Title

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Journal

Antimicrobial Agents and Chemotherapy, 58(8)

ISSN

0066-4804

Authors

Li, Mengjie
Gehring, Ronette
Tell, Lisa
et al.

Publication Date

2014-08-01

DOI

10.1128/aac.02806-14

Peer reviewed

AAC
Journals.ASM.org

Interspecies Mixed-Effect Pharmacokinetic Modeling of Penicillin G in Cattle and Swine

Mengjie Li,[a] Ronette Gehring,[a] Lisa Tell,[b] Ronald Baynes,[c] Qingbiao Huang,[a] Jim E. Riviere[a]

Institute of Computational Comparative Medicine, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA[a]; School of Veterinary Medicine, University of California, Davis, California, USA[b]; College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA[c]

Extralabel drug use of penicillin G in food-producing animals may cause an excess of residues in tissue which will have the potential to damage human health. Of all the antibiotics, penicillin G may have the greatest potential for producing allergic responses to the consumer of food animal products. There are, however, no population pharmacokinetic studies of penicillin G for food animals. The objective of this study was to develop a population pharmacokinetic model to describe the time-concentration data profile of penicillin G across two species. Data were collected from previously published pharmacokinetic studies in which several formulations of penicillin G were administered to diverse populations of cattle and swine. Liver, kidney, and muscle residue data were also used in this study. Compartmental models with first-order absorption and elimination were fit to plasma and tissue concentrations using a nonlinear mixed-effect modeling approach. A 3-compartment model with extra tissue compartments was selected to describe the pharmacokinetics of penicillin G. Typical population parameter estimates (interindividual variability) were central volumes of distribution of 3.45 liters (12%) and 3.05 liters (8.8%) and central clearance of 105 liters/h (32%) and 16.9 liters/h (14%) for cattle and swine, respectively, with peripheral clearance of 24.8 liters/h (13%) and 9.65 liters/h (23%) for cattle and 13.7 liters/h (85%) and 0.52 liters/h (40%) for swine. Body weight and age were the covariates in the final pharmacokinetic models. This study established a robust model of penicillin for a large and diverse population of food-producing animals which could be applied to other antibiotics and species in future analyses.

Penicillin G is a commonly used veterinary drug and is approved for use in cattle and swine in the United States (1). It has been used extensively in the treatment of bacterial pneumonia, upper respiratory infection, such as rhinitis or pharyngitis, and blackleg in ruminants (2, 3, 4). Penicillin G is used in the form of its sodium or potassium salts when the approved route of administration is intravenous (i.v.) or intramuscular (i.m.) (5, 6), while penicillin G procaine is approved for use via intramuscular and subcutaneous (s.c.) administration for cattle and swine (7).

Penicillin G is used extensively in food-producing animals to treat disease and maintain optimal health, all of which have the potential to result in drug residues in meat, milk, and eggs (8). Of all the antibiotics, penicillin G may have the greatest potential for producing allergic responses to the consumer of food animal products (9). To avoid tissue residues of penicillin G, the U.S. Food and Drug Administration (FDA) has established 0.05-ppm and zero-tolerance limits for penicillin G residues in edible tissues of cattle and swine, respectively (10). According to the tolerance limits, at least a 14-day withdrawal interval (WDI) is recommended for the labeled use of penicillin G. However, due to the development of microbial resistance since initial approval of these drugs decades ago, to remain effective penicillin is one of the drugs most commonly used at extralabel doses (11). A reason for the occurrence of violative residues may be the extralabel manner in which penicillin G often is administered to animals at a dose which is higher than the approved label dose, which may significantly influence the levels of antibiotic resistance (12, 13). Therefore, it is believed that more accurate information on the pharmacokinetics (PK) of penicillin G is important for selecting optimal therapy.

Many researchers have investigated the pharmacokinetic behavior and residues of penicillin G in blood, tissue, and milk for cattle and swine (14, 15, 16). A major concern of the use of penicillin G in food animals is that the extralabel use of antibiotics may cause the transfer of resistance via the food chain to humans (17). Penicillin G concentrations in tissue are also very important, because the meat may not be used for human food if the tolerance is exceeded. So far, studies have shown that the ratio of penicillin G concentration in the tissue to that in the blood was strongly influenced by the formulation of the drug and the time of sampling (11, 18). Individual PK studies cannot characterize the inter- and intrasubject variability. Thus, the establishment of a population pharmacokinetic model to gather integrated information in a large population, including all blood and tissue data, is essential.

Population pharmacokinetic analysis has been advocated to be used in veterinary medicine for many years (19, 20). The development of this technique allows us to explore the relationships between clinical factors (such as weight, age, gender, species, etc.) and drug disposition, which will facilitate the determination of efficacy and safety of drugs. So far, a stochastic pharmacokinetic model was successful in determining effects of variability in systemic pharmacokinetics on tissue depletion of sulfamethazine in swine (21). Wu et al. investigated the influence of PK parameters on flunixin tissue residue concentrations in livers using a population pharmacokinetic model (22). The objective of this study was to develop a population PK model to describe the complete PK profile in plasma/serum and tissues of penicillin G in cattle and swine based on data generated from a variety of studies.

