Mycoplasmas Isolated from Ruminants in Bosnia and Herzegovina between 1995 and 2015

Zinka Maksimović1, Azra Bačić2, Maid Rifatbegović1

Abstract
This study provides an overview of mycoplasma species isolated from ruminants in Bosnia and Herzegovina between 1995 and 2015. A total of 1442 samples from affected and asymptomatic cattle, sheep and goats were submitted for isolation of mycoplasmas. Mycoplasmas were isolated from 195 samples (13.5%). Mycoplasma bovis was the most frequently isolated species from pneumatic cattle and M. ovipneumoniae from affected small ruminants. The presence of some other important mycoplasmas, like M. conjunctivae, M. mycoides capri, M. capricolum and M. putrefaciens was confirmed.

Keywords
mycoplasmal diseases – ruminants – Bosnia and Herzegovina

Introduction
Mycoplasma diseases of livestock remain the major problems for animal health worldwide, causing large financial losses (17). Cattle, sheep and goats may all be infected with a wide range of different mycoplasma species (4).

Several of ruminant mycoplasmoses are listed by the OIE (Office International des Epizooties): Contagious bovine pleuropneumonia (CBPP), Contagious caprine pleuropneumonia (CCPP) and Contagious agalactia (CA). CBPP, caused by Mycoplasma mycoides subsp. mycoides (formerly “small colony” (SC) biotype), is eradicated from Europe, while it remains enzoootic in many African countries. CCPP is a severe disease of goats occurring in Africa and Asia, caused by Mycoplasma capricolum subsp. capripneumoniae (Mccp) (32). CA is a cosmopolitan disease, affecting primarily dairy sheep and goats, characterized by mastitis, arthritis, keratoconjunctivitis, pneumonia and abortion (2, 32). Mycoplasma agalactiae (Ma) is the main cause of the disease, but M. capricolum subsp. capricolum (Mcc), M. mycoides subsp. capri (Mmc) and M. putrefaciens also can be involved in the etiology of CA (6). In addition to diseases notifiable to the OIE, other mycoplasmoses are also economically important, and reported worldwide (1).

M. bovis is the most pathogenic bovine mycoplasma in the parts of the world that are free of CBPP, causing pneumonia, mastitis and arthritis (15, 17). Other mycoplasmas involved in bovine diseases include M. dispar, M. bovigenitalium, M. bovirhinis, M. canis, M. alkalescens and Ureaplasma diversum (17). M. ovipneumoniae (Mopv) causes atypical pneumonia in sheep (18), and recent reports confirm the involvement of Mopv in outbreaks of respiratory disease in goats (7,14,24). Infectious keratoconjunctivitis ("pink-eye"), caused by M. conjunctivae is also considered as one of important mycoplasmal diseases in small ruminants (17). Several mycoplasma species (Mmc, Mc, Ma, and M. putrefaciens) are involved in the MAKePS syndrome (mastitis, arthritis, keratitis, pneumonia and septicemia) of goats (27).

The intermingling of ruminant populations in Bosnia and Herzegovina (B&H) with imported animals and the relative absence of disease control measures contribute to the possibility for mycoplasmas to be introduced and spread among the herds, causing diseases alone or in conjunction with other bacteria (12). Mycoplasma species isolated from cattle, sheep and goats in B&H during the last two decades, are presented in this paper.

Material and Methods
A total of 1442 samples from the respiratory and reproductive systems, milk, ear and eye swabs were collected from cattle, sheep and goats from various regions in B&H (Table 1). The samples were obtained from asymptomatic and affected animals. Most samples were cultured in TH medium (28). In addition, Medium B (5) was also used for the isolation. All isolates were triple cloned and incubated in a 95% N2 and 5% CO2 prior to further examination.

Preliminary identification of the isolates was performed on the basis of colony morphology (22), sensitivity to digitonin, fermentation of glucose, hydrolysis of arginine, phosphatase activity and film and spot production (20). In addition, the growth inhibition test (21) was used for identification of M. bovis, M. bovirhinis, Mopv, Mmc, A. oculi, M. conjunctivae, Mcc, M. arginine and M. putrefaciens. PCR assays for Mycoplasma group (30), M. bovis (19, 25, 26), M. bovirhinis (11), Mopv (13), M. arginine (29), and Mmc (31) were performed in a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems), or Stratagene Mx3005P (Agilent Technologies) in a total volume of 25 µl. The M. bovigenitalium strain was identified by the Animal Health and Veterinary Laboratories Agency, Mycoplasma Group.
**Table 1.** The type and number of samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goat</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal swab</td>
<td>474</td>
<td>121</td>
<td>150</td>
<td>745</td>
</tr>
<tr>
<td>Lungs</td>
<td>93</td>
<td>77</td>
<td>17</td>
<td>187</td>
</tr>
<tr>
<td>Milk*</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Sperm</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Ear swab</td>
<td>0</td>
<td>31</td>
<td>28</td>
<td>59</td>
</tr>
<tr>
<td>Eye swab</td>
<td>0</td>
<td>210</td>
<td>63</td>
<td>273</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>0</td>
<td>44</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>685</td>
<td>483</td>
<td>274</td>
<td>1442</td>
</tr>
</tbody>
</table>

