

Pharmacokinetics and Distribution of Florfenicol in Bronchial Secretions of Healthy and *Pasteurella multocida* Infected Calves

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Abstract

Florfenicol was administered intravenously and intramuscularly at a dose rate of 20 mg/kg bwt to determine its concentration in blood and bronchial secretions as well as kinetic behavior in healthy and diseased calves. Severe acute bronchopneumonia was induced via inoculating the animals with *Pasteurella multocida*. Following intravenous (i.v.) administration, the serum concentration - time curve indicated a two compartment open model with a mean elimination half-lives ($t_{1/2\beta}$) of 4.10 and 4.84 h in healthy and infected calves, respectively. The mean volumes of distribution at steady state (V_{dss}) were 0.68 and 0.63 L/kg and the total body clearances (C_{cl}) were 0.15 and 0.11 L/kg/h with mean residence time (MRT) of 0.05 and 0.05 h, respectively. Florfenicol was slowly eliminated from serum and bronchial secretions with elimination half-lives ($t_{1/2el}$) of (12.43 and 17.23 h) and (13.74 and 22.46 h), respectively, following intramuscular (i.m.) injection. The peak concentrations (C_{max}) in serum and bronchial secretions were (3.70 and 4.06 $\mu\text{g/ml}$) and (6.88 and 7.62 $\mu\text{g/ml}$) attained at (3.07 and 3.01 h) and (1.54 and 1.70 h), respectively. The drug is extensively distributed to bronchial secretions with $AUC_{\text{bronchial secretion}} / AUC_{\text{serum}}$ ratio of 1: 2.53 and 1: 2.03, respectively. The clinical and hematological parameters in calves treated intramuscularly returned to normal faster than those treated intravenously. These results suggest that i.m. injection of florfenicol could be used for treatment of acute *P. multocida* bronchopneumonia in calves.

Keywords: Florfenicol; Disposition kinetics; Calves; Healthy; Diseased, Bronchial secretion

Introduction

Bronchopneumonia is one of the most economically important respiratory disorders affecting calves reared indoors [1,2]. The etiology of calf bronchopneumonia is complicated and could involve viruses, Mycoplasmas, and bacteria [3]. Bacteria, particularly *Pasteurella* species, play an important role in many outbreaks of calf bronchopneumonia. All *Pasteurella* species occur as commensals in the upper respiratory and alimentary tracts of their various hosts. In turn, they might increase the severity of the primary lung damage caused by viruses and exacerbate the clinical signs, frequently with fatal outcome (i.e. secondary to other infections). Varieties of some species, including *P. multocida* and / or *P. haemolytica* could act as primary pathogens and would produce severe acute pneumonia in calves [4-7].

Florfenicol is a broad spectrum antibiotic structurally related to thiamphenicol, which differ in substitution of a fluorine atom for a hydroxyl group. Because of this substitution, the antibacterial spectrum activity of florfenicol is superior to that of thiamphenicol [8,9]. It is active against many chloramphenicol-resistant strains of organisms, including *Pasteurella multocida*, *Pasteurella haemolytica*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli*, and *Haemophilus somnus* [8,10,11]. As it has an excellent antibacterial activity against a number of bacteria affecting cattle including *Pasteurella multocida*, *Pasteurella haemolytica*, and *Haemophilus somnus*, this could make the drug an ideal candidate for treatment of primary bacterial pathogens involved in bovine shipping fever [12-14].

While work undertaken in healthy animals can give a guide to the distribution of an antibiotic in the body, most calves receiving florfenicol will be clinically ill with pneumonia [9,15]. The role of alveolar macrophages in the clearance of bacteria from lungs has attracted an interest [16]. Some of the antibiotics are accumulated in

bronchial secretions and leukocytes and in turn, their penetration into the sub cellular compartments (lysosomes) would increase their clinical efficacies [7,16]. To guide effective therapy of pneumonia, this study was undertaken to investigate the disposition, distribution and the penetration pattern of florfenicol into respiratory tract secretions of clinically healthy and experimentally *P. multocida*-infected calves following i.v. and i.m administration at a dose rate of 20 mg/kg bwt.

Material and Methods

Drug

Florfenicol (Nuflor[®]) was supplied by Schering-Plough Animal Health, La Grindoliere, France.

