Pulmonary mycobacteriosis caused by *Mycobacterium haemophilum* and *M marinum* in a royal python

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- Mycobacteriosis is an uncommon and sporadic pyogranulomatous disease of reptiles.
- *Mycobacterium* spp cause a variety of diseases and have been isolated from reptiles at necropsy.
- *Mycobacterium haemophilium* infection is zoonotic.

An adult female royal python (*Python regius*) was referred with an 18-month history of chronic respiratory tract disease. Previously, the snake had been subjected to 3 pulmonary lavage procedures.[1] Cytologic evaluations of Romanowsky-, gram- and Ziehl-Neelsen-stained smears had revealed evidence of inflammation and bacterial infection.[1] *Pseudomonas* spp, susceptible to ceftazidime, were cultured; however, despite prolonged antimicrobial treatment (ceftazidime, 20 mg/kg [9 mg/lb], IM, q 72 h), the snake failed to recover and was referred for further investigation. The owner reported increased respiratory rate and respiratory noise, along with intermittent lethargy and anorexia. The snake was maintained in a vivarium situated inside a child's bedroom. A review of the history revealed that management during captivity was appropriate for this species.[2] At the time of initial examination, the snake was active and responsive with a heart rate of 52 beats/min and respiratory rate of 18 breaths/min. The snake was considered to be in poor body condition on the basis of length, palpation, and body weight of 1.4 kg (3.1 lb).[2] Examinations of the mouth and glottis did not reveal any abnormalities, and results of auscultation were inconclusive.

The snake was admitted and maintained in a hospital vivarium with a thermal gradient of 27 to 32 C (80 to 90 F). Blood was collected via cardiocentesis and submitted for routine hematologic assessment with standard techniques.[1,3] Anemia (PCV, 15%) was evident and considered to be nonregenerative[1,3] on the basis of lack of reticulocytes and erythrocyte morphologic characteristics. Moderate leukocytosis (WBC count, 18.4 × 10^6 cells/µl) with heterophilia (11.2 × 10^6 cells/ml) and monocytosis (2.6 × 10^6 cells/µl) were interpreted as evidence of a chronic inflammatory condition.[1,3]

Anesthesia was induced by use of propofol (10 mg/kg [4.5 mg/lb], IV). The snake was intubated with a 2-mm internal diameter endotracheal tube. Anesthesia was maintained by use of 2 to 3% isoflurane and 100% oxygen at 1.5 L/min, with intermittent positive-pressure ventilation every 15 seconds by use of an electrical ventilator.[a] Evaluation of lateral and dorsoventral radiographic views revealed multiple opacities of soft-tissue density within the cranial lung fields, extending from 35 to 65% of the snout-vent length (**Fig 1**).[4]

The anesthetized snake was temporarily extubated to permit endoscopic examination with a 2.5-mm flexible endoscope, xenon light source, and video camera.[b] The unique respiratory system of snakes lends itself to endoscopic examination.[1] The glottis is situated within the cranial region of the buccal cavity and provides access to the trachea, which is composed of incomplete cartilaginous rings. The trachea terminates directly into 2 lungs; there are no primary bronchi or bronchioles. In members of the Boidae (boas and pythons), left and right lungs are functional and of comparable size, with a large central cavity but no alveoli. The cranial aspect of the lung is normally highly vascular, and the surface has a reticulated pattern. Caudally, the lungs become much thinner and avascular. Endoscopic examinations of the trachea and caudal lung fields (air sacs) of the left and right lungs were unremarkable; however, changes were noticed throughout the cranial areas of both lungs. The normal reticulated pattern on the surface of the lung had been largely replaced by diffuse, granulomatous tissue (**Fig 2**). There was no visible exudate. Endoscopic tissue biopsy specimens were collected for histologic and microbiologic examination.

The python recovered from anesthesia without

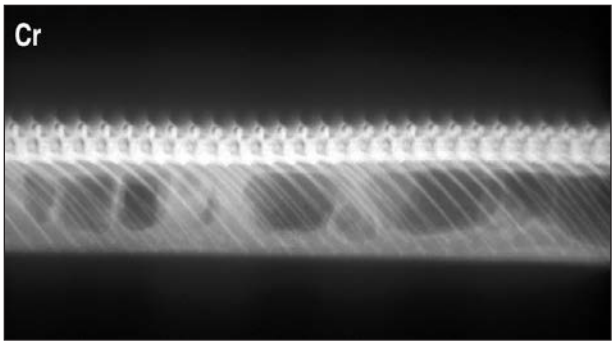

Figure 1—Right (horizontal beam) lateral radiographic view of a python with mycobacteriosis; the view represents the area from 35 to 65% of the snout-vent length. Notice multiple areas of increased soft-tissue opacity within the lungs. Cr = Cranial.

