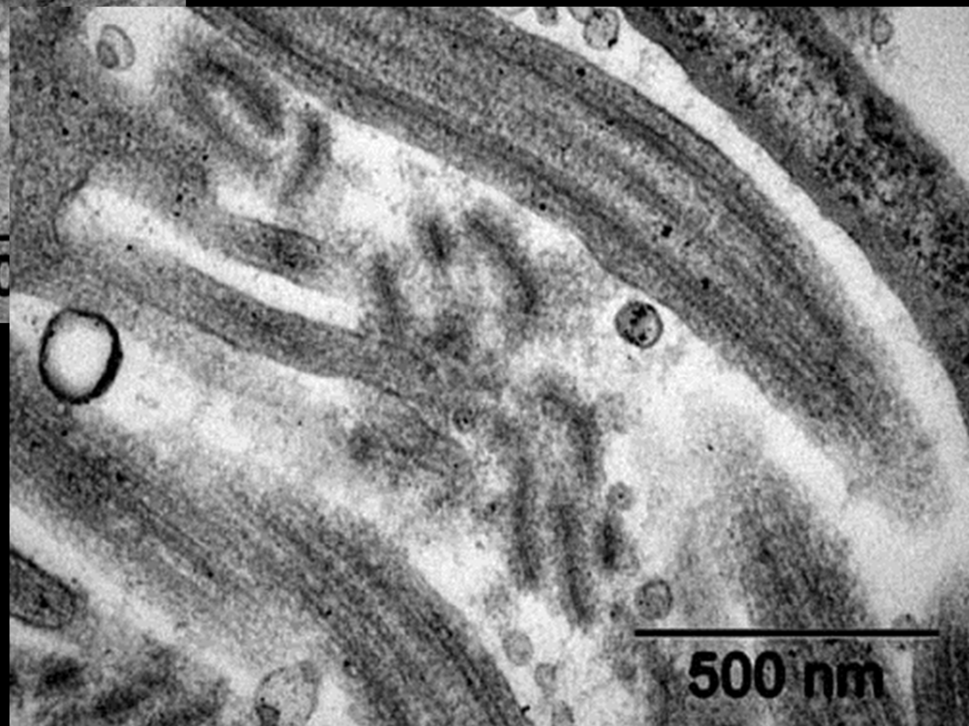


Pulmonary Disease of Ball Pythons



Pulmonary Disease of Ball Pythons





Pulmonary Disease of Ball Pythons

Cynthia Goldsmith and Charles Humphrey, CDC:

- **Filamentous virus in lung closely resemble either rhabdoviruses or filoviruses.**
- **While the morphogenesis is more similar to the filoviruses, the size (50-180 nm) is more in keeping with a rhabdovirus.**
- **Possibly within the genera Vesiculovirus or Novirhabdovirus.**

OUTBREAK OF WNV INFECTION IN FARMED AMERICAN ALLIGATORS IN FLORIDA

West Nile Virus Infection in American Alligators

Miller DL, Mauel MJ, Baldwin C, Burtle G, Ingram D, Hines II ME, Frazier KS. 2003. West Nile Virus in farmed alligators. Emerg Inf Dis 9:794-799.

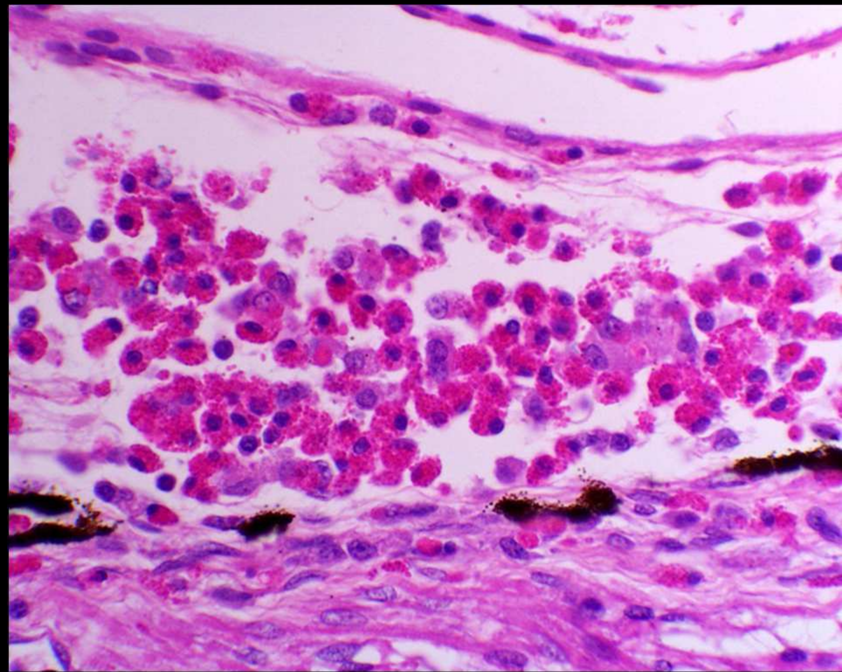
Jacobson ER, Ginn PE, Troutman JM, Farina L, Stark L, Klenk K, Komar N. 2005b. West Nile Virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida. J Wildl Dis 41:96-106.

Jacobson ER, Johnson AJ, Hernandez JA, Tucker SJ, Dupuis AP, Stevens R, Carbonneau D, and Stark K. 2005a. Use of an indirect enzyme-linked immunosorbent assay for detection of antibodies to West Nile Virus in American alligators (*Alligator mississippiensis*). J Wildl Dis 41:107-114.

