Effects of experimentally-induced respiratory disease on the pharmacokinetics and tissue residues of tulathromycin in meat goats

Jonathan P. Mochel, Iowa State University

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Abstract

Tulathromycin is a macrolide antibiotic commonly used for the treatment of respiratory disease in food animal species including goats. Recent research in pigs has suggested that the presence of disease could alter the pharmacokinetics of tulathromycin in animals with respiratory disease. The objectives of this study were (a) compare the plasma pharmacokinetics of tulathromycin in healthy goats as well as goats with an induced respiratory disease; and (b) to compare the tissue residue concentrations of tulathromycin marker in both groups. For this trial, disease was induced with Pasteurella multocida. Following disease induction tulathromycin was administered. Samples of plasma were collected at various time points up to 312 hours post-treatment, when study animals were euthanized and tissue samples were collected. For PK parameters in plasma, V_z (control: 28.7 ± 11.9 mL/kg; experimental: 57.8 ± 26.6 mL/kg), was significantly higher (P = 0.0454) in the experimental group than the control group, and non-significant differences were noted in other parameters. Among time points significantly lower plasma concentrations were noted in the experimental group at 168 hours (P = 0.023), 216 hours (P = 0.036), 264 hours (P = 0.0017), 288 hours (P = 0.0433), and 312 hours (P = 0.0486). None of the goats had tissue residues above the US bovine limit of 5 µg/g at the end of the study. No differences were observed between muscle, liver, or fat concentrations. A significantly lower concentration (P = 0.0095) was noted in the kidneys of experimental goats when compared to the control group. These results suggest that the effect of respiratory disease on the pharmacokinetics and tissue residues appear minimal after experimental P. multocida infection, however as evidenced by the disparity in Cmax, significant differences in plasma concentrations at terminal time points, as well as the differences in kidney concentrations, there is the potential for alterations in diseased vs clinical animals.
INTRODUCTION

Tulathromycin is a macrolide antibiotic widely used for respiratory disease in cattle and pigs due to its broad spectrum of antimicrobial activity. The broad spectrum of activity and long-acting formulation also make this an ideal antibiotic for treating respiratory disease in goats, along with its efficacy against caprine respiratory isolates of *Mannheimia hemolytica*, *Pastuerella multocida*, and *Bibersteinia trehalosi* (Clothier, Kinyon et al., 2012). Due to the importance of broad spectrum antimicrobials for veterinary as well as human medicine, the World Health Organization has classified macrolide antibiotics on the list of the highest priority, critically important antimicrobials for the preservation of human health. (WHO, 2017) This prioritization from the WHO is based on 1) the high number of people affected by diseases for which a macrolide is the sole or one of the few therapies, 2) the high frequency of use in human medicine, and 3) the potential transmission of *Campylobacter spp* from non-human sources. (WHO, 2017)

In the United States tulathromycin can be legally used in goats in an extra-label fashion when approved drugs have been deemed clinically ineffective. In addition to efficacy for the treatment of caprine respiratory disease, tulathromycin has been utilized as therapy for caseous lymphadenitis (Washburn, Bissett et al., 2009). When used in cattle, tulathromycin also possesses activity against *Fusobacterium necrophorum*, *Porphyromonas levii*, and *Moraxella bovis*. Tulathromycin has demonstrated pharmacokinetics in goats similar to what has been reported for cattle and swine (Clothier, Leavens et al., 2011). These parameters indicate rapid absorption, with maximum concentrations being reached approximately one hour after administration and a long
plasma elimination half-life (Romanet, Smith et al., 2012). Additionally, tulathromycin has demonstrated similar tissue elimination in goats when compared to cattle (Romanet, Smith et al., 2012).

In other veterinary species the presence of infectious respiratory disease has demonstrated alterations in the pharmacokinetics of tulathromycin. Recently, in pigs infected with *Actinobacillus pleuropneumoniae*, tulathromycin demonstrated both a slower elimination half-life as well as a longer drug persistence when compared to healthy pigs (Gajda, Bladek et al., 2016). However, currently no studies demonstrate the effect of respiratory disease on the pharmacokinetics and tissue residue marker concentrations of tulathromycin in goats. The increasing size of the US meat goat herd, as well as the potential for residues in goat products, presents a food safety issue. Withdrawal times are calculated based on healthy animals and the presence of disease may influence pharmacokinetics and tissue residue concentrations. The objective of this study was to determine the pharmacokinetics and tissue residue concentrations of tulathromycin in goats with experimentally-induced respiratory disease. Our hypothesis was that the presence of disease would result in altered plasma and tissue concentrations of tulathromycin when compared to healthy goats.

**MATERIALS AND METHODS**

**Experimental animals**

This study was completed at the Iowa State University (ISU) Livestock Infectious Disease Isolation Facility (LIDIF). Twelve healthy 8-10 month old female meat goats (Boer and Boer-cross) weighing $34.7 \pm 4.6$ kg, were enrolled in the study. Eligible goats had no prior drug administration and no history of respiratory disease. Goats were then randomly assigned by weight
into one of two groups: control (N=6) vs. experimental (N=6, details below). Each cohort was
group-housed in individual climate controlled rooms at the LIDIF.
During each treatment segment, goats were housed in raised group pens. Each pen had individual
access to feed and water. Goats were fed a mixed hay ration and water *ad libitum*. Ration
parameters met or exceeded those recommended by the NRC guidelines (NRC, 2001). In addition,
animal housing and management met the recommendations listed in the *Guide for Care and Use
of Agricultural Animals in Research and Teaching* (FASS, 2010). The research protocol was
approved by the ISU Institutional Animal Use and Care Committee prior to commencement of
trial procedures (protocol number-5-17-8517-F).

*Experimental design – Respiratory Challenge*

Three days prior to treatment, the six experimental group goats were administered *P. multocida*
strain P1062 (type A3) via intratracheal and intranasal inoculation as described by Smith et al.
(manuscript pending). The isolate was grown from stock culture and standardized as previously
reported (Elazab, Schrunk et al., 2018). Goats were assessed every 12 hours and deemed to be
infected when tachypnea (respiratory rate greater than 20% of that recorded at intake), abnormal
lung sounds (defined as harsh bronchovesicular sounds, crackles, and wheezes) were noted along
with infectious changes on the leukogram. Following confirmation of infection, the experimental
group of goats was treated with tulathromycin.
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102 **Drug administration**

For treatment and sample collection, goats were restrained via halter. At time 0 (T0), all goats received tulathromycin (Draxxin®; Zoetis Inc., New York, NY), at 2.5 mg per kg of body weight administered subcutaneously in the left neck as described on the package insert for beef cattle. No further medications were administered throughout the remainder of the experiment.

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108 **Collection of blood samples**

Prior to tulathromycin administration (T0), a 10-mL blood sample was collected from the jugular vein via vacutainer (BD Vacutainer; Franklin Lake, NJ) into blood tubes containing freeze-dried heparin (Becton, Dickinson and Co, Franklin Lakes, NJ). Subsequent blood samples were collected from alternating jugular veins into heparinized tubes at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24 hr and then every 24 hr after drug administration through 312 hr. Within 2 h of collection, blood samples were centrifuged for 20 min at 1000 g at 4 °C, then 5 mL of plasma was harvested and frozen at -70°C until analyzed for drug concentration.

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118 **Daily observations**

Study goats were observed daily for physical examination parameters relevant to respiratory disease (pyrexia, tachypnea, abnormal respiratory noise, discharge and tachycardia) and were assessed twice daily for general parameters of health such as appetite and responsiveness to stimuli.
To conserve animal resources, two control goats were enrolled in a separate, unrelated study at T312. At T312 hr, all remaining goats were humanely euthanized with a captive bolt as described by Plummer (Plummer, Shearer et al., 2018) followed by exsanguination. Following euthanasia, kidney, liver, skeletal muscle, and fat were collected, and then frozen at -70 °C until analyzed for common fragment concentration.

