Received: 19 July 2022 | Revised: 1 December 2022 | Accepted: 25 December 2022

DOI: 10.1111/jvp.13110

JOURNAL OF Veterinary Pharmacology and Therapeutics WILEY

ORIGINAL ARTICLE

Comparative pharmacokinetics of florfenicol in heifers after intramuscular and subcutaneous administration

Renat Selimov | Elizaveta Goncharova | Pavel Koriakovtsev | Diana Gabidullina | Julia Karsakova | Sergey Kozlov | Alexander Komarov | Ekaterina Engasheva | Sergey Engashev

AVZ, Ltd, Moscow, Russia

Correspondence
Elizaveta Goncharova, AVZ, Ltd, Moscow 129329, Russia.
Email: goncharova.betty@ya.ru

Funding information
AVZ Ltd

Abstract

Florfenicol was administered to five heifers intramuscularly at a dose rate of 20 mg/kg bwt and following wash-out period, subcutaneously at a dose rate of 40 mg/kg bwt. Blood plasma samples were collected from heifers before injection of florfenicol and up to 120 h after intramuscular (IM) injection and up to 264 h after subcutaneous (SC) injection. Florfenicol concentrations in plasma were measured by high-performance liquid chromatography with mass-spectrometric detection. Pharmacokinetics of florfenicol was estimated using non-compartment analysis. Mean maximum plasma concentration, area under the concentration–time curve and elimination half-life for florfenicol were 3.2 μg/ml, 101.5 μg×h/ml and 24.5 h, respectively, after IM injection at 20 mg/kg bwt, and 2.7 μg/ml, 194.5 μg×h/ml and 103.8 h, respectively, after SC injection at 40 mg/kg bwt. The obtained results indicated that both administration routes provided comparable bioavailability, whereas SC route was attributed with lower peak levels and markedly slower absorption of florfenicol from injection site. Both administration routes provided plasma florfenicol levels which are expected to be effective against prevalent infectious agents of cattle.

KEYWORDS
comparative, florfenicol, intramuscular, pharmacokinetics, subcutaneous

1 | INTRODUCTION

Florfenicol is a broad-spectrum antibiotic which is effective against numerous pathogens of livestock animals. The mechanism of florfenicol action is based on binding to the 50S ribosomal subunit of a bacterial cell, which inhibits protein synthesis in pathogenic organisms (Cannon et al., 1990; Plumb, 2004). This mechanism is also typical for related amphenicol antibiotics such as chloramphenicol and thiamphenicol. However, florfenicol exhibits greater antibacterial activity compared to these two compounds, and is effective against a number of pathogens that have developed resistance to chloramphenicol and thiamphenicol (Papich, 2020). In addition, chloramphenicol is banned from the use for farm animals in many countries, which further limits the choice of available amphenicols for treatment.

A number of studies of bacterial isolates from cattle have demonstrated that many types of bacteria are sensitive to florfenicol, in particular, prevalent bacteria that cause respiratory disease in cattle (BRD), namely *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* (de Jong et al., 2014; EMA, 2017; Kehrenberg et al., 2004; Plumb, 2004; The United States Pharmacopeial Convention, 2007; Wentzel, 2012), as well as other pathogens such as *Bacteroides melaninogenicus* and *Fusobacterium necrophorum* (The United States Pharmacopeial Convention, 2007).

These properties predetermine the area of clinical applications of florfenicol-containing drugs, which are extensively used to treat

respiratory disease in cattle (Aslan et al., 2002; Catry et al., 2008; The United States Pharmacopeial Convention, 2007; Varma et al., 1998). In addition, florfenicol is used to treat pododermatitis and infectious keratoconjunctivitis (Dowling, 2013). Also, florfenicol is reported to be effective in the treatment of septic arthritis due to its ability to penetrate into the synovial fluid (Jones et al., 2015).

The action of florfenicol is facilitated by its high lipophilicity, due to which it is capable of intensive penetration from blood into tissues (Anadón et al., 2008; Papich, 2020). It is also facilitated by the low degree of florfenicol's binding to plasma proteins, which, according to literature sources, ranges from 1% to 5% (Foster et al., 2016).

Florfenicol was classified as a time-dependent antibiotic in numerous publications (Dumka & Singh, 2014; Hesje et al., 2007; Holmes et al., 2012; Lacroix et al., 2011; Martinez et al., 2013). However, several authors suggested florfenicol to be concentration dependent or co-dependent (dependent both on concentration and time) relying on its time–kill curves against bovine respiratory pathogens (de Haas et al., 2002; Illambas et al., 2013; Sidhu, et al., 2014).

The most common routes for administration of florfenicol-based drugs to animals are intramuscular (IM) and subcutaneous (SC) injections. In the literature, there are numerous publications on pharmacokinetics of florfenicol in different animals such as cattle (Rassouli et al., 2011; Sidhu et al., 2014; Soback et al., 1995), pigs (Castillo et al., 2020; Liu et al., 2003), sheep (Birdane et al., 2015; Lane et al., 2004; Shen et al., 2004), elk (Alcorn et al., 2004), llamas (Pentecost et al., 2013), dogs (Birdane & Birdane, 2016).

However, regarding comparative pharmacokinetic studies for IM and SC administration of florfenicol, there are few published studies. To our knowledge, there are published data only for sheep (Balcomb et al., 2018; El-Sheikh et al., 2009) and alpacas (Holmes et al., 2012). In addition, one study was also published comparing the pharmacokinetics of IM and SC administration in cattle, but the drug used for this study contained two active substances—not only florfenicol, but also flunixin (Lacroix et al., 2011). It is important to note that in the works cited above, there is a general tendency that florfenicol is absorbed slower, reaches lower maximum plasma and is excreted slower after SC administration than after IM administration. However, in one above-mentioned study on sheep (El-Sheikh et al., 2009), the comparable elimination half-life values were reported for both routes of administration, and similar results were obtained in another study (Balcomb et al., 2018) for one of the two drugs studied. The aim of our work was to study and compare pharmacokinetics of florfenicol in heifers after SC and IM administration of a drug containing florfenicol as the only active substance.

2 | MATERIALS AND METHODS

2.1 | Animals

For the purpose of study, five clinically healthy Russian Black Pied heifers aged 12 months and weighing from 260 to 290 kg were selected. No drugs were administered to the heifers in the previous 30 days. The health status of animals was monitored daily. Throughout the study, animals were kept indoors in separate animal paddocks with ad libitum access to water and fed with age-appropriate rations. Veterinary care was available throughout the study.