Animals and husbandry

Ten calves aged one-and-a-half month and weighing from 65 to 75 kg were used in the experimental work. The calves were housed individually in adjacent boxes that had a floor area of 4 m² and a volume of 6 m³. The animals were bedded on straw. During acclimatization for one week and the subsequent treatment periods, calves 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.

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Received December 02, 2010; Accepted December 14, 2010; Published February 26, 2011

Citation: Ramadan A, Abd El-Aty AM (2011) Pharmacokinetics and Distribution of Florfenicol in Bronchial Secretions of Healthy and *Pasteurella multocida* Infected Calves. Pharm Anal Acta 2:117. doi:10.4172/2153-2435.1000117

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Drug administration

The calves were allocated randomly to two groups, 5 calves each. Florfenicol was administered to each calf of the first group by intravenous (i.v.) route into the left jugular vein at a dose rate of 20 mg/kg b wt. The calves of the second group were given a single dose of florfenicol, 20 mg/kg b wt, intramuscularly (i.m.) into the right cervical musculature (the manufacturer's recommended site of injection to avoid irritation). After injection, the site was massaged vigorously to enhance distribution of the drug solution into the surrounding musculature [9].

Experimental infection

Two weeks later after the i.v. and i.m. administration of florfenicol, the calves were subjected to physical stress of a two-hour journey before they were inoculated. On day 0 at 22.00 (approximately six hours after journey), all calves were inoculated intratracheally with *P. multocida*. A first-pass culture of *P. multocida* was inoculated into 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 [7]. Afterwards, the approximate bacterial count of each broth was 5×10^9 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 inoculums consisted of 5 mL of the six-hour culture of *P. multocida* diluted with 5 mL of sterile 0.9% sodium chloride.

Treatment schedule

The first and second group was treated with florfenicol (20 mg/kg/bwt) as aforementioned above. Treatment was initiated when the calves had a body temperature of more than 39.5°C and a respiratory rate of more than 52/min.

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.

Blood samples

Blood samples were obtained from the catheterized right jugular vein before and after infection. Samples were collected immediately before and at 0.25, 0.5, 1, 2, 4, 6, 8, 24, 36, and 48 h following i.v. and i.m. injections. Samples were centrifuged at 3000 rpm for 15 min and sera were used for estimation of florfenicol concentration. The serum samples were stored at -80°C pending analysis and the assay was performed within a week of obtainment.

Cytological analysis

Another blood samples were collected in vacutainer tubes (Venoject, Terumo) containing EDTA as an anti-clotting agent, once a day 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.

Bronchial secretions

Bronchoalveolar fluid samples were obtained from calves on day 0, 3, 4, and 5 after florfenicol 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 genital suction; approximately 75% of the infused

fluid could be retrieved [7]. The neutrophil/macrophage ratio was determined in the bronchoalveolar fluid. Cytological specimens were prepared by cytocentrifugation (Cytocentrifuge, Shandon Scientific) and standard Wright Giemsa stains were used.

From each bronchoalveolar 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 on 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 per cent Tween 80) [7]. The blood plates were evaluated after 24 or 48 h of incubation at 37°C in a carbon dioxide-enriched atmosphere. Mycoplasma plates were evaluated after two days and, if negative, daily until 14 days after inoculation. The bacteria were identified by the techniques described earlier [17].

Analytical method

The free florfenicol concentrations in serum and bronchial secretions were measured by a microbiological assay technique [18] using *Bacillus subtilis* (ATCC 6633) as the test organism [19]. Standard curves of florfenicol were prepared in pooled antibacterial free serum and bronchial secretions. Serum and bronchial secretions were directly added to the culture plates. The mean correlation coefficient (r^2) of the standard curves was found to be 0.998 and the lower limit of quantification both in serum and bronchial fluid was 0.01 µg/ml.

Pharmacokinetic analysis

The concentrations of florfenicol in serum and bronchial fluid were subjected to kinetic analysis, and the pharmacokinetic parameters were calculated by equations [20,21]. The non-compartmental parameters, including volume of distribution at steady state ($V_{d(ss)}$), body clearance ($Cl_{(B)}$), and the mean residence time (MRT) were calculated according to the statistical moment theory [21]. 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 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 florfenicol, 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 mean residence time (MRT) was calculated from AUC and AUMC ($MRT = AUMC / AUC$) [9].

