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Original article

Impact of milk yield on pharmacokinetics of six intramammary drugs – a population approach

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Abstract

The aim of the research was an examination of potential impact of milk yield on the intercompartmental clearance – distribution clearance as well as determination of the variability of obtained pharmacokinetic parameters by the population approach using a two-compartmental structural model. Blood perfusion has a considerable impact on physiology of the udder and kinetics of drugs that are distributed in this organ. The research was performed on healthy Holstein-Friesian and Polish Black-White cows at the age of 4-10 years. Determination of antibiotics (ampicillin, amoxicillin, cefoperazone, penicillin G prokaine, cloxacillin, cefacetril) concentration was carried out after their every intramammary administration to one quarter of the udder. A population pharmacokinetic model was created to fit milk concentration data. General milk yield of a single cow was used as a variable. A population analysis was conducted using non-linear mixed-effect modeling. The impact of milk productivity was set solely by reference to intercompartmental clearance only in case of penicillin G, cloxacillin and ampicillin. It, has been found that milk yield, depending on a drug, influenced the distribution clearance of the drug to varying degrees. It means indirectly that increased perfusion of the udder has a different impact on drug distribution from the udder to the bloodstream.

Key words: intramammary, population, pharmacokinetics, antibiotic

Introduction

Intramammary administration (IMM) of veterinary drugs has been widely applied especially in dairy cows on breeding farms. Currently, observational analysis founded on frequent sampling and calculations, is based exclusively on a structural model (e.g. compartmental model) without simultaneous variables analysis that can modify pharmacokinetic parameters of IMM drugs, and dominates (Allore and Erb 1999, Stockler et al. 2009). One of the pharmacometric methods combining the impact of dependent and independent variables is population pharmacokinetics. One of its applications is the usage of structural model as well as typical values

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to test the impact of variables on selected pharmacokinetic parameters. One of significant variables related to milk production is blood flow through the udder. To produce one liter of milk, about 450 L of blood must pass through the cow udder (Franscini et al. 2006, Berger et al. 2016). Currently, the influence of milk yield on such parameters as distribution clearance or microconstants illustrating the transfer of drug from milk to udder tissue and from udder tissue to milk after IMM application is thought to be ambiguous. Taking into account the presence of IMM drugs residues in the udder tissue as well as in milk, this is a key information. Thus far, the population approach is rarely used in tests on pharmacokinetics of IMM drugs (Whittem 1999). In this paper, the population analysis on six antibiotics in IMM formulations has been performed.

The aim of the research was an examination of the potential impact of milk yield on the intercompartmental clearance – distribution clearance as well as a determination of the variability of obtained pharmacokinetic parameters by the population approach using a two-compartmental structural model. A possible impact of milk productivity on a reduction of unexplained variability of analyzed pharmacokinetics parameters between drugs was subjected to analysis.

