Susceptibility of *Mycoplasma bovis* field isolates to antimicrobial agents

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ABSTRACT: The purpose of this study was to determine the antibacterial susceptibility of field isolates of *Mycoplasma bovis* originating from the upper respiratory tract of cattle of different ages. Bacteriological examination of 90 nasal swabs collected from calves at three months of age identified *M. bovis* in 31 (34.44%) samples. Seventeen (18.88%) of these animals still housed *M. bovis* in their nasal cavity at nine months and five animals (5.55%) still at seventeen months of age. *M. bovis* were confirmed by biochemical and antigenic methods. To confirm that these belonged to the *M. bovis* species isolated mycoplasmas were tested using the PCR method. Fifteen field strains of *M. bovis* isolated from the same cattle at three, nine and seventeen months (five strains from each age group) were selected for antibacterial susceptibility testing against six groups of antimicrobial agents using an agar dilution method. The MIC_{90} ranges established for tylosin, tulathromycin, enrofloxacin, florfenicol and lincomycin were 0.39–0.78 μg/ml, 0.50–1.00 μg/ml, 0.78–1.56 μg/ml, 3.12 μg/ml and 0.39–0.78 μg/ml, respectively. The range of MIC_{90} for oxytetracycline was from 50 to 100 μg/ml. Preliminary examination of the antimicrobial susceptibility of field strains of *M. bovis* did not reveal significant differences between different age groups of cattle. After evaluation of the MIC_{90} data with the SPSS 13.0 statistical package it was found that *M. bovis* isolates from animals at three, nine and seventeen months were similarly susceptible to tylosin and tulathromycin. Statistically significant differences in susceptibility of *M. bovis* isolated from cattle of different ages were found to florfenicol compared with tulathromycin ($P < 0.01$), lincomycin ($P < 0.01$) and enrofloxacin ($P < 0.05$). The susceptibility of all *M. bovis* isolates to oxytetracycline and penicillin G significantly differed from the sensitivity to all other antimicrobial agents used in the present study ($P < 0.05$). The *in vitro* susceptibility test showed that field isolates of *M. bovis* isolated from cattle of different ages were similarly sensitive to tylosin, tulathromycin, lincomycin and enrofloxacin. It was also determined that the field strains are resistant to oxytetracycline.

Keywords: *Mycoplasma bovis*; cattle; minimal inhibitory concentration

The most commonly encountered pathogenic mycoplasma in cattle that causes significant commercial losses in the cattle industry is *Mycoplasma bovis* (*M. bovis)* (Francoz et al. 2005). Several studies have shown that *M. bovis* is the most common bacterium identified in feedlot cattle affected by chronic pneumonia and in veal calves with fatal bronchopneumonia (Gerchman et al. 2009). *M. bovis* is also present in the respiratory tract of healthy cattle where it causes a subclinical upper respiratory infection and may provoke mastitis and metritis in caws and arthritis or tenosinovitis in fattening cattle (Fox et al. 2005; Gagea et al. 2006). From 2007 through to 2009, studies in Lithuania revealed that *M. bovis* was present in the upper respiratory tract of 52.5 percent (%) of healthy cattle (Gabinaitiene et al. 2011).

Although appropriate vaccines are available to reduce the consequences of infection, antimicrobial cure it is still a common practice for treating and controlling cattle infection. Infection with Mycoplasma is very difficult to cure because many commonly used antibiotics are not effective against the bacterium, as Mycoplasma do not have a normal cell wall. However, like other mycoplasmas, *M. bovis* is sensitive to antibiotics which inhibit protein or nucleic acid synthesis (Tenk 2005). Antimicrobial drugs known to be effective against Mycoplasma include macrolides, fluoroquinolones, tetracyclines, lincosamides, aminoglycosides and chloramphenicol (Maunsell et al. 2009). In the past decade, *in vitro* susceptibility studies on *M. bovis* have shown an increased resistance to the

antibiotics that are commonly used for treatment of infection, particularly erythromycin, spectinomycin and tilmicosin. Intermediate occurrence of resistance to oxytetracycline and chlortetracycline, and infrequent or no resistance to enrofloxacin, florfenicol was reported (Hirose et al. 2003; Francoz et al. 2005; Rosenbusch et al. 2005; Gerchman et al. 2009). Antimicrobial susceptibility of *M. bovis* field isolates determined by Ter Laak et al. (1993), Ayling et al. (2000), Hirose et al. (2003), Francoz et al. (2005) and Gerchman et al. (2009) showed that the use of antimicrobials results in the selection of resistant and susceptible bacteria.

