In vitro antimicrobial susceptibility of *Mycoplasma mycoides mycoides* large colony and *Arcanobacterium pyogenes* isolated from clinical cases of ulcerative balanitis and vulvitis in Dorper sheep in South Africa

A Kidanemariam[a], J Gouws[b], M van Vuuren[b*] and B Gummow[c]

ABSTRACT

The *in vitro* activities of enrofloxacin, florfenicol, oxytetracycline and spiramycin were determined against field isolates of *Mycoplasma mycoides mycoides* large colony (*Mmm*LC) by means of the broth microdilution technique. The minimum inhibitory concentrations (MICs) of these antimicrobial drugs were determined for a representative number of 10 isolates and 1 type strain. The susceptibility of *Arcanobacterium pyogenes* to enrofloxacin, oxytetracycline and tilmicosin was determined by means of an agar disk diffusion test. The MICs of enrofloxacin, florfenicol, oxytetracycline and spiramycin were within the ranges of 0.125–0.5, 1.0–2.0, 2.0–4.0 and 4.0–8.0 μg/mℓ, respectively. This study has shown that resistance of *Mmm*LC against enrofloxacin, florfenicol, oxytetracycline and spiramycin was negligible. All the field strains of *A. pyogenes* that were tested were susceptible to enrofloxacin, oxytetracycline and tilmicosin with mean inhibition zones of 30.6, 42.3 and 35.8 mm, respectively. Although there is lack of data on *in vivo* efficacy and *in vitro* MIC or inhibition zone diameter breakpoints of these antimicrobial drugs for *Mmm*LC, the MIC results indicate that these 4 classes of antimicrobial drugs should be effective in the treatment of ulcerative balanitis and vulvitis in sheep in South Africa.

Key words: *Arcanobacterium pyogenes,* Dorper sheep, minimum inhibitory concentrations, *Mycoplasma mycoides mycoides,* ulcerative balanoposthitis and vulvovaginitis.

Kidanemariam A, Gouws J, van Vuuren M, Gummow B ***In vitro* antimicrobial susceptibility of *Mycoplasma mycoides mycoides* large colony and *Arcanobacterium pyogenes* isolated from clinical cases of ulcerative balanitis and vulvitis in Dorper sheep in South Africa**. *Journal of the South African Veterinary Association* (2005) 76(4): 204–208 (En.). Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Private Bag X04, Onderstepoort, 0110 South Africa.

INTRODUCTION

Ulcerative balanoposthitis and vulvovaginitis of sheep is a venereal disease characterised by erosion and ulceration of the glans penis and vulval labia. It has been described in several countries[6,11,14,19,20,29,30] and has been recognised in South Africa since 1979[29,30] where sheep of the Dorper breed appear to be the most frequently affected[6,15,29]. Its distribution in South Africa therefore tends to be the drier parts of the country. It is of major concern to Dorper sheep breeders and farmers because of the detrimental effect it has on conception rates.

Various names have been given to the syndrome or similar syndromes. These include vulvovaginitis[10], balanitis and vulvovaginitis[31], granular vulvovaginitis[12], vulvitis[5], ulcerative balanitis and vulvitis[11,13,14], ulcerative balanoposthitis and vulvovaginitis[29,30] which indicate, if they are all indeed the same disease, that its clinical features and lesions require further elucidation. In a study of the disease in Dorper sheep on 15 farms in South Africa, the typical ulcerative lesions were found to be confined to the glans penis of rams and the mucocutaneous junction of the vulval labia in ewes[19]. The investigator therefore considered that a more appropriate name for the disease in South Africa would be ulcerative balanitis and vulvitis (UBV). In addition, the aetiology of the disease has not been conclusively resolved. In South Africa, Trichard *et al.*[29] isolated mollicutes from naturally infected ewes and rams showing lesions and suggested that *Mycoplasma mycoides mycoides* large colony biotype (*Mmm*LC) could be incriminated as the major cause of UBV in Dorper flocks in South Africa. They were able to reproduce the disease by application of *Mmm*LC intravaginally into healthy ewes. In another study Kidanemariam[19] found that *Mmm*LC and *Arcanobacterium pyogenes* were the 2 most common bacterial species isolated from the lesions of clinically affected cases, the former being isolated from 61.5% and the latter from 44.2%. He suggested a possible synergistic role between these 2 organisms in the disease process and found that the odds ratio analysis was that when the 2 organisms were present together they were 53.5 times more likely to occur in clinically affected sheep than in unaffected sheep.

