time during the year, and usually affects lactating cattle.[9] The gross and histopathologic appearance of the mammary gland in this doe was similar to that reported in cattle with *A. pyogenes* mastitis.[4] Clinically, *Actinomyces* mastitis in cattle is described as a peracute infection with a systemic response that includes pyrexia, tachycardia, anorexia, depression, and weakness. One quarter is generally affected, and abscessation occurs if the cow survives the peracute stages.

Actinomyces pyogenes is considered one of the most important organisms involved in pathologic changes of the postpartum bovine uterus.[5] In the deer of this study, *A. pyogenes* was isolated from the uterus, but the inflammatory response in the endometrium was relatively mild. November is breeding season for white-tailed deer, which have a gestation period of 201 days.[1] The doe was found in late May, which is the month in which most deer births occur; however, the uterus was small, and there was no gross or histologic evidence of mammary secretion, suggesting that parturition had not occurred in the recent past. We cannot rule out the possibility that normal parturition or abortion had occurred earlier and that the uterus was involuting normally. Because *A. pyogenes* is usually an opportunist, parturition or abortion may have served as a stressor, providing an opportunity for the dissemination of the bacterium. Another potential stessor in this doe was the presence of radio collar. The mammary gland and/or uterus probably were the primary sites of infection in this doe; initial infection probably was followed by multisystemic dissemination.

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Diagnosis of tuberculosis in two snow leopards using polymerase chain reaction

R. G. Helman, W. C. Russell, A. Jenny, J. Miller, J. Payeur

The incidence of tuberculosis in zoological animal collections is low, and the disease is monitored through skin testing primarily in primates and artiodactylids.[15,16] Other exotic animals are clearly at risk; tuberculosis has been described in elephants (*Mycobacterium tuberculosis, M. bovis*), rhinoceros (*M. bovis*), felids (*M. bovis*), foxes (*M. bovis*), birds (*M. avium* complex, *M. tuberculosis, M. bovis*), and reptiles, amphibians, and fish (cryophilic *Mycobacterium* species).[1,2,4,6,8–10,13,14,17] Mycobacterial infections in mammals and birds serve as a potential source of disease that can spread to other animals and to humans.[7,15,16] In humans, *M. bovis* and *M. tuberculosis* are the most important mycobacteria in the USA.

The rapid growth of molecular biologic techniques in medicine has been valuable for diagnosticians. These techniques enable scientists to make accurate identification of a variety of types of microorganisms in animal tissues, body fluids, or samples that have been chemically processed for pathological studies.[3,5] Moreover, samples for these molecular diagnostic techniques may be quite small compared with the more conventional samples used in diagnostic labs.

Recently, a diagnostic assay for *Mycobacterium* utilizing the polymerase chain reaction (PCR) was described.[11] This assay uses formalin-fixed tissue as the DNA source, and 3 insertion sequences allow classification of the mycobacteria into the *M. tuberculosis* group, *M. paratuberculosis,* or the *M. avium* complex. Here, we describe the diagnosis of tuberculosis using PCR on formalin-fixed tissues of 2 snow leopards (*Panthera uncia*).

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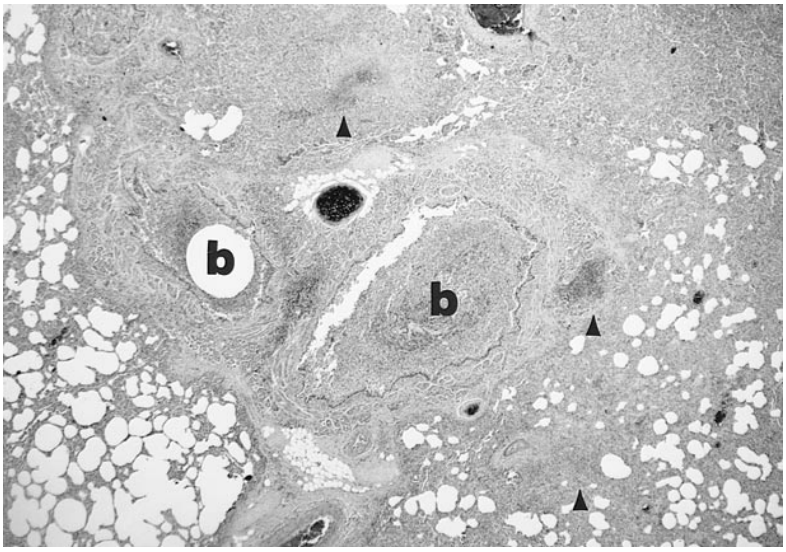

Figure 1. Lung of female snow leopard showing a multifocal to coalescing pattern of pneumonia. Two bronchi (b) with suppurative exudate are surrounded by several foci of granulomatous inflammation (arrowheads). Darker foci in centers of lesions are caseous necrosis. Hematoxylin and eosin.

