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# The Pharmacokinetics of Tulathromycin Following Subcutaneous Administration in Rhesus Macaques (*Macaca mulatta*)

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***Campylobacter jejuni* is a pathogenic bacterium commonly associated with enteritis and diarrhea in rhesus macaques (*Macaca mulatta*). The standard therapy at the California National Primate Research Center is oral azithromycin, a second-generation macrolide, given daily for 5 d. Oral treatment administration can be difficult with some animals. Poor oral compliance for antibiotics can result in treatment failure and potentially select for antibiotic resistance. Tulathromycin, a newer-generation macrolide, may offer an injectable alternative to azithromycin. The aim of the current study is to quantify the pharmacokinetics of tulathromycin in plasma in rhesus macaques. Six rhesus macaques were each given a single 2.5 mg/kg dose SC of tulathromycin, and serial blood samples were collected at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 8, 12, 24, 48, 72, 96, 120, and 168 h, to quantify the concentration of tulathromycin in plasma over time. Results show that $C_{max}$ = 1,280 ± 395 ng/mL, $T_{max}$ = 1.25 ± 0.5 h, $t_{1/2}$ = 77.2 ± 15.4 h, and $AUC_{0\text{-}168}$ = 6,557.4 ± 875.4 h·ng/mL. There are no published Clinical and Laboratory Sciences Institute breakpoints for tulathromycin against *C. jejuni*, but based on an independently established minimum inhibitory concentration of 500 ng/mL, these data suggest that 2.5 mg/kg tulathromycin can be given subcutaneously to achieve potential therapeutic levels in rhesus macaques, possibly providing an alternative to oral azithromycin.**

**Abbreviations and Acronyms:** CNPRC, California National Primate Research Center; MIC, minimum inhibitory concentration

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## Introduction

*Campylobacter jejuni* is a helical, microaerophilic, nonspore-forming, motile, gram-negative bacterium[1] that causes several disease conditions in a variety of mammalian and avian species. Transmission of this pathogen to humans, dogs, poultry, nonhuman primates, among others, occurs via ingestion of contaminated feed or water typically, or direct contact is possible. For humans, the reservoir most implicated in *C. jejuni* transmission is contaminated meat, especially chicken,[2] but other animals like wild rodents[3] and boars[4] can act as reservoirs for animal-to-animal transmission, with outdoor animal housing being especially susceptible to contamination. Disease is characterized by gastroenteritis with clinical signs including vomiting and diarrhea in numerous animals as well as abortion in cattle[5] and sheep.[6,7]

In rhesus macaques, *C. jejuni* can cause prolific diarrhea, with or without blood and/or mucus, often followed by dehydration and electrolyte deficiencies that can progress to significant morbidity and mortality.[8] Estimates of campylobacteriosis vary between 45% and 97%, depending on the rhesus colony observed, with *Campylobacter coli* being most prevalent, followed by *C. jejuni* and *C. lari*.[9] Despite the presence of subclinical carriers, research has demonstrated that *Campylobacter* spp. infection has the potential to both cause diarrheal disease in rhesus and stunt infant growth.[10,11]

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Previous research has demonstrated the sensitivity of various isolates of *Campylobacter* spp. to macrolide antibiotics, including azithromycin and erythromycin on cultures tested in vitro[12] and azithromycin in *C. jejuni* isolates harvested from beagles, cynomolgus macaques, and rhesus macaques.[13] Other studies have demonstrated that macrolides like tylosin and azithromycin have not produced any adverse effects in macaques.[14,15]

Animals admitted to the California National Primate Research Center (CNPRC) hospitals with diarrhea have a rectal swab performed for bacterial culture upon presentation. They are then cultured at the on-site clinical pathology laboratory with agars and/or broths to identify all possible pathogens that are known to cause diarrhea in rhesus, for example, *Shigella* spp., *Yersinia* spp., and in this case, *Campylobacter* spp. (Campy CVA Agar with 5% Sheep's Blood; Hardy Diagnostics, Springboro, OH). Further techniques speciate between *C. coli* compared with *C. jejuni* because *C. coli* is often found in both healthy rhesus macaques and those with diarrhea, so it is not considered a pathogenic bacterium. A positive *C. jejuni* diarrhea diagnosis is made with ongoing clinical signs during admission plus a positive rectal culture result. The current treatment of choice for *C. jejuni* infection in rhesus macaques (*Macaca mulatta*) at the CNPRC is azithromycin, a second-generation macrolide, given orally at 40 mg/kg once daily for 5 d. This treatment is empirical in rhesus macaques but is based on the literature on humans with campylobacter diarrhea.[16,17] There are currently no research publications demonstrating the therapeutic levels of azithromycin against *C. jejuni* infection in rhesus macaques.

