



HAL
open science

In vitro antibiotic susceptibility of Dutch *Mycoplasma synoviae* field isolates originating from joint lesions and the respiratory tract of commercial poultry

Wil J.M. Landman, Dik J. Mevius, Kees T. Veldman, Anneke Feberwee

► **To cite this version:**

Wil J.M. Landman, Dik J. Mevius, Kees T. Veldman, Anneke Feberwee. In vitro antibiotic susceptibility of Dutch *Mycoplasma synoviae* field isolates originating from joint lesions and the respiratory tract of commercial poultry. *Avian Pathology*, 2008, 37 (04), pp.415-420. <10.1080/03079450802216637>. <hal-00540122>

HAL Id: hal-00540122

<https://hal.science/hal-00540122v1>

Submitted on 26 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



***In vitro* antibiotic susceptibility of Dutch *Mycoplasma synoviae* field isolates originating from joint lesions and the respiratory tract of commercial poultry**

Journal:	<i>Avian Pathology</i>
Manuscript ID:	CAVP-2008-0028.R1
Manuscript Type:	Short Communication
Date Submitted by the Author:	15-Apr-2008
Complete List of Authors:	Landman, Wil; Animal Health Service (GD) Mevius, Dik; Institute for Animal Disease Control (CDIC-Lelystad) Veldman, Kees; Institute for Animal Disease Control (CDIC-Lelystad) Feberwee, Anneke; Animal Health Service (GD)
Keywords:	Mycoplasma synoviae, MIC, antibiotic, in vitro

SCHOLARONE™
Manuscripts

Cavp-2008-0028.R1

In vitro antibiotic susceptibility of Dutch *Mycoplasma synoviae* field isolates originating from joint lesions and the respiratory tract of commercial poultry

W.J.M. Landman¹, D.J. Mevius², K.T. Veldman² & A. Feberwee¹

¹*Animal Health Service (GD), P.O. Box 9, 7400 AA Deventer, the Netherlands,* ²*Central Institute for Animal Disease Control (CDIC-Lelystad), Lelystad, the Netherlands*

Running title: antibiotic susceptibility of *M. synoviae*

*To whom correspondence should be addressed. Tel: +31 5 70660386. Fax: +31 5 70660354.

E-mail: w.landman@gddeventer.com

Received: 14 February 2008

Cavp-2008-0028.R1

In vitro antibiotic susceptibility of Dutch *Mycoplasma synoviae* field isolates originating from joint lesions and the respiratory tract of commercial poultry

W.J.M. Landman¹, D.J. Mevius², K.T. Veldman² & A. Feberwee¹

Abstract

The *in vitro* susceptibility of 17 Dutch *Mycoplasma synoviae* isolates from commercial poultry to enrofloxacin, difloxacin, doxycycline, tylosin and tilmicosin was examined. Three isolates originated from joint lesions and 14 were from the respiratory tract. The type strain *M. synoviae* WVU 1853 was included as a control strain. Antibiotic susceptibility was tested quantitatively using the broth microdilution test. Based on initial and final MIC values, all tested isolates were susceptible to doxycycline, tylosin and tilmicosin. Two isolates from the respiratory tract were resistant to enrofloxacin and showed intermediate resistance to difloxacin.

Introduction

M. synoviae infections in poultry occur worldwide and most prominently in commercial layer chickens, turkeys and broilers although their incidence in breeding stock has decreased considerably due to eradication programmes. *M. synoviae* can induce infectious synovitis in chickens and turkeys, and subsequently cause considerable economic loss to the poultry industry. This mycoplasma species has also been associated with respiratory disease and condemnations due to airsacculitis (Kleven *et al.*, 1972; Mohammed *et al.*, 1987; Stipkovits and Kempf, 1996; Kleven, 2003) although subclinical infections seem predominant.