**Plasma tulathromycin concentration analysis**

Plasma concentrations of tulathromycin were determined using high-pressure liquid chromatography with mass spectrometry detection (LC-MS/MS) after precipitation of plasma proteins with acetonitrile as described previously for cattle (Coetzee, Kleinhenz et al., 2018). LC-MS/MS was performed using an Agilent 1100 Pump, column compartment, and autosampler (Santa Clara, CA, USA) coupled to an ion trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA, USA). Sequences consisting of plasma blanks, calibration spikes, QC’s, and caprine plasma samples were batch processed with a processing method developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). 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 tulathromycin in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. Twelve calibration spikes were prepared in blank caprine plasma covering the concentration range of 1 to 5,000 ng/mL. Calibration curves exhibited a correlation coefficient (R²) exceeding 0.993 across the concentration range. QC samples at 15, 150, and 1500 ng/mL were within ±15% of the nominal value with most of the QC’s within ±5%
of the nominal value. The limit of quantitation (LOQ) of the analysis was 1 ng/mL with a limit of
detection (LOD) of 0.2 ng mL.

Tissue CP-60,300 concentration analysis

Tissue concentrations (liver, kidney, muscle, fat) of tulathromycin were determined using high-
pressure liquid chromatography with mass spectrometry detection (LC-MS/MS) after acidic
hydrolysis of tissue residues of tulathromycin to the common hydrolytic fragment, CP-60,300. LC-
MS/MS was performed using an Agilent 1100 Pump, column compartment, and autosampler
(Santa Clara, CA, USA) coupled to an ion trap mass spectrometer (LTQ, Thermo Scientific, San
Jose, CA, USA). Homogenized tissue samples, tissue spikes, and caprine tissue blanks, 1 gram,
were hydrolyzed with 2 N hydrochloric acid (HCl), 4 mL, for 1 hour at 60°C. A second addition
of 3.5 mL of HCl to the tissue samples was performed after centrifugation of the tissue digest and
removal of the supernatant. The samples were then vortexed and shaken followed by
centrifugation. The supernatant from this second extraction was combined with the supernatant
from the first digestion and the volume was adjusted to 8 mL For LC-MS/MS analysis the samples
and spikes/blanks were diluted 1:20 with a 0.1 M potassium acetate buffer, pH 5.0 in autosampler
vials. The buffer contained an internal standard of roxithromycin at a concentration of 50 ng/mL.
The vials were then centrifuged at 2,400 rpm prior to analysis.

For LC-MS/MS analysis the injection volume was set to 12.5 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.
Separation was achieved with an ACE 3 C18 column, 150 mm x 2.1 mm, 3 μm particles (Mac-
Mod Analytical, Chadds Ford, PA, USA) maintained at 40°C. The analysis was performed starting
at a solvent composition of 5% B which was increased linearly to 95% B in 8 minutes after injection. The solvent composition was maintained at 95% B for 2 minutes prior to equilibration to 5% B. The flow rate during this time period was 0.325 mL/min. The tulathromycin marker, CP-60,300, and roxithromycin eluted from the ACE 3 C18 column at 5.81 ± 0.05 and 8.29 ± 0.05 minutes, respectively. Full scan positive ion MS of the precursor ions of the analytes was used for residue detection. The doubly charged precursor ion of CP-60,300 (m/z 289.4) and singly charged roxithromycin (m/z 837.3) were used for MS fragmentation in the tulathromycin analysis. The fragment ions of the doubly charged CP-60,300 marker precursor at m/z 289.4 were 158.2, 231.3, and 420.3 m/z. The fragment ions of the roxithromycin precursor ion at m/z of 837.3 were at 522.3, 558.3, and 679.3 m/z.

Each set of tissue samples was run with six calibration spikes (tulathromycin) prepared in the corresponding blank caprine tissue matrix along with tissue blank. The calibration spikes covered a range from 0.2 to 10 ug/g or 0.5-20 ug/g (caprine liver). After a set of tissue samples were run the results were batch processed with a processing method developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). 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. Tissue concentrations of the CP-60,300 marker in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. All calibration curves exhibited a correlation coefficient (R²) exceeding 0.998 across the concentration range. All of the calibration spikes in each tissue were within ± 7% of the nominal concentration with the majority of the spikes within ± 3%. The
limit of quantitation (LOQ) of the analysis was 0.2 µg/g with a limit of detection (LOD) of 0.02 µg/g.

Pharmacokinetic analysis

Pharmacokinetic analysis of total tulathromycin plasma concentration was completed using a statistical moment (i.e. non-compartmental) approach in commercial software (Phoenix WinNonlin 8.1, Certara, Princeton, NJ, USA). Time versus concentration figures for tulathromycin were produced via a commercial program (GraphPad Prism 8.0, GraphPad Software, Inc, La Jolla, CA, USA).

Standard PK parameters were generated for individual goats, as follows:

- Maximum tulathromycin concentration, $C_{\text{max}}$;
- Time of maximum tulathromycin concentration, $T_{\text{max}}$;
- Area under tulathromycin concentration-time curve, $AUC_{\text{last}}$;
- Area under the moment curve, $AUMC_{\text{inf}}$;
- Tulathromycin mean residence time, $MRT = \frac{AUMC_{\text{inf}}}{AUC_{\text{inf}}}$;
- Slope of the elimination phase $\lambda_z$, computed by linear regression of the logarithmic concentration vs. time curve during the elimination phase;
- Tulathromycin terminal half-life, $T1/2(\lambda_z) = \frac{\ln(2)}{\lambda_z}$;
- Tulathromycin apparent clearance, $CL/F = \frac{\text{Dose}}{AUC_{\text{inf}}}$;
- Apparent volume of distribution of tulathromycin during the elimination phase, $V_z/F = \frac{\text{Dose}}{(AUC_{\text{inf}} \times \lambda_z)}$;
For data analysis, the first value below the LLOQ was inferred to be LLOQ/2, and subsequent data points were excluded from the analysis. A linear/log trapezoidal rule was used to estimate the area under the tulathromycin time-curves. Summary statistics on the individual PK parameters were performed thereafter to derive the geometric mean, median and (min-max) range.

Data analysis

Drug concentrations were compared at each time point using contrasts. Comparison of variables between treatment groups that were single observations (i.e., enrollment variables and PK parameters) were made using a paired t-test when data were normally distributed and with a Wilcoxon signed rank test when distributions were not normally distributed. Comparisons of tissue marker residue (common fragment CP-60,300) concentrations at 312 hours were made using the Wilcoxon signed ranked test. Statistical significance was established when $P < 0.05$.

Statistical Analysis

Data distributions for all pharmacokinetic parameters were normality assessed by Shapiro-Wilk tests. Comparisons between the two experimental groups were performed via unpaired t tests for normally distributed parameters and Mann-Whitney tests for nonparametric parameters via a commercial program (GraphPad Prism 8, GraphPad Software, Inc, La Jolla, CA, USA).

RESULTS

3.1 Animal health
At enrollment, all study subjects were assessed to be healthy and to have parameters within the normal limits for goats of their respective ages. The injections were well tolerated by all goats, although three goats from each group vocalized during the injection. For heart rate, respiratory rate and temperature, no significant elevation or depression from baseline was reported amongst the control group. The experimental group had elevations above the normal baseline in rectal temperature and respiratory rate. Hematologically, the experimental group had elevations above caprine normal as well as pre-induction baselines for serum fibrinogen. Five of the six experimental goats had toxic changes present in their neutrophils at the time of treatment. No differences in body weight were noted between the control (34.3 ± 4.1 kg) and experimental (35.1 ± 5.5 kg) groups (P = 0.77).