2.2 | Drug administration

Florfenicol (2,2-Dichloro-N-[(1S,2R)-1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl)phenyl]ethyl]acetamide, CAS No 73231-34-2) injectable solution "Florfenicol 40%" (400 mg florfenicol per ml) produced by "AVZ S-P Ltd" (Moscow region) was used in the experiment. The chemical structure of florfenicol is presented in Figure 1. Due to poor solubility of florfenicol in water, N-methyl-2-pyrrolidone was used as solvent in the preparation. The solution was administered once intramuscularly to five animals at a dosage rate of 1 ml per 20 kg bodyweight (corresponding to 20 mg florfenicol per kg bodyweight). Then, after 30-day washout period, the drug was administered subcutaneously at a dosage rate of 2 ml per 20 kg bodyweight (corresponding to 40 mg florfenicol per kg bodyweight). IM injection was carried out into the muscles at the left side of the neck, SC injection was carried out into the skin fold at the right shoulder (ANADA, 2021).

2.3 | Collection of blood samples

Blood samples were collected for both (SC and IM) routes of administrations before administration and at 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, 48, 54, 72, 96, 120 h after administration. At each time point, 3 ml of blood per animal was collected from jugular vein. In addition, after the analysis of collected samples, a set of samples was additionally taken after SC administration at 264 h after injection. Blood samples were collected into heparin-containing tubes, centrifuged (3400 RCF, 4°C) and plasma was separated and stored at −20°C. During the method validation, it was found that storage at −20°C ensures the stability of florfenicol in plasma for at least 3 months.

2.4 | Sample analysis

The concentration of florfenicol was determined by HPLC-MS/MS using an in-house validated method. The measurement was

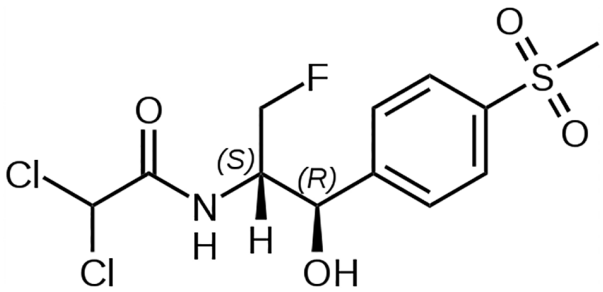

FIGURE 1 Chemical structure of florfenicol

performed using liquid chromatograph-mass spectrometer LCMS-8050 (Shimadzu Corporation, Japan) with negative electrospray ionization. For chromatographic separation, Zorbax Eclipse Plus C18 2.1×50 mm, 5 µm column (Agilent Technologies, USA) equipped with guard column was used. The column temperature was set at 30°C, the flow rate was 0.2 ml/min. Deionized water and 5 mM ammonium formate in methanol were used as mobile phases A and B, respectively. The separation was performed in a gradient elution mode with concentration of mobile phase B changing from 0% to 100% for 3 min. For quantification, matrix-matched calibration samples spiked with florfenicol (TRC, Canada) were used. Florfenicol-D3 (TRC, Canada) was used as the internal standard. Data acquisition was performed in the multiple reaction monitoring mode with the following quantification transitions quantitation: 356 > 336 for florfenicol; 359 > 188 for florfenicol-d3.

The previously published method (Shen et al., 2002) with some modifications was used for the extraction of florfenicol. Sample preparation was carried out as follows: 0.05 ml of florfenicol-D3 in methanol was added to 0.3 ml sample of plasma, extraction was performed with 2.5 ml of ethyl acetate for 10 min. The extract was evaporated to dryness under nitrogen stream at 50°C. The dry residue was dissolved in 1 ml of deionized water. The resulting sample was filtered and used for HPLC-MS/MS analysis.

The applied method is simple, reproducible under normal laboratory conditions and specific. No interferences from six independent blank bovine plasma samples were found during validation. Accuracy, precision and linearity were tested in three separate batches at six concentration levels. Method validation parameters are shown in Table 1. The method proved to be robust over time and was also applicable to samples from other species, namely porcine plasma.

2.5 | Data analysis

Individual time–concentration plots were built for each animal after both IM and SC administrations. The obtained data were evaluated using non-compartmental analysis (NCA) and also sequentially fitted to one- and two-compartment models. Pharmacokinetic parameters including maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the plasma concentration versus time curve from time zero to the last measure concentration (AUC_{0-t}), area under the plasma concentration versus time curve extrapolated to infinity ($AUC_{0-\infty}$), apparent clearance (Cl/F), terminal elimination rate constant (λ_z), terminal half-life ($t_{1/2}$), mean residence time (MRT) were calculated using the PK Solver software (Zhang et al., 2010). The statistical significance of the differences between results obtained in IM and SC experiments was calculated with Statistica 13 package using paired *t*-test, and defined at $p < .05$.

TABLE 1 Parameters of analytical method

Parameter	Value
Lower limit of quantification	0.03 µg/ml
Upper limit of quantification	3.00 µg/ml
Absolute recovery	75%
Within-run precision	6%
Between-run precision	4%
Accuracy	From −13% to 14%

3 | RESULTS

No adverse effects were observed during or after drug administration in any of the heifers.

The individual time–concentration results were fitted to one- and two-compartment models, and fitness was evaluated using visual inspection of the distribution of the residuals and Akaike information criteria (AIC). For SC data, two-compartment model provided a good fitness regarding both AIC and distribution of residuals. For IM data, one-compartment model provided better AIC values. Nevertheless, for IM data, the distribution of residuals was uneven both using one- and two-compartment models. Due to this, it was decided to use NCA results for comparison.

Concentrations of florfenicol in cattle plasma after IM and SC administration are presented in the form of an arithmetic graph (Figure 2) and a semi-logarithmic graph (Figure 3). The pharmacokinetic parameters obtained by NCA are shown in Table 2.

It can be seen that mean C_{max} after IM administration was close to mean C_{max} after SC administration, despite the fact that the dose of the drug was two times higher for SC administration.

The T_{max} appeared to be higher following IM administration than following SC administration (6.8 ± 1.1 vs 4.4 ± 0.9 h respectively). It is also evident from the graphs that the terminal slopes obviously differ, resulting in significant difference between IM and SC route in $t_{1/2}$ (24.5 ± 3.7 vs 103.8 ± 24.1 h respectively).

