

Sonographic findings in an alpaca with *Mycobacterium kansasii* infection

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Summary

This case report describes the clinical, ultrasonographic and postmortem findings in an alpaca with *Mycobacterium kansasii* infection. The alpaca was referred because of chronic weight loss and weakness. The results of clinical examination, haematology and serum biochemistry were not diagnostic. Ultrasonography of the liver revealed multiple, hyperechogenic lesions with a diameter of 1 to 3 cm. Histological evaluation of a liver biopsy sample showed acute, multifocal, suppurative, necrotising hepatitis. Despite treatment with antibiotics, the alpaca died. Postmortem examination revealed nodular to coalescing lesions in the liver, lungs, mediastinum, pleura and greater omentum, which could not be differentiated macroscopically or histologically from lesions caused by tuberculosis. Ziehl-Neelsen staining showed massive numbers of rods within epithelioid macrophages, which were identified as *Mycobacterium kansasii* by polymerase chain reaction analysis.

Keywords: alpaca, *Mycobacterium kansasii*, liver, lungs, ultrasonography

Sonographische Befunde in der Leber bei einem Alpaka mit *Mycobacterium kansasii*-Infektion

In diesem Fallbericht wird ein Alpaka mit einer *Mycobacterium kansasii*-Infektion beschrieben. Das Tier zeigte chronische Abmagerung und Schwäche. Die Blutuntersuchung war unauffällig. Bei der sonographischen Untersuchung der Leber wurden multiple, echogene, fokale Veränderungen mit einem Durchmesser von 1 bis 3 cm festgestellt. Die Leberbiopsie ergab eine akute multifokale, kleinherdförmige, eitrig-nekrotisierende Hepatitis. Bei der Sektion wurden multiple, knotige bis konfluierende Veränderungen in Leber, Lunge, Mediastinum, Pleura und grossem Netz festgestellt, die makroskopisch und histologisch nicht von Tuberkulose zu unterscheiden waren. In der Ziehl-Neelsen-Färbung wurde ein hochgradiger Gehalt an säurefesten Stäbchen in den epitheloiden Makrophagen festgestellt, bei denen es sich aufgrund der PCR-Analysen um *Mycobacterium kansasii* handelte.

Schlüsselwörter: Alpaka, *Mycobacterium kansasii*, Leber, Lunge, Sonographie

Introduction

Mycobacterium kansasii is a saprophytic atypical mycobacterium found in soil and water (Selbitz, 2006). It is of significant importance in HIV-infected patients (Sherer et al., 1986), in which it is estimated to be 250 times more prevalent than in healthy humans (Santin and Alcaide, 2003). Clinical infection with *M. kansasii* is seen in immunosuppressed patients with advanced AIDS (Yano et al., 2004), and macroscopic postmortem findings cannot always be differentiated from those of tuberculosis. *Mycobacterium kansasii* infection is much less common in animals. Case reports of *M. kansasii* infection in a dog

with granulomatous pleuropneumonia (Pressler et al., 2002) and a llama with tuberculosis-like lesions in the lungs and liver (Johnson et al., 1993) have been published. A rhesus monkey euthanased because of a positive tuberculosis test was shown to have granulomatous pneumonia caused by *M. kansasii* infection (Jackson et al., 1989). *Mycobacterium kansasii* was found in lymph nodes with tuberculosis-like lesions in six cattle (Jarnagin et al., 1983). Experimental infection of healthy calves with *M. kansasii* did not result in clinical infection or pathological lesions (Waters et al., 2006); however, various tests for tuberculosis were positive in the calves later. Non-specific reaction to tuberculin testing impedes

accurate confirmation or absence of tuberculosis in cattle populations. *Mycobacterium kansasii* and other atypical mycobacteria possess antigens that are similar to those of other mycobacteria causing tuberculosis, which may result in cross-reactions in tuberculin tests (Hughes et al., 2005; Waters et al., 2006). In Northern Ireland, *M. kansasii* was isolated in only 14 of 16'506 bovine tissue samples, which underscores the infrequency of this organism in cattle. There are no data on the number of asymptomatic carriers (Waters et al., 2006). To the authors' knowledge *M. kansasii* infection has not been reported in an alpaca. The goal of the present case report was to describe the clinical and postmortem findings in an alpaca with *M. kansasii* infection, and to highlight unusual ultrasonographic findings in the liver that, to our knowledge, have not been described in association with this infection.

