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Identification of *Chlamydophila pneumoniae* in an emerald tree boa, *Corallus caninus*

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Abstract. Tissues were evaluated from emerald tree boas, *Corallus caninus*, from a collection in which chlamydiosis was diagnosed. To determine the strain of chlamydia infecting these snakes, tissue samples from 5 frozen snakes were tested by a quantitative *Taq*Man polymerase chain reaction (PCR) test and a PCR sequence analysis test. Of the 22 samples tested, 9 were categorized as either positive or weakly positive with the *Taq*Man test, and 6 yielded an amplicon using a serial PCR test that amplified a portion of the 23S ribosomal RNA gene. A PCR product suitable for sequencing was obtained from the heart of one of the snakes. Sequence analysis showed that the snake had been infected with *Chlamydophila pneumoniae*. These findings show that *C. pneumoniae* can infect emerald tree boas, broadening the range of reptiles known to be infected by this primarily human pathogen.

Chlamydiois has been reported in several species of reptiles, including puff adders (*Bitis arietans*),[4] a flap-necked chameleon (*Chameleo dilepis*),[5] green turtles (*Chelonia mydas*),[7] Nile crocodiles (*Crocodylus niloticus*),[8] green iguanas (*Iguana iguana*),[1] and Burmese pythons (*Python molurus bivittatus*).[1] Lesions varied and included histiocytic granulomas (puff adders), granulomatous inflammation (flap-necked chameleon), necrotizing enteritis (green iguanas), necrotizing myocarditis (green turtles), and proliferative pneumonia (Burmese python). Polymerase chain reaction (PCR) amplification of the family-specific 16S ribosomal RNA (rRNA) gene and the *C. pneumoniae*–specific *ompA* gene was performed on paraffin-embedded tissues of several cases; the following specific identifications were made: *C. abortus* (puff adder, Burmese python, and green turtle), *C. felis* (iguana), and *C. pneumoniae* (puff adder, green iguana, Burmese python, chameleon, and green turtle).[1]

In a recent epidemic of regurgitation in emerald tree boas (*Corallus caninus*) in a private collection, snakes were submitted for pathologic evaluation and were found to have either histiocytic granulomas containing basophilic, centrally located inclusions in multiple tissues or more widespread lymphocytic and plasmacytic inflammation throughout the gastrointestinal tract.[3] Electron microscopy of intestinal histiocytic granulomas of 1 snake revealed that inclusions consisted of stages of an organism consistent with chlamydiae. Two monoclonal antibodies specific for chlamydial group–specific lipopolysaccharide (LPS) antigen were evaluated by immunoperoxidase staining; positive staining was seen at the center of the histiocytic granulomas and in macrophages surrounded by lymphocytes and plasma cells throughout the gastrointestinal tract. To identify the chlamydiae present in the emerald tree boas, tissues from several snakes that died in the epidemic were evaluated using 2 PCR tests. The first test was a highly specific and sensitive *Taq*Man test, a quantitative PCR-based test, that targeted the 23S ribosomal DNA (rDNA).[2] The second test consisted of a standard qualitative PCR-based test that targeted the 23S rRNA gene, producing a 600-bp amplicon suitable for sequence analysis and strain identification.[2]

Five dead emerald tree boas were frozen. Portions of the following tissues were collected on thawing: esophagus (5), small intestine (5), colon (5), spleen (5), heart (1), and pancreas (1). The heart had multiple white lesions near the base of the great vessels; gross lesions were not seen in any other tissues of the snakes. Samples were refrozen and shipped to the National Animal Disease Center, Ames, Iowa; DNA was prepared using a commercial extraction kit.[a] Samples were prepared by grinding tissues as a 20% suspension in phosphate-buffered saline, and 500 μl was pelleted by centrifugation at 15,000 × *g* for 40 minutes. The pellet was processed using the directions and materials supplied in the DNA preparation kit. The R27 strain of *Chlamydia suis* was processed similarly and used as a positive control. The negative control was the PCR mixture to which no template was added.

