Pneumatic dart delivery of tulathromycin in calves results in lower antimicrobial concentrations and increased biomarkers of stress and injection site inflammation compared with subcutaneous injection

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ABSTRACT: Remote drug delivery (RDD) using pneumatic darts has become more prevalent in situations where cattle handling facilities are not available. The objective of this study was to compare the effect of pneumatic dart delivery and subcutaneous injection of tulathromycin on plasma pharmacokinetics and biomarkers of inflammation, stress, and muscle injury in calves. Twenty-three castrated-male Holstein calves, approximately 10 mo of age with an average weight of 378 ± 6.49 kg, were randomly assigned to 1 of 2 groups. Calves in the RDD group ($n = 15$) received 10 mL of tulathromycin (2.42 to 2.93 mg/kg) delivered into the left neck using a Type U 10.0 mL 1.9-cm 14 G Needle pneumatic dart administered with a breech loading projector. With the exception of 1 light weight calf that received 7 mL (2.53 mg/kg), calves in the injection group (INJ) ($n = 8$) also received 10 mL of tulathromycin (2.34 to 2.68 mg/kg) administered as a single subcutaneous injection in the left neck using a 14 G, 1.9-cm needle and a 12-mL syringe. Serum tulathromycin, cortisol, creatine kinase (CK), and aspartate aminotransferase (AST) concentrations were determined in combination with other biomarkers of inflammation including mechanical

nociceptive threshold (MNT), infrared thermography (IRT), and swelling at the injection site over 432 h after administration. Pneumatic darts failed to deliver the required dose of tulathromycin in 4 of 15 calves evidenced by heavier dart weights post-administration (24 vs. 13.5 g). When these 4 calves were removed from the analysis, calves in the RDD group were found to have a smaller area under the tulathromycin concentration curve (AUC) ($P = 0.005$) and faster clearance ($P = 0.025$) compared with the INJ group. Furthermore, the RDD group recorded a greater difference in MNT between the treated and contralateral neck compared with the INJ group at 12 h ($P = 0.016$), 216 h ($P = 0.024$), and 288 h ($P = 0.0494$) after administration. Serum CK was elevated at 24 h ($P = 0.03$) and AST was greater at 24 h ($P = 0.024$) and 48 h ($P = 0.037$) after RDD. Serum cortisol concentrations were also greater at 0.5 h ($P = 0.02$) after RDD. These findings suggest that RDD is associated with reduced total body exposure to tulathromycin and increased acute stress, muscle damage, and pain at the injection site. Furthermore, the failure of darts to consistently deliver antimicrobial therapy has a negative impact on the welfare of sick animals treated with RDD technologies.

Key words: animal welfare, muscle damage, remote drug delivery, stress, tulathromycin

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INTRODUCTION

Remote drug delivery (RDD) technologies are commonly used in wildlife medicine to deliver anesthetic products for chemical restraint (Bush, 1992). RDD is typically accomplished through the use of 2-chambered compressed gas darts or powder explosive-powered darts that are delivered using either compressed gas projectors or powder load powered rifles (Isaza, 2014). Recently the use of pneumatic darts to deliver antimicrobial drugs to livestock has become more common in beef production systems in the United States. It is estimated that at least 4 million pneumatic darts were sold in the United States in 2015 (personal communication, Pneu-Dart Technical Services, Williamsport, PA). This application is especially prevalent in situations where handling facilities are not available or where it is perceived that RDD will reduce time to treatment and stress on the animal.

RDD of antimicrobial drugs requires the use of high-capacity pneumatic darts that typically deliver up to a 10-mL injection, to ensure that the administered dose is in a range that can achieve therapeutic concentrations in the target animal. High-capacity darts are typically prefabricated powder explosive powered darts in which the anterior chamber holds the drug while the posterior chamber contains a firing mechanism and an explosive cap (Isaza, 2014). When the dart strikes the animal, the forward momentum of the dart is carried to the firing pin causing the cap to explode producing compressed gases that force the plunger forward to deliver the medication through an injection needle (Isaza, 2014). Although darts and their projectors are considered indispensable tools in zoological medicine, anecdotal reports suggest that darting problems are not uncommon (Bush, 1992; Isaza, 2014). Drug delivery problems that have been reported include dart failure, drug failure (lack of sedation), challenges with dart aerodynamics, and animal injury (Cattet et al., 2006; Isaza, 2014). Specific descriptions of the impact of these issues on animal welfare are deficient in the published literature.

