Pharmacokinetic modeling of in-feed tetracyclines in pigs using a meta-analytic compartmental approach

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Summary

Objective: To assess effect of pharmacokinetic differences between chlortetracycline (CTC) and oxytetracycline (OTC), the effect of day/night variations in ad libitum intake of medicated feeds on variation in plasma and tissue concentrations of both drugs, and the influence on the overall efficacy of metaphylactic strategies for bacterial respiratory disease in growing pigs.

Methods: A multidosage pharmacokinetic model was created for feed-administered CTC and OTC in pigs. Parameters obtained by meta-analysis of published pharmacokinetic articles and a computerized iteration procedure were used to find minimum CTC or OTC in-feed dosage to maintain a selected plasma drug concentration in pigs incorporating feeding behavior variables.

Results: The model accounted for daily feed consumption, ad libitum feeding behavior, time-kill activity of tetracyclines, and the span of their postantibiotic effect. The model successfully passed an external validation procedure. The bioavailability (P=.0001), apparent volume of distribution (P=.001), and elimination constant rate (P=.03) differed between CTC and OTC. When identical in-feed dosages are consumed, plasma concentrations of CTC were twice the concentrations of OTC. Ad libitum feeding behavior, which becomes increasingly diurnal as pigs grow from weaning to finishing, induced major daily variations in plasma concentrations of both drugs. We devised two equations that easily calculate any specific result generated by the computerized iteration procedure for CTC and OTC.

Implications: The use of either equation will allow swine practitioners to create more precise CTC or OTC metaphylactic strategies from weaning to finishing. Matching daily antibiotic dosages to what is needed can improve the efficacy of metaphylaxis.

Controlling respiratory diseases is a major health concern for intensively raised weaner, grower, and finisher pigs. Often, the only realistic short-term option to prevent outbreaks of respiratory disease is to control infection pressure in colonized, subclinically infected animals (i.e., **metaphylaxis**—see glossary beginning page 200). Typically, metaphylaxis is accomplished through the large-scale use of antibiotics, which are delivered in feed and/or drinking water to minimize animal stress and labor and drug costs. However, while cost is an important factor in choosing a medication and administration route, oral **bioavailability** must also be considered. Tetracyclines, the most frequently used drugs for treating respiratory disease, inhibit bacterial protein synthesis (Figure 1).[1] The number of bacterial cells that die when exposed to greater than the minimum inhibitory concentrations (MIC) of tetracyclines is related to the duration of the exposure, not to drug concentration (e.g., time-dependent **kinetics of bactericidal activity**) (Figure 2).[2] With non-intestinal diseases, the antibiotic must be absorbed in the intestinal lumen before it can reach the targeted tissue. Thus, bioavailability is a factor in drug waste. The plasma concentration of a drug is influenced by three factors:

- bioavailability,
- dosage, and
- pig feeding behavior.

If any of these three factors is not addressed, then drug concentrations in plasma and tissue, when delivered in feed or water, are unlikely to meet the MIC for the targeted pathogens.[3]

A number of feed factors and characteristic pig behaviors affect the intake of medicated feeds. For example, when drugs are administered in moist or liquid feeds,[4] top dressing, or drinking water, the drug residence in the stomach, bioavailability, and/or **absorption** rate of drugs can be different than when they are administered in dry feed.[5] Supplemental citric acid in feeds medicated with tetracyclines is known to interfere with unavailable calcium-drug complexes that form in the intestines. The bioavailability of orally delivered tetracyclines, especially chlortetracycline (CTC), is thus enhanced by citric acid.[6]

Feed intake[7] and ad libitum feeding behavior are also both influenced by pig age.[8] Feed consumption in pigs is related to metabolic weight[9] ($W^{0.75}$) rather than crude weight (W); thus, ad libitum ingestion of a given medicated feed will result in higher drug dosages and higher plasma concentrations in 10-kg (22-lb) pigs than in 40-kg (88-lb)

JREdC, GPM: Université de Montréal, Faculté de Médecine Vétérinaire. CP 5000 St-Hyacinthe Quebec, J2S-7C6 Canada; JE: Ayerst Laboratories Inc.

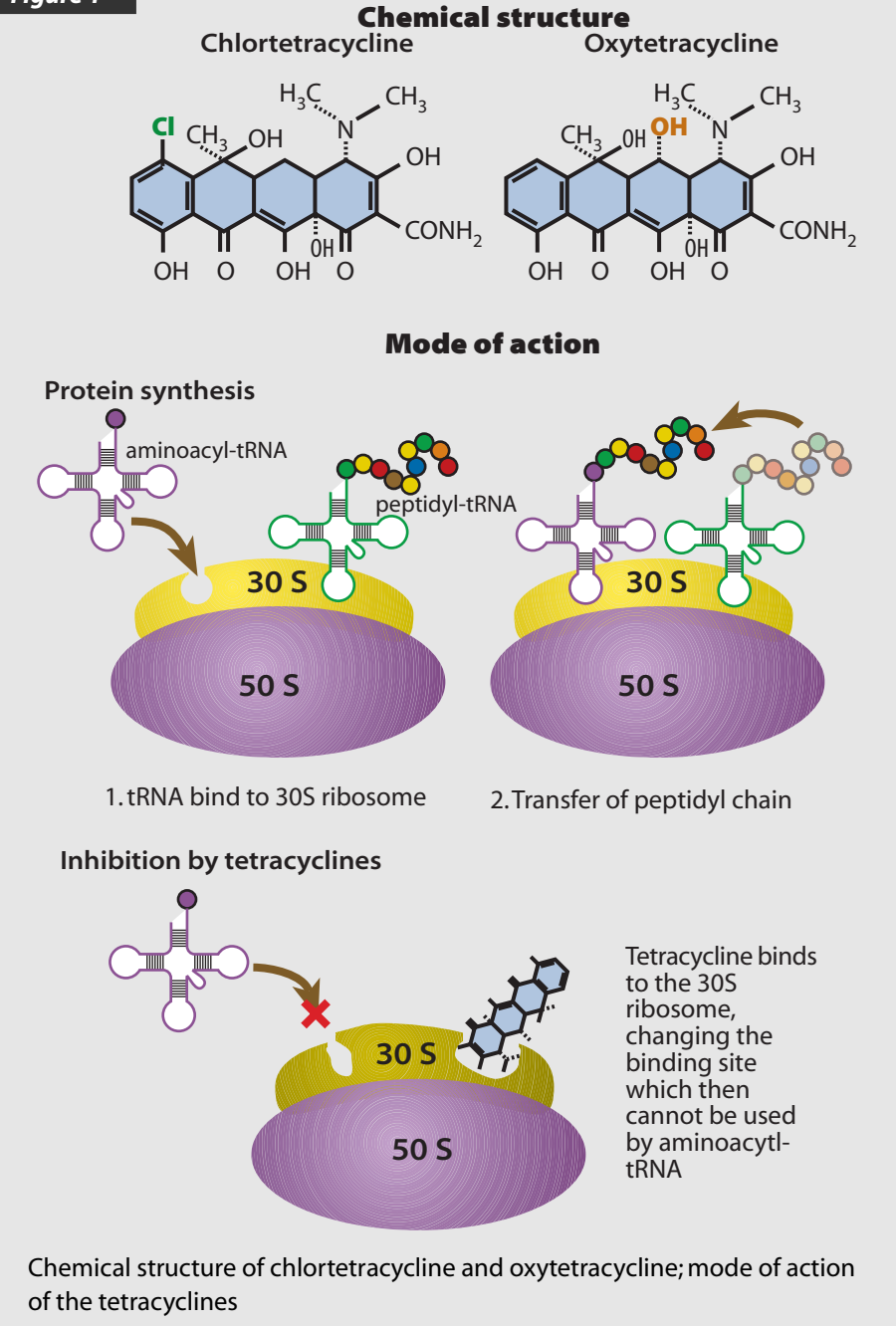This article is available online at **http://www.aasp.org/shap.html**

pigs. Moreover, Labroue, et al.,[8] have observed that as pigs get older, they consume an increasing percentage of their overall feed intake during the day (Table 1). This suggests that feeding behavior might be less affected by day/night cycles in 10-kg (22-lb) piglets than in 40-kg (88-lb) pigs. For a given in-feed tetracycline dosage, the daily variations in plasma concentrations of the drug are positively associated with the age of the pigs.

