Use of Monte Carlo simulation to determine pharmacodynamic cutoffs of amoxicillin to establish a breakpoint for antimicrobial susceptibility testing in pigs

Julien F. Rey, DVM; Céline M. Laffont, PhD; Siska Croubels, Pharm D; Patrick De Backer, DVM; Claudine Zemirline, DVM; Eric Bousquet, DVM; Jérôme Guyonnet; Aude A. Ferran, DVM; Alain Bousquet-Mélou, DVM; Pierre-Louis Toutain, DVM

Objective—To determine pharmacodynamic cutoffs with pharmacokinetic-pharmacodynamic principles and Monte Carlo simulation (MCS) for use of amoxicillin in pigs to set interpretive criteria for antimicrobial susceptibility testing.

Sample—191 plasma disposition curves of amoxicillin obtained from 21 IV, 104 IM, and 66 PO administrations corresponding to 2,098 plasma concentrations.

Procedures—A population model of amoxicillin disposition in pigs was developed for PO and IM administration. The MCS method was then used to determine, for various dosage regimens, the proportion of pigs achieving plasma amoxicillin concentrations greater than a selection of possible minimal inhibitory concentrations (MICs) ranging from 0.0625 to 4 mg/L for at least 40% of a 24-hour period.

Results—A target attainment rate (TAR) of 90% was never achieved with the breakpoint recommended by the Clinical and Laboratory Standards Institute (0.5 mg/L) when the usual recommended dosage (20 mg/kg/d) was used. Only by dividing the orally administered daily dose into 12-hour administration intervals was a TAR > 90% achieved when the total dose was at least 40 mg/kg for a pathogen having an MIC ≤ 0.0625 mg/L. For the IM route, the TAR of 90% could only be achieved for MICs of 0.0625 and 0.125 mg/L with the use of 15 and 30 mg/kg doses, respectively.

Conclusions and Clinical Relevance—Population kinetics and MCS are required to determine robust species-specific interpretive criteria (susceptible, intermediate, and resistant classifications) for antimicrobial susceptibility testing breakpoints (taking into account interanimal variability). (Am J Vet Res 2014;75:124–131)
ate, or resistant for a given antimicrobial and should be at least established for the targeted animal species for the claimed pathogens and take into account the routine dosage regimen of that antimicrobial. Presently, a limited number of veterinary breakpoints have been properly established, and even when they are available, many testing laboratories continue to routinely use human breakpoints as interpretative criteria, which are scientifically questionable. This most likely explains why in veterinary medicine the predictive value of AST can be rather disappointing and the use of susceptibility testing for antimicrobial selection can be challenged. Even when breakpoints are soundly determined, the predictive value of AST for clinical resistance is rather low, as recently reviewed for human medicine, and there is an urgent need to take into account these limiting issues for the prudent use of antimicrobials in veterinary medicine.

No official regulatory body exists in Europe to establish veterinary breakpoints, and presently, the CLSI is the only international organization with a veterinary working party (the SVAST). The SVAST has formally qualified the means to establish veterinary breakpoints (also named interpretative criteria), and the CLSI document M31-A3 lists the largest collection of approved clinical veterinary breakpoints. The SVAST approach to determine breakpoints is explained in a guideline (the so-called M37-A3). Briefly, to establish the published breakpoints, the SVAST considers 3 working cutoffs: the clinical cutoff, which corresponds to the value selected by inspecting clinical-microbiological outcome versus MIC from prospective clinical studies; a microbiological cutoff called the wild-type cutoff, which separates populations on the basis of MIC distributions; and a COPD value that can be calculated with PK-PD parameters and MCS. The latter is established solely on the basis of the relationship between drug concentrations in the biophase or its surrogate (eg, blood, urine, or milk) and possible MICs. For long-available antimicrobials such as amoxicillin, the SVAST acknowledges that the development of interpretive criteria is problematic because efficacy and pharmacokinetic data may be outdated and isolated from clinical trials may not be available (hence, there can be no clinical cutoff), but the establishment of veterinary-specific breakpoints for these compounds must include PK-PD data. For amoxicillin, a time-dependent antimicrobial, the appropriate PK-PD index to be considered is the cumulative time that the plasma free amoxicillin concentration is greater than the targeted MIC (ie, T% > MIC) during a period of 24 hours in steady-state conditions. The means to achieve this index depends on the pathogen, and typically a T% > MIC of at least 40% (ie, free plasma concentration greater than the MIC for at least 10 hours during a 24-hour period) is predictive of appropriate clinical efficacy. For amoxicillin, Schwarz et al suggested a breakpoint in pigs for respiratory tract pathogens. To obtain this, they first reviewed the published literature with regard to swine-specific pharmacokinetic data, clinical efficacy, and the in vitro susceptibility of the putative target pathogens. On the basis of existing associations between the MIC of the pathogens and the pharmacokinetics of amoxicillin with clinical or microbiological outcomes, they concluded that porcine respiratory tract pathogens that have MICs for amoxicillin < 0.5 mg/L can be classified as susceptible, those with MICs of 1 mg/L as intermediate, and those with MICs > 2 mg/L as resistant. The CLSI recently adopted these breakpoints.