History and clinical findings

A 10-year-old female alpaca was referred to the Department of Farm Animals because of weight loss, weakness and difficulty rising. The alpaca originated from a farm with 12 alpacas, which had had no previous health problems. The animals were housed in a free-stall barn with access to pasture. They were fed hay and a small amount of concentrate, and had been dewormed six months previously with triclabendazole and levamisole (Endex®, Novartis). The alpaca had progressively lost weight over a period of one month despite a good appetite. The general demeanour and condition were moderately abnormal. The alpaca weighed 61 kg and was thin and weak. Bruxism, reduced skin turgor and a tense abdominal wall were also noted. The rectal temperature was 37.8 °C, the heart rate was 80 beats per minute and the respiratory rate was 28 breaths/min. Mildly increased breath sounds were heard on auscultation of the lungs. There was no gastrointestinal motility, and only a small amount of well-formed, dark faeces was in the rectum.

Haematological and biochemical findings

The most important haematological and biochemical findings were mild anaemia with a haematocrit of 23% (normal 27–35%, Hengrave Burri et al., 2005), mild hypocalcaemia with a calcium concentration of 2.01 mmol/l (normal 2.30–2.60 mmol/l) and metabolic acidosis with a blood pH of 7.21 (normal 7.40–7.50) and a base deficit of –8.6 mmol/l (normal –2 to +2 mmol/l). The activities of γ -glutamyl transferase (γ -GT; 46 U/l, normal 15–43 U/l, Hengrave Burri et al., 2005), glutamate dehydrogenase (GLDH; 60.5 U/l, 4–21.2 U/l) and sorbit dehydrogenase (SDH; 2.8 U/l, 1–2 U/l) were elevated. The remaining variables, which included total leukocyte count and the concentrations of total protein, fibrinogen, bilirubin, sodium, potassium, chloride, magnesium and phosphorus, were normal. Parasitological examination of the faeces revealed trichostrongyle and strongyle eggs.

Ultrasonographic findings

Ultrasonographic examination of the abdomen using a 5.0 MHz linear transducer revealed multiple intensely echogenic foci with a diameter of 1 to 3 cm in the liver (Fig. 1). The lesions were round to crescent-shaped, non-encapsulated, and had an irregular surface. Larger lesions were accompanied by a distal acoustic shadow. Histological examination of an ultrasound-guided percutaneous liver biopsy sample revealed acute, multifocal necrosis of hepatocytes with moderate infiltration of randomly distributed neutrophils. A granulomatous reaction was not seen in the biopsy sample. Based on these findings, a histological diagnosis of multifocal, suppurative, necrotising hepatitis, most likely of bacterial origin, was made.

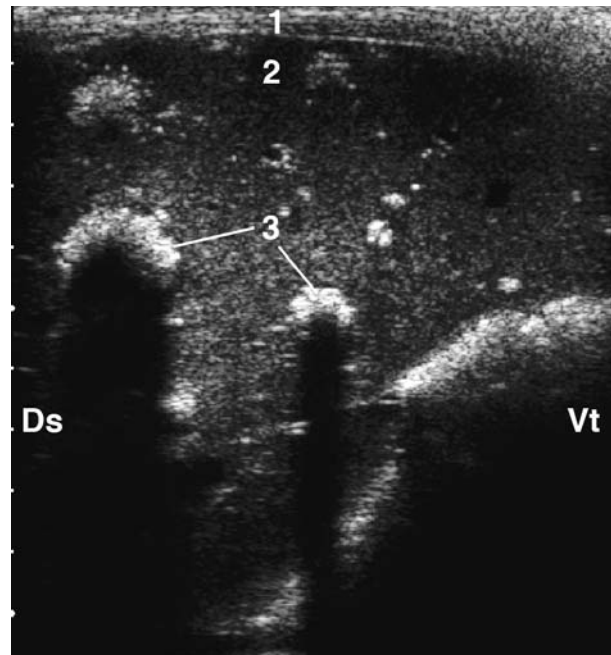


Figure 1: Ultrasonogram of the liver of an alpaca with *Mycobacterium kansasii* infection showing multiple hyperechogenic lesions. A 5.0 MHz linear transducer was used to image the liver from the costal part of the right abdominal wall. 1 Abdominal wall, 2 Liver, 3 Hyperechogenic focal lesions with distal acoustic shadows, Ds Dorsal, Vt Ventral.

Treatment

The alpaca was dewormed with 1 g netomibin per os (Hapadex®, Provect) and given 400 mg amoxicillin and 100 mg clavulanic acid (Synulox®, Pfizer) per os, BID. After initial improvement, the condition of the alpaca deteriorated and it died 14 days after the start of treatment.