Feeding behavior of pigs and the amount of feed ingested per meal are also affected by the size of groups placed within a pen,[10] even when pen density is equal. Pigs housed in groups of ≤ 15 pigs per pen eat approximately twice as often as those penned in groups of ≥ 20.[10] To maintain the same daily feed consumption, then, pigs housed in groups of ≥20 must eat twice as much at each meal. These feed characteristics and feeding behaviors may have an effect on the drug concentrations in plasma and tissues and on the degree of protection against disease. No pharmacokinetic study has taken into account actual pig feeding behavior.

Pharmacokinetics is what the body does to the drug and **pharmacodynamics** is what the drug does to the body (both of the animal and of the bacterium).[11] It is important to consider both pharmacokinetics and pharmacodynamics when designing metaphylactic strategies. Although both CTC and oxytetracycline (OTC) have been the subject of pharmacokinetic studies in pigs, the pigs were given a single oral treatment,[1,4,6,12,13] or the drug plasma concentrations were modeled over time in a population of pigs fed according to a fixed-interval schedule.[4] Little is known, therefore, about the pharmacokinetics of tetracyclines when medicated feeds are consumed ad libitum. It is risky to generalize from any one of these studies to a population of pigs that have ad libitum access to feed; however, by using meta-analysis we can synthesize the findings of many studies to obtain an average that can allow us to generalize more reliably to a wide variety of herd situations.

Meta-analysis is a process whereby a collection of research results from individual studies are statistically analyzed in a way that allows one to integrate their findings.[14] Meta-analysis is currently used in epidemiological studies to obtain stronger, more reliable information that can be applied to a wider range of situations than can the findings of individual studies. It produces a set of parameters that represents average, not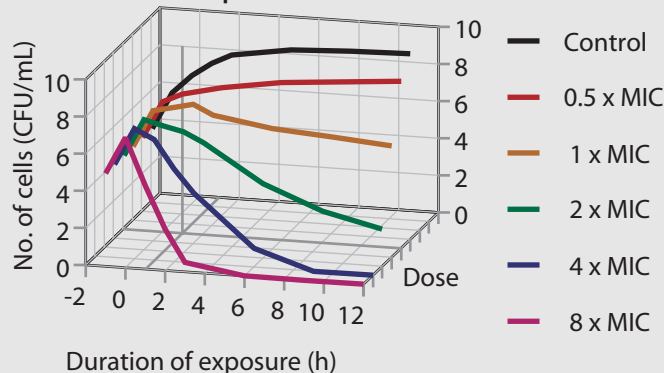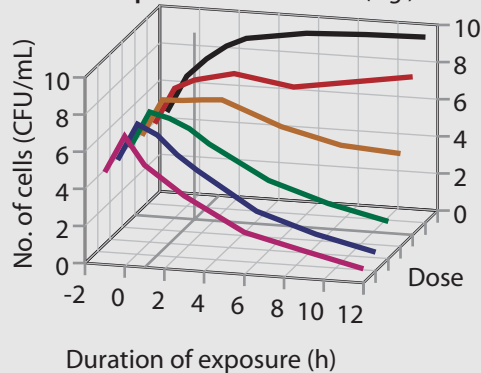 individual, findings across studies. This type of analysis can determine associations between disease and risk factors, risk estimates, or other parameter estimates when the original studies do not have adequate statistical power to report such findings.

Figure 1

Chemical structure

Chlortetracycline   Oxytetracycline

Mode of action

Protein synthesis

aminoacyl-tRNA

peptidyl-tRNA

30 S   30 S
50 S   50 S

1. tRNA bind to 30S ribosome   2. Transfer of peptidyl chain

Inhibition by tetracyclines

30 S
50 S

Tetracycline binds to the 30S ribosome, changing the binding site which then cannot be used by aminoacytl-tRNA

Chemical structure of chlortetracycline and oxytetracycline; mode of action of the tetracyclines

Table 1

Proportion of diurnal daily meals in pigs of distinct growth stages and fed ad libitum.[8]

Weight (kg)	Diurnal daily meals
40	67.5%
60	74.0%
90	83.3%

Figure 2

Kinetics of bactericidal activity for concentration-dependent antibiotics and time-dependent antibiotics

The objectives of the present investigation were to:

- perform meta-analysis on a number of pharmacokinetic studies to allow us to obtain reliable pharmacokinetic parameters for CTC and OTC, and then use these parameters in a pharmacokinetic model that describes the ad libitum feeding behavior of growing pigs;

- compare the meta-analytically derived pharmacokinetic parameters of feed-administered CTC and OTC in pigs;

- assess the validity of our meta-analytic model by comparing its predictions to actual observations of the plasma concentrations of CTC and OTC that appear in the literature; and

- assess the influence of feeding behaviors (diurnal patterns of feeding behavior and the number of pigs per pen) on daily variations of CTC and OTC concentrations in plasma.

Materials and methods

Meta-analysis and statistical analysis

Meta-analysis was performed with all available studies treating the pharmacokinetics of in-feed tetracyclines in pigs to obtain reliable parameters that could be further used in a multiple-dosage pharmacokinetic model, using the Mantel-Haenszel method.[14] Briefly, Mantel-Haenszel meta-analysis consists of a series of steps:

1. The materials and methods of the set of independent research studies are critically evaluated. Evaluation criteria can be both objective and subjective. Possible confounding factors and effect modifiers are identified and the studies are ranked according to quality based on those evaluation criteria.

2. The relevant results (e.g., means and variances; risk ratios; *P* values) are extracted from each of the studies.

3. Exploratory data analysis to assess the link between the results and any confounding factors is performed. If the results correlate with a confounding factor (e.g., age of animals, calcium content in feed,[7,9] type of analysis used), a weight is given to this variable, so that its confounding effects will be accounted for in the calculations.

4. An estimate of the results of all studies, as well as a global variance, is calculated. In this study, we calculated an inverse-variance weighted average (Mantel-Haenszel estimate) and a global variance of the pharmacokinetic parameters.

Compartmental pharmacokinetic analysis[15] was performed on all but one[12] of the articles included in the meta-analysis; the statistical moment kinetic approach[16] was used in the later study. A one-compartment kinetic analysis[15] was thus performed with the individual sets of plasma CTC concentrations reported in this latter article.[12] Experiments[6,13] using feeds supplemented with 1.4% of calcium were included in the meta-analysis, because somewhat lower concentrations of calcium are recommended in Europe for weaned pigs,[9] but their reported bioavailabilities were weighted at a 50% level.

To be included in our meta-analysis, a study had to meet several criteria:

- antibiotics had to have been administered to clinically healthy animals. Diseased pigs have been observed to have reduced feed and water intake compared to clinically healthy pigs.[1] In addition, the pharmacokinetic profile is completely different in diseased pigs;[1]

- the OTC and CTC had to have been delivered in medicated dry feed (as opposed to wet or moist feed);

- the feed had to meet both American and European calcium requirements;[7,9] and

- the feed had to be free of supplemental citric acid.

Studies excluded from meta-analysis were reports of experiments performed on disease-challenged animals,[1] those where moist or liquid medicated-feeds were used,[4] those with citric acid-supplemented feed,[6,13] and those that did not investigate the individual evolution of plasma concentrations of the drug over time.[17,18]

Five studies, two reporting on OTC[1,13] and three reporting on CTC,[4,6,12] met all the criteria and were included in the meta-analysis. To derive mean parameter values to use in calculations that describe the fluctuations in drug concentrations over time, two separate Mantel-

Table 2

Estimates (mean ± SD) of the pharmacokinetic parameters of feed-administered chlortetracycline in pigs reported in the literature and average values obtained with a Mantel-Haenszel meta-analysis

| Parameter | Units | Reference | | | | Meta-analysis |
		4	6 Ca=0.7%	6 Ca=1.4%	12	
F (absolute bioavailability)	%	17.6 ±4.0	12.6 ±1.3	9.5 ±4.0	17.9 ±5.3	13.6 ±6.17
$V_{(area)}$ (apparent distribution volume)	L/kg	2.03 ±0.66	1.76	2.34	1.29 ±0.21	1.854 ±0.444
k_a (absorption rate constant)	hours^{-1}	1.30 ±0.50	2.03 ±2.15	1.57 ±1.51	0.92 ±0.92	1.270 ±1.666
k (elimination rate constant)	hours^{-1}	0.15 ±0.02	0.20 ±0.05	0.25 ±0.05	0.17 ±0.05	0.169 ±0.073
$t_{1/2}$ (elimination half-life)	hours	4.62	3.46	2.77	3.99	4.08
CL_b (systemic clearance)	L/hour/kg	0.304	0.411	0.467	0.224	0.314

