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Tulathromycin in the Treatment of Respiratory Infections in Sheep

[1]V. Naccari, [2]F. Giofrè and [3]F.Naccari
[1]Veterinarian specializing in animal reproduction, University of Messina (Italy)
[2]ASP n. 8, Vibo Valentia (Italy)
[3]Department of Veterinary Sciences, University of Messina (Italy)

Abstract: In this study the effectiveness of tulathromycin, a new semi-synthetic macrolide, was assessed in treatment of sheep respiratory infection. The research was carried out on 36 half-breed sheep with clinical signs of bacterial respiratory infection. Specimens of nasal discharge (40-45 mL) from all animals were collected for bacteriological tests, before treatment and 2, 5, 7 and 15 days after the drug injection. Bacteriological investigations showed the presence of gram-negative strains of *Mannheima* (*Pasteurella*) *haemolytica*, *P. multocida*, *Mycoplasma ovipneumoniae* and *Pseudomonas spp*. The susceptibility of the isolated microorganisms to tulathromycin and other antimicrobials drugs used in veterinary medicine was estimated by *in vitro* test. A single dose of tulathromycin (DRAXXIN, Pfizer, Milan Italy) (2.5 mg/kg b.w.) was injected subcutaneously in the neck of each sheep. In treated animals, the symptomatology decreased rapidly 2 days after treatment and completely after 5-7 days, with remission and normal functioning of respirator activity. Actually, no literature data are present on tulathromycin treatment in sheep; therefore this research describes the first therapeutic use in this specie.

Keywords: Effectiveness, gram-negative, respiratory infections, sheep, tulathromycin

INTRODUCTION

The respiratory infections continue to be one of the most economically significant problems for health of calves, swine, sheep and goats breeding. In particular, *Pasteurella multocida*, *Mannheimia haemolytica*, often complicated by Mycoplasma colonization, are the most common causes of respiratory disease in sheep and goats (Brogden *et al*., 1998; Ackermannand Brogden, 2000; Zamri-Saadand and Mera, 2001; Berge *et al*., 2006; Washburn *et al*., 2007; Yener *et al*., 2009). Management of this complex disease involves preventive and therapeutic administration of various antimicrobials. Actually, options for approved antibiotic therapy to antagonize these bacterial infections are severely limited (Berge *et al*., 2006). Ceftiofur, is the only antibiotic labeled in goat (Washburn *et al*., 2007; Clothier *et al*., 2012); however, successful treatment relies on daily therapy which may be difficult to accomplish in field conditions (Courtin *et al*., 1997; Webb *et al*., 2004; Washburn *et al*., 2007). Additionally, cephalosporins are not active against *Mycoplasma* species, important pathogens of sheep and goats (Rosenbusch *et al*., 2005; Clothier *et al*., 2011). The Food and Drug Administration (FDA 1994) permits the use of certain approved animal and human drugs in extra-label manner under a valid veterinary relationship for sheep and goats (Fajt, 2001; Berge *et al*., 2006; Clothier *et al*., 2012). The veterinaries are responsible of choosing an appropriate drug, dose and withdrawal period in absence of label directions for the species being treated and Minimum Inhibitory Concentrations (MIC's) for antimicrobials most likely to be effective against bacterial pathogenic isolates could prove useful to clinicians making these decisions.

Macrolide antibiotics, such as tilmicosin and tylosin, rapidly distributed from plasma to pulmonary parenchyma, are active against bacterial pneumonia in various animal species (Williamsand and Sefton, 1993; Naccari *et al*., 2001), however these drugs are required to be administrated repeatedly over several days.