Azithromycin can be difficult to administer in macaques due to its bitter taste and typically needs to be hidden in highly palatable

foods. Furthermore, macaques experiencing enterocolitis may have decreased food intake due to gastrointestinal tract signs like hyporexia, nausea, and vomiting. In addition, food selectivity in general can be highly variable between individual animals. In the case of oral azithromycin, this can lead to poor oral compliance, failure to meet therapeutic levels, and potentially result in antimicrobial resistance. Alternative methods for oral administration, such as oral gavage or oral gastric lavage can be time consuming, require sedation of the animal, and can increase the risk of vomiting and aspiration. Injectable antibiotics mitigate many of the complications associated with oral antibiotics and only require momentary restraint. Tylosin is an injectable macrolide that has the potential to treat diarrhea in rhesus macaques,[14] but the treatment course is longer than other available options and in recent times has been intermittently unavailable.

Tulathromycin, a newer generation triamilide macrolide antibiotic, is an injectable medication that is effective against many gram-positive bacteria and a select number of gram negatives,[18] inhibiting bacterial protein synthesis through binding to the ribosomal 50S subunit.[19] It was originally licensed by the U.S. Food and Drug Administration in 2005 for the treatment of respiratory infections in beef cattle (including suckling calves), nonlactating dairy cattle (including dairy calves), veal calves, and swine,[20] but extralabel use is common. Many pharmacokinetic and pharmacodynamic studies have been published for numerous off-label species including goats,[21] bison,[22] rabbits,[23] guinea pigs,[24] mice,[25] and even salmon.[26] One recent study[27] demonstrated that tulathromycin can be effective in treating sheep (*Ovis aries*) that have been experimentally inoculated with *C. jejuni*.

Tulathromycin has a concentration-dependent mode of action that requires its presence to be above a minimum inhibitory concentration (MIC) for full antibiotic efficacy. The higher the peak concentration within the therapeutic range, the greater the bacteriostatic effect.[18] Currently, there are no published data showing a Clinical and Laboratory Sciences Institute-derived breakpoint for tulathromycin against *C. jejuni*. However, one research group[27] performed agar dilution studies and published an MIC of 500 ng/mL for tulathromycin against *C. jejuni* IA3902, a *C. jejuni* isolate cultured from an aborted ovine fetus.

Currently, there are no published studies to demonstrate whether other macrolides like tulathromycin would be effective and safe against *C. jejuni* in rhesus macaques. The purpose of this study was to characterize the pharmacokinetic profile of injectable tulathromycin in rhesus macaques. We hypothesized that a single 2.5 mg/kg SC dose of tulathromycin would meet or exceed potential therapeutic plasma concentrations of 500 ng/mL, based on the MIC found in previous agar dilution studies.[27] This dose is recommended by Zoetis and is the most common dose used in multiple pharmacokinetic studies in other species.[20–23,27–31]

## Materials and Methods

**Animals.** Six rhesus macaques were selected for this study ranging from 3 to 6 y of age with body condition scores[32] of 1.5 to 2.5 out of 5. Those 2 factors were important in our selection criteria to be as representative as possible of the typical *C. jejuni* diarrhea patient at the CNPRC: subadult or younger, with a body condition score of 2.5 or lower. Because there has been no sex predilection demonstrated for *C. jejuni* infection, 3 males and 3 females were chosen to maximize sampling diversity (Table 1). Subject randomization was not used for this phase one pharmacokinetic study. All animals, members of the CNPRC breeding colony, were housed in accordance with the Institute for Laboratory Animal Research's *Guide for the Care and Use of Laboratory Animals*,[33] the Public Health Service Policy, and the Animal Welfare Act[34] and Animal Welfare Regulations.[35] This study was conducted in accordance with the IACUC of the University of California at Davis, which is an AAALAC International-accredited and USDA-registered facility.

**Table 1.** Individual animal information

| Animal ID | Age (y) | Weight (kg) | BCS (×/5.0) | Sex |
|---|---|---|---|---|
| A | 3.2 | 4.26 | 2.5 | M |
| B | 3.2 | 5.49 | 2.5 | M |
| C | 4.0 | 4.74 | 2 | F |
| D | 5.2 | 7.02 | 2.5 | F |
| E | 5.5 | 5.7 | 2 | M |
| F | 6.2 | 5.4 | 1.5 | F |

BCS, body condition score.

All study animals were maintained within the indoor-housed SPF colony at the CNPRC, having tested negative for macacine herpesvirus-1, simian immunodeficiency virus, simian T-lymphotropic virus, and simian retrovirus type D consistently via serology, as described by established methods for pathogen exclusion at the CNPRC.[36] All animals received annual physical exams and semiannual tuberculosis screening as a part of routine colony veterinary care. In addition, all animals received measles and tetanus vaccinations at or after 6 mo of age. Preproject health assessments were performed on all enrolled animals, which included a full physical exam, CBC, and serum chemistry.

Animals were housed in a standard quad-unit stainless steel caging system under a 12:12-h light-dark cycle (0600 to 1800), between an ambient temperature range of 20 to 26 °C, and 30% to 70% relative humidity. Commercial monkey chow (LabDiet Monkey Diet 5047; Purina Mills International, Richmond, IN) was provided twice daily, and they also received a forage allotment of roughly one-fourth cup of mixed dried oats and peas as forage once daily. Additional enrichment included hanging mirrors, a rotation of manipulanda and enrichment objects, and constant visual and auditory contact with conspecifics. They also received fresh produce twice weekly as per the CNPRC husbandry standards and had unrestricted access to potable water obtained from the University of California at Davis campus domestic water system.