Ulcerative balanitis and vulvitis is at present controlled by application of antimicrobial drugs. The objective of this study was to determine the *in vitro* sensitivity of *Mmm*LC and *A. pyogenes* isolated from field cases of UBV to a variety of these drugs in order to assist in the selection of those most appropriate for the treatment of clinical cases.

At present there are no internationally accepted protocols for testing the susceptibility of mycoplasmas for antimicrobials which is ideally done by determination of their minimum inhibitory concentrations (MICs). Different methods and media are used in different laboratories. However, Hannan[18] in his review article described the general principles and guidelines. In mycoplasmology the MIC is defined as the lowest concentration of an antimicrobial drug that will inhibit visible growth of the mycoplasma under review as judged by the colour change of the medium due to metabolism of the substrate[18]. This method was followed in this study.

The antimicrobial drug susceptibility of *A. pyogenes* was determined using an agar disc diffusion method.

MATERIALS AND METHODS

Cultures of MmmLC

Ten mycoplasma field isolates obtained from affected sheep during an investigation of UBV in Dorper sheep[19,20] and the type strain, *Mmm*LC (Y-Goat) (NCTC 11706), were selected for the study. Purifi-

[a]National Animal Health Research Centre, PO Box 04, Sebeta, Ethiopia.

[b]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, Private Bag X04, 0110 South Africa.

[c]Department of Production Animal Studies, Faculty of Veterinary Science, Onderstepoort, Private Bag X04, 0110 South Africa.

*Author for correspondence.
E-mail: moritz.vanvuuren@up.ac.za

Received: June 2005. Accepted: September 2005.

cation of the cultures was based on the descriptions of several authors[2,7]. Mycoplasma colonies with morphological differences were located and a block of agar containing what appeared to be a single colony was transferred into separate tubes of Hayflick's broth. After incubation for 3 days at 37 °C, 10-fold dilutions were made and loopfuls of each were streaked onto plates containing Hayflick's agar. These were incubated and single-colony picks were made from those plates on which the colonies showed consistent morphological resemblance. Purification of strains was necessary to ensure that only pure cultures were used as inocula in the microdilution tests. The cloned colonies were confirmed as *Mmm*LC using the indirect immunofluorescent antibody test (IFAT). The strains were stored at –80 °C until used for determination of their susceptibility to antimicrobial drugs.

Culture of A. pyogenes

The *A. pyogenes* strains used in this study were field isolates obtained from genital swabs of affected sheep. Nine representative isolates were selected for the test and were grown on horse blood agar at 37 °C for 24 hours.

Media for broth microdilution tests

For the microdilution test, the purified strains of *Mmm*LC were grown at 37 °C aerobically in an atmosphere containing 5 % CO_2. The medium used was Hayflick's broth, pH 7.6, containing glucose (1 %, w/v) and 2 mℓ of 1 % (v/v) phenol red. Incubation was continued until a colour change from pink to orange-yellow was evident as a result of the fermentation of glucose during mycoplasma growth.

Standardisation of inocula

The 10 isolates and type strain of *Mmm* LC were removed from cryostorage and allowed to thaw at room temperature. A panel of 9 tubes each containing 3.6 mℓ of glucose-Hayflick's broth (pH 7.6) was prepared for each isolate, and 0.4 mℓ of the thawed aliquot was added to the 1st tube. A 10-fold dilution was made up to the 9th tube by transferring 0.4 mℓ of the suspension at each step. The tubes were incubated at 37 °C until an acidic reaction (colour change from pink to yellow) was observed. The time taken for this reaction to occur was recorded, and the lowest dilution to show a colour change represented the reciprocal of the number of colour changing units (ccu) in the undiluted mycoplasma culture. The inoculum size was determined to be 10^4 ccu/mℓ for all strains tested. The acceptable number of organisms for MIC tests is 10^3 to 10^5 ccu per mℓ[18].

Table 1: **Antimicrobial drugs tested and the class to which they belong.**

Antimicrobial drug	Class
Enrofloxacin	Fluoroquinolones
Florfenicol	Amphenicols
Oxytetracycline	Tetracyclines
Spiramycin	Macrolides

Antimicrobial drugs

The 4 antimicrobials tested and the classes to which they belong are given in Table 1. They were included on the basis of their known activity against *Mycoplasma* spp., their authorisation for local use in South Africa and clinical considerations.

