A Framework for Meta-Analysis of Veterinary Drug Pharmacokinetic Data Using Mixed Effect Modeling

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ABSTRACT: Combining data from available studies is a useful approach to interpret the overwhelming amount of data generated in medical research from multiple studies. Paradoxically, in veterinary medicine, lack of data requires integrating available data to make meaningful population inferences. Nonlinear mixed-effects modeling is a useful tool to apply meta-analysis to diverse pharmacokinetic (PK) studies of veterinary drugs. This review provides a summary of the characteristics of PK data of veterinary drugs and how integration of these data may differ from human PK studies. The limits of meta-analysis include the sophistication of data mining, and generation of misleading results caused by biased or poor quality data. The overriding strength of meta-analysis applied to this field is that robust statistical analysis of the diverse sparse data sets inherent to veterinary medicine applications can be accomplished, thereby allowing population inferences to be made. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association

Keywords: meta-analysis; nonlinear mixed-effect modeling; pharmacokinetics; population pharmacokinetics; veterinary medicine; drug depletion; drug withdrawal time; clearance; distribution; formulation

INTRODUCTION

In both human and veterinary medicine, pharmacokinetics (PKs) describes the absorption, distribution, metabolism, and elimination (ADME) of drugs in the body. Unlike human PKs, which focuses primarily on differences between individuals, veterinary medicine also gives consideration to differences between species and breeds.1 Veterinary and human PK also differ in the extent and variety of data collected from clinical trials that is much less comprehensive in veterinary studies. Another major difference is that a drug's depletion must be studied in the edible tissues of food-producing animals to ensure that human consumers of meat, eggs, and milk are not exposed to harmful drug residues. Moreover, PK studies in exotic animals are lacking, and therefore, the dosage regime for those species is mostly based on empirical knowledge.

Statistically, meta-analysis is a tool designed to summarize the results of multiple studies.2 It has been utilized in human drug development to assess the clinical effectiveness of healthcare interventions by combining data from different trials.3,4 For veterinary medicine, it is, however, impossible to accomplish this because of the lack of available data. Meta-analysis may only be performed based on the average reported data.5 However, combined data can be analyzed using nonlinear mixed effect (NLME) modeling approaches not specifically designed for this purpose. This review outlines the procedures and some of the differences and challenges in studying the PKs of drugs in veterinary species. We also discuss several advanced PK techniques that can be used to conduct meta-analysis and improve the interpretation of these data combined from several studies.

DEVELOPING A META-ANALYSIS AND NLME MODEL FRAMEWORK

A framework is needed to describe the performance of PK data in veterinary medicine. Figure 1 illustrates the basic components of such a framework for data collection, modeling, and interpretation.

Data Collection

There are two kinds of data amenable to meta-analysis: individual participant data (IPD) and aggregate data (AD). It is easier to obtain IPD from human clinical trials, whereas AD is more common for veterinary studies. For PK studies, AD represents the mean value of concentration at different time points from a group of animals with standard deviation. In fact, for most cases, we cannot get standard deviation as the sample sizes of most PK studies are relatively small, and we do not know the true population mean. Instead, we use standard error to describe how far our sample mean is likely to be from the true population mean. Generally, we can get such information from tables or graphs of time-concentration profiles in the literature using available software such as UN-SCAN-IT (Silk Scientific, Inc., Orem, Utah). Collected data should be clarified into corresponding groups such as control versus experimental variable. One should collect as much available supplementary information as possible about the data. Supplementary information includes variables such as dosage, dosing route, drug formulation, matrix and animal conditions (species, weight, age, sex, and disease), and so on.
Figure 1. The workflow of meta-analysis and population-based NLME modeling for veterinary drug development.

