CASE REPORT

Food/farmed animals



Septic tendosynovitis in a breeding ram and isolation of Mycoplasma arginini

Eva Roden¹ Marcel Suchowski² Katja Voigt¹ Joachim Spergser³ Julia M. Riehm²

¹Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich, Oberschleißheim,

²Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

³Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria

Correspondence

Eva Roden, Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich. Sonnenstraße 16, 85764 Oberschleißheim, Germany.

Email: evamariaroden@gmail.com

Abstract

A breeding ram was submitted to a veterinary hospital due to persistent pneumonia, emaciation and high fever. Upon submission, it also showed severe weight-bearing lameness on all four legs with highly fluid-filled digital flexor tendon sheaths. Additionally, a mild brisket sore was diagnosed. The postmortem examination revealed a chronically active fibrinosuppurative inflammation of the digital flexor tendon sheaths on all four legs. Additionally, a distinctive chronically active necrotising, suppurative and abscess-forming soft tissue inflammation was identified in the sternal area. Microbiological examination of tissue samples from different locations revealed a mixed bacterial colonisation including Escherichia coli, Klebsiella oxytoca and Trueperella pyogenes. Additionally, Mycoplasma arginini was detected in samples of the lung and tendon sheaths. Genome sequencing and characterisation of M. arginini revealed the presence of a prophage associated with arthritogenesis in related Mycoplasma species. However, the contribution of M. arginini to the diagnosed tendosynovitis cannot be conclusively clarified.

KEYWORDS

brisket sore, Mycoplasma, sheep, tenosynovitis

BACKGROUND

Septic tendosynovitis is a frequent finding in cattle. However, to the authors' knowledge, there are no published reports of septic tendosynovitis in sheep. In a retrospective study of 39 adult sheep with joint infections, none of the cases exhibited the clinical complication of tendosynovitis. Moreover, none of the 34 animals displaying chronic septic arthritis in this study showed fluid-filled distention of the affected joints. These authors described septic arthritis as a rare condition in adult sheep, which mostly occurs following bacteraemia.2

There are few publications describing medical conditions in rams that do not influence semen quality. Brisket sores are, however, a common medical problem in rams. The tissue necrosis and inflammation severely affect the ram's libido and is known to respond poorly to treatment.³ However, studies focusing on the impact of brisket sores on animal welfare, or covering prophylaxis, optimised treatment or common bacterial wound infections seem to be missing. Difficulties in the accessibility of the ventral thorax in rather heavy animals often lead to inadequate examination of the brisket by farmers or veterinarians .

Due to their high economic impact, Mycoplasma infections are of great concern in the small ruminant industry worldwide. 4-6 Therefore, diseases such as contagious agalactia and contagious caprine pleuropneumonia are listed by the World Organisation of Animal Health (WOAH).⁶ In the European Union, highly pathogenic Mycoplasma species, such as Mycoplasma agalactiae, are mainly identified in Mediterranean countries.4,7

As life-threatening Mycoplasma infections are considered rare in Germany and the organism cannot be identified by routine diagnostic techniques, true infection rates may currently be underdiagnosed. The relatively low value of sheep and goats, the comparatively elaborate diagnostic methods and a low number of specialised laboratories add to the assumingly insufficient appraisal of Mycoplasma infections.^{4,6} In addition, according to European Regulation (EU) 2016/429, contagious agalactia is no longer a notifiable disease in the EU. This fact might negatively impact future national monitoring, research and control efforts. 4,8 Nonetheless, Mycoplasma species should always be considered in small ruminant diagnostics, especially for disease complexes including mastitis, polyarthritis, pneumonia, keratoconjunctivitis, abortion or septicaemia. 5,7,9 For instance, Mycoplasma mycoides

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subspecies capri has recently been described in a German goat herd for the first time.