### 3.2 Pharmacokinetics of tulathromycin

No goat had detectable tulathromycin in plasma at time zero. The mean time-course of tulathromycin total concentrations in plasma can be found in Figure 1. Geometric mean profiles are presented in Table 1 for both groups. Among individuals in each group, there appears to be limited variation of time vs. concentration data noted by moderate variations of AUC\textsubscript{last} CV% amongst groups (control: 47.3%; experimental: 59.6%). When both groups are compared, there appears to be variation in the initial curve (Figure 2.), however elimination appears to be similar for each group approximated by similar slopes of the terminal phase. Non-significant differences were found on comparison of pharmacokinetic parameters (mean ± SD) between groups of C\textsubscript{max} (control: 3111.0 ± 2451.4 ng/mL; experimental: 1295.5 ± 630.2 ng/mL; P = 0.17), T\textsubscript{max} (control: 0.37 ± 0.14 hr; experimental: 0.54 ± 0.25 hr; P = 0.36), CL/F (control: 0.21 ± 0.06 mL/hr/kg; experimental: 0.31 ± 0.11 mL/hr/kg; P = 0.09), T1/2(λ\textsubscript{z}) (control: 90.7 ± 24.6 hr; experimental:...
125.7 ± 38.6; P = 0.13), AUC_{last} (control: 12630.9 ± 5972.6 hr*ng/mL; experimental: 8873.3 ± 
5290.7 hr*ng/mL; P = 0.06), and MRT (control: 85.6 ± 24.6; experimental: 93.0 ± 23.3; P = 
0.39). A significant difference was found between groups for V_z (control: 28.7 ± 11.9 mL/kg; 
experimental: 57.8 ± 26.5 mL/kg; P = 0.045).

Time point comparisons for plasma tulathromycin concentrations are presented in Table 2. With 
the exception of the +1 hour timepoint, the experimental group displayed an apparent decrease in 
plasma tulathromycin concentrations when compared to the control group. The initial time point 
concentrations are much greater for the control group (Table 2) when compared to the 
experimental group. All time point differences were compared amongst groups. Significant 
differences were noted in time points at: 168 hours (control: 15.2 ± 3.0 ng/mL; experimental:
7.7 ± 3.4 ng/mL; P = 0.02), 216 hours (control: 11.2 ± 3.3 ng/mL; experimental: 7.2 ± 4.4 
ng/mL; P = 0.03), 264 hours (control: 9.6 ± 2.5 ng/mL; experimental: 4.6 ± 1.4 ng/mL; P = 
0.0017), 288 hours (control: 6.1 ± 1.4 ng/mL; experimental: 4.1 ± 1.8 ng/mL; P = 0.043), and 
312 hours (control: 5.5 ± 0.7 ng/mL; experimental: 4.0 ± 1.4 ng/mL; P = 0.048).
3.3 Tissue residue concentrations of tulathromycin marker

All tissues contained detectable amounts of CP-60,300. When compared amongst groups no statistically significant concentration differences were found between muscle (control: 0.40 ± 0.045 µg/g; experimental: 0.34 ± 0.045 µg/g; P = 0.21), liver (control: 2.63 ± 0.28 µg/g; experimental: 2.28 ± 0.49 µg/g; P = 0.35), and fat (control: 0.12 ± 0.03 µg/g; experimental: 0.14 ± 0.09 µg/g; P = 0.66). A statistically significant difference was found between kidney tissues of each group (control: 1.56 ± 0.15 µg/g; experimental: 1.20 ± 0.16 µg/g; P = 0.009). Tissue concentration values for each group, of muscle and fat, as well as liver and kidney are presented in Figures 3A and 3B respectively.

DISCUSSION

To the author’s knowledge, this is the first report discussing the pharmacokinetics of tulathromycin in the context of goat respiratory disease. Although the research housing could potentially be a source of bias for this study, it was thought to be minimal as the goats were sourced from the same herd and were group-housed in two separate rooms that had identical temperature, humidity, ventilation, and light control settings. The age, breed, and size of the goats used for this study were designed to mimic young market goats that commonly enter the food chain.

In the United States tulathromycin is not currently labelled for use in goats. However, it may be used in an extralabel manner under a veterinary-client-patient relationship and the AMDUCA guidelines. Currently only two antibiotics, ceftiofur and neomycin are labelled for goats in the United States, so extra-label use is common when treating small ruminants for respiratory
disease if the approved drug is clinically ineffective. Tulathromycin has been shown to be an ideal antimicrobial for the treatment of respiratory disease in goats, based on 100% susceptibility of isolates of *M. haemolytica*, *P. multocida*, and *B. trehalosi* taken from goats with pneumonia (Clothier, Kinyon et al., 2012). It could be argued that the experimental group in our study exhibited successful treatment with tulathromycin to our isolate of *P. multocida* due to the lack of mortality and resolution of morbidity after treatment in our experimental group.

One recent study has determined altered pharmacokinetics and tissue disposition of tulathromycin with respiratory disease in pigs (Gajda, Bladek et al., 2016). In that study differences in the plasma peak concentrations as well increased levels of tulathromycin in the lungs of pigs infected with *Actinobacillus pleuropneumoniae* was observed. A notable difference in the plasma $C_{\text{max}}$ was also noted in the goats of our study (control: 3111.1 ± 2451.4 ng/mL; experimental: 1295.5 ± 630.2 ng/mL), but this difference was not statistically significant ($P = 0.11$). Studies in swine with infectious and inflammatory respiratory disease have also noted decreased maximal plasma concentrations in experimental vs control groups. (Villarino, Lesman et al., 2013; Gajda, Bladek et al., 2016)

The pharmacokinetic parameters of tulathromycin reported in our study varied slightly from those previously reported in the literature. Specifically, The $C_{\text{max}}$ from our control group (3111.1 ± 2451.4 ng/mL) was significantly higher than the $C_{\text{max}}$ (1000 ± 420 ng/mL) reported in healthy goats by Romenet et al (Romanet, Smith et al., 2012), despite similar sampling timepoints. In addition, the estimated $C_{\text{max}}$ from our study varied significantly from that reported in dairy goats (121.5 ± 19.0 ng/mL), however this study collected plasma samples every 12 hours, so a significant reduction in maximum concentration would be expected (Grismer, Rowe et al., 2014).

When compared to the 12 hour plasma concentrations of our control goats (225.3 ± 245.6
ng/mL) more similarities were observed. When the 12 hour time point of one outlier goat (721.5 ng/mL) is removed, the 12 hour time points from our study (126.0 ± 39.3 ng/mL) align very closely to the reported 12 hour timepoint plasma concentrations for dairy goats (121.5 ± 19.0 ng/mL). Variation in the elimination half-life has been noted in infected pigs (Gajda, Bladek et al., 2016) and this was also noted in our study (control: 90.69 ± 24.63 hr; experimental: 125.75 ± 38.57).