WNV INFECTION IN ALLIGATORS

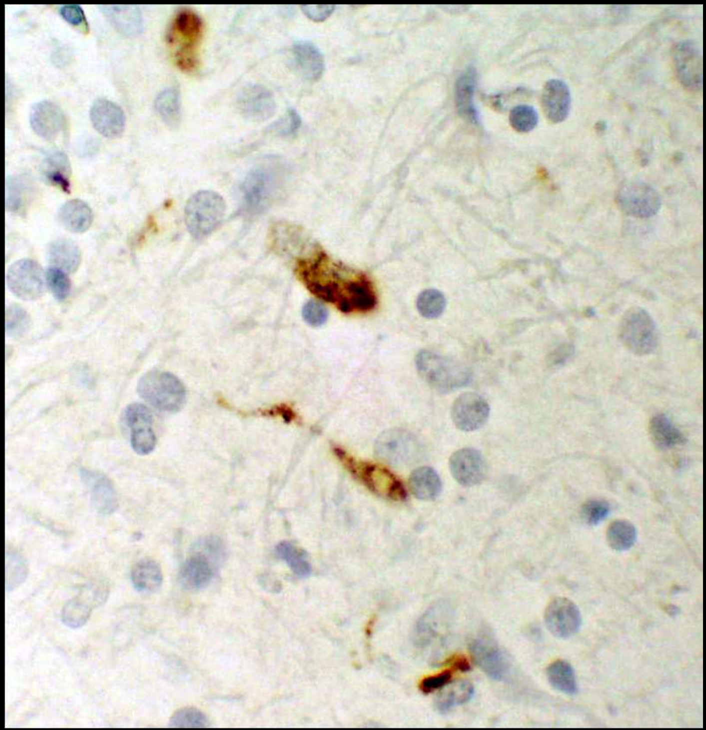
WNV INFECTION IN ALLIGATORS

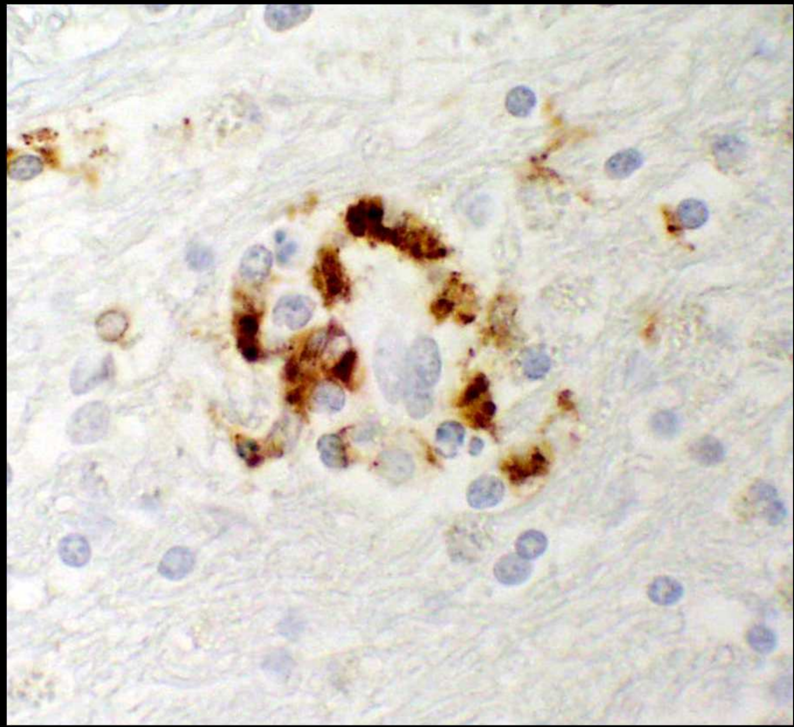
Meninges - Spinal Cord



WNV INFECTION IN ALLIGATORS

Immunoperoxidase - Brain





LYMPHOHISTIOCYTIC PROLIFERATIVE SYNDROME OF ALLIGATORS (*ALLIGATOR MISSISSIPPIENSIS*): A CUTANEOUS MANIFESTATION OF WEST NILE VIRUS

A Dissertation

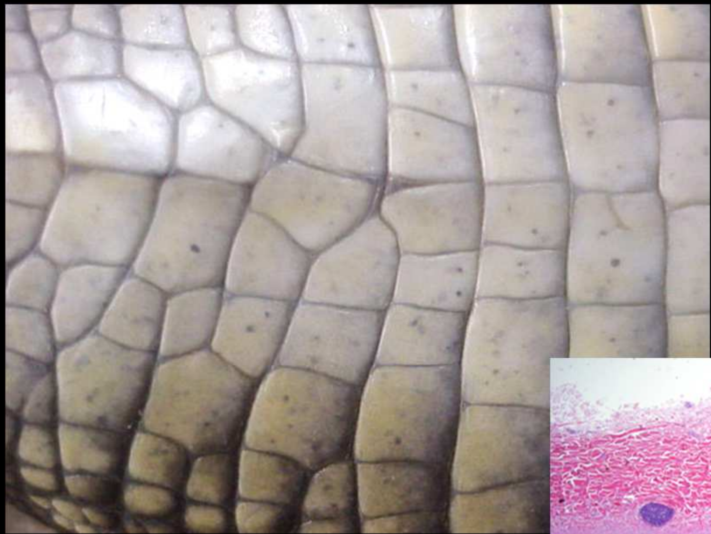
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in fulfillment of the
requirements for the degree of
Doctor of Philosophy

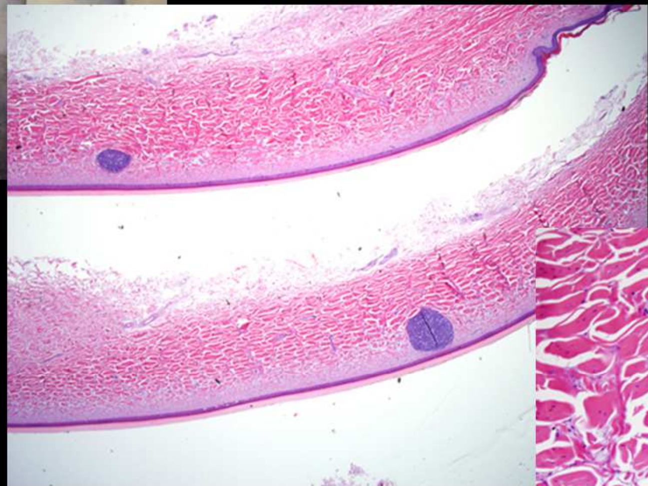
in

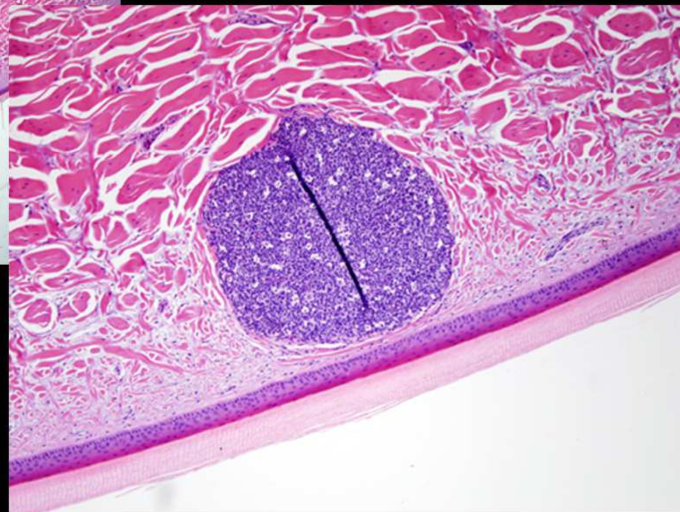
The Interdepartmental Program in
Veterinary Medical Sciences through
the Department of Veterinary Clinical Sciences

by
Javier G. Nevarez
B.S., Louisiana State University, 1998
D.V.M., Louisiana State University, 2001
May 2007

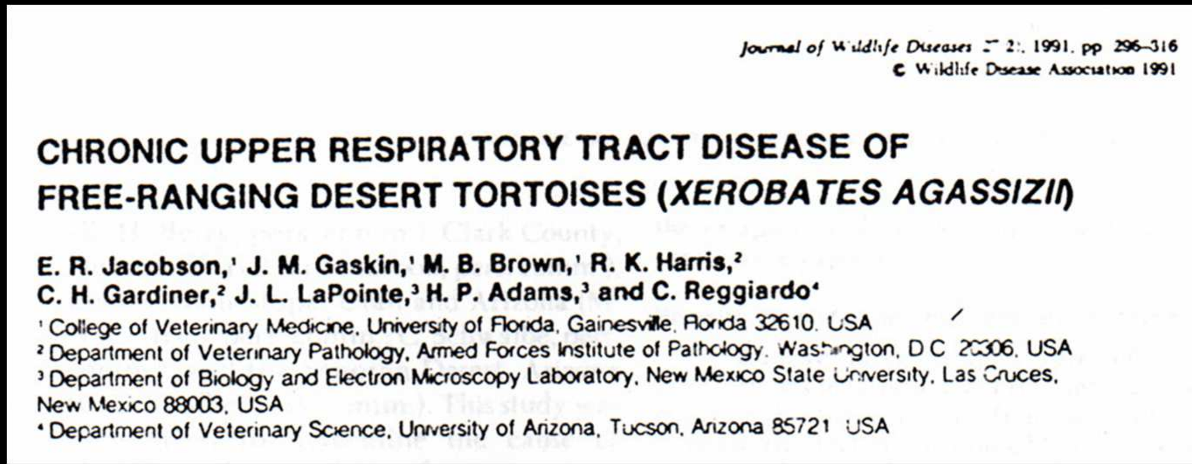
Lymphohistiocytic lesions in skin of American alligators - images courtesy of Dr. Javier Nevarez







