

## In vitro antimicrobial inhibition profiles of *Mycoplasma bovis* isolates recovered from various regions of the United States from 2002 to 2003

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**Abstract.** Antimicrobial therapy continues to be important in reducing losses due to pneumonic forms of *Mycoplasma bovis* disease in beef and dairy calves. Although *M. bovis* diseases have been documented as frequent and economically important in the United States, there are no published reports on the antimicrobial activity of approved compounds against US strains. In this study, the authors report on the activity of 9 different antimicrobials against 223 recently recovered isolates of *M. bovis*. These isolates represent accessions from 5 geographic regions of the United States and were grouped by 4 tissues of origin (milk, respiratory, joint, or ear and eye). A broth microdilution test was used to determine minimum inhibitory concentration (MIC) values by reading redox changes detected in broth with alamarBlue (resazurin) indicator. For each antimicrobial, the median, MIC<sub>50</sub>, MIC<sub>90</sub>, mode, and range were calculated, and the values used for comparisons. In the absence of accepted breakpoint values, published MIC cutoff values for animal mycoplasmas as well as Clinical Laboratory Standards Institute interpretive criteria were used as a reference to define in vitro activity. The MIC values from active antimicrobials were found to distribute independently of region of origin of the isolates or of tissue of origin. Enrofloxacin, florfenicol, and spectinomycin were found to be active compounds in vitro. Oxytetracycline and chlortetracycline were active against more than half of the isolates. Very few isolates were inhibited by tilmicosin and none by erythromycin, ampicillin, or ceftiofur. The antimicrobial profiles determined for these US strains were remarkably similar to those reported for European isolates. However, unlike in Europe, there appears to be no diversity of profiles when US isolates are grouped by region or tissue of origin.

**Key words:** Antimicrobial, in vitro testing, *Mycoplasma bovis*.

### Introduction

*Mycoplasma bovis* is recognized as a causative agent of mastitis, pneumonia, polyarthritis, tenosynovitis, middle-ear infections, skin abscesses, and genital-tract infections that include oophoritis, salpingitis, endometritis, abortion, and seminovesiculitis.<sup>9</sup> Historically, the first descriptions of *M. bovis* disease were those of mastitis,<sup>4</sup> and since early years, antimicrobial treatment of these infections was shown to be unsuccessful.<sup>3,6</sup> In contrast, antimicrobial treatment of pneumonia, polyarthritis, and middle-ear infections in young calves has been attempted with some success and may result in significant reduction of economic losses for the farmer.<sup>9</sup> In European studies, calves with respiratory disease involving mycoplasmas responded to high-dose macrolide antimicrobial treatment with recovery from clinical signs.<sup>11</sup> Valnemulin, a pleuromutilin, approved for use in cattle in Europe was

shown to control disease in calves infected under field conditions with *M. bovis*.<sup>16</sup> Calves infected experimentally by the intratracheal route and then medicated with spectinomycin had reduced lung mycoplasma burden but no change in clinical course.<sup>12</sup> In the United States, a common clinical indicator of *M. bovis* involvement in respiratory disease of young calves is the lack of response to treatment with broad-spectrum antimicrobials.<sup>1</sup>

Whereas in vitro activity of antimicrobials has been reported and reviewed for European strains of *M. bovis*,<sup>2,9,13,17,20</sup> there is paucity of information about the effect of antimicrobials on American strains. Because regional differences were noted in some European reports, such differences could also be apparent in North America. This study reports on the in vitro antimicrobial activity of selected antimicrobials approved for use in calves in the United States. Recently recovered strains from various regions representing various clinical conditions were included.

### Materials and methods

**Isolates and grouping assignments.** Isolates of *M. bovis* were obtained from 5 US regions as low passage uncloned cultures from case submissions received largely during 2002

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**Table 1.** Distribution of 233 *Mycoplasma bovis* isolates among geographic regions and tissues of origin.