Economic losses caused by *M. synoviae* with an affinity for the respiratory tract or for joints can be reduced by antibiotic treatment at the beginning of infection. However, the antibiotic susceptibility profiles of the mycoplasma strains involved should be determined if treatment efficacy is to be maximized. During the past decades a number of studies describing the *in vitro* antibiotic susceptibility of *M. synoviae* strains have been published (Jordan and Knight, 1984; Bradbury *et al.*, 1994; Jordan and Horrocks, 1996; Wang *et al.*, 2001; Cerda *et al.*, 2002). Nevertheless, recent reports on minimum inhibitory concentrations (MICs) of *M. synoviae* isolates from European countries are scarce. In a more recent study by Dufour-Gesbert *et al.* (2006), *M. synoviae* strains with intermediate susceptibility for enrofloxacin were described; however, the study only involved *M. synoviae* isolates originating from the respiratory tract without pathological lesions.

The emergence of *M. synoviae* infectious synovitis in both chickens and turkeys in the Netherlands (Landman and Feberwee, 2001; Van Beek *et al.*, 2002) with associated economic losses prompted the need for efficacious antibiotic treatments. In order to provide the optimum choice for antibiotic therapy knowledge of the susceptibility profiles of available compounds was needed. The present study was carried out due to the scarcity of recent data

on antibiotic susceptibility of *M. synoviae* isolates from European countries and the complete lack of data on amyloidogenic *M. synoviae* strains. Thus the antibiotic susceptibility of Dutch *M. synoviae* field isolates originating from both joint lesions and the respiratory tract of commercial poultry was determined against difloxacin, enrofloxacin, doxyxycine, tylosin and tilmicosin.

Materials and Methods

***M. synoviae* strains, culture and identification.** *M. synoviae* type strain WVU 1853^T (ATCC 25204) was used for control purposes and 17 *M. synoviae* field isolates gathered during the period 2000-2004 were included. The isolates were obtained from the trachea (n = 14) and joints (n = 3) of commercial poultry using sterile cotton swabs, which were dipped beforehand in Mycoplasma Experience (ME) broth (Mycoplasma Experience, Reigate Surrey, UK). The swabs were plated on ME agar which was incubated under aerobic conditions at 37°C and examined for colonies every 2 days up to 28 days. If colony growth occurred, one separate colony was plated out on a fresh ME agar and approximately 2 x 0.5 cm² of agar with positive clones was transferred to 10 ml ME broth and incubated at 37°C as described above. Subsequently, 1 ml ME broth was used for identification of *M. synoviae* by real time PCR. The real time *M. synoviae* PCR was performed following the *M. gallisepticum* PCR protocol described by Mekkes and Feberwee (2005). As forward primer 5' GAG AAG CAA AAT AGT GAT ATC A and as reverse primer 5' CAG TCG TCT CCG AAG TTA ACA (Genbank accession no. X52083) were used. These primers amplify a 211 bp sequence from the 16S ribosomal RNA gene of *M. synoviae*. Finally, isolates were freeze dried and stored at 2-8 °C as seed culture pending analysis.

Preparation of *M. synoviae* inocula. Each freeze dried culture was suspended in 1 ml distilled water and transferred to 25 ml ME broth, which was then incubated at 37°C. When colour change from red to yellow occurred, the ME broth was divided in portions of 1 ml each and stored at -70°C. Throughout the experiment ME broth was used without bacterial inhibitors.

In order to determine the concentration of the *M. synoviae* cultures, frozen *M. synoviae* stock was thawed and 10-fold dilutions were prepared in ME broth. Tubes were incubated for 14 days at 37°C and observed daily for colour change. The final *M. synoviae* concentration was determined as the highest dilution where colour change was observed and expressed as colour changing units per ml (CCU/ml) according to the Spearman-Kärber method for quantal data (Finney, 1952). Inocula with a concentration of 10^3 - 10^5 CCU/ml were used for the broth microdilution test.

Tested antibiotics and quality control of ME medium. The antibiotics tested were enrofloxacin (Lot no. 258623a. Bayer B.V., Mijdrecht, the Netherlands), difloxacin HCL (Lot no. 62-114-GS Profarmaco Lot no. 0103. Solvay Duphar B.V., Weesp, the Netherlands), doxycycline (Lot no. 022H0119. Sigma Aldrich.), tylosin tartrate (Lot no. 085H1016. Sigma-Aldrich, Zwijndrecht, the Netherlands) and tilmicosin (Lot no. 072KE5. Elanco Animal Health, Nieuwegein, the Netherlands).