Table 3

Estimates (mean ± SD) of the pharmacokinetic parameters of feed-administered OTC in pigs reported in the literature and average values obtained with a Mantel-Haenszel meta-analysis

| Parameter | Units | Reference | | | Meta-analysis |
		1	13 Ca=0.7%	13 Ca=1.4%	
F (absolute bioavailability)	%	4.8 ±1.7	3.6 ±0.9	3.7 ±0.9	3.80 ±1.53
$V_{(area)}$ (apparent distribution volume)	L/kg	1.44	1.43	1.61	1.495 ±0.099
k_a (absorption rate constant)	hours^{-1}	1.89 ±0.29	4.54 ±4.56	3.19 ±3.35	1.910 ±3.901
k (elimination rate constant)	hours^{-1}	0.12 ±0.03	0.13 ±0.03	0.14 ±0.05	0.127 ±0.048
$t_{1/2}$ (elimination half-life)	hours	5.68	5.33	4.95	5.33
CL_b (systemic clearance)	L/hour/kg	0.176	0.187	0.225	0.194

Haenszel meta-analyses were then performed on the sets of studies (Tables 2–3). These pharmacokinetic parameters were compared statistically using the Mann-Whitney U tests[19] at a .05 significance level.

The multiple-dosage pharmacokinetic model

The fluctuations of drug concentrations in plasma over time for one single dosage can be calculated with the following nonlinear equation:[20]

$$Cp(t) = \frac{F \times Dose}{V} \times \frac{k_a}{(k_a - k)} \times \left(e^{-k \times t} - e^{-k_a \times t} \right)$$

(Equation 1)

Where:

- $Cp(t)$ is drug plasma concentration at time t,
- F is the absolute bioavailability
- Dose is the administered dosage of drug,
- k_a is the **absorption rate constant**,
- k is the **elimination rate constant**,
- e is the natural base of logarithms, i.e., 2.71828…, and
- V is the apparent **distribution volume**.

To calculate V, the equation is:

$$V_{(area)} = \frac{F \times Dose}{AUC \times k}$$

(Equation 2)

Where AUC is the area under the curve of plasma drug concentration over time measured with the linear trapezoidal rule.[20]

To conduct the meta-analysis, we used a crude average estimate of $V_{(area)}$ instead of a Mantel-Haenszel[14] weighed average, because a majority of authors did not report the variance associated with their estimate (Tables 2–3).

Systemic **clearance** (CL_b) and elimination half-life ($t_{1/2}$) of CTC and OTC were calculated with the following equations:

$$CL_b = V_{(area)} \times k$$

(Equation 3)

$$t_{1/2} = \frac{0.693}{k}$$

(Equation 4)

We derived the estimates of CL_b and $t_{1/2}$ with parameters derived from the meta-analysis.

The multiple-dosage pharmacokinetic model was devised from equation 1 using the method of **superposition**;[21] e.g., repeatedly adding Equation 1 (Figure 3) based on the proportion of daily meals taken during daytime (Table 1),[8] and the amount of feed consumed ad libitum per meal.

Feeding behavior

Based on observations of the influence of the size of the group of pigs within a pen (not pig density) on feed consumption and feeding behavior,[10] we modeled a scenario with ≤ 15 pigs per pen, and another scenario with ≥ 20 pigs per pen.

Computer iteration procedure

A computerized iteration procedure was devised to find the minimum in-feed dosage of either CTC or OTC (in ppm) that would maintain plasma MIC over a 20-hour interval (to allow for a 4-hour postantibiotic effect [PAE]). We focused on the minimum concentration of antibiotic in plasma to take into account the time-dependent antimicrobial effect of tetracyclines. A procedure was programmed in Turbo Basic (Corel Inc., Ottawa, Ontario). A Basic compiler is needed to run the program.
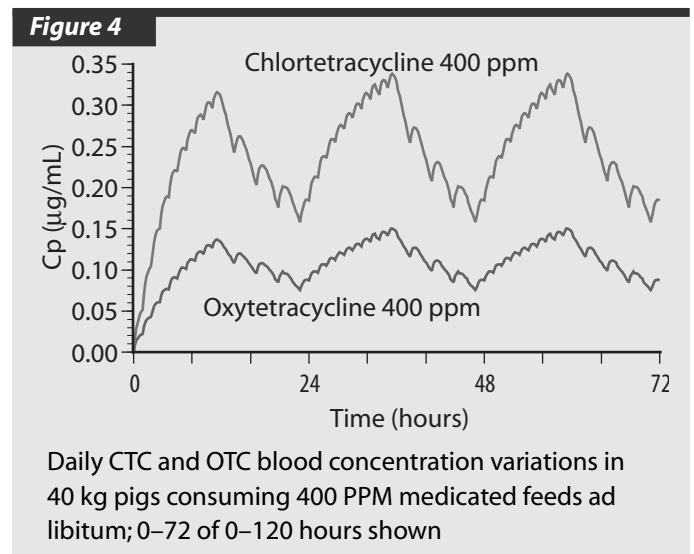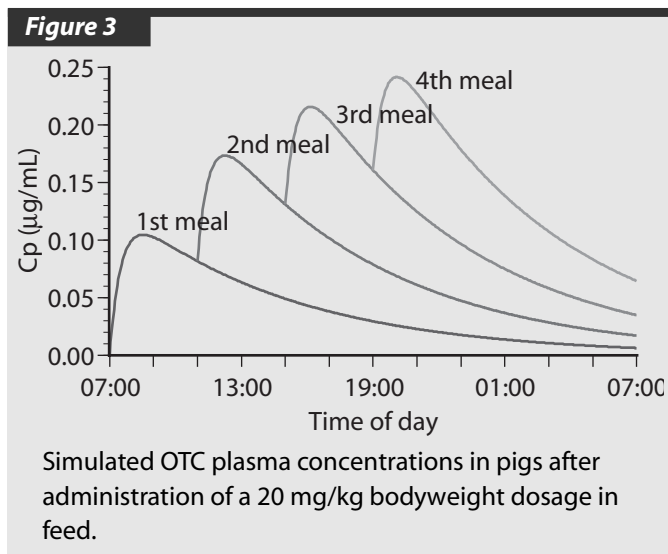
Variables we included in this program were the following:

- F,
- Vd,
- k_a and k,
- the targeted plasma concentration of drug (TPL),
- the weight of the average pig,
- the feed intake of the average pig during daytime,
- the number of daytime meals (this proportion can be estimated from Labroue's[8] data, Table 1),
- feed intake during nighttime, and
- the number of nighttime meals.

Begin and end values for the in-feed dosage and the iteration step are also variables in this program.

Values of the parameters derived from meta-analysis were then input into Equation 1, which was superposed at even intervals according to the number of daytime meals during the 0- to 12-hour (daytime) period. Thus, if the pigs were assumed to eat 4 meals during the day, the equation was superposed 4 times at 4-hour intervals (e.g., 07:00, 11:00, 15:00, 19:00) (Figure 3). During the 12- to 24-hour (nighttime) period, Equation 1 was superposed at even intervals according to the number of nighttime meals. Daytime and nighttime superpositions were repeated for a continuous 120-hour period (Figure 4). Dose was determined by dividing respective feed intakes (i.e., daytime or nighttime) by the number of meals assumed to have been taken during daytime and nighttime.

A pharmacokinetic profile was then generated for 200 hours (to ensure that the data would include the period of equilibrium) with 0.2-hour intervals. The minimum in-feed dosage (in ppm) required to achieve TPL was found by backward iteration according to the

Figure 3

Simulated OTC plasma concentrations in pigs after administration of a 20 mg/kg bodyweight dosage in feed.

Figure 4

Daily CTC and OTC blood concentration variations in 40 kg pigs consuming 400 PPM medicated feeds ad libitum; 0–72 of 0–120 hours shown

following criterion: the resulting plasma concentration profile had to fall below TPL for exactly 4 hours (the span of post-antibiotic effect with tetracyclines) somewhere between 36 and 60 hours after the medicated feed is first offered, the time at which equilibrium of drug between plasma and tissue has been achieved. Finally, the program generated an ASCII file with the final pharmacokinetic profile, giving pairs of time and plasma drug concentration values in the 0- to 200-hour period in 0.2-hour intervals.