Tissue*	Midwest	North East	South Central	South East	South West	Unknown region
Respiratory	58	6	18	11	4	1
Joint	12	1	0	3	2	0
Ear and eye	5	0	0	8	1	0
Milk	21	32	0	2	7	0
Unknown	2	18	2	2	4	3

\* Isolates from 3 cases culture positive in joint and lung were grouped as joint cases. One isolate grouped as respiratory was from a case with lung and ear recoveries.

and 2003. With each isolate, a report of location of case (locality or state, or both) and of tissue of origin was secured. Each isolate was assigned to 1 of 5 geographic regions (Midwest, North East, South Central, South East, South West, and a group of unknown origin). Mid West isolates included those from Illinois, Iowa, Kansas, Minnesota, Missouri, Nebraska, and Wisconsin. North East isolates included those from Massachusetts, Maryland, New York, Ohio, Pennsylvania, and Vermont. South Central isolates were those from Oklahoma and Texas. Isolates from Alabama, Georgia, Florida, Kentucky, Tennessee, and Virginia were grouped as South East origin. California and New Mexico isolates were included in the South West group. Each isolate was also assigned a tissue category (respiratory, milk, joint, ear and eye, or unknown tissue). In a few cases, isolations were made from more than one tissue source, and the isolate used for the case was categorized according to the tissue that yielded it. In all instances, a single isolate was included from each individual field case. The geographic and tissue distribution of the 223 isolates available for testing is shown in Table 1.

Test culture batches were prepared for each isolate by growing to log phase in PPLO Broth,<sup>a</sup> (formulated with 20% fetal calf serum, 500 U/ml penicillin G, 200 µg/ml thallos acetate, and 2.5 µg/ml amphotericin B) supplemented with 5% alamarBlue<sup>b</sup> as color redox indicator. Aliquots of each batch were stored frozen at -70°C until needed. Each frozen batch was titrated by preparing 10-fold dilutions in PPLO Broth with 5% alamarBlue, placing a 100-µl volume of each dilution in 2 replicate round-bottom wells (96-well microtiteration plates), and adding 100 µl of PPLO Broth with 5% alamarBlue before sealing the plate. Titration endpoints were read visually for up to 96 hr of incubation at 37°C in a 5% CO<sub>2</sub> atmosphere by color change (blue to red shift).

**Minimum inhibitory concentration test.** Isolates were tested for antimicrobial activity using "Sensititre" plates custom prepared by drying doubling dilutions of antimicrobials on round-bottom 96-well plates.<sup>c</sup> Nine different antimicrobials were tested, including ampicillin, chlortetracycline, ceftiofur, enrofloxacin, erythromycin, florfenicol, oxytetracycline, spectinomycin, and tilmicosin. The cell wall active antimicrobials ampicillin and ceftiofur were not expected to show activity against a mycoplasma, and they were included in this study as negative controls. Test dilutions were 0.06–32 µg/ml for ampicillin, erythromycin, and florfenicol; 0.03–32

