Mycobacterium paratuberculosis infection in two llamas

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- Mycobacterium paratuberculosis should be considered as a cause of chronic diarrhea and emaciation in llamas.
- Necropsy findings in llamas infected with M paratuberculosis are similar to findings reported in cattle and sheep.
- Bacteriologic culturing of feces is the most sensitive and specific diagnostic test for M paratuberculosis, although DNA probes and serologic tests may be of value in the diagnosis.

A 12-year-old female llama (Lama glama; llama 1) was examined because she was found recumbent in the pasture. She reportedly had a hemorrhagic vaginal discharge the preceding evening. The llama was pastured with other females. She was pregnant with parturition anticipated in 4 to 6 weeks. The llama was vaccinated against clostridial infections.

The llama was lethargic, thin, and unable to stand, even with assistance. Mucous membranes were pink (capillary refill time, < 2 seconds), and rectal temperature was 37 C. Green, watery diarrhea was observed. A large fetus was palpated per rectum. Abnormal laboratory results included leukopenia with a degenerative left shift (WBC count, 6,000 cells/µl, with 900 band neutrophils/µl and 200 metamyelocytes/µl), anemia (PCV, 28%), hypoproteinemia (plasma protein, 3.9 g/dl), hypokalemia (3.2 mEq/L), and ketonuria. Arterial blood gas results revealed hypoxemia with a combined respiratory alkalosis and metabolic acidosis (pH, 7.418; PaO₂, 64.8 mm of Hg; PaCO₂, 28.6 mm of Hg; HCO₃⁻, 18.4 mm/L). Blood and fecal samples were obtained for bacteriologic culturing. A tentative diagnosis of septicemia and pregnancy toxemia was made.

Treatment included iv administration of 2.5% dextrose solution and 20 mEq of KCL at 250 mL/h, potassium penicillin (22,000 U/kg of body weight, iv, q 6 h), ceftiofur sodium (4.4 mg/kg, iv, q 12 h), and flunixin meglumine (0.25 mg/kg, iv, q 8 h). Pure O₂ was administered via nasal tube at 5 L/min. During the next 8 hours, the llama’s condition did not improve and the decision was made to induce parturition and attempt resolution of the ketosis. Dexamethasone (20 mg) was administered iv.

Quantitative fecal examination revealed 9,800 Strongyle spp eggs/g of feces and > 2 coccidia/field of view at 10X magnification. A fetal heartbeat was not detected via ultrasonography. Subsequent urinalysis revealed decreased ketone concentrations, but glucosuria was detected. Serum biochemical analysis was repeated, revealing persistent hypokalemia, hyperglycemia, and decreasing total protein concentrations (3.3 g/dl; albumin, 1.5 g/dl). The next day, the llama gave birth to a dead 9.1-kg male fetus, and her uterus prolapsed immediately after parturition. Caudal epidural anesthesia was administered, and the uterus was cleaned and replaced. Flunixin meglumine was discontinued, and cimetidine (5 mg/kg, iv, q 8 h) was instituted as prophylaxis for ulcerations of the stomach. Diarrhea was persistent, and 4 days after admission serum total protein concentration was 3.1 g/dl. On the basis of this result, 1 L of llama plasma was administered iv to increase serum protein concentration. Bacteriologic culturing of blood samples yielded no growth, and bacteriologic culturing of fecal material failed to detect Salmonella spp. Serum was obtained for agar gel immunodiffusion (AGID) detection of antibodies directed against Mycobacterium paratuberculosis. The test was conducted, using a commercially available antigen preparation and a 6-well antiserum pattern with a centrally located antigen well. After 48 hours of incubation, an agar gel prepared with a pH of 7.2 and without NaCl yielded a weak line of identity approximately 1 mm from the test-serum well. Two other gel formulations with a pH range of 8.6 to 9.0 that contained 7.5 to 8.5% NaCl yielded negative results for the AGID test.¹²

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Four times daily, physical therapy was performed and the llama was encouraged to stand. Although the ketosis resolved, dependent edema was observed, and pure 
O₂ administered per nasal tube was reinstituted 2 days later when the llama became hypoaxemic because of respiratory alkalosis. The llama’s condition deteriorated and she was euthanatized.