After a minimum acclimation period of 2 wk following enrollment into the study, all subjects were acclimated to routine small-volume blood collection via cage-side cephalic venipuncture without the need for sedation. This entailed repeated, cage-side, positively reinforced sessions of arm presentation and sham sample collection, followed by dry and fresh fruit rewards. The number of sessions varied per animal and continued enrollment in the study depended on their cooperative arm presentation and sample collection. If the subjects were pair housed, they were temporarily separated when sedated for test article administration and for all subsequent study-related blood draws. Each animal also received fresh fruit and vegetable rewards immediately following each cage-side blood sample collection.

**Experimental design.** The first subject was anesthetized with ketamine hydrochloride (Zetamine; MWI Veterinary Supply, Boise, ID) at approximately 10 mg/kg IM so an accurate weight could be collected and used to calculate an accurate test article dose. A patch of hair was clipped in the interscapular region measuring approximately 5 × 5 cm, which allowed for easier monitoring of adverse reactions at the administration site while awake and moving about their cage. The skin was wiped with a 70% ethanol-soaked gauze, and a single 2.5 mg/kg

dose of tulathromycin (Draxxin; Zoetis, Kalamazoo, MI) was administered subcutaneously in the center of the hairless patch. A 100 mg/mL formulation of tulathromycin was supplied in a 50 mL light-resistant, amber glass vial with a multipuncture rubber seal and was stored in a refrigerator at or below 25 °C, per manufacturer recommendations. A single baseline blood sample (1.0 mL) was collected immediately before tulathromycin administration ($t = 0$) as the animal's own negative control sample. This was followed by serial (1.0 mL) blood draws performed at $t = 0.25$, 0.5, 0.75, and 1.0 h under sedation, and then by nonsedated cage-side collection at $t = 1.5$, 2, 4, 8, 12, 24, 48, 72, 96, 120, and 168 h.

Blinding was not necessary because this study was using objective measures, with a focus on drug properties, safety, and tolerability, and no emphasis on clinical efficacy. Two trained research support staff were assigned to test article administration and all subsequent blood collections under sedation. Seven trained research support staff were assigned to cage-side blood collections. Whole blood samples were placed into sodium heparin tubes by trained personnel and centrifuged at a minimum of 1,300 × *g* for 15 min at room temperature. Plasma was aliquoted into separate vials and stored in a freezer at –80 °C until further analysis.

The 5 remaining animals underwent the same regimen approximately 2 wk after the first animal, to allow time for monitoring, identifying, and treating any adverse reactions in the first animal. All animals were monitored daily for appetite, hydration, and stool quality as well as mentation and local inflammation at the site of injection.

**Tulathromycin assay.** Plasma samples were analyzed using an ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) assay technique. Analysis was performed based on modification of a previously described method used for equine plasma[37] using tulathromycin-d7 (Td7) as an internal standard. The analytical system consisted of a Sciex 6500+ QTRAP triple quadrupole mass spectrometer with a turbo ionspray source coupled to the Sciex Exion UPLC system with a cooled autosampler (Applied Biosystems, Foster City, CA). Samples were chromatographed on an Acquity UPLC BEH Phenyl column (1.7 µm, 100 mm × 2.1 mm; Waters Corporation, Milford, MA) with a matching precolumn. A liquid chromatography gradient was employed with mobile phase A consisting of Milli-Q water with 1% formic acid and mobile phase B consisting of acetonitrile with 1% formic acid. Chromatographic separation was achieved by holding mobile phase B steady at 10% from 0 to 0.5 min, increasing linearly from 10% to 98% between 0.5 and 2.25 min, holding steady at 98% until 3.25 min, and decreasing linearly to 10% between 3.25 and 3.5 min followed by equilibrating at 10% until 5 min. The sample injection volume was 5 µL, and the analysis run time was 5 min. The retention time for tulathromycin and Td7 was 2.28 min. Tulathromycin was quantified using the internal standard reference method monitoring the doubly charged tulathromycin parent ion (*m/z*: 403.9) and 2 singly charged fragment ions (*m/z*: 577.2 and 158.2) along with the doubly charged Td7 parent ion (*m/z*: 407.9) and singly charged fragment ion (*m/z*: 158.2) in multiple reaction monitoring (MRM) mode. Scan times were 75 ms, and quadrupoles 1 and 3 were both operated in unit resolution mode.