Statistical analysis

Data obtained were analyzed by the analysis of variance, and the mean values were compared by students "t" test. LSD was calculated by the SAS statistical analysis program [22]. The results are given as mean \pm SD.

Results

Pharmacokinetic studies

The mean concentrations of florfenicol in serum and bronchial secretions following i.v. and i.m injection in healthy and *P. multocida*-infected calves are shown in (Figure 1 and 2) respectively.

Values for the kinetic parameters describing the disposition of the drug are given in (Table 1). Following i.v. administration, the drug concentration decreased in a biexponential manner that could be described into a two-compartment open model. Florfenicol was slowly

distributed and eliminated with mean half-lives of (1.09 and 1.25 h) and (4.10 and 4.84 h) for the distribution and elimination phases in healthy and infected calves, respectively. The volume of distribution was less than 1 L/kg, indicating lower penetration into tissues. The total body clearance and the MRT were 0.15 and 0.11 L/kg/h and 0.05 and 0.04 h, respectively.

The mean peak concentration of florfenicol in serum (C_{max} , 3.70 and 4.06 $\mu\text{g/mL}$) and bronchial secretions (6.88 and 7.62 $\mu\text{g/mL}$) was reached (3.07 and 3.01 h) and (1.54 and 1.70 h) following i.m. injection in healthy and infected calves, respectively. *P. multocida* infection was significantly increased the mean peak concentration of florfenicol in bronchial secretions compared with pre-infection. (Table 2, Figure 2), complied that the drug was absorbed slowly from the site of injection, as indicated by its long absorption half-lives ($t_{1/2ab}$, 4.44 and 4.11 h), respectively. The mean elimination half-life ($t_{1/2el}$) was 12.43 and 13.74 h, indicating that the drug was slowly eliminated.

Clinical findings

During the five days before the infection and on the day of inoculation (day 0), none of the calves had suffered from fever and their

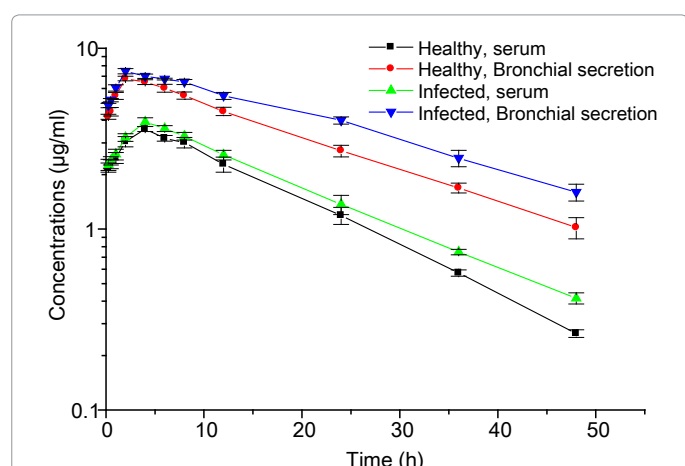


Figure 1: Semilogarithmic plot of the mean serum, and bronchial secretion concentrations ($\mu\text{g/ml}$) of florfenicol following intravenous injection of 20 mg/kg b.wt in healthy and *P. multocida* infected calves.

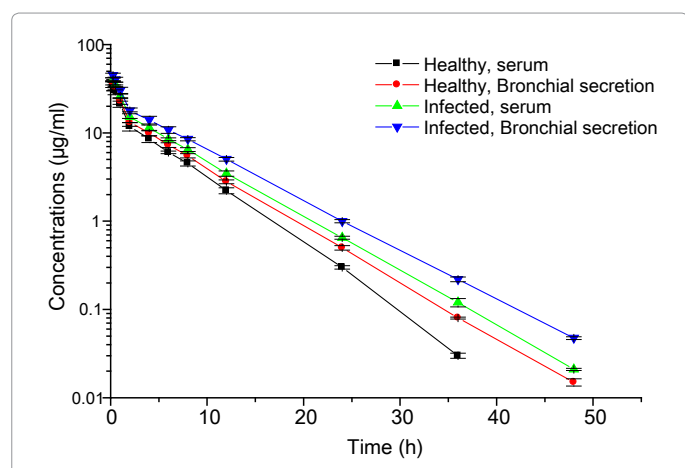


Figure 2: Semilogarithmic plot of the mean serum, and bronchial secretion concentrations ($\mu\text{g/ml}$) of florfenicol following intramuscular injection of 20 mg/kg b.wt in healthy and *P. multocida* infected calves.

respiratory rates were fluctuated between 25 and 31/min. On the first day (12 h after the intratracheal challenge) the body temperatures as well as the respiratory rates were significantly higher than before infection (Table 3). The body temperatures of calves treated intramuscularly remained significantly higher than before infection on day 1, 2, 3 and 4. However, on day 5 their body temperatures were significantly lower than the other days. On the contrary, the respiratory rates returned to the pre-inoculation values on day 4 and 5.