Received 18 March 2014 **Returned for modification** 19 April 2014
Accepted 18 May 2014
Published ahead of print 27 May 2014
Address correspondence to Jim Riviere, jriviere@ksu.edu.

doi:10.1128/AAC.02806-14

TABLE 1 Summary of PK studies of penicillin in cattle and swine from which PK data were collected and used in population PK modeling

Species	*N*	Route	Formulation	Dose(s) (mg/kg)	Matrix	WT (kg)	Age (yr)	Sex[a]	Source or reference
Cattle	6	i.v.	Penicillin sodium	4	Plasma		0.01/0.09		5
Cattle	9	i.v.	Penicillin sodium	6.25/7.1/8.8	Plasma	49.2	0.02		27
Cattle	11	i.v.	Penicillin sodium	1	Plasma	102	0.42	F	28
Cattle	5	i.v.	Penicillin sodium	10	Serum	148	0.46		29
Cattle	5	i.v.	Penicillin sodium	10	Serum	633.4	5.8	F	30
Cattle	6	i.v.	Penicillin sodium	6.4	Plasma	531.5		F	31
Cattle	3	i.m.	Penicillin sodium	10	Serum	150		F	15
Cattle	9	i.m.	Penicillin sodium	12.1/11.9	Plasma	149	0.25	F	32
Cattle	4	i.m.	Penicillin sodium	30	Serum	153	0.49	F	33
Cattle	4	i.m.	Penicillin sodium	6.3/30	Serum	179	0.41	Both	6
Cattle	7	i.m.	Penicillin procaine	20	Serum	65	0.12		34
Cattle	3	i.m.	Penicillin procaine	11	Blood			F	35
Cattle	4	i.m.	Penicillin procaine	9.3	Serum/kidney	537			18
Cattle	4	i.m.	Penicillin procaine	9.5	Plasma			F	36
Cattle	4	i.m.	Penicillin procaine	20	Plasma				37
Cattle	3	i.m.	Penicillin procaine	24/66	Plasma	480		M	12
Cattle	4	i.m.	Penicillin procaine	10.26	Plasma				38
Cattle	12	i.m.	Penicillin procaine	7	Plasma/kidney	193		M	11
Cattle	6	i.m.	Penicillin procaine	20	Serum			F	39
Cattle	3	i.m.	Penicillin procaine	9.26	Serum/kidney	500		F	40
Cattle	4	i.m.	Penicillin procaine	11	Serum			F	41
Cattle	4	i.m.	Penicillin procaine	6.76/13.3/18.53	Serum			F	42
Cattle	7	i.m.	Penicillin procaine	20	Serum	65	0.12		43
Cattle	5	i.m.	Penicillin procaine	30	Serum		0.21		14
Cattle	6	i.m.	Penicillin procaine	41	Serum	92	0.15	M	44
Cattle	15	i.m.	Penicillin procaine	12/24	Kidney/liver	553		M	45
Cattle	6	i.m.	Penicillin procaine	8	Kidney				46
Cattle	18	i.m.	Penicillin procaine	24	Kidney/liver	485	1	M	47
Cattle	3	i.m.	Penicillin procaine	3.75/7.5/15	Liver	104		M	48
Cattle	6	p.o	Penicillin procaine	4	Plasma		0.01/0.1		5
Cattle	19	s.c	Penicillin procaine	1.5	Plasma	43.5		M	49
Swine	8	i.v.	Penicillin potassium	7.64	Plasma/kidney/muscle	20.9	0.153		50
Swine	6	i.v.	Penicillin potassium	10.4/51.8	Plasma	27	0.259		51
Swine	6	i.v.	Penicillin potassium	10.4/52.6	Plasma	29.5			52
Swine		i.v.	Penicillin potassium	7.64	Kidney/muscle				53
Swine	5	i.v./i.m.	Penicillin potassium	10	Serum	150		F	15
Swine	6	i.m.	Penicillin potassium	15	Serum	35	0.25	M	54
Swine	9	i.m.	Penicillin procaine	15.5/11.7	Plasma	3.3	0.02		7
Swine	5	i.m.	Penicillin procaine	15.9	Plasma	70			55
Swine	2	i.m.	Penicillin procaine	8.47	Serum	53.4			56
Swine	8	i.m.	Penicillin procaine	21.3	Serum	49	0.25	Both	57
Swine		i.m.	Penicillin procaine	15	Kidney/muscle	100		Both	58
Swine		i.m.	Penicillin procaine	15	Kidney/muscle	100			59
Swine	3	i.m.	Penicillin procaine	12.3	Kidney/muscle	95			60
Swine	2	i.m.	Penicillin procaine	9	Muscle				61
Swine	126	i.m.	Penicillin procaine	33	Plasma/kidney/muscle	221.1			Shelver et al. (unpublished data)[b]

[a] F, female; M, male.
[b] Weilin L. Shelver, Sara J. Lupton, David J. Newman, and David J. Smith, unpublished data.

MATERIALS AND METHODS

Data collection. A literature search was conducted to obtain concentrations of penicillin G in the serum (plasma), liver, muscle, and kidney from original PK studies conducted in cattle and swine. The selected data for model development included time-concentration profiles of penicillin G for young and adult animals and the residues of penicillin G in serum (plasma), liver, kidney, and muscle. Based on these data, the plasma model was fit first, and then the tissue data were incorporated. In the tissue data, the residue data for muscle of cattle were rare, and no residue data for liver of swine were recorded. Therefore, none of the muscle data for cattle and liver data for swine were included in the model-building process. Data of animals in various diseased conditions were excluded. A PK study of penicillin G in bovine plasma was discarded because its calculated PK parameters were outliers compared to other data (23). Body weight, age, species, and sex also were recorded according to the original study. The individual estimated PK parameters were collected from the original literature or from that collected and entered into the Food Animal Residue Avoidance Databank (FARAD). A summary of the data sources is represented in Table 1. If the concentrations at each sampling time point were not listed in the original article, the graphs from references were

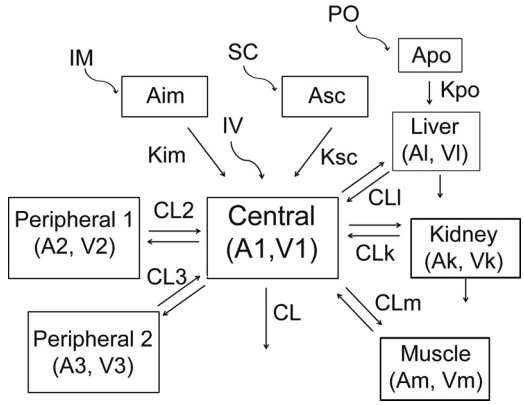

FIG 1 Schematic representation of the final pharmacokinetic (PK) model for pooled data set of blood and tissue concentrations of penicillin in cattle and swine.

scanned with the software UN-SCAN-IT (version 6.0; Silk Scientific, Orem, UT, USA) to extract the mean time-concentration data ($n = 3$ to 19 animals). All animals in healthy status, including steers, heifers, cows, and piglets, were pooled for the population model. Overall, a combination of a 100-cattle data set and an 89-swine data set was used in the population pharmacokinetic analysis.