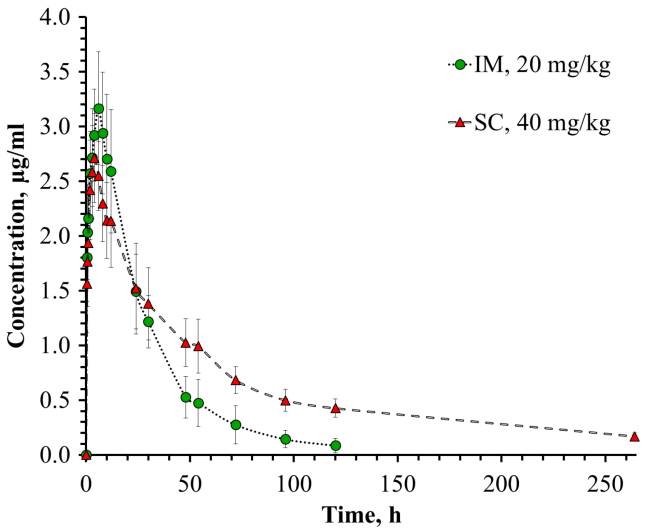

FIGURE 2 Arithmetic graph of mean plasma concentration of florfenicol versus time following intramuscular (IM) administration at a dose of 20 mg/kg and SC administration at a dose of 40 mg/kg.

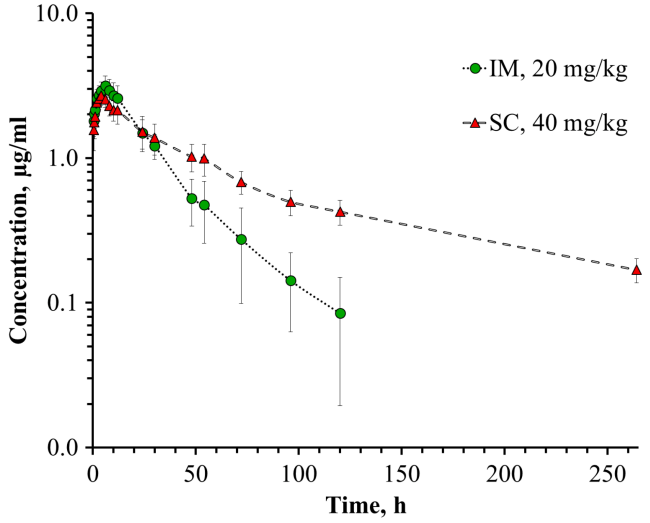

FIGURE 3 Semi-logarithmic graph of mean plasma concentration of florfenicol versus time following intramuscular (IM) administration at a dose of 20 mg/kg and subcutaneous (SC) administration at a dose of 40 mg/kg.

TABLE 2 Pharmacokinetic parameters of florfenicol after intramuscular and subcutaneous administration (NCA)

		20 mg/kg IM	40 mg/kg SC
Parameter	**Unit**	**Mean (SD)**	**Mean (SD)**
C_{max}	µg/ml	3.2 (0.5)	2.7 (0.4)
T_{max}	*t*	6.8 (1.1)	4.4 (0.9)
$AUC_{0\text{-}t}$	µg/ml*h	98.2 (14.7)	168.6 (26.8)
$AUC_{0\text{-}\infty}$	µg/ml*h	101.5 (16.9)	194.5 (24.9)
$AUC_{0\text{-}t}/AUC_{0\text{-}\infty}$		0.97 (0.02)	0.87 (0.06)
V_z/F	L/kg	7.0 (0.8)	31.4 (9.3)
Cl/F	L/kg/h	0.20 (0.03)	0.21 (0.03)
λ_z		0.029 (0.004)	0.007 (0.001)
$t_{1/2}$	h	24.5 (3.7)	103.8 (24.1)
MRT	h	30.4 (7.3)	119.0 (32.0)

Abbreviations: AUC, area under the curve; IM, intramuscular; MRT, mean residence time; NCA, non-compartmental analysis; SC, subcutaneous.

4 | DISCUSSION

The pharmacokinetic profiles of florfenicol after IM and SC administration of cattle appeared to be significantly different, although some parameters were comparable when normalized for dose. In particular, the average value of $AUC_{0\text{-}\infty}$ after SC injection was almost two times higher than after IM injection, and the $AUC_{0\text{-}\infty SC}/AUC_{0\text{-}\infty IM}$ ratios calculated for each animal individually and then averaged were 1.94 ± 0.21. However, since the SC dose was two times higher, these results indicate a comparable bioavailability of florfenicol in both routes of administration. Absolute bioavailability could not be estimated because the experiment did not include intravenous administration.

Contrary to expectations, mean T_{max} after SC injection was lower than after IM injection, 4.4 ± 0.9 h and 6.8 ± 1.1, respectively, though one could expect that maximum plasma level of florfenicol would be reached faster following IM injection. Nevertheless, these differences in T_{max} should be treated with caution due to relatively large sampling intervals around obtained T_{max}, namely 3, 4, 6, 8 h points, which might lead to skewing of calculated T_{max} in relation to true T_{max}. It should be also noted that almost all obtained PK profiles from both IM and SC experiments were not attributed with pronounced peak concentrations: despite large sampling intervals all five SC profiles and four of five IM profiles included 3 or 4 consecutive timepoints (corresponding intervals lasted from 3 to 6 h) around T_{max}, at which concentrations accounted 90%–100% of corresponding C_{max}. Such continuous persistence of high concentrations was likely to be stipulated by flip-flop effect when rate of absorption from the injection site is slower than the rate of excretion (Toutain & Bousquet-Mélou, 2004a; Yáñez et al., 2011).

The obtained values of C_{max} appeared to be comparable and amounted to 3.2 ± 0.5 µg/ml after IM administration and 2.7 ± 0.4 µg/ml after SC administration; however, it should be noted that the dosage with SC administration was two times higher. These results were in agreement with the results obtained in the previously published experiment on cows (Lacroix et al., 2011), in which C_{max} for IM administration was 1.5 times higher than for SC administration at equal dosages. Similar data were obtained in comparative experiments on sheep (Balcomb et al., 2018) and alpacas (Holmes et al., 2012), where C_{max} was higher for IM administration with a twofold lower dose compared to SC administration, as well as in the experiment on sheep (El-Sheikh et al., 2009), where at equal dosages C_{max} for IM administration was 4.5 times higher than for SC administration.

