Relative oral bioavailability of two amoxicillin – clavulanic acid formulations in healthy dogs: a pilot study

Jonathan P. Mochel, Iowa State University

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Relative Oral Bioavailability of Two Amoxicillin–Clavulanic Acid Formulations in Healthy Dogs: A Pilot Study

Jennifer Moczarnik, DVM, Darren J. Berger, DVM, DACVD, James O. Noxon, DVM, DACVIM, Dana N. LeVine, DVM, PhD, DACVIM, Zhoumeng Lin, BMed, PhD, DABT, Johann F. Coetzee, BVSc, Cert CHP, PhD, DACVCP, DACAW, DECAWBM, Jonathan P. Mochel, DVM, MSc, PhD, DECVPT

ABSTRACT

The use of human generic amoxicillin–clavulanic acid formulations in veterinary medicine is currently lacking supportive evidence. This pilot study was conducted to determine preliminary pharmacokinetic parameters and relative oral bioavailability of a human generic and veterinary proprietary 4:1 amoxicillin–clavulanic acid formulation in healthy dogs to evaluate whether drug exposure was similar and to determine if further comparative investigation is warranted. Each dog received a single oral dose of each formulation containing 500:125 mg of amoxicillin–clavulanic acid at two separate instances with a 2 wk washout period between product administration. Following drug administration, blood was collected at fixed times over 24 hr to measure plasma amoxicillin and clavulanic acid concentrations using liquid chromatography–mass spectrometry. There were no statistically significant differences between pharmacokinetic parameters of either formulation. Clavulanic acid showed greater between-dog variation in drug exposure between formulations compared with amoxicillin and was also observed to be more variable within the veterinary proprietary formulation. The average relative oral bioavailability was 98.2% (23.6% coefficient of variation) for amoxicillin and 152.6% (64.3% coefficient of variation) for clavulanic acid between formulations. This pilot investigation supports the need for further bioequivalence studies regarding these formulations before commenting on product interchangeability. (J Am Anim Hosp Assoc 2019; 55:14–22. DOI 10.5326/JAAHA-MS-6872)

Introduction

Oral amoxicillin (AMX)–clavulanic acid (CA) formulations exist in both the human and veterinary market in the United States, with a readily available 4:1 AMX–CA veterinary proprietary formulation licensed for use in dogs and cats, and many 2:1–7:1 AMX–CA proprietary and generic formulations are licensed for use in humans. The veterinary proprietary AMX–CA formulation is listed as a first-tier systemic antimicrobial agent for the treatment of pyoderma in addition to being labeled for the treatment of urinary tract infections in both dogs and cats and periodontal disease in dogs. AMX is a time-dependent, bactericidal beta-lactam amnopenicillin that targets gram-positive and some gram-negative organisms, excluding beta lactamase–producing pathogens. CA is used in conjunction with AMX to expand the spectrum of activity via beta lactamase inhibition.

Despite the availability of a veterinary proprietary AMX–CA formulation, veterinary referral cases continually present to the authors’ institutions with historical use of human generic AMX–CA formulations.

From the Lloyd Veterinary Medical Center (J.M.), Department of Veterinary Clinical Sciences (D.J.B., J.O.N., D.N.L.), and Department of Biomedical Sciences (J.P.M.), College of Veterinary Medicine, Iowa State University, Ames, Iowa; and Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas (Z.L., J.F.C.).

Correspondence: djberger@iastate.edu (D.J.B.)
formulations of varying ratios for the treatment of various conditions including canine pyoderma. To date, there are no published studies evaluating the oral bioavailability of a human generic AMX–CA formulation relative to the veterinary proprietary formulation in dogs. Therefore, the objectives of this pilot study were to (1) determine if similarities in pharmacokinetic (PK) parameters and relative oral bioavailability (ROB) were present, which would warrant further investigation with a larger study population, and (2) generate preliminary data for both formulations in non-laboratory-purpose–bred healthy adult dogs. This preliminary data provides insight into the expected differences and variability in drug exposure between each formulation in a clinically relevant population. It also allows for appropriate evidence-based size calculations for the design of formal relative bioavailability, bioequivalence, and pharmacodynamic studies to conclude on formulation interchangeability in dogs.6

Materials and Methods
The procedures performed in this study were approved by the Institutional Animal Care and Use Committee (log #4-16-8253-K) at Iowa State University. Informed consent was obtained from the owners of all dogs prior to study enrollment.