This iteration procedure was run twice, once for the OTC profile and once for the CTC profile.

Model validation

To assess the accuracy of the meta-analytic model, we used an external validation procedure to compare the antibiotic plasma concentrations the models predicted to actual plasma concentrations reported by Black and Gentry,[22] Asanuma, et al.,[17] Mevius, et al.,[23] Reichert,[24] Andrews, et al.,[18] Hall, et al.[25] and Hunneman, et al.[26] In some papers,[18,25] the mean ± SD of plasma concentrations was reported graphically; thus, we used approximate estimations of the mean and confidence intervals in our validity calculations. "Agreement" between the models and the literature was defined as ≥ 50% overlap between

their respective 95% confidence intervals (CI).

The predicted confidence intervals of CTC and OTC plasma concentrations were obtained by using F and k values equal to the upper and lower 95% confidence limits in the models instead of average Mantel-Haenszel[14] estimates. The upper limit represents a model with greater bioavailability (i.e., F at the upper limit) and slower elimination (i.e., k at the lower limit), whereas the lower limit represents a model with lesser bioavailability (i.e., F at the lower limit) and faster elimination (i.e., k at the upper limit) (Figure 5).

Results

Comparing CTC and OTC pharmacokinetics

The absolute bioavailability of CTC (13%) was significantly greater than that of OTC (4%) (P =.0001) (Table 4). Peak plasma concentration (C_{max}) of OTC was half of the C_{max} of CTC (Figure 6). The apparent distribution volumes ($V_{(area)}$) of CTC and OTC were also significantly lower for OTC than for CTC (P =.0014) (Table 4). The speed of intestinal uptake of the drugs, measured by k_a, was not significantly different (P =.9). On the other hand, CTC was eliminated

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Figure 5
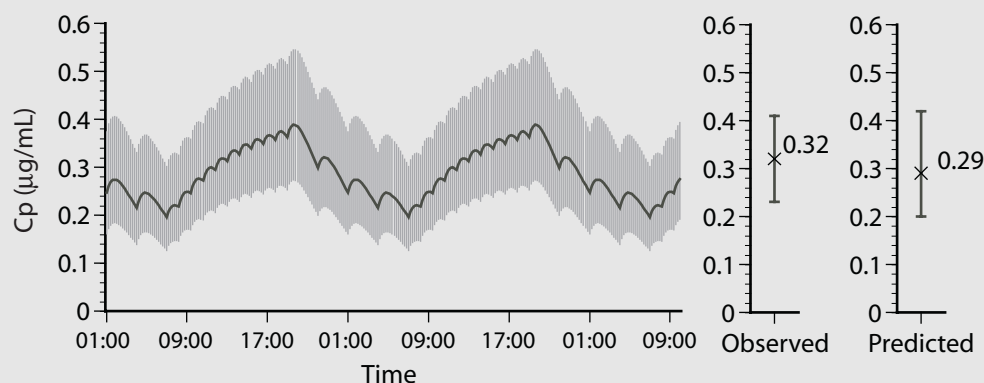
Observed (Andrews, et al.)[18] and predicted 95% confidence intervals of CTC plasma concentrations in pigs consuming a 220 PPM medicated feed ad libitum.


Table 4

Statistical comparison of mean pharmacokinetic parameters of CTC and OTC obtained with a Mantel-Haenszel meta-analysis

Parameter	Units	CTC	OTC	P
F (absolute bioavailability)	%	13.16 ±6.17	3.78 ±1.54	.0001
$V_{(area)}$ (apparent distribution volume)	L/kg	1.854 ±0.444	1.495 ±0.099	.001
k_a (absorption rate constant)	hours^{-1}	1.270 ±1.666	1.910 ±3.901	.9
k (elimination rate constant)	hours^{-1}	0.169 ±0.073	0.1277 ±0.048	.03
$t_{1/2}$ (elimination half-life)	hours	4.10	5.46	–
CL_b (systemic clearance)	L/hour/kg	0.314	0.194	–
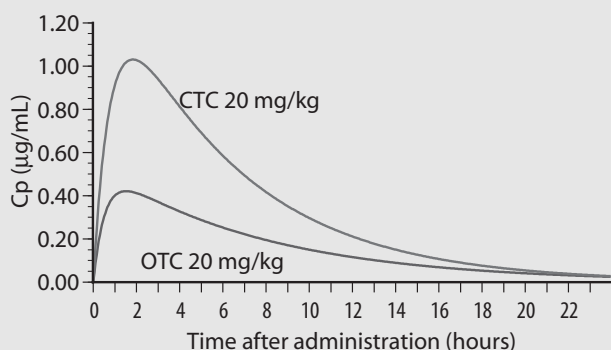
Simulated chlortetracycline (CTC) and oxytetracycline (OTC) plasma concentrations in pigs after administration of a 20 mg/kg bodyweight dose in feed

more quickly than OTC, as shown by the statistically significant difference in their respective k (P =.033). The elimination half-life ($t_{1/2}$) of CTC is thus 75% of OTC. Because CTC is absorbed more slowly and eliminated more quickly than OTC, the models predicted slightly greater daily variations in plasma concentrations of CTC compared to OTC (Figure 4).

Feeding behavior

Diurnal feeding patterns

Ad libitum feeding behavior of pigs induced a marked daily variation in both CTC and OTC plasma concentrations. In-feed dosages of both CTC and OTC differ between more-diurnal pigs and less-diurnal pigs when a targeted plasma drug concentration is required (Table 5). Because 40-kg (88-lb) pigs consume about 70% of their daily meals during the daytime,[8] the model should predict that the drug accumulated in the body during the day and was eliminated during the night. The model did predict that drug plasma concentrations were lowest by the beginning of the diurnal period (approximately 7:00 a.m.), and highest at its end (7:00 p.m.) (Figure 4).

Pig numbers in pen

The number of pigs in the pen (holding pen density constant) did not prove to be a significant factor in determining plasma drug concentration over time (data not shown).

Model validation

In general, our model generated predictions that were consistent with the literature (Figures 7–8). The model for OTC was in slightly better agreement with reported plasma concentrations (Figure 7) than was the model for CTC (Figure 8).

Summarizing equation

The simulations were used to devise an equation where the drug dosage to be given to pigs is calculated by the antibiotic concentration in plasma, the daily feed intake of pigs, as well as their feeding behavior, in the nonlinear equation:

$$D_{CTC} = 69 \times TPL - 35 \times e^{DFIR} + 18 \times e^{DDMR}$$

(Equation 5)

$$D_{OTC} = 141 \times TPL - 62 \times e^{DFIR} + 32 \times e^{DDMR}$$

(Equation 6)

Where:

- D_{CTC} and D_{OTC} are the dosage concentrations of OTC and CTC (in mg per kg bodyweight) to be administered in feed to pigs achieve the targeted plasma concentration *TPL* (in µg per mL),
- *DFIR* is daily feed intake of pigs, which is represented as a BW ratio (i.e., if the amount of feed ingested daily is equivalent to 4% of BW, then a value of 0.04 is given to *DFIR*), and
- *DDMR* is the ratio of daily meals eaten during the day by the pigs (i.e., if pigs eat nine of their 12 daily meals during the day, then *DDMR* has a value of 0.75).

Discussion

Model validation

The plasma concentrations of OTC and CTC predicted by our models were in close agreement with those reported by Asanuma, et al.,[17] Mevius, et al.,[23] and Hunneman, et al.,[26] strongly indicating that our model is valid. Moreover, the models made surprisingly accurate predictions for CTC and OTC plasma concentrations given by the 7-day use of medicated drinking water.[24] However, the latter findings are not adequate to support the application of the models to administration of medicated water.