Materials and Methods

Animals

The research was conducted on the basis of the authorization of Bioethical Committee, No. 34/2009 of 19 May 2009, Local Ethics Committee for Animal Experiments in Lublin, Poland. The study was performed on healthy Holstein-Friesian and Polish Black-White cows at the age of 4-10 years. The animals were fed farming feed concentrates comprising commercial (wheat, rye) and fodder (oats, barley) grain, alternately with raw corn and pasture grazing. Access to food and water was provided ad libitum. The animals with parameter of SCC < 300 000/mL were assigned to the study (EMA 2017). We utilized cows without chronic mastitis in the past. Evaluation of the animals was based on veterinary examination (udder palpation). Determination of antibiotics concentration was carried out after every IMM administration to one quarter of the udder at following doses: ampicillin (AMP; Lactaclox, ScanVet) 75 mg, amoxicillin (AMX; Synulox L.C, Pfizer) 200 mg, cefoperazone (CEF; Pathozone, Pfizer.) 250 mg, penicillin G prokaine (PEN; Albadry Plus, Pfizer) 200 000 IU, cloxacillin (CLO; Syntarpen, Biowet Pulawy) 500 mg, cefacetril (CTR; Masticef, Biowet Drwalew) 250 mg. The research was performed on cows assigned to 4 grups: CEF (10 cows), AMP/CEF (9 cows), AMX (8 cows), CLO (7 cows). Different groups of animals were used for each drug. All the cows were in the mid-lactation period. One quarter was selected from each cow for sampling. Milk samples for drugs quantification were harvested by hand collection. All the samples were taken before milking. First 4-5 mL of stripped milk was excluded from the analysis. For final analysis, 5 mL of milk was taken from fore-quarters. The analyses were carried out between March and September. The cows came from different farms in Lublin Province area. Milk samples in case of AMP, AMX treatment were collected for chemical analyses just before the drug administration (t=0) and: 2, 4, 6, 8, 10, 24, 36, 48, 60 h after administration; in case of PEN treatment just before the drug administration (t=0) and: 2, 4, 6, 8, 10, 24, 36, 48, 72 h; in case of CEF treatment just before the drug administration (t=0) and: 2, 4, 6, 8, 10, 24, 36, 48, 72 h, 84 h; in case of CTR treatment just before the drug administration (t=0) and: 2, 3, 6, 8, 10, 24, 28, 36, 48, 72, 96 h. The analysis of antibiotics concentration was performed using previously described method (Błądek et al. 2011). Antibiotics were extracted from milk samples, suspended in acetonitrile and their concentration was analysed by Agilent 1200 Series LC system (Agilent Technologies, Santa Clara, USA) connected to API 4000 mass spectrometer (AB SCIEX, Ontario, Canada). Sample analysis was performed by Luna C18 RP column 3 μm, 2.0 × 150 mm (Phenomenex, Torrance, CA, USA) with the mobile phase consisting of acetonitrile and 0.025% heptafluorobutyric acid and gradient elution. Quantification was obtained using multiple-reaction monitoring transition. The utilised method was validated in accordance with current requirements (EC 2002). Matrix-matched calibration curves were utilized for drugs quantification. The specificity of the method was verified by analysis of 20 blank milk samples.

Model development and analysis

A population pharmacokinetic model was created to fit milk concentration data, based on full pharmacokinetic screen sampling (FDA 1999). As a variable, general milk yield (L/24h) of the single cow in mid-lactation period was utilized. Model creation was based on the values acquired from total milk yield. A population analysis was conducted using non-linear mixed-effect modeling implemented in Phoenix WinNonlin v. 7.0 (Certara L.P., Cary, NC, USA). Initial values of structural model selection (multiplicative – $C_{obs,M}$ and log-additive – $C_{obs,A}$) for compartmental models were guided by goodness of fit plots observed *versus* predicted milk concentrations.

$$C_{obs,M} = C \times [1 + (C_\varepsilon)] \qquad C_{obs,A} = C \times \exp(C_\varepsilon)$$

where C_{obs} – observed concentration, C – predicted concentration, and C_ε – residual error.

The covariance structure selection, and final model were fitted by first order conditional estimation method (FOCE-Hess). Imprecision in parameters estimate was assessed using output obtained from Phoenix WinNonlin and simple run mode with 1000 iterations. The percentile bootstrap confidence interval was performed by taking the lower 2.5% and the upper 97.5% of value of each parameter estimated from runs (Mould and Upton, 2013). Naïve pooled analysis, sandwich method and standard error analysis were performed with central difference. A stiff numerical ordinary differential equation (ODE) solver was used for analysis. A two-compartment model with clearance parameterization was used as a structural model.

Final model selection was based on: goodness of fit analysis, precision of model parameters estimates and likelihood analysis. The 2 log-likelihood (-2LL), Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to test different models. The final model was chosen on the basis of lesser values of AIC, better precision of estimate, and lowest percentage coefficient of variation (CV%) of typical values (tv). Drop in AIC or BIC of 2 was a threshold for considering one model over another (Mould and Upton 2013). A reduction in the objective function -2LL by more than 3.84 units (for one added covariate) represented a statistically significant improvement in model fit ($p<0.05$). The variable (general milk yield of a cow) was added to a model if the value of minimum objective function -2LL was reduced by more than 3.84. The variable significantly contributed to the model if the value of minimum objective function increased by more than 7.88 ($p<0.005$) (Laffont et al. 2016). Presented population analysis reports CV% as the measure between animals variability. Parameters calculated in the study were: tvV1 – typical value of volume of distribution in milk compartment, tvV2 – typical value of volume of distribution in udder tissue compartment, tvCL1 – typical value of milk compartment clearance, tvCL2 –typical value of udder tissue compartment clearance, dCL2 – udder tissue compartment clearance calculated with milk productivity variable, tvKe – typical value of elimination rate from milk compartment, tvK12 – typical value of rate constant from milk to udder tissue compartment, tvK21 – typical value of rate constant from udder tissue to milk compartment, stdev0 – estimated residuals.