Animals and Housing
Six volunteered veterinary staff- and student-owned adult dogs of any breed or sex were considered for study inclusion. In the absence of information on between-dog variability in PK for the human generic AMX–CA formulation, sample size was selected based on similar, previously published prospective PK studies.7–9 Inclusion criteria were as follows: (1) dogs were to be considered in good health based on reported history and physical examination; (2) weights between 35 and 50 kg for ease of whole tablet drug administration; (3) no evidence of systemic disease based on laboratory diagnostics including complete blood cell count, serum chemistries, urinalysis, thyroid screening, heartworm test, and fecal flotation; (4) no recent medication administration within the past month other than routine heartworm, flea, and tick prevention; and (5) no known adverse reactions to AMX–CA. Prior to study inclusion, all owners provided informed written consent after reviewing printed information outlining study design, which included (1) the purpose of the study, (2) drug administration details, including details on each AMX–CA formulation and the need for drug administration to occur at two separate instances, (3) a list of all potential adverse reactions related to drug administration from available human and veterinary literature, (4) the measures that would be taken should any adverse events occur, and (5) the ability to withdraw their dog at any time from this voluntary study.

Experimental Protocol
A single-dose, randomized crossover study design was employed for this pilot study. Dogs were assigned to receive two different 4:1 AMX–CA formulations in a sequential order between two periods according to a computer-generated random sequence established prior to the study. The two 4:1 AMX–CA formulations administered included (1) the veterinary proprietary formulation and (2) the human generic formulationb. Each dog received 500 mg AMX and 125 mg CA at each period regardless of formulation. Dosage of each drug formulation was based on previously published AMX dosages of 11–15 mg/kg.1 A minimum 2 wk wash-out period was upheld between study periods to assure sufficient elimination of either formulation based on the known half-lives of both AMX and CA and to prevent adverse hemodynamic effects by allowing recovery time following serial blood collection.10,11

Dogs were fasted for 12 hr prior to drug administration at each period. On the day of dosing per period, physical examination and aseptic intravenous cephalic catheterplacement was performed for each dog. The following parameters were monitored hourly for the first 6 hr, then every 2 hr thereafter throughout the first 12 hr hospitalized period, and again at t = 24 hr when the dog returned for the last venipuncture: vital parameters (heart rate, respiratory rate and effort, capillary refill time and mucous membrane character, temperature); attitude, anxiety level, mentation; and possible adverse effects such as gastrointestinal upset.

Drug Administration
For each study period, a single oral dose of either the veterinary proprietary (375 and 250 mg tablet concurrently) or human generic (625 mg tablet) formulation was administered at t = 0 min alongside a commercially available maintenance dog foodd based on individualized maintenance calorie requirements. If a dog did not complete their meal voluntarily within 10 min of medication administration, gruel feeding (60 mL) was performed to ensure consistent food ingestion between any dogs requiring syringe feeding.12 This was performed to mimic clinical scenarios in which patients are recommended to receive a meal alongside medication to reduce gastrointestinal upset.

Sample Collection and Processing
All venous blood samples were 3 mL in volume per collection, assuring cumulative blood volumes were within published guidelines to avoid adverse hemodynamic effects.11 Serial blood samples were collected directly from the catheter using a needle holder, blood collection needle, and 3.0 mL plastic lithium heparin tube at t = 0, 20, 40, 60, and 90 min and at 2, 4, 6, 8, 10, and 12 hr. Heparinized saline flushes were performed following each blood sample collection.
via catheter to ensure patency. To assure sample dilution did not occur secondary to routine catheter flushing, a standard 1 mL sample was obtained via catheter and discarded prior to each time point blood sample collection. Catheters were removed following blood collection at t = 12 hr to allow each dog to return home, reducing the stress of an overnight hospital stay. Each dog then returned at t = 24 hr for final blood collection via venipuncture using a jugular or a noncatherized cephalic vein. All blood samples were immediately centrifuged for 15 min × 1000g at 25°C. All individual plasma samples were harvested in sterile vials and stored at −80°C until analysis. Analyses were performed within 3 mo of sample collection.