NLME Modeling

The difference between PK and clinical trial data is PK data consist of a set of dependent variables determined by a function of time. But for most meta-analysis, clinical trial data means single individual data from treated or untreated group. Therefore, when dealing with PK data, we need a time-dependent structure model to describe the kinetic process of the drug. NLME modeling is the primary technique available for the analysis of integrated PK data.6 “Nonlinear” implies that the dependent variable is nonlinearly related to the model parameters and independent variables. “Fixed effect” refers to the parameters that do not vary across individuals, whereas “random effect” refers to those parameters that vary across individuals.7 Basically, a NLME model contains three components: (1) structural model, (2) statistical model, and (3) covariate model as depicted in Figure 1.

The structural model is developed to describe the PKs of a drug after dosing. A typical structural model is represented by one, two, or three compartments (depending on the time–concentration profile) with an absorption compartment for extravascular administration. The statistical model explains the variability around the structural model. There are two major sources of variability: between-subject variability (BSV) and residual variability. BSV is the variance of a parameter across individuals and would be represented as:

$$\log(P_i) = \log(P_{pop}) \exp(\eta_i)$$  (1)

where $P_i$ is the parameter of the $i$th subject, $P_{pop}$ is the population parameter, and $\eta_i$ is the deviation from the population value for the $i$th subject and is assumed to be normally distributed with a mean of 0 and variance $\omega^2$ for parametric methods. It should be noted that there are also nonparametric approaches to mixed-effect modeling.6 Residual variability, also referred to as residual error, generally arises from assay variability or model misspecification. There are several functions for describing residual errors. Additive, proportional, or combined additive and proportional error model functions are the most commonly used in NLME model. Covariate model specification is important for developing the correct population PK model as it identifies which covariates are highly correlated with PK parameters. Potential covariates can include any available physiological parameters influencing ADME process such as weight, age, gender, liver enzyme activity, and so on. The covariates are classified into continuous or discrete. The typical continuous covariates are expressed using functions as:

$$\log(P_i) = \log(P_{pop}) \left( \frac{Cov}{Cov_{avg}} \right)^{\theta} \exp(\eta_i)$$  (2)

where $P_i$ is the parameter of the $i$th subject, $P_{pop}$ is the population parameter, $Cov$ is a covariate factor centered by average mean or a reference value, and $\eta_i$ is the deviation from the population value for the $i$th subject. In some situations, $\theta$ can be fixed to a certain value to account for changes in PK parameters. The discrete covariates are separated as dichotomous (gender) or polychotomous covariates (race). The values of such covariates are usually set as 0 for reference and 1 or more for the other classification. The typical discrete covariate is set as following:

$$P_i = \theta (1 + \theta Cov) \exp(\eta_i)$$  (3)

Simulation

Simulation is used to assess the effect of individual independent variability in each separate PK parameter on the overall model output. We obtain the PK model from the NLME model. This model is then used to simulate data that is an
important component for model evaluation and prediction.\textsuperscript{9} Monte Carlo simulation is a technique designed to study the impact of the population distribution properties of estimators on overall model output. After the model is established, the PK parameters of each individual can be described using a series of mathematical equations with the identified parameter value and individualized uncertainties. Then, simulation can be applied to maximize the information from the estimates to guide the new study design.

The detailed simulation process is described as follows: suppose you have $N$ subjects with 1000 replicates, then each subject will be simulated for 1000 times. For subject 1, we freeze all the fixed and random effects values and covariates values to what we obtain from the final model. As we know all the information relevant to simulation, we can start to create a new copy of subject 1 with the identified fixed effect values, but different random effect values. We repeat such a procedure until we get 1000 simulated datasets for subject 1. The same procedure should be performed for the rest of the subjects. However, we can change the dosing scheme or sampling time to what we like. The interpolation or extrapolation of new dosage PK profile can be simulated, which is more economic than actually redoing the design and testing it. Smaller trials could then be conducted to validate the simulation.

