Rhodococcus equi infection in a dairy goat from a herd in northeastern Italy

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SUMMARY

A 3-year-old Chamois Colored dairy goat from a herd in Treviso province (northeastern Italy) was examined for progressive weight loss and enlargement of cervical lymph nodes. The general condition rapidly worsened to severe weakness and death within few days. Multiple nodular caseous pyogranulomas were found in the lymph nodes, lung, liver, and spleen. Numerous intracytoplasmic Gram-positive coccobacilli were found within macrophages and neutrophils. Aerobic bacterial cultures yielded pure cultures of *Rhodococcus equi,* identified with MALDI-TOF mass spectrometry and 16S rDNA sequencing. The isolated strain resulted resistant to trimethoprim/sulfamethoxazole and susceptible to tetracycline (MIC values for horses). Other infectious diseases including *Mycobacterium* spp., small ruminant lentiviruses (SRLV), and pestiviruses were excluded. In recent years, several reports have described *Rhodococcus equi* infections in ruminants worldwide, especially after the recognition of the ruminant-associated virulence plasmid VapN. *Rhodococcus equi* might represent an unrecognized pathogen in goats that should be considered in the differential diagnosis of caprine tuberculosis and pseudotuberculosis, also in view of its zoonotic potential.

KEY WORDS

Caprine Rhodococcosis; *Rhodococcus hoagii; Rhodococcus equi.*

INTRODUCTION

Rhodococcus equi, also known as *Rodococcus hoagii* and *Prescottella equi*, is an aerobic, Gram-positive pleomorphic coccobacillus, that is a soil saprophyte and facultative intracellular bacterium. *Rhodococcus spp.* is gaining increasing significance as a zoonotic pathogen, particularly in immunocompromised HIV/AIDS patients or in transplant recipients.
R. equi is a primary pathogen of horses, typically causing chronic pyogranulomatous pneumonia and enterocolitis in foals. *R. equi* infection is occasionally reported in swine, cattle, and goats, with sporadic reports of the disease in camelids, and companion animals.
Caprine rhodococcosis has been described in different countries worldwide, but it has been rarely reported in northern Europe and the United Kingdom. Lesions in goats are characterized by disseminated abscesses and pyogranulomas of lymph nodes, spleen, kidney, and liver. A purulent chronic pneumonia is also recorded.
To the authors' knowledge rhodococcosis in goats has never been reported in Italy. In this report, we describe the anatomopathological and bacteriological findings in a case of rhodococcosis in a goat from a herd in Treviso, northeastern Italy.

CASE DESCRIPTION

In March 2020 a 3-year-old Chamois Colored goat was referred due to a 2-week history of severe, progressive weight loss with conserved appetite and milk production. The goat belonged to a farm located in the province of Treviso, in northeastern Italy. The herd was composed of 160 Chamois Colored goats in general good health. The animals were held in a stable with no outdoor access. Two adult horses were housed in a separated pen in the same property.
At clinical evaluation, a moderate enlargement of the cervical lymph nodes was detected. The body temperature was normal (38.4°C) and no respiratory, cardiovascular, nor gastrointestinal clinical signs were observed. The animal condition worsened

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rapidly, showing severe hind limb weakness, recumbency and spontaneous death within 4 days after the initial clinical evaluation.

At gross post-mortem examination, multicentric lymphadenopathy was observed, with mediastinal and abdominal lymph nodes, being the most severely enlarged (8x3cm) and characterized by a firm consistency. Multifocal to coalescing whitish, well demarcated and raised nodules, of 2 to 10 cm in diameter were distributed in pulmonary (Fig.1A), hepatic (Fig. 1B) and splenic parenchyma. On cut section, the lymph nodes and nodules contained caseous, white material arranged in concentric layers and surrounded by a fibrous capsule (Fig. 2A).

Samples of lung, thoracic lymph nodes, liver and spleen were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin-eosin.

Histologically, the nodules observed in the lung (Fig.2B), liver (Fig.3A) and spleen, corresponded to large pyogranulomas with central cellular debris and multifocal dystrophic mineralization. An abundant mixed inflammatory infiltrate, composed of epithelioid macrophages, degenerated neutrophils and rare bi- /multinucleated histiocytic giant cells, surrounded the central necrotic core. Lesions were lined by moderate numbers of mature lymphocytes and plasma cells and a slightly to moderately thick fibrous capsule. In affected lymph node, the tissue architecture was completely replaced by a diffuse pyogranulomatous inflammation and extensive necrosis. Numerous basophilic coccobacilli filling the cytoplasm of macrophages, neutrophils and multinucleated giant cells were seen (Fig.3A). Special histochemical stains included Gram (Artisan Gram Stain kit, Dako, Carpinteria, CA, USA) and acid-fast bacillus stain (Artinas AFB stain kit, Dako, Carpinteria, CA, USA). Bacteria resulted Gram-positive and acid-fast negative (Fig.3B).