Postmortem examination

Postmortem examination showed multiple, white, nodular to coalescing lesions, 0.5 to 7.0 cm in diameter, in the liver, lungs, mediastinum, pleura and greater omentum (Fig. 2, 3). Some of the lesions were hard and on cut surface had dystrophic calcification, often in a radial pattern, while other nodules had a homogeneous caseous cut surface with multifocal calcification. Histological evaluation revealed granulomatous inflammation with formation of characteristic tubercles (Fig. 4). Single nodules consisted of granulomas, which varied in size, sometimes coalesced and had a central area of caseous necrosis with varying degrees of mineralisation. The lesions were surrounded by predominantly epithelioid macrophages and a few multinucleated Langhans' cells (tubercles). Islands of normal and suppurative liver tissue were seen between coalescing granulomas, which, macroscopically, formed a single nodule. Numerous acid-fast rods were seen in epithelioid macrophages using Ziehl-Neelsen stain (Fig. 5), and polymerase chain reaction analysis (Lee et al., 2000) identified them as *M. kansasii*.



Figure 2: The cut surface of the liver of an alpaca with *Mycobacterium kansasii* infection showing a round, white, nodular lesion with an irregular margin.



Figure 3: The lungs of an alpaca with *Mycobacterium kansasii* infection; round, white, nodular lesions are seen in the lungs and mediastinum.

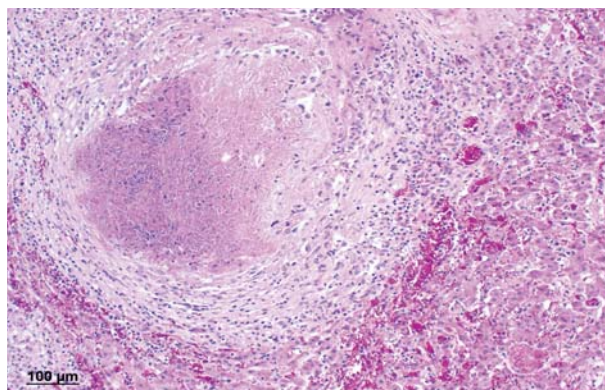


Figure 4: A granuloma measuring a little less than 1 mm with central necrosis surrounded by a layer of epithelioid macrophages, which sporadically form Langhans' giant cells. Interspersed lymphocytes are seen in the periphery, and the transition from granulomatous inflammation to normal liver tissue is abrupt (hematoxylin and eosin stain, 400× magnification).

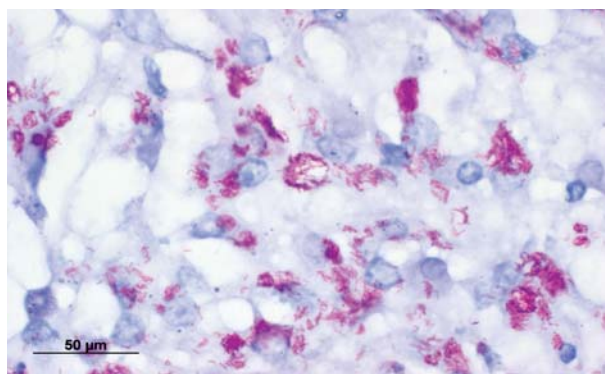


Figure 5: Capsule composed of epithelioid macrophages, which contain massive numbers of delicate acid-fast bacteria (Ziehl-Neelsen stain; 1000x magnification).

Discussion

The lead clinical findings were weight loss and weakness. Despite the severity of pulmonary lesions determined at necropsy, there was no clinical evidence of pulmonary disease and thus, radiography and ultrasonography of the thorax were not carried out. In all likelihood thoracic imaging would have revealed the lesions. Except for GLDH, the activities of the liver enzymes were only mildly elevated, which was surprising considering the severity of the liver lesions on ultrasonograms. Before necropsy, we had difficulty interpreting the liver lesions because similar changes had never been seen before during ultrasonographic evaluation of thousands of cows in our clinic. The lesions differed from the appearance of liver abscesses (Braun et al., 1995a), bile duct calcification (Braun et al., 1996), cholestasis (Braun et al., 1995b), liver tumours (Braun et al., 2005) and seneciosis (Braun et al.,

1999). Unfortunately, instead of revealing granulomatous lesions, the biopsy procedure produced a sample of adjacent tissue with suppurative necrotising inflammation. Treatment with amoxicillin and clavulanic acid resulted in only transient improvement and the alpaca relapsed and died 14 days after the start of therapy. It is plausible that immunosuppression predisposed our patient to *M. kansasii* infection. The concerns of the owner regarding infection of other individuals in the herd were allayed by the fact that *M. kansasii* infection is not known to be transmissible in humans (Bloch et al., 1998). This is supported by the finding that *M. kansasii* infection in a llama did not result in infection of any of 44 herd members 15 months later (Johnson et al., 1993).

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