CP-60,300 was utilized for tissue concentration as this is the FDA-approved regulatory method with respect to isoforms of tulathromycin, and the assay utilized has been validated for goats (Clothier, Leavens et al., 2012). This technique detects any isoform of tulathromycin and as such, will give the most conservative level of residue concentrations in tissue samples (Romanet, Smith et al., 2012). Muscle, liver, kidney and fat were chosen for analysis as these represent common edible tissues. For cattle in the United States the approved tolerance limit of CP-60,300 is 5 µg/g. Currently for cattle in the European Union the maximum residue limit (MRL) is 4.5 µg/g for liver, 3.0 µg/g for kidney, 0.3 µg/g for muscle and 0.2 µg/g for fat (EMA, 2015). Among our goats all tissue levels of all groups were below the US bovine tolerance level, and among the EU MRLs only the muscle levels were above the EU Bovine MRL. Since tulathromycin is currently not labelled for goats in the US, the withdrawal interval would be calculated based on the FDA recommendations (MacLachlan & Mueller, 2012), which would be based on the lower limit of detection, and has been reported by another study to be 34 days for goats with a lower limit of detection of 0.3 ppm (Romanet, Smith et al., 2012). Our equipment was more sensitive than used in that study, with a lower limit of detection of 0.02 ppm.
The goats in our study had higher tissue levels when compared to healthy goats administered a 2.5 mg/kg dose of tulathromycin subcutaneously and tested at 12 days post-injection, despite being collected 24 hours later. Clothier et al. found liver values of 1.18 ± 0.42 µg/g (Clothier, Leavens et al., 2012) which was lower compared to our goats (2.63 ± 0.28 µg/g; experimental: 2.28 ± 0.49 µg/g). That study also had muscle concentrations measuring < LOD (0.24 µg/g), which would be lower when compared to the muscle concentrations of the goats in our study (control: 0.40 ± 0.045 µg/g; experimental: 0.34 ± 0.045 µg/g). The levels of tulathromycin in fat found in our goats (control: 0.12 ± 0.03 µg/g; experimental: 0.14 ± 0.09 µg/g) could be similar to what was determined in the Clothier study, as no detectable levels were determined, but the level of detection utilized was 0.14 µg/g, which was close to our results. The kidney concentrations detected in our goats would also be higher than those determined by Clothier et al, at 12 days as they found levels also below the level of detection (0.29 µg/g) and our study goats had levels (control: 1.56 ± 0.15 µg/g; experimental: 1.20 ± 0.16 µg/g; P = 0.0095) higher than those reported. These differences could be due to differences in age, sex, breed and weight of the goats used in both studies as Clothier et al used 5-6 month old, male (castrated and intact), goats of dairy and meat breeds that weighed 13.8-27.4 kg, and our study utilized 8-10 month old, female goats, weighing 34.7 ± 4.6 Kg, of meat breeds. These differences could be due to breed, as differences in tulathromycin clearance has been observed between dairy calves (CL/F 0.33 L/h/kg) (Mzyk, Bublitz et al., 2018) and beef calves (CL/F 0.18 L/h/kg) (Nowakowski, Inskeep et al., 2004).

In other studies tulathromycin has been shown to concentrate in the lung, and swine studies suggest that the lung is the target organ for the drug. (Gajda, Bladek et al., 2016) In an intranasal challenge model using the Escherichia coli lipopolysaccharide in mice, tulathromycin had a 1.7-
2.8 times higher exposure in the lungs of mice treated with lipopolysaccharide compared to controls (Villarino, Brown et al., 2012). While plasma levels of macrolide antibiotics have not been shown to correlate with lung concentrations in animals with respiratory disease (Toutain, Potter et al., 2017). It is possible that the significant differences in plasma concentration noted at approximately 168 hours post injection is due to more drug residing in the lungs of the experimental group.

An unexpected finding of our study was the reduced renal concentrations in the experimental group when compared to the controls. In pigs it has been noted that tulathromycin is excreted by biliary and renal excretion (Villarino, Brown et al., 2013), and in cattle the major route of excretion is thought to be is biliary excretion (Villarino, Brown et al., 2014), these reduced renal concentrations could suggest increased renal excretion by the goat.

Limitations

A limitation of this study was the relatively small number of goats utilized, which could have hampered the statistical power of some of our comparisons. Also, while 4-6 animals are commonly used for PK studies (Riviere, 2011), this numbers utilized might not account for all population variability. This limitation is evident by the maximum concentrations observed in mean Cmax values of each group being 2.4 times greater for the control group, but this difference not being statistically significant. This intrinsic high variability of the studied goats, specifically the control goats, with respect to Cmax would prevent the obtaining of a statistically significant result even though there was a 2.4 fold difference in the Cmax of the control and experimental groups. All of the animals were of approximately the same age, which may not be
reflective of all meat goat populations. An additional limitation of this study is the reduced number of control goats that had tissue samples collected. While our study focused on edible tissues (liver, muscle, kidney and fat) future studies should consider differences in lung concentration in diseased goats. Since our study evaluated tissue residues at one time point, future studies should consider multiple tissue sampling to determine the effect of respiratory disease on tissue residue disposition in meat goats.

CONCLUSIONS

In conclusion, while there appears to be no overall statistically significant differences in the pharmacokinetics of tulathromycin between healthy and diseased goats, we did observe significant differences in tulathromycin plasma concentrations at multiple time points (168-312 hr). Specifically, goats infected with *P. multocida* demonstrated decreased plasma concentrations compared to healthy goats at approximately 168 hours after administration. There do not appear to be significant differences in edible tissue residues, with the exception of decreased kidney concentrations, amongst groups at 13 days post-injection Similarly, a significant difference was identified in kidney tissue levels, which when considered with the differences in plasma concentration at later time points may suggest a difference in the terminal depletion process. While tulathromycin is currently used in an extralabel manner in goats, the results of this study suggest that experimental *P. multocida* respiratory disease may have the potential to alter pharmacokinetics or tissue residue concentrations of tulathromycin in meat goats. Further studies including larger numbers of animals are warranted to confirm these preliminary observations.
Conflicts of Interest

The authors report no conflict of interest.

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Author contributions

JSS was involved in study design and execution, sample collection and analysis, manuscript preparation and submission. JPM was involved in study design, sample analysis and manuscript preparation. DJB was involved in study design, method development, sample analysis and manuscript preparation. RWG was involved in study design, sample analysis and manuscript preparation. All authors have read and approved the final manuscript.

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Effects of experimentally-induced respiratory disease on the pharmacokinetics and tissue residues of tulathromycin in meat goats

J. S. Smith\textsuperscript{1*}, J. P. Mochel\textsuperscript{1}, D. J. Borts\textsuperscript{1} and R. W. Griffith\textsuperscript{2}

Affiliations

\textsuperscript{1}Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, 50010, US.

\textsuperscript{2}Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, 50010, US.

*Corresponding author (J.S. Smith, jss303@iastate.edu, 515-294-1500)
Abstract

Tulathromycin is a macrolide antibiotic commonly used for the treatment of respiratory disease in food animal species including goats. Recent research in pigs has suggested that the presence of disease could alter the pharmacokinetics of tulathromycin in animals with respiratory disease. The objectives of this study were (a) compare the plasma pharmacokinetics of tulathromycin in healthy goats as well as goats with an induced respiratory disease; and (b) to compare the tissue residue concentrations of tulathromycin marker in both groups. For this trial, disease was induced with Pasteurella multocida. Following disease induction tulathromycin was administered. Samples of plasma were collected at various time points up to 312 hours post-treatment, when study animals were euthanized and tissue samples were collected. For PK parameters in plasma, $V_z$ (control: $28.7 \pm 11.9$ mL/kg; experimental: $57.8 \pm 26.6$ mL/kg), was significantly higher ($P = 0.0454$) in the experimental group than the control group, and non-significant differences were noted in other parameters. Among time points significantly lower plasma concentrations were noted in the experimental group at 168 hours ($P = 0.023$), 216 hours ($P = 0.036$), 264 hours ($P = 0.0017$), 288 hours ($P = 0.0433$), and 312 hours ($P = 0.0486$). None of the goats had tissue residues above the US bovine limit of 5 µg/g at the end of the study. No differences were observed between muscle, liver, or fat concentrations. A significantly lower concentration ($P = 0.0095$) was noted in the kidneys of experimental goats when compared to the control group. These results suggest that the effect of respiratory disease on the pharmacokinetics and tissue residues appear minimal after experimental P. multocida infection, however as evidenced by the disparity in $C_{max}$, significant differences in plasma concentrations at terminal time points, as well as the differences in kidney concentrations, there is the potential for alterations in diseased vs clinical animals.
INTRODUCTION