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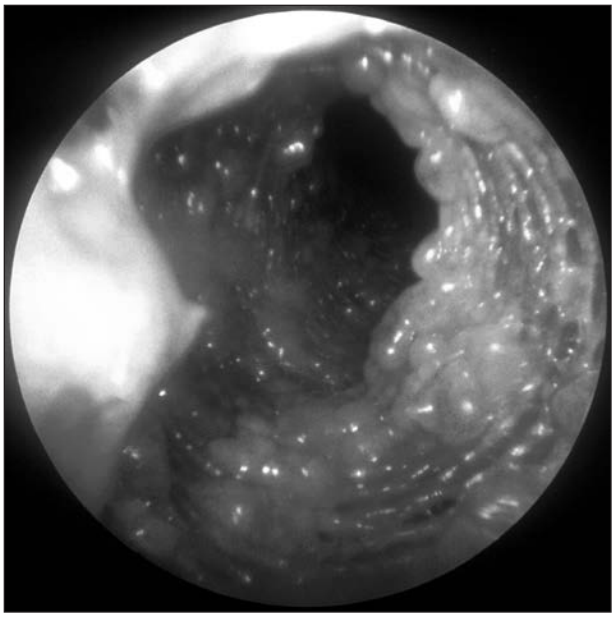

Figure 2—Endoscopic view of the left lung of a python with mycobacteriosis. Notice widespread loss of normal lung tissue and extensive granulomatous changes throughout the cranial portion of the lung.

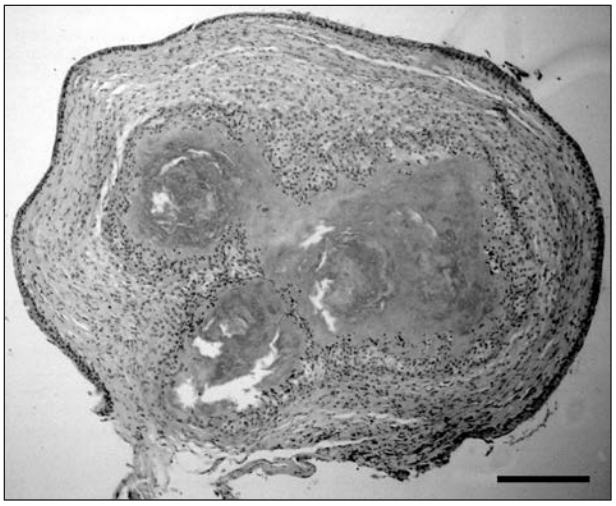

Figure 3—Photomicrograph of a section of lung from a python with mycobacteriosis. Notice the typical pyogranuloma (tubercle) formation. The necrotic core is surrounded by a moderate inflammatory infiltrate and a connective tissue capsule. H&E stain; bar = 200 μm.

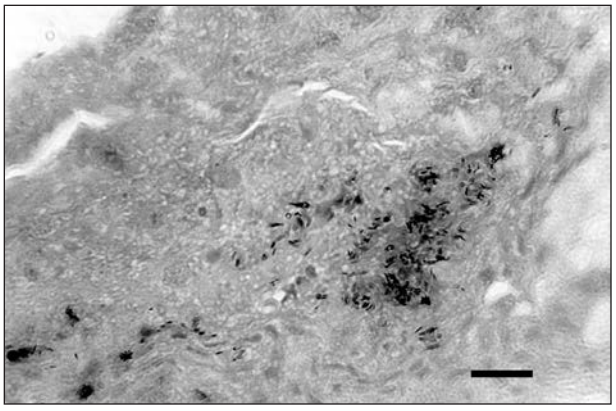

Figure 4—Photomicrograph of a section of lung from a python with mycobacteriosis. Notice the numerous acid-fast bacilli consistent with the genus *Mycobacterium*. Ziehl-Neelsen stain; bar = 20 μm.

complications and was maintained in isolation at 27 to 32 C (80 to 90 F). Treatment with enrofloxacin (5 mg/kg [2.3 mg/lb], IM, q 48 h) was initially prescribed until laboratory results were obtained. Carprofen (1 mg/kg [0.45 mg/lb], IM, q 72 h) was prescribed for its analgesic and anti-inflammatory properties. Fluid and nutritional support was provided by administering a solution[c] that contained glucose-maltodextrin, protein, and amino acids (25 ml/kg [11.4 ml/lb], PO, q 48 h).

No growth was obtained by use of standard aerobic and anaerobic bacteriologic cultures or fungal cultures incubated at 25 C (77 F) and 37 C (98.6 F).[3] Histologic examination of the biopsy specimens revealed respiratory epithelium overlying connective tissue containing granulomas. Classic pyogranulomas containing necrotic debris and surrounded by a moderate inflammatory infiltrate were apparent (**Fig 3**).[5,6] Ziehl-Neelsen stains revealed numerous acid-fast bacilli consistent with *Mycobacterium* spp (**Fig 4**). Given the advanced stage of the disease, histopathologic diagnosis, and potential risk of zoonosis, the snake was euthanatized.