The analytical standards for tulathromycin (Millipore-Sigma, Burlington, MA) and Td7 (Toronto Research Chemical, North York, ON) were dissolved in DMSO to make stock solutions at 10 and 1 mg/mL, respectively, and stored at –20 °C. Calibration standards were made fresh from stock solutions on the day of analysis and calibration curves were generated by fortifying blank rhesus macaque plasma with concentrations ranging from 12.5 to 2,500 ng/mL (10 nonzero concentrations), and quality control (QC) samples were made at 12.5, 75, and 500 ng/mL (3 per concentration). Plasma calibrators, QCs, and study samples (100 µL) were subjected to protein precipitation by adding acetonitrile with 1% formic acid (300 µL containing 500 ng/mL Td7), followed by vortex mixing for 5 min and centrifugation at 16,000 × *g* for 10 min at room temperature. Two hundred microliters of supernatant were then added to glass autosampler vials containing 300 µL of Milli-Q water with 1% formic acid for injection onto the UPLC system. This assay had a limit of quantitation of 12.5 ng/mL (signal to noise [S/N] ratio greater than 10) and a limit of detection of 2.5 ng/mL (S/N > 3). The calibration curve was linear between 12.5 and 2,500 ng/mL with a coefficient of determination ($R^2$) of 0.999 and an accuracy greater than 94% at all concentrations. Accuracy of QCs was within 4% for all 3 concentrations and precision was within 10%. Interday and intraday coefficient of variation of QCs was within 7% for all 3 concentrations, and samples were stable for greater than 24 h in the cooled autosampler (15 °C).

**Pharmacokinetic analysis.** Plasma tulathromycin concentrations were plotted on semilogarithmic graphs and were analyzed using noncompartmental analysis methods on commercially available software (Phoenix WinNonlin, v8.3.3; Certara, Radnor, PA). $C_{max}$ and $T_{max}$ were reported directly from the data. The $t_{1/2}$ was calculated from the slope of the terminal elimination phase determined using 5 time points. Area under the plasma tulathromycin concentration–time curve (AUC) was calculated using the linear trapezoidal/linear interpolation method and was calculated up to the last measured time point (168 h; $AUC_{0\text{-}T}$) and extrapolated to infinity ($AUC_{inf}$) for determination of percent extrapolation, required to be less than 20% for accurate prediction of terminal slope. Pharmacokinetic parameter estimates were reported as mean with SD except for elimination rate ($K_{el}$) and $t_{1/2}$, which were reported as harmonic mean and pseudo-SD. Nonparametric superposition was used to estimate the plasma tulathromycin concentration–time curves for alternative dosing regimens using the noncompartmental analysis results, and they were graphed as the mean plasma concentration along with the 95% CI of the mean.

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism v9.1.2 (GraphPad Software, San Diego, CA). The differences between reported $C_{max}$ and AUC values for male and female monkeys were tested using a 2-tailed, Mann–Whitney test. Significance was set at $P < 0.05$.

## Results

No adverse effects were noted at any point during the study. All blood samples were obtained at every time point. For all 6 animals, the average $C_{max}$ of tulathromycin with SD was 1,280 ± 395 ng/mL, and the time it took to reach $T_{max}$ was 1.25 ± 0.5 h. The $t_{1/2}$ of tulathromycin in plasma was 77.2 ± 15.4 h. The average elimination rate ($K_{el}$) was 0.0090 ± 0.0016 per h. The total drug exposure over 168 h ($AUC_{0\text{-}168}$) was 6,557 ± 875.4 h·ng/mL, and the total drug exposure from time point 0 extrapolated to infinity ($AUC_{inf}$) was 7,248.8 ± 948.8 h·ng/mL, with the percentage of extrapolated AUC ($AUC_{extrap}$) averaging 10% ± 2%. The apparent volume of distribution (Vz/F) equaled an average of 40.0 ± 8.5 L/kg, and apparent clearance (CL/F) averaged 5.8 ± 0.8 mL/kg/min. The mean residence time was 26.3 h on average with an SD of 3.3 h (Table 2).

A linear graph depicting $C_{max}$ with SD across all time points is included (Figure 1). Because there were no statistically significant differences between males and females, the data were

**Table 2.** Individual and group pharmacokinetic information

| Animals | $C_{max}$ (ng/mL) | $T_{max}$ (h) | $t_{1/2}$ (h) | $K_{el}$ (1/h) | $AUC_{0\text{-}t}$ (h·ng/mL) | $AUC_{0\text{-}inf}$ (h·ng/mL) | $AUC_{extrap}$ (%) | Vz/F (L/kg) | CL/F (mL/kg/h) | MRT (h) |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 1,130 | 0.75 | 75.7 | 0.0092 | 5,443.8 | 5,931.1 | 8 | 46.1 | 7 | 22.5 |
| B | 921 | 1.5 | 58.2 | 0.0119 | 6,262.5 | 6,791.6 | 8 | 30.9 | 6.1 | 31.8 |
| C | 1,070 | 1.5 | 71 | 0.0098 | 6,827.9 | 7,484.9 | 9 | 34.2 | 5.6 | 28 |
| D | 2,040 | 1 | 85.2 | 0.0081 | 7,890 | 8,664 | 9 | 35.4 | 4.8 | 24 |
| E | 1,210 | 2 | 101.2 | 0.0068 | 5,895.8 | 6,815.7 | 13 | 53.6 | 6.1 | 26.1 |
| F | 1,310 | 0.75 | 86 | 0.0081 | 7,024.1 | 7,805.6 | 10 | 39.7 | 5.3 | 25.5 |
| Mean | 1,280 | 1.25 | 77.2 | 0.0090 | 6,557.4 | 7,248.8 | 10 | 40.0 | 5.8 | 26.3 |
| SD | 395 | 0.5 | 15.4[a] | 0.0016[a] | 875.4 | 948.8 | 2 | 8.5 | 0.8 | 3.3 |