Samples of lung, liver, mediastinal lymph nodes, central nervous system, and mammary gland were collected aseptically for bacteriology by inoculation in brain-heart infusion broth (BHI) and plated on Blood-Agar with Esculin (ASE), chocolate agar (AC), Columbia agar (CA), and eosin-methylene blue agar (EMB). The cultures were incubated in aerobic (ASE; EMB), microaerophilic (AC; CA) and anaerobic (ASE) conditions at 37 °C and examined after 24 and 48 hours. A pure salmon pink

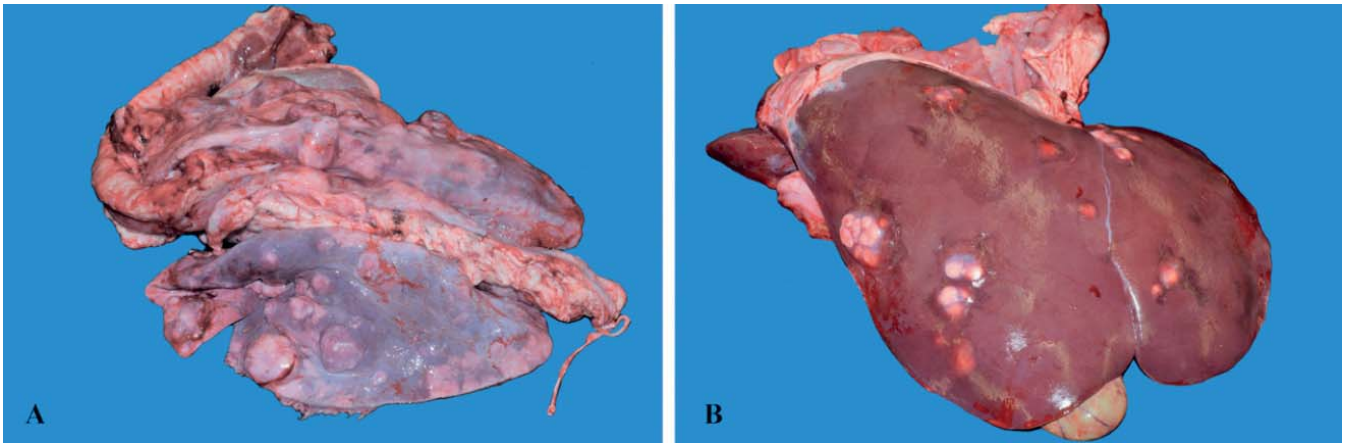

Figura 1 - Gross pathological picture of lesions in the lungs and the liver.
Lungs (A) Severe, chronic, multifocal to coalescing bilateral pyogranulomatous pneumonia with severe generalized lymph adenomegaly. Liver (B) Severe, chronic, multifocal to coalescing pyogranulomatous hepatitis.

