

Pharmacokinetics and Efficacy of Tilmicosin in the Treatment of *Pasteurella haemolytica* Bronchopneumonia in Calves

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Abstract

Tilmicosin was administered intravenously and subcutaneously at a dose rate of 10 mg/kg bwt to determine its concentration in blood and bronchial secretion as well as its kinetic behavior in healthy and *Pasteurella haemolytica* type A1-infected calves. Sever acute bronchopneumonia was induced in 10 calves by inoculating them intra-tracheally with *P. haemolytica* type A1. The calves were treated with tilmicosin; 5 of these received the drug intravenously and the other 5 were injected subcutaneously. After a slow intravenous injection, the serum concentration-time curve indicated a two compartment open model with a mean elimination half-lives ($t_{1/2\beta}$) of 22.09 and 22.14 hours before and after infection, respectively. The mean residence time (MRT) corrected for a bolus injection was 2.25 and 2.20 hours and the mean MRT_{inf} was 25.27 and 25.46 hours in healthy and *P. haemolytica*-infected calves, respectively. After subcutaneous injection, the drug was eliminated more slowly (before and after infection) from serum and bronchial secretions, with $t_{1/2\beta}$ of (24.60 and 25.85 hours) and (33.74 and 31.78 hours), respectively. The apparent volume of distribution ($V_{d(area)}$) of tilmicosin was more than 1 litre·kg⁻¹. The peak serum and bronchial secretions of tilmicosin concentration were (1.33 and 1.36 µg·ml⁻¹) and (1.40 and 1.70 µg·ml⁻¹) attained at (7.21 and 7.15 hours) and 7.11 and 7.10 hours) after subcutaneous injection, respectively. Tilmicosin was good secreted into bronchial secretions having $AUC_{bronchial\ secretion}/AUC_{serum}$ ratio of approximately 1:1.24 and 1:1.22 in healthy and *P. haemolytica*-infected calves, respectively. The clinical and hematological parameters of calves treated with subcutaneous injection returned to normal values significantly faster than those treated intravenously.

Keywords

Tilmicosin, Disposition Kinetics, Calves, Healthy, Diseased, Bronchial Secretion

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1. Introduction

Bronchopneumonia is one of the most economically important respiratory diseases of calves reared indoors [1] [2]. Its etiology is complex and can involve viruses, mycoplasmas and bacteria [3]. Bacteria, particularly *Pasteurella* species, play an important role in many outbreaks of calf bronchopneumonia. They increase the severity of the primary lung damage caused by viruses and exacerbate the clinical signs, frequently with fatal outcome. Furthermore, experimental studies indicate that *P. haemolytica* can act as a primary pathogen, producing severe acute pneumonia in calves [4]-[7].

Micotil injection, 300 mg tilmicosin per ml, is administered as a single subcutaneous injection at a dose rate of 10 mg/kg body weight. Tilmicosin has proven particularly effective in the treatment of bovine respiratory disease (BRD) [8]-[10]. While work undertaken in healthy animals can give a guide to the distribution of an antibiotic in the body, most cattle receive tilmicosin are clinically ill ones with pneumonia. The role of alveolar macrophages in the clearance of bacteria from lungs has recently attracted renewed interest [8]. Macrolide antibiotics are known to accumulate in leukocytes, bronchial secretions and penetrate into the subcellular compartments (lysosomes), thus contribute to the efficacy of the antibiotic [8]. Tilmicosin has an *in vitro* antibacterial spectrum that is primarily against Gram-positive, *Pasteurella* and *Mycoplasma* [11].

This study was undertaken to investigate the efficacy, disposition, distribution pattern and the penetration of tilmicosin into respiratory tract secretions of clinically healthy and experimentally *P. haemolytica*-infected calves after its subcutaneous and intravenous administration.

2. Materials and Methods

2.1. Drug

Tilmicosin (Micotil injection, 300 mg/ml) was supplied by Elanco Animal Health, England.

2.2. Animals and Husbandry

Ten calves aged one-and-a-half months, and weighing 60 to 70 kg were used in the experimental work. The calves were housed individually in adjacent boxes which had a floor area of 4 m² and a volume of 6 m³. The animals were bedded on straw. They were fed on milk replacer (free of antimicrobial substances twice daily at 09.00 and 18.00) and alfalfa with drinking water available *ad libitum*. The animals were shaved over jugular vein to facilitate the collection of blood samples.