$K_{el}$, elimination rate; $AUC_{0\text{-}t}$, area under the concentration–time curve from time 0 to last time point; $AUC_{0\text{-}inf}$, area under the concentration–time curve from time 0 extrapolated to infinity; $AUC_{extrap}$, percent of AUC that was extrapolated; Vz/F, apparent volume of distribution; CL/F, apparent clearance; MRT, mean residence time.
[a]Harmonic mean with pseudo-SD reported for $K_{el}$ and $t_{1/2}$.

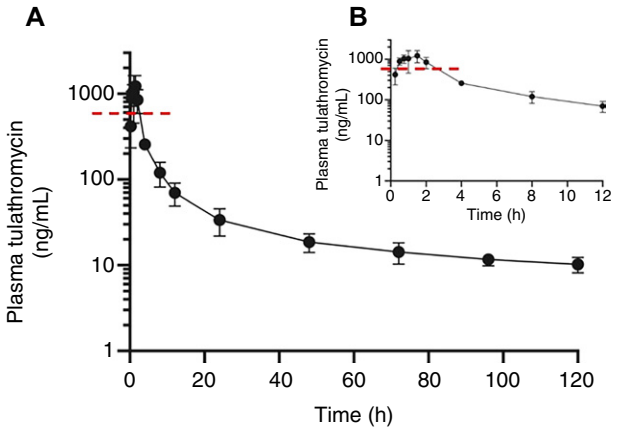


**Figure 1.** (A) Mean with SD bars showing concentration in plasma over time after subcutaneous injection of a single dose of tulathromycin (2.5 mg/kg) to 6 rhesus macaques (*Macaca mulatta*). Tulathromycin administration was designated as time 0. Peak plasma concentration (ng/mL; mean ± SD) was 1,280 ± 395. (B) Mean with SD bars showing tulathromycin concentrations in plasma over just the first 12 h. The red dashed lines on both plots represent the MIC of 500 ng/mL.

combined for all 6 animals and presented in the manuscript. A nonparametric superposition model (Figure 2) was created to demonstrate plasma tulathromycin concentrations following a 2.5 mg/kg/day dosing regimen given over 5 d.

## Discussion

Azithroymcin is the treatment of choice for *C. jejuni* diarrhea at the CNPRC, an oral macrolide that can be challenging to administer in rhesus macaques. The injectable macrolide tulathromycin has demonstrated efficacy against *C. jejuni* in livestock. We concluded that the pharmacokinetics of a single dose of tulathromycin (2.5 mg/kg SC) in rhesus macaques reached well above a potential therapeutic concentration of 500 ng/mL, an MIC established with an agar dilution study in a recent publication.[27] Furthermore, the pharmacokinetic findings have many similarities to those seen in multiple other species, for example, goats, rabbits, and cattle.

The $C_{max}$ demonstrated in this study (1,280 ± 395 ng/mL) is similar to, but greater than, those seen in meat goats (987 ± 227 ng/mL[28] and 1,185 ± 482 ng/mL[28]) and rabbits (882 ± 30 ng/mL[23]). The $C_{max}$ in this study was also significantly higher than the $C_{max}$ seen in bison (195 ± 157 ng/mL[22]), cattle (500 ± 400 ng/mL[38] and 300 ± 400 ng/mL[39]), and another study with meat goats (633 ± 300 ng/mL[21]) and significantly lower than the $C_{max}$ seen in nonpregnant ewes (3,598 ± 2,344 ng/mL[27]), with all groups receiving a single 2.5 mg/kg SC dose.

The $T_{max}$ demonstrated in this study (1.25 ± 0.5 h) was comparable to that seen in rabbits (1.55 ± 0.26 h[23]), and similar to nonpregnant adult ewes (1.6 ± 2.2 h[27]), but was roughly twice as long as those found in meat goats (0.6 ± 1.0 h[28] and 0.4 ± 0.26 h[21]) and cattle (0.71 ± 3 h[39]). The average $t_{1/2}$ found in our study (77.2 ± 15.4 h) was roughly double that found in rabbits (36.2 ± 2.26 h[23]), slightly higher than those found in 2 goat studies (45[28] and 60 h[30]), and roughly in between values found in 2 cattle studies (58[38] and 100 h[39]). Total drug exposure over 168 h ($AUC_{0\text{-}168}$) found in this study (6,557.4 ± 875.4 h·ng/mL) was much lower than the $AUC_{0\text{-}168}$ seen in meat goats (24,000 ± 6,900 h·ng/mL[21]) but was comparable to sheep (7,956.4 h·ng/mL[27]). The $AUC_{inf}$ seen in our study (7,248.8 ± 948.8 h·ng/mL) was very similar to the $AUC_{inf}$ found in rabbits (7,324.5 ± 304 h·ng/mL[23]). Having comparable $T_{max}$, $t_{1/2}$, and AUC values