Tulathromycin is a macrolide antibiotic of the subclass triamilide that is indicated for the treatment of bacterial infections associated with bovine respiratory disease complex, infectious bovine keratoconjunctivitis, and interdigital necrobacillosis (Cox et al., 2010). When tulathromycin is administered subcutaneously at the label dose of 2.5 mg/kg (1 mL/45 kg), this compound demonstrates a long elimination half-life of 2.75 d in plasma and 8.75 d in the lung tissue. The low injection volume and long duration of activity after a single injection

have resulted in tulathromycin becoming a popular choice for RDD in cattle (Pneu-Dart, 2018). Data describing the impact of RDD on drug pharmacokinetics (PK), injection site tolerance, and tissue residue depletion are deficient in the published literature.

The U.S. National Cattleman's Beef Association (NCBA) in 2016 issued a Beef Quality Assurance (BQA) Advisory Statement that highlighted concerns about the potential negative animal welfare and food safety implications of RDD in cattle (BQA, 2016). Specific challenges identified in this statement include the risk of 1) inaccurate dosage or unapproved route of administration that may prolong tissue residues or promote antimicrobial resistance; 2) misidentification of treated animals resulting in violative tissue residues; 3) bruising, injection site lesions, and damage to sensitive tissues or joints causing pain and distress; and (4) darts that remain attached to the animal for a period of time that may become a hazard to other livestock or personnel when they subsequently become dislodged in the environment. To assist in addressing these concerns, the present study was conducted to compare the PK profile, serum biomarkers for inflammation, and pain tolerance tests between RDD and subcutaneous injection of tulathromycin.

MATERIALS AND METHODS

This project was approved by the Iowa State University (ISU) Institutional Animal Care and Use Committee (IACUC) on December 3, 2015 and logged under IACUC no. 11-15-8131-B.

Animals and Housing

Twenty-three Holstein male castrated calves, aged approximately 10 mo and weighing between 341 and 413 kg, were enrolled in a study to 1) compare the PK of tulathromycin and 2) determine the differences in biomarkers of inflammation, stress, and muscle injury when administered via subcutaneous injection or RDD. Calves had no record of previous tulathromycin treatment for at least 60 d prior to study commencement. Calves were weighed on arrival, individually identified with ear tags, and randomly allocated into groups. Calves were housed at the Beef Nutrition Farm at ISU where drug administration and sample collection took place. Calves were housed in groups of 5 to 6 calves per pen with treatments distributed evenly between groups. Floor space per animal exceeded the requirements set forth in the Guide for the Care and Use of Agricultural Animals in Agricultural

Use and Research and Teaching 3rd Edition (FASS, 2010). Calves were fed a diet comprised of cracked corn, protein supplement, and prairie hay that meet NRC nutrient requirements for growing cattle (NRC, 2016) and had free access to water.

Acclimatization and Baseline Data Collection

Calves were acclimated for 7 d prior to study commencement. At 24 h before drug administration (T-24), 16 gauge catheters (SURFLO, Terumo Medical Corp., Somerset, NJ) were placed in the jugular vein for blood sample collection over the first 24 h of the study. For catheter placement, the hair over the jugular groove was clipped with electric clippers and the clipped area was aseptically prepared using Chlorhexidine scrub and 70% isopropyl alcohol and infiltrated with 1 mL of 2% lidocaine (Hospira Inc., Lake Forest, IL). The catheter was secured with #0 nylon suture (Ethilon, Ethicon, San Lorenzo, PR). With the exception of dart delivery or injection, all procedures occurred in the calf pens. Calves had been previously halter trained so that they were restrained with a rope halter. The targeted injection site on the left neck was also clipped and blood samples were collected from the jugular catheter for baseline determination of serum tulathromycin, cortisol, creatine kinase (CK), and aspartate aminotransferase (AST) concentrations.