In their survey, Schwarz et al critically discussed PK-PD results that were published without supplementary data analysis, modeling, or simulations. The limit of such a deterministic approach is that it only permits expression of an expert opinion on the possible value of the breakpoint. A more refined approach involves first considering the numerical values of the relevant PK-PD index (ie, the T% > MIC [the percentage of time the antimicrobial concentration is greater than the MIC during a 24-hour period]) and its interanimal variability to obtain and properly integrate the PK information needed to establish a probable (rather than possible) breakpoint. Thus, a clinical breakpoint can be derived by a probabilistic approach rather than a deterministic approach, as reviewed by Dalhoff et al. Presently, MCS is a tool that allows integration of previous knowledge on PK and PD (which are probabilistic in nature) and explicitly brings the variability of PK and PD into the establishment of a breakpoint. Such an approach was first described by Drusano et al. The use of MCS for the establishment of breakpoints has been described in detail. In the context of antimicrobial use at the herd level, this approach enables the user to qualify the so-called COPD value as a stochastic variable. The purpose of the study reported here was to determine a COPD value by use of PK-PD principles and MCS for use of amoxicillin in pigs to set interpretive criteria for AST.

Materials and Methods

Amoxicillin raw data—Ten sets of raw data were obtained from 3 pharmaceutical companies and 1 academic laboratory after PO or IM administration of amoxicillin in pigs. All plasma concentrations were normalized to a standard dose of 20 mg/kg to illustrate the variability in the data under the assumption that the pharmacokinetics of amoxicillin are linear (Figure 1). In total, 191 disposition curves of amoxicillin obtained from 21 IV, 104 IM, and 66 PO administrations and corresponding to 2,098 amoxicillin plasma concentrations were available. One of the data sets included both IV and PO data that have been published. From the 4 sources of data, 10 formulations were grouped by route of administration (IV [formulations 1 to 3], PO [formulations 4 to 8], or IM [formulations 9 and 10]). The data for the IV route (formulations 1 to 3) were not included in the population analysis in the study reported here but were evaluated to assist in the selection of a population pharmacokinetic model for IM administration.

The mean number of data points per individual was 13 and 12.2 for the PO and IM routes, respectively. All data were obtained under conditions of good laboratory practice and were used to support marketing authorization of amoxicillin in Europe. The analytic techniques were validated with the LLOQ ranging from 0.025 to 0.1 mg/L or a lowest level of detection of 0.005 mg/L.
Population pharmacokinetic analysis—Two population pharmacokinetic models were developed for disposition of amoxicillin in pigs: 1 for PO administration (5 formulations [4, 5, 6, 7, and 8], 66 subjects, and 832 observations including 120 data points less than the LLOQ) and 1 for IM administration (2 formulations [9 and 10], 104 subjects, and 1,266 observations). Data analysis was performed with software dedicated to the estimation of nonlinear mixed-effects models. Because a nonnegligible percentage of plasma amoxicillin concentrations (14.4%) were less than the LLOQ (0.025 or 0.05 mg/L) for PO administration, this was taken into account when performing model estimations by use of an appropriate method for left-censored data implemented in the software.