Mycoplasmosis in Tortoises



Journal of Wildlife Diseases 27(2), 1991, pp. 296–316

CHRONIC UPPER RESPIRATORY TRACT DISEASE OF FREE-RANGING DESERT TORTOISES (*XEROBATES AGASSIZII*)

E. R. Jacobson,[1] J. M. Gaskin,[1] M. B. Brown,[1] R. K. Harris,[2] C. H. Gardiner,[2] J. L. LaPointe,[3] H. P. Adams,[3] and C. Reggiardo[4]

[1] College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610, USA
[2] Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306, USA
[3] Department of Biology and Electron Microscopy Laboratory, New Mexico State University, Las Cruces, New Mexico 88003, USA
[4] Department of Veterinary Science, University of Arizona, Tucson, Arizona 85721 USA

Mycoplasmosis in Tortoises

INFECTION AND IMMUNITY, Oct. 1994, p. 4580–4586 Vol. 62, No.
0019-9567/94/$04.00+0

Mycoplasma agassizii Causes Upper Respiratory Tract Disease in the Desert Tortoise†

MARY B. BROWN,[1*] ISABELLA M. SCHUMACHER,[2] PAUL A. KLEIN,[3] KEITH HARRIS,[4] TERRIE CORRELL,[5] AND ELLIOTT R. JACOBSON[2]

Departments of Infectious Diseases[1] and Small Animal Clinical Sciences,[2] College of Veterinary Medicine, and Department of Pathology and Laboratory Medicine, College of Medicine,[3] University of Florida, Gainesville, Florida; Armed Forces Institute of Pathology, Washington, D.C.[4]; and The Living Desert Museum, Palm Springs, California[5]

Received 18 March 1994/Returned for modification 17 May 1994/Accepted 26 July 1994

The desert tortoise is listed by the United States government as a threatened species in part of its range. A major contributing factor in the decline of this animal has been the presence of an upper respiratory tract disease (URTD) which is characterized by a chronic disease which eventually leads to severe occlusion of the nares with viscous exudate and destruction of the respiratory epithelium. Electron microscopy of infected tissues demonstrated the presence of a mycoplasma-like organism attached to the respiratory surfaces. The mycoplasma was isolated and designated as a new species, with the proposed name *Mycoplasma agassizii*. The current study was designed to fulfill Koch's postulates and determine if *M. agassizii* was the etiologic agent of URTD. Clinically healthy animals with known antibody status were infused intranasally with pooled exudate ($n = 8$) from ill donor animals, with *M. agassizii* alone ($n = 9$) or in combination with *Pasteurella testudinis* ($n = 8$), with *P. testudinis* alone ($n = 9$), or with sterile broth ($n = 12$). The pooled exudate was culture positive for *M. agassizii*. Tortoises which received exudate or *M. agassizii* alone or in conjunction with *P. testudinis* were significantly more likely to develop clinical disease ($P < 0.0004$) than animals which received *P. testudinis* alone or the broth controls. Tortoises demonstrated a strong immune response to *M. agassizii*, and seroconversion was seen in all groups with clinical disease. *M. agassizii* was isolated from the upper respiratory tracts of clinically ill animals up to 6 months postinfection. On the basis of the results of these transmission studies, we conclude that *M. agassizii* is an etiologic agent of URTD in the desert tortoise.

JOURNAL OF CLINICAL MICROBIOLOGY, July 1999, p. 2262–2269 Vol. 37,
0095-1137/99/$04.00+0

Upper Respiratory Tract Disease in the Gopher Tortoise Is Caused by *Mycoplasma agassizii*†

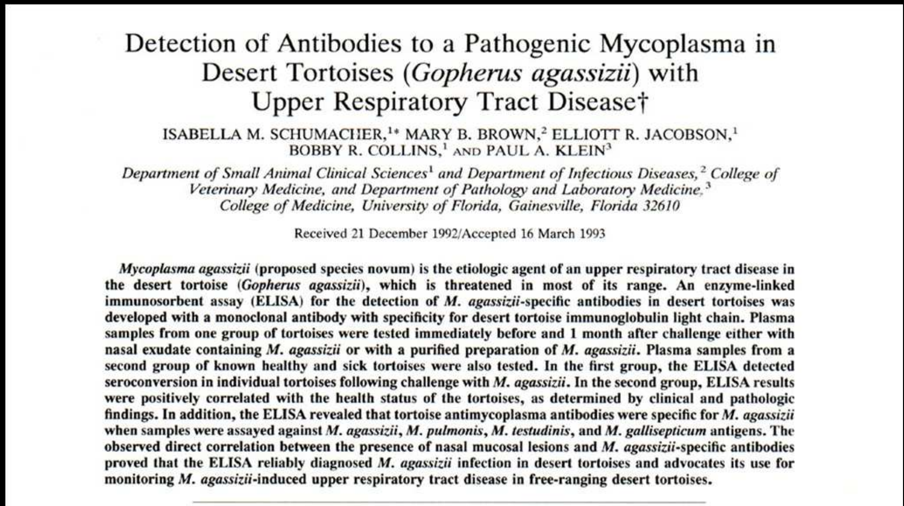
M. B. BROWN,[1*] G. S. MCLAUGHLIN,[2,3] P. A. KLEIN,[4] B. C. CRENSHAW,[1] I. M. SCHUMACHER,[4] D. R. BROWN,[1] AND E. R. JACOBSON[2]

Department of Pathobiology and Division of Comparative Medicine[1] and Department of Small Animal Clinical Sciences,[2] College of Veterinary Medicine, Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences,[3] and Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine,[4] University of Florida, Gainesville, Florida

Received 19 November 1998/Returned for modification 18 February 1999/Accepted 31 March 1999

