MYCOPLASMA ISOLATED FROM CATTLE LUNGS
AND THEIR PATHOGENICITY STUDY

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Abstract
A total of 170 lung samples, 85 of which from Balady cattle and 85 from Friesian cattle, revealed 30 mycoplasma isolates (17.6%). The Balady lung samples revealed 19 mycoplasma isolates, 5 of which were from normal lungs, typed as 2 M. bovis, 3 M. bovirhinis, and 3 M. marginits. While 14 mycoplasma isolated pneumonia lungs were typed as 5 M. bovis, 3 M. bovirhinis, 3 M. bovirhinis and 3 M. marginits.

The Friesian lung samples revealed 11 mycoplasma isolates, 3 of which were isolated from apparently normal lungs, typed as 1 M. bovirhinis and 2 were M. marginits, while 8 mycoplasma isolated from pneumonia lungs were typed as 4 M. bovis, 2 M. bovirhinis, 1 M. bovirhinis and 1 M. marginits.

Antibiogram was made for the isolated mycoplasma species against 17 antibiotics. They were highly sensitive to Troleandomycin sulphate, Enrofloxacin, Norfloxacine, Tiamulin and Ciprofloxacin.

Pathogenicity study was made by injecting the isolated mycoplasma strains intraperitoneally into mice. The test resulted in congestion of the lungs injected with M. bovis and M. bovirhinis after one week of infection, and mycoplasmas could be reisolated from the lungs.

INTRODUCTION

Respiratory diseases, especially, pneumonia represent a serious economic important problem affecting calves due to morbidity and mortality (Sood et al., 1986).

Mycoplasma is one of the causative agents of pneumonia in calves and in adult cattle and buffaloes.

So far, M. mycoides was known to be the cause of pneumonia in bovine
The culture medium was heart infusion broth and agar, and the culture procedure was as described by Sabry and Ahmed (1975) as follows:

0.5 g of lung tissue was aseptically placed into a sterile mortar, cut by sterile scissors and ground using storie sand. Five ml of broth were added, then, from the mixture, direct plating was made, and 0.2 ml were transferred into broth which was incubated at 37°C for three days. Then, 0.2 ml broth were dispensed into 1 ml broth and plating was made. This step was repeated 3 times. The agar plates were incubated at 37°C under reduced oxygen tension in humidified candle jars. The plates were examined after 48 hours under a stereomicroscope for the presence of Mycoplasma colonies having typical fried egg appearance.

Purification and maintenance of the isolates

These were made according to Sabry and Ahmed (1975). Genus determination was carried out as described by Frendt et al. (1973) using digitonin sensitivity test in which the agar plate was inoculated with 0.2 ml of the test culture using the running drop technique. A disc impregnated in 1.5% ethanol was pressed in the middle of the inoculated area. The plate was incubated at 37°C to be examined for the presence of inhibition zone which indicated a positive test.

Biochemical characterization was adopted according to Erno and Stipkovits (1973) as follows.

Glucose fermentation test: 2.9 ml of glucose medium was inoculated with 0.1 ml of the suspected mycoplasma culture, incubated aerobically at 37°C and examined daily for seven days. A yellow colour indicates a positive test.

Arginine deamination test: 2.9 ml of arginine medium was inoculated with 0.1 ml of the suspected mycoplasma culture, and incubated aerobically at 37°C for 7 days. A red colour indicates a positive test.

Tetrazolium reduction test: 2.9 ml of tetrazolium medium was inoculated with 0.1 ml of the suspected mycoplasma culture and incubated aerobically at 37°C for 7 days. A pink colour indicates a positive test.

Film and Spot formation test: 0.2 ml of mycoplasma broth culture was cultivated on heart infusion agar plate which was incubated at 37°C. After 3 days, a glistening layer appeared owing to the presence of lipase, indicating a positive test.

Serotyping of the mycoplasma isolate: Was applied using, (1) growth inhibition test as described by Clyde (1964) using a disc saturated with antisera
that was placed in the middle of suspected agar culture. The presence of inhibition zone indicates a positive result, (2) Growth precipitation test according to Kroghgaard-Jensen (1972). The reference antisera were kindly supplied by Dr. Shin Diagnostic Lab. Cornell University, Ithaca, NY, USA.