Hematological 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 counts returned to the pre-inoculation values on day 2 after infection in calves treated intramuscularly and on day 3 in intravenous -injected calves.

The means and ranges of granulocyte/agranulocyte (G/AG) ratio recorded in calves of both groups on the day of intratracheal challenge onwards had demonstrated severe granulocytosis on the first day after infection, compared with the pre-inoculation data (Table 4).

Bronchoalveolar lavage (BAL)

The mean neutrophil/macrophage (N/M) ratio in the BAL fluid is shown in (Table 4). There were significant increases in the N/M ratios on day 4 and 5 after infection, in calves treated intramuscularly.

P. multocida was not isolated from the BAL fluid samples taken from any day of the animals before the intratracheal challenge. Furthermore, no other respiratory pathogens were isolated from any calves before infection. *Mycoplasma bovis* was isolated from two of the five intravenous-treated calves on day 5 after inoculation. On all occasions the strains of *P. multocida* isolated was the same as that inoculated.

Discussion

This study used the bioassay to determine the pharmacokinetics and bronchial secretion concentrations of florfenicol in healthy and infected calves. The bioassay did not distinguish the active metabolite from the parent compound. Since the metabolites are microbiologically active, their presence may not necessarily interfere with determination of a therapeutic dosage regimen [23]. Lavy et al. [24] found that chemical (HPLC) and microbiological assay methods for florfenicol gave very similar test results.

The intrinsic antibacterial activity of the drug together with its pharmacokinetic properties after subcutaneous injection suggest that systemic florfenicol bronchopneumonia calves therapy should be explored, in field efficacy trials, as a means of eliminating *P. haemolytica* lung infections.

The results of this study had shown that the serum and bronchial secretions concentrations of florfenicol in experimentally *P. haemolytica*-infected calves remained above the minimum inhibitory concentrations (MICs) for the most sensitive bacteria (*P. multocida* and *P. haemolytica* (0.25 to 1 $\mu\text{g/mL}$), and *A. pleuropneumoniae* (0.2 to 1.56 $\mu\text{g/mL}$) isolated from cattle [13,25] for 24 and 36 h post i.v. and i.m. injection. Similar results have been reported by Adams et al. [26], Lobell et al. [9], and Varma et al. [15], who found that free florfenicol concentrations in the serum of calves were remained above the MIC of the drug for one to two days.

The disposition kinetic of florfenicol in calves after a single i.v. injection was adequately described by a biexponential terms with a slow

distribution and elimination phases. Our findings were similar to those reported in calves [12,26] and cattle [15,27].

The apparent volume of distribution at steady-state (V_{dss}) is an indication of its diffusion into body tissues [28]. Florfenicol showed a low volume of distribution (0.68 L/kg), indicating a lower penetration extent into tissues [20]. Our results showed that the volume of distribution is lower and the clearance rate is slower in bronchial secretions of infected calves compared to healthy ones. These findings were consistent with the higher serum and bronchial secretions

concentrations recorded in infected calves compared to healthy ones. Similar results were observed by Thompson et al. [16] who found a high serum and lung concentrations of tilmicosin in acute pneumonic cattle than in healthy animals. On the other hand, florfenicol showed high body clearance rate (0.15 L/kg/h) in calves, the finding which is at variance to those reported by Afifi and Abo El-Sooud [19] in chicken. This could be attributed to the anatomical and physiological variations between different species.