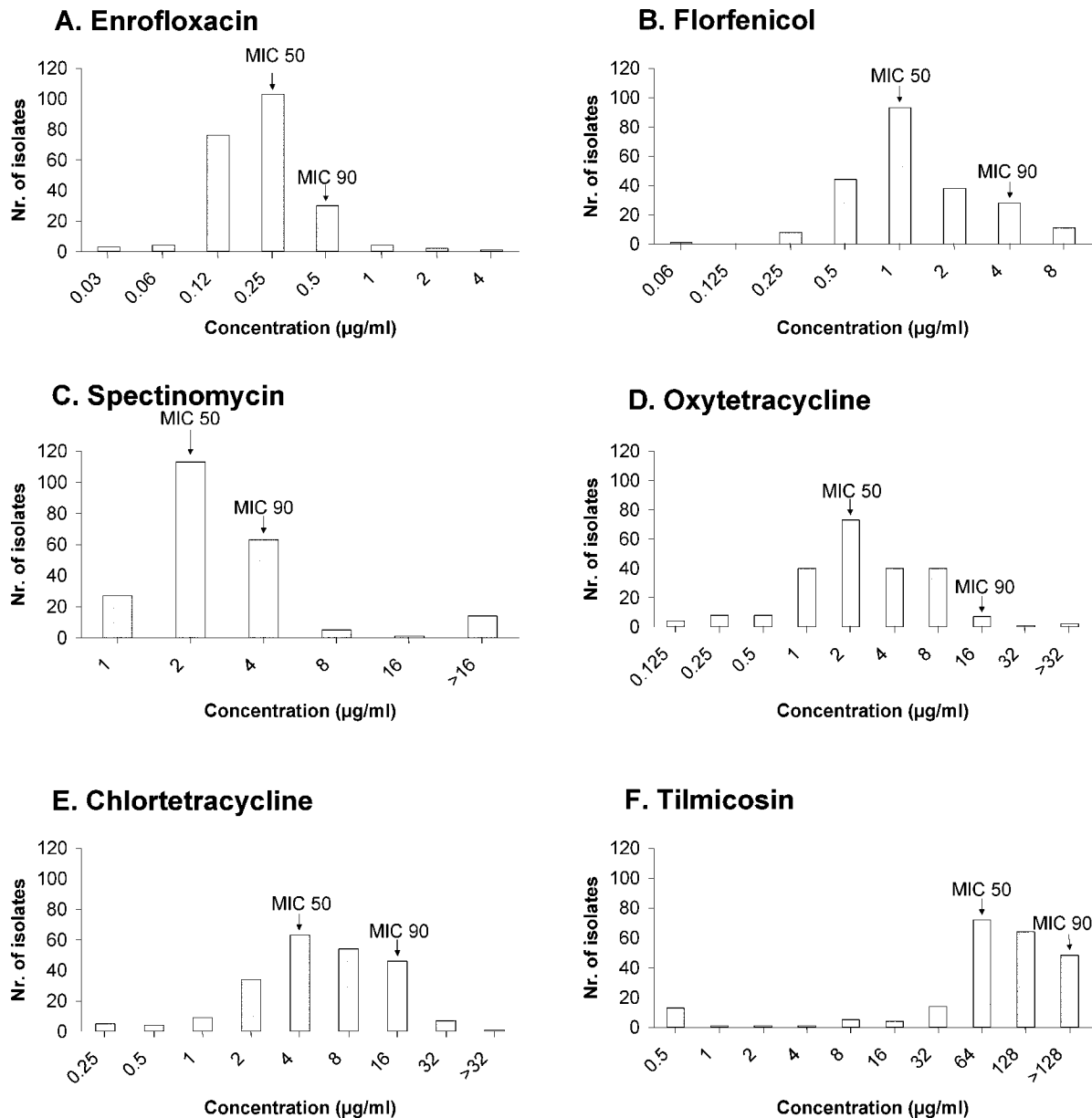
µg/ml for chlortetracycline and oxytetracycline; 0.125–64 µg/ml for ceftiofur; 0.03–4 µg/ml for enrofloxacin; 0.125–16 µg/ml for spectinomycin; and 0.5–128 µg/ml for tilmicosin. A standard dilution of culture was added to each well so that  $2 \times 10^3$  to  $2 \times 10^5$  color-changing units per well were delivered in a final 200-µl volume. Ten-fold dilutions of the challenge culture were also plated into wells without antimicrobial to confirm that the challenge was within the acceptable range of color-changing units per well. After sealing the plates, they were incubated at 37°C in a 5% CO<sub>2</sub> atmosphere for 48 hr. The lowest concentration of antimicrobial suppressing growth, as expressed by blue to red shift, was recorded as the minimum inhibitory concentration (MIC) for that antimicrobial. For each antimicrobial, the range of results, MIC<sub>50</sub>, and MIC<sub>90</sub> were obtained for all strains, as well as for strains grouped by geographic region and tissue site.