In order to prepare the stock solutions of enrofloxacin 0.1 M NaOH was used, while for the stock solution of tilmicosin 0.1 M HCl was necessary. Therefore, the influence of these solvents on the pH of the ME medium as well as in the same dilutions as those used later on in the broth micro-dilution test was assessed. The induced pH variation did not exceed 0.1, making corrections unnecessary.

Also, beforehand a potential effect of the media and growth conditions used was studied by determining MICs for two reference strains (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213) in ME broth. The accepted quality control (QC) ranges of MICs for the tested antibiotics are published by the National Committee for Clinical Laboratory Standards, now Clinical and Laboratory Standards Institute (NCCLS, 2002).

Preparation of microtitre plates and broth microdilution test. Broth microdilution tests were performed in two different studies. First a pilot study, which included three joint isolates, was performed once. In a second study, susceptibility tests were performed in duplicate and included 14 isolates originating from the respiratory tract. In both studies *M. synoviae* ATCC 25204 (WVU 1853) was also added as a control strain and to determine the reproducibility of the test. Susceptibility tests were performed following the guidelines described by Hannan (2000). In short, the wells of each microtitre plate (eight rows of 12 wells) (Greiner, Omnilabo B.V., Breda, Netherlands) were filled with 50 µl ME broth. For each isolate-antibiotic combination two rows were used. In the first row a two-fold dilution of the antibiotic was prepared, whereas in the second row a two-fold dilution of the solvent was prepared. This second row was used as a growth control row prepared to exclude potential antimicrobial effects of the solvents on the growth of *M. synoviae*. Plates were sealed after preparation and stored at -70°C pending the test, which was carried out within 4 weeks. Immediately before inoculation sufficient volumes of *M. synoviae* culture were thawed and diluted to a concentration of 10⁴ CCU/ml. As inoculum 50 µl per well of each *M. synoviae* culture was used. Immediately after inoculation the concentration of viable mycoplasmas was determined as described above.

The final test concentrations of all antibiotics were 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03 and 0.015 µg/ml (excluding the rows used for control for *M. synoviae* growth).

Plates were sealed and incubated aerobically at 37°C. They were examined after 1, 2, 3, 4, 7, 8, 9, 10, 11 and 14 days of incubation. The initial MIC was defined as the lowest antibiotic concentration to show no change in colour when the colour of the mycoplasma growth control changed (Hannan, 2000). The final MIC was read when no further colour change was observed (Bradbury *et al.*, 1994; Hannan, 2000).

As the interpretation of the MIC depends on the reproducibility of the test, this was assessed using *M. synoviae* WVU 1853. The MIC breakpoints of avian mycoplasmas for enrofloxacin, doxycycline and tylosin were based on those determined by Hannan (1997, 2000). As the MIC breakpoints for avian mycoplasma of difloxacin and tilmicosin have not been given in literature, those derived from the NCCLS were used. They are based on the MIC for other pathogens in other animal species (Table 1).

Results and Discussion

Reports on MIC profiles of *M. synoviae* field isolates are scarce both in Europe and elsewhere. During the last decade only one recent study has focussed on the assessment of MICs of European *M. synoviae* field isolates (Dufour-Gesbert *et al.*, 2006).

The antibiotics tested in the present study were chosen based on their availability in the Netherlands for commercial meat turkeys because outbreaks of *M. synoviae*-associated joint pathology and associated economic losses were increasing in such birds from 2000 to 2004. In contrast to Dufour-Gesbert *et al.* (2006) we also examined the susceptibility of *M. synoviae* for difloxacin and tilmicosin. Another difference was that we included *M. synoviae* isolates from joints in addition to isolates from the respiratory tract. A third difference was that our study included *M. synoviae* isolates from different types of poultry (i.e. layers, meat type breeders and meat-turkeys).