The IM $AUC_{0\text{-}t}/AUC_{0\text{-}\infty}$ ratio was 0.97 ± 0.02, meaning that with t_{last} at 120 h only 3% of $AUC_{0\text{-}\infty}$ was obtained by extrapolation, which maintains high precision of predicted $AUC_{0\text{-}\infty}$. In SC experiment, the sampling was initially planned to last up to 120 h, with exactly same schedule as in IM experiment. However, the analysis of plasma samples revealed that after SC injection the plasma concentrations of florfenicol at 120 h timepoint were still relatively high and accounted from 10% to 21% of corresponding maximum concentrations. Interim PK analysis showed that $AUC_{0\text{-}120}/AUC_{0\text{-}\infty}$ was from 0.70 to 0.86 for different animals, meaning that substantial part of $AUC_{0\text{-}\infty}$ accounting up to 30% was obtained by extrapolation. Due to this, it was immediately decided to collect additional plasma samples at 264 h after injection. Plasma florfenicol levels at 264 h point were at 4%–8% of corresponding maximum concentrations, and subsequent $AUC_{0\text{-}\infty}$ calculations using C_{last} from 264 h showed that extrapolated part of AUC decreased to 9%–12% for 4 animals, which generally provided reliable estimation of $AUC_{0\text{-}\infty}$. However, extrapolated part of AUC was still high for one animal (24%), despite the extended sampling time.

Significant differences were noted in the elimination half-life $t_{1/2}$, which was 24.5 ± 3.7 h after IM administration and 103.8 ± 24.1 h after SC administration. A similar trend, although less pronounced, was described in the previously published experiment on cows

(Lacroix et al., 2011), where $t_{1/2\ IM}$ was 25.5 h and $t_{1/2\ SC}$ was 39.6 h. The $t_{1/2\ IM}$ value was comparable with the data from other studies with cattle (18.3 h, Lobell et al., 1994) or exceeded it (12.4 h, Ramadan & Abd El-Aty, 2011). The $t_{1/2\ SC}$ value was higher than those reported in other sources (27.5 h, Sidhu, et al., 2014; 28.5 h, Resflor Gold Product Information, 2009).

In comparative experiments on other animals, similar results were observed for alpacas (Holmes et al., 2012), where $t_{1/2\ IM}$ was 17.6 h and $t_{1/2\ SC}$ was 99.7 h. Regarding published comparative experiments on sheep, it is impossible to determine the general trend of the half-life dependence on the route of administration. For example, one study (El-Sheikh et al., 2009) revealed no significant differences in $t_{1/2}$ with IM and SC administration. However, this may be due to the fact that the above-mentioned experiment involved sampling only up to 24 h after administration, when relatively high plasma concentrations of FF were still observed. Therefore, the description of the terminal stage of pharmacokinetics could be difficult, since the concentration values obtained in the terminal stage can dramatically affect the $t_{1/2}$ value (Toutain & Bousquet-Mélou, 2004a). Another trial on sheep (Balcomb et al., 2018) included a comparative test of two formulations of florfenicol. For one of the formulations, clear differences in $t_{1/2}$ were observed depending on the route of administration (6.0 h for IM, 16.6 h for SC), while for the other formulation the results were comparable (13.4 and 12.5 h). Nevertheless, that experiment also implied sampling only up to 24 h.

It should be noted that $t_{1/2}$ values were significantly influenced with extended sampling in SC experiment. The interim PK analysis at t_{last} = 120 h resulted in $t_{1/2}$ values at 55.7 ± 13.5 h, whereas final calculation with t_{last} = 264 h resulted in $t_{1/2}$ values at 103.8 ± 24.1 h. The results indicate that for SC injection of florfenicol it is advisable to prolong sampling beyond 120 h to achieve better accuracy of $t_{1/2}$. Generally, in current study the additional sampling at 264 h has significantly impacted on both $AUC_{0-\infty}$ and $t_{1/2}$ values. The relevance of sampling duration for accurate determination of above-mentioned parameters was demonstrably described using Monte-Carlo simulations (Colucci et al., 2011). Nevertheless, the obtained high $t_{1/2}$ values must be treated carefully, because in flip-flop situation which is discussed below, terminal half-life provided by NCA more likely represents absorption phase than elimination phase. Another limitation of NCA is related to computation of apparent volume of distribution (Vz/F), which in flip-flop conditions may be overestimated due to low value of λz which is used as denominator in calculation of Vz/F (Toutain & Bousquet-Mélou, 2004b).

The $t_{1/2}$ values obtained after both administration routes were much higher compared to the published $t_{1/2}$ values after intravenous administration to cattle (2.7 h, Lobell et al., 1994; 2.9 h, Soback et al., 1995; 3.2 h, De Craene et al., 1997; 4.0 h, Ramadan & Abd El-Aty, 2011). This indicates that after IM and SC administration, a flip-flop effect is observed for florfenicol. Similar assumptions about the nature of the high $t_{1/2}$ values of florfenicol are given in a number of works (Balcomb et al., 2018; Holmes et al., 2012; Lacroix et al., 2011; Varma et al., 1998). The suggestion about occurrence of flip flop is supported by the obtained MRT values, which can provide approximate evaluation of absorption time. Despite that this experiment did not include IV administration, the literature sources report MRT_{IV} for florfenicol in cattle in range 2.5–4 h (Dumka & Singh, 2014; Lobell et al., 1994; Soback et al., 1995) with maximum value at 6.6 h (De Craene et al., 1997). Given that mean absorption time (MAT) = $MRT_{non\text{-}IV}$–MRT_{IV}, the obtained high MRT values (MRT_{IM} = 30.4 h, MRT_{SC} = 119 h) indicate that MAT after both IM and SC administration is obviously higher than MRTIV. Hence, the absorption is the limiting process for elimination which corresponds to flip-flop phenomenon. In addition, the MRT values indicate that MAT_{SC} is markedly higher that MAT_{IM}, that is, flip-flop effect is more expressed (absorption is pronouncedly slower) when florfenicol is administered subcutaneously, which can be explained by a less intense metabolism in the SC tissue compared to muscle tissue which is more abundantly supplied with blood vessels (Yáñez et al., 2011).