**Control and validation of MIC test.** The MIC test using alamarBlue as reported in this study is a novel development for mycoplasma MIC testing. Test conditions were therefore subjected to control and preliminary validation. The Sensititre plates and incubation conditions were confronted weekly to test cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 using Clinical Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards—NCCLS) approved methods. Test control results from 19 weekly tests were within acceptable CLSI limits. In addition, a historical strain of *M. bovis* (ATCC 25025) was used 27 times and generated MIC values that ranged within a 2-fold dilution of the mode value for florfenicol, tilmicosin, enrofloxacin, and spectinomycin. Values ranged within a 4-fold dilution of the mode value for oxytetracycline and chlortetracycline and were all above 32 µg/ml for erythromycin, ampicillin, and ceftiofur. The type culture strain ATCC 25523 of *M. bovis*, also used in this study, gave MIC values of 0.25 µg/ml for enrofloxacin and 0.125 µg/ml for oxytetracycline, matching values obtained by others using a different test.<sup>17</sup> Although this does not constitute validation of the MIC test reported in this study, it provides evidence that the test is comparable with other reported tests.

**Statistical analysis.** Only data for antibiotics that demonstrated activity against at least some isolates were considered for statistical analysis. Isolates of unknown geographic location or unknown tissue were also excluded from statistical analysis. The MIC<sub>50</sub> and MIC<sub>90</sub> values were analyzed separately in  $r \times c$  contingency tables, testing the independence of active antibiotics and grouping of isolates by region or tissue of origin.<sup>10</sup> Contingency table comparisons between tissue and region for each active antibiotic could not be performed because not all tissues had isolates from each region.

## Results

Ampicillin and ceftiofur did not demonstrate activity against any of the 223 isolates in the range of concentrations tested. Consequently, the values obtained for these antibiotics were excluded from statistical analysis. Considering the 7 active antimicrobials, comparison of the antimicrobial activity profiles among the



**Figure 1.** Distribution of isolates by MIC value (in µg/ml) for each antimicrobial. The MIC<sub>50</sub> and MIC<sub>90</sub> values are marked with arrows.

isolates obtained from respiratory tissues, milk, joint fluid, or ear and eye showed remarkable similarity (no significant interaction between antimicrobials and tissue of origin was found at  $P < 0.05$ ). The antimicrobial activity profiles were also similar for various US regions (no significant interaction between antimicrobials and region of origin was found at  $P < 0.05$ ).

Enrofloxacin, florfenicol, and spectinomycin were found to be active compounds in vitro (Fig. 1; Table 2). Single dilutions separated the MIC<sub>50</sub> and MIC<sub>90</sub> values for enrofloxacin and spectinomycin, indicating a tight distribution of MIC values for these antimicrobials. A small population of isolates had high MIC

values for spectinomycin (>16 µg/ml), and these tended to come from milk submissions (6 of 14) but without any specific regional focus. Chlortetracycline and oxytetracycline were found to give a more dispersed distribution of MICs, with chlortetracycline yielding a slightly higher MIC<sub>50</sub> value than oxytetracycline. Tilmicosin MICs clustered into 2 distinct populations, with 8 of 223 isolates yielding MICs of ≤4 µg/ml and the rest clustering with an MIC<sub>50</sub> of 64 µg/ml. There was no discernible pattern to tissue or region distribution among the 8 inhibited isolates. The MICs for erythromycin clustered broadly around the MIC<sub>50</sub> of 32 µg/ml, with no isolates inhibited.

**Table 2.** Minimum inhibitory concentration (MIC) values of 223 isolates of *Mycoplasma bovis*.\*

	Range†	Mode‡	Median and MIC <sub>50</sub> §	MIC <sub>90</sub>
Chlortetracycline	0.25 to >32	4	4	16
Enrofloxacin	0.03 to 4	0.215	0.25	0.5
Erythromycin	4 to >32	32	32	>32
Florfenicol	0.06 to 8	1	1	4
Oxytetracycline	0.125 to >32	2	2	16
Spectinomycin	1 to >16	2	2	4
Tilmicosin	0.5 to >128	64	64	>128
Ampicillin	>32	>32	>32	>32
Ceftiofur	64 to >64	>64	>64	>64

\* All MIC values are expressed as µg/ml.

† Range of MIC results for each antibiotic.

‡ Mode, the most frequent result.

§ Median and MIC<sub>50</sub>, the central result.

|| MIC<sub>90</sub>, 90% of isolates have MIC below the value.