2.3. Drug Administration

The calves were allocated, randomly to two groups of 5 animals each. The first group was treated with tilmicosin by the intravenous route and the second group was treated with tilmicosin subcutaneously. Tilmicosin solution (300 mg/ml) was diluted in saline to 2.5 mg/ml and the diluted solution was administered by slow intravenous infusion into the left jugular vein for 20 minutes at the rate of 0.5 mg/kg/min at a total dose of 10 mg/kg [12]. Each of the calves of the second group was given a single dose of 10 mg/kg of tilmicosin subcutaneously in the left dorso-lateral chest wall. All calves received tilmicosin on the same day.

2.4. Experimental Infection

Two weeks later after the intravenous and subcutaneous injection of tilmicosin, the calves were subjected to physical stress of a two-hour journey before they were inoculated with the pathogen. On day 0, approximately six hours after journey, all the calves were inoculated intratracheally with *P. haemolytica* type A1 (LPB 1419) [7]. A first-pass culture of *P. haemolytica* type A1 was inoculated onto 10 ml quantities of brain-heart-infusion broth (Oxoid), enriched with 5% fetal calf serum and incubated at 37°C in a shaking water bath for six hours. The approximate bacterial count of each broth, after six hours of incubation, was 1×10^9 cfu/ml. A polyethylene catheter (Intramedic, VEL) was inserted through the right nostril and advanced into trachea until it was 5 cm proximal to the bifurcation. The inoculum consisted of 5 ml of the six-hour culture of *P. haemolytica* type A1 diluted with 5 ml of sterile 0.9% sodium chloride.

2.5. Treatment Schedule

The first and second groups were treated with tilmicosin (10 mg/kg body weight) by the intravenous infusion and subcutaneous injections as mentioned before. Treatment was initiated when the calves had a body temperature of more than 39.5°C and a respiration rate of more than 52/min.

2.6. Clinical Examination

The calves were examined twice a day for 10 days (5 days before and 5 days after infection) at 09.00 and 18.00. Body temperature and respiratory rate were recorded daily at 10.00.

2.7. Blood Samples

Blood samples were obtained from the catheterized right jugular vein before and after infection. Samples were collected in heparinized tubes immediately before and at 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours following i.v. and s.c injections. Samples were centrifuged at 3000 rpm for 15 minutes and the obtained sera were used for the estimation of tilmicosin concentration. The serum samples were stored at -80°C until analysis, and the assay was performed within a week of obtainment.

2.8. Bronchial Secretions

Bronchial secretions were collected from the calves at the same intervals of the blood samples following tilmicosin administration before and after infection. Bronchial secretions (0.5 ml) were collected by a tampon-device introduced into the principal bronchi through the tracheal tube [13]. The bronchial fluid was used for estimation of tilmicosin concentration.

2.9. Cytological Analysis

Another blood samples were collected in vacutainer tubes (Venoject, Terumo) containing EDTA as an anti-clotting agent, once daily on the day of the inoculation (day 0) and on the following five days at 10.00 after drug administration. Total white cell counts and granulocyte/agranulocyte ratios were determined by standard methods.

2.10. Bronchoalveolar Lavage (BAL)

BAL fluid samples were obtained from infected animals on day 0, 3, 4 and 5 after tilmicosin administration to infected animals. A polyethylene tube (Intramedic, VEL) with an external diameter of 4.8 mm was inserted through the right nostril and advanced into trachea and the bronchial tree until an elastic resistance felt. Fifty ml of a sterile 0.9% solution of sodium chloride at 37°C was injected and aspirated immediately by gentle suction; approximately 75% of the infused fluid could be retrieved [7]. The neutrophil/macrophage ratio was determined in the BAL fluid. Cytological specimens were prepared by cytocentrifugation and standard WrightGiemsa stains were used.

From each BAL fluid sample, 10 ml was stored in sterile plastic tubes at 4°C and examined for the presence of bacteria and mycoplasmas within two hours after collection. All the samples were inoculated onto three media: Colombia blood agar (Oxoid), PPLO agar (Difco) enriched with 25% inactivated horse serum, 7% yeast extract, 400 µg/ml ampicillin, 0.05% thallium acetate and 1% glucose and Tween 80 PPLO agar (the same enriched and selective medium with 0.1% Tween 80) [7]. The blood plates were evaluated after 24 or 48 hours of incubation at 37°C in a carbon dioxide-enriched atmosphere, and the mycoplasma plates were evaluated after two days, and if negative, daily until 14 days after inoculation. The bacteria were identified by the techniques described by Carter (1984) [14].