Mycoplasma arginini is frequently isolated from the respiratory⁵ and genital tract⁹ of both healthy and diseased small ruminants. It has also been isolated from cases of ovine keratoconjunctivitis and mastitis, but until recently M. arginini has not been considered a primary pathogen but rather a contributor to disease, exacerbating clinical signs in mixed bacterial pulmonary or urogenital infections.⁵ Recent research results indicate that it may be more pathogenic than widely believed. Fernández et al. 10 showed a statistical association between M. arginini and the presence of pulmonary lesions. As described by Pavone et al.,5 typical histological lesions for M. arginini and M. ovipneumoniae infections are bronchiolar epithelial hyperplasia, desquamative alveolitis, presence of a variable number of alveolar syncytial cells, spots of type II pneumocyte hyperplasia and peribronchial lymphoid hyperplasia/lymphoid cuffing. No statistically significant differences in the type and severity of histological lesions between M. ovipneumoniae and M. arginini infections were found in this publication, suggesting no difference in their pathogenicity.

CASE PRESENTATION

A breeding ram of the Alpine breed 'Schwarzes Bergschaf' was submitted to the Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich, because of persistent pneumonia with high fever and significant weight loss. The 2-year-old ram had previously been serving around 20 sheep on pasture. The animal weighed 92 kg and was fed additional hay, mineral lick and small amounts of beet pulp. It had not been vaccinated against clostridial diseases. One week before presentation, the farmer noticed a reduced general demeanour and diarrhoea. He therefore treated the ram with vitamin E/selenium and a combination of closantel and mebendazole. Three days later, the ram was presented to a veterinarian who diagnosed high fever and pneumonia. The ram was treated with oxytetracycline and a combination of metamizole, meloxicam and dexamethasone. In addition, the anthelmintic treatment was repeated with eprinomectin. Despite the combination of steroidal and non-steroidal anti-inflammatory drugs and the antimicrobial therapy, its measured body temperature never dropped below 40°C during the following 5 days. The ram was subsequently presented to the veterinary hospital for a second opinion.

Upon submission, the ram exhibited a body condition score of 1.5 on a scale of 1–5, pale and dry mucous membranes, sunken eyes and a rectal body temperature of 40.5°C. Rumen contractions were reduced, auscultation of bowel motility was unremarkable and the faeces were formed. Tachypnoea was present and bilateral harsh inspiratory breathing sounds were noted cranioventrally. The ram was apathetic, reluctant to stand or walk, showed bruxism and reduced appetite. In recumbent position, it would stretch the front legs and shift its weight to the side. When walking, it showed a severe weightbearing lameness on all four legs (Video 1). All four feet and especially the area of all four fetlock joints were pressuresensitive and warm to the touch. The digital flexor tendon sheaths of all four legs were fluid filled to a high degree (Figure 1A). The ram also showed a mild brisket sore in the

LEARNING POINTS/TAKE-HOME MESSAGES

- Brisket sores can be an entrance point for bacterial pathogens and should be a focus of attention in the healthcare of rams.
- The severity of a brisket sore infection can be concealed by skin closure, which should be considered in every clinical examination.
- Involvement of Mycoplasma spp. infections should be considered in cases of tendosynovitis in sheep, especially in combination with clinical signs such as polyarthritis, keratoconjunctivitis, pneumonia, abortion, mastitis or septicaemia.

form of a hand-sized hairless spot with small crusts and no visible exudation (Figure 2A). In addition, the consistency of both testicles was abnormally softened.

INVESTIGATIONS

A blood sample was taken for a haemogram and faeces for a parasitological examination including a modified McMaster technique, Baermann-Wetzel technique and a sedimentation technique. The haemogram revealed a slightly elevated haematocrit, low albumin, high globulin and moderately elevated liver enzymes. The glutathione peroxidase activity showed no indication of selenium deficiency, and the faecal sample was negative for gastrointestinal nematode, trematode and tapeworm eggs as well as lungworm larvae. Ultrasonographic examination of the significantly extended tendon sheaths was performed. A predominantly anechoic effusion with hypoechoic structures, presumably fibrin, was identified. Ultrasonographically assisted aspiration resulted in the extraction of light yellow, turbid synovial fluid with small fibrin clots, supporting the clinical diagnosis of severe tendosynovitis.