Different structural models were evaluated for each route of administration. All models were parameterized in apparent clearances and apparent volumes of distribution. Different variance models were tested for the residual error (ie, additive, proportional, and 3 to 4 combined error models). Unless otherwise specified, interindividual variability was assessed assuming a log-normal distribution of individual pharmacokinetic parameters. For example, the CL/F was modeled according to the following equation:

\[
(CL/F)_i = \theta_{CL} \times \exp(\eta_{CL,i})
\]

where \((CL/F)_i\) is the CL/F in the \(i^{th}\) animal, \(\theta_{CL}\) is the population geometric mean, and \(\eta_{CL,i}\) is a random variable following a normal distribution with a mean of 0 and variance \(\omega_{CL}^2\). A covariate analysis was performed, taking into account the formulation as the main covariate able to account for a part of the interindividual variability. For clearance, for example, the following equation was used:

\[
(CL/F) = \theta_{CL} \times \exp(\text{Formulation}_j) \times \exp(\eta_{CL,i})
\]

where Formulation\(_j\) accounts for the effect of the \(j^{th}\) formulation (\(j = 1\) to 5 for the PO route and \(j = 1\) or 2 for the IM route). A diagonal \(\Omega\) matrix was assumed, meaning that the covariance between individual pharmacokinetic parameters was null; the appropriateness of each model was checked by standard and advanced diagnostic plots. It should be stressed that the so-called formulation effect can actually encompass not only the formulation per se that was administered but also other confounding factors when only 1 formulation was tested in a given experimental setting. For a given route of administration, vari...
ances for interindividual variability were assumed to be equal for all the tested formulations.

Model selection was performed with the likelihood ratio test for nested models and the Bayesian information criterion for nonnested models. At each step of the model selection, the model was evaluated with standard goodness-of-fit plots and VPCs, all displayed by the software. At each time point, VPCs were used to compare the empirical distribution of the observations with their theoretical distribution given by the model (computed from simulations in observation times). The theoretical distribution was summarized by 3 percentiles, the 50th percentile (ie, the median) and the 5th and 95th percentiles, which delineated a 90% prediction interval. Therefore, if the model adequately described the data, it was expected, at each time point, that there would be an even distribution of observations around the median and approximately 90% of the observations would be within the theoretical 90% prediction interval given by the model.

MCS—The MCSs were performed with appropriate software by use of the final population pharmacokinetic models with the formulations as covariates, to cover a broad range of situations. Plasma amoxicillin concentrations were simulated every 15 minutes over 24 hours. Linear kinetics for amoxicillin were assumed, given that dose linearity in diseased pigs was established in a dose range of 4 to 18 mg/kg; however, because the numerical value of the PK-PD index that should be selected for a time-dependent antimicrobial (ie, T% > MIC) behaves nonlinearly with the dosage regimen and that T% > MIC values rely heavily on the shape of the disposition curve (PO vs IM route), a series of different scenarios was simulated separately for the PO and the IM routes of administration. For the PO route, simulations were performed for a total daily dose of 10, 15, 20, or 40 mg of amoxicillin/kg of body weight administered in 2 equal doses at 12-hour intervals; in 3 equal doses at 0, 5.5, and 11 hours (reproducing a typical schedule for feed distribution); in 4 equal doses every 3 hours followed by a 12-hour washout period; or as a 15-hour infusion administered PO (to obtain the maximal time with plasma concentration greater than the MIC) followed by a 9-hour washout period. For the IM route, single-dose administrations of 15 and 30 mg/kg were simulated. For each scenario, a hypothetical population of 1,000 pigs was generated for each formulation investigated by use of the final population pharmacokinetic model. To be consistent with MICs that are homogeneous for free drug concentrations, free amoxicillin concentrations in plasma were computed, taking into account that the bound fraction of amoxicillin in pigs was 17% as reported for diseased pigs. By use of a standard spreadsheet program, the time for which the free amoxicillin concentrations were greater than a given MIC ranging from 0.0625 to 4 mg/L was computed over 24 hours to report the percentage of pigs

![Diagram of pharmacokinetic models of disposition of amoxicillin in pigs.](https://example.com/diagram.png)

Figure 2—Diagram of pharmacokinetic models of disposition of amoxicillin in pigs. The left side of the figure illustrates a 2-compartment model with a zero-order input rate (K0) between lag time (Tlag) and end time (Tend) for PO administration. The right side of the figure illustrates a 1-compartment model with 2 parallel first-order Ka (Ka1 and Ka2, with Ka1 > Ka2), with one fraction of the bioavailable dose (f) being absorbed following Ka1 and the other fraction (1 - f) following Ka2, for IM administration. CL = Clearance of elimination equal to K10 X Vc, K10 = First order rate constant of elimination from the central compartment. Vc = Volume of central compartment. CLd = Clearance of distribution equal to K12 X Vc or K21 X Vp. K12 and K21 = First-order rate constants of exchange between the central compartment and the peripheral compartment. Vp = Volume of peripheral compartment.