2.11. Analytical Methods

The free tilmicosin concentrations in serum and bronchial secretions were measured by a microbiological assay technique [15] using *Micrococcus lutea* (ATCC 9431) as test organism [16]. The limit of detection of tilmicosin in serum and bronchial fluid was 4 ng/ml.

2.12. Pharmacokinetic Analysis

The concentrations of tilmicosin in serum and bronchial fluid were subjected to kinetic analysis and the pharmacokinetic parameters were calculated for each animal by classical methods [17] [18]. The non-compartmental pharmacokinetic parameters, volume of distribution at steady state ($V_{d(ss)}$), body clearance ($Cl_{(B)}$) and the mean residence time (MRT) were calculated according to standard methods using statistical moment theory [18]. The area under the blood concentration (C_{pt}) time (t) curve to infinity (AUC), and the area under the first moment curve ($AUMC = C_{pt} \times t^2$) were calculated from the first to the last blood and bronchial sample by using the trapezoidal rule, and an estimate of the residual area under the curve was obtained from $C_{p(t^*)}/\beta$, where $C_{p(t^*)}$ is the last measured blood and bronchial secretions concentration of tilmicosin, and β the overall elimination rate constant. An estimate of the residual part of the AUMC curve was obtained from $C_{p(t^*)} \times t^*/\beta$. The MRT, corrected for the duration of the infusion to give a corresponding value for a bolus injection, was calculated from AUC and AUMC ($MRT = AUMC/AUC - T/2$), where T is the infusion time. The MRT for the infusion (MRT_{inf}) was calculated from AUC and AUMC ($MRT_{inf} = AUMC/AUC + T/2$), where T is the infusion time (PCNONLIN, SCI, USA).

2.13. Statistical Analysis

Obtained data was analyzed by analysis of variance, and the mean values were compared by Duncan's Multiple Range test and student's "t" test (LSD) using the SAS statistical analysis program [19]. The results are given as mean \pm SD.

3. Results

3.1. Pharmacokinetic Studies

The slow intravenous infusion of a dilute solution of tilmicosin resulted in clinical signs suggesting acute cardiac toxicity which disappeared 25 minutes later. No side effects were observed after the subcutaneous injection.

The mean concentrations of tilmicosin in serum and bronchial secretions after tilmicosin injection in healthy and *P. haemolytica*-infected calves are shown in Figure 1 and Figure 2, respectively.

Values for the kinetic parameters describing the disposition of the drug are given in Table 1. Following intravenous injection of tilmicosin (10 mg/kg body weight), its concentration decreased in a biexponential manner that could be described in a two-compartment open model. Tilmicosin was rapidly distributed and slowly eliminated with mean half-lives of (2.70 and 2.76 hours) and (22.09 and 22.14 hours) for the distribution and elimination phases in healthy and *P. haemolytica*-infected calves, respectively. The volume of distribution was

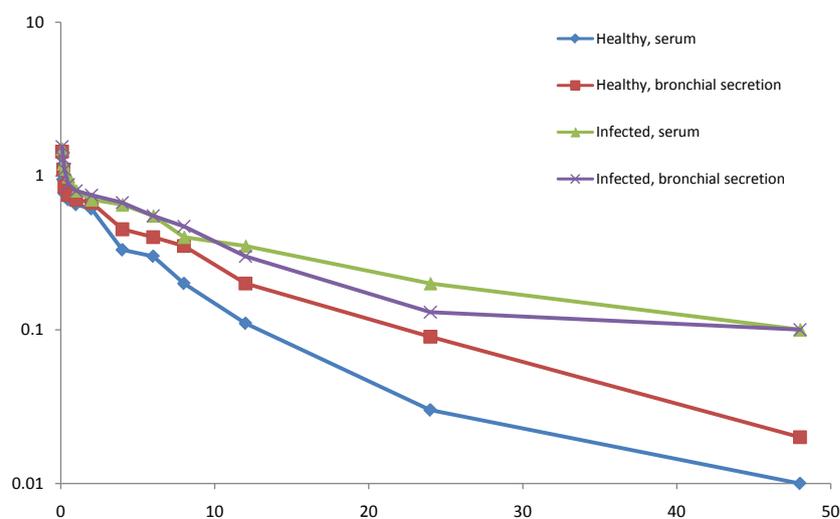


Figure 1. The serum and bronchial secretions concentration ($\mu\text{g/ml}$)-time profile of tilmicosin (10 mg/kg bw) after IV administration after tilmicosin injection in healthy and *P. haemolytica*-infected calves. Data are presented as the mean \pm SD.