Recently, tulathromycin, a novel semisyntetic triamilide antibiotic in the macrolide class, particularly active against gram-negative bacteria with low MIC values (1-4 µg/mL), has been shown to be safe and efficacious in the treatment of bacterial respiratory disease in cattle, swine and goats (Benchaoui *et al*., 2004; Nowakowski *et al*., 2004; Evans, 2005; Washburn *et al*., 2007; Clothier *et al*., 2011). Antimicrobials activity of this antibiotic is generally bacteriostatic and act by inhibiting protein biosynthesis through selective binding to bacterial ribosomes and stimulating dissociation of peptidyl-tRNA from ribosome during the traslocation process. A single intramuscular (i.m.) or subcutaneous (s.c.) tulathromycin injection provides therapeutic

Corresponding Author: F. Naccari, Department of Veterinary Sciences, University of Messina (Italy), V.le S. Annunziata, 98168 Messina (Italy),Tel.: +39 0903503780

concentrations in lung tissues for seven days. In particular, after treatment, tulathromycin is completely absorbed to reach maximal serum concentrations within half an hour. The systemic bioavailability following i.m. or s.c. administrations is >87% and tulatromycin is widely distributed and accumulates in lung tissue in various animals species (Nowakowski *et al*., 2004; Clothier *et al*., 2011; Wang *et al*., 2011). A single dose of tulatrhomycin is indicated for the treatment of respiratory infections in cattle, swine and goat, such as *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumoniae* and *Bordetella bronchiseptica* (Benchaoui *et al*., 2004; Nowakowski *et al*., 2004; Evans, 2005; Washburn *et al*., 2007; Clothier *et al*., 2011). Recently, it was also used in the treatment of abscessing pneumonia of foals caused by *Rhodococcus equi* (Scheuch *et al*., 2007).

Actually, no literature data are present on tulathromycin treatment in ovine specie. Therefore, the aim of this study was to evaluate the effectiveness of a single subcutaneous injection of 2.5 mg/kg body weight of tulatrhomycin in the treatment of sheep bacterial respiratory infections.

MATERIALS AND METHODS

Animals: The research was carried out on 36 half-breed dairy sheep affected by bacterial respiratory infections, between 3-5 year old, body weight (b.w.) around 50 kg. These animals were selected by a clinical study made on 460 sheep kept in loose housing on several farms in Calabria area (a southern region of Italy). The study took place over the winter when the sheep were housed.

Clinical signs: The sheep affected by bacterial respiratory infections showed clinical signs such as coughing, dyspnoea, nasal discharge and wheezing, rubbing vescicular and murmuring sounds on thoracic auscultation. Moreover, the animals presented slightly raised body temperature, anorexia and depression. Diagnosis of respiratory infections was made on clinical signs and by means of bacteriological investigations on nasal discharge samples.

Sample collection: Specimens of nasal discharge (40-45 mL each) were collected for bacteriological investigations from all animals by means of sterile plugs (CULTURETTZ, Becton Dickinson), before treatment (baseline) and 2, 5, 7 and 15 days after. The samples were collected after the nasal area had been carefully cleaned and disinfected and the initial nasal discharge had been discarded. The samples were kept at 4°C until they were taken under sterile conditions to the analytical laboratory within 24 h.

Bacteriological investigations: Specimens of nasal discharge were homogenised for 3 minutes with 0.01 mL in phosphate buffer (pH 7) with N-acetylcysteine (1:1, v:v) and incubated for 30 minutes at 37°C. The specimens were examined microscopically by Gram staining, enriched on Brain-Heart medium for 18 hours and then inoculated on MacConkey-agar (OXOID) and Chapman-Stone-agar (OXOID) for isolation of Gram-positive and Gram-negative bacteria, respectively, as well as on Pasteurella selective agar (OXOID). All plates were incubated for 48 h at 37°C and the microorganisms isolates were identified by a standard commercial fermentation test (API System, Bio-Merieux, Italy).