The choice of spiramycin as a representative for macrolide drugs was based on practical considerations. Apart from the fact that it is included in the VetMIC™ microwell plates, it can be used as a representative of the macrolides by virtue of the fact that the antimicrobial spectrum of spiramycin is similar to tylosin, erythromycin and tilmicosin (A Franklin, National Veterinary Institute, Uppsala, Sweden, pers. comm., 2002).

Representative drugs of all 4 classes are registered in South Africa. The commercially produced microtitre plates, VetMIC™, were obtained from the National Veterinary Institute, Uppsala, Sweden. The plates are provided with the drugs coated on the bottom of the wells at specific concentrations. The plates were designed to provide doubling dilutions of the antimicrobials when 50 µℓ of inoculum was added. The concentration ranges of the antimicrobial agents, after the addition of 50 µℓ inoculum, are listed in Table 2. Two wells of the microplates to which 50 µℓ of inoculum and 50 µℓ of sterile broth were added, respectively, were used for control of growth and sterility.

Determination of minimum inhibitory concentrations

The MICs were determined by a glucose metabolism inhibition method performed in 96-well microtitre plates. Two-fold dilutions of each drug were made. To each well of the microtitre plate, 50 µℓ of diluted culture containing 10^4 ccu/mℓ was added. The plates were sealed with transparent self-adhesive tape to prevent evaporation, and then incubated at 37 °C. The incubation time was controlled by observing the colour changes equivalent to the growth control well, and the plates were monitored twice daily until the required colour change was observed. The MIC was recorded as the lowest concentration of antibiotic that inhibited visible colour change of the medium at the time when a colour change could be observed in the growth control without antibiotic. MICs were obtained after 24 to 48 hours depending on the strains tested. All MICs were determined twice to confirm results and repeated a third time if the end points for any antibiotic differed by more than 1 dilution.

Table 2: **Concentration ranges of antimicrobials in the VetMIC™ microwell plates.**

Antimicrobial drugs	Concentration ranges (µg/mℓ)
Enrofloxacin	1.0–0.125
Florfenicol	16.0–2.0
Oxytetracycline	8.0–1.0
Spiramycin	32.0–4.0

Agar disk diffusion test

Susceptibility testing of *A. pyogenes* was performed using an agar disk diffusion method[4] on Columbia blood agar (Difco) supplemented with 6 % horse blood. The antimicrobial drugs tested were enrofloxacin, oxytetracycline and tilmicosin. Owing to the unavailability of florfenicol disks, the drug was not included in the test. After incubation for 24 hours, the diameters of the zones of inhibition were measured using a calliper. Each zone diameter was interpreted by reference to the zone diameter interpretive standards in NCCLS document M31-A[23].

RESULTS

The minimum inhibitory concentrations of the antibiotics to which the *Mmm*LC field isolates were susceptible are shown in Tables 3 and 4. Duplicate tests did not vary by more than 1 serial 2-fold dilution. For enrofloxacin 50 % of the isolates showed an MIC value of 0.25 µg/mℓ; 20 % of the isolates had an MIC value of 0.5 µg/mℓ. Thirty per cent of the isolates yielded a MIC value of ≤0.125 µg/mℓ. The MIC_{50} and MIC_{90} were 0.025 µg/mℓ and 0.35 µg/mℓ, respectively (Fig. 1).

The MIC range for florfenicol was 2.0–4.0 µg/mℓ, and the MIC_{90} was 2.8 µg/mℓ

Table 3: **Selected *Mmm*LC field strains and their minimum inhibitory concentrations for 4 antibiotics.**

Antibiotic	MIC (µg mℓ)	No. of isolates
Enrofloxacin	≤0.125	3
	0.25	5
	0.5	2
Florfenicol	2.0	8
	4.0	2
Oxytetracycline	≤1.0	9
	2.0	1
Spiramycin	≤4.0	7
	8.0	3

(Fig. 2). Oxytetracycline showed activity against all isolates of *Mmm*LC, with a range of MIC values between 1.0 μg/mℓ and 2.0 μg/mℓ, and a mean MIC value of 1.1 μg/mℓ (Table 5). Ninety per cent of the strains yielded an MIC ≤1.0 μg/mℓ (Fig. 3). The MIC_{90} of spiramycin for the isolated strains was 6.0 μg/mℓ (Fig. 4).