Treatment

At the time of study commencement (T0), calves in the RDD group ($n = 15$) were scheduled to receive 10 mL (between 2.4 and 2.9 mg/kg) of tulathromycin (Draxxin 100 mg/mL, Zoetis Animal Health, Kalamazoo, MI) injected using a Type U 10.0 mL 1.9-cm 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) in the left neck. Darts were identified on the barrel with the individual animal identification number using a permanent ink marker to facilitate recovery. The administration dose was rounded to 10 mL in accordance with current recommended best practices to ensure the correct functioning of the 10-mL capacity dart without adulterating the antibiotic with a diluent. Calves were individually restrained in a mobile chute with the neck extended using a rope halter. The dart was delivered over a fixed distance of 9.1 m (30 feet) using a Model 178B breech loading projector (Pneu-Dart, Williamsport, PA) that was charged with 7 pumps in accordance with the manufacturer's instructions (personal

communication, Pneu-Dart Technical Services). All darts were delivered by the same trained person (N.R.). After delivery, injection sites were photographed and darts were allowed to remain in situ until they were expelled as a result of calf activity. The time at which each dart was expelled was recorded and darts were recovered and retained for later examination.

Calves assigned to the subcutaneous injection group (INJ) ($n = 8$) were restrained in a chute and had 10 mL of tulathromycin (2.34 to 2.68 mg/kg) injected as a single subcutaneous injection in the left neck using a 14 G, 1.9 cm needle and a 12-mL syringe. One calf was a light weight calf that had 7-mL (2.53 mg/kg) tulathromycin manually injected as described above.

Concerning the PK analysis of tulathromycin, a sample size of 6 to 8 animals is adequate to describe the PK of a drug based on guidelines outlined in Comparative Pharmacokinetics: Principles, Techniques, and Applications; Riviere J.E. John Wiley and Sons, 2011, ISBN0813829933, 9780813829937: "a broad examination of the comparative pharmacokinetic literature suggests that the typical size of a pharmacokinetic trial is approximately 6 animals." Specifically a sample size of 15 calves in the RDD group and 8 calves in the INJ group provided a statistical power of 0.8 at an α of 0.05 assuming that the true difference between the means of the outcome measures was 0.65 and the σ of 0.5.

Posttreatment Data Collection

Calves were individually restrained in a pen with rope halters for data collection. Starting at 24 h after tulathromycin administration, animals were assembled in a chute and measurements were taken and sample collected while calves were individually restrained in a head gate.

Injection Site and Dart Examination

Injection sites were photographed and tissue reactions were measured prior to drug administration and at 24 h after tulathromycin administration using a Westward Polycarbonate Caliper (Grainger International, Inc., Lake Forest, IL). The caliper had a range of 0 to 150 mm with a resolution of 0.01 mm and an accuracy of 0.20 mm. Cranial to caudal and dorsal to ventral measurements were multiplied to provide an estimate of the area of the swelling at the injection site. Darts that were recovered from the calves were weighed at 24 h

after RDD using a calibrated compact scale (A&D EK-1200i Compact Balance, A&D Engineering, INC, San Jose, California) with a calibration range up to 1,200 g and a sensitivity of 0.1 g.

Thermography Assessment

Changes in skin temperature that would indicate inflammation at the injection site were measured after tulathromycin administration using a commercial infrared thermography (IRT) camera (ThermaCAM FLIR SC 660, FLIR Systems, Nashua, NH) with a thermal sensitivity of 0.05 °C, 320 × 240 pixel display, and precision >98%. The camera was internally calibrated to ambient temperature prior to image collection. Images were obtained from the left side of the calf, at an approximately 90° angle, and 0.5-m distance from the neck. Images were collected in the pen at 0.25, 0.5, 1, 3, 12, and 24 h after drug administration. Mean, maximum, and minimum temperature (°C) within a circumferential area at the injection site was obtained. The temperature at the center of the circumferential area was recorded as it approximated the injection site temperature. Following collection, images were analyzed using FLIR ExaminIR PRO (FLIR Systems, Danderyd, Sweden).