For isolation of *Mycoplasma spp*., the samples were inoculated into Mycoplasma-broth (Axcell Biotechnologies SA, Lyon, France) complemented with ampicillin (2 mg/mL; Sigma Aldrich, Germany) and incubated aerobically at 37°C for 3-7 days and then sub-cultured onto Mycoplasma-agar (Axcell) with ampicillin and re-incubated at 37°C in an atmosphere of 5% CO_2 for 5 days. Mycoplasma were confirmed to species level by a PCR specific for *Mycoplasma ovis* (Subramaniam *et al*., 1998; Bashiruddin *et al*., 2005).

***In vitro* antimicrobial activity:** The antibacterial activity of tulathromycin and other antimicrobials agents was evaluated by *in vitro* bacteriological test using the agar disk diffusion method (Kirby-Bauer) and measuring the diameter of the growth inhibition area, as recommended by Clinical Laboratory Standards Institute (2008). In particular, *in vitro* activity of tulathromycin on the bacterial strains isolated from nasal discharge samples was evaluated in comparison to some antimicrobials drugs widely used in veterinary medicine: ampicillin (10 µg), amoxicillin (30 µg), amikacin (30 µg), thiamphenicol (20 µg), erythromycin (15 µg), enrofloxacin (10 µg) and oxytetracycline (30 µg). Specifically, paper disks (6 mm in diameter) were loaded with 20 µL (18 µg) of antimicrobial drugs, mixed with Tween 80 (Sigma Tau) (1:1 v/v) to enhance its solubility and were placed on the surface of the Muller-Hinton agar inoculated with a suspension of the various isolated microorganisms (0.1 mL of 10^8 cfu/mL). The plates were incubated at 37°C for 24 h. Thereafter, the diameter (mm) of the growth inhibition areas was measured. All the assays were performed in duplicate.

Pharmacological treatment: The present investigation with an off-label use of tulathromycin in sheep, approved by commission on drug experimentation of Messina University and supported by Pfizer (Milan Italy), was conducted in accordance with local ethical regulations for veterinary practice (Recommendation, 2007 /526/CE; Legislative Decree 1992/116; Directive 2010/63/EU), prior informed consent of the breeders.

The dosage of tulathromycin was prepared in accordance with the manufacturer's instructions. For these trials the use of a positive control group (treated

with oxytetracycline) rather than an untreated group was applied according to regulations for veterinary practice.

Animals were blocked in pairs and each animal assigned to one or other group in order to clinical examination. The two groups were treated with a single dose of Oxytetracycline (OTC) at 20 mg/kg for intramuscular injection (group 1) and tulathromycin (DRAXXIN, Pfizer, Milan Italy) at 2.5 mg/kg body weight for subcutaneous injection (group 2), in the neck of each sheep, respectively.

Clinical score: The clinical signs showed by sheep suffering from bacterial respiratory infections were evaluated using a scores system, assessed on a four-point scale as follows: 0 Absent; 1 Moderate; 2 Severe and 3 Very Severe. The effectiveness of oxytetracyclin (group 1) and tulathromycin (group 2) treatments was evaluated by comparing the sheep's clinical signs before treatment and to 2, 3, 5, 7 and 15 days later and evaluating the presence of microorganisms in nasal discharge collected at the same times, as previously describes. All sheep were kept under clinical observation during the experimental period to check for any drug-related side effects.

Statistics: The data were expressed as mean value ± Standard Deviation (S.D.). An Analysis of Variance (ANOVA) was applied to assess the significance of the difference between the scores recorded before the drug treatment and 2, 3, 7, 5 and 15 days later in both groups treated with oxytetracylcine and tulathromycin, respectively. Difference with $p<0.05$ were considered significant. In Stat 3.0 software (GraphPad) was used to make the statistical analyses.

RESULTS

Bacteriological test: The bacteria isolated by microbiological test from the nasal discharge samples of 36 sheep affected by bacterial respiratory infections were: *Mannheima* (*Pasteurella*) *haemolytica* (16 animals), *P. multocida* (8 animals), *Mycoplasma ovipneumoniae* (6 animals) and *Pseudomonas spp.* (6 animals) (Table 1).