Once the simulation is complete, statistical analysis should be applied to interpret the results. Each replication of the simulation can be seen as an outcome of the random variable. Theoretically, the more replicates, the better the simulation. In general, the sample size should be large enough to cover the stochastic distribution of the output measurements [e.g., at least 1000 replicates for calculating the confidence intervals (CIs)]. A simulation result can be summarized as mean values and SD. The results can also be demonstrated as CIs.

$$\text{CIs}_{ij} = \text{Mean}_{ij} \pm \tau_{n/2 \alpha} \sqrt{\text{MSE}_{ij}}$$  \hspace{1cm} (4)

where CIs$_{ij}$ is the CIs at $i$th time point, mean$_{ij}$ is the average concentration at $i$th time point, $\tau_{n/2 \alpha}$ is a standard normal random variable of probability $\alpha$, and MSE$_{ij}$ is the mean standard error at $i$th time point.

APPLICATION OF META-ANALYSIS AND NLME MODEL IN VETERINARY DRUGS

Classic PK analysis has been applied to veterinary drugs for many years.\textsuperscript{10,11} The subjects of classic PK are restrictively selected according to the specific experimental treatments being studied and the sample size is generally very small. Therefore, the impact of interindividual variability is reduced and the intraindividual variability cannot be considered. In fact, information on the source and magnitude of variability is crucial for assessing drug efficacy and safety. Meta-analysis using the NLME model approach can help one identify ignored information from the existing PK data, which would be valuable to veterinary drug regulation.

Covariates Effects

One objective of the NLME model approach is to identify the appropriate covariates affecting the PK properties of a subpopulation and to explain the intersubject and intrasubject variability in terms of these covariates, which is helpful to optimize the initial dosage regimen to meet the therapeutic window and safety criterion of a certain subpopulation of animals.\textsuperscript{12,13} As described by Cox et al.,\textsuperscript{14} the NLME model approach was successfully implemented in the study of mavacoxib in osteoarthritic dogs. A previous PK study suggested a 4-mg/kg oral administration with 2-week interval between the first and second doses, but with monthly dosing thereafter, would achieve the 0.4-μg/mL steady-state concentration for therapeutic efficacy.\textsuperscript{15} The PK characteristics of mavacoxib significantly differed between healthy dogs and osteoarthritic patients, which were geriatric large-breed dogs. After building a PK model taking into consideration weight, age, sex, and breeds, plasma mavacoxib concentration profiles of more than 10,000 dogs were simulated. The simulation results indicated that a reduced dosage of 2 mg/kg was appropriate for 85% of patients to achieve the therapeutic concentrations. On the basis of the above result, the reduced dosage of 2 mg/kg was approved supporting that NLME model is a powerful tool for dosage regimen optimization.\textsuperscript{16}

Pharmacokinetic/Pharmacodynamic Modeling

The NLME model approach and simulation method can also be applied to determine the pharmacodynamic (PD) cutoffs for dosage regimens especially for antimicrobial agents. For antibiotics, breakpoints are the concentrations at which bacteria are susceptible to successful treatment.\textsuperscript{17} Veterinary breakpoints are sometimes unclear and, therefore, need reevaluation. Clinical breakpoints can be evaluated by deterministic or probabilistic approaches. Traditionally, a deterministic approach is used to set the breakpoints. However, this approach fails to consider the interanimal variability and can only provide a possible breakpoint value. The probabilistic approach appears better as reviewed by Dalhoff et al.\textsuperscript{18} The stochastic nature of NLME model and simulation makes it an ideal tool to integrate variability of PK and PD data to establish a breakpoint.

Rey et al.\textsuperscript{19} gave a detailed example on how to set amoxicillin breakpoint for the pig by using NLME approach and Monte Carlo simulation. The raw datasets were composed of individual plasma PK data from different routes of administration. Different structural models were developed and validated for each route of administration. Formulations were added as a covariate to cover a broad range of situations. The simulation was performed after the final models were established. A series of different scenarios was simulated for each route of administration. After the simulations were implemented, they found that a target attainment rate of 90% was never achieved with the recommended breakpoint of 0.5 mg/L by an oral dosage of 20 mg/kg. The result suggests that the breakpoint derived from deterministic approach can be high because it does not consider the interindividual variability. NLME model approach and simulation is recommended to evaluate the breaking points in order to enhance the predictive value of antimicrobial susceptibility testing.