Upper respiratory tract disease (URTD) has been observed in a number of tortoise species, including the desert tortoise (*Gopherus agassizii*) and the gopher tortoise (*Gopherus polyphemus*). Clinical signs of URTD in gopher tortoises are similar to those in desert tortoises and include serous, mucoid, or purulent discharge from the nares, excessive tearing to purulent ocular discharge, conjunctivitis, and edema of the eyelids and ocular glands. The objectives of the present study were to determine if *Mycoplasma agassizii* was an etiologic agent of URTD in the gopher tortoise and to determine the clinical course of the experimental infection in a dose-response infection study. Tortoises were inoculated intranasally with 0.5 ml (0.25 ml/nostril) of either sterile SP4 broth (control group; $n = 10$) or 10^8 color-changing units (CCU) (total dose) of *M. agassizii* 723 (experimental infection group; $n = 9$). *M. agassizii* caused clinical signs compatible with those observed in tortoises with natural infections. Clinical signs of URTD were evident in seven of nine experimentally infected tortoises by 4 weeks postinfection (p.i.) and in eight of nine experimentally infected tortoises by 8 weeks p.i. In the dose-response experiments, tortoises were inoculated intranasally with a low (10^1 CCU; $n = 6$), medium (10^3 CCU; $n = 6$), or high (10^5 CCU; $n = 5$) dose of *M. agassizii* 723 or with sterile SP4 broth ($n = 10$). At all time points p.i. in both experiments, *M. agassizii* could be isolated from the nares of at least 50% of the tortoises. All of the experimentally infected tortoises seroconverted, and levels of antibody were statistically higher in infected animals than in control animals for all time points of >4 weeks p.i. ($P < 0.0001$). Control tortoises in both experiments did not show clinical signs, did not seroconvert, and did not have detectable *M. agassizii* by either culture or PCR at any point in the study. Histological lesions were compatible with those observed in tortoises with natural infections. The numbers of *M. agassizii* 723 did not influence the clinical expression of URTD or the antibody response, suggesting that the strain chosen for these studies was highly virulent. On the basis of the results of the transmission studies, we conclude that *M. agassizii* is an etiologic agent of URTD in the gopher tortoise.

Tortoise Mycoplasmosis



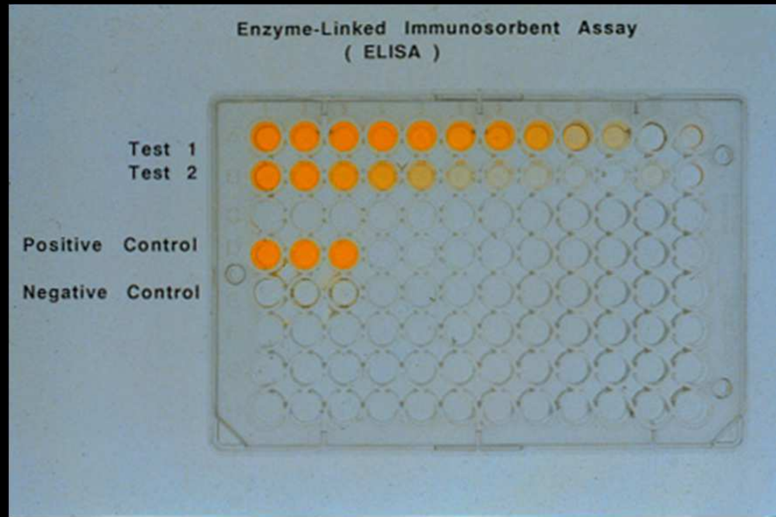
Detection of Antibodies to a Pathogenic Mycoplasma in Desert Tortoises (*Gopherus agassizii*) with Upper Respiratory Tract Disease†

ISABELLA M. SCHUMACHER,[1]* MARY B. BROWN,[2] ELLIOTT R. JACOBSON,[1] BOBBY R. COLLINS,[1] AND PAUL A. KLEIN[3]

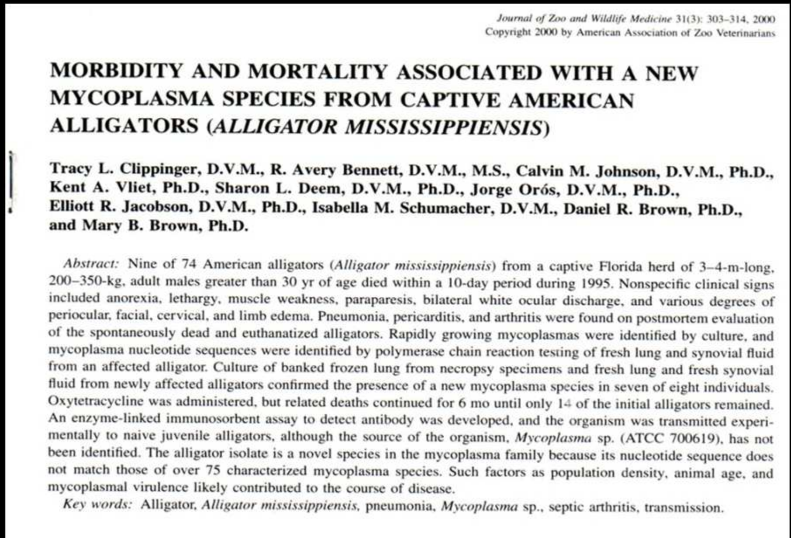
Department of Small Animal Clinical Sciences[1] and Department of Infectious Diseases,[2] College of Veterinary Medicine, and Department of Pathology and Laboratory Medicine,[3] College of Medicine, University of Florida, Gainesville, Florida 32610

Received 21 December 1992/Accepted 16 March 1993

Mycoplasma agassizii **(proposed species novum) is the etiologic agent of an upper respiratory tract disease in the desert tortoise (*Gopherus agassizii*), which is threatened in most of its range. An enzyme-linked immunosorbent assay (ELISA) for the detection of *M. agassizii*-specific antibodies in desert tortoises was developed with a monoclonal antibody with specificity for desert tortoise immunoglobulin light chain. Plasma samples from one group of tortoises were tested immediately before and 1 month after challenge either with nasal exudate containing *M. agassizii* or with a purified preparation of *M. agassizii*. Plasma samples from a second group of known healthy and sick tortoises were also tested. In the first group, the ELISA detected seroconversion in individual tortoises following challenge with *M. agassizii*. In the second group, ELISA results were positively correlated with the health status of the tortoises, as determined by clinical and pathologic findings. In addition, the ELISA revealed that tortoise antimycoplasma antibodies were specific for *M. agassizii* when samples were assayed against *M. agassizii*, *M. pulmonis*, *M. testudinis*, and *M. gallisepticum* antigens. The observed direct correlation between the presence of nasal mucosal lesions and *M. agassizii*-specific antibodies proved that the ELISA reliably diagnosed *M. agassizii* infection in desert tortoises and advocates its use for monitoring *M. agassizii*-induced upper respiratory tract disease in free-ranging desert tortoises.**



Mycoplasmosis in American Alligators



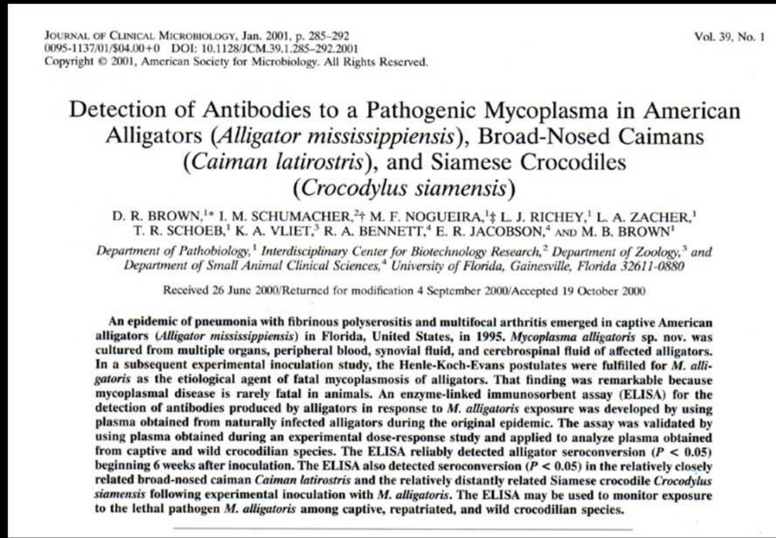
Journal of Zoo and Wildlife Medicine 31(3): 303–314, 2000

MORBIDITY AND MORTALITY ASSOCIATED WITH A NEW MYCOPLASMA SPECIES FROM CAPTIVE AMERICAN ALLIGATORS (*ALLIGATOR MISSISSIPPIENSIS*)

Tracy L. Clippinger, D.V.M., R. Avery Bennett, D.V.M., M.S., Calvin M. Johnson, D.V.M., Ph.D., Kent A. Vliet, Ph.D., Sharon L. Deem, D.V.M., Ph.D., Jorge Orós, D.V.M., Ph.D., Elliott R. Jacobson, D.V.M., Ph.D., Isabella M. Schumacher, D.V.M., Daniel R. Brown, Ph.D., and Mary B. Brown, Ph.D.