The *in vivo* activity of antimicrobial agents for the treatment of *M. bovis*-induced pneumonia has been reported frequently, some of these values are comparable with *in vitro* susceptibility data (Stipkovits et al. 2005). The efficacy of tulathromycin and tilmicosin for naturally occurring acute bovine respiratory disease treatment was also confirmed (Godinho et al. 2005; Caswell and Archambault 2008).

The purpose of this study was to determine the antibacterial susceptibility of field isolates of *M. bovis* originating from the upper respiratory tract of cattle of different ages.

MATERIAL AND METHODS

Mycoplasma bovis isolation

The study was carried out from 2008–2009. Nasal swabs for the isolation of Mycoplasma were collected three times at a cattle breeding farm in Lithuania from 90 healthy cattle: when the cattle were aged three, nine and seventeen months, respectively. Fifteen field strains of *M. bovis* isolated from the same cattle were selected for antibacterial susceptibility testing. Mycoplasma isolation procedures and the testing of susceptibility to antimicrobial agents using an agar dilution method were carried out in the microbiology laboratory of the Lithuanian University of Health Science, Veterinary Academy, Department of Infection Diseases.

Mycoplasma cultivation procedures were performed according to the method described by Friis (1975). Isolation of *Mycoplasma bovis* was performed in NHS20 broth media by carrying out 10-fold dilutions from 10^{-1} to 10^{-4} and inoculating the last dilution onto NHS20 solid media agar. Inoculated broth media were cultivated under aerobic conditions. Solid media were incubated under microaerophillic conditions for 7 to 14 days. All media were incubated at 37 °C.

To identify microorganisms of the *Mollicutes* class, a polymerase chain reaction (PCR) method was applied. DNA from isolated microorganisms was extracted with a 5% Chelex solution (Sigma, USA). Isolated microorganisms were analyzed by PCR using the forward primer, MW28 (5'-CCAGACTCCTACGGGAGGCA-3') and reverse oligonucleotide primer MW29 (5'-T GCGAGCATACTACTCAGGC-3') (Grida Lab, Lithuania) that are specific for the *Mollicutes* class (Bashiruddin et al. 2005; Gabinaitiene et al. 2011). This primer pair generates a 560 bp product. The purity of Mycoplasma cultures was determined according to the recommendations of Goll (1994). PCR-positive Mycoplasma cultures were inoculated onto NHS-20 agar plates. Five Mycoplasma colonies were picked from each plate after three to seven days of cultivation and subcultured in NHS-20 broth. This procedure was repeated three times.

In order to separate *Mycoplasma bovis* strains from others species of Mycoplasma, isolates were tested for their biochemical properties (glucose fermentation, arginine hydrolysis, phosphatase activity, tetrazolium reduction and production of spots and films) while the disc growth inhibition (DGI) test was also used (Clyde 1964; Aluotto et al. 1970). Paper discs with antiserum against the following reference strains were used: *Mycoplasma arginini* G230, *Mycoplasma bovigenitalium* PG11, *Mycoplasma bovirhinis* PG43, *Mycoplasma bovis* Donetta and *Mycoplasma dispar* 462/2. All strains biochemically and antigenically classified as *Mycoplasma bovis* were confirmed by the amplification of a 734 bp amplicon. Two oligonucleotide primers targeting the 16S rRNA gene, that are specific for *M. bovis* species were used: forward primer MboF2 (5'-GAAGAAAAAGTAGCATAGGAAATGAT-3') and reverse primer MboR2 (5'-CGTCGTCCC-CACCTTCCTCCCG-3') (Timenetsky et al. 2006).

For further analysis pure *M. bovis* culture colonies were then picked from plates and transferred to NHS-20 broth medium. Isolates were stored at –70 °C until used.

To prepare a suitable Mycoplasma inoculum concentration for minimal inhibitory concentration (MIC) testing, a thawed pure culture of Mycoplasma was inoculated onto NHS-20 agar plates and incubated for 72 h. Then, five colonies of each isolate were selected, aggregated and incubated for 48 h in NHS-20 broth medium. Attention was paid to

the growth-induced pH shift exchange in the broth medium (from red to orange due to fermentation of glucose) (Francoz et al. 2005). After incubation 1 ml of fresh Mycoplasma cultures were added to 9 ml of selective medium and vortexed. Then, 0.1 ml of vortexed suspensions were diluted ten-fold from 10^{-2} to 10^{-9} in 0.9 ml of selective medium. For Mycoplasma colony counting 2 µl of each Mycoplasma dilution were inoculated onto the surface of freshly prepared Mycoplasma agar plates (in triplicate) and incubated at 37 °C under microaerophillic conditions. Inoculated plates were incubated until the plates with the lower dilutions of the Mycoplasma suspensions had well-developed Mycoplasma colonies. The number of colonies was counted using a microscope. For antimicrobial susceptibility testing a 10^5 CFU/ml Mycoplasma inoculum was prepared (Hannan 2000).