Population pharmacokinetic analysis. A population pharmacokinetic model was developed to fit to whole-blood and tissue concentration data, as illustrated in Fig. 1. Population analysis was conducted using nonlinear mixed-effect modeling as implemented in Phoenix NLME (version 1.2; Certara, Cary, NC, USA). The extended least-squares, first-order conditional estimation method (FOCE-ELS) with interaction was used to derive population pharmacokinetic parameters. Mean population pharmacokinetic variables, interindividual variability (IIV), and residual error were assessed in model development (24). Exponential models were used to describe between-individual and between-occasion variability, while additive, proportional, and combined error models were tested for residual variability. Model selection for compartmental models was guided by goodness-of-fit plots (e.g., observed versus predicted plasma concentrations, weighted residuals versus predicted concentrations, and weighted residuals versus time). The −2 log-likelihood (−2LL), the Akaike information criterion (AIC), and Bayesian information criterion (BIC) were used to test different hypotheses regarding the final model (25). The model was chosen on the basis of lesser values of AIC, better precision of estimate, and superior goodness-of-fit plots.

Simultaneous modeling of plasma data and tissue data. To establish the model for combined data of plasma/serum and tissue concentrations of penicillin G, first the best structural model for plasma/serum concentration of penicillin was selected. A one-, two-, or three-compartment model with first-order absorption and first-order elimination was used to analyze the concentration-time data. Given that different penicillin formulations were administered via different routes, several absorption compartments were added to the structural model to incorporate these data sets. Based on the best model for plasma concentrations of penicillin G, tissue compartments, including a liver compartment for cattle, muscle compartment for swine, and kidney compartments for both cattle and swine, subsequently were added. In this modeling process, it was assumed that penicillin was orally administered to the liver compartment directly (20). The final PK structural model developed for blood and tissue concentrations of penicillin is shown in Fig. 1. The model assumed that clearance (CL) of peripheral compartment 1 was consistent with the clearance of liver, muscle, and kidney compartments. The volume of distribution was partitioned between peripheral compartment 1 and the liver, muscle, and kidney compartments. The differential equation describing the PK model of blood and tissue concentrations of penicillin is the following:

$$\frac{dA_1}{dt} = A_{\mathrm{im}_1} \times \ k_{\mathrm{im}_1} + A_{\mathrm{im}_2} \times K_{\mathrm{im}_2} + A_{\mathrm{sc}} \times K_{\mathrm{sc}} - \mathrm{CL} \times C - [\mathrm{CL}_2 \times (C - C_2)] - [\mathrm{CL}_3 \times (C - C_3)] - [Q_{\mathrm{L}} \times (C - C_{\mathrm{L}})] - [Q_{\mathrm{K}} \times (C - C_{\mathrm{K}})] - [Q_{\mathrm{M}} \times (C - C_{\mathrm{M}})] \quad (1)$$

where $dA_{\mathrm{im1}}/dt = -A_{\mathrm{im1}} \times K_{\mathrm{im1}}$, $dA_{\mathrm{im2}}/dt = A_{\mathrm{im2}} \times K_{\mathrm{im2}}$, $dA_{\mathrm{sc}}/dt = -A_{\mathrm{sc}} \times K_{\mathrm{sc}}$, $dA_{\mathrm{po}}/dt = -A_{\mathrm{po}} \times K_{\mathrm{po}}$, $dA_2/dt = \mathrm{CL}_2 \times (C - C_2)$, $dA_3/dt = \mathrm{CL}_3 \times (C - C_3)$, $dA_{\mathrm{L}}/dt = A_{\mathrm{po}} \times K_{\mathrm{po}} + Q_{\mathrm{L}} \times (C - C_{\mathrm{L}}) - \mathrm{CL}_{\mathrm{L}} \times \mathrm{CL}$, $dA_{\mathrm{K}}/dt = Q_{\mathrm{K}} \times (C - C_{\mathrm{K}}) - \mathrm{CL}_{\mathrm{K}} \times C_{\mathrm{K}}$, $dA_{\mathrm{M}}/dt = Q_{\mathrm{M}} \times (C - C_{\mathrm{M}})$, $C = A_{\mathrm{iv}}/V$, $C_2 = A_{\mathrm{iv}}/V$, $C_3 = A_{\mathrm{iv}}/V$, $C_{\mathrm{L}} = A_{\mathrm{iv}}/V$, $C_{\mathrm{K}} = A_{\mathrm{iv}}/V$, and $C_{\mathrm{M}} = A_{\mathrm{M}}/V_{\mathrm{M}}$. A_1 is the total penicillin amount in the central compartment, A_{im1} is the penicillin sodium or potassium amount in the absorption compartment via i.m. dosing, A_{im2} is the procaine penicillin amount in the absorption compartment via i.m. dosing, A_{sc} is the procaine penicillin amount in the absorption compartment via s.c. dosing, A_{po} is the procaine penicillin amount in the absorption compartment via per os (p.o.) dosing, A_2 is the total penicillin amount in peripheral compartment 1, A_3 is the total penicillin amount in peripheral compartment 2, A_{L} is the total penicillin amount in the liver compartment, A_{K} is the total penicillin amount in the kidney compartment, A_{M} is the total penicillin amount in the muscle compartment, C is the penicillin concentration in the central compartment, C_2 is the penicillin concentration in the peripheral compartment 1, C_3 is the penicillin concentration in peripheral compartment 2, C_{L} is the penicillin concentration in the liver, C_{K} is the penicillin concentration in the kidney, and C_{M} is the penicillin concentration in muscle. The definitions for PK parameters in the final model are listed in Table 2.