Susceptibility of the microorganisms to tulathromycin and other antibiotics: The susceptibility of the isolated microorganisms in sheep affected by bacterial respiratory infections to tulathromycin and other antimicrobials widely used in veterinary medicine (penicillin G, ampicillin, gentamicin, oxytetracycline, thiamphenicol, tilmicosin and enrofloxacin) is reported in Table 1. Antimicrobial activity *in vitro* test has showed that tulathromycin is the antibacterial drug most active in comparison to other antibiotics studied.

Bacteria findings in nasal discharge samples during pharmacological treatment: Table 2 and 3 show the bacteriological findings in the nasal discharge, taken before the treatment (baseline) and 2, 3, 5, 7 and 15 days later the single injection of oxytetracycline (20 mg/kg b.w., i.m.) and tulathromycin (2.5 mg/kg b.w., s.c.), respectively. The severity of the respiratory infection was expressed in the conventional manner (from + to ++++) in relation to the quantity of bacteria present. No microorganisms were isolated in any nasal discharge samples at 7 and 15 days after tulathromycin administration.

Variations in clinical scores recorded during antibiotic treatments: In Table 4 are reported the mean body temperatures and scores for clinical signs of anorexia, coughing, nasal discharge, dyspnoea and wheezing, rubbing vesicular and murmuring sounds in the sheep affected by respiratory infection before and at various time after the treatment with a single dose of oxytetracyclin (i.m.) and tulathromycin (s.c.), respectively. The treatment with a single dose of oxytetracyclin (20 mg/kg i.m.,) (group 1) have not showed the remission of respiratory infection in sheep and the clinical signs continued until to 15 days. In sheep treated with a single dose of tulathromycin (2.5 mg/kg s.c.) (group 2), instead, it is possible to observe that after 2 days the severity of clinical signs was

Table 1: Susceptibility to antibacterial drugs of the microorganism strains isolated from nasal discharge samples collected from sheep with Gram-negative respiratory infections

	Bacterial strains			
Drugs	*Manheima haemolitica*	*Pasteurella multocida*	*Mycoplasma ovipneumoniae*	*Pseudomonas spp.*
Tulathromycin	+++	+++	+++	+++
Ampicillin	+	+	++	-
Gentamicin	-	-	+	++
Amikacin	+	+	-	+
Thiamphenicol	+	+	+	-
Oxytetracyclin	+	-	+	-
Tilmicosin	+	+	+	-
Enrofloxacin	++	+	-	+

+++: Very susceptible (≤0.56 µg/mL); ++: Susceptible (0.57 to 6.25 µg/mL); +: Poorly susceptible (6.26 to 12.5 µg/mL); -: Resistant (≥12.5 µg/mL)

Table 2: Bacteria isolated from nasal discharge samples taken before (baseline) and 2, 3, 5, 7, 15 days after the intramuscolar injection of a single dose of oxytetracyclin (20 mg/kg b.w.) in 36 sheep with respiratory infection

Strain isolated	T 0	T 1 (48 h)	T 2 (72 h)	T 3 (5 gg)	T 4 (7 gg)	T 5 (15 gg)
M. (*Pasteurella*) *haemolytica* (16 samples)	++++	++++	+++	++	++	+
P. multocida (8 sample)	++++	+++	++	+	+	-
Mycoplasma ovipneumoniae (6 samples)	++++	+++	++	++	+	-
Pseudomonas sp. (6 samples)	++++	++++	++++	++++	++++	++++

++++: 10^6 colony forming units (CFU); +++ = 10^5 CFU; ++: 10^4CFU; +: 10^3CFU

Table 3: Bacteria isolated from nasal discharge samples taken before (baseline) and 2, 3, 5, 7 and 15 days after the subcutaneosly injection of a single dose (2.5 mg/kg) of tulathromycin in 36 sheep with respiratory infection