Drug Withdrawal Time Estimation

One important issue in veterinary PK is to calculate the drug withdrawal time (WDT) for use in food-producing animals. To ensure the safety of food obtained from animals treated with veterinary drugs, there are regulations imposed on veterinary drug usage in food-producing animals, which require additional safety and tissue PK studies be undertaken.\textsuperscript{20} According to US
FDA guidelines, an approved drug dosage regimen must include a WDT, which is the time following the last dose during which edible tissues may not be harvested from the treated animal. The WDT calculation is based on data from experiments conducted in small groups of animals with similar physiological characteristics and may therefore fail to reflect the drug’s kinetic properties across various populations, field usage, and disease conditions.

Extralabel use of drugs is also very common in veterinary practice. This practice was legalized when the US congress passed the Animal Medicinal Drug Use Clarification Act (AMDUCA) in 1994, followed by its implementation with regulations published in 1996 by Food and Drug Administration Center for Veterinary Medicine. AMDUCA allows veterinarians to prescribe extralabel uses of certain approved animal and human drugs for animals under specified conditions. One of these specific conditions is the availability of sufficient scientific information on which to base a recommendation for an extended WDT to prevent violative drug residues in food-producing animals.

Extralabel (higher) doses of older antimicrobial drugs are often required as targeted pathogens are no longer sensitive to concentrations achieved by the label dose. For the last few decades, the Food Animal Residue Avoidance Databank (FARAD) has provided recommendations for extended WDTs (called withdrawal intervals (WDIs)) for such extralabel drug use in various species. The recommendations are based on published PK data from studies that match the specific conditions of the presented case. If there are no matching conditions in the published literature, PK simulations need to be used.

Wu et al. developed a NLME-based population PK model to describe the complete distribution and elimination profiles of flunixin as well as predict WDIs in cattle. Data used in this study were divided into two sets. One set was made up of time-concentration data in plasma from various sources. Another dataset consisted of time-concentration data in liver during the depletion phase. Large numbers of plasma samples per individual from different assays (rich data) were collected in order to cover the limited data in liver samples (sparse data). Figure 2 illustrates the schematic representation of the population PK model for the pooled data set of plasma and liver. An independent liver compartment was separated from peripheral compartment, but still linked by the same elimination rate constant from the central compartment to facilitate incorporation of the measured liver samples. After model validation, Monte Carlo simulation for flunixin liver data was performed to estimate WDIs. The WDI was estimated to be 7 days following intravenous (i.v.) administration of flunixin, which is 3 days longer than the current label WDT. This is not a surprise as the 5-hydroxy-flunixin (metabolite of flunixin) concentration was found to be higher than the tolerance limit in previous studies using current label WDT. The advantage of the NLME approach was that effects of animal variability and disease on plasma flunixin profiles, that are captured in small classic PK trials, and that ultimately modulate the downstream tissue deposition, could be analyzed and integrated to WDT predictions without conducting large-scale tissue depletion trials in diseased animals.

A similar application was also applied to penicillin G in cattle and swine. The formulations of penicillin G are more complex than flunixin. Different formulations of penicillin G may cause the absorption to vary from one to the other. Previous 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. Therefore, the absorption phase of the model was divided according to not only the dosing routes, but also the formulations. Another area worthy of note is that this model describes the complete distribution and elimination profiles of penicillin G across two different species. Figure 3 demonstrates the simulation result of the tissue residue predictions.

The advantage of NLME approach to estimate WDTs is that it pools all data to provide a wide range of WDTs that suit a population of diverse animals rather than classic PK targets that are appropriate only for specific animals studied. The technique could also be sued to estimate WDTs based on existing PK datasets gathered under realistic field use conditions.