As expected, these models were not able to predict plasma concentrations of OTC in fasted piglets dosed with a medicated drench.[22,23] Plasma OTC concentrations found with medicated drench administration to fasted[22,23] or fed[22] pigs were considerably higher than both concentrations predicted with the equations and those from their experiments using medicated feeds.[25,26] Intestinal absorption of OTC is reduced by the presence of food.[5]
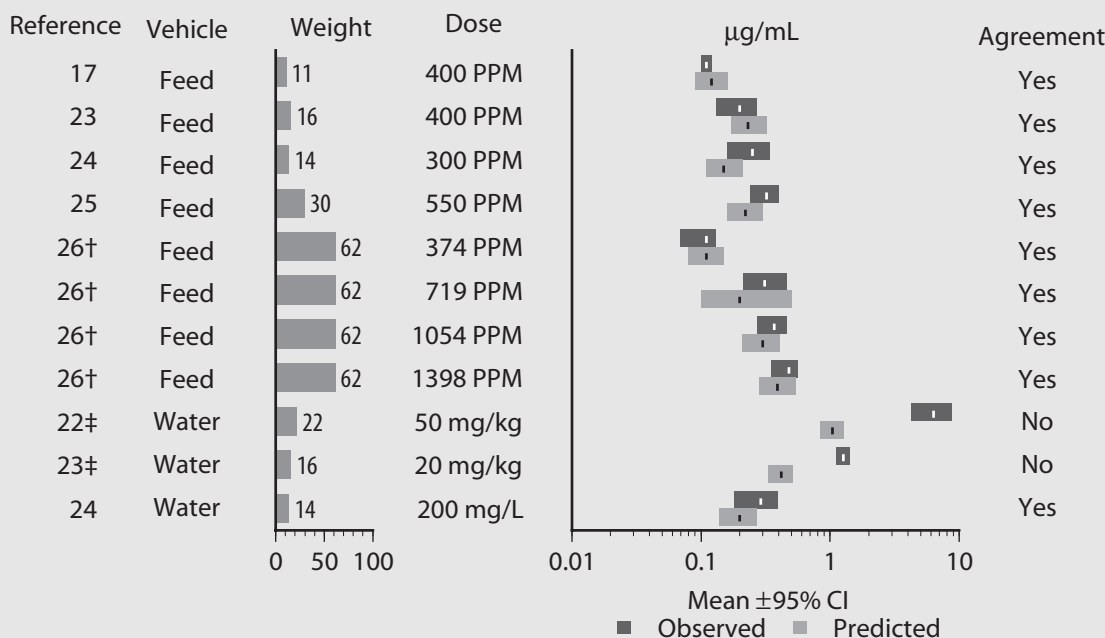
Our model for CTC was also in close agreement to observations reported by Andrews, et al.[18] in pigs that were challenged with *Pas-*

Dosages of CTC and OTC (in mg/kg bodyweight) to be given in feed to provide selected targeted plasma concentrations for 20 hours per day in pigs weighing 40 kg with a daily feed intake equivalent to 4% of their body weight, and distinct diurnal feeding behaviors

Drug type:	CTC		OTC	
% of meals eaten during the day:	65%	85%	65%	85%
Targeted plasma concentration (µg/mL)	Dose required (mg/kg bodyweight)			
0.10	5	11	11	21
0.25	15	21	32	42
0.50	33	38	67	77
0.75	50	55	103	112
1.00	67	73	138	148

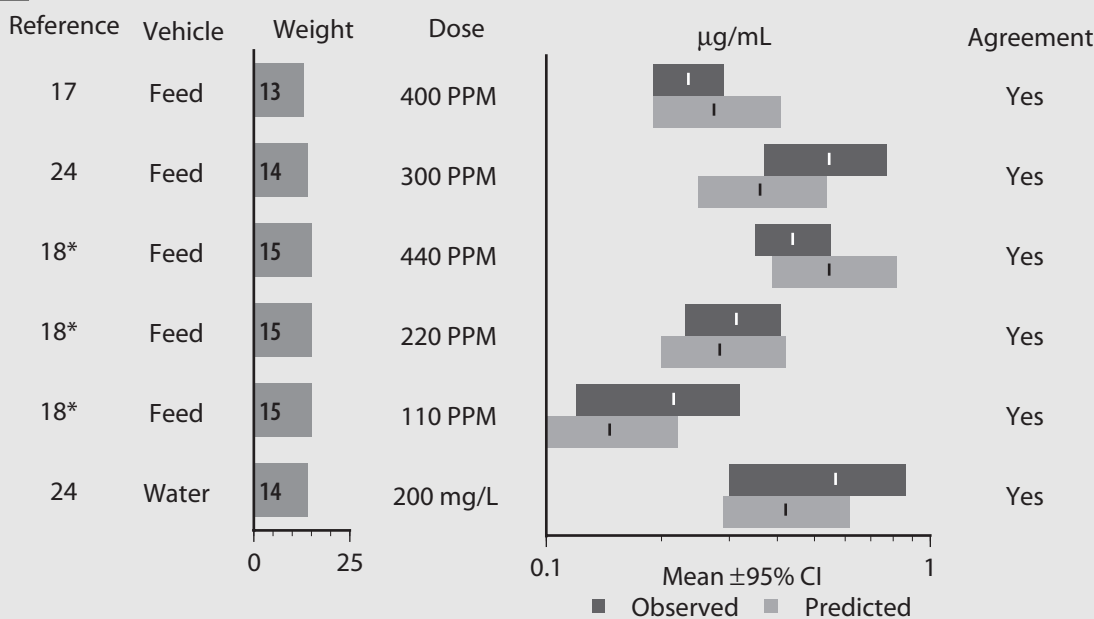| Reference	Vehicle	Weight	Dose	µg/mL	Agreement
17	Feed	11	400 PPM		Yes
23	Feed	16	400 PPM		Yes
24	Feed	14	300 PPM		Yes
25	Feed	30	550 PPM		Yes
26†	Feed	62	374 PPM		Yes
26†	Feed	62	719 PPM		Yes
26†	Feed	62	1054 PPM		Yes
26†	Feed	62	1398 PPM		Yes
22‡	Water	22	50 mg/kg		No
23‡	Water	16	20 mg/kg		No
24	Water	14	200 mg/L		Yes

Mean ±95% CI
■ Observed   ■ Predicted

Plasma concentrations of OTC (mean ± 95% CI) reported in the articles used for the validation procedure, predicted blood concentrations (mean ± 95% CI), and agreement between them

* Values in this study were graphically reported

† OTC blood levels reported here are from samples taken before *Actinobacillus pleuropneumoniae* experimental challenge.

‡ Values reported are the range of peak OTC serum concentrations obtained with single administration of a medicated drench. Comparison is made with predicted Cmax for the given dosage.

Figure 8

Reference	Vehicle	Weight	Dose	µg/mL	Agreement
17	Feed	13	400 PPM		Yes
24	Feed	14	300 PPM		Yes
18*	Feed	15	440 PPM		Yes
18*	Feed	15	220 PPM		Yes
18*	Feed	15	110 PPM		Yes
24	Water	14	200 mg/L		Yes

Mean ±95% CI
■ Observed   ■ Predicted

Plasma concentrations of CTC (mean ± 95% CI) reported in the articles used for the validation procedure, predicted blood concentrations (mean ± 95% CI), and agreement between them

* Pigs were experimentally infected with *Pasteurella multocida*, and CTC blood concentrations were graphically reported.

teurella multocida and then received CTC-medicated feed. This agreement is somewhat surprising, because the disease challenge is known to modify drug disposition in animals.[1]