Results of the initial MICs and results read at day 7 and 14 of the *M. synoviae* WVU 1853 (Table 2) showed a variation of one to two dilutions for all antibiotics except for tilmicosin at day 7 and for doxycycline at day 14 where a difference of more than two dilutions was found 0.06-0.5 and <0.015-0.25, respectively. The occurrence of MIC profiles differing in more than two dilutions using mycoplasma inocula ranging from 10^3 to 10^5 CCU/ml was described previously by Hannan (2000) for *M. hyopneumoniae* and tylosin. Differences in mycoplasma growth were put forward as a possible explanation. In our reproducibility study only in two cases did MIC profiles differ in more than two dilutions, so we concluded that the overall reproducibility of the microdilution test was good.

The initial and final MICs of all isolates were outlined in Table 3. The MIC results of the control *E. coli* and *S. aureus* ATCC strains were within the accepted QC-range of the NCCLS, indicating that the growth conditions had no effect on the potency of the antibiotics. After thawing the plates no change in broth colour due to possible pH changes was observed. The change in colour noted in the control rows was therefore attributed to mycoplasma growth. The results showed that all strains were susceptible to tylosin, doxycycline and tilmicosin. Two isolates from the respiratory tract were resistant to enrofloxacin; for these isolates initial MICs varying from 2 to 4 $\mu\text{g/ml}$ were found. Dufour-Gesbert *et al.* (2006) recently found MICs of enrofloxacin varying from 0.5 to 1 $\mu\text{g/ml}$ for French *M. synoviae* field isolates and concluded that there was intermediate susceptibility to this antibiotic. Moreover, these strains also showed intermediate susceptibility to difloxacin.

In general the reproducibility of the duplicate MIC values of the respiratory strains (both initial and final MICs) was good with 53/70 (76%) of initial MICs and 69/70 (99%) of final MICs showing no more than one dilution step difference. As the test reproducibility of enrofloxacin, difloxacin, tylosin, doxycycline and tilmicosin was acceptable, i.e. within the range of one to two dilutions (Table 3), explanation for this variation could be, as already

mentioned, a difference in growth rate of the same strain in each test despite a standardized inoculum of 10^3 - 10^5 CCU/ml (Hannan, 2000). Although the occurrence of a mixture of strains with different susceptibility could theoretically have occurred this is not considered likely because all isolates were cloned prior to testing.

Several times the final MICs for enrofloxacin and difloxacin differed from that of the initial MICs (Table 3), including changes of profile from sensitive to intermediate (difloxacin) or resistant (enrofloxacin and difloxacin). There was little difference between initial and final MICs of most isolates, which is consistent with a mycoplasmacidal antibiotic. However, in two cases (isolate 2000.05 and 2003.08) there was a large difference between the initial and final MIC for enrofloxacin changing from sensitive to resistant. The difference between the initial and final MIC could not be attributed to slow mycoplasma growth as the initial MIC was read at day 2 and day 3, indicating rapid growth of these isolates. An alternative explanation is that resistance is developed *in situ* as described earlier by Hannan *et al.* (1989), who showed a similar effect for *M. hyopneumoniae* versus gentamicin.

The last representative study which reported full susceptibility to the newer quinolones (enrofloxacin and danofloxacin) for all *M. synoviae* isolates analyzed dated from 1997 by Hannan *et al.* However, five years later Stanley *et al.* (2001) reported the occurrence of reduced effectivity of enrofloxacin for the treatment of an experimentally induced *M. synoviae* infection with a field isolate. The results of our study and the paper by Dufour-Gesbert *et al.* (2006) suggest an increase in the occurrence of quinolone resistant *M. synoviae* field isolates.

Descriptions of mechanisms involved in quinolone resistance in veterinary medicine are scarce (Reinhardt *et al.*, 2002; Le Carrou *et al.*, 2006). Reinhardt *et al.* (2002) showed the presence of alterations on the four target genes encoding DNA gyrase and topoisomerase IV in *M. gallisepticum* enrofloxacin mutants. The studies of Le Carrou *et al.* (2006) showed a

significant increase of the resistance level of *M. synoviae* isolates under experimental conditions after a therapeutic dose of enrofloxacin. The development of resistance was associated with a *parC* gene substitution. This was also found for quinolone-resistant mutants of *M. gallisepticum* (Reinhardt *et al.*, 2002).