After i.m. injection, the mean apparent elimination half-lives of

Parameter	Healthy		Infected		LSD (Pr > F)
	S	BS	S	BS	
C _i (g/ml)	42.35(4.20) ^b	41.65(2.12) ^b	45.05(3.88) ^b	51.60(2.95) ^a	4.536(0.001)
A (μg/ml)	26.22(2.60) ^{ab}	24.70(1.60) ^b	25.40(2.27) ^{ab}	27.70(1.35) ^a	2.705(0.148)
α (h ⁻¹)	0.639(0.04) ^a	0.591(0.05) ^{ab}	0.560(0.06) ^{bc}	0.512(0.02) ^c	0.061(0.003)
t _{1/2(α)} (h)	1.089(0.08) ^c	1.178(0.09) ^{bc}	1.248(0.13) ^{ab}	1.354(0.05) ^a	0.125(0.002)
B (μg/ml)	16.13(1.69) ^c	16.95(1.93) ^c	19.65(1.75) ^b	23.27(1.99) ^a	2.469(0.0001)
β (h ⁻¹)	0.169(0.004) ^a	0.149(0.004) ^b	0.144(0.008) ^b	0.127(0.003) ^c	0.007(0.0001)
t _{1/2(β)} (h)	4.099(0.10) ^c	4.669(0.12) ^b	4.841(0.25) ^b	5.460(0.12) ^a	0.215(0.0001)
V _c (L/kg)	0.467(0.05) ^a	0.481(0.02) ^a	0.447(0.04) ^a	0.389(0.02) ^b	0.045(0.002)
V _{d(area)} (L/kg)	0.875(0.11) ^a	0.870(0.10) ^a	0.769(0.09) ^{ab}	0.658(0.06) ^b	0.124(0.005)
V _{d(ss)} (L/kg)	0.681(0.09) ^a	0.694(0.07) ^a	0.628(0.09) ^{ab}	0.545(0.05) ^b	0.101(0.026)
Cl _(B) (L/kg/h)	0.148(0.02) ^a	0.129(0.01) ^b	0.110(0.008) ^c	0.083(0.006) ^d	0.014(0.0001)
AUC (μg/ml. h ⁻¹)	0.137(0.01) ^c	0.156(0.01) ^c	0.183(0.01) ^b	0.241(0.02) ^a	0.020(0.0001)
AUMC (μg/ml.h ²)	6.337(0.29) ^c	4.838(0.40) ^d	7.026(0.22) ^b	17.913(0.38) ^a	0.443(0.0001)
MRT (h)	0.047(0.003) ^b	0.031(0.001) ^d	0.039(0.004) ^c	0.075(0.004) ^a	0.004(0.0001)

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

Table 1: Mean (SD) pharmacokinetic parameters of florfenicol in serum (S) and bronchial secretions (BS) after intravenous injection of 20 mg/kg b wt in healthy and *Pasteurella multocida*-infected calves (n = 5).

Parameter	Healthy		Infected		LSD
	S	BS	S	BS	
t _{1/2(ab)} (h)	4.44(0.09) ^a	2.37(0.20) ^b	4.11(0.50) ^a	2.58(0.09) ^b	0.372
C _{max} (μg/mL)	3.70(0.08) ^d	6.88(0.23) ^b	4.06(0.24) ^c	7.62(0.26) ^a	0.289
t _{max} (h)	3.07(0.21) ^a	1.54(0.10) ^b	3.01(0.14) ^a	1.70(0.08) ^b	0.190
t _{1/2(el)} (h)	12.43(0.90) ^d	17.23(1.25) ^b	13.74(0.50) ^c	22.46(0.96) ^a	1.262
AUC (μg/ml. h ⁻¹)	0.096(0.009) ^c	0.195(0.02) ^b	0.108(0.007) ^c	0.263(0.03) ^a	0.025
AUC _{BS} /AUC _S	-	2.529(0.04) ^a	-	2.034(0.10) ^a	0.111
F (AUC _{i,m} /AUC _{i,v})	0.704(0.01) ^c	1.253(0.02) ^a	0.591(0.04) ^d	1.132(0.008) ^b	0.030

(a, b, c, d) Means with the same letter in the same row are not significantly different at (Pr > F 0.0001). LSD = Least significant difference.

Table 2: Mean (SD) pharmacokinetic parameters of florfenicol in serum (S) and bronchial secretions (BS) after a single intramuscular injection 20 mg/kg b wt in healthy and *Pasteurella multocida*-infected calves (n = 5).