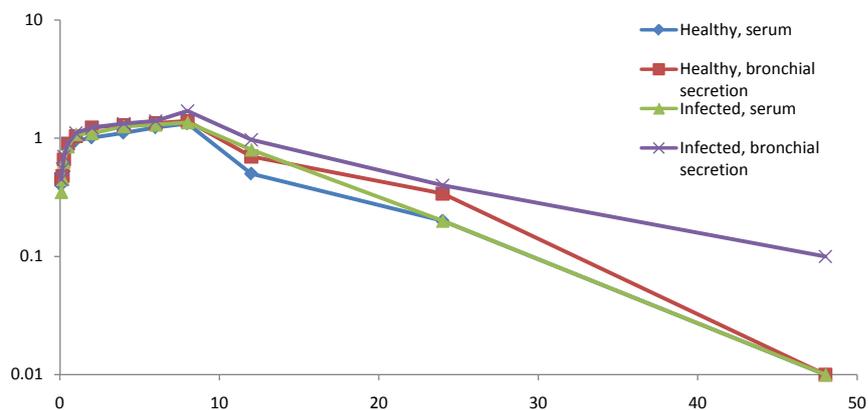


Figure 2. The serum and bronchial secretions concentration ($\mu\text{g/ml}$)-time profile of tilmicosin (10 mg/kg bw) after SC administration after tilmicosin injection in healthy and *P. haemolytica*-infected calves. Data are presented as the mean \pm SD.

Table 1. Mean (SD) pharmacokinetic parameters of tilmicosin in serum (S) and bronchial secretions (BS) after a slow intravenous infusion of $10 \text{ mg}\cdot\text{kg}^{-1}$ body weight in healthy and *Pasteurella haemolytica*-infected calves ($n = 5$).

Parameter	Healthy		Infected		LSD (Pr > F)
	S	BS	S	BS	
C° ($\mu\text{g}\cdot\text{ml}^{-1}$)	6.47 (0.38) ^c	7.43 (0.12) ^a	6.96 (0.14) ^b	7.47 (0.24) ^a	0.323 (0.0001)
A ($\mu\text{g}\cdot\text{ml}^{-1}$)	5.27 (0.29) ^c	6.12 (0.10) ^a	5.81 (0.07) ^b	5.79 (0.16) ^b	0.234 (0.0001)
α (h^{-4})	0.71 (0.03) ^a	0.64 (0.02) ^b	0.70 (0.02) ^a	0.56 (0.01) ^c	0.026 (0.0001)
$t_{1/2(\alpha)}$ (h)	2.70 (0.10) ^c	2.99 (0.07) ^b	2.76 (0.09) ^c	3.49 (0.05) ^a	0.105 (0.0001)
B ($\mu\text{g}\cdot\text{ml}^{-1}$)	1.20 (0.12) ^{bc}	1.29 (0.07) ^b	1.15 (0.08) ^c	1.69 (0.09) ^a	0.122 (0.0001)
β (h^{-2})	0.32 (0.03) ^a	0.32 (0.01) ^a	0.32 (0.02) ^a	0.34 (0.01) ^a	0.025 (0.2311)
$t_{1/2(\beta)}$ (h)	22.09 (1.96) ^a	21.72 (0.99) ^a	22.14 (1.10) ^a	20.53 (0.33) ^a	1.658 (0.1821)
V_c (Litre $\cdot\text{kg}^{-1}$)	1.55 (0.09) ^a	1.35 (0.02) ^c	1.44 (0.03) ^b	1.34 (0.04) ^c	0.071 (0.0001)
$V_{d(\text{area})}$ (Litre $\cdot\text{kg}^{-1}$)	4.28 (0.12) ^a	3.29 (0.08) ^c	3.83 (0.06) ^b	2.84 (0.10) ^d	0.124 (0.0001)
$V_{d(\text{ss})}$ (Litre $\cdot\text{kg}^{-1}$)	1.90 (0.09) ^a	1.63 (0.02) ^c	1.72 (0.02) ^b	1.72 (0.05) ^b	0.090 (0.0001)
$\text{Cl}_{(\text{B})}$ (Litre $\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)	0.14 (0.01) ^a	0.11 (0.004) ^c	0.12 (0.01) ^b	0.10 (0.003) ^d	0.009 (0.0001)
AUC ($\mu\text{g}\cdot\text{h}^{-1}\cdot\text{Litre}^{-1}$)	73.95 (6.18) ^d	95.19 (3.66) ^b	83.50 (3.43) ^c	104.35 (2.76) ^a	5.643 (0.0001)
AUMC ($\mu\text{g}\cdot\text{h}^{-1}\cdot\text{Litre}^{-1}$)	155.1 (44.01) ^a	185.1 (51.78) ^a	169.6 (32.65) ^a	202.0 (18.53) ^a	52.04 (0.2934)
AUMC _{inf} ($\mu\text{g}\cdot\text{h}^{-1}\cdot\text{Litre}^{-1}$)	2941 (240.17) ^a	2118 (525.58) ^{bc}	2525 (186.16) ^{ab}	1860 (189.82) ^c	427.5 (0.0004)
MRT (h)	2.25 (0.44) ^a	2.11 (0.49) ^a	2.20 (0.33) ^a	2.11 (0.18) ^a	0.508 (0.9107)
MRT _{inf} (h)	25.27 (1.45) ^a	26.60 (4.72) ^a	25.46 (1.41) ^a	28.36 (1.48) ^a	3.58 (0.2752)