Antimicrobial agents

The minimal inhibitory concentration of each antimicrobial agent was determined by applying an agar dilution method with some modifications and in accordance with the recommendations of Hannan (2000) and Kobayashi et al. (1996). Seven antimicrobial agents were included in the testing: tulathromycin (Pfizer, United States), tylosin (Chemifarma, Italia), lincomycin (Pfizer, United States), enrofloxacin (Vetoquinol, Austria), florfenicol (Krka, Slovenia), oxytetracycline (Chemifarma, Italia),) and penicillin G (Actavis Baltics, Lithuania). The absence of a cell wall protects Mycoplasma from the influence of beta lactam antibiotics. Consequently, penicillin G was included in this study as a negative control. Antimicrobial agent concentrations ranged from 0.39 to 100 µg/ml for lincomycin, enrofloxacin, tylosin, florfenicol, oxytetracycline, penicillin G, and for tulathromycin concentrations from 0.0625 to 64 µg/ml were tested. The procedures were performed as described by Hannan (2000). All antimicrobial agents were dissolved in the optimum diluent at 100 µg/ml, except tulathromycin (at 64 µg/ml) that was subjected to a two-fold serial dilution in test tubes containing NHS-20 broth without any selective supplement.

Agar dilution test

One millilitre of each antimicrobial dilution was mixed with 9 ml of NHS-20 agar medium without selective supplement. NHS-20 agar plates without antimicrobial agents served as controls. Prepared agar plates with different antimicrobial concentrations were inoculated with 5 µl 10^5 CFU/ml of the Mycoplasma culture. When the inoculum droplets had been absorbed the plates were incubated at 37 °C under microaerophilic condition. Inoculated plates were observed daily for a period of three to five days, by which time Mycoplasma species formed clearly visible colonies. The MIC for each isolate was defined as the lowest concentration of antibiotics to completely inhibit visible growth on agar. The MIC_{50} and MIC_{90} values were defined as the lowest concentrations capable of inhibiting the growth of 50% and 90% of isolates, respectively (Hannan 2000; Hirose et al. 2003; Gerchman et al. 2009). The tests were performed three times. To ensure the validity of the agar dilution method for determining antimicrobial susceptibility the *M. bovis* reference type strain Donetta was included in the tests.

Interpretation of MIC results

Clinical and Laboratory Standards Institute (CLSI, formerly known as NCCLS) criteria for veterinary pathogenic bacteria in cattle were used to interpret the results (NCCLS 2002). The MIC breakpoints of each antimicrobial agent group, tetracyclines, macrolides, quinolones, phenicols were 4–16, 8–32, 0.25–2 and 2–8 µg/ml, respectively. The MIC breakpoints for penicillin (0.25–8 µg/ml) were based on guidelines for testing the susceptibility of bacteria that affect humans (Ter Laak et al. 1993). The MIC breakpoints of lincosamides (1–2 µg/ml) were evaluated as recommended by Hirose et al. (2003). If the MIC of tetracycline was 4 µg/ml or lower, the isolate was considered susceptible; if the MIC was 16 µg/ml or higher, the isolate was considered resistant.

Statistical analysis

The antibacterial susceptibility of the *M. bovis* field isolates was evaluated using the SPSS 13.0 Windows statistical package (2004). The Wilcoxon two-sample-test and the Kruskal-Wallis test were used to examine the equality among the isolate medians for each antibacterial material. Data with a $P < 0.05$ were considered to be significant.

RESULTS

Mycoplasma was isolated from 41 (45.56%) nasal swabs collected from 90 calves at three months of age. Twenty four (26.67%) of these animals still housed Mycoplasma in their nasal cavity at nine and seventeen months of age. For each isolate, its identity as a member of the *Mollicutes* class was confirmed by PCR. Examination of the biochemical and antigenic characteristics of these isolates revealed that *M. bovis* was isolated from 31 (34.44%) nasal cavities of calves at three months of age. Seventeen (18.88%) of these animals still housed *M. bovis* in their nasal cavity at nine months and five animals (5.55%) still at seventeen months of age. All 53 (100%) isolates were confirmed as *M. bovis* by PCR, i.e., based on the amplification of a product of 734 bp (Figure 1).