![Visual predictive checks for plasma amoxicillin concentration data from 2 sources.](https://example.com/vpc.png)

Figure 3—Visual predictive checks for plasma amoxicillin concentration data from 2 sources. A—Formulation 6 (PO administration of amoxicillin in feed; 40 pigs). Arithmetic scale is used for the y-axis of the left graph; logarithmic scale is used for the y-axis of the right graph. B—Formulation 9 (IM administration of amoxicillin; 80 pigs). Arithmetic scale is used for the y-axis of the left graph; logarithmic scale is used for the y-axis of the right graph. Solid line indicates the median concentration and shaded area indicates 90% prediction interval (5th to 95th percentiles). Circles indicate data values.
for which the plasma concentration of free drug was greater than the tested MIC for at least 9.6 hours (ie, 40% of 24 hours).

Results

Population pharmacokinetic models—Plasma amoxicillin concentrations obtained for the PO route were best modeled with a 2-compartment model with a zero-order Ka, starting after a lag time and stopping after a given delay. Other pharmacokinetic parameters of the model were the CL/F, clearance of distribution per bioavailability, Vc/F, and Vp/F (Figure 2).

The IM data were best modeled with a 1-compartment model with 2 parallel first-order Ka (Ka1 and Ka2 [Ka1/time]), with a fraction of the dose bioavailable (f) absorbed following Ka1 and the remaining fraction (1-f) absorbed more slowly following Ka2 (Ka2 < Ka1). In contrast to other pharmacokinetic parameters, a logit-normal distribution was used for fractions of the bioavailable dose between 0 and 1. It should be noted that this structural model is not distinguishable from the classical 2-compartment model, but when compared with the IV data, the long terminal half-life observed after IM administration clearly distinguished the existence of a flip-flop phenomenon, with Ka2 determining the slope of the terminal phase.

Examples of the consistency between observations and the selected population models (Figure 3) are the VPC for formulation 6 (amoxicillin in feeds) and for formulation 9 (IM administration). Similar consistency was obtained with the other formulations. Final model parameter estimates were determined (Tables 1 and 2) for the PO and IM routes of administration.

MCS—For a selection of possible MICs ranging from 0.0625 to 2 mg/L and for different dosage regimens, the PO TARs for T% > MIC (ie, the percentage of pigs for which T% > MIC is ≥40% over the 24 hours of simulation) were determined for different dose-to-MIC ratios (Figure 4). This is the percentage of pigs with the PK-PD index value for which it is assumed that the amoxicillin treatment will be efficacious or, axiomatically, that the pathogen is clinically susceptible. With a single orally administered amoxicillin dose, the TAR could not be achieved whatever the simulated dose (from 10 to 40 mg/kg), but by splitting the daily dose into 12-hour intervals a TAR > 90% (ie, 96%) could be attained when the total dose was 40 mg/kg for a pathogen having an MIC of 0.0625 mg/L (ie, a dose-to-MIC ratio of 640 L/kg). The amoxicillin efficiency (the overall efficacy of an amoxicillin dose unit) increased as the regimen became more and more similar to that obtained by an infusion. For the 30 mg/kg dose administered IM, a TAR of 90% could only be achieved for an MIC of 0.125 mg/L to ensure a T% > MIC of at least 40% (Figure 5).

Considering the CLSI breakpoint for amoxicillin susceptibility (0.5 mg/L), the simulations indicated that the TAR of 90% could only be achieved with a daily dose of 40 mg/kg for the PO route, provided that the total dose was evenly divided over the 24 hours and that the maximum TAR was only 54% for a single 30 mg/kg dose of amoxicillin administered IM.