* only real time PCR detection in milk samples

(Weybridge, UK). To detect *M. bovis* in the bulk tank samples, DNA was extracted from the milk samples (10) and subjected to real time PCR (25).

## Results

A total of 195 samples tested positive for mycoplasmas (13.5%) (Table 2). In cattle, mycoplasmas were found exclusively in the respiratory system. They were isolated from 38 of 474 nasal swabs (8%), and from 16 of 93 lung samples (17%). *M. bovis* was the most commonly identified species (57.4%). Other mycoplasma species isolated from cattle were *M. bovirhinis* (7.4%) and *Acholeplasma sp.* (1.9%), both detected in nasal swabs.

Mycoplasmas were isolated from 90 of 483 sheep samples, and from 51 of 274 goat samples (18.6%, both). A total of 97 respiratory system samples were found to be positive for mycoplasmas (26.6%). *Movp* was the most commonly identified species in small ruminants (39%), being detected in 37 of 198 respiratory system samples obtained from sheep (18.7%), and 18 of 167 samples collected from goats (10.7%). It was isolated from 43 of 271 nasal swabs (15.9%), and from 12 of 26 lungs (12.8%). *Mmc* was the second frequently found mycoplasma, accounting for 6.4 percent of the isolates. It was detected in goat lungs (n=2), and in the eye and vaginal swabs (n=7) obtained from sheep. *M. arginini* was the next most commonly identified mollycute (5%), isolated only from the nasal swabs in goats. *M. bovigenitalium* were recovered from the nasal swab of goats (0.7%). Other mycoplasma species in small ruminants included: *M. conjunctivae* (2.8%), *A. oculi* (2%), *Mcc* (1.4%) and *M. putrefaciens* (0.7%).

## Discussion and conclusion

Mycoplasmas can be a predisposing factor for bacterial and viral infections, and as a result, mycoplasmoses are often overlooked (17). The presence of pathogenic mycoplasma species was confirmed in ruminant populations from different areas in B&H. *M. bovis* was the most commonly identified mycoplasma species from cattle. It was detected in animals showing clinical signs of respiratory infection, which included loss of appetite, depression, fever, nasal discharge, dyspnoea and coughing. Death cases associated with *M. bovis* were also recorded (23). Infections with *M. bovis* have been reported throughout the world, causing significant economic losses. It is responsible for at least a quarter to a third of all calf pneumonia (15). It can also cause arthritis, keratoconjunctivitis, infertility, abortion, and other pathogenic conditions (15). *M. bovis* was not found in the milk samples in B&H, although it is a major cause of mastitis worldwide (15). This is a significant finding considering that there is no effective treatment for mycoplasmal mastitis (8). The occurrence and spread of this mycoplasma may be due to the low efficiency of antibiotic treatments supported by the evidences of the strains becoming resistant to antibiotics, and/or the lack of commercial vaccines (15, 23). Related to this, consideration should be given to monitoring mycoplasma infections, especially on dairy farms.

Sheep and goat populations in B&H are managed under the extensive production system. Clinical respiratory diseases are very common in B&H herds, and may result in sudden death or protracted illness (17). In sheep and goats, the majority of the isolates were identified as *Movp*. Although *Movp* is frequently isolated from pneumonia sheep, it can also be found in the respiratory tracts of healthy animals (3). However, in this study *Movp* was isolated from the animals showing clinical signs of respiratory infection. The presence of *Movp* in affected goats supports the involvement of this mycoplasma in the outbreaks of respiratory disease in goats as was reported in some previous studies (7, 14, 24). Very similar to *M. bovis* case, there is no commercial vaccine available for *Movp*.

*Mmc* was isolated from the lungs of goats suffering from the respiratory disease prior to death. *Mmc* has one of the widest geographic distributions of ruminant mycoplasmas (17), and can cause high mortality outbreaks in goats, as described previously (9). *Mmc* was also detected in two vaginal swabs, related to its occasional isolation from sheep with the reproductive system disease (17).