Mechanical Nociceptive Threshold (MNT) Assessment

MNT was measured at the injection site at 0, 2, 6, 24, 48, 72, 96, 120, 144, 168, 192, 216, 264, 288, 312, 336, 360, 384, 408, and 432 h after tulathromycin administration using a pressure algometer (Wagner Force Ten FDX 25 Compact Digital Force Gage, Wagner Instruments, CT, USA) as described previously (White et al., 2013; Stock et al., 2015; Stock et al., 2016). Briefly, the operator lightly placed their hand on the injection site and maintained this position until the calf no longer pulled away. The hand was removed and the rubber algometer tip was placed directly on the neck so that the tip aligned with the clipped area designated as the injection site and the screen was not visible to the operator to avoid bias. Increasing pressure was incrementally applied perpendicular to the neck until a withdrawal response was elicited. The digital output reflecting the kilograms of force applied to elicit the withdrawal response was then recorded by a second researcher to ensure that the algometer operator remained masked. Four sites, located at the 4 corners of the injection site, were measured in the same order for each calf. Similar measurements

were taken on the opposite, noninjected side of the neck to facilitate within animal comparisons. The order of measurement was randomized across time point. Data are presented as the difference in kilograms of force applied within animal between the measurements taken at the control or injection site on the right and left neck, respectively.

Blood Sample Collection, Processing, and Analysis

Blood samples for tulathromycin, cortisol, CK, and AST determination were collected from all the calves at 0, 0.25, 0.5, 1, 2, 3, 6, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 264, 288, 312, 336, 360, 384, 408, and 432 h after tulathromycin administration. For the first 12 h, up to 20 mL of blood was collected at each time point from the preplaced jugular catheter using a syringe. For blood samples collected at 24 h and later, calves were restrained in a chute with head gate, and blood was collected using a needle and syringe via venipuncture of the jugular vein. The whole blood sample was immediately transferred to 2 aliquot tubes (Vacutainer, BD Diagnostics, Sparks, MD) containing 1) lithium heparin anticoagulant for cortisol and tulathromycin determination and 2) no additive for AST and CK determination. Samples were stored on ice and processed within 2 h after collection. Blood samples were centrifuged at 1,500 g for 10 min and plasma or serum was transferred with a single-use plastic transfer pipette to duplicate labeled 2-mL cryovials. Samples for CK and AST determination were stored in a refrigerator and analyzed within 12 h of collection. The remaining samples were frozen at -70 °C until they were analyzed within 2 mo of collection.

Tulathromycin Analysis

Tulathromycin was analyzed in protein-precipitated bovine plasma by high-pressure liquid chromatography coupled to an ion trap mass spectrometer (LC-MS/MS). An internal standard, roxithromycin, was added to all plasma samples by including it at a concentration of 0.25 µg/mL in the acetonitrile used to affect the protein precipitation. A volume of 150 µL of samples, calibration spikes, quality control (QC) samples, and blank plasma were used in the analysis and precipitated with 600 µL of acetonitrile/0.1 % formic acid. The 1.5-mL microcentrifuge tubes were centrifuged at 7,500 rpm for 20 min in an Eppendorf 5417 C centrifuge. The supernatant was poured off into glass test tubes and dried down in a TurboVap (Biotage,

Charlotte, NC) at 48 °C. The dried residue was reconstituted with 100 μ L of 25% acetonitrile in water followed by 50 μ L of water. LC-MS/MS analysis was performed on a linear (LTQ) ion trap mass spectrometer (Thermo Scientific, San Jose, CA) coupled to an Agilent 1100 pump, autosampler, and column compartment. Separations were performed on a Hypersil Gold C18 column (50 $\times$ 2.1 mm, Thermo Scientific). The column temperature was 45 °C. Mobile phases A and B were 0.1% formic acid in LC-MS grade water and acetonitrile, respectively. The solvent gradient was from 10% acetonitrile to 95% acetonitrile in 4 min at 0.275 mL/min with a 4.5-min column flush/re-equilibration. The LC-MS/MS analysis utilized positive electrospray ionization (ESI⁺) for production of the parent ions of tulathromycin and roxithromycin at a mass-to-charge ratio (m/z) of 269.8 (triply charged parent) and 837. Five fragment ions were used for quantitation of tulathromycin. These ions were (m/z) 158, 231, 259, 289, and 325. The fragment ions for roxithromycin were (m/z) of 522, 540, 558, and 679. Calibration curves and QC results, as well as sample results, were automatically calculated by the Xcalibur software (Thermo Scientific, San Jose, CA) following analysis. The calibration range was 1 to 5,000 ng/mL with 12 calibration spikes. QC samples, like the calibration spikes, were prepared in blank bovine plasma. QC samples were prepared at 15, 150, and 1500 ng/mL. A weighted (1/X) quadratic fit was used to establish a calibration curve with a correlation coefficient of >0.998. The QC samples were within a 5% to 8% range of their nominal value.