Table 1—Pharmacokinetic parameters obtained for a population pharmacokinetic model comprising data from 5 of 10 formulations (formulations 4 to 8) developed for amoxicillin disposition in pigs after PO administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population geometric mean</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (L/kg/h)</td>
<td>3.1 (All formulations)</td>
<td>34.6</td>
</tr>
<tr>
<td>Ka1 (1/h)</td>
<td>0.343 (9); 0.275 (10)</td>
<td>28.8</td>
</tr>
<tr>
<td>Ka2 (1/h)</td>
<td>0.041 (9); 0.003 (10)</td>
<td>52.5</td>
</tr>
<tr>
<td>Fraction of dose absorbed following Ka1 (from 0–1)</td>
<td>0.161 (9); 0.403 (10)</td>
<td>52.2</td>
</tr>
<tr>
<td>Vc/F (L/kg)</td>
<td>3.56 (All formulations)</td>
<td>66.4</td>
</tr>
<tr>
<td>Vp/F (L/kg)</td>
<td>3.56 (All formulations)</td>
<td>66.4</td>
</tr>
</tbody>
</table>

Table 2—Pharmacokinetic parameters obtained for a population pharmacokinetic model comprising data from 2 of 10 formulations (formulations 9 and 10) developed for amoxicillin disposition in pigs after IM administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population geometric mean</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka1 (1/h)</td>
<td>0.343 (9); 0.275 (10)</td>
<td>28.8</td>
</tr>
<tr>
<td>Ka2 (1/h)</td>
<td>0.041 (9); 0.003 (10)</td>
<td>52.5</td>
</tr>
<tr>
<td>Fraction of dose absorbed following Ka1 (from 0–1)</td>
<td>0.161 (9); 0.403 (10)</td>
<td>52.2</td>
</tr>
<tr>
<td>CL/F (L/kg/h)</td>
<td>0.388 (9); 0.279 (10)</td>
<td>14.1</td>
</tr>
<tr>
<td>Vc/F (L/kg)</td>
<td>0.252 (9 and 10)</td>
<td>52.1</td>
</tr>
</tbody>
</table>

Ka1 = Rapid Ka, Ka2 = Slow Ka.

Standard errors of estimates were < 24% for fixed and random effect parameters. When the effect of formulation was significant (P < 0.05), the formulation was included as a covariate in the model, which required division of data sets into different subgroups of formulations. Numbers in parentheses indicate formulation number.
distribution, such as for a specific region. The dynamic value at each MIC could be weighted by any MIC achieving the target pharmacokinetic and pharmacodynamic index. Subsequently, these cumulative probabilities of pigs in the herd level because ignoring this variability can lead to the pharmacodynamic variability (the clinically relevant MIC distributions of the targeted pathogens). 11

Instead, the goal was to determine how a PK-PD analysis could help those selecting a clinical breakpoint by quantifying the pharmacokinetic variability of amoxicillin that needs to be taken into account and expressing it in a user-friendly way. To do this, we computed the probability that the antimicrobial concentration was greater than the MIC in a 24-hour period in 10% increments from 100% (lower dotted line) to 10% (upper continuous line). The continuous thick black line corresponds to a T% > MIC of 40%.

Discussion

To the authors’ knowledge, this study was the first attempt in veterinary medicine to provide pharmacodynamic cutoffs based on a population kinetic analysis of a target species. It should be understood that the goal was not to derive a PK-PD breakpoint because to propose a PK-PD breakpoint of the same status as a clinical breakpoint it would have been necessary to also consider the pharmacodynamic variability (the clinically relevant MIC distributions of the targeted pathogens). 11

Instead, the goal was to determine how a PK-PD analysis could help those selecting a clinical breakpoint by quantifying the pharmacokinetic variability of amoxicillin that needs to be taken into account and expressing it in a user-friendly way. To do this, we computed the probability that the antimicrobial concentration was greater than the MIC in a 24-hour period in 10% increments from 100% (lower dotted line) to 10% (upper continuous line). The continuous thick black line corresponds to a T% > MIC of 40%.