A 14-year-old male snow leopard had been on breeding loan to the Tulsa Zoological Park since 1982. The cat had a 5.5-month history of intractable cough and weight loss. Chest radiographs showed extensive consolidation of the lungs with cavitation. The cat was found dead in its enclosure.

A 44-month-old female offspring of the dead male leopard had a 2-month history of a persistent cough that did not respond to antibiotic therapy. Radiographic changes were similar to those of the male leopard. This leopard was also found dead. Both cats had shared housing during periods of captivity. Diets consisted of commercially prepared horsemeat rations, bones from a local custom-slaughter plant, and rare access to fresh carcasses from local wild or captive ungulates, including exotic ungulates within the zoo.

A necropsy was performed on each cat. The male leopard was markedly emaciated and had bilateral alopecia in the flanks and lumbosacral region. The thorax contained approximately 800 ml of clear, straw-colored, thin fluid. Approximately 75% of the right lung was firm, uncollapsed, and discolored tan to grayish yellow with multiple areas of cavitation filled with a purulent exudate. The left lung had nodules of various sizes distributed throughout the parenchyma and interspersed with areas of emphysema. The lymph nodes in the mediastinum were enlarged approximately 2–3 times normal. The heart was mildly enlarged and flabby. Other organ systems were unremarkable.

The female leopard had fair to good body condition. The right and left lungs had firm nodules of various sizes distributed throughout all lobes, similar to those seen in the male. The right caudal lobe had more extensive consolidation with marked cavitation. The mediastinal nodes were enlarged 2–3 times normal size. All other organ systems were unremarkable.

Fresh lung (male leopard) and formalin-fixed tissues (both cats) were forwarded to the Oklahoma Animal Disease Diagnostic Laboratory for bacterial culture and histopathology.

Formalin-fixed tissues were processed and stained in a routine manner. Microscopic lesions were similar in both animals. The lungs had multifocal to locally extensive inflammation from purely granulomatous, involving broad regions of alveoli, to a suppurative reaction involving the bronchi. The granulomatous reaction consisted of zones of caseous necrosis surrounded by a mixture of macrophages, lymphocytes, neutrophils, and organizing fibrous tissue (Fig. 1). The recognizable lung tissue external to the granulomatous inflammation had alveolar edema, a mixed macrophage–neutrophil infiltrate, and some fibrin accumulation. Acid-fast bacteria were present in areas of granulomatous inflammation but were more easily recognized in early lesions. Bacteria were few; isolated macrophages contained 1 or 2 large rods of uniform size (Fig. 2).

There was necrosis of segments of the walls of bronchi with extension of exudate into surrounding alveoli. In some sections there were abscesses within the lung apart from the bronchi. In many regions of the lung, the suppurative inflammatory reaction was the primary lesion.

The mediastinal nodes in both leopards had marked lymphoid hyperplasia of both cortical and paracortical compartments. Increased numbers of macrophages and neutrophils were distributed throughout the subcapsular sinuses. Within the cortex and medulla, there were multifocal nodular accumulations of relatively large epithelioid macrophages. Rare macrophages contained 1 or 2 acid-fast bacteria similar to those described in granulomata in the lung.

Blocks containing acid-fast organisms were forwarded to the National Animal Disease Center for PCR identification of the organism. Sample preparation and PCR were performed according to previously described methods.[11] The primers used to identify *M. tuberculosis* complex amplified

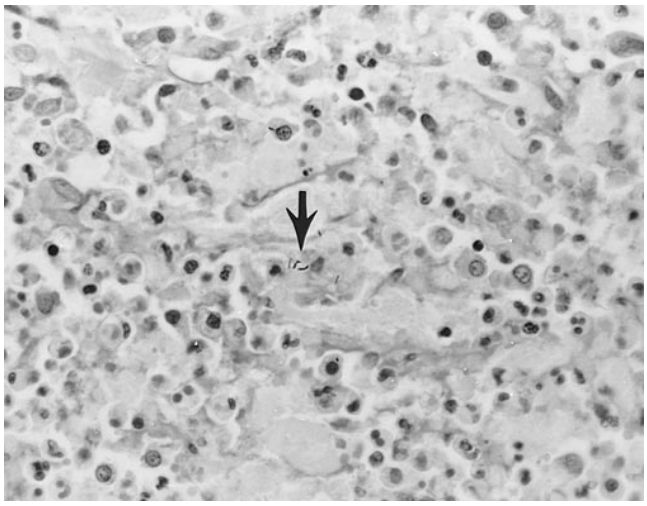

Figure 2. Lung of snow leopard showing acid-fast bacilli in macrophage within granulomatous lesion (arrow). Zeihl-Neelson.