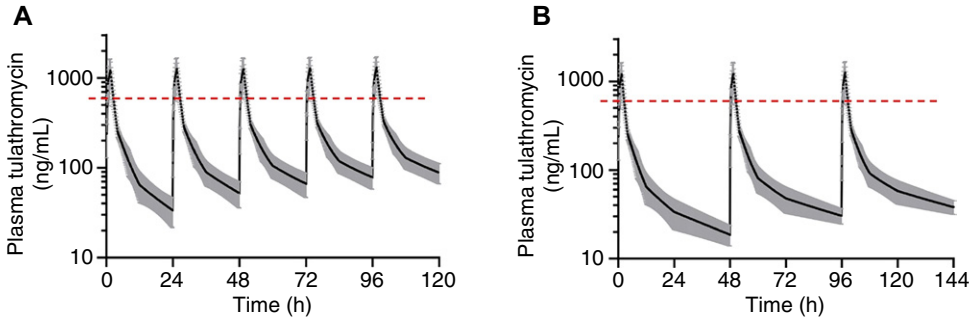


**Figure 2.** Nonparametric superposition models demonstrating predicted plasma concentrations. The dark line is mean, and the shaded regions represent 95% CI of the mean. (A) Following daily administration of tulathromycin at 2.5 mg/kg SC. (B) Following every other day administration of tulathromycin at 2.5 mg/kg SC. The red dashed lines on both plots represent the MIC of 500 ng/mL.

indicates similar absorption, distribution, metabolism, and excretion (ADME) characteristics to other animals, many of whom tulathromycin is already commonly prescribed.

It is important to consider that despite concentrations reaching potential therapeutic levels[27] within plasma, concentrations in other tissues or organs (that is, the gastrointestinal tract) may differ from those in plasma. However, given that previous studies have demonstrated that tulathromycin is mostly excreted biologically unchanged via the biliary system,[40] it is reasonable to consider that sufficient concentrations could be achieved within the gastrointestinal tract. In addition, this drug has clinically useful pharmacokinetic characteristics such as slow elimination and extensive distribution with no or minimal adverse effects.

Another consideration is that the *C. jejuni* isolates at the CNPRC are not the same isolates that were used in the agar dilution studies[27] referenced in this paper. Individual susceptibility can vary between isolated populations within the same bacterial species. However, the manufacturer's recommended dose of tulathromycin reached a plasma concentration over 2.5 times above the experimentally established MIC (500 ng/mL); this point supports the clinical application of tulathromycin to effectively combat the *C. jejuni* population at the CNPRC.

Tulathromycin's application may be a practical and effective alternative in future research for treating *C. jejuni* diarrheal infection in nonhuman primates compared with azithromycin, the current standard of care. Average plasma concentrations of tulathromycin, at 1,280 ng/mL, have the potential to reach peak levels 2.5 times greater than the MIC of 500 ng/mL, established in previous agar dilution studies[27] for tulathromycin against *C. jejuni*.

Future studies in rhesus macaques should also consider comparing other parameters of tulathromycin administration like dose, frequency, route, duration, etc. Triamilides have 3 amine sites built within their structure, with $pK_{a1}$ = 8.49, $pK_{a2}$ = 9.28, and $pK_{a3}$ = 9.80. In general, the basic nature, limited degree of ionization, and lipophilicity found in macrolides equips the drug for high penetration into tissues and fluids, resulting in large volumes of distribution.[41–43] It is conceivable that tulathromycin's extended half-life compared with older generation macrolides may allow for less frequent dosing while still achieving the same level of efficacy.

As examples, two nonparametric superposition models are included. One demonstrates a 2.5 mg/kg dose given every day for 5 d (Figure 2A), and the other demonstrates every other day (EOD) for 6 d (Figure 2B). These models are valuable for predicting plasma concentrations while minimizing handling, allowing adherence to the Replacement, Reduction, and Refinement (3Rs) principle of Refinement.[33,44] Some of the other advantages to using this model include that because it is a conservative method that reduces incorrect conclusions, results are not greatly affected by outliers, and modeling can be done with small sample sizes.[45] The results in both of our models show similar peak concentrations and elimination characteristics to each other, possibly justifying reduced administration frequency.

The main shortcomings of the current pharmacokinetics study are small sample size and the paucity of published data for comparison to our own data, largely published MIC data in particular. Future studies should also include MIC determinations for site-specific *C. jejuni* isolates.

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## Conflict of Interest

The authors have no conflicts of interest to declare.