The *Taq*Man test used 2 primers and a detection probe.[b] The primers and procedure have been previously described,[2] except that the results were monitored real-time using a *Taq*Man ABI Prism® 7700 Sequence Detector.[b] The *Taq*Man primers targeted the 23S rDNA amplifying a 132-bp PCR product. A sample was considered positive if signal levels were detected by 35 cycles. Weak positive samples showed detectable results between 36 and 40 cycles. Samples with no positive signal by 40 cycles were considered negative. The results are given in Table 1.

Of the samples that tested strongly or weakly positive with the *Taq*Man test, only 2 samples resulted in amplicons using the serial PCR test. Amplicons were produced from 4 samples that were negative using the *Taq*Man test. The PCR products were resolved in VisiGel separation matrix and observed with ethidium bromide. The 6 amplicons produced a detectable band at the proper molecular weight for the 23S rRNA gene amplification product (Table 1). Polymerase chain reaction products from these 6 samples were submitted for sequence analysis.[c] One of the products from heart tissue

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Table 1. Results of tissue testing multiple tissues from 5 emerald tree boas for chlamydiae, using 2 PCR tests.

	Test 1*		Test 2
	*Taq*Man 23S rDNA		23S rRNA
Tissue	P	WP	Amplicons
Esophagus	1/5†	2/5	3/5
Small intestine	0/5	1/5	0/5
Colon	1/5	1/5	2/5
Spleen	1/5	1/5	0/5
Heart	1/1	0/1	1/1
Pancreas	0/1	0/1	0/1

* P = positive; WP = weak positive.
† Number of snakes tested.

produced a readable code, the only sample with a strong positive signal using the *Taq*Man test. The code was analyzed using a Basic Local Alignment Search Tool (BLAST) search of the GenBank database. The amplicon had a 100% homology with the AR39 strain of *C. pneumoniae.*

The lack of useable codes from the other samples could be due to weakness of signal or to mixed infections. Qualitative PCR is not as sensitive or specific as quantitative *Taq*Man PCR.[2] The 5 samples were from the intestinal or esophageal samples, where a wide variety of organisms would be expected. The primers amplify some nonchlamydial bacterial templates.[2] The freeze–thawing of the samples would tend to degrade the larger templates faster than the template needed for the *Taq*Man test.

Generally, the diagnosis of chlamydiosis in reptiles has been made by using electron microscopy and observing the characteristic developmental stages in lesions. In some cases pathognomonic histiocytic granulomas have been seen,[3,4] whereas in other cases the lesions were rather nonspecific.[1,3,7] In a recent report, immunoperoxidase staining of sections with 2 antichlamydial LPS antigen monoclonals demonstrated chlamydial antigen in both granulomas and diffuse inflammatory infiltrates in the gastrointestinal tract of emerald tree boas dying in an epizootic. Polymerase chain reaction amplification of the 23S rRNA gene is an additional potential tool for diagnosing chlamydial infection in reptiles.

Amplification of chlamydial 16S RNA and *ompA* genes from paraffin-embedded tissues was recently reported.[1] This study documented the presence of multiple species of chlamydiae in reptiles. A puff adder, green turtle, and Burmese python with *C. abortus* and *C. pneumoniae,* a green iguana with *C. felis* and *C. pneumoniae,* and a chameleon with *C. pneumoniae* only were identified. This report demonstrates that *C. pneumoniae* infection is not restricted to humans but also occurs in lower vertebrates. Similarly, sequencing of a PCR-amplified product identified *C. pneumoniae* in an emerald tree boa. Whether the snake acquired the pathogen from humans or other animals or whether humans can be infected with the strain in emerald tree boas is yet to be determined. Although reptiles can harbor a variety of pathogens that can potentially infect humans,[6] the authors are not aware of any pathogen, primarily of humans, that infects reptiles.

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Sources and manufacturers

a. QIAmp DNA Mini Kit, Qiagen, Inc., Santa Clarita, CA.
b. Applied Biosystems, Foster City, CA.
c. Iowa State University DNA Sequencing and Synthesis Facility, Ames, IA.

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