Tulathromycin PK Analysis

Tulathromycin PK analysis was conducted using noncompartmental analysis using computer software (Phoenix WinNonLin 7.0, Princeton, NJ). The mean, standard error, and 95% confidence intervals were adjusted for dose and calculated for each calf.

CK Analysis

Serum CK concentrations were measured using a Vitros 5.1 Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ). A drop of blood was deposited on a slide pretreated with N-acetylcysteine to activate the CK which catalyzed the conversion of creatine phosphate to creatine and ATP. The ATP in turn phosphorylated glycerol kinase to produce L- α -glycerophosphate which was then oxidized to

produce hydrogen peroxide. The hydrogen peroxide then oxidized a leuco dye during incubation causing a change in reflection density that was correlated with enzyme activity.

AST Analysis

Serum AST concentrations were measured using a Vitros 5.1 Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ). For the AST analysis, the amino group of L-aspartate is transferred to α -ketoglutarate to produce glutamate and oxaloacetate in a drop of blood placed on a slide. The oxaloacetate is then converted by oxaloacetate decarboxylase to pyruvate which is oxidized by pyruvate oxidase to hydrogen peroxide. The hydrogen peroxide then oxidized a leuco dye during incubation causing a change in reflection density that is proportional to enzyme activity.

Plasma Cortisol Analysis

Plasma cortisol concentrations were determined using a commercial radioimmune assay kit (MP Biomedical RIA kit, Irvine, CA) previously validated for use in bovine plasma (Stock et al., 2015; Stock et al., 2016). The range of detection was from 2.79 to 279 nmol/liter. Samples were assayed in duplicate with the reported concentration equaling the average cortisol concentration between duplicates. The intra- and inter-assay coefficients of variation were 11.02% and 9.09%, respectively.

Statistical Analysis

Plasma cortisol, CK, and AST were log transformed for normality prior to analysis. Responses were analyzed using mixed-linear effects models with the calf as the experimental unit. Animal nested in treatment group was designated as a random effect with treatment and time designated as fixed effects. Response variables included plasma cortisol, CK, AST, MNT, injection site temperatures via IRT, and plasma tulathromycin concentrations. PK variables between the RDD and INJ groups were analyzed using paired *t*-test. A nonparametric test (Wilcoxon test) was used for non-normal data specifically Tmax and half-life. All statistics were performed using statistical software (JMP Pro. 13.0, SAS Institute, Cary, NC). Interactions were investigated using Tukey's HSD test for multiple comparisons when $P \leq 0.1$. Statistical significance was set a priori at $P \leq 0.05$. Mean and 95% confidence intervals are reported for statistical differences.

RESULTS AND DISCUSSION

Individual calf information for the RDD and INJ groups is summarized in [Tables 1](#) and [2](#), respectively.

Injection Site and Dart Examination

The location of the dart entry sites relative to the sequence in which darts were delivered (1–15) is presented in [Figure 1](#). Five of the 15 darts were delivered outside the injection triangle for subcutaneous injection recommended by the Beef Quality Assurance (BQA) Manual ([National Cattleman's Beef Association, 2014](#)). The injection triangle is bordered by the ligamentum nuchae dorsally, the jugular groove ventrally, and the shoulder blade caudally ([Figure 1](#)). Darts remained in situ for between 27 and 120 min after RDD with an average time to expulsion of 67 min (95% CI: 53 to 81 min) ([Table 1](#)). Examination of the injection site at 24 h after RDD revealed that 4/15 calves did not develop significant swelling at the injection site ([Figure 2F](#)). Given this observation, the individual darts that were recovered from these calves after RDD were weighed. Darts recovered from calves that did not develop a significant swelling at the injection site at 24 h after RDD were found to weigh 24 g, whereas darts recovered from calves that did develop swelling at the injection site were found to weigh 13.5 g. Examination of the individual darts also revealed that the clear cylinder that attaches the plastic flight