ADVANTAGES AND CHALLENGES FOR NLME APPLICATION IN VETERINARY MEDICINE

Data Selection

The application of NLME in veterinary medicine is not as widespread nor accepted as it is in human drug development. Table 1 illustrates some NLME model approach applications in the veterinary field over the last few decades. According to the table, most of the applications focus on covariate effects. There are several tentative applications on drug residue studies, but only one PK/PD related study. If we look into the data used, such application allows the combination of data from multiple trials, which means the data were obtained from animals with different husbandry conditions or analyzed in different laboratory settings. The dataset consists of a wide range of scenarios that allows one to interpret the interanimal variability. On the contrary, investigators seem to be conservative about the data they use. They are likely to choose individual raw data rather than the AD. Ideally, individual raw data is more specific to characterize the intra-animal variability. However, it is unrealistic to conduct large-scale PK trials in animals to meet the NLME approach requirement in the veterinary field because of animal welfare consideration and extreme financial constraints. This appears to be a major reason the spread of NLME approach in veterinary medicine is slow. The ultraconservative mindset of regulatory agency to adopt modern PK approaches also

![Figure 2. Schematic representation of the final PK model for pooled data set of plasma and liver concentrations of flunixin in cattle.](image)
Figure 3. 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 (C_{Obs}) of the tissue residues are represented by closed circles. The solid lines are the tolerance limits of penicillin G in cattle and swine tissues.

Data Variability of Animal Versus Human

As mentioned above, it is common to conduct large-scale clinical trials in terms of funding and regulatory requirements to gain individual raw data for NLME implementation for human drug development. However, it is unrealistic to replicate the large-scale trials to obtain big datasets for veterinary medicine. Meta-analyses are therefore a necessary tool to bridge this data gap to combine different studies in order to gain a better understanding of the true PK profile, its variability in the target population, and effect of disease conditions on the drug label on PK behavior.

Another difference of data in veterinary PK occurs when exotic animals are studied. For those animals, such as reptile, birds, and amphibians, it is near impossible to obtain multiple sampling from one individual animal. One animal–one sample designed PK studies (naive pooled data approach) are used in these animals. Such design may also cause greater variability in the data, which cannot be divided into interindividual or intraindividual origins.

Species variation is another issue that veterinarians must take into account on a daily basis. For veterinary medicine, one drug can be used in many species. In comparative veterinary PKs, the wide range of animal species gives rise to species variations. Species variations in PK behavior can generally be attributed to the differences in the rate of absorption, metabolism, or elimination. The Word Watch List for Domestic Animal Diversity issued by Food and Agriculture Organization provides more than 40 domestic livestock species. Veterinary medicine encompasses many types of animals and has to take all variations result from anatomical, physiological, and behavioral aspects into consideration. One common phenomenon in veterinary PK is that for most drugs, lower clearance is observed in carnivores than that in herbivores while omnivores have an intermediary clearance. This may cause differences in
Table 1. Applications of NLME Approach in Veterinary Medicine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Dataset Number</th>
<th>Data Point</th>
<th>Data Type</th>
<th>Data Source</th>
<th>Matrix</th>
<th>Application</th>
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<td>145</td>
<td>489</td>
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<td>Single trial</td>
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<td>Covariates effects</td>
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<td>438/574</td>
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<td>Plasma</td>
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<td>Plasma</td>
<td>Covariates effects</td>
<td>Guo et al. 34</td>
</tr>
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<td>Martin-Jimenez et al. 36</td>
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terminal half-life of a drug. One classic example is salicylic acid. The terminal half-life of salicylic acid is 1 h in herbivores (cattle, horse), from 6 to 9 h in omnivores (pig, dog), and up to 22–45 h in carnivores (cat). The variation is caused by many factors including reduced function of hepatic microsomal enzymes in certain species and breeds, different activities of transporter proteins, different glomerular filtration rates, and so on.

Allometric scaling is one simple way to evaluate the species variations in PK parameters. In most cases, PK parameters (CL, \(V_{ss}\), \(t_{1/2}\)) of a certain species can be extrapolated with bodyweight according to the allometric equation:

\[ Y = aW^b \]  

where \(Y\) is the estimated parameter, \(a\) is an allometric coefficient, \(W\) is the average body weight of the species, and \(b\) is the allometric exponent. One limitation for allometric scaling is that it cannot be applied to extrapolate from ectothermic animals to endothermic animals as ectothermic animals have lower rates of metabolism. The major limitation in this approach is that animal estimate of variability below the species level is not available, and differences in experimental study designs cannot be accounted for. Allometric scaling can also be implemented with NLME approach. Martín-Jiménez and Riviere performed a NLME-based drug disposition model of gentamicin across species. Data of different species were collected from literature and pooled together. The data were analyzed using allometric scaling equations above to describe the interspecies PK parameters. After several adjustments, the
The best model was used to predict the time–concentration profile of gentamicin for swine.