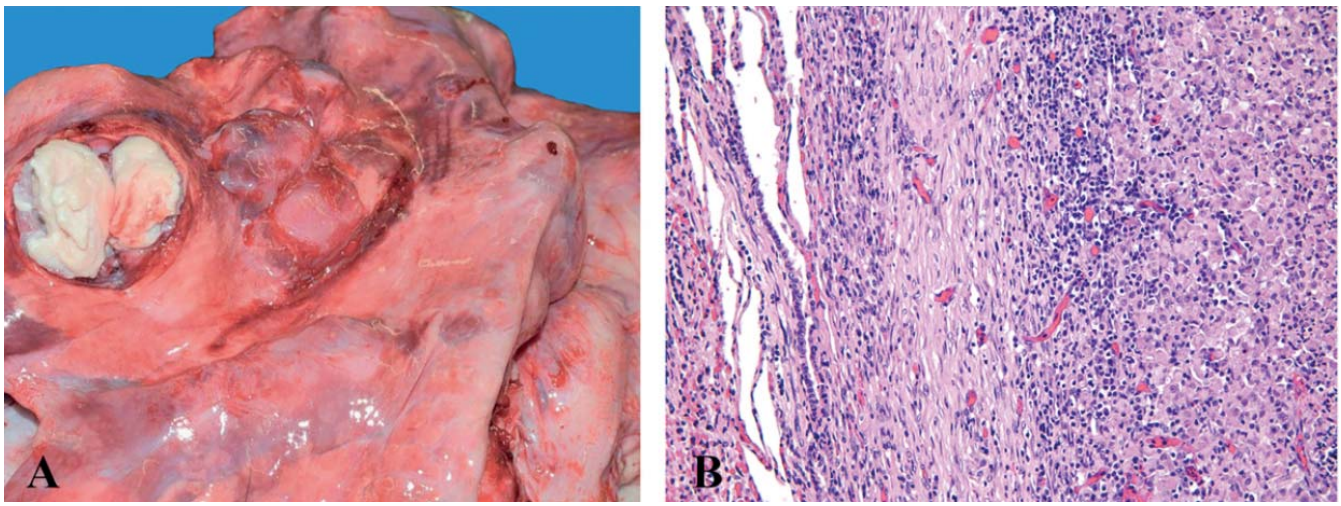

Figura 2 - Comparison between gross and histologic pictures of a lung pyogranuloma.
Lung (A), particular of a sectioned pyogranulomatous lesion. Lung H&E x20 (B), periphery of a pyogranuloma composed of epithelioid and foamy macrophages, neutrophils, fewer multinucleated giant cells, lymphocytes, and plasma cells, circumscribed by a fibrous capsule.

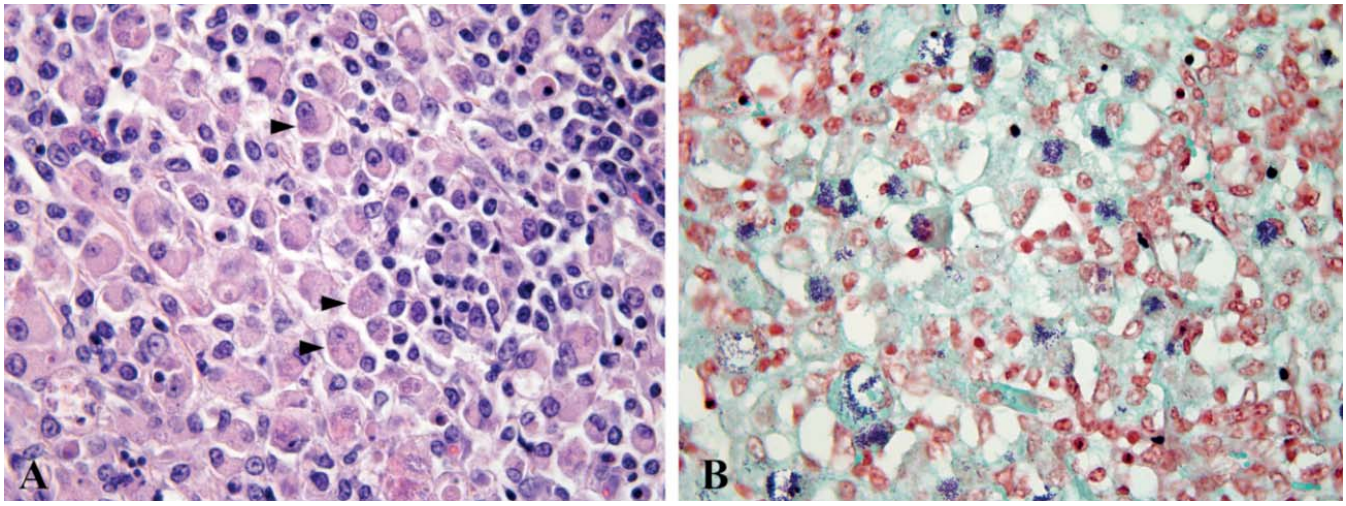

Figura 3 - **Comparison between H&E and Gram stain of a hepatic lesion.**
Liver H&E x63 (A), numerous basophilic coccobacillary bacteria were present in the cytoplasm of macrophages and multinucleated giant cells (arrows). Liver Gram Stain x63 (B), numerous Gram-positive coccobacilli within the macrophages.

mucus culture was isolated at 24 hours in ASE, CA, and AC in aerobic and microaerophilic conditions from lung, liver and lymph nodes. The colonies were composed of Gram-positive, catalase-positive and oxidase-negative, coccobacilli. The bacterial cultures were identified by means of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (Microflex LT MALDI-TOF MS; Bruker Italy, Milan, Italy).
Mass spectra were acquired and analyzed by MALDI Biotyper software package (version 3.0, Bruker Italy, Milan, Italy) that compares sample spectra with reference spectra, assigning a similarity score from 0 to 3. The bacterial isolate was identified as *Rhodococcus hoagii* with a score value of 2.13. Based on manufacturer's interpretation criteria, scores between 2.0 and 2.3 are reliable at the genus level and probable at the species level.
In addition, the bacteria 16S rRNA was sequenced using previously described primers and blasted on BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) showing a 100% homology with *Rhodococcus hoagii* (LC552072.1, CS402 gene for 16S rRNA, partial sequence). The sequence was aligned with *Rhodococcus equi* reference strain (ATCC 25729) showing 99. 1%. homology. This finding supports that *R. hoagii* and *R. equi* are the same bacterial species.
Considering the zoonotic potential of *R. equi*, antimicrobial susceptibility of the strain isolated was assessed by Antimicrobial Susceptibility test (AST) and determination of the minimum inhibitory concentration (MIC) of amikacin, amoxicillin/ clavulanic acid, ampicillin, cefazolin, ceftiofur, clindamycin, enrofloxacin, florfenicol, gentamicin, penicillin, spectinomycin, spiramycin, tetracycline, tilmicosin and trimethoprim/sul-