Results

The impact of milk productivity was solely confirmed with reference to one pharmacokinetic parameter – tvCL2 (forward covariate model) in case of three antibiotics: PEN, CLO and AMP. The significance of this observation at the level of $p<0.05$ confirmed the reduction of the objective function -2LL by more than 3.84 units in case of PEN (3.86 units) and CLO (6.67 units). It was stated that along with increasing difference between -2LL in an output model and model analyzing the influence of milk productivity, the value of ratio tvKe/tvK12 increased. CV% dCL2 also increased with reference to tvCL2. However, in case of PEN, CLO and AMP, it decreased by 10.95, 6.67, and 11.55%, respectively. In case of AMX, CTR and CEF, no considerable impact of milk yield on any of analyzed pharmacokinetic parameters was observed (Table 1, 2).

CV% in the basic population model was at low level, and in case of such parameters as tvK21, tvV1, tvCL1, it did not exceed 20%. The highest value of CV%, in case of all tested drugs, was noted with reference to tvCL2, tvV2 and tvK12. During the analysis, it was proved that the ratio tvKe/tvK12 is different for all tested antibiotics. This ratio with reference to PEN, CLO and AMP (significant impact of milk yield on tvCL2) was at least two times higher than in case of AMX, CTR, CEF where no significant impact of milk yield on was observed. The validation parameters of analytical method are presented in Table 3.

Discussion

It is known that in case of many antibiotics administered IMM, milk yield influences their elimination with milk (Perez-Marin 2012). However, the impact of milk yield on the elimination from the udder tissue and distribution clearance have not been examined so far. A key element of performed calculations was the application of two-compartmental model describing pharmacokinetics of analyzed drugs. On one hand, frequent sampling after single drug administration has an influence on pharmacokinetics of drugs administered IMM. On the other hand, it ensures the analysis of the distribution clearance by determining a microconstant model. It is not possible to determine the distribution clearance without the analysis of microconstants. In order to examine the distribution clearance of drugs administered IMM, frequent sampling of milk is essential during the first 24 hours or 48 hours after drug administration. It enables the increase of dynamic range and sensitivity of the model so that the microconstants can be determined.

Table 1. Arithmetic mean of milk yield, typical value (tv) and subject variability of key population parameters calculated using basic model.

Parameters	PEN	CLO	AMP	AMX	CTR	CEF
tvV1 [L]	3.86 (17.31)	9.12 (6.82)	1.60 (16.35)	4.57 (10.68)	6.08 (34.35)	1.16 (4.46)
tvV2 [L]	0.157 (32.35)	0.189 (17.00)	0.015 (33.73)	0.276 (14.64)	4.476 (40.99)	0.063 (13.21)
tvCL1 [mg/L/h]	2.412 (16.19)	2.725 (4.93)	0.876 (15.02)	2.961 (9.64)	2.005 (17.74)	0.293 (3.87)
tvCL2 [mg/L/h]	0.011 (35.86)	0.012 (23.73)	0.001 (37.60)	0.031 (16.71)	0.246 (50.23)	0.004 (16.44)
tvKe [h^{-1}]	0.6250 (2.71)	0. 2990 (2.11)	0.5485 (1.88)	0.6478 (1.33)	0. 3293 (25.91)	0. 2519 (6.65)
tvK12 [h^{-1}]	0.0029 (21.81)	0.0013 (21.77)	0.0008 (22.45)	0.0068 (10.81)	0. 0404 (44.85)	0.0033 (18.29)
tvK21 [h^{-1}]	0.0710 (4.93)	0.0648 (10.56)	0.0887 (11.37)	0.1135 (6.60)	0.0548 (12.90)	0.0606 (3.42)
stdev0	0.536	0.343	0.683	0.462	0.817	0.527
-2LL	100.10	48.74	168.04	93.06	2131.06	1873.07
AIC	110.10	58.74	178.04	103.06	2141.06	1883.07
BIC	120.81	69.98	190.01	114.44	2154.56	1895.57
Residual error	Log-additive				Multiplicative	
tvKe/tvK12 [h^{-1}]	216.20	221.95	647.47	94.58	8.16	76.26
Milk yield [L/24h]*	35.14 (±6.08)	37.57 (±6.07)	38.11 (±3.87)	38.75 (±3.34)	36.60 (±5.31)	36.67 (±5.23)