**NLME Approach Versus Other PK Approach**

The NLME approach should be used for population studies. Table 2 summarizes the differences between NLME approach and classic PK modeling approaches. In traditional PK, emphasis is placed on the PK properties of a certain study group. Strict criteria for sample selection are set in order to minimize the interanimal variability. As a result, the values of the same PK parameter could vary a lot between different studies. A problem also occurs on how to integrate the disparate PK parameters coming from different assays. NLME allows different assays or other animal characteristics to be taken into consideration. In fact, information on population variability is very useful when a drug is given to large herds of animals. Traditional PK provides less information on this issue. In veterinary drug development, investigators are inclined to use noncompartment analysis (NCA) modeling approach, which is simpler but restricts implementation of simulation afterwards. Overall, the NLME approach-based PK analysis can be used to adjust PK properties derived from traditional PK analysis.

Physiologically based pharmacokinetic modeling (PBPK) is another approach that could be employed for drug development. PBPK models are parameterized using known anatomy and physiology and consist of different organ or tissue compartments. PBPK models are parameterized using known anatomy and physiology and consist of different organ or tissue compartments. PBPK modeling approach can be used to extrapolate PK from healthy population to diseased population. The amount of binding sites for a certain drug is limited on available tissue protein. Saturability of tissue binding may occur when a high dosage is administered. A study was carried out to investigate the effects of dose on tissue residues of gentamicin in sheep. The terminal phase half-lives varied from 90 to more than 500 h in low- and high-dosage groups because of the slow release of drug from the tissue binding site at high dosage.

**Drug Depletion**

One of the major concerns of drug use in food producing animals is to monitor for potential violative residues to occur in meat, eggs, and dairy products. The depletion phase of a drug's disposition profile is the very terminal phase reflecting drug release from tissue. In contrast to earlier phases, for example, α, which reflects rapid distribution, the depletion phase can last for weeks or months depending upon the drug and assay sensitivity. Human PK studies pay no attention to this terminal phase as the plasma concentrations are extremely low and without therapeutic significance. However, when it comes to food-producing animals, this slowly depleting phase should be taken into account as it may reflect the potential for drug residue exposure to food consumers. However, the data we collect from a single depletion study are rare and sometimes even missing for a certain species. An appropriate method to utilize data from different studies and can be extrapolated between species, is urgent. Successful examples of NLME-based drug residue studies are provided in the section of drug withdrawal time estimation. Also, Buur et al. developed a PBPK model to estimate the WDIs of the chemical adulterant melamine in pigs.

**Biophysicochemical Factors**

A drug molecule can only be eliminated if it is released from tissue to plasma and excreted in urine, feces, or other fluids. The relationship between plasma and tissue concentration of the drug is controlled by the redistribution rate. One of the major factors affecting redistribution is the lipid solubility of the drug. Lipophilic drugs are inclined to penetrate across cell membranes and accumulate in tissue compartment. The WDIs of these drugs are expected to be longer than hydrophilic drugs as the redistribution rate constants from tissue to plasma are lower. To obtain a long period of therapeutic concentration, the residues are sustained in deep compartment. The release of the residues is controlled by the individual redistribution rate that makes the depletion more variable and unpredictable. Similarly, the molecular size, polarity, and pK value of the drug can also influence the depletion of drug residues.

The amount of binding sites for a certain drug is limited on available tissue protein. Saturability of tissue binding may occur when a high dosage is administered. A study was carried out to investigate the effects of dose on tissue residues of gentamicin in sheep. The terminal phase half-lives varied from 90 to more than 500 h in low- and high-dosage groups because of the slow release of drug from the tissue binding site at high dosage.