Covariate model building. To establish the possible relationships between the PK of penicillin and individual characteristics, the following covariates were defined: species, weight, and age. Species was set as a dichotomous variable in order to distinguish cattle and swine in the model using $P_i = [P_{\mathrm{pop\ swine}} \times (\mathrm{species} == 0) + P_{\mathrm{pop\ cattle}} \times (\mathrm{species} == 1)] \times e^{ni}$ for cattle and $P_i = [P_{\mathrm{pop\ swine}} \times (\mathrm{species} == 1) + P_{\mathrm{pop\ cattle}} \times (\mathrm{species} == 0)] \times e^{ni}$, where P_i is the individual parameter in the ith individual, P_{pop} is the estimate of the population parameter, and ni is the random between-subject variability. The double equals sign means a value is assigned to the object. The default covariate value of species is set as 1 for cattle and 0 for swine in the cattle population, with the values switched for the swine population.

Meanwhile, weight and age were set as continuous variables which are related to the population means as $P_i = [P_{\mathrm{pop}} \times (\mathrm{weight/mean})^{\theta}] \times e^{ni}$ and $P_i = [P_{\mathrm{pop}} \times (\mathrm{age/mean})^{\theta}] \times e^{ni}$, where θ is the estimate of the covariate factor.

The covariates were first individually assessed in univariate analyses. A covariate was included in an intermediate model if addition of the covariate resulted in a decrease in the objective function value (OFV) of >3.875 ($P < 0.05$). A stepwise backward elimination procedure was also performed in which each of the covariates was deleted sequentially. A covariate was retained only in the model when its influence was statistically significant and relevant.

Model validation and simulation. A bootstrap resampling technique was applied to assess the stability and performance of the final model (26). One thousand bootstrap data sets were generated by random sampling with replacement of the original data set using Phoenix NLME (version 1.2; Certara). If the parameter estimates fell into the 95% confidence intervals (CIs) from the bootstrap analysis, the model was considered unbiased. Monte Carlo simulations were conducted using the PK parameter estimates from the final model to simulate the time-concentration profile of residues in tissues of cattle and swine. Phoenix NLME was used to perform Monte Carlo simulation under a dosing regimen of 30 mg/kg of body weight daily for 5 days by i.m. administration. For each species, the Monte Carlo simulation generated concentration-time profiles of penicillin G in tissue for 100 replicates. The WDI was determined when the upper limit of the 95% confidence intervals of the 99th percentile of the tissue concentration-

TABLE 2 Population PK parameters obtained from the PK model for plasma and tissue concentrations of penicillin for cattle

Parameter	Description	Value (RSE [%])[a]			CI	
		Population mean	IIV	Bootstrap value	2.5%	97.5%
V_1 (liter)	Volume of distribution for the central compartment	3.45 (12)	9.59 (23)	3.44	3.39	3.47
V_2 (liter)	Volume of distribution for the peripheral compartment 1	20.8 (14)	0.89 (22)	21.9	21.6	22.2
V_3 (liter)	Volume of distribution for the peripheral compartment 2	30.3 (13)	0.45 (15)	30.4	29.9	30.7
V_L (liter)	Volume of distribution for the liver compartment	11.2 (29)	2.76 (31)	11.4	11.3	11.5
V_K (liter)	Volume of distribution for the kidney compartment	12.5 (25)	2.91 (13)	13.1	12.3	13.3
CL_1 (liters/h)	Central clearance	105 (32)	0.89 (23)	105	104	106
CL_2 (liters/h)	Clearance between the central and the peripheral 1 compartment	24.8 (13)	0.37 (11)	24.8	24.5	25.1
CL_3 (liters/h)	Clearance between the central and the peripheral compartment 2	9.65 (23)	NE (NA)	9.75	9.63	9.86
CL_L (liters/h)	Clearance between the central and the liver compartment	21.5 (37)	NE (NA)	21.7	21.4	21.8
CL_K (liters/h)	Clearance between the central and the kidney compartment	21.5 (24)	4.04 (12)	20.7	20.5	21.1
K_{im1} (liters/h)	Absorption rate constant after intramuscular (i.m.) injection of penicillin sodium	0.31 (14)	0.05 (5.7)	0.33	0.31	0.35
K_{im2} (liters/h)	Absorption rate constant after intramuscular (i.m.) injection of procaine penicillin	0.22 (17)	0.35 (17)	0.21	0.20	0.22
K_{sc} (liters/h)	Absorption rate constant after subcutaneous (s.c.) injection of procaine penicillin	0.56 (15)	NE (NA)	0.55	0.54	0.56
K_{po} (liters/h)	Absorption rate constant after oral administration (p.o.) of procaine penicillin	0.38 (11)	NE (NA)	0.38	0.37	0.39
F_{im1} (%)	Bioavailability of penicillin after i.m. injection of penicillin sodium	80.1 (7.3)	1.24 (16)	79.8	79.1	80.5
F_{im2} (%)	Bioavailability of penicillin after i.m. injection of procaine penicillin	91.3 (19)	1.84 (22)	91.0	90.1	91.8
F_{sc} (%)	Bioavailability of penicillin after s.c. injection of procaine penicillin	75.2 (12)	2.31 (21)	74.7	74.1	75.3
F_{po} (%)	Bioavailability of penicillin after p.o. injection of procaine penicillin	42.4 (14)	2.01 (15)	42.6	42.1	43.1
Covariate factors						
θ_1	Estimated covariate factor of wt on V_3	−0.005		−0.005	−0.0051	−0.0049
θ_2	Estimated covariate factor of wt on CL_L	0.008		0.008	0.0078	0.0082
θ_3	Estimated covariate factor of age on V_1	−0.011		−0.011	−0.011	−0.012
Residual errors (%)		23				

[a] RSE, relative standard error; NE, not estimated; NA, not applicable.

time data were below the target tolerance limit, set at 0.05 ppm and 0.025 ppm for cattle and swine, respectively.