The manufacturer of the tested drug suggested two schemes of administration: single SC injection at a dose rate of 40 mg/kg or two IM injections at a dose rate of 20 mg/kg with 48 h interval. Apart from evaluation of antibacterial efficacy, it is important to mention that both proposed routes of administration have practical advantages and disadvantages. In particular, the SC route of administration seems to be preferable from a veterinarian's point of view, since only one injection is required. Also, with single injection, there is less stress for animals. On the other hand, the depletion of florfenicol and its metabolites from edible tissues is significantly faster after IM injections than after SC injection, which is an undoubted practical advantage of the IM route (instructions for Floron 30%, Resflor Gold Product Information). The SC and IM injections are conventional routes for florfenicol administration to cattle. The oral route which is used for florfenicol administration to monogastric animals, for example, pigs, is usually not applied for ruminants. This is a common limitation for numerous drugs, which is related to physiological aspects of ruminants' digestive system. These aspects imply low bioavailability of drugs caused by their inactivation by rumen microflora, binding to ruminal content and their dilution in large medium (Hinchcliff et al., 1991). Moreover, it may be suggested that ruminant digestion may contribute to florfenicol retention and/or inactivation in rumen even in case of parenteral administration. This suggestion is based on two considerations. First, florfenicol being a highly lipophilic compound is likely to intensely penetrate into saliva and subsequently be transferred to rumen. Though we did not find published data about this feature directly for florfenicol, there is a report describing intense penetration of similar and also lipophilic chloramphenicol into saliva following SC injection to cows (Dotter et al., 1990). Second, florfenicol due to its lipophilic nature may presumably diffuse from plasma through the rumen membrane. Importantly, in this case, florfenicol being a weak base will ionize in acidic medium of rumen which will thereby favor further diffusion of the non-ionized form from the blood into the rumen (Hinchcliff et al., 1991).

The high susceptibility of bovine to BRD is predetermined by several inherent aspects of their anatomy and physiology, for example, large amount of respiratory dead space volume and poor collateral

ventilation (Ackermann et al., 2010). These features provide environment for development of respiratory infection, which, according to current theory regarding BRD pathogenesis, is caused by mixed interaction of bacteria and viruses being triggered by various stressors, including weaning, comingling, transportation and dietary changes (Chai et al., 2022). The localization of BRD infection requires intense delivery of drug into respiratory tissues. The specific features of florfenicol, namely lipophilic nature (Anadón et al., 2008; Papich, 2020) and low binding to plasma proteins (Foster et al., 2016) make it strongly advantageous in this regard, which was confirmed in studies showing high degree of florfenicol penetration into lung tissue, interstitial fluid and pulmonary epithelial lining fluid (Adams et al., 1987; Foster et al., 2016).

Regarding antibacterial efficacy, the obtained pharmacokinetic profiles allow to assume that any of suggested administration routes could maintain effective florfenicol levels against the bacteria causing respiratory disease in cattle, namely Mannheimia haemolytica (MIC90 1–2 μg/ml), Pasteurella multocida (MIC90 0.5 μg/ml), Histophilus somni (MIC90 0.25–0.3 μg/ml) (Blondeau et al., 2012; EMA, 2017; Goldspink et al., 2015; Kehrenberg et al., 2004; Shin et al., 2005; USP, 2007). Florfenicol is considered to be a time-dependent antibiotic, for which the time above minimum inhibitory concentration (T > MIC) is often considered to be the key efficacy parameter. Nevertheless, recent comprehensive publications suggested AUC/MIC to be preferable predictive index of efficacy (Toutain et al., 2019). Furthermore, target values for $AUC_{0\text{-}96}$/MIC were suggested at 115 h for *P. multocida* and 127 h for M. haemolytica (for 90% efficacy (in silico) with MIC of 1 mg/L for inoculum of 10^5 CFU/ml) by Veterinary European Committee on Antimicrobial Susceptibility Testing (Florfenicol (Cattle). Rationale for EUCAST clinical breakpoints, version 1.0, 10 August 2019). In our study, the SC experiment resulted in average $AUC_{0\text{-}96}$/MIC to be 115 ± 22 h, which was consistent with target value for *P. multocida* and close to target value for *M. haemolytica*. Regarding IM injection, with implication of similar bioavailability of florfenicol at both routes, it can be assumed that in case of second injection at 20 mg/kg after 48 h the AUC/MIC values would be comparable with those obtained after SC experiment. The suggested dosage regimes are also supported by previously published studies which have demonstrated florfenicol efficacy against BRD using either double 20 mg/kg IM injection (Aslan et al., 2002; Hoar et al., 1998; Jim et al., 1999; Thiry et al., 2011; Welling et al., 2020) or single 40 mg/kg SC injection (Bringhenti et al., 2021; Catry et al., 2008; Varma et al., 1998). Concerning other cattle pathogens which are more susceptible to florfenicol, namely Bacteroides melaninogenicus, Fusobacterium necrophorum with MIC90 0.25 μg/ml (US Pharmacopeial Convention, 2007), both injection routes apparently provide effective florfenicol amount against these agents. In conclusion, it can be suggested that both routes of administration provide relevant florfenicol exposure on prevalent bacteria causing bovine respiratory disease and for some other more susceptible pathogens. However, given the variation in antimicrobial resistance of different strains, preliminary assessment of bacterial susceptibility to florfenicol would be the crucial factor for the correct choice of treatment regimen.

AUTHOR CONTRIBUTIONS

RS carried out design of the study, pharmacokinetic analysis, data interpretation and writing of manuscript. EG carried out method development and contributed to writing of manuscript. PK carried out the data analysis and writing of manuscript. DG and JK contributed to sample preparation and analyses. SK contributed to the animal experiment, AK and EE contributed to study design and data interpretation, SE conceived the presented study and contributed to data interpretation. All authors contributed to and reviewed the manuscript.

ACKNOWLEDGMENTS

The study was funded by AVZ Ltd. The authors thank Mrs. N. Soloshenko, Mr. A. Nagonov, Mr. I. Stoyanov for their valuable assistance in conducting animal experiment.