^{a,b,c,d}Means with the same letter in the same row are not significantly different. LSD = Least significant difference.

more than 1 L/kg indicating good penetration into tissues and bronchial secretions. The total body clearance of the drug was 0.14 and 0.12 L/kg/h in healthy and *P. haemolytica*-infected calves, respectively. The mean MRT corrected for a bolus injection was 2.25 and 2.20 hours; the mean MRT_{inf} was 25.27 and 25.46 hours and the mean $V_{d(\text{ss})}$ was 1.90 and 1.72 L/kg in healthy and *P. haemolytica*-infected calves, respectively.

The mean peak concentration of tilmicosin in serum (C_{max} , 1.33 and 1.36 $\mu\text{g/ml}$) and bronchial secretions (1.4 and 1.7 $\mu\text{g/ml}$) was reached in (7.21 and 7.15 hours) and (7.11 and 7.1 hours) after subcutaneous injection in healthy and *P. haemolytica*-infected calves, respectively. *P. haemolytica* infection significantly increased the mean peak concentration of tilmicosin in bronchial secretions as compared with pre-infection values. **Table 2** and **Figure 2**, showed that tilmicosin was absorbed slowly from the injection site, as indicated by its long absorption half-lives ($t_{1/2\text{ab}}$, 7.72 and 6.87 hours) before and after infection. The mean elimination half-life ($t_{1/2\beta}$) after subcutaneous injection was 24.6 and 25.85 hours indicating that the drug was eliminated slowly.

3.2. Clinical Findings

The mean body temperature of the calves is shown in **Table 3**. During the five days before the infection and on the day of inoculation (day 0) none of the calves had a temperature exceeding 39°C. On the first day after inoculation (day 1), 12 hours after the intratracheal challenge, the body temperatures of all the calves were significantly higher than before the infection and ranged between 40°C and 40.2°C. The body temperatures of the calves treated subcutaneously remained significantly higher than before the infection throughout the experiment. However, on day 2, 3 and 4 their body temperatures were significantly lower than on day 1.

The mean respiratory rate of the calves is shown in **Table 3**. During the five days preceding the infection and on the day of inoculation, the respiratory rates of the 10 calves fluctuated between 24 and 32/min. By 12 hours after the intratracheal challenge the respiratory rate of all the calves was significantly higher than those before the infection. The respiratory rates returned to the pre-inoculation values on day 4 after the infection in the group treated subcutaneously.