Due to the painful physical condition of the animal, the severe clinical findings and an associated poor prognosis, the owner gave consent for euthanasia. The carcase was transferred to the Bavarian Health and Food Safety Authority in Oberschleissheim, Germany, for postmortem examination including routine microbiological testing as well as mycoplasma culture. Postmortem examination was performed with special attention to the musculoskeletal system. Tissue samples of macroscopically altered organs such as sternal skin and underlying soft tissue, lung and testicle, as well as swab samples of one digital flexor tendon sheath, were taken for microbiological investigation. In addition, specimens of the brain, heart, lung, liver, testicle, sternal soft tissue and digital flexor tendon sheath of the right forelimb and hindlimb were fixed in 4% formaldehyde solution and processed according to in-house standard protocols for routine histological examination (automated tissue processing, embedding in paraffin, preparing of 4 μm thick paraffin sections, staining with haematoxylin and eosin [HE] stain). The pathological examination of the animal revealed a reduced body condition. In the sternal region, the skin and subcutis showed a demarcated, chronically active,



VIDEO 1 Breeding ram of the Alpine breed 'Schwarzes Bergschaf' with severe weight-bearing lameness on all four legs.



FIGURE 1 Tendon sheath. (a) Prominent swelling proximal of and around fetlock joints. (b) Dissection reveals fibrinosuppurative inflammation of the digital flexor tendon sheath (arrowhead). (c) Histology shows a chronically active, fibrinosuppurative inflammation (asterisk—bacteria and necrotic debris, number sign—neutrophil granulocytes, dotted line—granulation tissue).

necrotising, suppurative to abscess-forming soft tissue inflammation (Figure 2B). In both lungs, small embolic-metastatic abscesses were present. A chronically active, fibrinosuppurative inflammation of the digital flexor tendon sheaths was diagnosed on all four legs (Figure 1). None of the examined joints showed signs of arthritis. The macroscopic diagnoses were confirmed by histology (Figures 1C and 2C).

Haemolytic *Escherichia coli* was isolated from tissue samples of the lung, testicle, digital flexor tendon sheaths and soft tissue covering the sternum. Furthermore, *Klebsiella oxytoca* colonisation of the lung and sternal soft tissue was identified. *Trueperella pyogenes* was isolated from samples of the sternal skin and soft tissue. Additionally, mycoplasmas were cultured from samples of the lung and digital flexor tendon sheath.

Analysis of the *Mycoplasma* isolates was performed at the Institute of Microbiology at the University of Veterinary Medicine in Vienna, Austria. The cultured isolates (Figure 3), tentatively named LGL1 (tendon sheath isolate) and LGL2 (lung isolate), were identified as Mycoplasma arginini using MALDI-ToF mass spectrometry, as previously described.¹¹ Whole genome sequencing of the isolates was performed by employing Nanopore and Illumina sequencing followed by hybrid assembly and PGAP annotation, 12 resulting in two circular and almost identical genomes with a size of 0.666 Mb, indicating clonality between LGL1 and LGL2. Genome comparison between LGL1 (GenBank accession CP165990) and M. arginini G230T (type strain CP143577) disclosed a prophage sequence integrated into the chromosome of LGL1 that closely resembles φ MAV1 (Figure 4), which has previously been associated with arthritogenic changes in rodents infected with M. arthritidis. 13 The genome comparison also revealed that unlike M. arginini G230T, LGL1 lacks an adaptive CRISPR/Cas immune system for the protection against invading bacteriophages. A generated phylogenetic tree (Figure 5) based on

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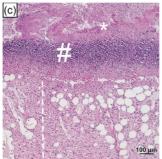


FIGURE 2 Sternal region. (a) Mild brisket sore. (b) Dissection of the skin reveals necrotic and purulent inflammation of the underlying soft tissue (arrowheads). (c) Histology shows a marked chronically active, necrotising to abscess-forming soft tissue inflammation (asterisk—bacteria and necrotic debris, number sign—neutrophil granulocytes, dotted line—granulation tissue).