RESULTS

Structural PK model development. A 3-compartment model with first-order adsorption and first-order elimination best characterized the data of plasma/serum concentrations only. A total of 368 plasma/serum concentrations of penicillin in cattle and 443 plasma concentrations in swine were simultaneously modeled. We combined several dosing routes of different penicillin formulations into the same model. The PK parameters of the plasma PK model are listed in Tables 2 and 3. A proportional error model most adequately characterized the distribution of residual variability. Based on the plasma model of penicillin, a total of 26 liver concentrations and 13 kidney concentrations in cattle, as well as 97 kidney concentrations and 84 muscle concentrations in swine, were added to develop the tissue population PK model.

Covariate model development. The final PK model was significantly improved by introduction of the covariate weight and age. Both body weight and age showed an influence on penicillin clearance and volume of distribution in cattle and swine. However, considering the magnitude of the changes in objective function, body weight was found to be more influential than age on clearance while age had more impact on volume of distribution. Weight was significantly correlated with the distribution clearance between the central and liver compartments and volume of distribution for peripheral compartment 2, while age was strongly associated with volume of distribution for the central compartment in cattle. For swine, a close relationship was found between weight and volume of distribution for peripheral compartment 1. The covariate factors in the final model are listed in Tables 2 and 3 for cattle and swine, respectively.

Model validation. The validation of the final PK model was based on graphical and statistical methods. After adding the covariates, the AIC values of cattle and swine models declined from 255.3 to 240 and 1,064 to 941.8, respectively. Goodness-of-fit plots from the final PK model are shown in Fig. 2 and 3 for cattle and swine. The representative real concentration-time profile versus the individual predicted concentration-time profile in tissue are presented in Fig. 4. The plots displayed a good agreement between the model-predicted and observed mean data. The PK parameter estimates obtained from the final model and the bootstrap analysis are provided in Table 2. Bootstrap analysis suggested that the 95% CIs generally were narrow and centered around the parameter estimates. Model robustness of the final model was assessed by bootstrapping, with the mean values obtained from the bootstrap being comparable to parameter estimates from the final model.

Simulations. Using the final model parameter estimates, the simulated time-concentration profile of tissue residues for cattle and swine are shown in Fig. 5. According to the time-concentration profiles and tolerance limit, the WDI of cattle was estimated to be

TABLE 3 Population PK parameters obtained from the PK model for plasma and tissue concentrations of penicillin for swine

Parameter	Description	Value (RSE [%])[a]			CI	
		Population mean	IIV	Bootstrap value	2.5%	97.5%
V_1 (liter)	Volume of distribution for the central compartment	3.05 (8.8)	0.13 (15)	3.01	2.76	3.26
V_2 (liter)	Volume of distribution for the peripheral compartment 1	1.65 (12)	0.03 (10)	1.66	1.58	1.75
V_3 (liter)	Volume of distribution for the peripheral compartment 2	4.65 (17)	NE (NA)	4.61	4.51	4.70
V_K (liter)	Volume of distribution for the kidney compartment	4.38 (14)	0.02 (15)	4.38	4.15	4.57
V_M (liter)	Volume of distribution for the muscle compartment	1.10 (12)	0.03 (17)	0.99	0.93	1.04
CL_1 (liters/h)	Central clearance	16.9 (14)	0.12 (14)	17.2	15.8	18.8
CL_2 (liters/h)	Clearance between the central and the peripheral compartment 1	13.7 (85)	NE (NA)	13.9	12.7	14.8
CL_3 (liters/h)	Clearance between the central and the peripheral compartment 2	0.52 (40)	NE (NA)	0.54	0.52	0.55
CL_K (liters/h)	Clearance between the central and the kidney compartment	12.1 (53)	0.06 (15)	12.2	11.4	13.1
CL_M (liters/h)	Clearance between the central and the muscle compartment	14.8 (64)	0.06 (13)	14.9	14.0	15.8
K_{im1} (liters/h)	Absorption rate constant after i.m. injection of penicillin potassium	3.03 (27)	0.04 (15)	3.00	2.84	3.18
K_{im2} (liters/h)	Absorption rate constant after i.m. injection of procaine penicillin	0.48 (23)	0.13 (15)	0.48	0.44	0.54
F_{im1} (%)	Bioavailability of penicillin after i.m. injection of penicillin potassium	73.3 (17)	0.02 (10)	71.1	68.2	73.6
F_{im2} (%)	Bioavailability of penicillin after i.m. injection of procaine penicillin	64.2 (15)	0.14 (14)	65.1	58.5	73.3
Covariate factor						
θ_1	Estimated covariate factor of wt on V_2	0.132		0.133	0.131	0.135
Residual errors (%)		35				

[a] RSE, relative standard error; NE, not estimated; NA, not applicable.

7 days, which is shorter than the current recommendation of 14 to 21 days. The WDI of swine was estimated to be at least 30 days, while 50 days of drug withdrawal time is recommended.

DISCUSSION

The intention of this study was to describe the population pharmacokinetics of penicillin G in cattle and swine, utilizing both dense and sparse data from different data sources, a typical scenario encountered in the veterinary literature. First, we built a three-compartment model with first-order absorption and first-order elimination to describe the various formulations of penicillin G. Liver, kidney, and muscle compartments then were added sequentially in order to depict the physiologic distribution of penicillin G in cattle and swine. After incorporating covariates into clearance and volume parameters, the effects of weight and age on the clearance and volume parameters were evaluated using a full-covariate-model approach. The best model describing the PK behavior of penicillin G was selected according to several accepted criteria for model validation. Finally, the model was used to simulate the time-concentration profile of tissue and successfully predicted the WDI of cattle and swine.