The susceptibility to 15 field isolates of *M. bovis* from the same cattle at three, nine and seventeen months of the age, respectively, to seven different antimicrobial agents is shown in Table 1.

All (100%) isolates of *M. bovis* were observed to be susceptible to tylosin. The MIC_{90} for tylosin of field strains of *M. bovis* isolated from cattle aged three and seventeen months was identical (0.39 μg/ml), while for nine months the MIC_{90} was 0.78 μg/ml. One hundred percent of isolates were found to be sensitive to tulathromycin. The MIC_{90} range determined for tulathromycin was 0.50–1.00 μg/ml. According to the breakpoints of the MICs for quinolone, 100% of isolates could be considered sensitive to enrofloxacin, too. The MIC_{90} range determined for enrofloxacin was 0.78–1.56 μg/ml. All (100%) field strains of *M. bovis* isolated from cattle of different ages were found to be sensitive to florfenicol and lincomycin. The range of MICs for florfenicol and lincomycin was 0.78–3.12 μg/ml and 0.39–1.56 μg/ml.

All (100%) field strains of *M. bovis* isolated from cattle of different ages were determined to be resistant to oxytetracycline. The range of MICs for oxytetracycline was 6.25–100 μg/ml and the MIC_{90} was from 50 to 100 μg/ml. This was higher than the breakpoints for the tetracyclines (4–16 μg/ml). All (100%) field strains of *M. bovis* were found to be resistant to penicillin G and no growth inhibition with penicillin G was observed. In all cases the MIC_{90} was >100 μg/ml.

To ensure the credibility of our antimicrobial susceptibility test, procedures were performed initially with the Donetta *M. bovis* reference type strain. MIC_{90} values obtained by agar dilution method of tylosin (0.78 μg/ml) and enrofloxacin (0.39 μg/ml), lincomycin (0.39 μg/ml), oxytetracycline (1.56 μg/ml) and penicillin G (> 100 μg/ml) were comparable to previously reported MIC values for the same antimicrobials against this same reference type strain by Ter Laak et al. (1993) using a serial broth dilution test.

Preliminary examination of the antimicrobial susceptibility of field strains of *M. bovis* did not reveal any significant differences between different age groups of cattle. Upon evaluation of the MIC_{90} data with the SPSS 13.0 statistical package it was found that *M. bovis* isolates from animals aged three and

Table 1. The MICs of antimicrobial agents used against field isolates of *M. bovis* from cattle at 3, 9 and 17 months of age, respectively. The MIC values were estimated for five different isolates from each age group

Antimicrobial agent	At the age of 3 months			At the age of 9 months			At the age of 17 months		
	field strains (n = 5)								
	MIC_{50}	MIC_{90}	range	MIC_{50}	MIC_{90}	range	MIC_{50}	MIC_{90}	range
	(μg/ml)			(μg/ml)			(μg/ml)		
Tulathromycin	0.06	0.50	0.06–0.50	0.50	1.00	0.06–1.00	0.25	0.50	0.25–1.00
Tylosin	0.39	0.39	0.39–1.56	0.39	0.78	0.39–0.78	0.39	0.39	0.39 –0.78
Lincomycin	0.39	0.78	0.39–0.78	0.39	0.78	0.39–1.56	0.39	0.39	0.39
Enrofloxasin	0.39	1.56	0.39–1.56	0.39	0.78	0.39–3.12	0.78	1.56	0.78–3.12
Florfenicol	0.78	3.12	0.78–3.12	1.56	3.12	0.78–3.12	1.56	3.12	0.78–3.12
Oxytetracycline	12.5	50	6.25–50.0	6.25	100	6.25–100	25	50	25.0–100
Penicillin G	> 100	> 100	25.0–100	> 100	> 100	25.0–100	100	100	50.0–100

MIC_{50} of antimicrobial agent to more than 50% of field isolates
MIC_{90} of antimicrobial agent to more than 90% of field isolates