to the aluminum body of the dart was transparent in the darts weighing 24 g and opaque in the darts weighing 13 g. This suggested that the explosive cap required to force the plunger forward to deliver the medication through a hollow needle had not been detonated in 4/15 darts and not changing the opacity of the cylinder from clear to opaque. It was therefore concluded that RDD was unsuccessful in these 4 calves and data from these calves were not included in subsequent data analysis.

The PK parameters for the 4 calves after unsuccessful RDD are presented in [Table 3](#). A statistical analysis of these data was not conducted because randomization and allocation of animals to either the dart failure or success group was not performed to test an a priori hypothesis. This violates the assumptions required for valid statistical inferences to be made. The measured outcome variables of CK, tulathromycin, AST, the difference in MNT between the injection-site vs. control site, plasma cortisol and injection site area for calves experiencing successful RDD, unsuccessful RDD, and the subcutaneous injected controls are presented in [Figure 2A–F](#). For all outcomes, calves in which RDD was unsuccessful tended to have lower measurements of the outcome variables compared with the 2 groups in which drug delivery was successful.

The PK parameters of tulathromycin in calves following a single subcutaneous injection at 2.5 mg/kg bodyweight have been reported ([Cox et al., 2010](#)). In this study, a mean peak plasma concentration (C_{max}) of 277 ng/mL was reported at 3 h

Table 1. Individual calf information and remote drug delivery (RDD) outcomes following administration of 10 mL of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector (Pneu-Dart, Williamsport, PA)

RDD sequence	Bodyweight at day 0 (kg)	Tulathromycin dose (mg/kg)	Time to dart recovery (min)	Injection site area at 24 h (cm ²)	Dart weight at 24 h (g)	RDD outcome
1	370	2.7	59	4.5	24	Failure
2	413	2.4	48	187.2	13.5	Success
3	387	2.6	62	12.4	24	Failure
4	341	2.9	60	76.26	13.5	Success
5	381	2.6	59	79.2	13.5	Success
6	404	2.5	58	166.92	13.5	Success
7	359	2.8	59	59.86	13.5	Success
8	401	2.5	49	158.05	13.5	Success
9	386	2.6	109	134.16	13.5	Success
10	364	2.8	99	85.5	13.5	Success
11	391	2.6	60	109.22	13.5	Success
12	384	2.6	120	0	24	Failure
13	341	2.9	55	166.92	13.5	Success
14	356	2.8	27	0	24	Failure
15	395	2.5	87	177.8	13.5	Success

Table 2. Individual calf information following administration of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected as a single subcutaneous injection in the left neck using an 14 G, 3/4 inch needle and a 12-mL syringe

Injection sequence	Body weight at day 0 (kg)	Tulathromycin dose (mg/kg)	Injection site area at 24 h (cm ²)
1	377	2.7	243.36
2	374	2.7	218.4
3	408	2.4	137.5
4	404	2.5	0
5	386	2.6	138.43
6	427	2.3	129.72
7	384	2.6	243.36
8	276	2.5	0

Figure 1. Location of the dart entry sites by remote drug delivery (RDD) sequence (1–15; Table 1) relative to the injection triangle for subcutaneous injection recommended by the Beef Quality Assurance (BQA) Manual. Darts were delivered over a fixed distance of 9.1 m (30 feet) using a Model 178B breech loading projector (Pneu-Dart, Williamsport, PA) that was charged with 7 pumps in accordance with the manufacturer's instructions (personal communication, Pneu-Dart Technical Services).

after injection with a plasma elimination half-life of 64 h. It is noteworthy that the calves in which RDD was unsuccessful demonstrated detectable plasma tulathromycin concentrations although these concentrations peaked below 100 ng/mL at 30 min after administration. This is likely due to compound leaking through the needle over the 27- to 120-min period between dart delivery and expulsion. This is a concern due to the potential for treatment failure and exposure of bacteria to subtherapeutic drug concentrations that could contribute to the emergence of antimicrobial resistant bacteria. Furthermore, calves in which RDD was

unsuccessful may potentially have violative tissue residues if slaughtered prior to the approved meat withhold period.