We used both dense data and sparse data to build the structural model. Sparse data consisted mainly of the concentrations of penicillin G tissue residues, which were determined at least 1 h after the drug administration. In previous studies, the PK profile of penicillin G was described by a one- or two-compartment open model (54, 62). Given that previous sampling periods were relatively short, a three-compartment open model was applied in our study in order to account for the slower terminal depletion phase of the later time-concentration data profile. During data collection, it was found that some concentrations of penicillin residues were at the limit of detection (LOD) of the original studies. To discover whether this affected the structural model building, we constructed the model using data sets with and without LOD concentration data. The results showed that the data set containing LOD did not influence the PK parameters of penicillin G but sig-

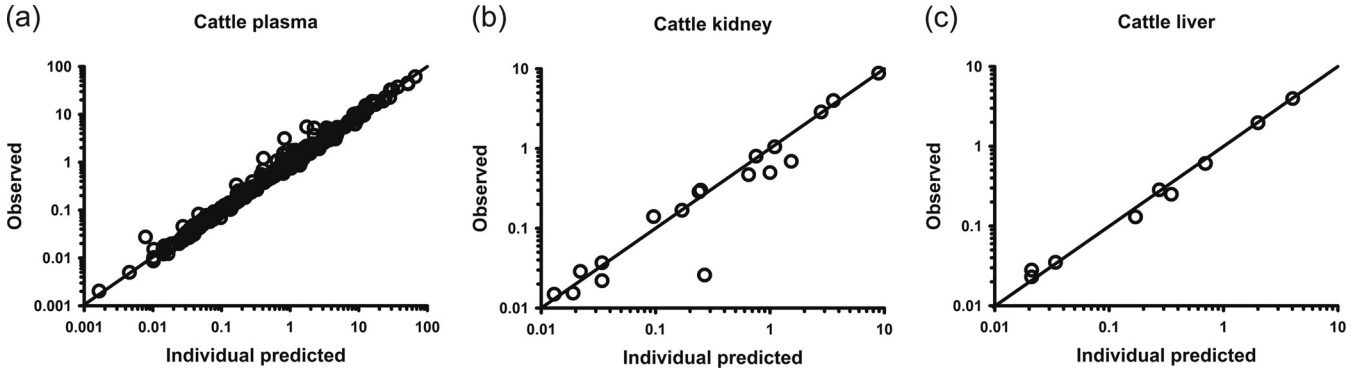

FIG 2 Goodness-of-fit plots for the final pharmacokinetic (PK) model of pooled blood and tissue data for cattle. Scatter plots of observed versus individual predicted penicillin concentrations in blood (a), kidney (b), and liver (c) for cattle.

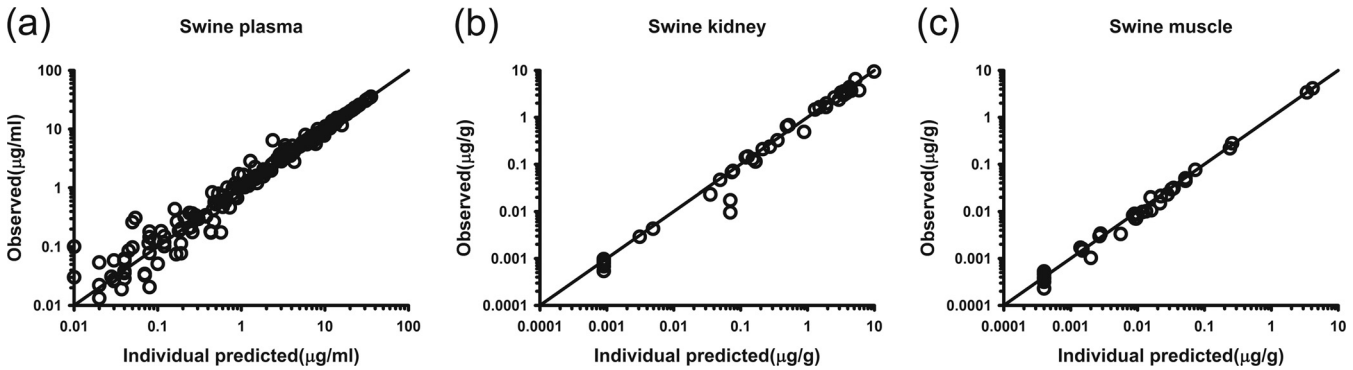

FIG 3 Goodness-of-fit plots for the final pharmacokinetic (PK) model of pooled blood and tissue data for swine. Scatter plots of observed versus individual predicted penicillin concentrations in blood (a), kidney (b), and muscle (c).

nificantly enhanced the bioavailability of procaine penicillin, since the area under the concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) was larger. Considering the LODs cannot represent the actual concentrations of drug residues, our structural model was built based on the data set without LODs.

For both cattle and swine models, the central volumes of distribution for penicillin were significantly decreased when the tissue compartments were added. The choice to incorporate tissue compartments into the structural model was based on the assumption that the CL between the central and tissue compartments was consistent with the CL between the central and the peripheral compartments. According to the results, the CLs of liver, kidney, and muscle compartments all were in good agreement with the CL of peripheral compartment 1, which implies the tissue disposition of penicillin is similar to the PK behavior of peripheral compartment 1. The purpose of adding a tissue com-