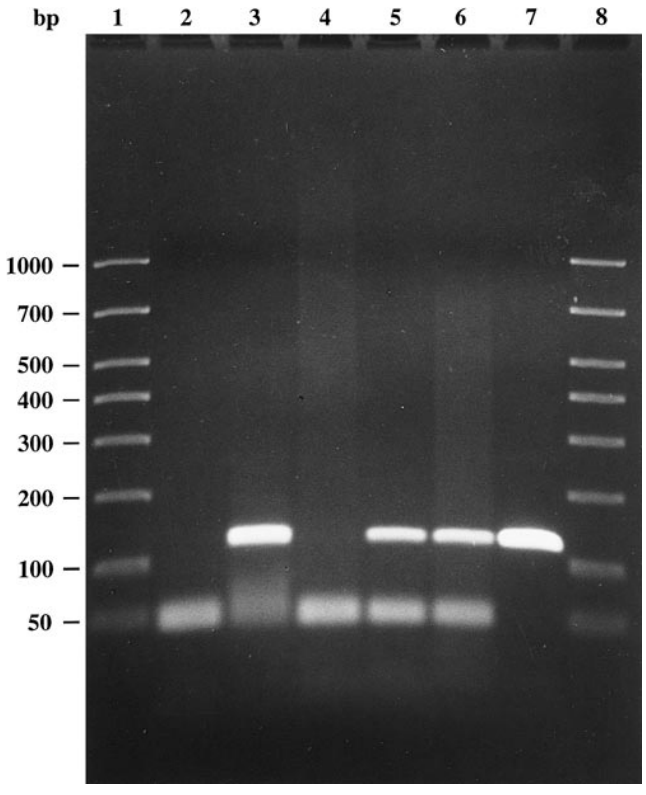

Figure 3. Agarose gel stained with ethidium bromide showing identification of DNA recovered from acid-fast bacteria in the lungs of 2 snow leopards with granulomatous pneumonia. Lane 2 = reagent control (no template); lane 3 = lung from female leopard; lane 4 = negative control tissue; lane 5 = lung from male leopard; lane 6 = lymph node from male leopard; lane 7 = positive control taken from *M. bovis* cultures. Amplified DNA from tissues of both leopards comigrated with the DNA from *M. bovis.*

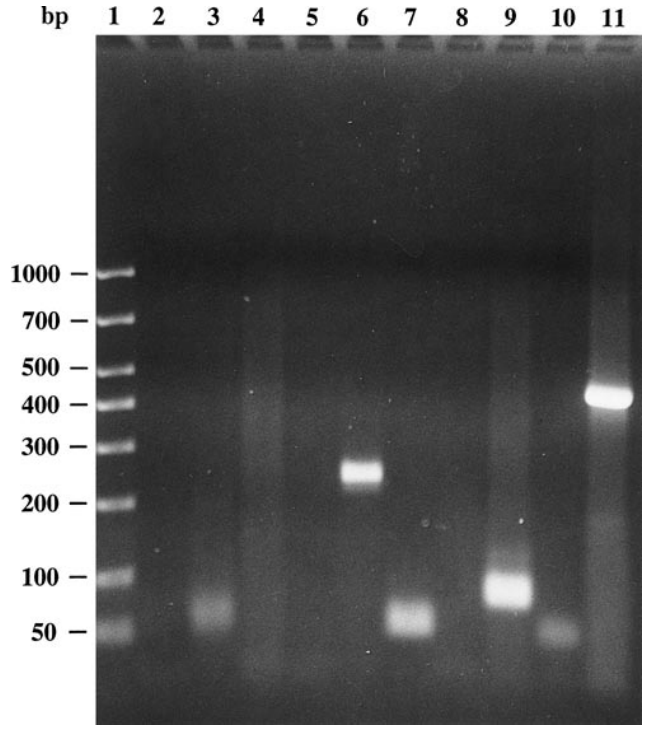

Figure 4. Agarose gel stained with ethidium bromide. DNA from both snow leopards did not comigrate with DNA from either *M. paratuberculosis* (lane 6) or *M. avium*, serotype 2 (lane 11). Lane 2 = control checking for PCR reagent contamination. Lanes 3–5, 7–9 = tissue samples from the 2 snow leopards.

a portion of IS6110, those to identify *M. paratuberculosis* amplified a segment of IS900, and those to identify *M. avium* complex amplified a segment of IS1245. Amplification products were separated by electrophoresis in 1.5% agarose and stained with ethidium bromide. Appropriate controls were used to guarantee there was no DNA contamination of the PCR reagents, and purified DNA from *M. bovis, M. paratuberculosis,* and *M. avium* (serotype 2) were available for positive identification of different subgroups used in this method.