**Antibiogram**: was performed according to Cyde (1964) using 17 different antibiotics, applying growth inhibition test as follows:

Each of the isolated mycoplasmas was cultivated on agar plate using single drop technique, then, a disc saturated with the standard concentration of antibiotic was pressed in the middle of the mycoplasma culture. The plates were placed in the candle jar and incubated at 37°C. The plates were examined daily under stereomicroscope. The inhibition zone was measured to indicate the degree of sensitivity to the used antibiotic.

**Pathogenicity test**: was made according to Singh and Prasad (1994) as follows:

Ten albino mice, 1 month old, proved to be free from mycoplasma by culture method were divided into 5 groups, each of 2 mice. Each group was inoculated intraperitoneally with one of the isolated mycoplasma species, *M. bovis*, *M. bovigenitalium*, *M. bovirisin* and *M. arginini*, and group 5 was considered as a control group. All the 4 mycoplasma isolates 1x10⁹ C.F.U were grown in heart infusion agar at 37°C for 4 days under reduced oxygen tension. One typical colony of each isolate was picked up and grown in 10 ml heart infusion broth and incubated at 37°C for 48 h in a lighted candle jar. A mixture was prepared using 1 ml of inoculum and 4 ml of 5% sterile mucin. 0.5 ml of this mixture was used for intraperitoneal inoculation in mice.

One mouse of the control group was inoculated with 0.5 sterile heart infusion broth, and the second was inoculated with 0.5 ml of 1:4 mixture (v/v) of broth and 5% mucin.

The inoculated mice were kept under observation for 6 days, then, sacrificed and mycoplasma was reisolated from lungs and confirmed.

**RESULTS**

**Mycoplasma isolation and serotyping**

From the results recorded in Table 1, it is clear that, 5 mycoplasmas (2.9%) could be isolated from 5 Balady cattle normal lung samples, 2 of which were
antigenically related to *M. bovis* (6.6%), and 3 were *M. marginini* (10%). On the other hand, 14 mycoplasmas could be isolated from pneumatic lungs (8.2%), 5 of which typed as *M. bovis* (16.0%), 3 were *M. bovigenitalium* (10%), 3 were *M. bovirhinis* (10%), and 3 were typed as *M. marginini* (10%).

It was also found that, 3 mycoplasmas (1.7%) were isolated from 55 normal Friesian cattle lung samples, one of which was typed as *M. bovirhinis* (5.3%), and 2 were *M. marginini* (6.6%), while, 8 mycoplasmas (4.7%) were recovered out of 30 pneumatic lungs, 4 of which were typed as *M. bovis* (13.3%), 2 were *M. bovigenitalium* (6.6%), 1 was *M. bovirhinis* and 1 was *M. marginini* (3.3%).

Table 1. Mycoplasma isolated from cattle lungs.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Type of sample</th>
<th>No. of sample exam.</th>
<th>No. of Mycoplasma isolated</th>
<th>Mycoplasma serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M. bovis</td>
<td>M. bovigenitalium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Balady cattle</td>
<td>Normal lungs</td>
<td>50</td>
<td>5</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Pneumonic lungs</td>
<td>35</td>
<td>14</td>
<td>8.2</td>
</tr>
<tr>
<td>Friesian cattle</td>
<td>Normal lungs</td>
<td>55</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Pneumonic lungs</td>
<td>30</td>
<td>8</td>
<td>4.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>170</td>
<td>30</td>
<td>17.6</td>
</tr>
</tbody>
</table>

Percentages were calculated from the total Mycoplasma isolated.

**Antibiogram**

The results of antibiogram recorded in Table 2 showed that the isolated mycoplasmas were highly sensitive to Enrofloxacin, Trospectomycin sulphate, Tiamulin, Norfloxacin and Ciproflaxacin. Oxytetracylin and Gentamycin were of intermediate activity, while, the isolated Mycoplasmas were resistant to Amoxycillin and Erythromycin.

**Pathogenicity test**

The results of pathogenicity test showed that the lungs of mice inoculated with *M. bovis* and *M. bovigenitalium* were congested compared with the lungs of mice infected with *M. bovirhinis* and *M. marginini*. Moreover, *M. bovis* and *M. bovigenitalium* could be resolated from the lungs infected with these mycoplasmas.
DISCUSSION

From the data recorded in Table 1, it could be concluded that the rate of mycoplasma recovery was higher in pneumonia-lungs with apparently normal ones. It was also noticed that M. arginini and M. bovirhinis were the mycoplasma species that could be isolated from both Balady and Friesian cattle apparently normal lungs. This agreed with (Soury et al., 1970) who mentioned that M. bovirhinis has its natural habitat in the respiratory tract, and with Mosher (1997) who could isolate M. bovirhinis and M. arginini from apparently normal calf lungs.