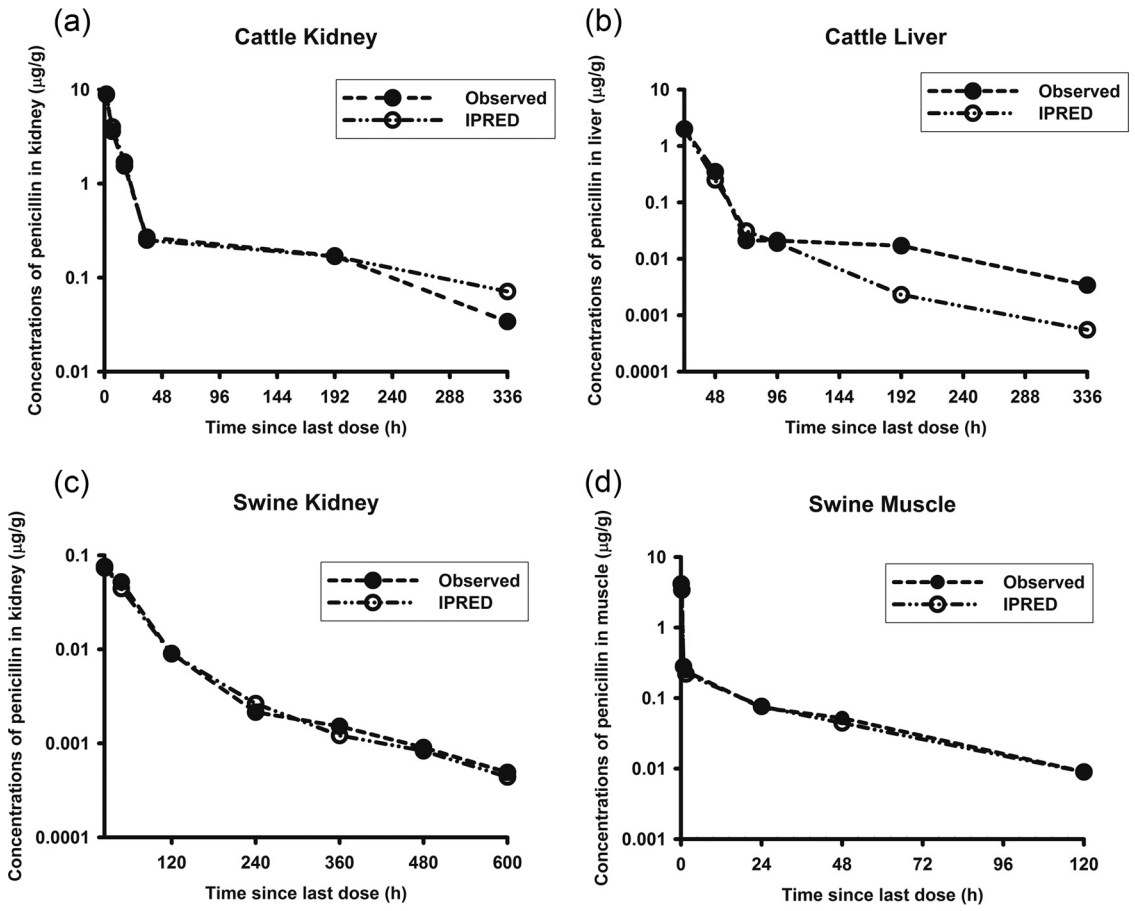

FIG 4 Real concentration-time profile (dot) versus individual predicted concentration-time profiles (blank dot) in cattle kidney (a), cattle liver (b), swine kidney (c), and swine muscle (d).