Abstract: Nine of 74 American alligators (*Alligator mississippiensis*) from a captive Florida herd of 3–4-m-long, 200–350-kg, adult males greater than 30 yr of age died within a 10-day period during 1995. Nonspecific clinical signs included anorexia, lethargy, muscle weakness, paraparesis, bilateral white ocular discharge, and various degrees of periocular, facial, cervical, and limb edema. Pneumonia, pericarditis, and arthritis were found on postmortem evaluation of the spontaneously dead and euthanatized alligators. Rapidly growing mycoplasmas were identified by culture, and mycoplasma nucleotide sequences were identified by polymerase chain reaction testing of fresh lung and synovial fluid from an affected alligator. Culture of banked frozen lung from necropsy specimens and fresh lung and fresh synovial fluid from newly affected alligators confirmed the presence of a new mycoplasma species in seven of eight individuals. Oxytetracycline was administered, but related deaths continued for 6 mo until only 14 of the initial alligators remained. An enzyme-linked immunosorbent assay to detect antibody was developed, and the organism was transmitted experimentally to naive juvenile alligators, although the source of the organism, *Mycoplasma* sp. (ATCC 700619), has not been identified. The alligator isolate is a novel species in the mycoplasma family because its nucleotide sequence does not match those of over 75 characterized mycoplasma species. Such factors as population density, animal age, and mycoplasmal virulence likely contributed to the course of disease.

Key words: Alligator, *Alligator mississippiensis*, pneumonia, *Mycoplasma* sp., septic arthritis, transmission.



JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2001, p. 285–292 Vol. 39, No. 1
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.1.285–292.2001

Detection of Antibodies to a Pathogenic Mycoplasma in American Alligators (*Alligator mississippiensis*), Broad-Nosed Caimans (*Caiman latirostris*), and Siamese Crocodiles (*Crocodylus siamensis*)

D. R. BROWN,[1*] I. M. SCHUMACHER,[2†] M. F. NOGUEIRA,[1‡] L. J. RICHEY,[1] L. A. ZACHER,[1] T. R. SCHOEB,[1] K. A. VLIET,[3] R. A. BENNETT,[4] E. R. JACOBSON,[4] AND M. B. BROWN[1]

Department of Pathobiology,[1] Interdisciplinary Center for Biotechnology Research,[2] Department of Zoology,[3] and Department of Small Animal Clinical Sciences,[4] University of Florida, Gainesville, Florida 32611-0880

Received 26 June 2000/Returned for modification 4 September 2000/Accepted 19 October 2000

An epidemic of pneumonia with fibrinous polyserositis and multifocal arthritis emerged in captive American alligators (*Alligator mississippiensis*) in Florida, United States, in 1995. *Mycoplasma alligatoris* sp. nov. was cultured from multiple organs, peripheral blood, synovial fluid, and cerebrospinal fluid of affected alligators. In a subsequent experimental inoculation study, the Henle-Koch-Evans postulates were fulfilled for *M. alligatoris* as the etiological agent of fatal mycoplasmosis of alligators. That finding was remarkable because mycoplasmal disease is rarely fatal in animals. An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies produced by alligators in response to *M. alligatoris* exposure was developed by using plasma obtained from naturally infected alligators during the original epidemic. The assay was validated by using plasma obtained during an experimental dose-response study and applied to analyze plasma obtained from captive and wild crocodilian species. The ELISA reliably detected alligator seroconversion ($P < 0.05$) beginning 6 weeks after inoculation. The ELISA also detected seroconversion ($P < 0.05$) in the relatively closely related broad-nosed caiman *Caiman latirostris* and the relatively distantly related Siamese crocodile *Crocodylus siamensis* following experimental inoculation with *M. alligatoris*. The ELISA may be used to monitor exposure to the lethal pathogen *M. alligatoris* among captive, repatriated, and wild crocodilian species.

Chlamydophilosis in Puff Adders

Journal of Zoo and Wildlife Medicine 20(3): 364–369, 1989

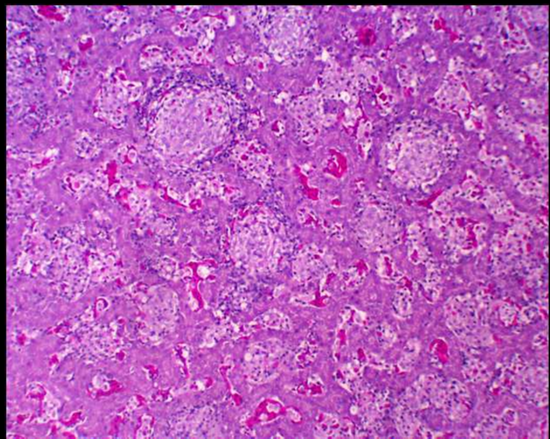
CHLAMYDIAL INFECTION IN PUFF ADDERS (*BITIS ARIETANS*)

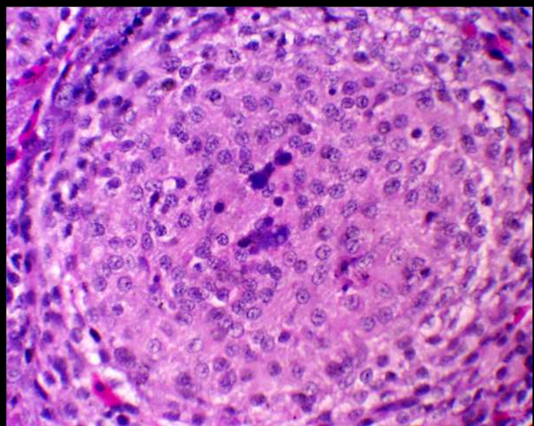
Elliott R. Jacobson, D.V.M., Ph.D., Jack M. Gaskin, D.V.M., Ph.D., and Joanne Mansell, D.V.M., M.S.