Necropsy findings confirmed the diagnosis made via endoscopic biopsy. Pathologic changes were restricted to the cranial portions of the lung only, and no gross lesions were detected in the trachea, caudal air sacs, or any other visceral organs. Postmortem specimens of affected lung tissue were sent to the Scottish Mycobacteria Reference Laboratory[d] and Scottish Agricultural College[e] for culture and species identification. Separate acid-fast organisms were isolated on glycerol egg medium after approximately 26 weeks of incubation at 25 C (77 F)[d] and on Middlebrook 7H11 medium supplemented with mycobactin J at 28 C (82 F).[e] The 2 isolates did not grow sufficiently well on subculture to permit typical bacteriologic identification tests. Molecular methods including polymerase chain reaction restriction assays and DNA sequencing[7,8] confirmed the identification of *M haemophilum* and *M marinum*.

Mycobacteriosis is an uncommon and sporadic pyogranulomatous disease of reptiles.[5,6,9,10] A variety of *Mycobacterium* spp that cause a variety of diseases have been isolated from reptiles at necropsy.[5,6] Despite the previous isolation of *M marinum* from various reptile species, to the authors' knowledge there are no previous reports of isolation of *M haemophilum*.[5,6]

There does not appear to be any particular species susceptibility to mycobacteriosis. Individual susceptibility does exist and is believed to be related to diminished host immune response.[6] The infection is likely to be acquired by the ingestion of infected material or via defects in the respiratory, integumentary, or urogenital systems. In necropsy reports[5,6,9] of mycobacteriosis in reptiles, pyogranulomas have been most commonly associated with the lung, liver, spleen, CNS, gonads, bone, and subcutaneous locations. The acute extracellular form of mycobacteriosis is rare.[6] The histologic

lesions observed in this snake were consistent with those previously reported,[5,6] specifically the characteristic pyogranulomas that contained a necrotic center, acid-fast bacilli, and moderate peripheral inflammatory response.

Hematologic evaluation provided useful indicators of chronic inflammatory disease, but none were considered specific for mycobacteriosis.[1,3] Clinical diagnosis of mycobacteriosis relies on detection of acid-fast organisms in exudates, fine-needle aspirates, or tissue biopsy specimens.[6] Bacteriologic culture is prolonged, and species identification is technically demanding. Pulmonary mycobacteriosis induces a granulomatous rather than an exudative response.[6] The lack of exudate was believed to be responsible for the absence of acid-fast organisms in the initial pulmonary lavage samples. Collection of tissue biopsy specimens permitted rapid antemortem diagnosis of pulmonary mycobacteriosis, despite the lack of bacteriologic culture results at that time. Endoscopic evaluation of the lungs is therefore recommended for snakes with respiratory tract disease.

The zoonotic potential of *M marinum* has been well documented, and this organism generally causes dermatologic disease in aquarists. Sources of human infection are mainly aquarium fish tanks, although infection after a dolphin bite has also been reported.[11-14] No reports of zoonotic infection with *M marinum* from a reptile source have been published.

The zoonotic potential of *M haemophilum* has also been documented.[15] Most human infections have been limited to the cutaneous or subcutaneous tissues. Septic arthritis, osteomyelitis, and pneumonia are restricted to immunocompromised patients (eg, organ transplant recipients or those concurrently infected with human immunodeficiency virus).[16] Of great concern and particular relevance to this case, *M haemophilum* has been associated with lymphadenitis in apparently immunocompetent children.[17] Fortunately, despite the snake being housed in a child's bedroom for the previous 2 years, the child associated with this snake remained healthy.

In most cases of reptile mycobacteriosis, treatment is not advised because of the chronic and often advanced stage of the disease, long-term and expensive nature of potential treatment regimens, and the risk of spread to other animals as well as humans.[1,6] Furthermore, no successful treatment regimen has yet been reported for reptiles. Therefore, euthanasia is generally recommended.[1,6]

[a]Small Animal Ventilator Mark 3, Vetronic Services, Watcombe, Torquay, England.

[b]100-cm 2.5-mm flexible fiberscope with 1.2-mm biopsy forceps (60003VB), xenon light source (69131501), 4.8-mm-diameter 180-cm length fiber-optic light cable (69495NB), and endovision veterinary video camera (69230001), Karl Storz Veterinary Endoscopy-America Inc, Goleta, Calif.

[c]Critical Care Formula, Vetark Professional, Winchester, Hampshire, England.

[d]Scottish Mycobacteria Reference Laboratory, City Hospital, Edinburgh, Scotland.

[e]Scottish Agricultural College, Perth, Scotland.

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