Sample Processing and Analysis
Plasma concentrations of AMX and CA were determined using high-performance liquid chromatography (LC)\(^1\) with mass spectrometry (MS)\(^1\) detection. LC-MS is the standard way of measuring the concentration of AMX and CA within biological fluids.\(^{13}\) Plasma samples, plasma spikes, plasma quality controls (QC), and canine plasma blanks, 200 μL, were mixed with 800 μL of acetonitrile to precipitate plasma proteins. The acetonitrile contained amoxicillin-d\(_4\) as an internal standard at a concentration of 2000 ng/mL. The samples were vortexed for 5 s and centrifuged for 20 min at 7500 rpm to sediment the protein pellet. The supernatant was poured off into dry down tubes and evaporated at 50°C with a flow of nitrogen in a solvent evaporator\(^1\). The pellets were reconstituted with 125 μL of water. The samples were transferred to autosampler vials fitted with a glass insert and centrifuged at 2000xg prior to analysis.

For LC-tandem MS analysis, the injection volume was set to 25 μL. The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at a flow rate of 0.25 mL/min. The mobile phase began at 10% B, held for 1 min with a linear gradient to 75% B in 8.0 min, which was maintained for 2 min, followed by re-equilibration to 10% B. Separation was achieved with a column\(^1\) maintained at 45°C. AMX, amoxicillin-d\(_4\), and CA were all eluted at 3.0 min. The MS was operated in negative ion detection mode with three product ions for each analyte. The multiple reaction monitoring ions for AMX were m/z 93, 206, and 320, whereas the ions for amoxicillin-d\(_4\) were m/z 97, 210, and 324. The product ions for CA were m/z 82, 108, and 136.

Sequences consisting of plasma blanks, calibration spikes, QCs, and canine plasma samples were batch processed with use of a software program\(^1\). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) linear fit. Plasma concentrations of AMX and CA in unknown samples were calculated by this software program based on the calibration curve. Results were then viewed in the Quan Browser portion of this software program. Twelve calibration spikes were prepared in blank canine plasma covering the concentration range of 10–50,000 ng/mL. Calibration curves for AMX exhibited a correlation coefficient exceeding 0.995 across the usable concentration range. The highest calibrator at 50 ng/mL was excluded from the calibration fit as the high concentrations of analytes resulted in ion suppression of the internal standard. Three QC samples were performed in quadruplicate for both AMX and CA at the following values: low QC (150 ng/mL), mid QC (1500 ng/mL), and high QC (15,000 ng/mL). There were 24 total QC samples run, 12 for AMX and 12 for CA. The QC samples were almost all within ±5% of the nominal value. Three of the QC samples for AMX were out of the ±5% nominal value by the following values: one low-QC sample by 0.9%, one mid-QC sample by 5.6%, and one high-QC sample by 3.1%. Six of the 12 QC samples for CA were out of the ±5% nominal value by the following values: 1 low-QC sample by 3.3%; 3 mid-QC samples by 6.2, 9.3, and 27.2%; and 3 high-QC samples by 6.1, 13.5, and 13.6%. Calibration curves for CA exhibited a correlation coefficient exceeding 0.99 across the usable concentration range. QC samples at 150, 1050, and 16,000 ng/mL were within ±15% of the nominal value with a few exceptions.

The limit of quantification (LOQ) of the AMX analysis was 10 ng/mL with a limit of detection (LOD) of 3 ng/mL. The LOQ of the CA analysis was 30 ng/mL with a LOD of 10 ng/mL.

Pharmacokinetic Analysis
Plasma concentrations of AMX and CA were analyzed using a noncompartmental approach\(^1\). The following variables were calculated for both AMX and CA: elimination rate constant (λz), elimination half-life (T\(_{1/2z}\)), time to observed maximum plasma concentration after drug administration (T\(_{\text{max}}\)), observed maximum plasma concentration (C\(_{\text{max}}\)), area under the plasma concentration versus time curve to the last measurable value (AUC\(_{\text{last}}\)), area under the plasma concentration versus time curve extrapolated to infinity (AUC\(_{\text{inf}}\)), and percentage of AUC\(_{\text{inf}}\) as a result of extrapolation from last measurable time to infinity (AUC\(_{\%}\) Extrap).