### Discussion

There are no CLSI-approved MIC breakpoint values for mycoplasmas of animals or humans. In addition, there are no standard methods for testing because mycoplasma species vary widely in nutritional and cultural requirements.<sup>5,21</sup> For antimicrobials demonstrating inhibitory activity in this study, interpretive criteria from the CLSI M31-A2 document are shown in Table 3.<sup>8</sup> These interpretive criteria have been developed for the disease and pathogen applications described in the table. They are valid only when the susceptibility data being interpreted were generated using standardized methods specified by the CLSI. Although they are not advocated in this study as also being valid criteria for interpretation of *M. bovis* data, they are presented as examples of when clinical efficacy is expected for these drugs against other pathogens. The authors suggest that when the *M. bovis* susceptibility data are significantly higher than the susceptible criteria established for other pathogens, there is reasonable doubt that the antimicrobial will demonstrate clinical efficacy. In contrast, the authors may be more optimistic

regarding efficacy when the *M. bovis* susceptibility distribution is close to that of other pathogens where efficacy has been demonstrated. This approach requires clinical validation before acceptance as predictive of clinical efficacy. Alternatively, MICs of ≤1 and up to 2 µg/ml have been considered predictive of potential usefulness for treatment. Segregation of mycoplasma isolates into 2 distinct populations has also been taken as evidence of separation between susceptible and resistant populations.<sup>22</sup> Combining all these criteria, ampicillin, ceftiofur, erythromycin, and to a large extent tilmicosin would not be considered active compounds against the 223 US isolates tested in this study.

Several methodologies have been used to report MIC values for *M. bovis*. Among the more recent studies, the preference has clearly been to use a broth microdilution method. Methods of measuring growth endpoint are varied because of the characteristic lack of acid production from sugars by *M. bovis*. They range from a redox indicator,<sup>18</sup> to growth pellet formation,<sup>2</sup> to acid production from pyruvate.<sup>19</sup> Stabilized resazurin (alamarBlue) is used as growth indicator for MIC studies with mycobacteria,<sup>20</sup> as well as gram-negative and gram-positive bacteria.<sup>15</sup> In this study, the authors report on the use of alamarBlue as a sensitive redox indicator with no toxicity for *M. bovis* strains.

There are numerous reports about antimicrobial inhibition of isolates of *M. bovis*. In the United States, 36 *M. bovis* isolates from 1979 to 1996 cases of calf pneumonia in the Midwestern states were not inhibited by ampicillin, spectinomycin, tylosin, tilmicosin, ceftiofur, and erythromycin. They were inhibited by tetracycline and lincomycin. In contrast, 5 of 9 isolates from calf pneumonia and polyarthritis cases of the same origin and time span were not inhibited by the antibiotics tested.<sup>14</sup> In that report, a cutoff value of ≤2.5 µg/ml was used to define susceptibility. In contrast, several recent or benchmark reports were produced, studying European strains. An early study re-

**Table 3.** National Committee for Clinical Laboratory Standards interpretive criteria for selected drugs against disease/pathogen combinations. When interpretive criteria are validated for veterinary applications, they are valid only for the stated organisms.

Drug—disease—pathogen(s) for which interpretive criteria are validated	Susceptible (≤µg/ml)	Intermediate (µg/ml)	Resistant (µg/ml)
Enrofloxacin-bovine respiratory disease- <i>Pasteurella multocida</i> , <i>Mannheimia haemolytica</i> , <i>Haemophilus somnus</i>	0.25	0.5–1	2
Florfenicol-bovine respiratory disease- <i>Pasteurella multocida</i> , <i>Mannheimia haemolytica</i> , <i>Haemophilus somnus</i>	2	4	8
Spectinomycin-bovine respiratory disease- <i>Pasteurella multocida</i> , <i>Mannheimia haemolytica</i> , <i>Haemophilus somnus</i>	32	64	128
Tilmicosin-bovine respiratory disease- <i>Mannheimia haemolytica</i> (also applicable for <i>Pasteurella multocida</i> )	8	16	32
Tetracycline-adapted from human medicine and used as interpretive criteria for the tetracycline group in veterinary medicine	4	8	16