The effect of OTC and CTC dosage on consumption of medicated feed by pigs has not been considered in the models. We do not know whether higher in-feed dosages of CTC or OTC would significantly change the palatability of medicated feeds offered to pigs, but Pijpers, et al.[27] reported that pigs offered feed with 2400 ppm of OTC showed similar appetites to those of pigs fed with a much lower dosage of OTC-medicated feeds. Also, the rate of feed intake of 10-kg pigs offered a
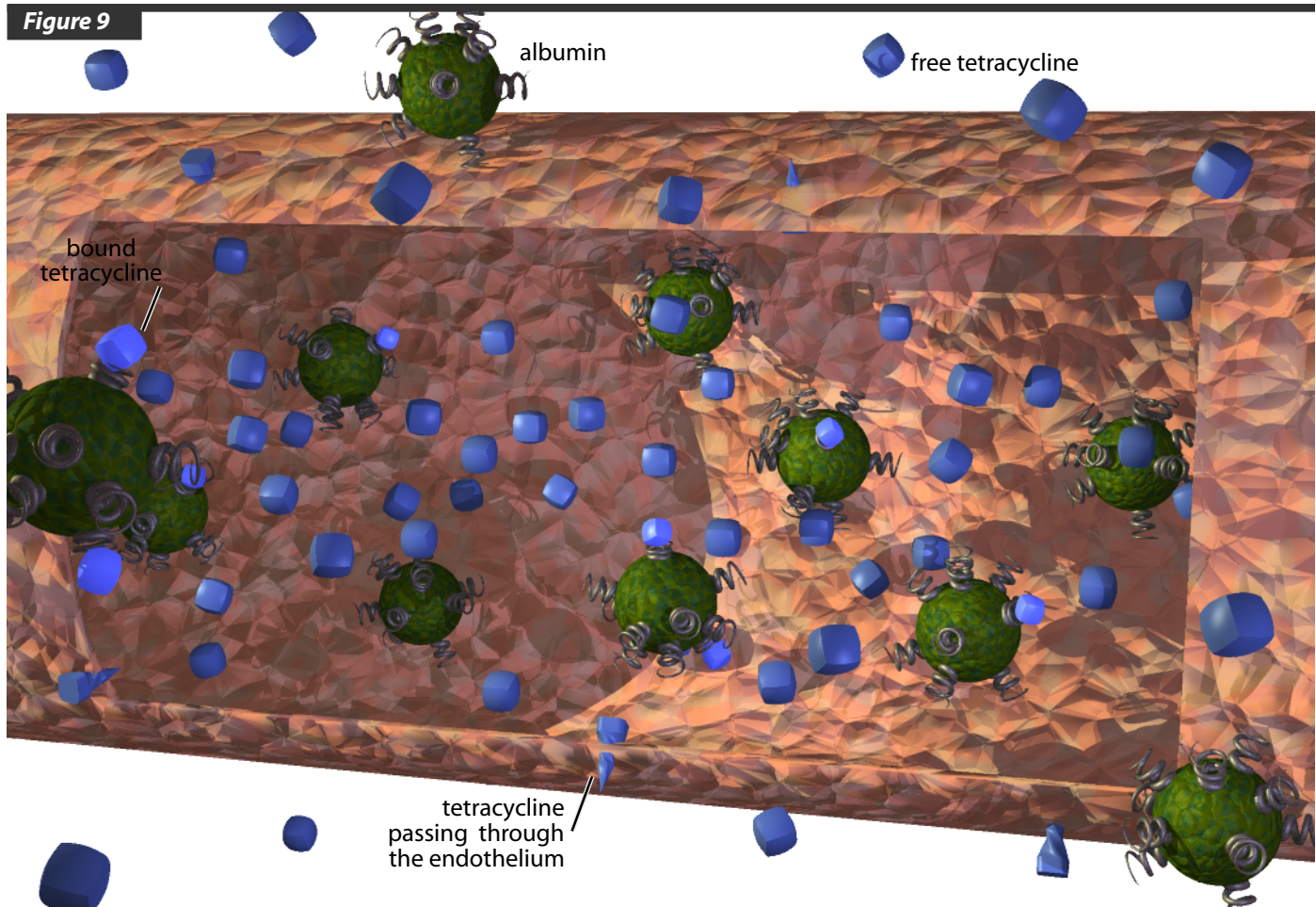
Illustration of binding of drug molecules to plasma and extravascular albumin at a given instant, and diffusion across endothelium in a small blood vessel.

diet fortified with 3600 ppm of CTC was identical to that of a drug-free diet.[28]

The diurnal pattern of drug plasma concentrations that our model predicted is consistent with the observation of fluctuations in tetracycline plasma concentrations in pigs offered medicated drinking water for ad libitum consumption.[29]

Labroue, et al.,[8] and Nielsen, et al.[10] used an electronic monitoring device placed on each individual pig that allowed them to monitor feeding frequency and individual feed intake in a nonintrusive way. In their studies, then, normal feeding behaviors were not altered by human interaction. Unfortunately, in the studies used for model validation,[17,18,22-26] feed intake of pigs was monitored by a stocksperson who entered the facility. This intrusive method of monitoring pig intake and plasma concentration is a potential confounder of pig feeding behavior. Factors related to feed characteristics (i.e., citric acid supplementation, calcium content, etc.) and pig feeding behaviors (i.e., diurnal feeding patterns, intrusive management) might be responsible for the differences between CTC and OTC plasma concentrations that were predicted by the models and those reported in some papers[24,25] used for the validation procedure.

Pharmacokinetics

The pharmacokinetics of both CTC and OTC are linear. The time-evolution concentration parameters are thus assumed to remain constant throughout a wide range of in-feed dosages. The superposition method we used is valid and the drug plasma concentrations it predicts are directly related to the administered dosage, which is consistent with reports in literature.[12,27] The better agreement between our model and the OTC literature than for the CTC literature may be due to the greater variation in bioavailability of CTC. The Mantel-Haenszel estimate of F for CTC may appear conservative, however, because two independent studies reported mean values near 17%. Large variance is associated with these F values.

Plasma concentrations are not necessarily equivalent to concentrations of drug in target tissue. Diffusion of antimicrobials to extra-vascular fluids is restricted to free molecules, whereas molecules transiently bound to proteins remain in plasma[30] (Figure 9). Plasma-protein binding rate is negatively related to drug distribution to lung and other tissues in a nonlinear way; only drugs possessing plasma protein binding rates over 85%-90% have significantly reduced free drug-plasma concentrations.[30] On the other hand, the rate of protein binding of tetracyclines is positively correlated to lipid solubility,[30] which enhances

tissue penetration. Consequently, the clinical significance of this balance between protein binding and lipid solubility is difficult to assess. As 20%–25% of less lipid-soluble OTC and 50%–70% of more lipid-soluble CTC are bound to plasma proteins,[24] similar free drug concentrations should be found with identical plasma concentrations,[31] and free CTC tissue penetration might be greater than that of OTC. Lung CTC and OTC concentrations reported by Asanuma, et al.,[17] were higher than plasma concentrations and the mean of tissue:plasma concentration ratios of CTC is about 30% greater than that of OTC. Nonetheless, these findings are affected by last-meal-to-slaughter intervals, which were not specified. Concentrations of CTC in lung reported by Andrews, et al.,[18] were also greater than plasma concentrations, but data represented in this article was from *P. multocida*-challenged pigs, and distribution kinetics could also be affected by this situation. The clinical significance of the comparison of CTC and OTC plasma and lung pharmacokinetic profiles at a given time is difficult to assess, because both functions are nonlinear and have different parameter values. In this case, however, it is less important than bioavailability; the dosage to be administered to pigs to obtain a given concentration of OTC in plasma is twice that of CTC (Figure 4). The greater lipid solubility of CTC is also likely to enhance diffusion to the site of infection.

Pharmacodynamics and antibiotic sensitivity testing

Tetracyclines achieve full antimicrobial activity when supraMIC concentrations are reached at the site of potential infection: the prevalence of signs and lesions in pigs experimentally infected with pathogenic *Actinobacillus pleuropneumoniae* decreased as the steady-state OTC plasma concentrations approached the MIC.[26] Andrews, et al.,[18] performed a similar study with *P. multocida*, but the assessment of a prophylactic effect of subMIC concentrations of CTC was not included.

It is important, then, that the MICs of CTC and OTC serve as the basis for the design of strategies that ensure optimal prophylactic efficacy. Although this information is reported for several swine respiratory pathogens, only semi-quantitative information (sensitive, intermediate,

Putting it into practice...

It is easy to calculate daily CTC and OTC feed dosages for metaphylaxis of swine respiratory diseases. Practitioners can predict plasma drug concentrations in pigs offered medicated feeds, according to daily feed intake and nycthemeral feeding behavior, and correlate this and the MIC for targeted bacteria to reduce the incidence or severity of clinical disease. These equations might be of special interest to practitioners in countries where antibiotic prescription is left to the practitioner's judgement.

Given the importance of the diurnal feeding behavior we observed in this study, we propose the following equations to calculate in-feed dosages of CTC and OTC dosages, from nursery to finishing phases:

$$D_{CTC} = 69 \times TPL - 35 \times e^{DFIR} + 18 \times e^{DDMR}$$

(Equation 5)

$$D_{OTC} = 141 \times TPL - 62 \times e^{DFIR} + 32 \times e^{DDMR}$$

(Equation 6)

Where:

- D_{CTC} and D_{OTC} are the dosage concentrations of OTC and CTC (in mg per kg BW) to be administered in feed to pigs in order to achieve the targeted plasma concentration *TPL* (in μg per mL).

- *DFIR* is daily feed intake of pigs, which is represented as a BW ratio (i.e., if the amount of feed daily ingested is equivalent to 4% of BW, then a value of 0.04 is given to *DFIR*).
- *DDMR* is the ratio of daily meals eaten during the day by the pigs (i.e., if pigs eat nine of their 12 daily meals during the day, then *DDMR* has a value of 0.75).