In the Netherlands, clinically relevant *M. synoviae* isolates were not detected for decades until recently when amyloid-inducing joint isolates causing considerable economic losses were observed (Landman and Feberwee, 2001; Van Beek *et al.*, 2002). This prompted a substantial increase in antibiotic treatments as such treatment may reduce the economic impact of the disease if given at the beginning of infection. This increased use of antibiotics may at least explain in part the occurrence of antibiotic resistant *M. synoviae* isolates. It is well known that there is a close relationship between antibiotic misuse and bacterial resistance. The use of sub-inhibitory concentrations but also the long-term use of therapeutic doses can contribute to the induction of antibiotic resistance. In an experimental *M. synoviae* infection of hens Le Carrou *et al.* (2006) demonstrated a significant increase in the resistance to enrofloxacin of five re-isolated *M. synoviae* clones after a second therapeutic treatment with this drug. Although *in vitro* resistance of *M. synoviae* strains to tylosin and oxytetracycline has been reported (Hannan *et al.*, 1997; Cerda *et al.*, 2002; Gautier-Bouchardon *et al.* 2002), all strains tested in our study were still susceptible to tylosin and doxycycline.

Based on the MIC breakpoints for *M. synoviae* therapeutic failures are more likely to occur with quinolones than with oxytetracycline or tylosin. The MIC values found in this study further emphasize that *M. synoviae* infections should not be treated with quinolones. The application of quinolones may not only contribute to the selection of *M. synoviae* resistant strains but may also affect other avian pathogens including zoonotic bacteria such as *Campylobacte* spp. Periodic assessment of the MICs of *M. synoviae* field isolates and other

bacteria in general, may contribute to a more rational and efficient use of antibiotics in the treatment of *M. synoviae* affected flocks.

References

- Bradbury, J.M., Yavari, C.A. & Giles, C.G. (1994). *In vitro* evaluation of various antimicrobials against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by micro-broth method, and comparison with a commercially prepared test system. *Avian Pathology*, 23, 105-115.
- Cerda, R.O., Giacoboni, G.I., Xavier, J.A., Sansalone, P.L. & Landoni, M.F. (2002). *In vitro* antibiotic susceptibility of field isolates of *Mycoplasma synoviae* in Argentina. *Avian Diseases*, 46, 215-218.
- Dufour-Gesbert, F., Dheilly, A., Marois, C. & Kempf, I. (2006). Epidemiological study on *Mycoplasma synoviae* infection in layers. *Veterinary Microbiology*, 114, 148-154.
- Finney, D. J. (1952). *Statistical Method in Biological Assay* 1st edn. (pp. 524-530). Charles Griffin and Co. Ltd. London.
- Gautier-Bouchardon, AV., Reinhardt, A.K., Kobisch, M. & Kempf, I. (2002). *In vitro* development of resistance to enrofloxacin, erythromycin, tylosin, tiamulin and oxytetracycline in *Mycoplasma gallisepticum*, *Mycoplasma iowae* and *Mycoplasma synoviae*. *Veterinary Microbiology*, 88, 47-58.
- Hannan, P.C., O'Hanlon, P.J., & Rogers, N.H. (1989). *In vitro* evaluation of various quinolone antibacterial agents against veterinary mycoplasmas and porcine respiratory bacterial pathogens. *Research in Veterinary Science*, 46, 202-211.

- Hannan, P.C., Windsor, G.D., de Jong, A., Schmeer, N. & Stegeman, M. (1997). Comparative susceptibilities of various animal pathogenic mycoplasmas to fluoroquinolones. *Antimicrobial Agents and Chemotherapy*, *41*, 2037-2040.
- Hannan, P.C. (2000). Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. *Veterinary Research*, *31*, 373-395.
- Jordan, F.T.W. & Horrocks, B.K. (1996). The minimum inhibitory concentration of tilmicosin and tylosin for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and a comparison of their efficacy in control of *Mycoplasma gallisepticum* infection in broiler chicks. *Avian Diseases*, *40*, 326-334.
- Jordan, F.T.W. & Knight, D.L. (1984). The minimum inhibitory concentration of kitasamysine, tylosin and tiamulin for *Mycoplasma gallisepticum* and their protective effect on infected chicks. *Avian Pathology*, *13*, 151-162.
- Kempf, I., Ollivier, C., L'Hopitalier, R., Guittet, M. & Bennejean. (1989). Concentrations minimales inhibitrices de 13 antibiotiques vis-à-vis de 21 souches de mycoplasmes des volailles. *Le Point Vétérinaire*, *20*, 935-940.
- Kleven, S., King, D. & Anderson, D. (1972). Airsacculitis in broilers from *Mycoplasma synoviae*: effect on air-sac lesions of vaccinating with infectious bronchitis and Newcastle virus. *Avian Diseases*, *16*, 915-924.
- Kleven, S.H. (2003). *Mycoplasma synoviae* infection. In: Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald, & D.E. Swayne (Eds.), *Diseases of Poultry* 11th edn (pp. 756-766). Ames: Iowa State Press.
- Landman, W.J.M. & Feberwee, A. (2001). A field-study on the association between amyloid arthropathy and *Mycoplasma synoviae* infection, and experimental reproduction of the condition in brown layers. *Avian Pathology*, *30*, 629-639.