Tulathromycin is a macrolide antibiotic widely used for respiratory disease in cattle and pigs due to its broad spectrum of antimicrobial activity. The broad spectrum of activity and long-acting formulation also make this an ideal antibiotic for treating respiratory disease in goats, along with its efficacy against caprine respiratory isolates of *Mannheimia hemolytica*, *Pasteurella multocida*, and *Bibersteinia trehalosi* (Clothier, Kinyon et al., 2012). Due to the importance of broad spectrum antimicrobials for veterinary as well as human medicine, the World Health Organization has classified macrolide antibiotics on the list of the highest priority, critically important antimicrobials for the preservation of human health. (WHO, 2017) This prioritization from the WHO is based on 1) the high number of people affected by diseases for which a macrolide is the sole or one of the few therapies, 2) the high frequency of use in human medicine, and 3) the potential transmission of *Campylobacter spp* from non-human sources. (WHO, 2017)

In the United States tulathromycin can be legally used in goats in an extra-label fashion when approved drugs have been deemed clinically ineffective. In addition to efficacy for the treatment of caprine respiratory disease, tulathromycin has been utilized as therapy for caseous lymphadenitis (Washburn, Bissett et al., 2009). When used in cattle, tulathromycin also possesses activity against *Fusobacterium necrophorum*, *Porphyromonas levii*, and *Moraxella bovis*. Tulathromycin has demonstrated pharmacokinetics in goats similar to what has been reported for cattle and swine (Clothier, Leavens et al., 2011). These parameters indicate rapid absorption, with maximum concentrations being reached approximately one hour after administration and a long
plasma elimination half-life (Romanet, Smith et al., 2012). Additionally, tulathromycin has demonstrated similar tissue elimination in goats when compared to cattle (Romanet, Smith et al., 2012).

In other veterinary species the presence of infectious respiratory disease has demonstrated alterations in the pharmacokinetics of tulathromycin. Recently, in pigs infected with *Actinobacillus pleuropneumoniae*, tulathromycin demonstrated both a slower elimination half-life as well as a longer drug persistence when compared to healthy pigs (Gajda, Bladek et al., 2016). However, currently no studies demonstrate the effect of respiratory disease on the pharmacokinetics and tissue residue marker concentrations of tulathromycin in goats. The increasing size of the US meat goat herd, as well as the potential for residues in goat products, presents a food safety issue. Withdrawal times are calculated based on healthy animals and the presence of disease may influence pharmacokinetics and tissue residue concentrations. The objective of this study was to determine the pharmacokinetics and tissue residue concentrations of tulathromycin for goats with experimentally-induced respiratory disease. Our hypothesis was that the presence of disease would result in altered plasma and tissue concentrations of tulathromycin when compared to healthy goats.

**MATERIALS AND METHODS**

**Experimental animals**

This study was completed at the Iowa State University (ISU) Livestock Infectious Disease Isolation Facility (LIDIF). Twelve healthy 8-10 month old female meat goats (Boer and Boer-cross) weighing 34.7 ± 4.6 kg, were enrolled in the study. Eligible goats had no prior drug administration and no history of respiratory disease. Goats were then randomly assigned by weight
into one of two groups: control (N=6) vs. experimental (N=6, details below). Each cohort was
group-housed in individual climate controlled rooms at the LIDIF.
During each treatment segment, goats were housed in raised group pens. Each pen had individual
access to feed and water. Goats were fed a mixed hay ration and water *ad libitum*. Ration
parameters met or exceeded those recommended by the NRC guidelines (NRC, 2001). In addition,
animal housing and management met the recommendations listed in the *Guide for Care and Use
of Agricultural Animals in Research and Teaching* (FASS, 2010). The research protocol was
approved by the ISU Institutional Animal Use and Care Committee prior to commencement of
trial procedures (protocol number-5-17-8517-F).

**Experimental design – Respiratory Challenge**

Three days prior to treatment, the six experimental group goats were administered *P. multocida*
strain P1062 (type A3) via intratracheal and intranasal inoculation as described by Smith et al.
(manuscript pending). The isolate was grown from stock culture and standardized as previously
reported (Elazab, Schrunk et al., 2018). Goats were assessed every 12 hours and deemed to be
infected when tachypnea (respiratory rate greater than 20% of that recorded at intake), abnormal
lung sounds (defined as harsh bronchovesicular sounds, crackles, and wheezes) were noted along
with infectious changes on the leukogram. Following confirmation of infection, the experimental
group of goats was treated with tulathromycin.
Drug administration

For treatment and sample collection, goats were restrained via halter. At time 0 (T0), all goats received tulathromycin (Draxxin®; Zoetis Inc., New York, NY), at 2.5 mg per kg of body weight administered subcutaneously in the left neck as described on the package insert for beef cattle. No further medications were administered throughout the remainder of the experiment.

Collection of blood samples

Prior to tulathromycin administration (T0), a 10-mL blood sample was collected from the jugular vein via vacutainer (BD Vacutainer; Franklin Lake, NJ) into blood tubes containing freeze-dried heparin (Becton, Dickinson and Co, Franklin Lakes, NJ). Subsequent blood samples were collected from alternating jugular veins into heparinized tubes at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24 hr and then every 24 hr after drug administration through 312 hr. Within 2 h of collection, blood samples were centrifuged for 20 min at 1000 g at 4 °C, then 5 mL of plasma was harvested and frozen at -70°C until analyzed for drug concentration.

Daily observations

Study goats were observed daily for physical examination parameters relevant to respiratory disease (pyrexia, tachypnea, abnormal respiratory noise, discharge and tachycardia) and were assessed twice daily for general parameters of health such as appetite and responsiveness to stimuli.
Trial conclusion

To conserve animal resources, two control goats were enrolled in a separate, unrelated study at T312. At T312 hr, all remaining goats were humanely euthanized with a captive bolt as described by Plummer (Plummer, Shearer et al., 2018) followed by exsanguination. Following euthanasia, kidney, liver, skeletal muscle, and fat were collected, and then frozen at -70 °C until analyzed for common fragment concentration.

Plasma tulathromycin concentration analysis

Plasma concentrations of tulathromycin were determined using high-pressure liquid chromatography with mass spectrometry detection (LC-MS/MS) after precipitation of plasma proteins with acetonitrile as described previously for cattle (Coetzee, Kleinhenz et al., 2018). LC-MS/MS was performed using an Agilent 1100 Pump, column compartment, and autosampler (Santa Clara, CA, USA) coupled to an ion trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA, USA). Sequences consisting of plasma blanks, calibration spikes, QC’s, and caprine plasma samples were batch processed with a processing method developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). 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 tulathromycin in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. Twelve calibration spikes were prepared in blank caprine plasma covering the concentration range of 1 to 5,000 ng/mL. Calibration curves exhibited a correlation coefficient ($R^2$) exceeding 0.993 across the concentration range. QC samples at 15, 150, and 1500 ng/mL were within ±15% of the nominal value with most of the QC’s within ±5%.
of the nominal value. The limit of quantitation (LOQ) of the analysis was 1 ng/mL with a limit of
detection (LOD) of 0.2 ng/mL.