Necropsy findings of the fetus were unremarkable. Necropsy of the adult llama revealed marked subcutaneous edema, especially along the ventral portion of the abdominal and cervical areas and the intermandibular space. Approximately 8 L of clear fluid was found in the peritoneal cavity. Segments of the glandular portion of compartment 3 of the stomach, jejenum, colon, and all of the ileum and cecum were thick. The mucosal surface of the thick regions had an irregular rugose appearance, and on cut section the thickening was attributable to an expansion of the mucosal and submucosal layers (Fig 1). The mesenteric lymph nodes were 2 to 4 times normal size, and on cut surface were yellow-white and soft, with partial effacement of the corticomedullary junction. There was marked serous atrophy of fat surrounding the coronary vessels. The lungs were congested and edematous, but foci of inflammation or mineralization were not evident.

On histologic evaluation, the glandular portions of compartment 3 of the stomach, jejenum, ileum, and colon were characterized by mild-to-marked macrophagic, lymphocytic, and plasmacytic infiltrates within the lamina propria, extending into the submucosa (Fig 2). Macrophages had an epitheloid appearance characterized by abundant amphophilic-to-eosinophilic cytoplasm and central euchromatic nuclei with small, multiple nucleoli. Acid-fast staining (Kinyoun’s technique) revealed numerous acid-fast bacilli within the macrophage population of all intestinal segments except for the glandular portion of compartment 3 of the stomach. Moderate, multifocal, granulomatous lymphadenitis was detected in all mesenteric lymph nodes examined. Numerous intracytoplasmic acid-fast bacilli were detected in the granulomatous inflammation in the lymph nodes.

Other important findings included multifocal, discrete granulomas with mineralization but a lack of acid-fast bacteria within the liver, mesometrium, and cervical lymph nodes; endometritis; membranous glomerulonephropathy; and fibrinopurpurative anterior uveitis.

Specimens of lymph node and small intestine were obtained for bacteriologic culturing on Herrold’s egg yolk medium, with and without mycobactin, and also on 7H10 media. After a 1-month incubation, pinpoint colonies were observed on the bacteriologic culture slant (with mycobactin) of the lymph node specimen, and by 9 to 10 weeks after inoculation, the colonies were larger. These bacteria stained acid-fast, but were believed to be atypical because their structure was larger and more filamentous than the structure of organisms isolated from bovine feces that contained M. paratuberculosis. The growth was subcultured and sent to a diagnostic laboratory where it was identified by means of bacteriologic culturing as M. bovis. Detection using the DNA probe, with primers derived from the insertion element IS900 that is specific for M. paratuberculosis, and a polymerase chain reaction to amplify the DNA was attempted on 4 preparations of tissue (lymph node and small intestinal segments). All tissues were strongly positive for M. paratuberculosis. Polymerase chain reaction amplification tests, with primers specific for the gene or portion of the gene that codes for the protein MPB70, were performed on the tissues. The MPB70 protein is produced by M. bovis and, to a

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lesser extent, by *M tuberculosis*, but not by *M paratuberculosis*. Polymerase chain reaction amplification results were indicative that *M bovis* was not in the culture sample. Controls for the polymerase chain reaction were: reaction mixture (negative control); *M paratuberculosis* (negative control); *M bovis* 1877 DNA (positive control), and bacille Calmette-Guerin cells (positive control). Cells collected from the organism grown on bacteriologic culturing were tested against the control samples.

Results of radiometric assays and guinea pig inoculation for *M bovis* complex were negative; results of radiometric assays for *M avium* complex were positive.8

A 16-month-old 77-kg male llama (llama 2) from the same farm was examined 49 weeks after llama 1 for evaluation of persistent diarrhea. Clostridial vaccinations and deworming treatments were current. The llama had been observed eating pasture grass, but was emaciated. Abnormalities in hematologic values included a leukocytosis with toxic neutrophils and a left shift (WBC count, 29,600 cells/μl, with 1,500 band neutrophils/μl), hyponatremia (141 mEq/L), hypokalemia (3.3 mEq/L), hypochloremia (97 mEq/L), azotemia (BUN 48 mg/dl), and hypoprothrombinemia (total protein, 3.7 g/dl; albumin 1.8 g/dl). Fecal examination revealed strongyle, nematodirus, trichuris, and coccidia eggs. Feces also were obtained for bacteriologic culturing. The llama continued to eat well, but died despite IV treatment with fluids and antibiotics. Necropsy findings were similar to the results of llama 1.