- Le Carrou, J., Reinhardt, A.K., Kempf, I. & Gautier-Bouchardon, A.V. (2006). Persistence of *Mycoplasma synoviae* in hens after two enrofloxacin treatments and detection of mutations in the *parC* gene. *Veterinary Research*, 37, 145-54.
- Mekkes, D.R. & Feberwee, A. (2005). Real-Time PCR for the qualitative and quantitative detection of *Mycoplasma gallisepticum*. *Avian Pathology*, 34, 348-354.
- Mohammed, H.O., Carpenter, T.E. & Yamamoto, Y. (1987). Economic impact of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial layer flocks. *Avian Diseases*, 31, 477-482.
- National Committee for Clinical Laboratory Standards (2002). Performance standards of antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A2 .Second Edition. NCCLS (now Clinical and Laboratory Standards Institute), Wayne, PA, USA.
- Reinhardt, A.K., Béb ar, C.M., Kobisch, M., Kempf, I. & Gautier-Bouchardon, A.V. (2002). Characterization of mutations in DNA gyrase and topoisomerase IV involved in quinolone resistance of *Mycoplasma gallisepticum* mutants obtained *in vitro*. *Antimicrobial Agents and Chemotherapy*, 46, 590-593.
- Stanley, W.A., Hofacre, C.L., Speksnijder, G., Kleven, S.H. & Aggrey, S.E. (2001). Monitoring *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in breeder chickens after treatment with enrofloxacin. *Avian Diseases*, 45, 534-539.
- Stipkovits, L. & Kempf, I. (1996). Mycoplasmosis in poultry: an overview. *Revue Scientifique et Technique* (International Office of Epizootics), 15, 1495-1525.
- Van Beek, P.G.M., Feberwee, A., Fabri, T.H.F. & Heijmans, M.J.H.M. (2002). Longitudinal field-study on the presence of *Mycoplasma synoviae* in meat-turkey flocks with arthritis. In M.H. Hafez (Ed.), *Proceedings of the IVth International Symposium on Turkey Diseases* (pp. 177-178), Berlin, Germany.

Wang, C., Ewing, M. & A'arabi, S.Y. (2001). *In vitro* susceptibility of avian mycoplasmas to enrofloxacin, sarafloxacin, tylosin and oxytetracycline. *Avian Diseases*, 45, 456-460.

For Peer Review Only

Table 1. MIC breakpoints of the tested antibiotics

Antibiotic	Susceptible µg/ml	Intermediate µg/ml	Resistant µg/ml
Enrofloxacin ^a	≤0.5	1	≥2
Oxytetracycline ^{a,b}	≤4	8	≥16
Tylosin ^a	≤1	2	≥4
Difloxacin ^c	≤0.5	1-2	≥4
Tilmicosin ^d	≤8	16	≥32

^aBreakpoints according to Kempf *et al.* (1989) and Hannan (1997).

^bBreakpoints of doxycycline were expected to be comparable to that of oxytetracycline according to Kempf *et al.* (1989) and Hannan (1997).

^cBreakpoints according to NCCLS standard M31-A2, based on *Enterobacteriaceae* and *Staphylococcus* spp. in dogs (skin).

