

Presence of mycoplasmas in the respiratory system of small ruminants managed under an extensive production system

Zinka MAKSIMOVIC¹, Christian DE LA FE², Maid RIFATBEGOVIĆ^{1*}

¹Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, University of Sarajevo, Sarajevo 71000, Bosnia and Herzegovina

²Department of Animal Health, Faculty of Veterinary Sciences, University of Murcia, Espinardo Campus, 30100 Murcia, Spain

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Abstract: The objective of this study was to investigate the presence of mycoplasmas in the respiratory system of sheep and goat populations managed under extensive production systems. Specific culture and polymerase chain reaction techniques were performed on a total of 88 lung tissue samples and 142 nasal swabs obtained from multiple herds in Bosnia and Herzegovina. A total of 29 samples (12.6%) were found to be positive for mycoplasmas. *Mycoplasma ovipneumoniae* was detected in the nasal swabs and lungs of affected sheep (n = 19) and in the lungs of asymptomatic goat kids (n = 2), while *M. arginini* (n = 5) and *M. bovis* (n = 1) were found in the goats' respiratory tracts. These data confirm the importance of *M. ovipneumoniae* in sheep raised under an extensive production system. This report also provides the first evidence of *M. bovis* isolation from the respiratory tract of goats.

Key words: *Mycoplasma ovipneumoniae*, *Mycoplasma bovis*, goat, sheep, extensive production

Respiratory disease in sheep and goats can result in sudden death or in protracted illness. Pneumonia may occur in all ages of sheep, but lambs require significant time for treatment and the severity of pneumonia is directly related to reduced growth and feed efficiency. In many sheep flocks pneumonia is a major cause of lamb mortality (1). The infecting agent could be one of a number of bacteria, chlamydia, mycoplasma, or viruses. The most common cause of pneumonia, particularly in sheep, is pasteurellosis or mannheimiosis, while mycoplasmal pneumonia is often overlooked. Other bacteria occasionally associated with respiratory disease in small ruminants are *Arcanobacterium pyogenes*, *Staphylococcus* spp., *Streptococcus* spp., *Haemophilus* spp., and *Klebsiella pneumoniae* (2). The mycoplasmas most commonly associated with disease in sheep and goats are *Mycoplasma capricolum* subsp. *capripneumoniae* (a causal agent of caprine contagious pleuropneumonia), *M. mycoides* subsp. *capri* (involved in contagious agalactia syndrome), and *M. ovipneumoniae*. Mycoplasma species occasionally associated with respiratory disease are *M. arginini*, *M. agalactiae*, *M. putrefaciens*, and *M. bovis* (2).

M. ovipneumoniae is considered to be one of the most important mycoplasmas involved in the respiratory diseases of sheep. Primary infection with *M. ovipneumoniae* may

predispose sheep to invasion of the lower respiratory tract by other organisms such as the parainfluenza-3 virus and *Mannheimia haemolytica* (2,3). This agent is frequently isolated from pneumonic sheep, but it can also be found in the respiratory tracts of healthy animals (4). There are few reports incriminating *M. ovipneumoniae* as a cause of severe respiratory disease in goats (5,6).

Clinical respiratory diseases are common in Bosnia and Herzegovina (B&H) herds. Reported risk factors include the intermingling of populations of autochthonous breeds (Pramenka sheep and Balkan goats) with imported animals of various breeds and origins. These factors, along with limited populations (1,000,000 sheep and 70,000 goats) and the relative absence of disease control measures enhanced our opportunity to study the involvement of these microorganisms in respiratory diseases in the existing extensive production systems.

This research reports the finding of mycoplasmas from the respiratory system of sheep and goats managed under extensive production system and the first isolation of *M. ovipneumoniae* and *M. bovis* from the goats in B&H. A total of 142 nasal swabs and 88 lung samples were collected from sheep and goat herds from various regions in B&H. Samples were obtained from animals in herds reared under extensive systems. To detect mycoplasmas,

* Correspondence: maid.rifatbegovic@vfs.unsa.ba

all samples were cultured in liquid and solid media (7) and incubated in a 95% N₂ and 5% CO₂ humidified atmosphere at 37 °C. Mycoplasma isolates were identified by colony morphology (8), biochemical testing (9), and the growth inhibition test (10). Polymerase chain reaction (PCR) tests for mycoplasma groups (11), *M. bovis* (12), *M. putrefaciens* (13), *M. ovipneumoniae* (14), and *M. arginini* (15) were performed for final identification. The *M. bovis genitalium* strain was identified by the Animal Health and Veterinary Laboratories Agency, Mycoplasma Group (Weybridge, UK) by PCR/denaturing gradient gel electrophoresis and confirmed using 16S rRNA gene sequencing.