As shown by the equation coefficients, the CTC dosage level needed to achieve a given plasma target concentration is almost 50% of the OTC level. On the other hand, coefficients of DFIR and DDMR in the equation to determine CTC dosage concentrations are 55% of those in the equation for OTC. This difference in the ratio of coefficients is associated with the faster clearance of CTC, as shown by *k* (Table 4).

Hypothetical example

You find pathogenic *Pasteurella multocida* is increasing the severity of respiratory signs caused by porcine reproductive and respiratory syndrome virus (PRRSV) in the growing section of a finisher operation. Affected pigs are 40 kg when they start to show clinical signs. Daily feed intake is 4.5%. You expect pigs at this weight to eat 67.5% of their daily meals during daytime (Table 1). The MIC of both CTC and OTC for the targeted bacterium

is 0.25 mg per mL.

You would solve Equation 6 to determine the in-feed dosage for OTC:

$$D_{OTC} = 141 \times 0.25 \text{ mg/mL} - 62 \times e^{0.045} + 32 \times e^{0.675}$$

$$D_{OTC} = 35.25 - 64.85 + 62.85$$
$$D_{OTC} = 33.25 \text{ mg/kg BW/day}$$

And the required feed dosage for this hypothetical situation will thus be:

$$D_{OTC} \text{ (feed)} = 33.25 \div 0.045$$
$$D_{OTC} \text{ (feed)} = 739 \text{ ppm}$$

In the United States, this dosage exceeds the approved prescription dosage and is therefore not legal. You would then consider the alternative use of CTC, using Equation 5:

$$D_{CTC} = 69 \times 0.25 \text{ mg/mL} - 35 \times e^{0.045} + 18 \times e^{0.675}$$

$$D_{CTC} = 17.25 - 36.61 + 35.35$$
$$D_{CTC} = 15.99 \text{ mg/kg BW/day}$$

And the required in-feed dosage for this hypothetical situation will thus be:

$$D_{CTC} \text{ (feed)} = 15.99 \div 0.045$$
$$D_{CTC} \text{ (feed)} = 355 \text{ ppm}$$

This prescription dosage is legal in the United States.

The approximate dosages of OTC and CTC needed to achieve some specific concentrations in plasma are listed in Table 5.

or resistant) is typically given to veterinary practitioners. For human bacteria other than *Neisseria* spp., and *Haemophilus* spp.,[32] the sensitivity breakpoint of tetracycline is equivalent to an MIC of 8.0 mg per mL. Hence, strains with MIC ≤ 4 µg per mL are sensitive, and strains with MIC ≥ 32 µg per mL are resistant. Many veterinary diagnostic laboratories use this breakpoint for veterinary pathogens. In the case of *A. pleuropneumoniae*, some veterinary laboratories[33] use the breakpoint for human *Haemophilus* spp.,[32] which is equivalent to 4 µg per mL. Strains with MIC ≤ 2 µg per mL are susceptible, while strains with MIC ≥ 8 µg per mL are resistant to tetracycline. Drug concentrations equivalent to these two breakpoints are easily exceeded with parenterally administered OTC, but rarely achieved with medicated feeds. The use of this breakpoint in diagnostic laboratories, consequently, creates misleading information for swine practitioners.[34]

Many papers report the MICs of CTC and OTC for *Actinobacillus pleuropneumoniae*,[35-39] *Bordetella bronchiseptica*,[35,36] *Haemophilus parasuis*,[35,40] *Mycoplasma hyopneumoniae*[35,37,41-43] and *P. multocida*.[35-37,39] This list is restricted to articles that report the sample distribution of MICs. Differences noted among these reports may be associated with geographic variations, but may also be due to the MIC determination technique chosen.[42]

The 90th percentile of the MIC distribution (MIC_{90}) is often considered as a target to reach. The use of such a parameter by itself is unfair, since it is often derived from bacterial isolates whose collection is based on taxonomy, with little consideration of their pathological and epidemiological importance as a cause of respiratory disease. Nonvirulent strains, or virulent ones that are seldom implicated with disease in the field, could be added to the test sample. This could lead to the inclusion of several subgroups of strains with different concentrations of both antimicrobial susceptibility and clinical interest. The MIC_{90} can thus be biased by strains possessing extreme MIC values but that are epidemiologically irrelevant, while much lower concentrations could inhibit a majority of more prevalent pathogenic strains.[44]

Precise evaluation of MIC sample distribution is necessary to discriminate between more- or less-sensitive subgroups of bacterial isolates and identify outlying values. The geometric dilution procedures that are used to determine MICs produce an increasing error to the right side of sample distributions, where the MIC_{90} is always located. Therefore, the bias created by outliers is exacerbated by the dramatic imprecision associated with its value.[34,44,45]

Another source of bias is when practitioners submit samples for bacteriological analysis to diagnostic laboratories only in cases of metaphylactic failure. The proportion of unusually resistant strains in the bacterial collection eventually increases, and inference of the MIC_{90} to the population of field strains will no longer be valid. The MIC target should be representative of field strains commonly implicated in disease outbreaks. Veterinary practitioners should routinely send animals or tissue specimens to diagnostic laboratories to maintain the relevance of the MIC target. Additionally, developing techniques to predict in vivo MIC would be of major clinical interest.

It is critically important to consider the type of activity provided by the antimicrobial to obtain effective metaphylaxis. So far, tetracyclines have been observed to exert a time-dependent bactericidal activity.[2] In addition, subMIC concentrations of drug could reduce the growth rate of exposed bacteria, but without any decrease in bacterial population being induced. Nevertheless, this effect could help the immune system in controlling infectious pressure.

The post-antibiotic effect (PAE) of tetracyclines has only been studied on human enterobacteria, but the extrapolation to other Gram-negative rods, such as swine respiratory pathogens, seems to be acceptable.[46] *Escherichia coli* cultures exposed for 1 hour to supraMIC concentrations of tetracycline did not show any noticeable in vitro growth for over 3 hours after drug had been washed off.[47] This PAE has been reported with other protein synthesis inhibitors for *E. coli* and other Gram-negative bacilli as well.[48] Theoretically, if in-feed medication regimens can deliver supraMIC concentrations of CTC and OTC to the site of infection for some period of time, their effect would be maintained for at least 3 hours even after an instantaneous and complete elimination.[47] This instant elimination does not exist in animals; CTC and OTC elimination half-lives are > 4 hours in pigs (Table 4). SubMIC concentrations of drug are thus found for several hours before complete elimination, which may increase the length of PAE effect.[48] However, the length of in vivo PAE effect caused by CTC and OTC is not yet known. Consideration of PAE is thought to be of major importance for the design of more effective treatment strategies. For example, schedules that give transient subMIC plasma concentrations of drug could be tolerated for antimicrobials possessing a PAE.[49] The computerized iterative procedure we used in our study took into consideration a 4-hour-long postantibiotic phase, which allowed short-term subMIC plasma concentrations of CTC and OTC.

Post-antibiotic leukocyte enhancement effect (PALE) has not been taken into account in our model.[46] After exposure to antibiotics, enhanced phagocytosis and intracellular killing of bacteria is noted during the antibiotic-free phase. Modifications in the bacterial cell surface caused by the drug may cause this phenomenon.[48] The in vivo extent of PALE, which has been noted in vitro, is unknown but likely plays a major role in the outcome of metaphylaxis with antimicrobials. The addition of subMIC concentrations of some antibiotics has also been reported to induce a PALE effect.[49] The PALE effect of the tetracyclines has not been studied to our knowledge, but is likely to exist as suggested by the sometimes successful results of metaphylaxis with low drug in-feed dosage concentrations.

Our observation that the number of pigs in the pen didn't affect plasma concentrations of OTC and CTC could be predicted by Nielsen's[10] observation that pigs housed in larger groups (with pen density kept constant) ate less frequently, but ate more at each meal. Thus, the frequency of feeding would have an effect on dosage and dosage interval, but not an overall effect on drug concentrations in plasma. In our simulations, we kept pen density constant and altered only the number of pigs in the pen. Our result therefore cannot be safely generalized to situations in which pig density has been increased beyond standard commercial guidelines.