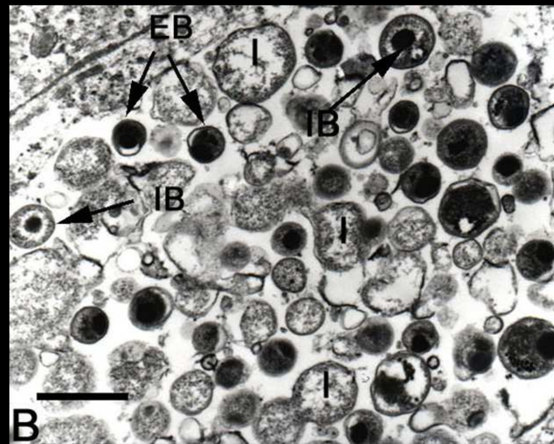
Abstract: Six captive-born puff adders (*Bitis arietans*), housed together in a fiberglass reptile cage, died within 4 mo of acquisition. All snakes occasionally regurgitated mice within 2 days of feeding and one snake manifested a mild respiratory disease preceding death. At necropsy, all snakes had exudate within the pericardial sac and two snakes had multifocal white nodules in their livers. Histologic examination revealed granulomatous peri- and myocarditis, pneumonia, hepatitis, splenitis, and enteritis with basophilic inclusion bodies of various sizes within the caseated centers of the granulomas. The inclusions were found by electron microscopy and consisted of pleomorphic bodies typical of the developmental stages of the genus *Chlamydia*.
Key words: *Chlamydia,* infection, puff adder, *Bitis arietans.*

Chlamydophilosis in Puff Adders

Granulomatous Hepatitis

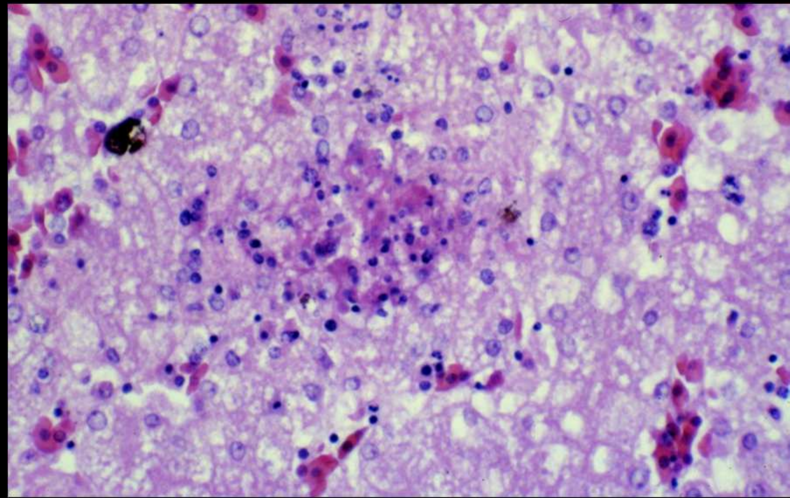


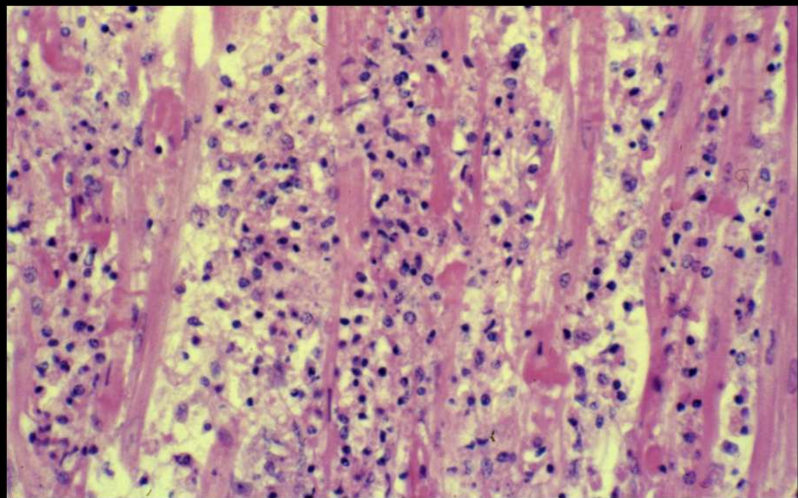




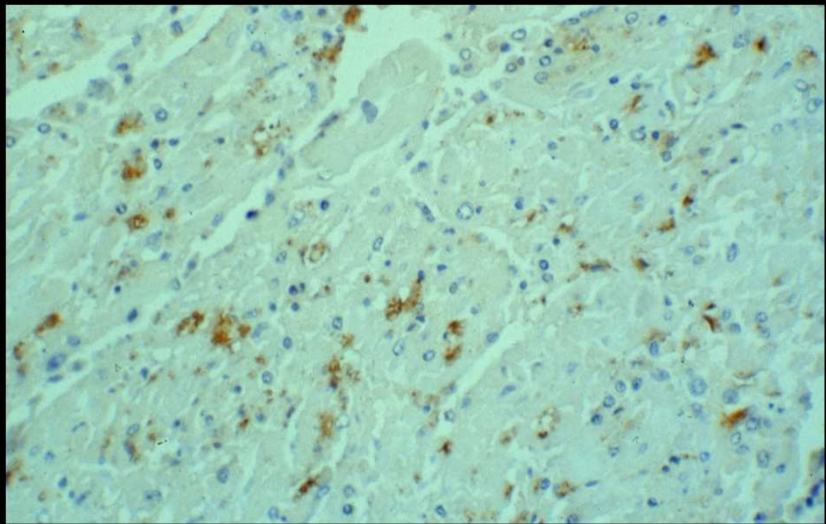
- Initial bodies
- Intermediate bodies
- Elementary bodies