In the present study, M. bovis was the predominating mycoplasma species isolated from pneumonia calf lungs (30%), followed by M. bovigenitalium (16.6%). Pfutzner et al. (1980), Shimizu et al. (1981) and Deverse et al. (1988) diagnosed calf pneumonia associated with M. bovis. Moreover, Liberal (1989) detected mycoplasmosis carriers in bovine herds by the isolation of M. bovis from calves nasal exudate, while, Bois et al. (1993) and Doherty et al. (1994) could isolate M. bovis from pneumonia lungs of calves.

The present study, also, coincides with that of Srivastava and Uppal (1985) who isolated M. bovigenitalium from lungs of calves showing signs of respiratory infection, and also, with Laak et al. (1992) who could isolate M. bovigenitalium from lungs of pneumonia calves.

In the present study, M. arginini was isolated from pneumonia Friesian cattle lungs at the rate of (3.3%). M. arginini could also be isolated from pneumonia lungs of calves by Muenster Matsuoka (1979).

The results of the antibiogram showed that the isolated mycoplasmas were highly sensitive to Trospectomycin sulphate, Enrofloxacin, Norfloxacin and Tiamulin. Our results agreed with those of Yancey and Klein (1988) who found that Trospectomycin sulphate was active in vitro against a variety of human and veterinary pathogens.

The present study, also, coincides with Devriese and Haesebrock (1991) who found that M. bovis was sensitive to Oxytetracycline, Tylosin, Lincomycin, Gentamycin and Enrofloxacin. In addition, our results agreed with Allan and Prie (1981) who stated, in vitro, the activity of Tiamulin against bovine respiratory tract mycoplasmas.

Regarding the pathogenicity study, it was found that the lungs of mice after
one week infection with *M. bovis* and *M. bovigenitalium* were congested, and the injected Mycoplasmas could be reisolated from the lungs, while, *M. arginini* and *M. boviriinis* could not be reisolated from mice infected with these mycoplasmas. Singh and Prasad (1994) could not, also, reisolate *M. arginini* from infected mice.

Pathogenicity study was also previously applied by Tomas and Howard (1974) to know the effect of different mycoplasmas including *M. boviriinis* on explant cultures of bovine trachea which proved cytopathic effect represented by sloughing and flattening of the epithelial layer.

From the results of pathogenicity study of the present investigation, it could be concluded that *M. bovis* and *M. bovigenitalium* were more pathogenic to the lungs than *M. boviriinis* and *M. arginini*. It is known that *M. boviriinis* and *M. arginini* have their natural habitat in the lungs, and when the animal becomes understress or with the aid of other pathogenic organisms, they become able to produce the disease.