Time (days)	Measurement	Intravenous	Intramuscular
0	TEMP	38.20 (0.16)(38.0-38.4) ^d	38.20 (0.10)(38.1-38.3) ^a
	RESP	31.40 (3.36)(27-35) ^e	31.60 (2.70)(28-35) ^d
1	TEMP	40.80 (1.30)(39.0-42.0) ^a	40.08 (0.19)(39.8-40.3) ^a
	RESP	85.80 (3.49)(81.0-90.0) ^a	83.00 (2.24)(80.0-86.0) ^a
2	TEMP	40.00 (1.22)(39.8-40.1) ^b	39.10 (0.16)(38.9-39.3) ^b
	RESP	66.00 (4.12)(61.0-71.0) ^b	51.80 (2.59)(48.0-55.0) ^b
3	TEMP	39.02 (0.24)(38.7-39.3) ^c	38.78 (0.19)(38.5-39.0) ^c
	RESP	53.80 (2.86)(50.0-57.0) ^c	41.20 (2.39)(38.0-44.0) ^c
4	TEMP	38.70 (0.16)(38.5-38.8) ^c	38.56 (0.11)(38.4-38.7) ^d
	RESP	42.80 (2.39)(40.0-46.0) ^d	34.00 (2.55)(31.0-37.0) ^d
5	TEMP	38.50 (0.26)(38.2-38.8) ^{cd}	38.32 (0.13)(38.2-38.5) ^e
	RESP	32.80 (2.49)(29.0-35.0) ^e	32.00 (1.58)(30.0-34.0) ^d
LSD	TEMP	0.732	0.119
	RESP	4.148	3.094

(a, b, c, d, e) Means with the same letter in the same column measurement are not significantly different at (Pr > F 0.0001). 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 multocida* and treated with florfenicol either intravenously or intramuscularly.

Time (days)	Measurement	Intravenous	Intramuscular
0	WBC	14.30(2.41)(10.0-16.0) ^c	13.30(2.59)(9.0-15.5) ^b
	G/AG	0.81(0.21)(0.55-1.10) ^c	0.81(0.25)(0.50-1.10) ^c
	N/M	0.14 (0.03)(0.11-0.17) ^c	0.14 (0.02)(0.12-0.17) ^c
1	WBC	30.00(5.70)(23.0-37.0) ^a	27.60(4.93)(21.0-34.0) ^a
	G/AG	3.18(0.46)(2.60-3.80) ^a	2.90(0.60)(2.10-3.70) ^a
	N/M	NS	NS
2	WBC	19.8(3.83)(15.0-24.0) ^b	15.40(2.41)(12.0-18.0) ^b
	G/AG	1.98(0.30)(1.60-2.40) ^b	1.48(0.30)(1.10-1.90) ^b
	N/M	NS	NS
3	WBC	16.40(2.41)(13.0-19.0) ^{bc}	14.80(2.59)(11.0-18.0) ^b
	G/AG	2.12(0.49)(1.50-2.70) ^b	0.86(0.31)(0.50-1.20) ^c
	N/M	0.23(0.05)(0.17-0.29) ^b	0.20(0.04)(0.15-0.26) ^{bc}
4	WBC	16.00(2.74)(12.0-19.0) ^{bc}	13.80(2.86)(10.0-17.0) ^b
	G/AG	0.86(0.04)(0.80-0.91) ^c	0.78(0.28)(0.45-1.10) ^c
	N/M	0.30 (0.07)(0.21-0.39) ^{ab}	0.25 (0.02)(0.19-0.32) ^{ab}
5	WBC	14.60 (1.14)(13.0-16.0) ^c	12.80 (2.39)(10.0-16.0) ^b
	G/AG	0.80(0.16)(0.60-1.00) ^c	0.80(0.20)(0.55-1.00) ^c
	N/M	0.36 (0.05)(0.29-0.43) ^a	0.29 (0.06)(0.21-0.37) ^a
LSD	WBC	4.381	4.037
	G/AG	0.418	0.455
	N/M	0.069	0.062

(^{a, b, c})Means with the same letter in the same column measurement are not significantly different at (Pr>F 0.0001). NS = Not sampled. LSD = Least significant difference.