PEN – penicillin G prokaine; CLO – cloxacillin; AMP – ampicillin; AMX – amoxicillin; CTR – cefacetril; CEF – cefoperazone; * – arithmetic mean with standard deviation value; tvV1 – typical value of volume of distribution in milk compartment; tvV2 – typical value of volume of distribution in udder tissue compartment; tvCL1 – typical value of milk compartment clearance; tvCL2 – typical value of udder tissue compartment clearance; tvKe – typical value of elimination rate from milk compartment; tvK12 – typical value of rate constant from milk to udder tissue compartment; tvK21 – typical value of rate constant from udder tissue to milk compartment; -2LL – 2 log-likelihood; AIC – Akaike infLLormation criterion; BIC – Bayesian information criterion; stdev0 – estimated residuals.

As a result of performed analyses, a substantial impact of milk yield on the distribution clearance of selected IMM antibiotics in the analyzed set was observed. One of the limits of the research was a small sample size (n=7-10) (Duffull et al. 2011, Colby 2012). However, it should be noted that the calculations were performed on the basis of full pharmacokinetic screen (FDA 1999), which increases the quality of the proposed model. The results of research led to the conclusion that in case of only some IMM drugs, general milk yield can considerably diversify the level of drug residuals in the udder tissue. This means that for such drugs determination of the level of residuals in milk and tissues should consider the level of milk yield as the criterion for test inclusion. Hence, the question remains whether during determination of permissible limits of drugs' residuals in tissues and milk for this type of drugs, these limits should be dependent upon milk yield. Since the differences in milk production considerably influence distribution clearance, the level of *depot* in udder tissues should be very different for cows of productivity of 30 and 50 L of milk per 24 h. It should be borne in mind that in such case, blood flow through the udder should oscillate within the limits of 562-937 L/h. Thus, the differences in blood flow through udder tissues are reflected in the differences in milk yield, and this, in case of selected drugs, has a considerable impact on distribution clearance.

Simultaneously to the population analysis, pharmacokinetic parameters which would correlate with the influence of mild yield on distribution clearance, were searched. The compilation of parameters that differentiate between PEN, CLO, AMP (CL2 dependent on milk yield) and AMX, CTR, CEF (no impact) turned out to be speed constants. The ratio tvKe/tvK12 appears to be crucial in the light of performed tests. It was demonstrated that along with increasing distance between both values i.e. at tvKe reaching 1 and tvK12 reaching 0, the value of this ratio substantially increases in case of drugs which tvCL2 is dependent upon milk yield. The difference in value of ratio tvKe/tvK12 between both groups of antibiotics (PEN, CLO, AMP *ver-*

Table 2. Values of the distribution clearance and subject variability in the final model.

Parameters	PEN	CLO	AMP	AMX	CTR	CEF
dCL2 [mg/L/h]	0.019 (27.59)	0.061 (18.04)	1.62 (21.08)		na	
-2LL	96.24	42.07	144.20	1100.21	2131.05	1873.07
-2LL	3.86*	6.67*	23.85**		na	
AIC	108.24	54.07	156.20	1118.21	2141.05	1883.07
BIC	121.09	67.56	170.56	1139.76	2154.55	1895.57
Residual error	Log-additive				Multiplicative	

PEN – penicillin G prokaine; CLO – cloxacillin; AMP – ampicillin; AMX – amoxicillin; CTR – cefacetril; CEF – cefoperazone; dCL2 – udder tissue distribution clearance calculated with milk yield covariate; -2LL – 2 log-likelihood (objective function); -2LL – level of reduction of the objective function; AIC – Akaike information criterion; BIC – Bayesian information criterion; * p-value <0.05; ** p-value <0.005; na – data not available.

Table 3. The validation parameters of analytical method.

Validation parameters	PEN	CLO	AMP	AMX	CTR	CEF
Linearity (r)	0.9984	0.9995	0.9956	0.9984	0.9994	0.9984
LLOQ (ng/mL)	4.00				1.00	
HLOQ (ng/mL)	300.00		40.00		500.00	
Repeatability LQ (CV%)	14.20	15.20	12.30	14.90	16.70	15.70
Repeatability MQ (CV%)	5.70	5.10	9.80	12.20	12.20	12.20
Repeatability HQ (CV%)	9.10	8.10	7.60	9.20	9.60	9.60
Reproducibility LQ (CV%)	15.70	15.90	10.40	13.60	7.20	15.10
Reproducibility MQ (CV%)	9.40	7.30	9.30	12.60	13.40	13.40
Reproducibility HQ (CV%)	11.00	9.50	8.00	10.00	9.50	9.00
Recovery (%)	99.60	99.90	95.40	95.20	89.50	91.50