Besides these above factors inherent to the drug itself, formulation of a drug can also lead to changes in the rate and extent of absorption, which may ultimately result in the changes of tissue concentration. For some extravascularly administered drugs, a “flip-flop” phenomenon occurs when the absorption half-life is slower than the elimination half-life. This often happens in sustained release or long-acting depot formulations. A small fraction of the drug immediately releases with rapid absorption rate, whereas a large fraction slowly releases with

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**Table 2. Comparison of Different PK Modeling Approach**

<table>
<thead>
<tr>
<th>Compartmental Modeling</th>
<th>NCA</th>
<th>NLME</th>
<th>PBPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Specific selected</td>
<td>Specific selected</td>
<td>Large integrated population</td>
</tr>
<tr>
<td>Sample size</td>
<td>Small</td>
<td>Small</td>
<td>Large/integrated</td>
</tr>
<tr>
<td>Data</td>
<td>Dense</td>
<td>Dense</td>
<td>Dense/sparse</td>
</tr>
<tr>
<td>Interindividual variability</td>
<td>Minimized</td>
<td>Minimized</td>
<td>Demographics</td>
</tr>
<tr>
<td>Simulation</td>
<td>Simple simulation</td>
<td>No</td>
<td>Simple simulation/Monte Carlo simulation</td>
</tr>
</tbody>
</table>
a slower absorption rate. In this case, the terminal phase is not only the elimination but a combination of elimination and absorption.\textsuperscript{67} Liposome-encapsulated drug is a dedicated designed drug delivery system that helps to increase the therapeutic index and reduce the toxicity of the drug in veterinary therapeutic field.\textsuperscript{68,69} Generally, compared with free drugs, liposome-encapsulated drugs act as depot formulations in order to maintain sustained release. Depletion of these drugs is non-exponential and unpredictable whose PK properties differ a lot from free drugs.

Physiological States

Drug PK can vary among different physiological conditions. Thus, an appropriate adjustment of dosing regimen is needed for diseased animals, which can be optimized using NLME approach that has been discussed before. For the perspective of violative residues, physiological states can also be an important factor. According to previous research, the suspect cull cows have a significant higher possibility of violative flunixin concentrations than healthy dairy cows.\textsuperscript{70} Mastitis is another disease that has a great effect on antibiotic residues in milk. Administered veterinary drugs are inclined to persist longer in milk from mastitic cows.\textsuperscript{61,71} Therefore, disease-induced variability in individual animals should be taken into account in the various classes of population for WDI estimation. This is a crucial application of NLME models in estimating WDIs for diseased animals as the current US regulatory approach determines WDIs in healthy animals under controlled conditions often designed to minimize variability.

Age is another important factor affecting PK properties among individuals. Immature liver and kidney function is observed in young animals, whereas impaired kidney function appears to accompany aging. The age-related PK variance frequently occurred in food-producing animals.\textsuperscript{72,73} One of the most significant differences between newborns and adults is lack of fully developed renal function in the newborn. It usually takes 1 or 2 weeks to develop mature renal function for most ruminants.\textsuperscript{74} Nouws has pointed out that half-lives of ally takes 1 or 2 weeks to develop mature renal function for lack of fully developed renal function in the newborn. It usually takes 1 or 2 weeks to develop mature renal function for most ruminants.\textsuperscript{74} Nouws has pointed out that half-lives of

CONCLUSIONS

Meta-analysis allows the integration of multiple, small datasets that expend the inheritance of PK data. The NLME approach can utilize those datasets combing both dense and sparse data to interpret the interanimal variability for large population of animals. It allows disease effects determined in research or field studies to be integrated with existing datasets, a particular need for estimating WDIs in food-producing animals. This is crucial in veterinary medicine where large population studies with very dense data sets simply do not exist, yet inferences must be made across very large populations of animals. We believe meta-analysis in the context of NLME-based PK modeling is a powerful tool to integrate multiple datasets from different studies for veterinary PK.

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REFERENCES


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