3.3. Haematological Analysis

The means and ranges of the total WBC counts recorded in the two groups of calves on the day of the intratracheal challenge and on the five days after inoculation are shown in **Table 4**. The total WBC counts of all the calves were significantly higher on the first day after inoculation than before the intratracheal challenge. The

Table 2. Mean (SD) pharmacokinetic parameters of tilmicosin in serum (S) and bronchial secretions (BS) after a single subcutaneous injection of 10 mg·kg⁻¹ body weight in healthy and *Pasteurella haemolytica*-infected calves (n = 5).

Parameter	Healthy		Infected		LSD (Pr > F)
	S	BS	S	BS	
t _{1/2(ab)} (h)	7.72 (1.07) ^a	7.37 (0.19) ^{ab}	6.87 (0.38) ^b	8.11 (0.27) ^a	0.791 (0.0256)
C _{max} (µg·ml ⁻¹)	1.33 (0.25) ^b	1.40 (0.04) ^b	1.36 (0.05) ^b	1.70 (0.08) ^a	0.180 (0.0016)
t _{max} (h)	7.21 (0.11) ^a	7.11 (0.21) ^a	7.15 (0.16) ^a	7.10 (0.08) ^a	0.198 (0.6369)
t _{1/2(β)} (h)	24.60 (0.43) ^b	33.74 (1.86) ^a	25.85 (1.39) ^b	31.78 (2.05) ^a	2.096 (0.0001)
AUC (µg·h ⁻¹ ·l ⁻¹)	67.58 (4.16) ^c	83.7317.53) ^{ab}	70.24 (8.24) ^{bc}	84.91 (8.14) ^a	14.36 (0.0397)
AUC _{BS} /AUC _S		1.24 (0.26) ^a		1.22 (0.20) ^a	0.338 (0.9196)
F (AUC _{SC} /AUC _{IV})	0.916 (0.02) ^a	0.875 (0.16) ^a	0.839 (0.07) ^a	0.813 (0.06) ^a	0.122 (0.3401)

^{a,b,c}Means with the same letter in the same row are not significantly different. LSD = Least significant difference.

Table 3. Mean (SD) and [ranges] of body temperature (TEMP, °C) and respiratory rates (RESP, min⁻¹) in groups of 5 calves infected experimentally with *Pasteurella haemolytica* type A1 and treated with tilmicosin either slow intravenously or by subcutaneously.

Time (days)	Measurement	Intravenous	Subcutaneous
0	TEMP	38.00 (0.10) [37.9 - 38.1] ^c	38.00 (0.14) [37.8 - 38.2] ^e
	RESP	29.80 (1.48) [28 - 32] ^e	30.00 (1.23) [29 - 32] ^d
1	TEMP	40.16 (0.11) [40.0 - 40.3] ^a	40.10 (0.10) [40.0 - 40.2] ^a
	RESP	82.00 (1.58) [80 - 84] ^a	79.80 (2.39) [77 - 83] ^a
2	TEMP	39.72 (0.19) [39.5 - 40.0] ^b	39.28 (0.13) [39.1 - 39.4] ^b
	RESP	59.80 (3.83) [55 - 64] ^b	49.80 (2.86) [46 - 53] ^b
3	TEMP	39.40 (0.10) [39.3 - 39.5] ^c	39.00 (0.16) [38.8 - 39.2] ^c
	RESP	50.20 (2.39) [47 - 53] ^c	39.80 (2.86) [36 - 43] ^c
4	TEMP	39.30 (0.16) [39.1 - 39.5] ^{cd}	38.88 (0.19) [38.6 - 39.1] ^c
	RESP	44.40 (3.36) [40 - 48] ^d	31.60 (1.14) [30 - 33] ^d
5	TEMP	39.18 (0.15) [39.0 - 39.4] ^d	38.70 (0.70) [38.6 - 38.8] ^d
	RESP	42.60 (1.67) [40 - 44] ^d	29.80 (1.92) [27 - 32] ^d
MSE	TEMP	0.020	0.019
	RESP	6.533	4.767

^{a,b,c,d}Means with the same letter in the same column measurement are not significantly different at (P > 0.0001). MSE = Minimum significant error.

counts returned to the pre-inoculation values on day 2 after the infection in the calves treated subcutaneously and on day 3 after inoculation in the slow intravenously-infused calves.

Severe granulocytosis occurred on the first day after the infection in comparison with the pre-inoculation data, but it persisted for only two days after the inoculation in intravenously treated calves.

