JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2004, p. 1818–1821 Vol. 42, No. 4
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.4.1818–1821.2004

Generalized Tuberculosis in Llamas (*Lama glama*) Due to *Mycobacterium microti*

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Received 3 July 2003/Returned for modification 27 July 2003/Accepted 13 September 2003

Necropsy of two llamas revealed numerous caseous nodules containing abundant acid-fast bacilli (AFB) in various organs. The AFB were identified by spoligotyping as *Mycobacterium microti*, vole type. Infection caused by *M. microti* should be considered in the differential diagnosis of debilitating diseases in New World camelids.

CASE REPORTS

Case 1. An 8-year-old female llama (llama 1; weight, 60 kg) was presented to the Department of Fish and Wildlife Medicine, Institute of Veterinary Pathology, University of Bern, Bern, Switzerland, with a history of appetite loss over the previous few months, cachexia, and recumbency. According to its owner, the llama had always been healthy and gave birth to a sound cria every year. A blood sample was taken, and at the owner's request, the animal was immediately euthanized. The blood chemistry profile showed hypoproteinemia with hypoalbuminemia; increased urea, lactate dehydrogenase, and bilirubin levels; and decreased Fe levels (Table 1).

Case 2. Four months later, a 4-year-old female llama (llama 2; weight, 130 kg) from a different owner was presented to the Clinic for Ruminants, University of Bern, with a history of appetite loss, muscle weakness, and incoordination of 3 weeks' duration. Upon admission, the llama presented in a moderate body condition and was recumbent. It showed open-mouth breathing and bruxism. A white film covered the tongue, and a slight swelling was apparent in the retropharyngeal region. The rectal temperature was 38.6°C (normal temperature, 37.5 to 38.9°C), the heart rate was 88 beats per min (normal heart rate, 60 to 90 beats per min), and the respiratory rate was 68 breaths per min (normal respiratory rate, 10 to 30 breaths per min). Examination of the digestive tract showed poor C1 filling, decreased stomach activity, and intestinal borborygmi. The white and red blood cell counts for llama 2 revealed normal total leukocyte and neutrophil counts, toxic neutrophils with a left shift (band neutrophil count, 1.04×10^9/liter; normal count, 0 to 0.4×10^9/liter), and monocytosis (1.91×10^9/liter; normal count, 0.2×10^9 to 0.94×10^9/liter). The packed cell volume (23%; normal volume, 27 to 35%), hemoglobin level (5.64 mmol/liter; normal level, 6.9 to 9.3 mmol/liter), and erythrocyte count (8.42×10^{12}/liter; normal count, 9.7×10^{12} to 13.4×10^{12}/liter) were reduced. Blood biochemistry analysis revealed hypoproteinemia with marked hypoalbuminemia, increased creatinine and urea levels, and elevated liver enzyme activities (Table 1). Parasitologic examination of feces detected *Nematodirus* sp., *Strongylus* sp., and *Trichuris* sp. eggs. The llama died shortly after hospitalization.

Necropsy was performed on both animals. Llama 1 was cachectic, while llama 2 had good fat stores. Gross lesions consisted of multiple confluent, yellowish, caseous nodules (diameters, up to 10 cm) with friable centers in the lungs (llamas 1 and 2), livers (llamas 1 and 2), spleen (llama 1), bronchial lymph nodes (llamas 1 and 2), hepatic lymph nodes (llamas 1 and 2), mediastinal lymph nodes (llama 2), and mesenteric lymph nodes (llama 2). Similar nodules of a smaller size were observed in the adjacent serosa. By examination of cut sections, these nodules were yellowish and firm with an onion-skin-like structure and a partially mineralized center (Fig. 1). Additional findings for llama 2 included hydrothorax, ascites, hepatic lipidosis, splenomegaly, pulmonary edema and congestion, esophageal petechiae, intestinal hemorrhages, and cervical subcutaneous edema.