The 9 field strains of *A. pyogenes* were susceptible to all 3 antimicrobial drugs tested. The inhibition zone diameters of the tested drugs are presented in Table 6.

DISCUSSION

One of the aims of quantitative studies of antimicrobial sensitivity is to assist in choosing an effective antimicrobial to control an infection. *In vitro* antimicrobial activity, however, does not always correlate with the *in vivo* efficacy, although a drug showing little or no activity *in vitro* is unlikely to be effective in aiding the body's defences to eliminate the responsible organism[24].

Many methods have been used to obtain MIC data for veterinary *Mycoplasma* species, which make it difficult to compare the results reported from different laboratories. This lack of standardisation has been caused partly by the wide variation in nutritional requirements and culture conditions needed for different *Mycoplasma* spp. and partly by the lack of internationally agreed standards of performance and interpretation. The broth microdilution susceptibility testing system has been validated for use with human and animal bacterial pathogens[23], and MIC values for reference strains are recommended to be within ±1 dilution of the expected value.

The microdilution method used in this study was that recommended by Hannan[18]. However, apart from the colour change in the medium, the end point could also be determined using the extent of the mycoplasma growth (or lack of it), which was visible at the bottom of the plate as 'buttons'. This was made possible by the high growth rate of the mycoplasma isolates in the test, which made it easy to interpret the results.

The MICs for the antimicrobial drugs tested for mycoplasma isolates were generally in agreement with the MIC breakpoints of the same antibiotics against bacterial pathogens[23]. Furthermore, the MIC values of enrofloxacin, oxytetracycline and spiramycin obtained for the *Mmm*LC field isolates and type strain were lower than those reported by other investigators for different *Mycoplasma* spp.[3,9,16].

While no MIC breakpoints are available for mycoplasmas in general and for *Mmm*LC strain Y-Goat in particular, the

Table 4: **MIC_{50} and MIC_{90} of field isolates of *Mmm*LC and the type strain.**

Antimicrobial drug	MIC (μg/mℓ) for field strains (n = 10)		MIC (μg/mℓ) for type strain (Y-Goat)
	50 %	90 %	
Enrofloxacin	0.025	0.35	0.125
Florfenicol	ND*	2.8	2.0
Oxytetracycline	ND	1.0	1.0
Spiramycin	ND	6.0	4.0

ND = not determined.