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## References

1. Balaban M, Hendrixson DR. Polar flagellar biosynthesis and a regulator of flagellar number influence spatial parameters of cell division in *Campylobacter jejuni*. *PLoS Pathog*. 2011;7(12):e1002420.
2. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of *Campylobacter* infection. *Clin Microbiol Rev.* 2015;28(3):687–720.
3. Kim J, Guk JH, Mun SH, et al. The wild mouse (*Micromys minutus*): reservoir of a novel *Campylobacter jejuni* strain. *Front Microbiol.* 2019;10:3066.
4. Kerkhof PJ, Peruzy MF, Murru N, Houf K. Wild boars as reservoir for *Campylobacter* and *Arcobacter*. *Vet Microbiol*. 2022;270:109462.
5. Uzal F. Enteric campylobacteriosis in animals - digestive system. *Merck Veterinary Manual*. Accessed May 21, 2024. https://www.merckvetmanual.com/digestive-system/enteric-campylobacteriosis/enteric-campylobacteriosis-in-animals.
6. Sahin O, Plummer PJ, Jordan DM, et al. Emergence of a tetracycline-resistant *Campylobacter jejuni* clone associated with outbreaks of ovine abortion in the United States. *J Clin Microbiol.* 2008;46(5):1663–1671.
7. Yaeger MJ, Sahin O, Plummer PJ, Wu Z, Stasko JA, Zhang Q. The pathology of natural and experimentally induced *Campylobacter jejuni* abortion in sheep. *J Vet Diagn Invest.* 2021;33(6):1096–1105.
8. Laing ST, Merriam D, Shock BC, et al. Idiopathic colitis in rhesus macaques is associated with dysbiosis, abundant enterochromaffin cells and altered T-cell cytokine expression. *Vet Pathol.* 2018;55(5):741–752.
9. Bacon RL, Hodo CL, Wu J, et al. Diversity of *Campylobacter* spp. circulating in a rhesus macaque (*Macaca mulatta*) breeding colony using culture and molecular methods. *mSphere.* 2024;9(11):e0056024.
10. Hendrickson SM, Thomas A, Raué HP, et al. *Campylobacter* vaccination reduces diarrheal disease and infant growth stunting among rhesus macaques. *Nat Commun.* 2023;14(1):3806.
11. Quintel BK, Prongay K, Lewis AD, et al. Vaccine-mediated protection against *Campylobacter*-associated enteric disease. *Sci Adv.* 2020;6(26):eaba4511.
12. Taylor DE, Chang N. In vitro susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* to azithromycin and erythromycin. *Antimicrob Agents Chemother.* 1991;35(9):1917–1918.
13. Yoshimura M, Ebukuro M, Kagiyama N, Shimizu A, Saito Y. Isolation of *Campylobacter jejuni* from experimental dogs and monkeys in Japan. *Jikken Dobutsu*. 1984;33(2):209–212.
14. Blackwood RS, Tarara RP, Christe KL, Spinner A, Lerche NW. Effects of the macrolide drug tylosin on chronic diarrhea in rhesus macaques (*Macaca mulatta*). *Comp Med.* 2008;58(1):81–87.
15. Kelleher MA, Lee JY, Roberts VHJ, et al. Maternal azithromycin therapy for *Ureaplasma parvum* intraamniotic infection improves fetal hemodynamics in a nonhuman primate model. *Am J Obstet Gynecol.* 2020;223(4):578.e1–578.e11.
16. Nielsen HL, Kirk KF, Bodilsen J, Ejlertsen T, Nielsen H. Azithromycin vs. placebo for the clinical outcome in *Sampylobacter concisus* diarrhoea in adults: a randomized, double-blinded, placebo-controlled clinical trial. *PLoS One.* 2016;11(11):e0166395.
17. Hinterwirth A, Sié A, Coulibaly B, et al. Rapid reduction of *Campylobacter* species in the gut microbiome of preschool children after oral azithromycin: a randomized controlled trial. *Am J Trop Med Hyg.* 2020;103(3):1266–1269.
18. Villarino N, Brown SA, Martín-Jiménez T. The role of the macrolide tulathromycin in veterinary medicine. *Vet J.* 2013;198(2):352–357.
19. Patel PH, Hashmi MF. Macrolides. NIH.gov. Accessed May 23, 2024. https://www.ncbi.nlm.nih.gov/books/NBK551495/#_article-24625_s3_.