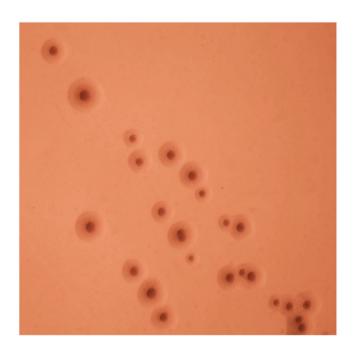


FIGURE 3 Colonies of *Mycoplasma arginini* LGL1 on SP4-Z (+ arginine) agar medium after 4 days of incubation at 37°C under 5% CO₂ atmosphere.

analysing 424 single copy coding sequences and including LGL1, LGL2, six *M. arginini* strains with publicly available genomes and two strains of related species (*M. gateae, M. canadense*) confirmed the phylogenetic positioning of the newly sequenced isolates within the species *M. arginini*, and depicted a close relationship of LGL1/2 to an *M. arginini* strain recently isolated from the eye of a goat in Germany.⁷

DISCUSSION

To the authors' knowledge, there are no published reports of septic tendosynovitis in small ruminants. In cattle, septic tendosynovitis is a relatively common condition, generally arising from penetrating wounds, sole ulcers, white-line abscesses or interdigital necrobacillosis. In this species, it is rarely a result of haematogenous spread, and surgical treatment has a relatively good success rate.¹

Likewise, very few publications address the condition of septic arthritis in adult small ruminants. There is most likely a low incidence in sheep given the lack of literature, and the condition predominantly seems to be a consequence of bacteraemia. Reported cases were often associated with endocarditis and were frequently unresponsive to joint lavage and antimicrobial treatment. Euthanasia is therefore recommended in all adult animals with severe lameness and thickened joint capsules that have been unresponsive to treatment for over 1 week.²

In a study including 293 rams in Greece, 49% of the examined animals displayed at least one abnormality with regard to general health, genital organs or libido, which highlights the importance of routine breeding soundness examinations in rams. Notably, brisket sores were present in 7% of the examined rams in that study.¹⁴ Although the primary route of infection could not be conclusively identified in the presented case, brisket sores have been previously described as a possible cause of septicaemia and arthritis in adult sheep.² In this particular case, the brisket sore had almost completely healed superficially, thus hiding the underlying site of infection, which could easily be missed in a clinical exam. The degree of bacterial colonisation in the presented case revealed a rather long-term inflammatory process. The identified bacterial species are opportunistic pathogens, commonly found in pneumonia, abscesses or septic disease. 15,16 In the presented case, the isolation of haemolytic E. coli from the brisket sore, the testicles, lung and tendon sheaths indicates a haematogenic spread. Endotoxemia and septicaemia caused by E. coli are among the most important causes of death in neonatal lambs.¹⁷ It has also been described as an important pathogen in lamb pneumonia.¹⁸ Although E. coli septicaemia has been identified in older lambs, 19 it has only rarely been reported in mature small ruminants where it is again mainly described as an opportunistic pathogen that can cause or complicate pneumonia in immunocompromised sheep and goats. 16 Unlike cattle, 20 to the authors' knowledge, there is no published report of E. coli isolated from the tendon sheaths or joints in adult small ruminants. In addition to haemolytic E. coli, K. oxytoca was isolated from the brisket sore and lung tissue. It is also considered an opportunistic pathogen¹⁵ and has been described in many mammalian infections such as pneumonia in adult cattle²¹ or meningitis in calves.22

M. arginini was isolated from the lung and the tendon sheaths of the ram. Although it is a common finding in small ruminants and has been isolated from the respiratory tract,⁵ genital tract, eyes and udder of sheep and goats,⁹ there seems to be no description of this organism in ruminant tendon sheaths. In cattle, it has been identified as the cause of mild to moderate arthritis. It is considered to