Table 2. Antibiotic sensitivity for Mycoplasma isolated from cattle lungs.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Antibiotic concentration</th>
<th><em>M. bovis</em></th>
<th><em>M. bovigenitalium</em></th>
<th><em>M. boviriinis</em></th>
<th><em>M. arginini</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>10 per g</td>
<td>3mm*xxx</td>
<td>2mm*xxx</td>
<td>2mm*xxx</td>
<td>2mm*xxx</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 per g</td>
<td>4mm*xxx</td>
<td>3mm*xxx</td>
<td>2mm*xxx</td>
<td>2mm*xxx</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>30 per g</td>
<td>17mm*</td>
<td>18mm*</td>
<td>17mm*</td>
<td>17mm*</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>15 per g</td>
<td>19mm*</td>
<td>20mm*</td>
<td>20mm*</td>
<td>20mm*</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>2 per g</td>
<td>20mm*</td>
<td>20mm*</td>
<td>21mm*</td>
<td>21mm*</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10 per g</td>
<td>17mm*</td>
<td>18mm*</td>
<td>18mm*</td>
<td>18mm*</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>10 per g</td>
<td>21mm*</td>
<td>22mm*</td>
<td>23mm*</td>
<td>23mm*</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20 per g</td>
<td>21mm*</td>
<td>22mm*</td>
<td>23mm*</td>
<td>23mm*</td>
</tr>
<tr>
<td>Canamycin</td>
<td>30 per g</td>
<td>20mm*</td>
<td>21mm*</td>
<td>21mm*</td>
<td>21mm*</td>
</tr>
<tr>
<td>Spiamycin</td>
<td>30 per g</td>
<td>20mm*</td>
<td>21mm*</td>
<td>21mm*</td>
<td>21mm*</td>
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<tr>
<td>Neomycin</td>
<td>30 per g</td>
<td>19mm*</td>
<td>20mm*</td>
<td>20mm*</td>
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</tr>
<tr>
<td>Vibramycin</td>
<td>30 per g</td>
<td>20mm*</td>
<td>20mm*</td>
<td>23mm*</td>
<td>20mm*</td>
</tr>
<tr>
<td>Trospenemycin</td>
<td>20 per g</td>
<td>23mm*</td>
<td>23mm*</td>
<td>21mm*</td>
<td>23mm*</td>
</tr>
<tr>
<td>Danofoxacin</td>
<td>30 per g</td>
<td>21mm*</td>
<td>21mm*</td>
<td>22mm*</td>
<td>22mm*</td>
</tr>
<tr>
<td>Trimulin</td>
<td>30 per g</td>
<td>22mm*</td>
<td>22mm*</td>
<td>23mm*</td>
<td>23mm*</td>
</tr>
<tr>
<td>Norfoxacin</td>
<td>20 per g</td>
<td>22mm*</td>
<td>22mm*</td>
<td>22mm*</td>
<td>23mm*</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>10 per g</td>
<td>16mm*</td>
<td>17mm*</td>
<td>17mm*</td>
<td>18mm*</td>
</tr>
</tbody>
</table>

Δ Zone of inhibition xxx resistant xx intermediate sensitivity x sensitive
REFERENCES


الميكوبلازما المعزولة من رئتين الأبقار يدراس تأثيرها الفرعي

لبنى مصطفى الشحيتي، وندا محمد أبو الكارم، وسماء سعد ندا

1 الخدمات البيئية - مركز البيئات النباتية - التربة - جيزان - السعودية
2 كلية الطب البيطرية - كلية الشهير - جامعة طنطا

أجري البحث على 70 عينة من رئتين الأبقار منها 38 عينة من رئتين الأبقار البديلة و 32 عينة من الأبقار الفرعيين. وقد أسفرت النتائج عن 20 عينة ميكوبلازما (M. \textit{hominis}) و 5 عزلات ميكوبلازما من رئتين الأبقار البديلة. كانت 9 عزلات ميكوبلازما من الأبقار الفرعيين، و 11 عزلة ميكوبلازما من الأبقار البديلة. بينما تم العزل من 14 عزلة ميكوبلازما من الأبقار التي بها التهابات رئوية، صفت 9 عزلات منها ميكوبلازما بوفيسي، 3 ميكوبلازما بوفيسياثالية، 2 ميكوبلازما بوفيسي، و 3 ميكوبلازما بوفيسياثالية.

وسفر فحص عينات رئتين الأبقار الفرعيين من 11 عزلة ميكوبلازما عزل منها 2 عزلات من الأبقار السليمة فلورهيا وصفت واحدة ميكوبلازما بوفيسيثالي و 2 ميكوبلازما بوفيسي. وتم العزل من 8 عزلات ميكوبلازما من الأبقار التي بها التهابات رئة. صفت 5 عزلات ميكوبلازما بوفيسي، 1 ميكوبلازما بوفيسياثالي و 1 ميكوبلازما بوفيسياثالي.

تعد اختبار الممارسة للمخاطب عليهم لعشرات الميكوبلازما الموزولة باستخدام 17 نوع مسما حيوي. وكانت عزلات الميكوبلازما شديدة الماسسة لسلفات النورفوكلستاسين، النورفوكلستاسين، العاملين، والسيروفلوكاسين.

وقد أجريت دراسة التأثير الفرعي لعشرات الميكوبلازما الموزولة بقياس هذه العزلات في النفايات البرية في الأبقار وأنتجت نتائج علاجات رؤية بالنسبة للأبقار، والتي تم السيطرة عليها بميكوبلازما بوفيسي و ميكوبلازما بوفيسياثالي بعد أسبوع من العزلة. تم إعادة عزل الميكوبلازما بوفيسي والميكوبلازما بوفيسياثالي.