Individual values for each of these PK variables between the two drug formulations were compared with Student t test using standard statistical software\(^1\). Comparisons were then made between the two study groups, obtaining the geometric mean values and ranges for the above PK parameters. Because PK parameters tend to be log-normally distributed, statistical comparisons were conducted based on log-transformed values of the parameters, and the geometric mean and geometric coefficient of variation (CV) were calculated. A P value of <.05 was considered statistically significant. Because the
amount of AMX and CA provided by the two formulations was identical, the relative oral bioavailability for each ingredient could be calculated as follows: \[ \frac{AUC_{\text{inf}} (\text{human generic})}{AUC_{\text{inf}} (\text{veterinary proprietary})} \times 100. \]

**Results**

**Patient Population and Safety**

All six dogs completed the study successfully, and no adverse events were observed following drug administration of either formulation. All dogs ate freely and completed their meals within 10 min after medication administration. The study population was made up of four mixed-breed dogs and two purebred dogs (Labrador retriever and American Staffordshire terrier, one each). There were four castrated male dogs and two spayed female dogs. The weight ranges within this study population varied from 37.2 to 50.5 kg, with an average weight of 41.3 kg. Ages ranged from 2.8 to 5.3 yr, with an average age of 3.67 yr. The mean dosage of AMX–CA was 15.3 mg/kg (range 14.3–16.8 mg/kg), with AMX reaching a mean dosage of 12.3 mg/kg (range 10.0–13.4 mg/kg) and CA reaching a mean dosage of 3.1 mg/kg (range 2.47–3.36 mg/kg).

**Pharmacokinetic Analysis**

There was no statistically significant difference for any of the PK parameters between the two formulations based on Student t test. PK parameters for each drug formulation based on active ingredient, AMX (Table 1) and CA (Table 2), and individual plasma drug concentration versus time curves for each dog (Figure 1, 2) have been summarized and displayed accordingly.

The time courses of AMX and CA agreed between both formulations and showed a monoeponential decline. Most of the samples were below the LOD (10 ng/mL) and LOQ (30 ng/mL) after 6 hr for CA for both drug formulations. Between the 8 and 24 hr time points, only two dogs (dog 1, human generic formulation; dog 2, veterinary proprietary formulation) showed measurable plasma concentrations at \( t = 8 \) hr, representing 4.2% of all 48 samples collected at 8, 10, 12, and 24 hr timepoints between both formulations.

**TABLE 1**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dog</th>
<th>( \lambda_z ), 1/hr</th>
<th>( T_{1/2\lambda_z} ), hr</th>
<th>( T_{\text{max}} ), hr</th>
<th>( C_{\text{max}} ), ng/mL</th>
<th>AUC_{\text{inf}}, \text{ng/mL} \times \text{hr}</th>
<th>AUC_{\text{last}}, \text{ng/mL} \times \text{hr}</th>
<th>AUC_{% \text{Extrap}}, %</th>
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<td>Veterinary proprietary*</td>
<td>1</td>
<td>0.35</td>
<td>2.00</td>
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<td>10,893</td>
<td>36687</td>
<td>37,193</td>
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<td></td>
<td>2</td>
<td>0.27</td>
<td>2.58</td>
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<td>2999</td>
<td>38080</td>
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<td>Human generic†</td>
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<td>0.24</td>
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<td>11,943</td>
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<td>13.18</td>
<td>13.18</td>
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<tr>
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<tr>
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<td>15.33</td>
<td>57.91</td>
<td>30.41</td>
<td>24.22</td>
<td>23.97</td>
<td>308.76</td>
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*P value*: .563 .568 .937 .911 .818 .818 .699

*Clavamox; Zoetis Inc., Kalamazoo, MI.*
†Amoxicillin-clavulanic acid 500 mg/125 mg tablets; Aurobinda Pharma Ltd., Hyderabad, India.

*P values of Student t test of the difference of each parameter between the two drug formulations.

\( \lambda_z \), elimination rate constant; AUC_{\% \text{Extrap}}, percentage of AUC_{\text{inf}} as a result of extrapolation from last measurable time to infinity; AUC_{\text{inf}}, area under the plasma concentration versus time curve extrapolated to infinity; AUC_{\text{last}}, area under the plasma concentration versus time curve to the last measurable value; \( C_{\text{max}} \), observed maximum plasma concentration; CV, coefficient of variation; \( T_{1/2\lambda_z} \), elimination half-life; \( T_{\text{max}} \), time to observed maximum plasma concentration after drug administration.
In contrast, only a fraction of plasma concentrations fell below the LOQ (10 ng/mL) and LOD (3 ng/mL) at 24 hr for AMX between both formulations as follows: 3 out of 12 samples (25%) yielded concentrations between the LOQ and LOD (dog 1, veterinary proprietary formulation; dogs 3 and 4, human generic formulation), whereas only 2 out of 12 samples (16.7%) yielded no measurable concentrations (dog 6, both formulations).