A total of 29 samples were positive for mycoplasmas (12.6%). *M. ovipneumoniae* was recovered from 21 samples (Table), and most strains (n = 17) were isolated from the area with the largest sheep population in B&H. *M. arginini*, *M. ovipneumoniae*, and *M. bovis genitalium* were isolated from the goat samples. One mycoplasma isolate was unidentified with the methods used for identification.

M. ovipneumoniae is known as the cause of atypical or ovine nonprogressive pneumonia, which is well recognized in different parts of the world (2). Our results appear to confirm the importance of this mycoplasma in an extensive production system. In this study *M. ovipneumoniae* was recovered from sheep that suffered from respiratory disease, but not from the lungs of apparently healthy sheep. All isolates were recovered in the area with the largest sheep population in B&H. This finding was very important since sheep are the most abundant domestic ruminant in B&H. Close and repeated contact or the absences of preventive measures were apparent risk factors for the occurrence and spreading of *M. ovipneumoniae* in different herds at this region.

In goats, *M. ovipneumoniae* was only isolated from the lungs of 2 asymptomatic kids. Curiously, this species of mycoplasma could not be recovered from adult goats in this herd or others. These findings suggest that possible early infection occurred in these asymptomatic goat kids. We found this interesting because these asymptomatic carriers could possibly spread this agent to other animals

through contact typical of extensive systems (communal pastures, watering holes, etc.) or through movement associated with trade (2). The asymptomatic presence of *M. ovipneumoniae* could also predispose goats to pneumonia caused by other organisms, as was previously observed in sheep (16). The lack of positive samples from adult goats could be explained by the capacity of some adult goats to clear the infection or at least inhibit bacterial growth (17). An outbreak of a severe respiratory disease occurred in the kids of other goat herds, and *M. arginini*, *M. haemolytica*, and *Streptococcus* sp. were identified in the lungs of the affected animals. In this outbreak, *M. haemolytica* was probably the primary cause of a respiratory disease in the kids. *M. arginini* was not considered a major pathogen, but it is known that this species can increase pathological damage (2). The lack of *M. ovipneumoniae* in this case cannot rule out the importance of this mycoplasma for goats because there are several reports of outbreaks of atypical nonprogressive pneumonia in goats (5,6). The presence of *M. ovipneumoniae* in animals with or without clinical respiratory disease provides the opportunity for this organism to be spread in extensive production systems and for it to cause severe disease alone or in association with other bacteria.

Our results underscore the need to develop control measures to combat respiratory diseases in small ruminants in B&H. Bought-in sheep or lambs should be isolated before mixing with the home flock. Lower stocking densities and improved ventilation are also important in reducing the spread of respiratory disease (2). Consideration should be given to vaccination with *M. ovipneumoniae* and *M. haemolytica* vaccines, since mixed vaccines provide greater protection against respiratory disease (14). An interesting finding of this research is the isolation of *M. bovis genitalium* from the nasal swab of goats. *M. bovis genitalium* was recently reclassified as a single species within *M. ovine/caprino* serogroup 11 (18), and it usually causes genital disorders in ruminants (19,20). There is no evidence that *M. bovis genitalium* has a pathogenic role in respiratory disease of goats. Since

Table. Presence of mycoplasmas in the samples tested.

Species	Nasal swabs			Lungs		
	Tested	Positive	Identification (n)	Tested	Positive	Identification (n)
Sheep	73	18	<i>M. ovipneumoniae</i> (17) <i>Mycoplasma</i> sp. (1)	77	2	<i>M. ovipneumoniae</i> (2)
Goat	69	6	<i>M. arginini</i> (4) <i>M. bovis genitalium</i> (1) <i>Mycoplasma</i> sp. (1)	11	3	<i>M. ovipneumoniae</i> (2) <i>M. arginini</i> (1)
Total	142	24		88	5	

experimental infection of cattle with this species results in a mild, subclinical pneumonia (21), we cannot exclude the possibility of a pathogenic effect in the respiratory tract of goats. With all due respect to the literature available, this is the first isolation report of this mycoplasma from the goat respiratory tract.

Additional work is necessary, both to elucidate the molecular characteristics of the *M. ovipneumoniae* strains and their relationship to the epidemiology and to research the potential pathogenicity of *M. bovis* for the goat respiratory tract.

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