**Tissue CP-60,300 concentration analysis**

Tissue concentrations (liver, kidney, muscle, fat) of tulathromycin were determined using high-pressure liquid chromatography with mass spectrometry detection (LC-MS/MS) after acidic
hydrolysis of tissue residues of tulathromycin to the common hydrolytic fragment, CP-60,300. LC-
MS/MS was performed using an Agilent 1100 Pump, column compartment, and autosampler
(Santa Clara, CA, USA) coupled to an ion trap mass spectrometer (LTQ, Thermo Scientific, San
Jose, CA, USA). Homogenized tissue samples, tissue spikes, and caprine tissue blanks, 1 gram,
were hydrolyzed with 2 N hydrochloric acid (HCl), 4 mL, for 1 hour at 60° C. A second addition
of 3.5 mL of HCl to the tissue samples was performed after centrifugation of the tissue digest and
removal of the supernatant. The samples were then vortexed and shaken followed by
centrifugation. The supernatant from this second extraction was combined with the supernatant
from the first digestion and the volume was adjusted to 8 mL For LC-MS/MS analysis the samples
and spikes/blanks were diluted 1:20 with a 0.1 M potassium acetate buffer, pH 5.0 in autosampler
vials. The buffer contained an internal standard of roxithromycin at a concentration of 50 ng/mL.
The vials were then centrifuged at 2,400 rpm prior to analysis.

For LC-MS/MS analysis the injection volume was set to 12.5 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.
Separation was achieved with an ACE 3 C18 column, 150 mm x 2.1 mm, 3 μm particles (Mac-
Mod Analytical, Chadds Ford, PA, USA) maintained at 40°C. The analysis was performed starting
at a solvent composition of 5% B which was increased linearly to 95% B in 8 minutes after
injection. The solvent composition was maintained at 95% B for 2 minutes prior to equilibration
to 5% B. The flow rate during this time period was 0.325 mL/min. The tulathromycin marker, CP-
60,300, and roxithromycin eluted from the ACE 3 C18 column at 5.81 ± 0.05 and 8.29 ± 0.05
minutes, respectively. Full scan positive ion MS of the precursor ions of the analytes was used for
residue detection. The doubly charged precursor ion of CP-60,300 (m/z 289.4) and singly charged
roxithromycin (m/z 837.3) were used for MS fragmentation in the tulathromycin analysis. The
fragment ions of the doubly charged CP-60,300 marker precursor at m/z 289.4 were 158.2, 231.3,
and 420.3 m/z. The fragment ions of the roxithromycin precursor ion at m/z of 837.3 were at 522.3,
558.3, and 679.3 m/z.

Each set of tissue samples was run with six calibration spikes (tulathromycin) prepared in the
corresponding blank caprine tissue matrix along with tissue blank. The calibration spikes covered
a range from 0.2 to 10 ug/g or 0.5-20 ug/g (caprine liver). After a set of tissue samples were run
the results were batch processed with a processing method developed in the Xcalibur software
(Thermo Scientific, San Jose, CA, USA). 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. Tissue concentrations of the CP-60,300 marker in unknown samples were calculated by
the Xcalibur software based on the calibration curve. Results were then viewed in the Quan
Browser portion of the Xcalibur software. All calibration curves exhibited a correlation coefficient
(R^2) exceeding 0.998 across the concentration range. All of the calibration spikes in each tissue
were within ± 7% of the nominal concentration with the majority of the spikes within ± 3%. The
limit of quantitation (LOQ) of the analysis was 0.2 µg/g with a limit of detection (LOD) of 0.02 µg/g.

**Pharmacokinetic analysis**

Pharmacokinetic analysis of total tulathromycin plasma concentration was completed using a statistical moment (i.e. non-compartmental) approach in commercial software (Phoenix WinNonlin 8.1, Certara, Princeton, NJ, USA). Time versus concentration figures for tulathromycin were produced via a commercial program (GraphPad Prism 8.0, GraphPad Software, Inc, La Jolla, CA, USA).

Standard PK parameters were generated for individual goats, as follows:

- Maximum tulathromycin concentration, $C_{\text{max}}$;
- Time of maximum tulathromycin concentration, $T_{\text{max}}$;
- Area under tulathromycin concentration-time curve, $AUC_{\text{last}}$;
- Area under the moment curve, $AUMC_{\text{inf}}$;
- Tulathromycin mean residence time, $\text{MRT} = \frac{AUMC_{\text{inf}}}{AUC_{\text{inf}}}$;
- Slope of the elimination phase $\lambda z$, computed by linear regression of the logarithmic concentration vs. time curve during the elimination phase;
- Tulathromycin terminal half-life, $T1/2(\lambda z) = \frac{\ln(2)}{\lambda z}$;
- Tulathromycin apparent clearance, $\text{CL/F} = \frac{\text{Dose}}{AUC_{\text{inf}}}$;
- Apparent volume of distribution of tulathromycin during the elimination phase, $Vz/F = \frac{\text{Dose}}{(AUC_{\text{inf}} \times \lambda z)}$;
For data analysis, the first value below the LLOQ was inferred to be LLOQ/2, and subsequent data points were excluded from the analysis. A linear/log trapezoidal rule was used to estimate the area under the tulathromycin time-curves. Summary statistics on the individual PK parameters were performed thereafter to derive the geometric mean, median and (min-max) range.

Data analysis

Drug concentrations were compared at each time point using contrasts. Comparison of variables between treatment groups that were single observations (i.e., enrollment variables and PK parameters) were made using a paired t-test when data were normally distributed and with a Wilcoxon signed rank test when distributions were not normally distributed. Comparisons of tissue marker residue (common fragment CP-60,300) concentrations at 312 hours were made using the Wilcoxon signed ranked test. Statistical significance was established when $P < 0.05$.

Statistical Analysis

Data distributions for all pharmacokinetic parameters were normality assessed by Shapiro-Wilk tests. Comparisons between the two experimental groups were performed via unpaired t tests for normally distributed parameters and Mann-Whitney tests for nonparametric parameters via a commercial program (GraphPad Prism 8, GraphPad Software, Inc, La Jolla, CA, USA).

RESULTS

3.1 Animal health
At enrollment, all study subjects were assessed to be healthy and to have parameters within the normal limits for goats of their respective ages. The injections were well tolerated by all goats, although three goats from each group vocalized during the injection. For heart rate, respiratory rate and temperature, no significant elevation or depression from baseline was reported amongst the control group. The experimental group had elevations above the normal baseline in rectal temperature and respiratory rate. Hematologically, the experimental group had elevations above caprine normal as well as pre-induction baselines for serum fibrinogen. Five of the six experimental goats had toxic changes present in their neutrophils at the time of treatment. No differences in body weight were noted between the control (34.3 ± 4.1 kg) and experimental (35.1 ± 5.5 kg) groups ($P = 0.77$).