Histologically, the caseous nodules presented as granulomas composed of large numbers of closely packed, epithelioid macrophages admixed with various numbers of lymphocytes, plasma cells, and neutrophils (Fig. 2). The larger granulomas showed central necrosis with foci of mineralization and fibrous capsules of various thicknesses. Epithelioid macrophages contained eosinophilic, fine granular cytoplasms. Ziehl-Neelsen and Fite-Faraco staining revealed abundant acid-fast bacilli (AFB) within the epithelioid macrophages throughout all layers of the granulomas. They appeared as irregular long, straight, or curved structures without branches. Direct molecular testing of lung, liver, kidney, and lymph node specimens by the amplified *Mycobacterium tuberculosis* Direct Test (Gen-Probe, San Diego, Calif.) yielded *M. tuberculosis* complex. Only one culture medium (Mycobacteria Growth Indicator Tube; Becton Dickinson, Sparks, Md.) showed weak growth of AFB, while the solid media (Löwenstein-Jensen, Middlebrook 7H10/sel7H11) remained negative for growth. Due to the very sparse growth, biochemical identification of the AFB was not possible. The diagnosis of *Mycobacterium microti* infection was finally achieved by spacer oligonucleotide typing (spoligotyping [14]). Both strains showed a two-spacer spoligotype (spacers 37

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TABLE 1. Blood chemistry profiles of llamas 1 and 2[a]

Parameter	Normal value[b]	Llama 1	Llama 2
Na (mmol/liter)	147–158	153	143
K (mmol/liter)	4–5.4	2.9	3.5
Ca (mmol/liter)	2.26–2.57	2.11	1.99
Mg (mmol/liter)	0.8–1.13	0.87	0.67
Cl (mmol/liter)	107–134	103	108
P (mmol/liter)	1.35–2.75	1.55	2.3
Fe (μmol/liter)	17–31.6	10.2	
Total protein (g/liter)	57.3–72.2	50.5	46.7
Albumin (g/liter)	30.1–39.8	13.1	14.4
BUN (mmol/liter)	5.82–11.48	19.72	30.84
Creatinine (μmol/liter)	121–203	111	373
Bilirubin, total (μmol/liter)	0.2–1.4	1.9	0.1
Glucose (mmol/liter)	5.4–6.6	3.31	
ASAT (IU)	167–302	294	361
AP (IU)	46–107	61	1,180
CK (IU)	56–663	199	275
γ-GT (IU)	29.8–56.4	41	227
GLDH (IU)	6–44.2	9	109
LDH (IU)	200–785	1,400	2,511
SDH (IU)	1–2	2	3

[a] Abbreviations: BUN, blood urea nitrogen; ASAT, Aspartate transaminase; AP, alkaline phosphatase; CK, creatine kinase; γ-GT, γ-glutamyltransferase; GLDH; glutamate dehydrogenase; LDH, lactate dehydrogenase; SDH, sorbitol dehydrogenase.

[b] I. Hengrave, doctoral thesis, in preparation.

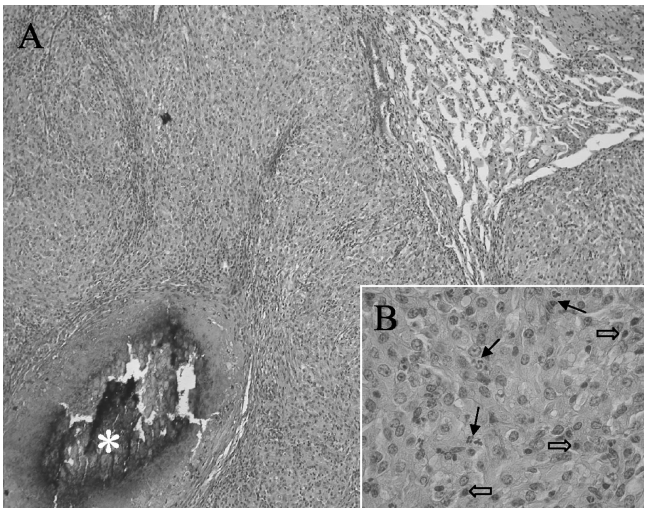

FIG. 2. Histologic features of the lung. (A) The lung parenchyma is obscured due to the presence of numerous granulomas with necrotic and partially mineralized centers (asterisk). Hematoxylin-eosin stain; magnification, ×81. (B) The granulomas are composed of epithelioid macrophages admixed with scattered neutrophils (solid arrows) and lymphocytes (open arrows). Hematoxylin-eosin stain; magnification, ×324.

and 38) (Fig. 3), which is a characteristic of *M. microti*, vole type (15).