Positive identification of the acid-fast bacteria as *M paratuberculosis* was obtained, using the DNA probe and routine mycobacterial culture methodology, as was done for llama 1. Agar gel immunodiffusion testing was performed, using serum obtained from llama 2. As with llama 1, positive results were observed using the gel formulation without NaCl (Fig 3).6 Results of the AGID were positive by both of the other methods.1,2

Results of necropsies of the adult llamas were similar to those reported for cattle and sheep infected with *M paratuberculosis*.6,7 *Mycobacterium paratuberculosis* in cattle is typified by a granulomatous enteropathy that results in a chronic, malabsorptive wasting syndrome.8 The disease primarily affects mature animals. The most dramatic clinical sign is persistent diarrhea. Lesions in cattle include serous atrophy of fat, edema, thickening of the intestinal wall resulting in a corrugated appearance, and large mesenteric lymph nodes.9 Lesions may be less prominent in small ruminants.7 The organism is a gram-positive, facultative, intracellular acid-fast bacillus, which may inhabit the entire intestinal tract, but is most commonly isolated from the ileocecal valve and ileocecal lymph node.9 Infiltrates of lymphocytes, epithelioid cells, and multinucleate giant cells are noticed in the lamina propria. Sinuses of regional lymph nodes may contain epithelioid macrophages or granulomatous lymphadenitis. Gross or histopathologic lesions were not indicative of infection with *M bovis*.6,8

Other *Mycobacteria* spp that infect the *Lama* spp include *M microti*. This organism has been detected in a vicuña (*L vicugna*) with clinical signs of pneumonia.10

*Mycobacterium paratuberculosis* infections have been reported in sheep, goats, deer, camels, some equine species, and cattle.6,11-13 In the 1950s, a suspected *M paratuberculosis* infection in a llama with acid-fast organisms in the gastrointestinal tract was not confirmed by bacteriologic culturing.14,15

Diagnostic tests for *M paratuberculosis* in cattle include conventional bacteriologic culturing of feces on Herrold's egg yolk medium, AGID, standard complement fixation, ELISA, radiometric culturing of feces, polymerase chain reaction, and a DNA probe kit.15,18 Of these tests, bacteriologic culturing of feces is considered the standard, but it can require up to 16 weeks for results.16 Biopsy specimens of intestinal mucosa and regional lymph nodes also may be obtained for bacteriologic culturing. Radiometric culturing of feces is a sensitive test and requires less incubation time than conventional bacteriologic culturing of feces.15,16 Detection of acid-fast bacteria and granulomatous histopathologic changes is suggestive, but not definitive, of infection with *M paratuberculosis*, because other

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acid-fast bacteria may cause similar lesions. The AGID test is specific but is not sensitive. Although DNA probe tests are rapid (48 hours) diagnostic tests, they cannot detect infected animals that are shedding low numbers of *M paratuberculosis* in the feces.\(^1\)

*Mycobacterium paratuberculosis* infection should be considered as a diagnosis for llamas with chronic weight loss and diarrhea. The organism can be cultured, using routine methods for *M paratuberculosis*; however, atypical morphologic features may result in confusion with other mycobacterial species. Radiometric assays do not always differentiate *M avium* from *M paratuberculosis*.\(^2\) The AGID test that detects *M paratuberculosis* infection in cattle and sheep with clinical signs of disease may be useful in the detection of serologic reactivity in llamas. Bacteriologic culturing of feces remains the most sensitive and specific of diagnostic tests. The DNA probe test is advantageous because results are available in approximately 12 hours, whereas radiometric or bacteriologic culturing may take 7 to 12 weeks, depending on culturing method.

The llama herd of this report has not had new animals added for approximately 12 years, but the original llamas of the herd were from all parts of North America. Because the 2 llamas had a diagnosis of infection with *M paratuberculosis*, the entire herd was tested. Fecal samples were tested for *M paratuberculosis*, using DNA probes,\(^4\) and sera were tested for *M paratuberculosis* antibodies, using an ELISA.\(^4\) Results of the DNA probe and ELISA tests revealed none of the llamas were positive. Fecal samples were collected for radiometric culture from 65 llamas of the herd, but *M paratuberculosis* was not isolated from any of these samples. Clinical signs consistent with infection attributable to *M paratuberculosis* have not been observed on this farm for the past 18 months.

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**References**


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