### 3.2 Pharmacokinetics of tulathromycin

No goat had detectable tulathromycin in plasma at time zero. The mean time-course of tulathromycin total concentrations in plasma can be found in Figure 1. Geometric mean profiles are presented in Table 1 for both groups. Among individuals in each group, there appears to be limited variation of time vs. concentration data noted by moderate variations of $\text{AUC}_{\text{last}} \text{CV\%}$ amongst groups (control: 47.3%; experimental: 59.6%). When both groups are compared, there appears to be variation in the initial curve (Figure 2.), however elimination appears to be similar for each group approximated by similar slopes of the terminal phase. Non-significant differences were found on comparison of pharmacokinetic parameters (mean ± SD) between groups of $C_{\text{max}}$ (control: 3111.0 ± 2451.4 ng/mL; experimental: 1295.5 ± 630.2 ng/mL; $P = 0.17$), $T_{\text{max}}$ (control: 0.37 ± 0.14 hr; experimental: 0.54 ± 0.25 hr; $P = 0.36$), $\text{CL/F}$ (control: 0.21 ± 0.06 mL/hr/kg; experimental: 0.31 ± 0.11 mL/hr/kg; $P = 0.09$), $T1/2(\lambda_z)$ (control: 90.7 ± 24.6 hr; experimental: 90.8 ± 24.7 hr; $P = 0.90$).
125.7 ± 38.6; P = 0.13), AUC\textsubscript{last} (control: 12630.9 ± 5972.6 hr*ng/mL; experimental: 8873.3 ± 5290.7 hr*ng/mL; P = 0.06), and MRT (control: 85.6 ± 24.6; experimental: 93.0 ± 23.3; P = 0.39). A significant difference was found between groups for V\textsubscript{z} (control: 28.7 ± 11.9 mL/kg; experimental: 57.8 ± 26.5 mL/kg; P = 0.045).

Time point comparisons for plasma tulathromycin concentrations are presented in Table 2. With the exception of the +1 hour timepoint, the experimental group displayed an apparent decrease in plasma tulathromycin concentrations when compared to the control group. The initial time point concentrations are much greater for the control group (Table 2) when compared to the experimental group. All time point differences were compared amongst groups. Significant differences were noted in time points at: 168 hours (control: 15.2 ± 3.0 ng/mL; experimental: 7.7 ± 3.4 ng/mL; P = 0.02), 216 hours (control: 11.2 ± 3.3 ng/mL; experimental: 7.2 ± 4.4 ng/mL; P = 0.03), 264 hours (control: 9.6 ± 2.5 ng/mL; experimental: 4.6 ± 1.4 ng/mL; P = 0.0017), 288 hours (control: 6.1 ± 1.4 ng/mL; experimental: 4.1 ± 1.8 ng/mL; P = 0.043), and 312 hours (control: 5.5 ± 0.7 ng/mL; experimental: 4.0 ± 1.4 ng/mL; P = 0.048).
3.3 Tissue residue concentrations of tulathromycin marker

All tissues contained detectable amounts of CP-60,300. When compared amongst groups no statistically significant concentration differences were found between muscle (control: 0.40 ± 0.045 µg/g; experimental: 0.34 ± 0.045 µg/g; P = 0.21), liver (control: 2.63 ± 0.28 µg/g; experimental: 2.28 ± 0.49 µg/g; P = 0.35), and fat (control: 0.12 ± 0.03 µg/g; experimental: 0.14 ± 0.09 µg/g; P = 0.66). A statistically significant difference was found between kidney tissues of each group (control: 1.56 ± 0.15 µg/g; experimental: 1.20 ± 0.16 µg/g; P = 0.009). Tissue concentration values for each group, of muscle and fat, as well as liver and kidney are presented in Figures 3A and 3B respectively.

DISCUSSION

To the author’s knowledge, this is the first report discussing the pharmacokinetics of tulathromycin in the context of goat respiratory disease. Although the research housing could potentially be a source of bias for this study, it was thought to be minimal as the goats were sourced from the same herd and were group-housed in two separate rooms that had identical temperature, humidity, ventilation, and light control settings. The age, breed, and size of the goats used for this study were designed to mimic young market goats that commonly enter the food chain.

In the United States tulathromycin is not currently labelled for use in goats. However, it may be used in an extralabel manner under a veterinary-client-patient relationship and the AMDUCA guidelines. Currently only two antibiotics, ceftiofur and neomycin are labelled for goats in the United States, so extra-label use is common when treating small ruminants for respiratory
disease if the approved drug is clinically ineffective. Tulathromycin has been shown to be an
ideal antimicrobial for the treatment of respiratory disease in goats, based on 100% susceptibility
of isolates of *M. haemolytica*, *P. multocida*, and *B. trehalosi* taken from goats with pneumonia
(Clothier, Kinyon et al., 2012). It could be argued that the experimental group in our study
exhibited successful treatment with tulathromycin to our isolate of *P. multocida* due to the lack
of mortality and resolution of morbidity after treatment in our experimental group.

One recent study has determined altered pharmacokinetics and tissue disposition of
tulathromycin with respiratory disease in pigs (Gajda, Bladek et al., 2016). In that study
differences in the plasma peak concentrations as well increased levels of tulathromycin in the
lungs of pigs infected with *Actinobacillus pleuropneumoniae* was observed. A notable difference
in the plasma $C_{\text{max}}$ was also noted in the goats of our study (control: $3111.1 \pm 2451.4$ ng/mL;
experimental: $1295.5 \pm 630.2$ ng/mL), but this difference was not statistically significant ($P =
0.11$). Studies in swine with infectious and inflammatory respiratory disease have also noted
decreased maximal plasma concentrations in experimental vs control groups (Villarino, Lesman
et al., 2013; Gajda, Bladek et al., 2016)

The pharmacokinetic parameters of tulathromycin reported in our study varied slightly from
those previously reported in the literature. Specifically, The $C_{\text{max}}$ from our control group ($3111.1
\pm 2451.4$ ng/mL) was significantly higher than the $C_{\text{max}}$ ($1000 \pm 420$ ng/mL) reported in healthy
goats by Romenet et al (Romanet, Smith et al., 2012), despite similar sampling timepoints. In
addition, the estimated $C_{\text{max}}$ from our study varied significantly from that reported in dairy goats
($121.5 \pm 19.0$ ng/mL), however this study collected plasma samples every 12 hours, so a
significant reduction in maximum concentration would be expected (Grismer, Rowe et al., 2014).
When compared to the 12 hour plasma concentrations of our control goats ($225.3 \pm 245.6$
ng/mL) more similarities were observed. When the 12 hour time point of one outlier goat (721.5 ng/mL) is removed, the 12 hour time points from our study (126.0 ± 39.3 ng/mL) align very closely to the reported 12 hour timepoint plasma concentrations for dairy goats (121.5 ± 19.0 ng/mL). Variation in the elimination half-life has been noted in infected pigs (Gajda, Bladek et al., 2016) and this was also noted in our study (control: 90.69 ± 24.63 hr; experimental: 125.75 ± 38.57).