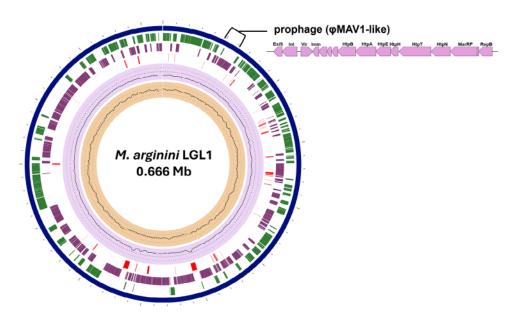


FIGURE 4 Circular graphical display of the completed genome of *Mycoplasma arginini* LGL1 and genomic position of an integrated prophage resembling φ MAV1. The circular plot includes, from outer to inner rings, the contig (blue), CDS on forward strand (green), CDS on reverse strand (purple), RNA genes (red), GC content, and GC skew.

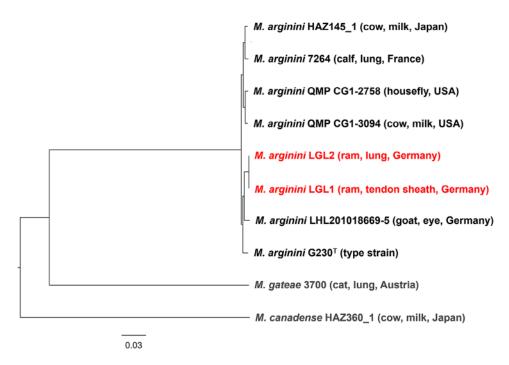


FIGURE 5 Phylogenetic tree based on genome comparison of *Mycoplasma arginini* strains and related species (*M. gateae*, *M. canadense*) using RAxML, analysing 424 single copy coding sequences (CDS). Bar, number of substitutions per site.

have a relatively low ability to induce joint inflammation in comparison to *M. bovis*.²³ Nonetheless, a predisposing and enhancing effect on bacterial infections has been described for mastitis in dairy cows.²⁴ Such an effect might have also been a factor in the development of tendosynovitis in the presented case. In addition, both identified *M. arginini* isolates carried a prophage sequence, which has previously been associated with arthritogenic changes in rodents infected with *M. arthritidis*.¹³ It can therefore be assumed that *M. arginini* substantially contributed to the described tendosynovitis.

To the authors' knowledge, this is the first description of septic tendosynovitis in sheep and also the first described isolation of *M. arginini* from tendon sheaths in small ruminants. It is important to stress that the contribution of *M. arginini* to the diagnosed tendosynovitis cannot be conclusively clarified. Nonetheless, this case report highlights the importance of a thorough diagnostic approach and draws attention to the possibly underestimated contribution of *Mycoplasma* spp. to small ruminant diseases. It also sheds light on the frequently neglected preventive healthcare of rams as an important but often underrated flock management aspect.

AUTHOR CONTRIBUTIONS

Eva Roden: clinician; manuscript preparation and editing based on co-authors' recommended edits. Marcel Suchowski:

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pathologist; writing and reviewing of the manuscript. Katja Voigt: clinician; writing and reviewing of the manuscript. Joachim Spergser: molecular microbiologist; writing and reviewing of the manuscript. Julia M. Riehm: bacteriology; manuscript preparation.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

FUNDING INFORMATION

The authors received no specific funding for this work.

ETHICS STATEMENT

This case report did not involve any experimental procedures requiring formal ethical approval. The principles of good veterinary practice were applied to this clinical case. Licensed veterinarians examined, treated and euthanased the diseased animal according to standard veterinary procedures and in accordance with European and German animal welfare legislation. The postmortem examination was performed by a licensed veterinary pathologist. Client consent was granted to the Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich, before submission of this manuscript.

ORCID

Eva Roden https://orcid.org/0009-0007-1901-8381

Julia M. Riehm https://orcid.org/0000-0002-0767-5217

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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