^dBreakpoints according to NCCLS standard M31-A2, based on *Mannheimia haemolytica* in cattle (respiratory disease) and *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* in swine (respiratory disease).

Table 2. Reproducibility of the test based on the initial MIC and antibiotic susceptibility profiles at day 7 and day 14 ($\mu\text{g/ml}$) of *M. synoviae*

WVU 1853

Strain	Enrofloxacin			Difloxacin			Doxycycline			Tylosin			Tilmicosin			
	Initial MIC	D7	D14	Initial MIC	D7	D14	Initial MIC	D7	D14	Initial MIC	D7	D14	Initial MIC	D7	D14	
	Joint ^a															
WVU 1853	0.25	0.5	1	0.5	1	1	≤ 0.015	0.06	0.25	≤ 0.015	≤ 0.015	≤ 0.015	0.06	0.5	0.5	
	Respiratory ^b															
WVU 1853	0.5	0.5	1	0.25	0.5	1	≤ 0.015	≤ 0.015	≤ 0.015	≤ 0.015	≤ 0.015	≤ 0.015	≤ 0.015	0.125	0.25	
WVU 1853	0.25	0.5	0.5	0.25	1	1	≤ 0.015	≤ 0.015	0.06	≤ 0.015	≤ 0.015	≤ 0.015	≤ 0.015	0.06	0.125	
WVU 1853	0.25	0.5	1	0.125	1	1	≤ 0.015	≤ 0.015	0.03	≤ 0.015	≤ 0.015	≤ 0.015	≤ 0.015	0.125	0.125	
WVU 1853	0.25	0.5	1	0.5	1	1	≤ 0.015	≤ 0.015	0.06	≤ 0.015	≤ 0.015	≤ 0.015	0.06	0.125	0.125	
WVU 1853	0.5	0.5	0.5	0.5	1	1	≤ 0.015	≤ 0.015	0.03	≤ 0.015	≤ 0.015	≤ 0.015	0.06	0.125	0.125	
WVU 1853	0.25	0.5	0.5	0.5	1	1	≤ 0.015	0.03	0.06	≤ 0.015	0.03	0.03	≤ 0.015	0.125	0.25	
WVU 1853	0.125	0.5	0.5	0.25	0.5	0.5	≤ 0.015	0.03	0.125	≤ 0.015	≤ 0.015	≤ 0.015	≤ 0.015	0.25	0.25	
WVU 1853	0.25	0.5	0.5	0.125	1	1	≤ 0.015	0.03	0.06	≤ 0.015	≤ 0.015	0.03	≤ 0.015	0.125	0.25	
WVU 1853	0.25	0.25	0.5	0.5	0.5	0.5	≤ 0.015	0.03	0.06	≤ 0.015	≤ 0.015	≤ 0.015	0.06	0.25	0.25	
WVU 1853	0.25	0.5	0.5	0.5	1	1	≤ 0.015	0.03	0.125	≤ 0.015	0.03	0.03	0.06	0.25	0.25	

^aWVU 1853 control strain pilot MIC study.^bWVU 1853 control strains main MIC study.

Table 3. Initial and final MIC ($\mu\text{g/ml}$) values and corresponding antibiotic sensitivity profiles (S = susceptible, I = intermediate and R = resistant) of all *M. synoviae* isolates tested