Table 4: Mean (SD) and ranges of total blood cell counts (WBC, × 10³ 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 multocida* and treated with florfenicol either intravenously or intramuscularly.

florfenicol in serum and bronchial secretions were (12.43 and 17.23 h) and (13.74 and 22.46) in healthy and infected calves, respectively. Similar half-life values (18 and 5.57 h) were previously reported in serum of healthy calves [9,26]. This strongly supports the idea that florfenicol partitions into lung secretions and possibly other tissues and becomes sequestered.

The bioavailability of florfenicol following i.m. injection was moderate with approximately 70.4 and 59.1 being absorbed in healthy and infected calves, respectively. These values were similar to those reported in calves (78.5 and 88.8% [9,26]) and in equine (81%, [29]).

The results described in this article were similar to an earlier observation, which indicated that *P. multocida* could be able to produce severe acute pneumonia in conventional calves [6]. The development of clinical signs (pyrexia, tachypnea, anorexia), hematological changes (leucocytosis, neutophilia) and bacteriological findings were very similar to those reported in clinical cases of “shipping fever” [5,30]. The principle method for treating pneumonic pasteurellosis is antibiotic therapy [31]. Florfenicol has been suggested as an appropriate antimicrobial drug for the treatment of respiratory disease in cattle [15,27], the suggestion which is supported by the results of the present study.

During the clinical efficacy experiments, no calves died in the treated groups. Body temperature, respiratory rate, hematological and bronchoalveolar-lavage fluid parameters returned to normal significantly faster in i.m. treated group than the i.v. Similar findings were reported by Lockwood et al. [14] and Navetat et al. [32] who concluded that treatment with single dose of florfenicol is effective in most cases of cattle or pigs bacterial pneumonia.

Conclusion

These results suggest that i.m. injection of florfenicol could be used for treatment of acute *P. multocida* bronchopneumonia in calves.

References

1. Vermeersch H, Vandebossche G, Remon JP, Vandendriessche K, Sustronck B, Muylle E, Deprez P (1994) Pharmacokinetics and efficacy of nebulized sodium ceftiofur in the treatment of *Pasteurella haemolytica* bronchopneumonia. Proceedings of the European Association of Veterinary Pharmacology and Toxicology, 6th International Congress, Edinburgh, pp 94-95.

2. Scott PR (1995) Efficacy of strategic tilmicosin injection during an outbreak of respiratory disease in housed beef calves. Br Vet J 151: 587-589.
3. Dyer RM (1982) The bovine respiratory disease complex: a complex interaction of host, environment and infectious factors. Compendium on Continuing Education for the Practicing Veterinarian 4: S296-S304.
4. Confer AW, Panciera RJ, Corstvet RE, Rummage JA, Fulton RW (1984) Bovine pneumonic pasteurellosis: effect of culture age of *Pasteurella haemolytica* used as a live vaccine. Am J Vet Res 45: 2543-2545.
5. Gibbs HA, Allan EM, Wiseman A, Selman IE (1984) Experimental production of bovine pneumonic pasteurellosis. Res Vet Sci 37: 154-166.
6. Ames TR, Markham RJ, Opuda-Asibo J, Leininger JR, Maheswaran SK (1985) Pulmonary response to intratracheal challenge with *Pasteurella haemolytica* and *Pasteurella multocida*. Can J of Comp Med 49: 395-400.
7. Sustronck B, Deprez p, Muylle E, Vermeersch G, et al. (1995) Evaluation of the nebulisation of sodium ceftiofur in the treatment of experimental *Pasteurella haemolytica* bronchopneumonia in calves. Res Vet Sci 59: 267-271.
8. Varma KJ, Adams PE, Lobell RD, Lamendola JF, Houdeshell JW, et al. (1986) Pharmacokinetics of florfenicol in veal calves J Vet Pharmacol Ther 9: 412-425
9. Lobell RD, Varma, KJ, Johnson JC, Sams RA, Gerken DF, et al (1994) Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. J Vet Pharmacol Ther 17: 253-258.
10. Neu HC, Fu KP (1980) *In vitro* activity of chloramphenicol and thiamphenicol analog. Antimicrob Agents Chemother 18: 311-316.
11. Syriopoulou VP, Harding AL, Goldmann DA, Smith AL (1981) *In vitro* antibacterial activity of fluorinated analogs of chloramphenicol and thiamphenicol. Antimicrob Agents and Chemother 19: 294-297.
12. Varma KJ, Adams PE, Powers TE, Powers JD, Lamendola JF (1986) Pharmacokinetics of florfenicol in veal calves. J Vet Pharmacol Ther 9: 412-425.
13. Hörmansdorfer S, Bauer J (1996) Resistance pattern of bovine *Pasteurella*. Berl Munch Tierarztl Wochenschr 109: 168-171.
14. Lockwood P, De Haas VD, Katz TMS, Varma KJ (1996) Clinical efficacy of florfenicol in the treatment of shipping fever in cattle in the USA. Medicina Veterinaria 13: 34-38.
15. Varma KJ, Walker RD, Sams RA, Holland RE, Trenti F (1994) Pharmacokinetics and distribution of florfenicol in bronchial secretions and tissue cage fluid in cattle. Proceedings 18th World Buiatrics Congress: 26th Congress of the Italian Association Buiatrics, Bologna, Italy, pp. 547-550.
16. Thompson TD, Lawrence K (1994) Micolil: pharmacokinetics of tilmicosin, a