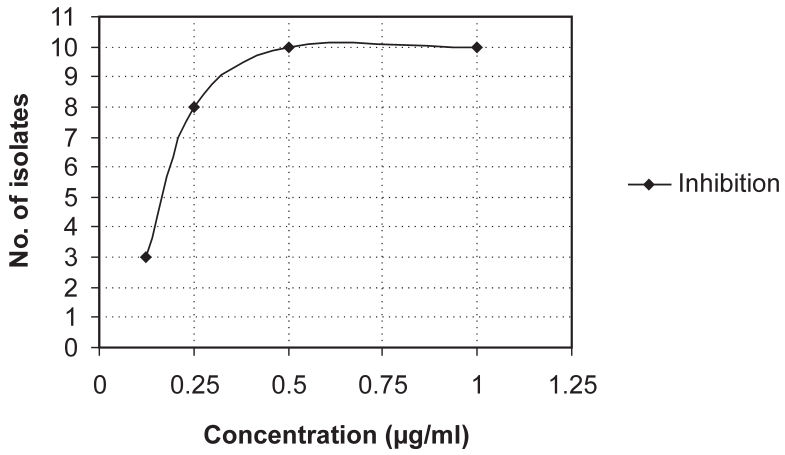

Fig. 1: **Probit graph to determine the MIC_{50} and MIC_{90} of enrofloxacin.**

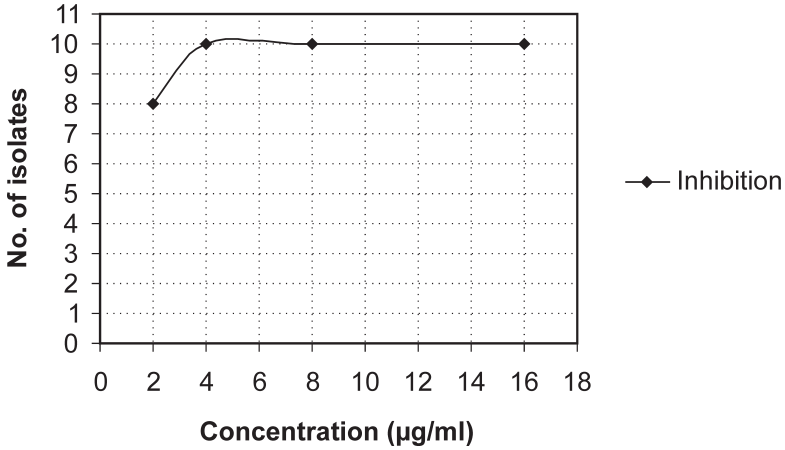

Fig. 2: **Probit graph to determine the MIC_{90} of florfenicol.**

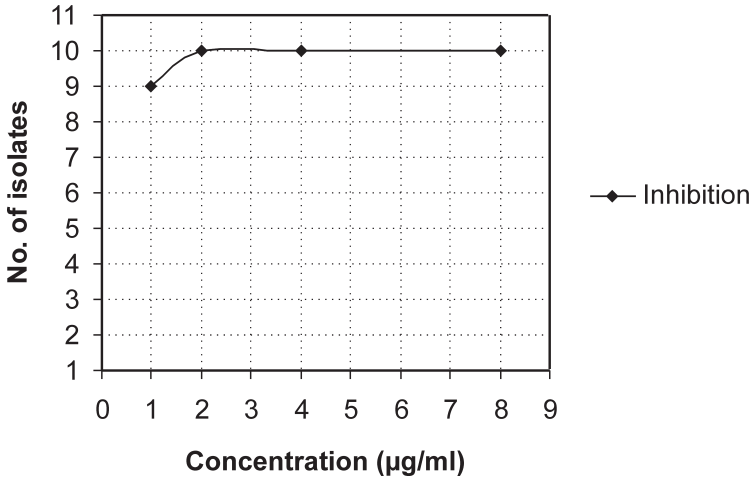

Fig. 3: **Probit graph to determine MIC_{90} of oxytetracycline.**

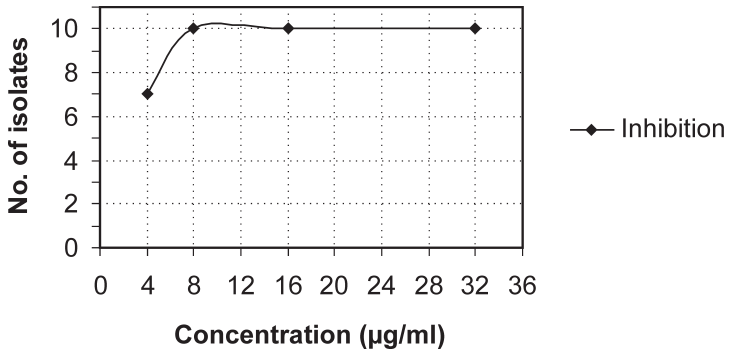

Fig. 4: **Probit graph to determine MIC_{90} of spiramycin.**

Table 5: **Mean MIC values for the tested drugs against *Mmm*LC field isolates.**

Antimicrobial drug	MIC values for field strains (n = 10)		
	Mean (µg/mℓ)	SEM*	Range (µg/mℓ)
Enrofloxacin	0.24	0.044	0.125–0.50
Florfenicol	2.4	0.155	2.0–4.0
Oxytetracycline	1.1	0.014	1.0–2.0
Spiramycin	5.0	0.440	4.0–8.0

*Standard error of the mean.

results of the test were determined in accordance with the NCCLS guidelines[23], and were similar to data for veterinary mycoplasmas. Unfortunately, an insufficient number of studies have been performed in small ruminants to determine the efficacy of antibiotics against mycoplasmas associated with genital diseases such as UBV.

The pharmacokinetic characteristics of an antimicrobial drug determine the concentrations of that drug that can be achieved in the blood and tissues. These can then be compared with the MICs of the various drugs against a particular pathogen. The effective concentration or breakpoint can be compared with the concentration in the target tissue. This should ideally be higher than the MIC_{90} for the particular organism so that there is a good chance of successful treatment. It is, for example, known that the tissue concentrations for enrofloxacin are considerably higher than 1 µg/mℓ[32], which is higher than the breakpoint for most bacterial pathogens. The fact that all 10 *Mmm*LC strains tested had MICs ≤0.5 µg/mℓ shows that enrofloxacin will likely be an effective drug for the treatment of UBV in Dorper sheep.