CONFLICT OF INTEREST

All the authors (Renat Selimov, Elizaveta Goncharova, Pavel Koriakovtsev, Diana Gabidullina, Julia Karsakova, Sergey Kozlov, Alexander Komarov, Ekaterina Engasheva, Sergey Engashev) are employees of AVZ Ltd., who funded the study and supplied the test item.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Supplemental Data. The further data are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, were followed. The Committee on Animal Care and Use of AVZ Ltd. approved (Approval No 24/06/20) the study protocol and its amendment according to provisions of the EC Directive 2010/63.

ORCID

Elizaveta Goncharova https://orcid.org/0000-0002-2207-3257

REFERENCES

Ackermann, M. R., Derscheid, R., & Roth, J. A. (2010). Innate immunology of bovine respiratory disease. *Veterinary Clinics: Food Animal Practice*, *26*(2), 215–228. https://doi.org/10.1016/j.cvfa.2010.03.001

Adams, P. E., Varma, K. J., Powers, T. E., & Lamendola, J. F. (1987). Tissue concentrations and pharmacokinetics of florfenicol in male veal calves given repeated doses. *American Journal of Veterinary Research*, *48*(12), 1725–1732.

Alcorn, J., Dowling, P., Woodbury, M., & Killeen, R. (2004). Pharmacokinetics of florfenicol in north American elk (*Cervus elaphus*). *Journal of Veterinary Pharmacology and Therapeutics*, *27*(5), 289–292. https://doi.org/10.1111/j.1365-2885.2004.00594.x

Anadón, A., Martínez, M. A., Martínez, M., Ríos, A., Caballero, V., Ares, I., & Martínez-Larrañaga, M. R. (2008). Plasma and tissue depletion of florfenicol and florfenicol-amine in chickens. *Journal of Agricultural and Food Chemistry*, *56*(22), 11049–11056. https://doi.org/10.1021/jf802138y

ANADA. (2021). *Freedom of information summary*. Original abbreviated new animal drug application, ANADA 200-588, Date of Approval: October 28, 2021.

Aslan, V., Maden, M., Erganis, O., Birdane, F. M., & Corlu, M. (2002). Clinical efficacy of florfenicol in the treatment of calf respiratory tract infections. *The Veterinary Quarterly*, *24*(1), 35–39. https://doi.org/10.1080/01652176.2002.9695122

Balcomb, C. C., Angelos, J. A., Chigerwe, M., Byrne, B. A., Lane, V. M., Wetzlich, S. E., Sahin, O., Holler, L., Zhang, S., & Tell, L. A. (2018). Comparative pharmacokinetics of two florfenicol formulations following intramuscular and subcutaneous administration to sheep. *American Journal of Veterinary Research*, *79*(1), 107–114. https://doi.org/10.2460/ajvr.79.1.107

Birdane, Y. O., & Birdane, F. M. (2016). Pharmacokinetics of florfenicol following intravenous and intramuscular administration in dogs. *Veterinární Medicína*, *60*(6), 323–329. https://doi.org/10.17221/8247-vetmed

Birdane, Y. O., Birdane, F. M., Özdemir, M., Kabu, M., & Yavuz, H. (2015). Pharmacokinetic of Florfenicol after Administration in Sheep. *Kocatepe Veterinary Journal*, *8*, 19–24. https://doi.org/10.5578/kvj.8739

Blondeau, J. M., Borsos, S., Blondeau, L. D., Blondeau, B. J. J., & Hesje, C. E. (2012). Comparative minimum inhibitory and mutant prevention drug concentrations of enrofloxacin, ceftiofur, florfenicol, tilmicosin and tulathromycin against bovine clinical isolates of *Mannheimia haemolytica*. *Veterinary Microbiology*, *160*(1–2), 85–90. https://doi.org/10.1016/j.vetmic.2012.05.006

Bringhenti, L., Pallu, M., Silva, J. C., Tomazi, T., Tomazi, A. C. C. H., Rodrigues, M. X., Cruzado-Bravo, M., Bilby, T. R., & Bicalho, R. C. (2021). Effect of treatment of pneumonia and otitis media with tildipirosin or florfenicol + flunixin meglumine on health and upper respiratory tract microbiota of preweaned Holstein dairy heifers. *Journal of Dairy Science*, *104*(9), 10291–10309. https://doi.org/10.3168/jds.2020-19945

Cannon, M., Harford, S., & Davies, J. (1990). A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated derivatives. *The Journal of Antimicrobial Chemotherapy*, *26*(3), 307–317. https://doi.org/10.1093/jac/26.3.307

Castillo, L. G., Marchetti, M. L., Buldain, D., Lozano, K. J., Aliverti, F., Buchamer, A., & Mestorino, N. (2020). Pharmacokinetics of florfenicol in healthy pigs after its intramuscular administration. https://doi.org/10.13140/RG.2.2.22118.24646

Catry, B., Duchateau, L., Van de Ven, J., Laevens, H., Opsomer, G., Haesebrouck, F., & De Kruif, A. (2008). Efficacy of metaphylactic florfenicol therapy during natural outbreaks of bovine respiratory disease. *Journal of Veterinary Pharmacology and Therapeutics*, *31*(5), 479–487. https://doi.org/10.1111/j.1365-2885.2008.00981.x

Chai, J., Capik, S. F., Kegley, B., Richeson, J. T., Powell, J. G., & Zhao, J. (2022). Bovine respiratory microbiota of feedlot cattle and its association with disease. *Veterinary Research*, *53*, 4. https://doi.org/10.1186/s13567-021-01020-x

Colucci, P., Turgeon, J., & Ducharme, M. P. (2011). How critical is the duration of the sampling scheme for the determination of half-life, characterization of exposure and assessment of bioequivalence? *Journal of Pharmacy and Pharmaceutical Sciences: A Publication of the Canadian Society for Pharmaceutical Sciences, Societe Canadienne Des Sciences Pharmaceutiques*, *14*(2), 217–226. https://doi.org/10.18433/j3fg63

de Craene, B. A., Deprez, P., D'Haese, E., Nelis, H. J., Van den Bossche, W., & De Leenheer, P. (1997). Pharmacokinetics of florfenicol in cerebrospinal fluid and plasma of calves. *Antimicrobial Agents and Chemotherapy*, *41*(9), 1991–1995. https://doi.org/10.1128/AAC.41.9.1991

de Haas, V., Bonnier, M., Gicquel, M., Etore, F., & Shuster, D. (2002). Florfenicol: a time- or concentration-dependent antibiotic? In *Newadvances in calf disease management. XXII World Buiatrics Congress, IV* (pp. 17–25). World Association for Buiatrics.