ported on the susceptibility of 16 Dutch isolates collected from 1983 to 1988 from mastitis, pneumonia, and polyarthritis cases.<sup>18</sup> They reported enrofloxacin and spectinomycin MIC<sub>50</sub> and MIC<sub>90</sub> values comparable with those reported in this study. Their oxytetracycline and chlortetracycline MIC<sub>50</sub> values were 8-fold and 4-fold higher, respectively, than those reported in this study. A British study reported on 62 pneumonia isolates from 1996 to 1997.<sup>2</sup> A fluoroquinolone (danofloxacin) gave results comparable with those the authors report for enrofloxacin, and spectinomycin values were comparable to those obtained in this study. As in the Dutch report, oxytetracycline MIC<sub>50</sub> values were much higher than in this study (16-fold). Florfenicol values were 4-fold higher than those reported in this study. They also reported total resistance to tilmicosin, in general concordance with data of this study. A Belgian report on 40 isolates from calf pneumonia cases (collected 1997–2000) reported danofloxacin and enrofloxacin susceptibility of isolates.<sup>19</sup> It should be noted that MIC<sub>90</sub> values of 4 µg/ml for enrofloxacin were much higher than those from previous studies or the current study. Spectinomycin and oxytetracycline MIC<sub>50</sub> values were comparable with those the authors report in this study. Overall, these recent European studies indicate few differences with the current study, primarily the high level of oxytetracycline/chlortetracycline resistance of the Dutch and British reports and the recovery of florfenicol-resistant isolates in the British report. Of note is an Italian report of susceptibility of *M. bovis* isolates to oxytetracycline.<sup>7</sup> This raises the question of existence of heterogeneity of antimicrobial profiles among European isolates of *M. bovis*, which the authors do not observe in this study of US isolates.

The chlortetracycline MIC<sub>50</sub> value reported in this study for *M. bovis* must be interpreted with caution. Underestimation of MIC<sub>50</sub> of mycoplasmas to chlortetracycline has been reported because of the in vitro thermal lability of aqueous solutions of this antimicrobial (Wu CC, Wolff, T: 2002, *In vitro* susceptibility of chlortetracycline vs. *Mycoplasma hyopneumoniae*. Proceedings of the Annual Meeting of the American Association of Swine Veterinarians, pp 149–151, Kansas City, MO). Because *M. bovis* is a fast-growing mycoplasma, the potential underestimation of MIC values should be less than that reported for *M. hyopneumoniae*.

The study of antimicrobial inhibition of 223 recent US isolates of *M. bovis* showed that all isolates were inhibited by enrofloxacin. Florfenicol was active on most isolates, although the MIC<sub>90</sub> value of 4 µg/ml indicated some isolates might be resistant. Spectinomycin showed excellent activity, although a few isolates, primarily from mastitis cases, separated into a

perhaps resistant population (Fig. 1 panel C). These isolates would be considered susceptible by comparison with CLSI criteria. Although the distribution of MIC values for oxytetracycline, and to a lesser degree chlortetracycline, would support the activity of these antimicrobials on many isolates, their use should be studied in vivo to validate the data. Tilmicosin, erythromycin, ampicillin, and ceftiofur could not be recommended for treatment of *M. bovis* infections. As pointed out by others,<sup>2</sup> antibiotics effective against gram-negative pathogens of the bovine respiratory tract should still be considered in mycoplasmal pneumonia cases because combined infections are usually observed.

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### Sources and manufacturers

- a. Remel Inc., Kansas City, KS.
- b. Biosource, Camarillo, CA.
- c. Trek Diagnostic Systems, Cleveland, OH.