In one study[3], danofloxacin, a fluoroquinolone, with an MIC of 0.25 µg/mℓ for *Mycoplasma mycoides mycoides* small colony (*Mmm*SC) was determined to be as effective for the treatment of pleuropneumonia in cattle. By analogy, the enrofloxacin MIC values of ≤0.25 µg/mℓ for *Mmm*LC indicate that it should be effective in the treatment of UBV in Dorper sheep.

The MIC_{50} of enrofloxacin against mycoplasma species has been shown to be 0.01–1.0 µg/mℓ[28]. The MIC_{50} for enrofloxacin in the present study was 0.025 µg/mℓ, which is within the range obtained by Spoo & Riviere[28]. Enrofloxacin was found to be 100 % effective at 1.25 mg per kg per day *per os* in pigs with experimentally induced *Mycoplasma hyopneumoniae* respiratory tract infections. It has also been shown that a mean plasma concentration of 0.6 µg/mℓ will be attained for enrofloxacin administered to pigs at a dose rate of 2.5 mg/kg body weight[32]. The same study showed that the mean tissue concentration of enrofloxacin after intramuscular administration will reach between 1.9 and 2.1 µg/mℓ. These results further support the use of enrofloxacin for the treatment of UBV where the MIC values were lower than the expected tissue concentrations.

The MIC values obtained for spiramycin and florfenicol were lower than the MIC breakpoints described for bacterial pathogens[23], and it seems, therefore, justifiable to claim that these drugs will be effective against *Mmm*LC infections.

Spiramycin has good tissue penetration ability, reaching concentrations of 25–60 times more than that of serum[25]. It has also been used successfully to treat contagious bovine pleuropneumonia caused by *Mmm*SC at a dose rate of 25 mg/kg[25]. It has also been reported that spiramycin has similar applications and effects as those of tylosin, and a much higher *in vivo* efficacy than that of erythromycin in small ruminants[25]. Due to the fact that macrolide antibiotics are highly lipid soluble and widely distributed in body fluids and tissues, spiramycin could effectively be used in combating mycoplasma-induced ulcerative genital infections. Although a value of MIC_{90} of 4.0 µg/mℓ for tilmicosin has been reported[8,21], it is slightly lower than the values for spiramycin. The MIC values for spiramycin in this study are comparable to the breakpoints for bacteria, and would be attainable in the blood and body tissues where the concentration markedly exceeds that of the MICs.

Florfenicol, an amphenicol, has a broad range of activity because of wide tissue distribution and high bioavailability[26]. The potential of this compound in the treatment of microbial infections in food animals intended for human consumption has been demonstrated[28]. It was initially used for the treatment of bovine respiratory disease caused by *Mannheimia haemolytica.*

The volume of distribution of oxytetracycline varies markedly (0.32–18.5 ℓ/kg) between animal species and in the different age groups within species[27]. Owing to their solubility in lipids, tetracyclines are capable of penetrating tissues and becoming widely distributed throughout the body. They have been shown to penetrate well into pulmonary and renal tissues, as well as into bronchial fluids. Concentrations within extracellular tissue fluids are expected to be similar or higher than those in the blood[27]. Higher concentrations of tetracyclines in tissues as such could dictate their increased usage in the treatment of infections caused by a wide variety of microorganisms. The MIC_{90} of tetracycline for the 10 selected isolates of *Mmm*LC tested in this study was

Table 6: **Zones of inhibition of *Arcanobacterium pyogenes* for the 3 drugs** (n = 9).

Antimicrobial drug	Mean (mm)	SEM*	Range	Zone diameter interpretive standard (NCCLS 1999)	
				Susceptible	Resistant
Enrofloxacin	30.6	1.8	26.8–32.4	≥20	≤16
Oxytetracycline	42.3	1.4	36.8–47.3	≥23	≤18
Tilmicosin	35.8	4.12	31.7–39.0	≥14	≤10

*Standard error of the mean.