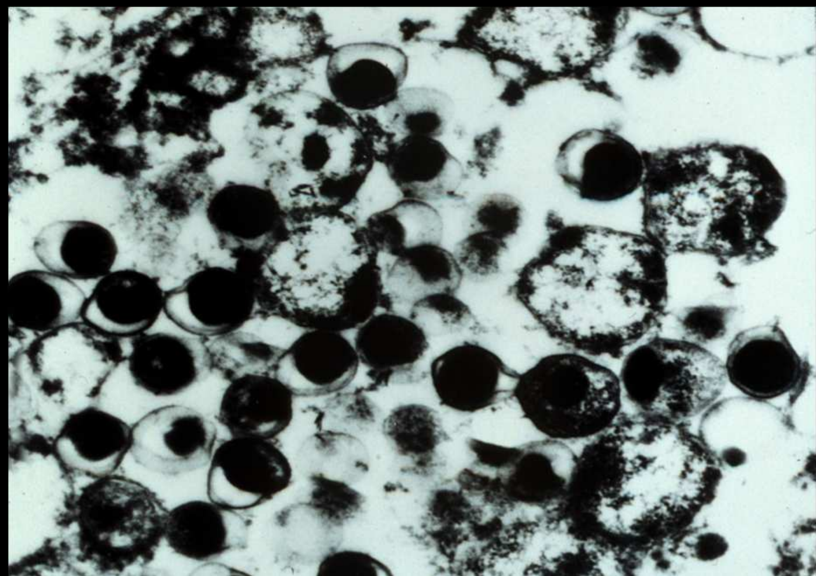
Chlamydophilosis in Green Turtles





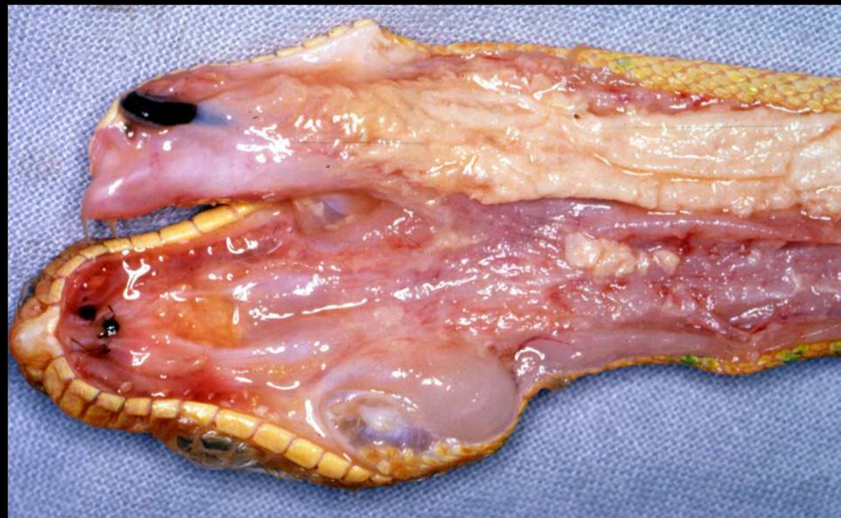
Chlamydophilosis in Green Turtles



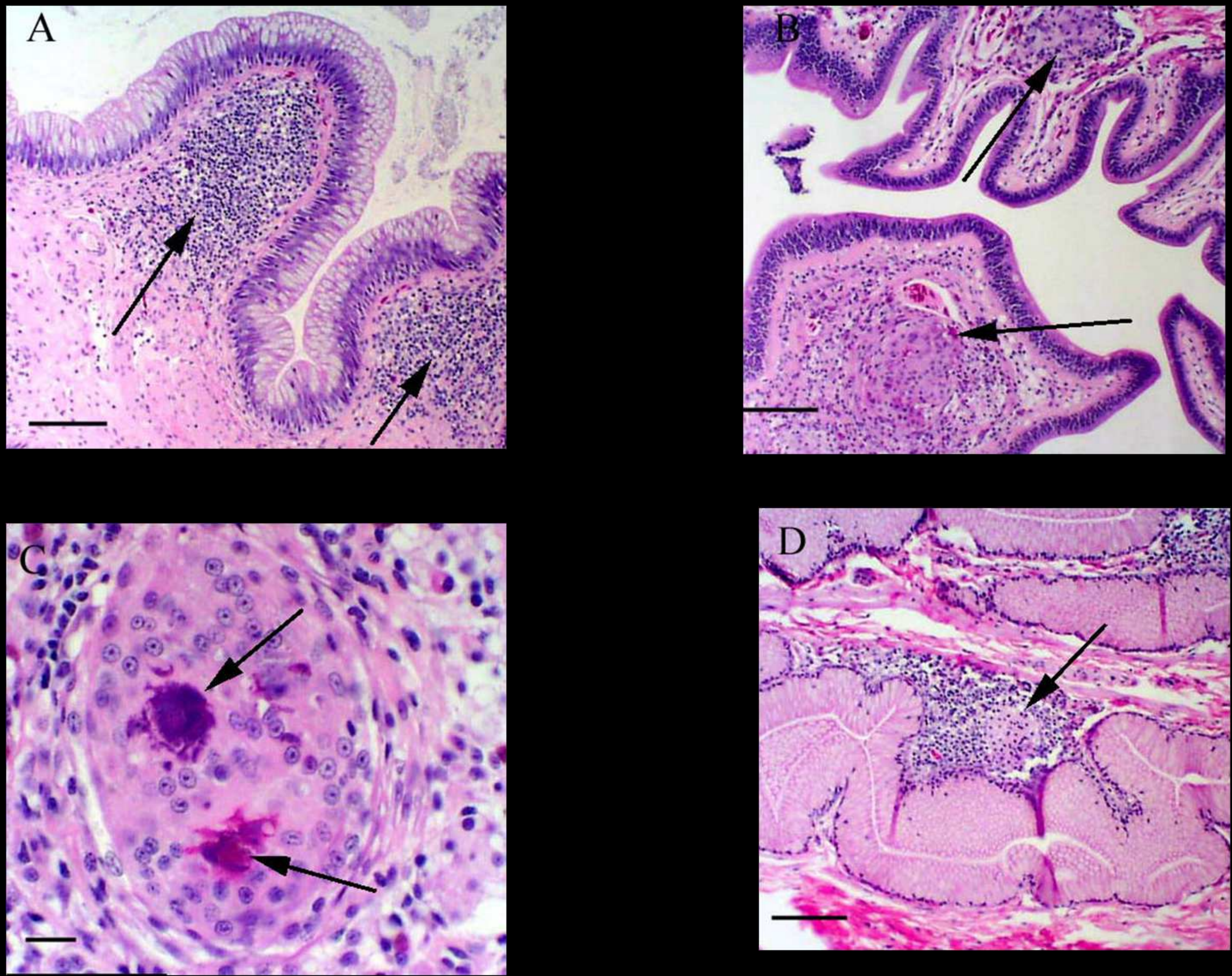


Chlamydophilosis in

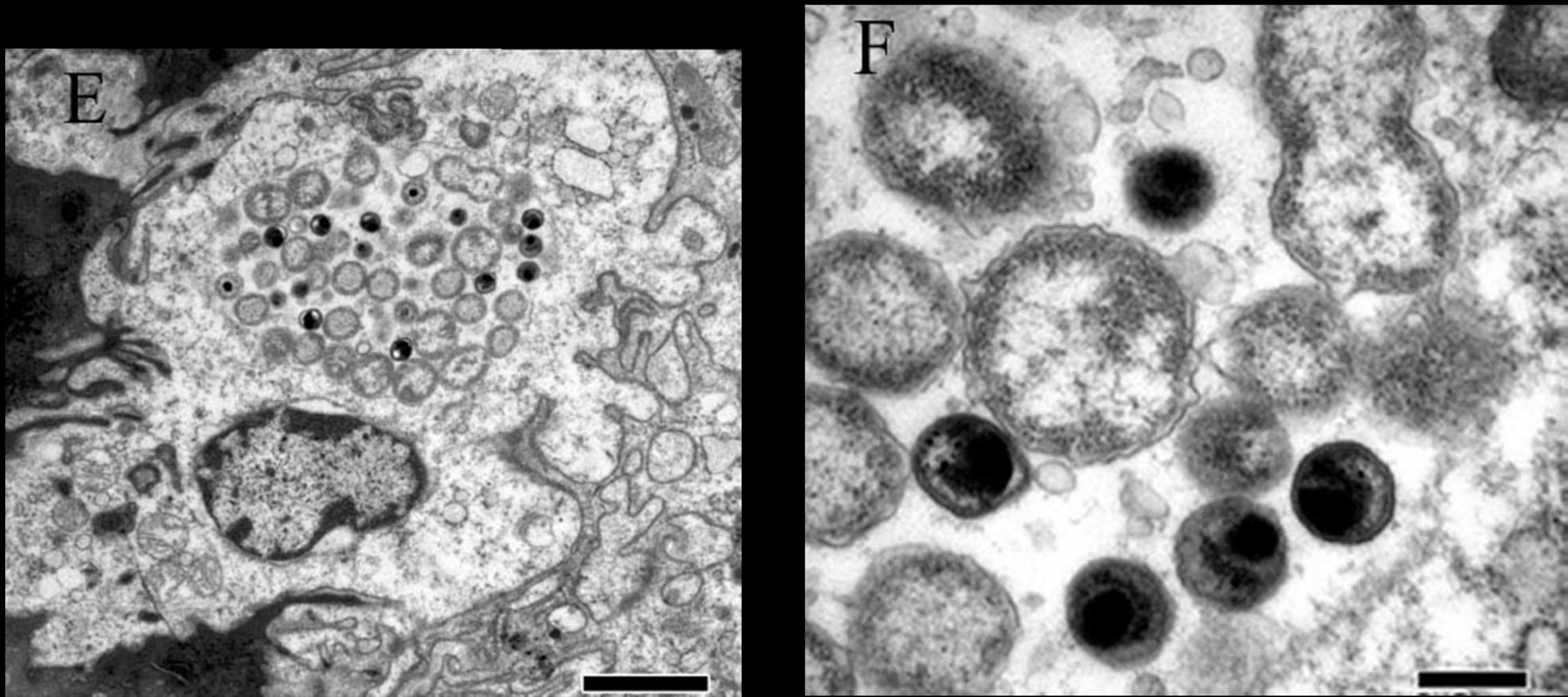
Emerald Tree Boas



Chlamydophilosis in Emerald Tree Boas

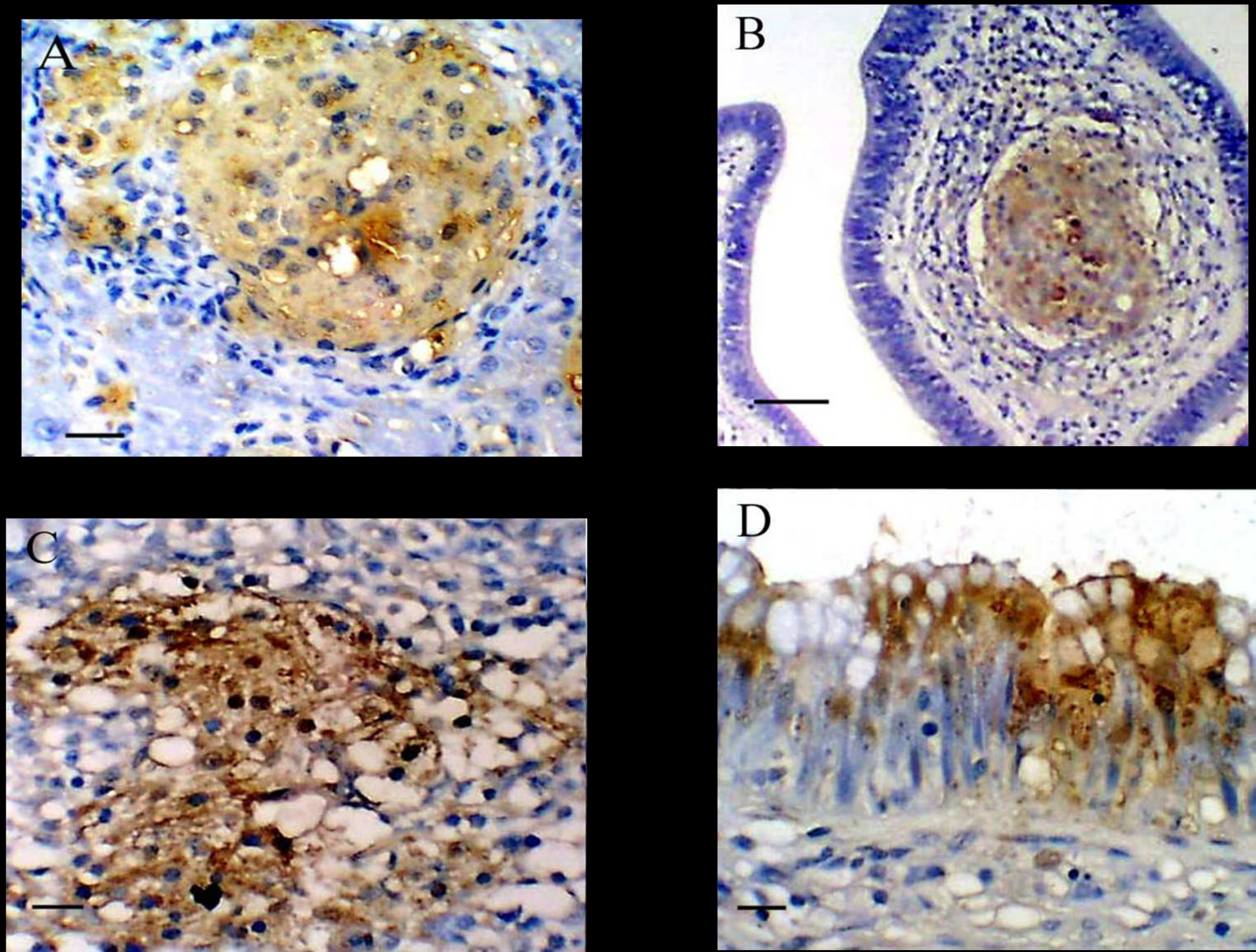


Chlamydophilosis in Emerald Tree Boas



Chlamydophilosis in

Emerald Tree Boas



Host range of the human pathogen Chlamydophila pneumoniae expanded to include reptiles and amphibians

Tracey J. Bodetti[1], Elliott Jacobson[2], Charles Wan[1], Louise Hafner[1], Andreas Pospischil[3], Karrie Rose[4], and Peter Timms[1]*.