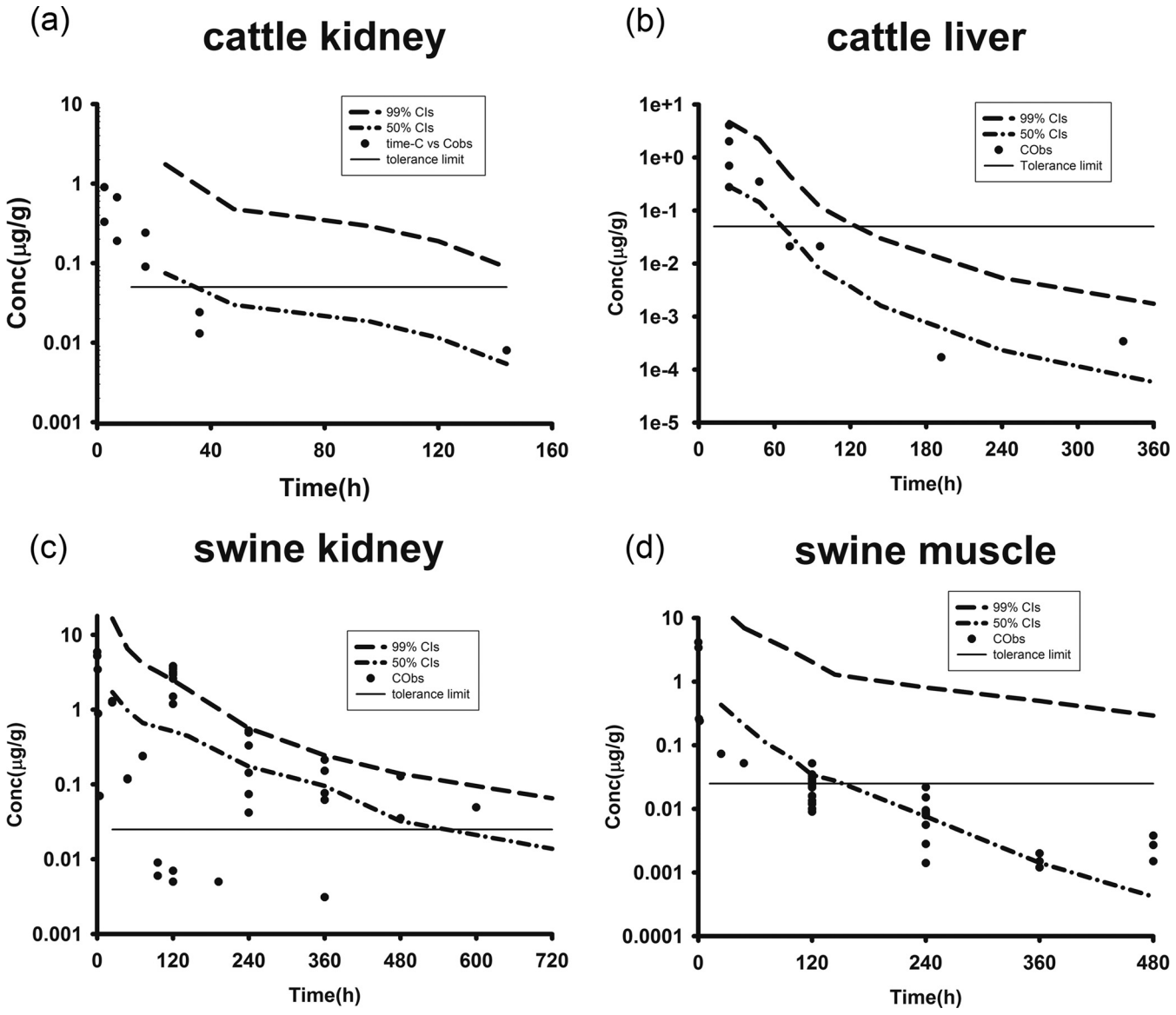

FIG 5 Simulated data for the tissue residues of penicillin G in cattle kidney (a), cattle liver (b), swine kidney (c), and swine muscle (d). The 99th percentiles of the simulated penicillin residues are represented by a dashed line. The 50th percentiles of the simulated penicillin residues are represented by a dash-dot line. The observed concentrations (CObs) of the tissue residues are represented by closed circles. The solid lines are the tolerance limits of penicillin G in cattle and swine tissues.

partment is to overcome the model misspecification from the plasma data only, as the elimination phase of penicillin G is always faster in plasma than in tissue (11, 40). The limitation for this model is the lack of tissue concentration collected from 0 to 24 h, which results in a blank time frame for plasma only (19). This situation is common in the veterinary literature, since plasma studies are often conducted in clinical research studies while tissue data are collected for food safety residue depletion trials. Overall, the model incorporating tissue is stable and adequate to describe the PK profiles of penicillin in plasma and tissue.

In this analysis, we used data from studies of penicillin from various weights and ages in both cattle and swine, because the disposition of penicillin can be altered in young versus adult animals. Ranheim et al. reported that the clearance of penicillin was lower in 1-week-old piglets than in adult pigs (7, 51). Musser et al. found that the volume of distribution at steady state (V_{ss}) of the calf was significantly higher than that of adult cattle (5, 30). The use of adult data only may not be able to describe all of the PK characteristics of penicillin G. Thus, we combined data from a wide range of weights and ages by adding them as covariates in order to illustrate their relationships with the basic PK parameters. According to the result, the covariates have more significant influence on PK parameters of volume of distribution than on those of clearance.

To our knowledge, this is the first time a population pharmacokinetic model across two species has been reported. The internal validation (Tables 2 and 3) suggested the model was unbiased. The goodness-of-fit plots (Fig. 2 and 3) of the final model demonstrated that there was good agreement between observed data and

individual predictions, especially for the tissue data. These findings support the similarity of penicillin disposition across two species. The plot of real concentration-time profile versus individual predicted concentration-time profile in tissue (Fig. 4) further supported the unbiased nature of the model. There is a slight bias toward predicting more rapid drug elimination from the bovine liver, which is mainly a result of the limited liver data available for cattle. It would be possible to improve the prediction if more data were available from the depletion phase. Furthermore, the simulation results (Fig. 5) support that our model is able to predict the withdrawal time for cattle and swine. The observed data on tissue residues was centered around the 50% confidence intervals of simulated time-concentration profiles. According to the tolerance limit, the predicted WDIs are more specific than the current recommendation, supporting the extremely conservative nature of official withdrawal times.

Conclusions. Avoiding violative tissue residues of drugs in the edible products of food-producing animals requires adherence to an appropriate withdrawal time. When the label dose is used, a specific residue depletion study in healthy animals is conducted by the sponsor as part of the regulatory approval package. However, the presence of systemic disease or the legal extralabel use of the drug, either via a different route or at a higher dose to treat infections caused by organisms with higher MICs, requires estimation of a prolonged withdrawal interval to ensure that edible products meet food safety guidelines (20, 22). In many cases, the label dose of a drug approved decades ago, such as penicillin G, must be increased to effectively treat bacteria with higher MICs without inducing resistance (16). Withdrawal times can be estimated using pharmacokinetic models; however, the data required to populate such models is varied (different doses, routes, disease states, and ages), and often plasma and tissue data are not collected in the same studies. Regulatory withdrawal time trials are conducted in healthy animals and do not require collection of the plasma data needed to define the structural pharmacokinetic model or to estimate disease effects on drug disposition. In order to accurately predict withdrawal intervals under conditions of field use, data from multiple studies reflecting these varied conditions must be analyzed to make sure predictions are within the inference space defined by these studies.

In summary, the present analysis clearly demonstrates the utility of using a mixed-effect pharmacokinetic model as a meta-analysis tool to link published penicillin pharmacokinetic data collected from both sparse and dense data sets covering a wide range of field conditions. In addition, we report the first population pharmacokinetic model able to describe the complete distribution and elimination profiles of penicillin across two different species and successfully applied it to predict the WDIs for tissues of cattle and swine. By incorporating tissue compartments, we clarified the tissue disposition of penicillin in cattle and swine, a necessary prerequisite to predicting tissue withdrawal times. Using a model across multiple species opens up the possibility of probing how disease factors influence disposition in a more mechanistic fashion. Additional species also could be added when data are available, allowing for a careful comparison of interspecies differences in drug disposition. This study established a robust model of penicillin G for a large and diverse population of food-producing animals which could be applied to other antibiotics and species in future analyses.

ACKNOWLEDGMENTS

This work was supported by USDA-NIFA funds for food animal research.

We thank Nitesh Verma for providing technical support for this study. We also thank David Smith, Weilin L. Shelver, and Sara J. Lupton of USDA-ARS Bioscience Research Laboratory for providing individual swine data.

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