Implications

- In-feed dosage concentrations currently used are related neither to targeted pathogens nor to populations at risk, and often provide much lower antibiotic concentrations to pigs than the MIC alone indicates. Consequently, the outcome of metaphylaxis is somewhat unpredictable.
- Effective metaphylaxis in the whole pig herd should be achieved by using the drug MIC for the targeted pathogen. This information allows practitioners to customize therapeutic strategies to what is really needed. Diagnostic laboratories could provide a restricted MIC estimate by the use of linear series of dilutions (Table 5) or at least use sensitivity breakpoints that could be obtained with oral administration of realistic antibiotic dosages.
- The computerized iteration procedure used in this study was the first multiple-dosage pharmacokinetic model of in-feed tetracyclines that took into effect actual ad libitum feeding behavior of pigs.
- The outcome of metaphylaxis could be affected by age, daily feed intake, day/night variations in feeding behavior, and pen density, all of which may produce variations in plasma drug concentrations. Improving our knowledge of these sources of variation might be helpful in the design of better medication strategies. The equations proposed here can be helpful in designing metaphylactic strategies by accounting for some of these factors.
- CTC plasma and lung concentrations obtained in our models with ad libitum intake of medicated feed were at least twice that achieved with OTC. The pharmacokinetic response for CTC and OTC has been observed in a number of studies to differ significantly. Therefore, it is likely that there can be an economic benefit to the proper antibiotic choice when designing a metaphylactic strategy in intensive swine production.

Acknowledgments

Glossary

ABSORPTION: The process by which unchanged drug proceeds from the site of administration to the site of measurement within the body (e.g., blood). Some of the drug may remain in the administration site (i.e., adsorbed by feed particles; or in insoluble salts when injected intramuscularly or subcutaneously) or may be metabolized during transit between the administration site and the measurement site (i.e., destroyed by the liver or other tissues that possess the appropriate enzymes). Drug absorption is described with two pharmacokinetic parameters: **BIOAVAILABILITY** and **ABSORPTION RATE CONSTANT**.

ABSORPTION RATE CONSTANT: The rate at which the drug is transferred from the administration site to the site of measurement. In other words, it is the proportion of the dose that proceeds to blood per unit of time. That is, $k_a = 0.75$ h^{-1} means that 75% of the dose remaining in the administration site is absorbed per hour of time.

BIOAVAILABILITY: The rate and extent of drug absorption. In other words, it is the fraction of the dose that will reach the site of measurement after extravascular administration, compared to a standard route. When the standard route is an intravascular administration, the result is called **ABSOLUTE BIOAVAILABILITY.** When the standard route is also extravascular, the result is called **RELATIVE BIOAVAILABILITY.**

BIOEQUIVALENCE: When two different products possess equal pharmacokinetic properties, so they can be used interchangeably. Tetracyclines are erroneously considered to be " bioequivalent ," and many practitioners switch from one to another according to economic criteria.

CLEARANCE: The loss of drug by the blood when passing across an organ of elimination. It is also defined as the volume of a body fluid (e.g., plasma) that is totally purified of drug molecules per unit of time.

DISTRIBUTION: The process of reversible transfer of a drug to and from the measurement site. Once in blood, the drug proceeds to tissues so an equilibrium between blood and tissues is achieved. Distribution is affected by the blood supply to the tissue, the ability of the drug to cross tissue membranes, binding of the drug within blood and tissues, and partitioning into fat. **DISTRIBUTION VOLUME** is a pharmacokinetic parameter describing the distribution of drug within the body.

ELIMINATION: The irreversible loss of drug from the site of measurement. Some drugs will be metabolized when passing through the liver or some other tissue that has the required set of enzymes. Others will be directly eliminated when passing through the kidney or other excretion tissues. **CLEARANCE, ELIMINATION RATE CONSTANT**, and **ELIMINATION HALF-LIFE** are pharmacokinetic parameters describing the elimination of drugs.

ELIMINATION RATE CONSTANT: The rate at which a drug disappears from the site of measurement. In other words, it is the proportion of the dose that proceeds outside the blood per unit of time (e.g., $k = 0.13$ h^{-1} means that 13% of the dose remaining in the site of measurement is eliminated per hour of time).

ELIMINATION HALF-LIFE: The amount of time required to eliminate half of the amount of drug remaining in the body.

EXCRETION: The irreversible loss of chemically unchanged drug (through target or nontarget tissues. This is one type of elimination.

KINETICS OF BACTERICIDAL ACTIVITY: Drugs are historically divided into bactericidals and bacteriostatics. This classification does not take into account the effect of drug concentration over time. When studying the effect of antibiotics on bacteria over time, it is apparent that drugs behave in two different ways that are not reflected by the old classificatory system. With drugs possessing concentration-dependent bactericidal activity (Figure 2), the number of bacterial cells that die

is direct function of how much the drug concentration exceeds the MIC (e.g., the more drug you add, the more cells die and the faster they die). Fluoroquinolones and some aminoglycosides, as well as metronidazole with anaerobic bacteria, show this type of bactericidal activity. With drugs possessing time-dependent bactericidal activity, the number of cells that die reaches a maximum (e.g., no further addition of drug will increase bacterial death or death rate). Cephalosporins, macrolides, and other bacteriostatic protein inhibitors show this type of activity. Many antibiotics exhibit either types of bactericidal activity, depending on the Gram type of the test bacteria (e.g., concentration-dependent with Gram-positive organisms and time-dependent with Gram-negative organisms).

LINEAR PHARMACOKINETICS: When pharmacokinetic properties of a drug remain constant throughout a wide range of dosages. Concentrations at the site of measurement are always directly related to dose, both for single and multiple doses.

METABOLISM: The conversion of one chemical species to another. Metabolism may have an effect on the biological activity of a drug (i.e., metabolism can decrease, increase, or have no effect on the activity of drugs). There are minor reactions (known as Phase I metabolism) and major reactions (Phase II metabolism) that increase the ability of the body to eliminate the drug. Enzymes such as the cytochrome P450 isoenzymes are responsible for the metabolism of drugs.

PHARMACOKINETICS: the study of the evolution of drug absorption and disposition by the living body over time and the use of mathematics to describe and predict the whole process. Drug disposition is characterized by two events that occur simultaneously: **DISTRIBUTION** through the body and **ELIMINATION** by means of **METABOLISM** or **EXCRETION**.

PHARMACODYNAMICS: The study of the relationship between drug concentrations at the site of action and drug effects, factors influencing this relationship, and the use of mathematics for the description of the process. Models that can both describe the observations and offer some insight into the underlying biological process are usually preferred. Determining the **POST-ANTIBIOTIC EFFECT**, the **POST-ANTIBIOTIC LEUKOCYTE ENHANCEMENT EFFECT**, and the **KINETICS OF BACTERICIDAL ACTIVITY** are different ways to study the pharmacodynamics of antimicrobial drugs. The minimum inhibitory concentration, the minimum bactericidal concentration, and antimicrobial sensitivity testing do not provide any information on the time course of antimicrobial activity or the persistent effects of antimicrobial agents, and thus are not considered to be pharmacodynamic parameters.

POST-ANTIBIOTIC EFFECT: The persistent suppression of bacterial growth after exposure to an antimicrobial. It can be conceived of as the time it takes for an organism to recover from the effects of exposure to an antimicrobial. The post-antibiotic effect has been observed both under in vitro and in vivo conditions. The span of the post-antibiotic effect is affected both by the concentration and the duration of exposure of bacteria to drug. SubMIC concentrations of antibiotics are known to slow growth, produce morphological changes, and prolong the duration of the in vivo post-antibiotic effect produced by supraMIC concentrations.

POST-ANTIBIOTIC LEUKOCYTE ENHANCEMENT EFFECT: The increase in susceptibilty of bacteria in the post-antibiotic phase to phagocytosis or intracellular killing by leukocytes. The inhibition of bacterial repair mechanisms or antiphagocytic substance production by antibiotics is thought to be the cause of this phenomenon.

PRINCIPLE OF SUPERPOSITION: For drugs that possess linear pharmacokinetics, all kinetic profiles corrected for the administered dose can always be superimposed—i.e., added to the concentration of the drug already available in plasma at the time of the next administration. When kinetic profiles fail to superimpose, the drug possesses nonlinear pharmacokinetics for either dose (e.g., saturation of transport across membranes, protein binding, etc) or time (e.g., induction/inhibition of metabolism).

VOLUME OF DISTRIBUTION: The apparent volume in which a drug distributes in the body at equilibrium of drug between blood and tissue. It is a direct measure of the extent of distribution, but it rarely corresponds to a real volume (e.g., plasma; extracellular water; total body water).

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