Strain isolated	T 0	T 1 (48 h)	T 2 (72 h)	T 3 (5 gg)	T 4 (7 gg)	T 5 (15 gg)
M. (*Pasteurella*) *haemolytica* (16 samples)	++++	++++	++	+	-	-
P. multocida (8 sample)	++++	+++	++	-	-	-
Mycoplasma ovipneumoniae (6 samples)	++++	+++	+	+	-	-
Pseudomonas sp. (6 samples)	++++	+++	+	-	-	-

++++: 10^6 colony forming units (CFU); +++: 10^5 CFU; ++: 10^4CFU; +: 10^3CFU

Table 4: Effects of a single dose of oxytetracyclin (20 mg/kg i.m.) and tulathromycin (2.5 mg/kgs.c.) on clinical signs of 36 sheep with naturally occurring respiratory infections

Respiratory infections and clinical signs	Oxytetracyclin Before treatment	After 2 days	After 5 days	After 7 days	After 15 days	Tulatromycyn Before treatment	After 2 days	After 5 days	After 7 days	After 15 days
Bronchiapneumoniae to:										
*M. Pasteurella haemolytica (*n. 16)										
Dispnea	2.39±0.65	2.54±0.52	1.98±0.68	1.86±0.75	1.45±0.52	2.29±0.46	1.50±0.52*	0.43±0.51**	--	--
Cought	2.22±0.73	2.01±0.36	1.87±0.61	1.79±0.53	1.25±0.46	2.50±0.73	1.50±0.46*	0.50±0.31**	--	--
Nasal discarge	1.97±0.53	1.86±0.49	1.67±0.41	1.48±0.29	1.09±0.70	2.37±0.63	0.92±0.61*	0.37±0.31**	--	--
Objective signs*	2.14±0.55	2.04±0.68	1.892±0.49	1.92±0.38	1.12±0.35	2.00±0.55	1.21±0.42*	0.25±0.31**	--	--
Temperature (C°)	40.89±0.60	40.38±0.48	39.87±0.30	39.78±0.38	39.50±0.38	40.63±0.49	39.42±0.38*	38.5±0.34**	38.65±0.62	38.5±0.45
Bronchitis to:										
Pasteurella multocida (n. 8)										
Dispnea	1.97±0.54	1.91±0.35	1.87±0.61	1.91±0.47	1.75±0.46	2.66±0.46	1.33±0.35*	0.50±0.38**	--	--
Cought	2.44±0.64	2.25±0.67	1.97±0.31	1.87±0.58	1.62±0.51	2.12±0.54	1.25±0.46*	0.37±0.31**	--	--
Nasal discarge	1.78±0.64	1.62±0.51	1.37±0.52	1.37±0.32	1.12±0.35	1.87±0.64	0.62±0.51*	0.37±0.42**	--	--
Objective signs*	2.27±0.36	2.12±0.35	1.97±0.24	1.83±0.29	1.53±0.32	2.50±0.36	1.12±0.35*	0.50±0.32**	--	--
Temperature (C°)	40.77±0.43	40.24±0.37	39.91±0.59	39.86±0.45	39.42±0.38	40.50±0.53	39.50±0.37*	38.5±0.37**	38.45±0.37	38.65±0.38
Respiratory syndrome to:										
Mycoplasma ovipneumoniae (n. 6)										
Dispnea	2.47±0.64	2.25±0.42	1.95±0.63	1.79±0.32	1.48±0.29	2.27±0.64	1.45±0.52*	0.45±0.32**	--	--
Cought	2.36±0.60	2.05±0.52	1.84±0.32	1.74±0.32	1.28±0.46	2.18±0.60	1.45±0.52*	0.54±0.42**	--	--
Nasal discarge	2.19±0.54	1.89±0.70	1.75±0.32	1.53±0.32	1.12±0.35	2.09±0.54	1.09±0.70*	0.37±0.22**	--	--
Objective signs*	2.08±0.60	2.02±0.46	1.85±0.32	0.65±0.35	0.54±0.32	2.18±0.60	1.27±0.46*	0.45±0.32**		--
Temperature (C°)	40.53±0.39	39.5±0.38	39.41±0.30	38.37±0.78	38.41±0.45	40.31±0.60	39.50±0.35	39.41±0.30**	38.65±0.62	38.37±0.51
***Pseudomonas sp.* (n.6)**										
Dispnea	2.50±0.73	2.75±0.54	2.44±0.46	2.66±0.46	2.35±0.35	2.66±0.64	1.60±0.54*	0.50±0.32**		
Cought	2.18±0.54	2.36±0.32	2.27±0.58	2.19±0.37	2.19±0.32	2.80±0.36	1.60±0.46*	0.43±0.51**		
Nasal discarge	1.87±0.46	1.75±0.46	1.65±0.51	1.78±0.29	1.62±0.41	2.40±0.54	1.40±0.54*	0.60±0.51**		
Objective signs*	2.27±0.36	2.02±0.68	2.19±0.54	2.08±0.60	2.14±0.54	2.40±0.54	1.20±0.64*	0.40±0.54**		
Temperature (C°)	40.5±0.51	40.28±0.37	39.86±0.49	39.55±0.55	39.42±0.4	41.5±0.35	38.6±0.41*	38.5±0.43**	38.37±0.51	38.40 ±0.38