Amplified DNA from formalin-fixed lung and lymph node from both cats colocated in agarose with a 123-bp IS6110 sequence specific for the *M. tuberculosis* complex. This process allowed identification of the bacterium as 1 of the following organisms: *M. tuberculosis, M. bovis, M. africanum,* or *M. microti* (Fig. 3). DNA fragments of *M. paratuberculosis* and the *M. avium* complex were not detected (Fig. 4).

Routine aerobic bacterial culture of the lung from the male cat revealed the presence of a mixed population of contaminant organisms. Fungal cultures yielded no growth. Specific mycobacterial culture positively identified the acid-fast organism as *M. bovis.*

Fresh lung was submitted to the National Veterinary Services Laboratories for mycobacterial culture and identification using previously established techniques.[12] Processed lung was placed in the following media: Lowenstein Jensen with and without glycerin, Middlebrook 7H10 with OADC and sodium pyruvate, Herrold egg yolk with malachite green, with and without glycerin, and with and without my-

cobactin, Stonebrink, and BACTEC Middlebrook 7H12 and 7H11. The inoculated media were incubated at 37 C for 8 weeks and examined for colony formation every 2 weeks. All acid-fast isolates were classified to mycobacterial species using standard growth and biochemical characteristics.[12]

The granulomatous lesions in the lungs of both leopards suggested the possibility of a mycotic, chronic bacterial, or mycobacterial infection. Bacterial pneumonia is uncommon in zoo cats, but secondary infections may occur with primary viral pneumonia. In zoo cats, feline viral rhinotracheitis (FVR) and calicivirus (FCV) infections produce upper and lower respiratory infections.[8] When there is more significant pulmonary damage by these viruses, bacteria such as *Streptococcus, Staphylococcus aureus,* or *Mycoplasma* may be responsible for a fatal bronchopneumonia. In these snow leopards, the marked suppurative reaction in the airways and surrounding alveolar beds plus the cavitation suggested a bacterial bronchopneumonia with abscessation. Routine aerobic cultures of lung from the male leopard yielded only an assortment of contaminant bacteria. This cat had been on antibiotics for many weeks, which may account for the inconclusive culture results.

FVR and FCV were considered unlikely causes of the initiating disease of these cats. Neither animal had ever displayed signs of upper respiratory disease such as sneezing and nasolacrimal discharge. The duration of clinical signs was 5.5 months in the male and 2 months in the female. Both cats had a deep cough consistent with lower respiratory disease. Neoplasia was considered a possibility in the male leopard, considering its age. Histopathology and histochemistry eliminated cancer and pulmonary mycosis and confirmed the presence of acid-fast bacteria.

The low numbers of organisms plus the morphology of the bacteria in lesions was suggestive of *M. bovis* infection. However, the prominence of the suppurative inflammation was not typical for tuberculosis.[7,10] This inflammation and the low numbers of acid-fast organisms present in both lung and regional lymph nodes made diagnosis of tuberculosis somewhat tentative.

Mycobacterial culture can require up to 8–10 weeks for isolation and definitive identification.[12] There was obvious concern at the zoo about the exact identity of the organism and whether it was a public health threat. The delay in waiting for culture results in potential cases of tuberculosis creates problems when human exposure is involved. The ability to more rapidly identify cases of tuberculosis in these situations is important in control of the disease. Appropriate measures then can be more quickly instituted to isolate infected animals and deal with contaminated housing, while making informed judgements on human health and infected animal disposition.[15,16]

The point source of mycobacterial infection was never determined. After the initial diagnosis of tuberculosis, 2 of the authors (WCR, RGH) reviewed 21 case reports and histopathologic specimens submitted to the Oklahoma Animal Disease Diagnostic Laboratory from the Tulsa Zoo. These cases covered a 10-year period (1986–1996) and dealt specifically with animal submissions where there was a diagnosis of granulomatous disease. The microscopic slides from these cases were reviewed and, where appropriate, acid-fast staining was performed. No evidence of acid-fast bacteria was found in any of the archived material or in material submitted to the lab since the discovery of tuberculosis in the snow leopards. To date, no zoo keeper has reacted positively to skin testing for tuberculosis.

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