de Jong, A., Thomas, V., Simjee, S., Moyaert, H., El Garch, F., Maher, K., Morrissey, I., Butty, P., Klein, U., Marion, H., Rigaut, D., … Vallé, M. (2014). Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased cattle and pigs across Europe: The VetPath study. *Veterinary Microbiology*, *172*(1–2), 202–215. https://doi.org/10.1016/j.vetmic.2014.04.008

Dotter, A., Kroker, R., & Arnold, D. (1990). The pharmacokinetics of chloramphenicol in plasma and saliva of dairy cows. *Journal of Veterinary Pharmacology and Therapeutics*, *13*(1), 81–85. https://doi.org/10.1111/j.1365-2885.1990.tb00751.x

Dowling, P. M. (2013). Chloramphenicol, thiamphenicol, and florfenicol. In *Antimicrobial therapy in veterinary medicine* (pp. 269–277). Wiley Online Library. https://doi.org/10.1002/9781118675014.ch16

Dumka, V. K., & Singh, I. (2014). Pharmacokinetics and dosage regimen of florfenicol in co-administration with paracetamol in cross bred calves. *Veterinarski Arhiv*, *84*(3), 229–339.

El-Sheikh, W., Shaheen, H., & El-Ghoneimy, A. (2009). Comparative pharmacokinetics of florfenicol after intravenous, intramuscular and subcutaneous injection in sheep. *Assiut Veterinary Medical Journal*, *55*(120), 1–11. https://doi.org/10.21608/avmj.2009.174182

EMA. (2017). *Zeleris. ANNEX I summary of product characteristics*. EMA.

Foster, D. M., Martin, L. G., & Papich, M. G. (2016). Comparison of active drug concentrations in the pulmonary epithelial lining fluid and interstitial fluid of calves injected with enrofloxacin, florfenicol, ceftiofur, or tulathromycin. *PLoS One*, *11*(2), e0149100. https://doi.org/10.1371/journal.pone.0149100

Goldspink, L. K., Mollinger, J. L., Barnes, T. S., Groves, M., Mahony, T. J., & Gibson, J. S. (2015). Antimicrobial susceptibility of Histophilus somni isolated from clinically affected cattle in Australia. *Veterinary Journal (London, England: 1997)*, *203*(2), 239–243. https://doi.org/10.1016/j.tvjl.2014.12.008

Hesje, C. K., Tillotson, G. S., & Blondeau, J. M. (2007). MICs, MPCs and PK/PDs: A match (sometimes) made in hosts. *Expert Review of Respiratory Medicine*, *1*(1), 7–16. https://doi.org/10.1586/17476348.1.1.7

Hinchcliff, K. W., Jernigan, A. D., Upson, D. W., & Constable, P. D. (1991). Ruminant pharmacology. *Veterinary Clinics of North America: Food Animal Practice*, *7*(3), 633–649. https://doi.org/10.1016/s0749-0720(15)31076-8

Hoar, B. R., Jelinski, M. D., Ribble, C. S., Janzen, E. D., & Johnson, J. C. (1998). A comparison of the clinical field efficacy and safety of florfenicol and tilmicosin for the treatment of undifferentiated bovine respiratory disease of cattle in western Canada. *The Canadian Veterinary Journal*, *39*(3), 161–166.

Holmes, K., Bedenice, D., & Papich, M. G. (2012). Florfenicol pharmacokinetics in healthy adult alpacas after subcutaneous and intramuscular injection. *Journal of Veterinary Pharmacology and Therapeutics*, *35*(4), 382–388. https://doi.org/10.1111/j.1365-2885.2011.01323.x

Illambas, J., Potter, T., Sidhu, P., Rycroft, A. N., Cheng, Z., & Lees, P. (2013). Pharmacodynamics of florfenicol for calf pneumonia pathogens. *The Veterinary Record*, *172*(13), 340. https://doi.org/10.1136/vr.101155

Jim, G. K., Booker, C. W., Guichon, P. T., Schunicht, O. C., Wildman, B. K., Johnson, J. C., & Lockwood, P. W. (1999). A comparison of florfenicol and tilmicosin for the treatment of undifferentiated fever in feedlot calves in western Canada. *The Canadian Veterinary Journal*, *40*(3), 179–184.

Jones, M. L., Washburn, K. E., Fajt, V. R., Rice, S., & Coetzee, J. F. (2015). Synovial fluid pharmacokinetics of tulathromycin, gamithromycin and florfenicol after a single subcutaneous dose in cattle. *BMC Veterinary Research*, *11*(1), 26. https://doi.org/10.1186/s12917-015-0346-4

Kehrenberg, C., Mumme, J., Wallmann, J., Verspohl, J., Tegeler, R., Kühn, T., & Schwarz, S. (2004). Monitoring of florfenicol susceptibility among bovine and porcine respiratory tract pathogens

collected in Germany during the years 2002 and 2003. *The Journal of Antimicrobial Chemotherapy*, *54*(2), 572–574. https://doi.org/10.1093/jac/dkh371

Lacroix, M. Z., Gayrard, V., Picard-Hagen, N., & Toutain, P. L. (2011). Comparative bioavailability between two routes of administration of florfenicol and flunixin in cattle. *Revista de Medicina Veterinaria*, *162*, 321–324.

Lane, V. M., Villarroel, A., Wetzlich, S. E., Clifford, A., Taylor, I., & Craigmill, A. L. (2004). Intravenous and subcutaneous pharmacokinetics of florfenicol in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, *27*(4), 191–196. https://doi.org/10.1111/j.1365-2885.2004.00580.x

Liu, J., Fung, K. F., Chen, Z., Zeng, Z., & Zhang, J. (2003). Pharmacokinetics of florfenicol in healthy pigs and in pigs experimentally infected with Actinobacillus pleuropneumoniae. *Antimicrobial Agents and Chemotherapy*, *47*(2), 820–823. https://doi.org/10.1128/AAC.47.2.820-823.2003

Lobell, R. D., Varma, K. J., Johnson, J. C., Sams, R. A., Gerken, D. F., & Ashcraft, S. M. (1994). Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. *Journal of Veterinary Pharmacology and Therapeutics*, *17*(4), 253–258. https://doi.org/10.1111/j.1365-2885.1994.tb00241.x

Martinez, M. N., Toutain, P., & Turnidge, J. (2013). The pharmacodynamics of antimicrobial agents. In *Antimicrobial therapy in veterinary medicine* (pp. 79–103). Wiley. https://doi.org/10.1002/9781118675014.ch5

Papich, M. G. (2020). *Papich handbook of veterinary drugs* (5th ed., p. 1056). Elsevier Health Sciences.