3.4. Bronchoalveolar Lavage (BAL)

The results of the mean Neutrophil/Macrophage (N/M) ratio in the BAL fluid are shown in **Table 4**. There were no significant changes in the N/M ratios in the subcutaneously treated calves.

P. haemolytica type A1 was not isolated from the BAL fluid samples before the intratracheal challenge. Furthermore, no other respiratory pathogens were isolated from of the calves before the infection. *P. haemolytica* type A1 was not isolated from BAL fluid samples of the subcutaneously-treated calves. *Mycoplasma bovis* was isolated from one of the five intravenously-treated calves on day 5 after inoculation. On all occasions the strains of *P. haemolytica* type A1 isolated were the same as that inoculated.

4. Discussion

The intrinsic antibacterial activity of tilmicosin together with its pharmacokinetic properties after subcutaneous injection suggests that they should be explored in field efficacy trials against *P. haemolytica* lung infection.

The results of this study showed that the serum and bronchial secretions concentrations of tilmicosin in experimentally *P. haemolytica*-infected calves remained above the minimum inhibitory concentrations (MICs) for the most sensitive bacteria (*A. pyogenes* and *S. aureus*) isolated from cattle, which range from 0.04 to 0.78 µg/ml [12] for three days after a slow intravenous or single subcutaneous administration. Similar results have been reported by Ramadan (1996) [20] who found that free tilmicosin concentrations in the serum of goats remained above the MIC of the drug for two days. [12] found that free tilmicosin concentrations in the udder of cows remained above the MIC of the drug for seven-and-a-half days after subcutaneous administration. The results are in accordance with those reported by Thompson and Lawrence (1994) [16] who attributed it to the basically and high lipid solubility of tilmicosin. Both of these characteristics indicate a large volume of distribution.

Table 4. Mean (SD) and [ranges] of total blood cell counts (WBC, $\times 10^3/\text{mm}^3$), blood granulocyte/agranulocyte ratios (G/AG) and BAL fluid neutrophil/macrophage ratios (N/M) in groups of 5 calves infected experimentally with *Pasteurella haemolytica* type A1 and treated with tilmicosin either slow intravenously or by subcutaneously.

Time (days)	Measurement	Intravenous	Subcutaneous
0	WBC	12.40 (2.41) [9.0 - 15.0] ^b	12.80 (2.39) [10.0 - 16.0] ^{bc}
	G/AG	0.92 (0.24) [0.6 - 1.2] ^b	0.84 (0.27) [0.5 - 1.1] ^{bc}
	N/M	0.15 (0.04) [0.10 - 0.21] ^b	0.31 (0.33) [0.12 - 0.90] ^a
1	WBC	27.20 (5.07) [21.0 - 34.0] ^a	25.60 (4.45) [20.0 - 31.0] ^a
	G/AG	2.62 (0.51) [2.0 - 3.3] ^a	2.80 (0.54) [2.1 - 3.5] ^a
	N/M	NS	NS
2	WBC	26.20 (4.66) [21.0 - 32.0] ^a	15.00 (4.74) [9.0 - 21.0] ^b
	G/AG	2.20 (0.57) [1.5 - 2.9] ^a	1.30 (0.51) [0.7 - 1.9] ^b
	N/M	NS	NS
3	WBC	17.80 (6.01) [10.0 - 25.0] ^b	11.20 (2.59) [5.0 - 15.0] ^{bc}
	G/AG	1.20 (0.46) [0.4 - 1.6] ^b	0.68 (0.30) [0.3 - 1.1] ^c
	N/M	NS	NS
4	WBC	14.60 (2.07) [12.0 - 17.0] ^b	9.20 (1.04) [8.0 - 10.5] ^c
	G/AG	0.78 (0.24) [0.5 - 1.1] ^b	0.60 (0.16) [0.4 - 0.8] ^c
	N/M	0.36 (0.08) [0.26 - 0.47] ^a	0.23 (0.04) [0.18-0.29] ^a
5	WBC	14.00 (1.58) [12.0 - 16.0] ^b	10.20 (1.30) [9.0 - 12.0] ^c
	G/AG	0.96 (0.21) [0.6 - 1.1] ^b	0.70(0.16) [0.5 - 0.9] ^c
	N/M	0.40 (0.07) [0.30 - 0.49] ^a	0.27 (0.06) [0.20 - 0.36] ^a
MSE	WBC	16.033	9.577
	G/AG	0.159	0.127
LSD	N/M	0.005	0.038

^{a,b,c}Means with the same letter in the same column measurement are not significantly different at ($P > 0.0001$ and $P > 0.805$ for N/M only). NS = Not sampled. MSE = Minimum significant error. LSD = Least significant difference.