Upon diagnosis of *M. microti* infection, the state veterinarian quarantined both herds. All remaining llamas were tested twice at 6-week intervals by intradermal tuberculin testing of the axillary space with bovine and avian purified protein derivatives (9). The quarantine was lifted after the two consecutive tuberculin tests were negative. The owners and their families were also tested and were negative.

Epidemiological investigations revealed that llama 1 had been imported from South America in 1994, together with 83 other animals. At that time, the whole group was kept in quarantine for 2 months and tested for tuberculosis by intradermal skin tests. Seventy-eight llamas proved to be negative by the tuberculin skin test, while six animals had ambiguous results. Four weeks later these animals with questionable results were negative by a second tuberculin skin test. Llama 2 was an offspring of one of the imported animals (but not of llama 1) and was subsequently sold to a second owner. We could not determine if llama 1 or the dam of llama 2 was one of the animals that had exhibited an ambiguous skin test result.

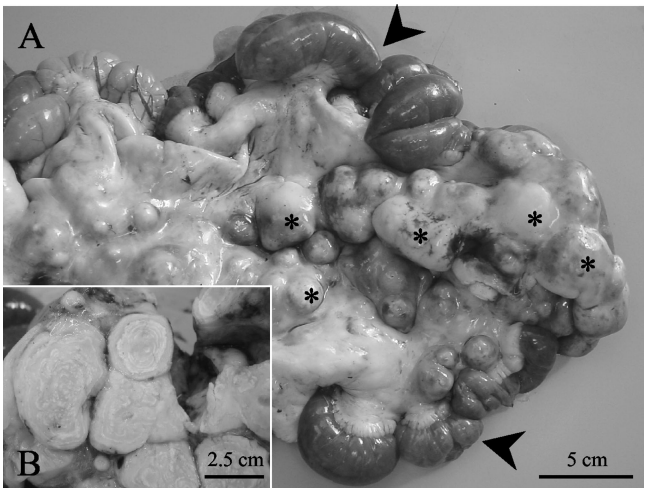

FIG. 1. (A) Jejunum (arrowheads) with enlarged mesenteral lymph nodes (asterisks); (B) cut section of mesenteric lymph nodes, which are enlarged due to the presence of partially mineralized granulomas with onionskin-like structures.

In humans, tuberculosis is the leading infectious cause of death worldwide, with one-third of the global population infected with *M. tuberculosis* (7, 21). In animals, tuberculosis is of primary importance for both its impact on the economy and its zoonotic potential. The majority of tubercular diseases in ungulates are caused by *Mycobacterium bovis* (18), which can also infect Old World and New World camelids (1, 3, 5, 17, 24, 28). Among the latter, infections due to other mycobacteria, including *M. tuberculosis*, *Mycobacterium paratuberculosis*, *Mycobacterium kansasii*, and *M. microti*, have been described (2, 9, 13, 20, 22, 23). Mostly, animals in zoos are affected, while infections in their natural habitat in South America are rarely reported, suggesting that under natural conditions lamoids are not highly susceptible to infections with mycobacteria.