### References

1. Adegbeye DS, Halbur PG, Nutsch RG, et al.: 1996, *Mycoplasma bovis*-associated pneumonia and arthritis complicated with pyogranulomatous tenosynovitis in calves. *J Am Vet Med Assoc* 209:647–649.
2. Ayling RD, Baker SE, Peek ML, et al.: 2000, Comparison of in vitro activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against recent field isolates of *Mycoplasma bovis*. *Vet Rec* 146:745–747.
3. Gonzales RN, Wilson DJ: 2003, Mycoplasmal mastitis in dairy herds. *Vet Clin N Am Food Anim Pract* 19:199–221.
4. Hale HH, Hemboldt CF, Plastring WM, Stula EF: 1962, Bovine mastitis caused by *Mycoplasma* species. *Cornell Vet* 52:582–591.
5. Hannan P: 2000, Guidelines and recommendations for anti-

- crobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. *Vet Res* 31:373–395.
6. Jasper DE: 1981, Bovine mycoplasmal mastitis. *Adv Vet Sci Comp Med* 25:121–159.
  7. Mazzolini E, Agnoletti F, Friso S: 1997, Pharmacological sensitivity of the respiratory mycoplasmas of the bovine. *Obiettivi Documenti Veterinari* 1:61–66.
  8. National Committee for Clinical Laboratory Standards (NCCLS): 2002, Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 2nd ed., approved standard NCCLS document M31-A2. NCCLS, Wayne, PA.
  9. Nicholas RA, Ayling RD: 2003, *Mycoplasma bovis*: disease, diagnosis, and control. *Res Vet Sci* 4:105–112.
  10. Ostle B, Malone LC: 1988, *Statistics in research*, 4th ed. Iowa State University Press, Ames, IA.
  11. Pignatelli P: 1978, Respiratory disease and the incidence of pulmonary mycoplasmosis in intensively-reared calves in Italy. *In: Respiratory diseases in cattle*, ed. Martin WB, pp. 284–294. Martinus Nijhoff, The Hague, The Netherlands.
  12. Poumarat F, Le Grand D, Philippe S, et al.: 2001, Efficacy of spectinomycin against *Mycoplasma bovis* induced pneumonia in conventionally reared calves. *Vet Microbiol* 80:23–35.
  13. Poumarat F, Martel JL: 1989, In vitro antibiotic sensitivity of French strains of *Mycoplasma bovis*. *Ann Rech Vet* 20:145–152.
  14. Rosenbusch RF: 1998, Antibiotic susceptibility of *Mycoplasma bovis* strains recovered from mycoplasmal pneumonia and arthritis in feedlot cattle. Beef Research Report, AS Leaflet R1548, Iowa State University, Ames, IA.
  15. Sreenivasan PK, Tambs G, Gittins E, et al.: 2003, A rapid procedure to ascertain the antimicrobial efficacy of oral care formulations. *Oral Microbiol Immunol* 18:371–378.
  16. Stipkovits L, Ripley PH, Varga J, Palfi V: 2001, Use of valnemulin in the control of *Mycoplasma bovis* under field conditions. *Vet Rec* 148:399–402.
  17. Tanner AC, Wu CC: 1992, Adaptation of the Sensititre broth microdilution technique to antimicrobial susceptibility testing of *Mycoplasma gallisepticum*. *Avian Dis* 36:714–717.
  18. ter Laak EA, Noordegraf JH, Verschure MH: 1993, Susceptibilities of *Mycoplasma bovis*, *Mycoplasma dispar*, and *Ureaplasma diversum* strains to antimicrobial agents in vitro. *Antimicrob Agents Chemother* 37:317–321.
  19. Thomas A, Nicolas C, Dizier I, et al.: 2003, Antibiotic susceptibilities of recent isolates of *Mycoplasma bovis* in Belgium. *Vet Rec* 153:428–431.
  20. Vanitha JD, Paramasivan CN: 2004, Evaluation of microplate alamarBlue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. *Diagn Microbiol Infect Dis* 49: 179–182.
  21. Wachowski C, Kirchhoff H: 1986, Sensitivity of *Mycoplasma bovis* field strains to various antibiotics and chemotherapeutic agents. *Berl Muench Tieraertzl Wochenschr* 99:41–44.
  22. Waites KB, Bebear CM, Robertson JA, et al.: 2001, Laboratory diagnosis of mycoplasmal infections. Ed. Nolte FS, pp. 16–20. ASM Press, Washington, DC.