Pentecost, R. L., Niehaus, A. J., Werle, N. A., & Lakritz, J. (2013). Pharmacokinetics of florfenicol after intravenous and intramuscular dosing in llamas. *Research in Veterinary Science*, *95*(2), 594–599. https://doi.org/10.1016/j.rvsc.2013.05.009

Plumb, D. C. (2004). *Veterinary drug handbook* (5th ed.). Blackwell Publication.

Ramadan, A., & Abd El-Aty, A. M. (2011). Pharmacokinetics and distribution of florfenicol in bronchial secretions of healthy and Pasteurella multocida infected calves. *Pharmaceutica Analytica Acta*, *2*(1), 1000117. https://doi.org/10.4172/2153-2435.1000117

Rassouli, A., Lees, P., & Sidhu, P. (2011). Florfenicol pharmacokinetics in calves following a single subcutaneous injection. *Journal of Veterinary Research*, *66*, 95–102.

FDA. (2009). *Resflor Gold Product Information, NADA 141–299, approved by FDA*.

Shen, J., Li, X., Jiang, H., & Hsu, W. H. (2004). Bioavailability and pharmacokinetics of florfenicol in healthy sheep. *Journal of Veterinary Pharmacology and Therapeutics*, *27*(3), 163–168. https://doi.org/10.1111/j.1365-2885.2004.00574.x

Shen, J., Wu, X., Hu, D., & Jiang, H. (2002). Pharmacokinetics of florfenicol in healthy and Escherichia coli-infected broiler chickens. *Research in Veterinary Science*, *73*(2), 137–140. https://doi.org/10.1016/s0034-5288(02)00033-4

Shin, S. J., Kang, S. G., Nabin, R., Kang, M. L., & Yoo, H. S. (2005). Evaluation of the antimicrobial activity of florfenicol against bacteria isolated from bovine and porcine respiratory disease. *Veterinary Microbiology*, *106*(1–2), 73–77. https://doi.org/10.1016/j.vetmic.2004.11.015

Sidhu, P., Rassouli, A., Illambas, J., Potter, T., Pelligand, L., Rycroft, A., & Lees, P. (2014). Pharmacokinetic-pharmacodynamic integration and modelling of florfenicol in calves. *Journal of Veterinary Pharmacology and Therapeutics*, *37*(3), 231–242. https://doi.org/10.1111/jvp.12093

Soback, S., Paape, M. J., Filep, R., & Varma, K. J. (1995). Florfenicol pharmacokinetics in lactating cows after intravenous, intramuscular and intramammary administration. *Journal of Veterinary Pharmacology and Therapeutics*, *18*(6), 413–417. https://doi.org/10.1111/j.1365-2885.1995.tb00618.x

The United States Pharmacopeial Convention. (2007). Florfenicol.

Thiry, J., Rubion, S., Sarasola, P., Bonnier, M., Hartmann, M., & de Haas, V. (2011). Efficacy and safety of a new 450 mg/ml florfenicol formulation administered intramuscularly in the treatment of bacterial bovine respiratory disease. *The Veterinary Record*, *169*(20), 526. https://doi.org/10.1136/vr.d5498

Toutain, P. L., & Bousquet-Mélou, A. (2004a). Plasma terminal half-life. *Journal of Veterinary Pharmacology and Therapeutics*, *27*(6), 427–439. https://doi.org/10.1111/j.1365-2885.2004.00600.x

Toutain, P. L., & Bousquet-Mélou, A. (2004b). Volumes of distribution. *Journal of Veterinary Pharmacology and Therapeutics*, *27*(6), 441–453. https://doi.org/10.1111/j.1365-2885.2004.00602.x

Toutain, P.-L., Sidhu, P. K., Lees, P., Rassouli, A., & Pelligand, L. (2019). VetCAST method for determination of the pharmacokinetic-pharmacodynamic cut-off values of a long-acting formulation of florfenicol to support clinical breakpoints for florfenicol antimicrobial susceptibility testing in cattle. *Frontiers in Microbiology*, *10*, 1310. https://doi.org/10.3389/fmicb.2019.01310

Varma, K. J., Lockwood, P. W., Cosgrove, S. B., & Rogers, E. R. (1998). Pharmacology, safety and clinical efficacy of Nuflor (florfenicol) following subcutaneous administration to cattle. Schering-Plough Animal Health Nuflor: New therapeutic applications. Proceedings of a Symposium held in conjunction with the XX World Buitarics Congress (pp. 13–19).

Welling, V., Lundeheim, N., & Bengtsson, B. (2020). A pilot study in Sweden on efficacy of benzylpenicillin, oxytetracycline, and florfenicol in treatment of acute undifferentiated respiratory disease in calves. *Antibiotics*, *9*(11), 736. https://doi.org/10.3390/antibiotics9110736

Wentzel, G. M. (2012). *A comparative study of the minimum inhibitory and mutant prevention concentrations of florfenicol and oxytetracycline for animal isolates of Pasteurella multocida and Salmonella typhimurium*. University of Pretoria.

Yáñez, J. A., Remsberg, C. M., Sayre, C. L., Forrest, M. L., & Davies, N. M. (2011). Flip-flop pharmacokinetics--delivering a reversal of disposition: Challenges and opportunities during drug development. *Therapeutic Delivery*, *2*(5), 643–672. https://doi.org/10.4155/tde.11.19

Zhang, Y., Huo, M., Zhou, J., & Xie, S. (2010). PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft excel. *Computer Methods and Programs in Biomedicine*, *99*(3), 306–314. https://doi.org/10.1016/j.cmpb.2010.01.007

How to cite this article: Selimov, R., Goncharova, E., Koriakovtsev, P., Gabidullina, D., Karsakova, J., Kozlov, S., Komarov, A., Engasheva, E., & Engashev, S. (2023). Comparative pharmacokinetics of florfenicol in heifers after intramuscular and subcutaneous administration. *Journal of Veterinary Pharmacology and Therapeutics*, *00*, 1–8. https://doi.org/10.1111/jvp.13110