The principle of the present study was rather simple but it required robust estimates of the distributions of pharmacokinetic parameters (population means and variances), which are complicated to obtain. It also required that some assumptions had to be accepted concerning the pharmacokinetic and pharmacodynamic relationship for amoxicillin. It should be acknowledged that the use of the T% > pharmacokinetic-pharmacodynamic index value to compare different dosage regimens is not without difficulties. 11 An initial difficulty is that the T% > MIC is not a parameter but a variable that depends on many factors including not only the administered dose but also, for a given dose, the shape of the amoxicillin disposition curve. Thus, a universal pharmacokinetic-pharmacodynamic index cannot be computed for a time-dependent antimicrobial, even for a fixed dose. A second difficulty is the relationship between the pharmacokinetic-pharmacodynamic index and the clinical efficacy. In a proposal of a clinical breakpoint for amoxicillin in pigs, Schwarz et al 2 exhaustively reviewed the literature and were able to draw qualitative conclusions, but from the same published data, we considered it was impossible to retrieve relevant pharmacokinetic parameter distributions for a realistic population pharmacokinetic model that would ultimately provide quantitative conclusions by use of MCS. This prompted us to directly model several sets of raw data that were used historically to support marketing authorizations for use of amoxicillin in pigs. Analyzed data (191 individual disposition curves) were obtained in different laboratory settings (in groups of pigs differing in their age, body weight, sex, and other factors) with different husbandry conditions and with amoxicillin administered in fed or food-withheld conditions, as a bolus or continuously in the food; additionally, plasma concentrations were analyzed with different analytical techniques (although all were validated). This heterogeneity of collected data was not necessarily a disadvantage because breakpoints are derived for neither a subset of animals nor for a given modality of amoxicillin administration; the data set can be viewed as a representing a worst-case scenario that allowed rather conservative (low) pharmacokinetic-pharmacodynamic cutoffs to be derived. The main limitation of the data set was that the pigs were healthy. This can be relevant for the prophylactic and metaphylactic uses of amoxicillin but is not likely to be fully representative of diseased pigs. We acknowledge that most pharmacokinetic data submitted for marketing authorization in veterinary medicine are obtained in healthy animals, and this is less than ideal when developing a population model for an anti-infective drug for which the infection is the main possible factor of variability of the drug disposition. 22 The pharmacokinetics of amoxicillin in healthy and Salmonella typhimurium–inoculated pigs have been compared. 22 A minimal difference in drug exposure was detected between the 2 conditions over the first 12 hours, but when the AUC was extrapolated to infinity, it was suggested that amoxicillin exposure was 54% greater in diseased pigs, with a much longer terminal half-life (mean = SD, 12 ± 10 hours vs 3.9 ± 1.6 hours) in diseased versus healthy pigs. In contrast, E coli–induced diarrhea decreased the AUC of amoxicillin by 50% over the first day of administration and reduced the duration for which the plasma concentration was > 0.025 mg/L. 23 The influence of a spontaneous respiratory tract disease on the disposition of amoxicillin in pigs has been investigated; 20 systemic amoxicillin exposure was significantly greater in diseased pigs, compared with healthy pigs, by a 3-fold difference in...
the AUC. This was attributed to a significantly greater amoxicillin bioavailability (44.7% vs 14.1%). In addition, a longer absorption period was observed in diseased pigs, indicating that PK-PD cutoffs can be different for curative, metaphylactic (control), or prophylactic treatments.

The other limit of the PK-PD approach is the definition of the TAR to be achieved (40% of 24 hours greater than the MIC for 90% of the pig population in the present study); 90% is a value that is widely used in TAR analysis, but this has not yet been validated in veterinary medicine. The relationship between % > MIC and antimicrobial efficacy has been determined in vitro in several animal studies and retrospective analysis of clinical trials in human medicine seems to confirm those findings. However, there is a complex quantitative relationship between the in vivo concentrations of an antimicrobial and the growth and death rates of bacteria, and antimicrobials with the same MICs and pharmacokinetics may differ profoundly in their microbiological efficacy; therefore, the % > MIC should be considered as a crude surrogate of clinical efficacy.

In the present study, use of the 90% value seemed to be a good compromise between a clinically acceptable result at the herd level and a realistic TAR (increasing the percentage of success to 95% or 99% would considerably reduce the critical MIC). However, the effect of use of this percentage on the possible promotion of AMR is unknown because promotion of AMR is not an individual animal issue but a collective issue. It can only be assumed that marketed dosage regimens with their corresponding mean or typical exposure values are appropriate and that large between-subject variability leading to underexposure of a fraction of the pig population is not desirable. More problematic is the selection of a pharmacodynamic target (ie, % > MIC = 40% of a daily [24-hour] treatment). This is a classic value used to obtain a bacteriostatic effect in an experimental animal infection study, but for gram-positive bacteria such as Staphylococcus aureus, the free β-lactam concentrations can exceed the MIC for as little as 23% of an administration interval for almost maximal bacterial killing in experimental in vivo and in vitro studies of infection, and nothing indicates that for prophylaxis or metaphylaxis in pigs in which even lower values would be appropriate (leading to an increase in the final critical MIC). The use of an MIC has inherent weaknesses.