LLOQ – lower limit of quantitation; HLOQ – higher limit of quantitation; LQ – low quality control level; MQ – medium quality control level; HQ – high quality control level; CV% – coefficient of variation (%).

sus AMX, CTR, CEF) is in this case significant. Without population analysis, the determination of impact of such covariates as milk yield on tvCL2 would be very difficult. Methods of population pharmacokinetics are perfect tools for analyzing such types of variables. Due to the limited number of raw data, only six drugs were subjected to analysis. Nevertheless, the results indicate that the value of ratio tvKe/tvK12 determined within the frame of two-compartment structural model (omitting population analysis) can constitute an index for tvCL2 dependent or independent on milk yield. As results from performed analyses, in case of PEN, the significance at the minimum level was reached in case of tvKe/tvK12 = 216.2. It should be noted that the drop of -2LL by 3.84 points, means reaching the significance at the level of $p<0.05$. In case of PEN the drop in value -2LL was 3.86. This means that in relation to the ratio tvKe/tvK1, it was close to the limit (216.2) from which occurs a considerable impact of milk yield on CL2. In practice, it can mean that if tvKe/tvK12, calculated on the basis of two-compartment model, is equal to or greater than the value 216.2, CL2 can significantly be dependent on milk yield. In the paper devoted to pharmacokinetics of pirlimycin, data referring to a two-compartmental structural model were presented (Whittem 2012). On their basis, it can be stated that the ratio tvKe/tvK12 for pirlimycin after IMM administration is about 115. This shall constitute the basis for posing a hypothesis that tvCL2 of pirlimycin is not dependent upon milk yield. However, this hypothesis requires verification by a separate analysis. In the paper devoted to pharmacokinetics of cephapirin, administered IMM, a two-compartmental model was also applied, yet the value of K12 was not published, which makes it impossible to calculate the ratio tvKe/tvK12 (Stockler et al. 2009). In relation to a lot of drugs, administered IMM, some

elements concerning drug distribution from the udder tissue to peripheral blood were discovered. IMM administered PEN could be redistributed into the blood circulation by the active transport (Schadewinkel-Scherkl et al. 1993). In case of CLO, the findings confirm, to some extent, previous results on good distribution of CLO in all regions of the mammary gland (Kietzmann et al. 2010). Systemic drug absorption of CEF after IMM administration was negligible in healthy animals (Cagnardi et al. 2010, Burmańczuk et al. 2011). Nevertheless, the majority of analyses of pharmacokinetic IMM drugs, do not consider the phenomena of drug transfer between compartments such as the udder tissue, milk or peripheral blood.

The present study has revealed that milk yield, depending on a drug, influences the distribution clearance of drugs to varying degrees. This means indirectly that increased perfusion of the udder has a different impact on the drug distribution from the organ to bloodstream. It is confirmed by observations made in this research, in which after IMM administration, the concentration of the drug was also analyzed in blood plasma. Depending on a drug, these concentrations can be relatively high or very low (Soback et al. 1995, Cagnardi et al. 2010, Li et al. 2014, Ray et al. 2014, López et al. 2015).

Conclusions

In conclusion, a considerable impact of milk yield on the distribution clearance of some drugs administered IMM was confirmed in this study. Moreover, the model enabling the differentiation of IMM drugs among themselves depending on the influence of milk yield on their distribution clearance was developed.

References

Allore HG, Erb HN (**1999**) Approaches to modeling intramammary infections in dairy cattle. Prev Vet Med 39: 279-293.

Berger H, Lietzau M, Tichy A, Herzog K (**2016**) Investigations of mammary and uterine blood flow in relation to milk yield, postpartum disease, and pregnancy result in dairy cows. Theriogenology. 86: 1906-1912.

Błądek T, Posyniak A, Gajda A, Gbylik M, Żmudzki J (**2011**) Multi-class procedure for analysis of antibacterial compounds in animal tissues by liquid chromatography tandem mass spectrometry. Bull Vet Inst Pulawy 55: 741-748.

Burmańczuk A, Roliński Z, Kowalski C, Zań R (**2011**) Concentration of cefacetril in milk after its intramammary administration to cows with healthy and inflammed mammary gland. Bull Vet Inst Pulawy 55: 685-688.