1.0 μg/mℓ. This value is similar to the observations obtained in other studies[1,8,17], in which a MIC_{90} of 1.0 μg/mℓ was reported. The extensive distribution of oxytetracycline and its *in vitro* effect against *Mmm*LC also makes this agent a suitable candidate for the treatment of ulcerative balanitis and vulvitis.

Several investigators have evaluated the susceptibility of *A. pyogenes* to different antimicrobial drugs[33]. The present study has shown that all the isolates were susceptible to oxytetracycline, enrofloxacin and tilmicosin. A study in Kenya revealed that *A. pyogenes* was susceptible to oxytetracycline[22]. Tylosin, erythromycin and enrofloxacin were also found to be effective against strains of *A. pyogenes* isolated from bovines[33].

Although data on the *in vivo* efficacy and *in vitro* breakpoints for mycoplasmas are incomplete, the MIC results of this study suggest that the 4 antimicrobial drugs will be effective in the treatment of *Mmm*LC infections of the genital tract of sheep. It should be borne in mind that although only 1 of the 4 antimicrobial drugs, namely oxytetracycline, is registered for use in small stock in South Africa, the other 3 have authorisation for use in other animal species such as cattle and pigs. Their use in small stock would therefore constitute extra-label use, which implies use of a drug in a manner or dosage different from the instructions on the manufacturer's label. These drugs should therefore be used in small stock only by, or under the supervision of, a veterinarian.

ACKNOWLEDGEMENT

The authors thank Prof. Roy C. Tustin for his valuable assistance with the editing of the manuscript.