- semi-synthetic macrolide antibiotic, in cattle. Proceedings of the European Association for Veterinary Pharmacology and Toxicology, 6th International Congress, Edinburgh, 7-11 August 1994. pp 55-56.
17. Carter GR (1984) "Diagnostic Procedure in Veterinary Bacteriology and Mycology", 4th edn., Springfield, Illinois, Charles C. Thomas, USA, pp. 129-142.
 18. Bennett JV, Brodie JL, Benner EJ, Kirby WM (1966) Simplified, accurate method for antibiotic assay of clinical specimens. *Appl Microbiol* 14: 170-177.
 19. Afifi, NA, Abo El-Sooud KA (1997) Tissue concentrations and pharmacokinetics of florfenicol in broiler chickens. *Br Poult Sci* 38: 425-428.
 20. Baggot J.D. (1978) Some aspects of clinical pharmacokinetics in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics* 1: 5-18.
 21. Gibaldi M (1984) *Biopharmaceutics and clinical pharmacokinetics*, 3rd edn., Philadelphia, Lea and Febiger. pp 45-111.
 22. SAS Institute (1982) *SAS User's Guide*. SAS Institute, Cary, NC. pp 191-193.
 23. Sams RA (1994) Florfenicol: Chemistry and metabolism of a novel broad-spectrum antibiotic. *International Symposium on bovine Respiratory Disease. New Therapeutic Advances* 13-17.
 24. Lavy E, Ziv G, Soback S, Gilckman A, Winkler M (1991) Clinical pharmacology of florfenicol in lactating goats. Proceedings of the European Association for Veterinary Pharmacology and Toxicology, 5th International Congress, Copenhagen, Denmark, 1991. *Act Vet Scandinavica Suppl.* 87: 133-136.
 25. Ueda Y, Suenaga I (1995) In vitro antibacterial activity of florfenicol against *Actinobacillus pleuropneumoniae*. *J Vet Med Sci* 57: 363-364.
 26. Adams PE, Varma KJ, Powers TE, Lamendola JF (1987) Tissue concentrations and pharmacokinetics of florfenicol in male veal calves given repeated doses. *American Journal of Veterinary Research* 48: 1725-1732.
 27. Soback S, Paape MJ, Filep R, Varma, KJ (1995) Florfenicol pharmacokinetics in lactating cows after intravenous, intramuscular and intramammary administration. *J Vet Pharmacol Ther* 18: 413-417
 28. Gilman AG, Goodman LS, Gilman A (1980) *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, 6th edn., Macmillan, New York, P 21.
 29. McKellar QA, Varma KJ (1996) Pharmacokinetics and tolerance of florfenicol in Equidae. *Equine Veterinary Journal* 28: 209-213.
 30. Allan EM, Gibbs HA, Wiseman A, Selman IE (1983) *Pasteurella haemolytica* pneumonia in weaned housed single suckled calves. *Vet Rec* 112: 327
 31. Whiteley LO, Maheswaran SK, Weiss DJ, Ames TR, Kannan MS (1992) *Pasteurella haemolytica* A1 and bovine respiratory disease: pathogenesis. *J Vet Intern Med* 6: 11-22.
 32. Navetat H, Concordet D, Lockwood P, Madelenat A, Espinasse (1995) Efficacy of florfenicol in the therapy of acute pneumonia in housed beef calves. *Revue de Medecine Veterinaire* 148: 567-574.