20. DailyMed - DRAXXIN- tulathromycin injection, solution. NIH.gov. 2022. Accessed August 21, 2023. https://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=258f413b-95e0-4082-a150-85af60b2af80.
21. Young G, Smith GW, Leavens TL, et al. Pharmacokinetics of tulathromycin following subcutaneous administration in meat goats. *Res Vet Sci.* 2011;90(3):477–479.
22. Bachtold K, Alcorn J, Matus J, Boison J, Woodbury M. Pharmacokinetics of tulathromycin after subcutaneous injection in North American bison (*Bison bison*). *J Vet Pharmacol Ther.* 2015;38(5):471–474.
23. A-El-Sooud K, Afifi N, Hassibelnaby A. Pharmacokinetics and bioavailability of tulathromycin following intravenous, intramuscular and subcutaneous administrations in healthy rabbits. *Vet World.* 2012;5(7):424.
24. Zhao Y, Guo LL, Fang B, Liu B. Pharmacokinetic/pharmacodynamic (PK/PD) evaluation of tulathromycin against *Haemophilus parasuis* in an experimental neutropenic guinea pig model. *PLoS One.* 2018;13(12):e0209177.
25. Villarino N, Brown SA, Martín-Jiménez T. Pharmacokinetics of tulathromycin in healthy and neutropenic mice challenged intranasally with lipopolysaccharide from *Escherichia coli*. *Antimicrob Agents Chemother.* 2012;56(8):4078–4086.
26. Elliott D. Efficacy of injectable tulathromycin for reduction of vertical transmission of *Renibacterium salmoninarum* in Spring Chinook Salmon *Oncorhynchus tshawytscha*. USGS.gov. 2018. Accessed October 9, 2024. https://pubs.usgs.gov/publication/70204521.
27. Yaeger M, Mochel JP, Wu Z, et al. Pharmacokinetics of tulathromycin in pregnant ewes (*Ovis aries*) challenged with *Campylobacter jejuni*. *PLoS One.* 2021;16(8):e0256862.
28. Clothier KA, Leavens T, Griffith RW, et al. Pharmacokinetics of tulathromycin after single and multiple subcutaneous injections in domestic goats (*Capra aegagrus hircus*). *J Vet Pharmacol Ther.* 2011;34(5):448–454.
29. Cox SR, McLaughlin C, Fielder AE, Yancey MF, Brown SA. Rapid and prolonged distribution of tulathromycin into lung homogenate and pulmonary epithelial lining fluid of Holstein calves following a single subcutaneous administration of 2.5 mg/kg body weight. *J Appl Res Vet Med.* 2010;8(3):129–137.
30. Romanet J, Smith GW, Leavens TL, et al. Pharmacokinetics and tissue elimination of tulathromycin following subcutaneous administration in meat goats. *Am J Vet Res.* 2012;73(10):1634–1640.
31. Washburn K, Fajt VR, Coetzee JF, Rice S, Wulf LW, Washburn S. Pharmacokinetics of tulathromycin in nonpregnant adult ewes. *J Vet Pharmacol Ther.* 2015;38(4):414–416.
32. Summers L, Clingerman KJ, Yang X. Validation of a body condition scoring system in rhesus macaques (*Macaca mulatta*): assessment of body composition by using dual-energy X-ray absorptiometry. *J Am Assoc Lab Anim Sci.* 2012;51(1):88–93.
33. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals*. 8th ed. National Academies Press; 2011.
34. Animal Welfare Act as Amended. 2013. 7 USC §2131–2159.
35. Animal Welfare Regulations. 2013. 9 CFR § 3129.
36. Yee JL, Vanderford TH, Didier ES, et al. Specific pathogen free macaque colonies: a review of principles and recent advances for viral testing and colony management. *J Med Primatol.* 2016;45(2):55–78.
37. Leventhal HR, McKenzie HC, Estell K, Council-Troche M, Davis JL. Pharmacokinetics and pulmonary distribution of Draxxin (tulathromycin) in healthy adult horses. *J Vet Pharmacol Ther.* 2021;44(5):714–723.
38. Nowakowski MA, Inskeep PB, Risk JE, et al. Pharmacokinetics and lung tissue concentrations of tulathromycin, a new triamilide antibiotic, in cattle. *Vet Ther.* 2004;5(1):60–74.
39. Gáler D, Hessong S, Beato B, et al. An analytical method for the analysis of tulathromycin, an equilibrating triamilide, in bovine and porcine plasma and lung. *J Agric Food Chem.* 2004;52(8):2179–2191.
40. Lewy K, Cernicchiaro N, Dixon AL, et al. Association between tulathromycin treatment for bovine respiratory disease and antimicrobial resistance profiles among gut commensals and foodborne bacterial pathogens isolated from feces of beef steers. *J Food Prot.* 2022;85(8):1221–1231.
41. Anadón A, Reeve-Johnson L. Macrolide antibiotics, drug interactions and microsomal enzymes: implications for veterinary medicine. *Res Vet Sci.* 1999;66(3):197–203.
42. Peters DH, Friedel HA, McTavish D. Azithromycin. A review of its antimicrobial activity, pharmacokinetic properties and clinical efficacy. *Drugs.* 1992;44(5):750–799.
43. Zhanel GG, Dueck M, Hoban DJ, et al. Review of macrolides and ketolides: focus on respiratory tract infections. *Drugs.* 2001;61(4):443–498.
44. Hubrecht RC, Carter E. The 3Rs and humane experimental technique: implementing change. *Animals (Basel).* 2019;9(10):754.
45. Nahm FS. Nonparametric statistical tests for the continuous data: the basic concept and the practical use. *Korean J Anesthesiol.* 2016;69(1):8–14.