The main goal of the present study was not to fully discuss the population model that was built for amoxicillin in pigs. The selected structural models were simple (a 2-compartmental model for the PO route as also indicated by the IV data [results not shown]), and a monocompartmental model for the IM data that were characterized by a long terminal phase indicated a flip-flop process (slow IM absorption for the largest fraction of bioavailable amoxicillin). The pharmacokinetics reported here was consistent with that reported in the literature, but the model parameters expressing dispersion (interindividual variability) were larger than those reported in the literature for a given trial because larger and more representative samples were used. Furthermore, substantial differences between the formulations were observed and accounted for with the model and in the MCS. This is precisely what is required to develop a generic PK-PD cutoff.

After an MCS has been performed, the results need to be discussed and interpreted; as noted by Mouton et al., this is a gray area where options differ and it is important to remember that the MCS is only a tool for the decision-making process and does not provide a final answer. The main results of the present study were that the PK-PD cutoffs (the critical MIC for which 90% of the pigs have plasma values greater than the MIC for 40% of the 24-hour period) were consistently less than the recommended CLSI breakpoint (0.5 mg/L) as proposed by Schwarz et al. This was expected, and when breakpoints are derived by use of a deterministic approach (with mean point estimates for PK parameters), they are often too high because this procedure does not take the interindividual variability of drug exposure into account; this can be a factor promoting AMR. The MCS results of the present study illustrate this point because the CLSI fixed the breakpoint at 0.5 mg/L, whereas such a concentration cannot be achieved in plasma for most pigs when amoxicillin is administered at the recommended dosage (often 20 mg/kg/d). Whether this difference between the PK-PD cutoffs and the final recommended breakpoint is relevant remains unclear because in many countries, it is the human breakpoint (often 4 mg/L) that is used for the susceptibility test interpretation of amoxicillin without any major concerns expressed by clinicians.

Conversely, the PK-PD approach used in the present investigation can be used to reconsider some dosage regimens established historically without specific knowledge of the PK-PD relationship. By use of population pharmacokinetics, the derived parameters allow probable drug exposures to be explored that can be obtained with any dosage regimen, thus enabling drug administration schedules to be optimized. This is especially true in the case of amoxicillin, a time-dependent antimicrobial for which the input rate (absorption) is at least as important as the total administered dose and not directly proportional to the dose (as it is for the AUC:MIC ratio as a PK-PD index). In pigs, for oral ad libitum administration, plasma concentration–time profiles are related to the feeding behavior, indicating that prudent use of an antimicrobial should take into account husbandry factors to improve amoxicillin efficiency (ie, to obtain the highest possible TAR for a total given daily dose). This highlights the fact that a breakpoint cannot be a robust universal value even when derived for a given species and a set of known pathogens because the shape of the antimicrobial exposure curve, which is dependent on the formulation and route of administration, has a large effect on the TAR of the PK-PD index; ideally, authorities setting breakpoints for AST should clearly specify the dosage regimens on which their breakpoints are based. Because many long-acting formulations exist in veterinary medicine, it could be wise, especially for time-dependent antimicrobials, to systematically explore short- versus long-acting formulations and express recommendations accordingly. Indeed, nothing guarantees that a breakpoint valuable for a short-acting formulation administered daily is equiv-
alent to a single-dose administration of a long-acting formulation.

The present study revealed how to extract useful pharmacokinetic information from data files submitted for marketing authorization. For old antimicrobials, such data are unlikely to exist unless required for generic product marketing. Ideally, a population kinetic analysis considering a variety of international and updated data sets could be performed and the results (i.e., the TAR) could be made public as a series of what-if scenarios, allowing any regulatory organization to take into account these cutoffs to select a regional or an international breakpoint.

References
14. Dudley MN, Ambrose PG. Pharmacodynamics in the study of drug resistance and establishing in vitro susceptibility break-