The average ROB of the human generic formulation to that of the veterinary proprietary formulation was 98.2% for AMX (23.6% CV) and 152.6% for CA (64.3% CV), with each active ingredient approaching or exceeding the ideal ROB of 100%.

**Discussion**

Veterinary prescribing behaviors, as observed from the authors’ referral population, have demonstrated the ongoing use of various human generic AMX–CA formulations and ratios in canine patients. Its use has created both an interest and need in investigating preliminary PK and ROB parameters, specifically between 4:1 human generic AMX–CA formulations to match the ratio of AMX–CA seen with that of the veterinary proprietary formulation. It is hypothesized that generic AMX–CA substitution may occur for a variety of reasons, including drug cost, accessibility, and convenience. For example, our study included large- to giant-breed dogs weighing between 35 and 50 kg, a population in which the veterinary proprietary formulation may be cost prohibitive, ultimately deterring veterinarians from prescribing this formulation. There have been no previous studies assessing the PK and ROB of a 4:1 human generic AMX–CA formulation relative to the veterinary proprietary formulation in dogs. This preliminary investigation is the first to evaluate human generic AMX–CA use in dogs within a clinically relevant scenario and yields results that prove a definite need to pursue further comparative PK, bioavailability, and bioequivalence studies before commenting on 4:1 human generic AMX–CA substitution in veterinary medicine.

We have shown that the drug exposure of AMX and CA from the human generic formulation overlapped with that of the veterinary proprietary formulation.
proprietary formulation, despite inter-dog variability, for both formulations and more so for CA compared with AMX. This study echoes previous findings in which CA demonstrated greater variations in plasma concentrations between patients. This variability in CA was previously established by Vree et al. in two separate studies, one in healthy humans and one in healthy dogs, showing AUC variations up to fivefold for CA when assessing differing dosages and formulations of AMX–CA.\textsuperscript{10,14} The average ROB for AMX indicates comparable drug exposure between the two formulations, whereas the ROB for CA displays more of a discordance between the two formulations. However, on an important note, one dog showed lower exposure to both CA and AMX when treated with the human generic formulation as compared with the veterinary proprietary formulation.

Within human medicine, generic medications can be substituted for innovator products after meeting similar pharmacokinetic and bioequivalence parameters.\textsuperscript{15} However, when human generics are substituted for veterinary products, these same assumptions do not hold true. Therefore, formal and properly powered studies are required before advocating interchangeability between 4:1 human generic and veterinary proprietary formulations. Interchangeability can only be demonstrated through bioequivalence studies (well-defined, controlled, and outlined studies.

**FIGURE 1** Individual summary time-courses for AMX and CA for dogs 1–3 and for each drug formulation, assessing the area under the plasma concentration (nanogram per milliliter) versus time curve (minute). The black line plots represent the veterinary proprietary formulation, whereas the red line plots signify the human generic formulation. Pane A (dog 1), C (dog 2), and E (dog 3) depicts the AMX time-courses; pane B (dog 1), D (dog 3), and F (dog 3) depicts the CA time-courses. AMX concentrations could be measured out to 24 hr, whereas CA concentrations dropped below quantification levels within the first 6–8 hr for both drug formulations. AMX, amoxicillin; CA, clavulanic acid.
by the FDA using genetically similar dogs in one location and within the same period.\textsuperscript{16,17}

There are shortcomings with this pilot study, which most importantly include the small study population. Although the study population size was selected based on previously published canine PK studies, our experiment was not designed to detect statistically significant differences between study groups but rather determine proof of concept by deriving preliminary data on the expected differences in drug exposures between each formulation and between-dog variability to allow for the sample size calculation of a follow-up bioequivalence study with adequate statistical power.\textsuperscript{7–9}

Also, this study only assessed healthy dogs, not those necessitating treatment for a recognized condition that would have indicated on-label use of the veterinary proprietary AMX–CA formulation; therefore, our findings cannot speak to therapeutic equivalence. Finally, there are several pharmaceutical companies that produce human generic AMX–CA formulations of varying ratios, and this study only assessed one human generic formulation and one AMX–CA ratio, 4:1. Thus, we cannot comment on the scope of all human generic formulations because of the fact that oral absorption is formulation dependent.\textsuperscript{18} Because of these expected limitations, in no way can the results of this study be used to justify or advocate