Among the other important mycoplasmas, *M. conjunctivae*, the cause of infectious keratoconjunctivitis was found in both sheep and goats with the clinical signs of “pink-eye”. An interesting finding is the detection of *M. bovigenitalium* in the respiratory system of goats. Although this mycoplasma is recognized as a cause of genital disorders in ruminants (17), the possibility of a pathogenic effect in the respiratory tract of goats cannot be excluded (12). The causative agents of CA were not isolated, which may be due to the limited number of dairy sheep and goat herds in B&H, as observed in some other countries (1).

This research reveals the importance of mycoplasmas as the etiological agents of ruminant diseases, particularly respiratory infections. Respiratory diseases in cattle caused by *M. bovis* and in small ruminants caused by *Movp* were frequently reported and confirmed during the past twenty years. Contagious agalactia of small ruminants and mastitis in cattle caused by *M. bovis* were not detected. These findings indicate the need to develop enhanced control measures, which will support rapid and accurate etiological diagnosis of mycoplasmoses, antibiotic susceptibility testing of the isolates, and the prevention of diseases through the control of animal movements. Vaccine development in the future will provide improved preventive measures and protection against severe mycoplasmal infections.
Table 2. Number of mycoplasmas isolated from cattle, sheep and goats in B&H between 1995 and 2015

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mycoplasma species</th>
<th>Sheep Number of isolates</th>
<th>Mycoplasma species</th>
<th>Goat Number of isolates</th>
<th>Mycoplasma species</th>
<th>Cattle Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal swab</td>
<td>M. ovipneumoniae</td>
<td>33</td>
<td>M. ovipneumoniae</td>
<td>10</td>
<td>M. bovis</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>M. capricolum subsp. capricolum</td>
<td>2</td>
<td>M. arginini</td>
<td>7</td>
<td>M. bovirhinis</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>A. oculi</td>
<td>1</td>
<td>M. bovigenitalium</td>
<td>1</td>
<td>Acholeplasma sp.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mycoplasma sp.</td>
<td>9</td>
<td>Mycoplasma sp.</td>
<td>9</td>
<td>Mycoplasma sp.</td>
<td>11</td>
</tr>
<tr>
<td>Lungs</td>
<td>M. ovipneumoniae</td>
<td>4</td>
<td>M. ovipneumoniae</td>
<td>8</td>
<td>M. bovis</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Mycoplasma sp.</td>
<td>4</td>
<td>M. m. capri</td>
<td>2</td>
<td>Mycoplasma sp.</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>M. m. capri</td>
<td>5</td>
<td>M. conjunctivae</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. putrefaciens</td>
<td>1</td>
<td></td>
<td>6</td>
<td>nt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. oculi</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mycoplasma sp.</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye swab</td>
<td>Mycoplasma sp.</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. m. capri</td>
<td>2</td>
<td></td>
<td>2</td>
<td>nt</td>
<td></td>
</tr>
<tr>
<td>Ear swab</td>
<td>Mycoplasma sp.</td>
<td>14</td>
<td></td>
<td>14</td>
<td>neg.</td>
<td>nt</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>Mycoplasma sp.</td>
<td>14</td>
<td></td>
<td>14</td>
<td>neg.</td>
<td>nt</td>
</tr>
</tbody>
</table>

nt*: not tested

References


Mikoplazme izolovane iz preživara u Bosni i Hercegovini u periodu od 1995. do 2015. godine

Sažetak

Uvod i ciljevi

Glavni zaključci
Provedena istraživanja ukazuju na potrebu poboljšanja preventivnih mjera za zaštitu od mikoplazemske bolesti. Rezultati istraživanja ukazuju na potrebu poboljšanja preventivnih mjera za zaštitu od mikoplazemske bolesti.

Materijal i metode
U cilju izolacije i identifikacije mikoplazm, obradeno je ukupno 1442 uzorka respiratornog i reproduktivnog sistema. Uzorci su izloženi na kultiviranje u respiratornom sistemu izloženih stada, te nastanku mikoplazmoza. Zajedno sa zaraznom pleuropneumonijom je to i najvažnije oboljenje preživara u BiH.

Rezultati i interpretacija
Mikoplazme su izolirane iz 195 (13.5%) uzorka. Uzorci su izloženi na kultiviranje u respiratornom sistemu izloženih stada, te nastanku mikoplazmoza.