[1]Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, Brisbane, Australia

[2]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, USA

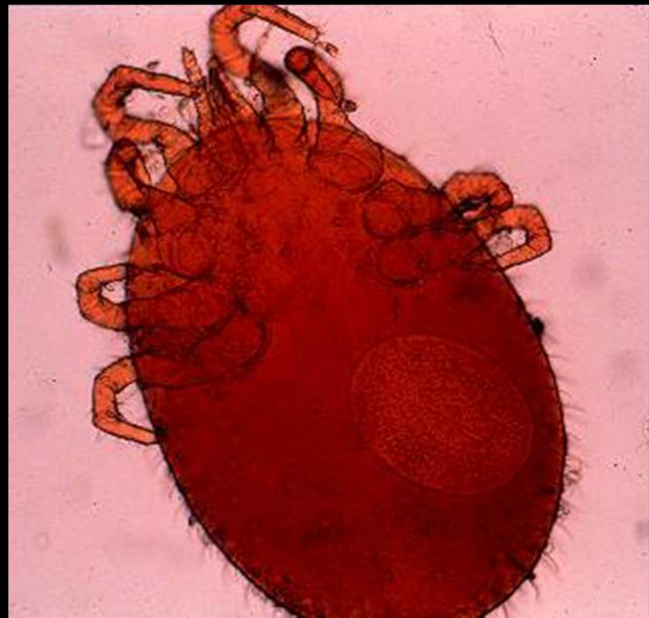
[3]Institute of Veterinary Pathology, University of Zurich, Switzerland

[4]Taronga Zoo, Sydney, Australia

Chlamydophila in reptiles identified by sequencing:

- Green Turtle - *Chlamydophila abortus; Neochlamydia*
- Flap-necked Chameleon - *Chlamydophila pneumoniae*
- Green Iguana - *Chlamydophila felis*
- Puff adder - *Chlamydophila pneumoniae*
- Burmese python - *Chlamydophila abortus*

REPTILE MITES



Leopard Tortoise - Ticks

African tortoise tick - *Amblyomma marmoreum*