Objective signs = wheezing, rubbing vescicular and murmuring sounds on auscultation; *: p< 0.01; **: p<0.05

decreased, after 5 days moderate respiratory signs were registered; after 7 days it is evident a complete remission of disease and after 15 days the complete recovery of respirator activity. No local or general signs of tulathromycin intolerance were recorded in any treated sheep and no relapses occurred in the month following treatment.

DISCUSSION

Many studies on the spectrum of antibacterial activity and on the pharmacokinetic characteristics of a single subcutaneously injection of tulathromycin showed how it can be used effectively and safety in respiratory infections of cattle and swine (Benchaoui *et*

al., 2004; Nowakowski *et al*., 2004; Evans, 2005; Clothier *et al*., 2011). Studies in goat have reported similar results, demonstrating a significant efficacy in respiratory infection and a mean lung/plasma ratio of 48h at 5 days after tulathromycin subcutaneously injection (Clothier *et al*., 2011, 2012; Washburn *et al*., 2007). Moreover, Young *et al*. (2011) demonstrated that the pharmacokinetic of tulathromycin after a single injection (2.5 mg/kg s.c.) in goats was similar to what has been previously reported in cattle. A comparative study on three treatment regimens for sheep and goats with *caseous lymphadenitis* showed acceptable alternative use of tulathromycin for treatment of these animal species (Washburn *et al*., 2009).

The present study conducted on sheep affected by respiratory infection indicates that a single dose of tulathromycin (2.5 mg/kg b.w. subcutaneosly), in comparation to oxytetracyclin treatment (single dose 20 mg/kg i.m.), can be useful in gram-negative bacterial infections caused by *M. haemolytica*, *P. multocida*, *Mycoplasma ovipneumoniae* and *Pseudomonas spp.*

In all treated animals, the symptomatology decreased 2 days after the drug injection and completely, with remission of disease and recovery of respirator activity after 7 days of the treatment. These data suggest that tulathromycin also in sheep reaches satisfactory concentration in the lung, which explains the swift resolution of clinical signs. No bacteria were isolated from nasal discharge 5-7 days after the treatment. No local or systemic side effects were registered in the treated animals and no relapses were observed during the 30 days after the treatment.

This study confirms the therapeutic effectiveness of tulathromycin in the ovine respiratory infection in comparison to other antimicrobial widely used in medicine veterinary. In particular a single dose was sufficient to remission of the disease, keeping drug and treatment costs contained. However, the tulathromycin treatment in sheep constituted an extra-label drug use and further studies are necessary to determine the concentration in milk to food safety.

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