M. microti belongs to the *M. tuberculosis* complex (26), whose members (*M. tuberculosis*, *M. bovis*, the *M. bovis* BCG vaccine strain, *Mycobacterium africanum*, *M. microti*, and *Mycobacterium canettii*) share an identical 16S rRNA gene and show a >90% relatedness (85 to 89% relatedness for *M. microti*) at the DNA level (12). The natural hosts and reservoirs of *M. microti*, first discovered by Wells and Oxen (27), are small rodents such as voles, wood mice, and shrews (16). A postmortem study of snap-trapped field voles in the United

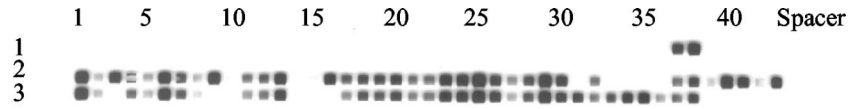

FIG. 3. Spoligotype patterns. Row 1, *M. microti*, vole type (isolated from llama 1); row 2, *M. tuberculosis*; row 3, *M. bovis*. The last two strains were isolated from human specimens and are shown for comparison.

Kingdom revealed a 21% prevalence of *M. microti* (4). Sporadic cases of *M. microti* infections were reported in cats, pigs, a ferret, a cow, a badger, and a captive vicuña (11, 15, 20). A very similar bacterium, the "Dassie bacillus," was identified in an imported Cape hyrax with granulomatous lesions of the lung, liver, kidney, and spleen (6). In contrast to *M. microti*, the "Dassie bacillus" does not cause infection in mice.

M. microti has long been considered an unimportant pathogen in mammalian species other than small rodents. However, the application of molecular methods to the identification of members of the *M. tuberculosis* complex resulted in the recognition of increased numbers of *M. microti* infections in animals and humans, suggesting that the prevalence of *M. microti* infections has been underestimated (4). In 1998, the bacterium was first detected in humans with pulmonary tuberculosis in The Netherlands (8, 25). Later, three cases were diagnosed in Germany (10, 19) and two cases were diagnosed in Switzerland. Immunocompromised as well as immunocompetent patients were affected.

Since small rodents are the natural hosts of *M. microti* and circumstantial evidence revealed human-rodent interactions in some reported cases, rodents have been discussed as a source of infection for humans, but this has not been confirmed with certainty (25). Human-to-human transmission may occur, but no specific *M. microti* strain adapted to the human host has been identified (8). In a vicuña born in a zoo in Belgium, infection by direct or indirect contact with wild mice was assumed (20). In the two cases described herein, the source of infection could not be determined, and there is no information on the prevalence of *M. microti* in small rodents in Switzerland. Interestingly, one llama had been imported from South America 7 years earlier. At that time, however, tuberculin tests were negative. Since llama 2 was an offspring of an animal within the same herd as llama 1, llama-to-llama transmission cannot be excluded.

The gross and histological lesions in both llamas were similar to those reported in 1970 in a vicuña with a *M. microti*, llama type, infection (20). As in the vicuña, granulomas were disseminated in multiple organs of the two llamas and were not limited to the lung, as is described in humans (8, 10, 19). The increases in the levels of specific liver enzymes, such as glutamate dehydrogenase, γ-glutamyltransferase, and sorbitol dehydrogenase, as well as the hypoalbuminemia, can be explained by the severe lesions found in the liver. Elevated blood urea nitrogen and creatinine values were likely due to a prerenal uremia.

The clinical diagnosis of *M. microti* infection remains a challenge. The use of intradermal tuberculin testing for the diagnosis of tuberculosis in camelids is controversial due to the false-positive and false-negative results that have been reported (9). Information on the sensitivity of tuberculin testing of animals infected with *M. microti* is lacking. Only one case report of a human infection with *M. microti* describes a positive tuberculin skin test result with a 7-mm induration (19). The laboratory diagnosis of *M. microti* is hampered by its sparse growth (25). Therefore, molecular methods which are able to discriminate members within the *M. tuberculosis* complex are indicated. Molecular spoligotyping, for instance, has proved to facilitate both the detection and the identification of a *M. microti* infection without the need to culture this slowly growing organism (25).

Since lamoids are increasing in popularity and often kept in close contact with humans (e.g., as trekking animals and in children's zoos), they may represent a potential source of zoonotic infection. Therefore, an infection caused by *M. microti* should be considered a differential diagnosis in debilitating diseases with or without respiratory signs in all New World camelids.

We thank A. von Graevenitz for revising the manuscript.

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