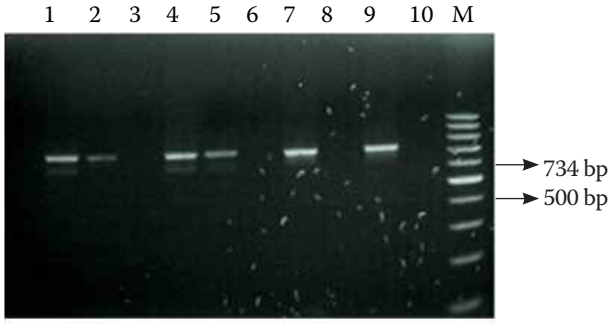

Figure 1. *Mycoplasma bovis* identification by PCR. Lane M = GeneRuler TM 100 bp DNA Ladder (MBI, Fermentas) marker; lanes 1, 2, 4, 5, 7 = *Mycoplasma bovis* isolates; lane 9 = positive control; lane 10 = negative control

nine months were similarly susceptible to tylosin, tulathromycin and enrofloxacin. The strains of *M. bovis* isolated from seventeen month old cattle were found to be the most susceptible to tulathromycin and tylosin. Statistically significant differences in susceptibility of *M. bovis* isolated from cattle of different ages were found for florfenicol compared to tulathromycin ($P < 0.01$), lincomycin ($P < 0.01$) and enrofloxacin ($P < 0.05$). The susceptibility of *M. bovis* isolates from seventeen month old cattle was also significantly different for enrofloxacin compared to tulathromycin ($P < 0.05$), lincomycin ($P < 0.01$) and tylosin ($P < 0.05$). The susceptibility of all *M. bovis* isolates to oxytetracycline and penicillin G significantly differed from the sensitivity to all other antimicrobial agents used in the present study ($P < 0.05$).

DISCUSSION

Mycoplasma bovis may be isolated from the upper respiratory tract, trachea, and lower respiratory tract of cattle without clinical disease or gross lesions, although its presence in the lower respiratory tract may cause subclinical inflammation. In this study the largest percentage (26.67%) of field strains of *M. bovis* was isolated from the upper respiratory tract of cattle at three months of age. Further investigation of the upper respiratory tract of these cattle showed that *M. bovis* is spread among animals which were the subject of this study. These observations are similar to those reported from several other countries where Mycoplasma have been isolated from 3 to 79% of the respiratory tracts of clinically healthy cattle (Ter Laak et al., 1992; Siugzdaite 2002; Thomas et al. 2002; Arcangioli et al. 2008).

In this study, the susceptibility of *M. bovis* field isolates was determined to seven antimicrobial agents *in vitro.* The field strains of *M. bovis* (100%) isolated from the cattle of different ages revealed comparable susceptibility to tylosin, tulathromycin and enrofloxacin.

Varying sensitivity ranges were observed for field strains of *M. bovis* to tylosin and tulathromycin for cattle at different ages. *M. bovis* isolates from three and seventeen month old cattle had lower MIC_{90} values for tylosin (0.39 μg/ml) than the strains isolated from the same cattle aged nine months (MIC_{90} 0.78 μg/ml). Similar results were obtained by valuation of Mycoplasma sensitivity to tulathromycin. However, in both cases the MIC_{90} value of *M. bovis* to tylosin and tulathromycin differs by one dilution and was lower than the breakpoint of the MIC value for macrolides (8–32 μg/ml). This confirms that *M. bovis* strains isolated from all age groups are sensitive to tylosin and tulathromycin. Gerchman et al. (2009) tested *M. bovis* strains isolated from cattle grown in Lithuania and examined respiratory tract sensitivity to antibacterials using a micro-broth dilution procedure for MIC detection. It was shown that, similarly to our investigated strains, 66.7% of *M. bovis* strains were sensitive to tylosin (MIC_{90} was 0.5 μg/ml).

The present study revealed that tylosin, the oldest antibiotic in veterinary medicine, can still be used for the treatment Mycoplasmal infections of cattle affecting the respiratory tract. Tulathromycin, a semisynthetic, tribasic, macrolide antimicrobial, was effective against all *M. bovis* isolates and its MIC_{90} for each isolate was low (0.5–1 μg/ml). The results of our study were comparable with those reported by Kilgore et al. (2005) and the MIC_{90} of *M. bovis* was established as 1 μg/ml. In *in vivo* investigations tulathromycin has also demonstrated a broad spectrum of activity in the therapy of bovine respiratory disease (Kilgore et al. 2005; Skogerboe et al. 2005).