The ion (pH) trapping of such basic molecules in areas of the body with a pH lower than the blood (7.4) can lead to high tissue concentration [21].

The subcutaneous administration of tilmicosin at 10 mg/kg resulted in free drug concentrations in serum and bronchial secretions which were under the MIC of the drug for *P. haemolytica* {0.78 to 6.25 or ≤ 2 $\mu\text{g/ml}$ [11], respectively}. Serum levels of macrolide antibiotics, such as tilmicosin, tend to be very low and are a poor indicator of total tissue concentrations [22]. Respiratory infections are effectively treated with doses producing serum concentrations lower than MIC for the pathogenic organisms because the lung/serum ratio can exceed 70:1 in cattle [8].

Following intravenous administration, the serum concentration data were best fitted to a two-compartment pharmacokinetic model. The slow intravenous infusion of a dilute solution of tilmicosin resulted in clinical signs suggesting acute cardiac toxicity but these side-effects were not observed after the subcutaneous injection. Similar clinical signs were also reported in cattle and goats [12] [20]. As a result, the subcutaneous route was found to be safer.

The apparent volume of distribution at steady-state of a drug ($V_{d(ss)}$) is an indication of its diffusion into body tissues [23]. The mean $V_{d(ss)}$ value of tilmicosin calves was 1.90 and 1.72 L/kg in healthy and *P. haemolytica*-infected calves, respectively, more than the 1.11 L/kg and lower than of 2.27 L/kg, reported for cows and goats [12] [20]. The higher values of $V_{d(are)}$, however, indicate that tilmicosin was widely distributed in the extravascular tissues. Our results showed that the volume of distribution is lower and the clearance rate is slower in infected calves as compared with healthy ones. These findings were consistent with the higher serum and bronchial secretions concentration of the drug recorded in diseased calves as compared to healthy ones. Similar results were observed by [8] who found that higher serum and lung concentrations of the tilmicosin in acutely pneumonic cattle than in healthy animals. In goats, sheep and cattle, [12] [20] [24] found that the half-lives of tilmicosin in milk (41.4 hours), lungs (26.9 hours) and udder (42.2 hours) was higher to its half-life in serum.

Following subcutaneous administration, the mean apparent elimination half-lives of tilmicosin in serum and bronchial secretions were (24.6 and 25.85 hours) and (33.74 and 31.78 hours) in healthy and *P. haemolytica*-infected calves, respectively. A similar half-lives (29.3 and 41.4 hours) was reported in serum and milk of healthy goats [20], in milk of cows (42.4 hours) [12], and in the serum of sheep (26.6 hours) [24]. This strongly suggests that tilmicosin partitions into lung secretions and possibly other tissues and becomes sequestered.

During the clinical efficacy experiments no calves died in the treated groups. Body temperature, respiratory rate, haematological and broncho-alveolar-lavage fluid parameters returned to normal significantly faster in subcutaneously treated group than in the intravenously treated calves. Similar findings were also observed for tilmicosin pneumonia of calves or pigs [25]-[32] who concluded that treatment with single dose of tilmicosin is effective in most cases of cattle or pigs bacterial pneumonia.

The results described in this paper are similar to earlier observations that *P. haemolytica* type A1 is capable of producing severe acute pneumonia in calves [5] [6]. The development of clinical signs (pyrexia, tachypnea, and anorexia), haematological changes (leucocytosis, neutophilia) and bacteriological findings were similar to those reported in clinical cases of "shipping fever" [5] [33]. The principle method for treating pneumonic pasteurellosis is antibiotic therapy [34]. Our results support previous reports that tilmicosin is an appropriate antimicrobial drug for the treatment of respiratory disease in cattle [32] [35]-[37] and this suggestion is supported by the results of the present study.

5. Conclusion

These preliminary results suggest that subcutaneous injection of tilmicosin can be effectively used in the treatment of acute *P. haemolytica* bronchopneumonia in calves.

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