REFERENCES

1. Aarestrup F M, Friis N F, Szancer J 1998 Antimicrobial susceptibility of *Mycoplasma hyorhinis* in a liquid medium compared to a disc assay. *Acta Veterinaria Scandinavica* 39: 145–147
2. Al-aubaid J M, Fabricant J 1971 The practical application of immunofluorescence (agar block technique) for the identification of mycoplasmas. *Cornell Veterinarian* 61: 519–542
3. Ayling R D, Baker S E, Nicholas R A J, Peek M L, Simon A J 2000 Comparison of *in vitro* activities of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against *Mycoplasma mycoides mycoides* small colony type. *The Veterinary Record* 146: 243–246
4. Bauer A W, Kirby W M M, Sherris J C, Turck M 1966 Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 36: 493–496
5. Ball H J, McCaughey W J 1982 Experimental production of vulvitis in ewes with *Ureaplasma* isolates. *The Veterinary Record* 110: 581
6. Bath G, De Wet J 2000 Pizzle disease. *Sheep and Goat Diseases.* Tafelberg Publishers Limited, Cape Town: 74–76
7. Carmichael L E, St. George T D, Sullivan N D, Horsfall N 1972 Isolation, propagation and characterization studies of an ovine mycoplasma responsible for proliferative interstitial pneumonia. *Cornell Veterinarian* 62: 654–679
8. Wu C C, Shyrock T R, Lin T L, Faderan M, Veenhuizen M F 2000 Antimicrobial susceptibility of *Mycoplasma hyorrhinis. Veterinary Microbiology* 76: 25–30
9. Cooper A C Fuller J R, Fuller M K, Whittlestone P, Wise D R 1993 *In vitro* activity of danofloxacin, tylosin and oxytetracycline against mycoplasmas of veterinary importance. *Research in Veterinary Science* 54: 329–334
10. Cottew G S, Lloyd L C, Parsonson I M 1974 Isolation of a *Mycoplasma* from vulvovaginitis in sheep. *Australian Veterinary Journal* 50: 576–577
11. Deas D W, 1983 Ulcerative balanitis and ulcerative vulvitis. In Martin W B (ed.) *Diseases of sheep.* Blackwell Scientific publications, Oxford: 144–146
12. Doig P A, Ruhnke H L, 1977 Isolation of *Ureaplasma* from sheep with granular vulvitis. *Veterinary Record* 100: 179–180
13. Dunn K, 1996 Vulvitis and balanitis in a low land flock. *Proceedings of the Sheep Veterinary Society* 20: 41–42
14. Greig A, 2000 Ulcerative balanitis and vulvitis. In Martin W B, Aitken I D (eds) *Diseases of sheep* (3rd edn) Blackwell Science, Cambridge: 119–121
15. Gummow B, Staley G P, 2000 A retrospective survey of ulcerative balanoposthitis and vulvovaginitis in South African Dorper sheep. *Livestock Health and Production* 12: 31–36
16. Hannan P C T, 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
17. Hannan P C T, Windsor H M, Ripley P H 1997 *In vitro* susceptibilities of recent field isolates of *Mycoplasma hyopneumoniae* and *Mycoplasma hyosynoviae* to valnemulin (Econor®), tiamulin and enrofloxacin and the *in vitro* development of resistance to certain antimicrobial agents in *Mycoplasma hyopneumoniae. Research in Veterinary Science* 63: 157–160
18. Hannan P C T 2000 Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary *Mycoplasma* species. *Veterinary Research* 31: 373–395
19. Kidanemariam A 2003 The identification and characterization of the primary infectious agents associated with ovine ulcerative balanoposthitis and vulvovaginitis in South Africa. MSc dissertation, University of Pretoria
20. Kidanemariam A, Van Vuuren M, Gummow B, Gouws J 2005 Ulcerative balanitis and vulvitis of sheep in South Africa: a study on its aetiology and clinical features. *Journal of the South African Veterinary Association* 76(4): 197–203
21. Kobayashi H, Morozumi T, Munthall G, Mitani K, Ito N, Yamamoto K 1996 Macrolide susceptibility of *Mycoplasma hyorhinis* isolated from piglets. *Antimicrobial agents and Chemotherapy* 40: 1030–1032
22. Mulei C M, Gitau G K 1993 Antibiotic sensitivity of aerobic bacterial organisms isolated from cows with *post-partum* vaginal discharges and their implication in therapy of uterine infections in Kenya. *Indian Veterinary Journal* 70: 999–1002
23. NCCLS 1999 Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard. M31-A. NCCLS, Wayne, Pennsylvania, USA
24. Nosanchuk J D, Cleare W, Franzot S P, Casadevall A 1999 Amphotercin B and fluconazole affect cellular charge, macrophage phagocytosis, and cellular morphology of *Cryptococcus neoformans* at subinhibitory concentrations. *Antimicrobial Agents & Chemotherapy* 43: 233–239
25. Prescott J F 2000 Lincosamides, macrolides and pleuromutilins. In Prescott J F, Baggot J D, Walker R D (eds), *Antimicrobial therapy in veterinary medicine* (3rd edn). Iowa State University Press, Ames: 229–262
26. Prescott J F 2000 Chloramphenicol, Thiamphenicol, and Florfenicol. In Prescott J F, Baggot J D, Walker R D (eds) *Antimicrobial therapy in veterinary medicine* (3rd edn). Iowa State University Press, Ames: 262–274
27. Riviere J E, Spoo J W 1995 Tetracycline antibiotics. In Adams H R (ed.) *Veterinary pharmacology and therapeutics* (7th edn). Iowa State University Press. Ames: 784–796
28. Spoo J W, Riviere J E 1995 Chloramphenicol, macrolides, lincosamides, fluroquinolones and miscellaneous antibiotics. In H R Adams (ed.) *Veterinary pharmacology and therapeutics* (7th edn). Iowa State University Press. Ames: 820–854
29. Trichard C J V, Jordan P, Prozesky L, Jacobsz E P, Henton M M 1993 The identification of *Mycoplasma mycoides mycoides* Large Colony as the aetiological agent of balanoposthitis and vulvovaginitis in sheep in South Africa. *Onderstepoort Journal of Veterinary Research* 60: 29–37
30. Trichard C J V, Van Tonder E M 1994 Ulcerative balanoposthitis and vulvovaginitis in sheep and goats. In Coetzer J A W, Thomson G R, Tustin R C (eds) *Infectious diseases of livestock with special reference to southern Africa* (1st edn). Oxford University Press, Cape Town: 1599–1602
31. Webb R F, Chick B F 1976 Balanitis and vulvovaginitis in sheep. *Australian Veterinary Journal* 52: 241–242
32. Wiuff C, Lykkesfeldt J, Aarestrup, F M, Svendsen O 2002 Distribution of enrofloxacin in intestinal tissue contents of healthy pigs after oral and intramuscular administrations. *Journal of Veterinary Pharmacology & Therapeutics* 25: 335–342
33. Yoshimura H, Kojima A, Ishimaru M 2000 Antimicrobial susceptibility of *Arcanobacterium pyogenes* isolated from cattle and pigs. *Journal of Veterinary Medicine B, Infectious Disease and Veterinary Public Health* 47: 139–143