We isolated some field strains of *M. bovis* sensitive to enrofloxacin. The MIC_{90} range of *M. bovis* was fractionally (by one dilution) different between the different age groups, but in all age groups the MIC value was lower than the breakpoints of quinolone (0.25–2 μg/ml). In a previous study (Gerchman et al. 2009), field strains of *M. bovis* isolated from cattle raised in Lithuania were also sensitive to quinolone. In our study, the MIC values of *M. bovis* to enrofloxacin were higher than those reported by Francoz et al. (2005) (0.19 μg/ml to 0.25 μg/ml), Rosenbusch et al.

(2005) (0.25 µg/ml), Hirose et al. (2003) (0.2 µg/ml) and Ayling et al. (2000) (0.5 µg/ml to 1 µg/ml). In Mycoplasma species acquired resistance is usually due to alterations of the target enzymes or the induction of active efflux systems (Reinhardt et al. 2002; Hirose et al. 2004). Thomas et al. (2003) isolated enrofloxacin-resistant strains from bovines. It has also been shown that, in the presence of enrofloxacin, *in vitro* passaging of Mycoplasmas results in the development of fluoroquinolone resistance (Gautier-Bouchardon et al. 2002).

The MIC_{90} value of *M. bovis* field isolates from catlle of different ages to lincomycin either did not differ or differed by only one dilution. It was determined that the MIC values of lincomycin for all *M. bovis* field strains were lower than the breakpoints of lincosamides (1–2 µg/ml) and lincomycin had a good effect against all field isolates. This is in agreement with the results of Hirose et al. (2003) and Ter Laak et al. (1993).

All field isolates of *M. bovis* were sensitive to florfenicol, as the MIC_{90} value (3.12 µg/ml) was lower than the breakpoints of phenicol (2–8 µg/ml). The MIC_{90} of florfenicol for all *M. bovis* field isolates in the present study was lower than the MICs to phenicol reported by Hirose et al. (2003). Florfenicol is exclusively used in veterinary medicine. The compound is analogous to chloramphenicol but does not cause irreversible depression of the bone marrow and can therefore be used in food-producing animals. Florfenicol is a strong inhibitor of microbial protein synthesis through irreversible binding with the 50S subunit of ribosomes, abolishing the activity of peptidyl transferase (Liu et al. 2003). No resistance against florfenicol associated with Mycoplasma infection has been described.

Tetracycline has been reported to be effective against Mycoplasmas (Hirose et al. 2003). Our study did not confirm that this antimicrobial agent is effective against field strains of *M. bovis*. In our study, the MICs for oxytetracycline of all isolates of *M. bovis* were higher than the breakpoint (4–16 µg/ml). In veterinary medicine, increases in tetracycline resistance have been described for *M. bovis*, *M. hyopneumoniae, M. bovirhinis* and *M. alkalescens* (Inamoto et al. 1994; Hirose et al. 2003; Thomas et al. 2003). Oxytetracycline-sensitive species of *M. bovis* isolated from 2005 to 2007 from Lithuanian cattle were reported by Gerchman et al. (2009). It is suggested that oxytetracycline-resistant Mycoplasmas are produced by the administration of oxytetracycline to cattle affected by respiratory disease. The results of the present study with regard to the resistance of *M. bovis* strains against tetracycline are in accordance with several previous studies, such as Hirose et al. (2003), Rosenbusch et al. (2005), Thomas et al. (2003) and Ayling et al. (2000). Resistance to antimicrobials is achieved by bacteria due to mutation. These abilities are typical for the mycoplasma (Kaluina 1998). Resistance is transferred by R-plasmids and mutant strains, especially with sub-therapeutic or sub-inhibitory concentrations. Resistant Mycoplasmas exhibit a reduced uptake of tetracycline into cells, lowering the initial concentration, and have acquired the ability to excrete the drug out of the cell, (Boothe 1998). A possible explanation for the high prevalence of oxytetracycline resistance in the present study may be the frequent use of oxytetracycline for the treatment of respiratory disease infection, gastroenteritis, metritis and mastitis in cattle.

In the present study, all *M. bovis* isolates were resistant to penicillin G. This confirms that the lack of a cell wall makes the Mycoplasma resistant to beta-lactam antimicrobials, and underlines the fact that penicillin G can be used in antimicrobial susceptibility testing only as a negative control.

CONCLUSION

In this study, the antibacterial susceptibility of field isolates of *M. bovis* originating from the upper respiratory tract of cattle of different ages was determined. *In vitro* susceptibility tests showed that field isolates derived from cattle of different ages were sensitive to tylosin, tulathromycin, lincomycin and enrofloxacin. It was also determined that the field strains are resistant to oxytetracycline.

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