CP-60,300 was utilized for tissue concentration as this is the FDA-approved regulatory method with respect to isoforms of tulathromycin, and the assay utilized has been validated for goats (Clothier, Leavens et al., 2012). This technique detects any isoform of tulathromycin and as such, will give the most conservative level of residue concentrations in tissue samples (Romanet, Smith et al., 2012). Muscle, liver, kidney and fat were chosen for analysis as these represent common edible tissues. For cattle in the United States the approved tolerance limit of CP-60,300 is 5 µg/g. Currently for cattle in the European Union the maximum residue limit (MRL) is 4.5 µg/g for liver, 3.0 µg/g for kidney, 0.3 µg/g for muscle and 0.2 µg/g for fat.(EMA, 2015) Among our goats all tissue levels of all groups were below the US bovine tolerance level, and among the EU MRLs only the muscle levels were above the EU Bovine MRL. Since tulathromycin is currently not labelled for goats in the US, the withdrawal interval would be calculated based on the FDA recommendations (MacLachlan & Mueller, 2012), which would be based on the lower limit of detection, and has been reported by another study to be 34 days for goats with a lower limit of detection of 0.3 ppm (Romanet, Smith et al., 2012). Our equipment was more sensitive than used in that study, with a lower limit of detection of 0.02 ppm.
The goats in our study had higher tissue levels when compared to healthy goats administered a 2.5 mg/kg dose of tulathromycin subcutaneously and tested at 12 days post-injection, despite being collected 24 hours later. Clothier et al. found liver values of 1.18 ± 0.42 µg/g (Clothier, Leavens et al., 2012) which was lower compared to our goats (2.63 ± 0.28 µg/g; experimental: 2.28 ± 0.49 µg/g). That study also had muscle concentrations measuring < LOD (0.24 µg/g), which would be lower when compared to the muscle concentrations of the goats in our study (control: 0.40 ± 0.045 µg/g; experimental: 0.34 ± 0.045 µg/g). The levels of tulathromycin in fat found in our goats (control: 0.12 ± 0.03 µg/g; experimental: 0.14 ± 0.09 µg/g) could be similar to what was determined in the Clothier study, as no detectable levels were determined, but the level of detection utilized was 0.14 µg/g, which was close to our results. The kidney concentrations detected in our goats would also be higher than those determined by Clothier et al, at 12 days as they found levels also below the level of detection (0.29 µg/g) and our study goats had levels (control: 1.56 ± 0.15 µg/g; experimental: 1.20 ± 0.16 µg/g; P = 0.0095) higher than those reported. These differences could be due to differences in age, sex, breed and weight of the goats used in both studies as Clothier et al used 5-6 month old, male (castrated and intact), goats of dairy and meat breeds that weighed 13.8-27.4 kg, and our study utilized 8-10 month old, female goats, weighing 34.7 ± 4.6 Kg, of meat breeds. These differences could be due to breed, as differences in tulathromycin clearance has been observed between dairy calves (CL/F 0.33 L/h/kg) (Mzyk, Bublitz et al., 2018) and beef calves (CL/F 0.18 L/h/kg) (Nowakowski, Inskeep et al., 2004).

In other studies tulathromycin has been shown to concentrate in the lung, and swine studies suggest that the lung is the target organ for the drug (Gajda, Bladek et al., 2016) In an intranasal challenge model using the *Escherichia coli* lipopolysaccharide in mice, tulathromycin had a 1.7-
2.8 times higher exposure in the lungs of mice treated with lipopolysaccharide compared to controls (Villarino, Brown et al., 2012). While plasma levels of macrolide antibiotics have not been shown to correlate with lung concentrations in animals with respiratory disease (Toutain, Potter et al., 2017). It is possible that the significant differences in plasma concentration noted at approximately 168 hours post injection is due to more drug residing in the lungs of the experimental group.

An unexpected finding of our study was the reduced renal concentrations in the experimental group when compared to the controls. In pigs it has been noted that tulathromycin is excreted by biliary and renal excretion (Villarino, Brown et al., 2013), and in cattle the major route of excretion is thought to be is biliary excretion (Villarino, Brown et al., 2014), these reduced renal concentrations could suggest increased renal excretion by the goat.

Limitations

A limitation of this study was the relatively small number of goats utilized, which could have hampered the statistical power of some of our comparisons. Also, while 4-6 animals are commonly used for PK studies (Riviere, 2011), this numbers utilized might not account for all population variability. This limitation is evident by the maximum concentrations observed in mean Cmax values of each group being 2.4 times greater for the control group, but this difference not being statistically significant. This intrinsic high variability of the studied goats, specifically the control goats, with respect to Cmax would prevent the obtaining of a statistically significant result even though there was a 2.4 fold difference in the Cmax of the control and experimental groups. All of the animals were of approximately the same age, which may not be
reflective of all meat goat populations. An additional limitation of this study is the reduced number of control goats that had tissue samples collected. While our study focused on edible tissues (liver, muscle, kidney and fat) future studies should consider differences in lung concentration in diseased goats. Since our study evaluated tissue residues at one time point, future studies should consider multiple tissue sampling to determine the effect of respiratory disease on tissue residue disposition in meat goats.

CONCLUSIONS

In conclusion, while there appears to be no overall statistically significant differences in the pharmacokinetics of tulathromycin between healthy and diseased goats, we did observe significant differences in tulathromycin plasma concentrations at multiple time points (168-312 hr). Specifically, goats infected with P. multocida demonstrated decreased plasma concentrations compared to healthy goats at approximately 168 hours after administration. There do not appear to be significant differences in edible tissue residues, with the exception of decreased kidney concentrations, amongst groups at 13 days post-injection. Similarly, a significant difference was identified in kidney tissue levels, which when considered with the differences in plasma concentration at later time points may suggest a difference in the terminal depletion process. While tulathromycin is currently used in an extralabel manner in goats, the results of this study suggest that experimental P. multocida respiratory disease may have the potential to alter pharmacokinetics or tissue residue concentrations of tulathromycin in meat goats. Further studies including larger numbers of animals are warranted to confirm these preliminary observations.
Conflicts of Interest

The authors report no conflict of interest.

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Author contributions

JSS was involved in study design and execution, sample collection and analysis, manuscript preparation and submission. JPM was involved in study design, sample analysis and manuscript preparation. DJB was involved in study design, method development, sample analysis and manuscript preparation. RWG was involved in study design, sample analysis and manuscript preparation. All authors have read and approved the final manuscript.

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Figure 1: Time vs Concentration data for plasma tulathromycin concentrations between control (top) and experimental (bottom) groups. *Indicates statistically significant differences.
Figure 2: Mean time vs concentration data for plasma tulathromycin concentrations between control (circle) and experimental (square) groups for the first 12 hours of the experiment.
Fat and Muscle Tissue Concentration

Tissue (C = Control; E = Experimental)

Liver and Kidney Tissue Concentration

Tissue (C = Control; E = Experimental)
Table 1: Pharmacokinetic parameters for control and experimental goats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Geomean</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>CV%</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>ng/mL</td>
<td>2298.6</td>
<td>2191.6</td>
<td>661.4</td>
<td>6232.9</td>
<td>78.8</td>
<td>2451.4</td>
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<tr>
<td>$T_{\text{max}}$</td>
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<td>0.35</td>
<td>0.375</td>
<td>0.25</td>
<td>0.5</td>
<td>37.8</td>
<td>0.14</td>
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<td>$\text{AUC}_{\text{last}}$</td>
<td>hr*ng/mL</td>
<td>11764.2</td>
<td>10257.2</td>
<td>8741.7</td>
<td>24395.8</td>
<td>47.3</td>
<td>5972.6</td>
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<tr>
<td>$\text{MRT}_{\text{last}}$</td>
<td>hr</td>
<td>59.6</td>
<td>61.6</td>
<td>38.7</td>
<td>76.8</td>
<td>28.7</td>
<td>24.6</td>
</tr>
<tr>
<td>CL/F</td>
<td>mL/hr/kg</td>
<td>0.199</td>
<td>0.225</td>
<td>0.101</td>
<td>0.266</td>
<td>28.6</td>
<td>0.06</td>
</tr>
<tr>
<td>$T_{1/2}$ (lambda_z)</td>
<td>hr</td>
<td>86.6</td>
<td>98.4</td>
<td>42.9</td>
<td>112.1</td>
<td>27.1</td>
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<td>$V_z$</td>
<td>mL/kg</td>
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<td>6.25</td>
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<td>CL/F</td>
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<tr>
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<td>88.4</td>
<td>46.0</td>
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Table 2. Mean concentrations and standard deviation per time point for control (left) and experimental (right) groups with $P$ value. $P < 0.05$ considered statistically significant.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Control Mean (ng/mL)</th>
<th>Control St. Dev (±)</th>
<th>Experimental Mean (ng/mL)</th>
<th>Exp St. Dev (±)</th>
<th>$P$ Value</th>
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<td>Time (min)</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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