Isolate	Source/year	Enrofloxacin		Difloxacin		Doxycyclin		Tylosin		Tilmicosin	
		Initial (Profile)	Final ^a	Initial (Profile)	Final ^a	Initial (Profile)	Final	Initial (Profile)	Final	Initial (Profile)	Final
Joint ^b											
2000.05	Layer, 2000	0.25 (S)	16 (R)	0.5 (S)	4 (R)	≤ 0.015 (S)	0.25	≤ 0.015 (S)	≤ 0.015	0.06 (S)	0.25
2001.14	Turkey, 2001	0.25 (S)	1 (I)	0.5 (S)	1 (I)	≤ 0.015 (S)	0.125	≤ 0.015 (S)	≤ 0.015	0.03 (S)	0.25
2002.02	Turkey, 2002	0.25 (S)	0.25	0.25 (S)	0.5	≤ 0.015 (S)	0.125	≤ 0.015 (S)	≤ 0.015	0.03 (S)	0.125
Respiratory ^c											
2001.38	Layer, 2001	0.25/0.125 ^d (S)	0.25	0.25 (S)	0.25/0.5	≤ 0.015 (S)	0.06/0.125	$\leq 0.015/0.06$ (S)	0.125/0.25	0.03/ ≤ 0.015 (S)	0.125
2001.39	Layer, 2001	0.125 (S)	0.25	0.25 (S)	0.25	≤ 0.015 (S)	0.06/0.125	$\leq 0.015/0.06$ (S)	0.5/0.25	$\leq 0.015/0.03$ (S)	0.125/0.06
2001.40	Breeder, 2001	0.125/0.03 (S)	0.25/0.125	0.25/0.06 (S)	0.5	≤ 0.015 (S)	0.06/0.03	≤ 0.015 (S)	0.03/ ≤ 0.015	$\leq 0.015/0.03$ (S)	0.125/0.25
2001.41	Breeder 2001	0.5/0.125 (S)	1/0.5 (I/S)	0.5/0.06 (S)	1 (I)	≤ 0.015 (S)	0.125/0.03	≤ 0.015 (S)	≤ 0.015	0.06/0.03 (S)	0.25
2001.43	Layer, 2001	0.125/0.25 (S)	0.5	0.5/ ≤ 0.015 (S)	1/0.5 (I/S)	≤ 0.015 (S)	0.06	≤ 0.015 (S)	$\leq 0.015/0.03$	0.125/0.03 (S)	0.25/0.125
2002.01	Layer, 2002	0.25 (S)	0.5/1 (S/I)	0.5 (S)	1 (I)	0.03/ ≤ 0.015 (S)	0.125/0.06	≤ 0.015 (S)	≤ 0.015	0.06 (S)	0.125/0.25
2002.05	Layer, 2002	0.06/ ≤ 0.015 (S)	0.125	0.125 (S)	0.5	≤ 0.015 (S)	0.03/0.06	≤ 0.015 (S)	0.03	$\leq 0.015/0.06$ (S)	0.125/0.25
2002.11	Layer, 2002	0.25/0.125 (S)	0.5/0.25	0.5/0.25(S)	1 (I)	≤ 0.015 (S)	0.03/0.125	≤ 0.015 (S)	0.03	$\leq 0.015/0.06$ (S)	0.125
2002.15	Breeder, 2002	0.25 (S)	0.5	0.5/0.25 (S)	0.5	≤ 0.015 (S)	0.03	≤ 0.015 (S)	≤ 0.015	$\leq 0.015/0.06$ (S)	0.125
2002.17	Layer, 2002	0.125/0.25 (S)	0.5	≤ 0.015 /0.5 (S)	1 (I)	≤ 0.015 (S)	$\leq 0.015/0.03$	≤ 0.015 (S)	≤ 0.015	$\leq 0.015/0.06$ (S)	0.25
2003.08	Breeder, 2003	0.5 (S)	8 (R)	0.25/0.5 (S)	2 (I)	≤ 0.015 (S)	0.06	≤ 0.015 (S)	0.06	0.06/0.03 (S)	0.5
2003.09	Breeder, 2003	4/2 (R)	8	0.5/2 (S/I)	4/2 (R/I)	≤ 0.015 (S)	0.03/0.06	≤ 0.015 (S)	≤ 0.015	0.06/ ≤ 0.015 (S)	0.125
2004.01	Breeder, 2004	0.06/0.5 (S)	0.5	0.25/0.5 (S)	0.5/1 (S/I)	0.03/ ≤ 0.015 (S)	0.125	≤ 0.015 (S)	0.03	0.03 (S)	0.5/0.25
2004.08	Breeder, 2004	2/4 (R)	4	0.5/1 (S/I)	2/1 (I)	$\leq 0.015/0.03$ (S)	0.06/0.03	≤ 0.015 (S)	≤ 0.015	0.125 (S)	0.5/0.25

^aOnly MIC profiles differing from the initial are mentioned.

^bIsolates tested once.

^cIsolates tested in duplicate.

^dMIC of both tests shown only if results were different from each other.