Table 1 - Results of antimicrobial susceptibility test on *Rhodococcus hoagii*.

Antimicrobial agent	MIC value (mg/L)	MIC (mg/L) interpretative categories and break points		
		S	I	R
amikacin	4	-	-	-
amoxicillin/clavulanic acid	1/0,5	-	-	-
ampicillin	2	-	-	-
cefazolin	4	-	-	-
ceftiofur	0,25	-	-	-
clindamycin	0,25	-	-	-
Enrofloxacin	0,25	-	-	-
Florfenicol	16	-	-	-
gentamicin	1	-	-	-
penicillin	1	-	-	-
spectinomycin	64	-	-	-
spiramycin	8	-	-	-
tetracycline	1	≤4	8	≥16
tilmicosin	64	-	-	-
trimethoprim/sulfamethoxazole	4/76	≤2/38	-	≥4/76

famethoxazole according to the Clinical and Laboratory Standards Manual (CLSI). The MIC values are summarized in Table 1. Clinical breakpoints for broth microdilution susceptibility testing are not available for the caprine species, but, according to the clinical breakpoints for horses, the strain resulted resistant to trimethoprim/sulfamethoxazole and susceptible to tetracycline (CLSI VET06, 1st edition, table 15).

Additional investigations were conducted in order to exclude the presence of other pathogens or immunosuppressive agents. Samples of liver, lung and lymph nodes were collected for culture and molecular identification of *Mycobacterium* spp. RT-PCR was employed for the detection of lentiviruses from the lung, and pestiviruses from the spleen. Serum from the cardiac blood clot was used for serological detection of small ruminant lentiviruses (SRLV) according to the World Organization for Animal Health (WOAH) Manual for Terrestrial Animals 2010, cap. 2.7.3/4, using a commercial ELISA kit (Eradikit SRLV, IN3 Diagnostic, Torino, Italy).

Analysis for mycobacteria, serological and molecular investigation of lentiviruses, RT-PCR for pestiviruses, and bacteriological analyses of the mammary gland and central nervous system were all negative. Thus, pure infection with *R. equi* was confirmed in this goat.

DISCUSSION AND CONCLUSION

R. equi is a common inhabitant of soil, feces and oral cavities of healthy animals. Studies worldwide have demonstrated an 80-100% prevalence in healthy mare feces, and from 4% to 24.3% prevalence in soils analyzed. No information is available concerning the prevalence of *R. equi* in healthy goats.

Rhodococcosis is considered a sporadic disease in farmed animals, and little is known about possible predisposing factors. In the case of caprine rhodococcosis described herein, the presence of horses in the same property may have resulted in contamination of the environment, although no investigations were conducted in this regard. However, as only one goat of the herd had clinical signs, immunosuppressive viral infections such as those caused by lentiviruses and pestiviruses, were taken into consideration but were ruled out.

R. equi is considered an emerging, invasive opportunistic zoonotic pathogen, causing pyogranulomatous bronchopneumonia and occasionally extrapulmonary infections in both immunocompetent and immunocompromised patients, particularly AIDS, and transplanted patients. To date, the route of infection for humans remains poorly understood, although inhalation and history of contact with infected livestock and/or contaminated environment are considered to be risk factors. For the case herein described, despite potential exposure to the pathogen, the farm workers exhibited no clinical signs and therefore did not undergo a clinical evaluation. Surveillance in domestic animals, particularly in multispecies farms, could help in the prevention of human clinical disease.

The pathogenicity of *R. equi* has been attributed to the presence of plasmid-encoded virulence-associated proteins (Vap) needed by the bacteria to survive within macrophages following phagocytosis. *R. equi* strains isolated from goats frequently carry the VapN plasmid. In this goat, characterization of the stain virulence was not conducted.

Practitioners and slaughterhouse veterinarians should consider *R. equi* infection in case of multiorganic granulomas or abscesses in goats, especially as a differential diagnosis for tuberculosis and pseudotuberculosis.

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