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Individual summary time-courses for AMX and CA for dogs 4–6 and for each drug formulation, assessing the area under the plasma concentration (nanogram per milliliter) versus time curve (minute). The black line plots represent the veterinary proprietary formulation, whereas the red line plots signify the human generic formulation. Pane A (dog 4), C (dog 5), and E (dog 6) depicts the AMX time-courses; pane B (dog 4), D (dog 5), and F (dog 6) depicts the CA time-courses. AMX concentrations could be measured out to 24 hr, whereas CA concentrations dropped below quantification levels within the first 6–8 hr for both drug formulations. AMX, amoxicillin; CA, clavulanic acid.}
\end{figure}
interchangeability between any AMX–CA formulations currently available in both human and veterinary markets.

It is important to note that, at the start of this study, there were no veterinary generic AMX–CA formulations available within the US market. Following completion of this study, a 4:1 veterinary generic AMX–CA product became available and offers an alternative to the veterinary proprietary formulation. This veterinary generic alternative did undergo blood-level bioequivalence testing alongside the veterinary proprietary formulation, using 125 mg tablets in dogs and 62.5 mg tablets in cats, concluding bioequivalence for these specific dosing tablets. A waiver in determining bioequivalence for the remaining 62.5, 250, and 375 mg tablets in dogs was granted after performing in vitro comparative dissolution studies. We suspect that the availability of this veterinary-specific generic formulation will not absolve the ongoing interest and prescribing habits of veterinarians regarding 4:1 human generic formulations within veterinary patient populations.

**Conclusion**

Although caution must be taken when interpreting the results of a small-sized study, this preliminary data shows drug exposures that are comparable between each formulation, despite observable between-dog variability. These results can be used to design a formal bioequivalence study for investigating formulation interchangeability. Until then, the use of 4:1 human generic AMX–CA formulations in veterinary medicine is deemed inappropriate.

The authors would like to thank Larry Wulf for his assistance in performing sample processing and analysis. The authors are also deeply grateful to the veterinary staff and their pets for their willing participation, enabling this study to be performed. This study was funded in part by the American College of Veterinary Dermatology Resident’s Research Award. Software license for Phoenix was provided by Certara USA, Inc. (Princeton, New Jersey) as part of their Centers of Excellence program.

**FOOTNOTES**

\(^a\) Clavamox Veterinary Tablets; Zoetis Inc., Kalamazoo, Michigan  
\(^b\) Amoxicillin–clavulinate potassium tablets, USP 500 mg/125 mg; Aurobinda Pharma Ltd., Hyderabad, India  
\(^c\) SURFLO ETFE I.V. 18-gauge x 2-inch catheters; Terumo Medical Corporation, Somerset, New Jersey  
\(^d\) Hill’s Healthy Advantage Adult Canine; Hill’s Pet Nutrition, Topeka, Kansas  
\(^e\) BD Vacutainer one-use needle holder; BD, Franklin Lakes, New Jersey  
\(^f\) BD Vacutainer 20-gauge x 1-inch blood collection needles; BD, Franklin Lakes, New Jersey  
\(^g\) BD Vacutainer Plus plastic lithium heparin tube; BD, Franklin Lakes, New Jersey  
\(^h\) Cryovial; Simport, Beloeil, Canada  
\(^i\) Agilent 1100 Pump, Column Compartment, and Autosampler; Agilent Technologies Inc., Santa Clara, California  
\(^j\) TSQ Vantage Mass Spectrometer; Thermo Scientific, San Jose, California  
\(^k\) TurboVap; Biotage, LLC, Charlotte, North Carolina  
\(^l\) ACE C18 column, 150 mm x 2.1 mm, 3 μm particles; Mac-Mod Analytical, Chads Ford, Pennsylvania  
\(^m\) Xcalibur software; Thermo Scientific, San Jose, California  
\(^n\) Phoenix WinNonlin, version 7.0; Certara USA, Inc., Princeton, New Jersey  
\(^o\) SigmaStat version 3.5; Systat Software, Inc., San Jose, California  
\(^p\) Amoxicillin trihydrate and clavulanate potassium veterinary tablets; Putney, Inc., Portland, Maine

**REFERENCES**