Cagnardi P, Villa R, Gallo M, Locatelli C, Carli S, Moroni P, Zonca A (**2010**) Cefoperazone sodium preparation behavior after intramammary administration in healthy and infected cows. J Dairy Sci 93: 4105-4110.

Colby EA (**2012**) Methods for population pharmacokinetics and pharmacodynamics. A dissertation, University of North Carolina, 1-109. https://cdr.lib.unc.edu/indexable-content/uuid:1dd46652-cb30-4b03-9c098b9f8e83fbb

Duffull SB, Wright DF, Winter HR (**2011**) Interpreting population pharmacokinetic-pharmacodynamic analyses - a clinical viewpoint. Brit J Clin Pharmacol 71: 807-814.

European Commission (**2002**) Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off J Eur Commun L 221: 8-36. http://eur-lex.europa.eu/eli/dec/2002/657/oj

European Medicines Agency (**2017**) Guideline for the conduct of efficacy studies for intramammary products for use in the cattle. 1-19. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2017/01/WC500220206.pdf

Franscini N. El Gedaily A, Matthey U, Franitza S, Sy M-S, Bürkle A, Groschup M, Braun U, Zahn R (**2006**) Prion Protein in Milk. PLoS One. 1: e71.

Kietzmann M, Niedorf F, Gossellin J (**2010**) Tissue distribution of cloxacillin after intramammary administration in the isolated perfused bovine udder. BMC Vet Res 6: 46.

Laffont CM, Gomeni R, Heidbreder C, Jones JP3rd, Nasser AF (**2016**) Population pharmacokinetic modeling after repeated administrations of RBP-6000, a new, subcutaneously injectable, long-acting, sustained-release formulation of buprenorphine, for the treatment of opioid use disorder. J Clin Pharmacol 56: 806-815.

Li Y, Wang L, Gu X, Zeng Z, He L, Yang F, Yuan B, Shu J, Ding H (**2014**) Pharmacokinetics and residues of cefquinome in milk of lactating chinese dairy cows after intramammary administration. J Integr Agr 13: 2750-2757.

López C, García JJ, Sierra M, Diez MJ, Pérez C, Sahagún AM, Fernández N (**2015**) Systemic and mammary gland disposition of enrofloxacin in healthy sheep following intramammary administration. BMC Vet Res 11: 88.

Mould DR, Upton RN (**2013**) Basic concepts in population modeling, simulation, and model-based drug development-part 2: introduction to pharmacokinetic modeling methods. CPT Pharmacometrics Syst Pharmacol 2: e38.

Perez-Marin CC (**2012**) A Bird's-Eye View of Veterinary Medicine. Pharmacokinetic – Pharmacodynamic Considerations for Bovine Mastitis Trea A Bird's-Eye View of Veterinary Medicine. Pharmacokinetic – Pharmacodynamic Considerations for Bovine Mastitis Treatment. Dr. Carlos C. Perez-Marin C.C. (ed.), pp 423-472. http://www.intechopen.com/books/a-bird-s-eye-view-of--veterinarymedicine/pharmacokinetic-pharmacodynamic--considerations-for-bovine-mastitis-treatment

Ray P, Knowlton KF, Shang C, Xia K (**2014**) Development and validation of a UPLC-MS/MS method to monitor cephapirin excretion in dairy cows following intramammary infusion. PLoS One. 9: e112343.

Schadewinkel-Scherkl AM, Rasmussen F, Merck CC, Nielsen P, Frey HH (**1993**) Active transport of benzylpenicillin across the blood-milk barrier. Pharmacol Toxicol 73: 14-19.

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.

Stockler RM, Morin DE, Lantz RK, Constable PD (**2009**) Effect of milking frequency and dosing interval on the pharmacokinetics of cephapirin after intramammary infusion in lactating dairy cows. J Dairy Sci 92: 4262-4275.

Whittem T (**1999**) Pharmacokinetics and milk discard times of pirlimycin after intramammary infusion: a population approach. J Vet Pharmacol Ther 22: 41-51.

US Food and Drug Administration (**1999**) Guidance for Industry Population Pharmacokinetics.1-35.https://www.fda.gov/downloads/drugs/guidances/UCM072137.pdf

Whittem, T, Whittem JH, Constable PD (**2012**) Modelling the concentration-time relationship in milk from cattle administered an intramammary drug. J Vet Pharmacol Ther 35: 460-471.