Although the experimental conditions established in this study were optimized for RDD by restraining calves at a fixed distance from the dart projector, RDD was unsuccessful in 4/15 calves and darts were delivered outside the injection triangle in 5/15 of the calves. Therefore, it could reasonably be anticipated that under field conditions, when animals are moving and the distance between the operator and the animal will vary, that delivery could be less accurate and dart failure rate increased. Taken together, this would have a negative impact on animal welfare and food safety due to treatment failure and increased risk of inappropriate dosing.

Plasma Tulathromycin Concentrations and PK

The PK outcomes following tulathromycin analysis after successful RDD ($n = 11$) and subcutaneous injection ($n = 8$) are presented in Table 4 and Figure 2B. The mean area under the drug concentration time curve (AUC) for calves in the RDD group was lower [8,433 h*ng/mL (95% CI: 6,626–10,241 h*ng/mL)] compared with the INJ group [11,485 h*ng/mL (95% CI: 10,419–12,553 h*ng/mL)] ($P = 0.005$). AUC provides an indication of the total body exposure to a drug. Therefore, the reduced AUC suggests that tulathromycin exposure was less in calves following dart administration compared with subcutaneous injection. This is further supported by the observation that mean tulathromycin clearance per dose fraction absorbed (Cl/F), which provides an indication of the volume of plasma from which drug is removed per unit time, was greater in the RDD calves [5.03 mL/min/kg (95% CI: 3.80–6.26 mL/min/kg)] compared with the INJ controls [3.56 mL/min/kg (95% CI: 3.26–3.85 mL/min/kg)] ($P = 0.025$). Taken together, these data suggest that tulathromycin concentrations were negatively affected by RDD compared with INJ.

The use of AUC above the minimum inhibitory concentration (MIC) for the target bacteria (AUC/MIC) is considered the primary PK/pharmacodynamic (PD) predictor for tulathromycin clinical efficacy (Toutain et al., 2017). Therefore, the lower AUC observed in the RDD group may contribute to diminished clinical effectiveness of tulathromycin and an increased risk of treatment failure. Further studies that compare treatment outcomes following RDD with INJ are necessary to clarify the clinical relevance of this observation.

Figure 2. Mean (± SEM) for the following: (A) serum creatine kinase (CK) concentrations (IU/L); (B) plasma tulathromycin (ng/mL); (C) serum aspartate aminotransferase (AST) (IU/L); (D) difference of mechanical nociception threshold (MNT) of left (inject-site) vs. right (control; KgF); (E) plasma cortisol (nmol/L); (F) injection site area (cm²) after successful (dots with circles; n = 11) remote drug delivery of 10 mL of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI); unsuccessful (square with dash; n = 4) remote drug delivery injected using a Type U 10.0 cc 3/4 inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector, or traditional hand-injection (triangles and solid line) subcutaneously (n = 8).

Infrared Thermography

The skin temperature at the dart or injection site after tulathromycin administration is presented in Table 5 and Figure 3. There were significant treatment effects ($P = 0.0002$), time effects ($P < 0.0001$), and treatment by time interactions ($P = 0.0034$) at the injection site. Specifically, RDD calves were observed to have greater mean temperatures at the injection site compared with INJ controls. However, for the maximum and minimum skin temperatures, there were no treatment effects ($P = 0.0814$ and 0.9444 , respectively) or treatment by time interactions ($P = 0.2179$ and 0.7756 , respectively), but

there was an effect of time for both outcome variables ($P < 0.0001$).

IRT is used to evaluate changes in surface temperature (Stewart et al., 2009). Alterations in cutaneous blood flow due to inflammation cause changes in skin temperature that can be quantified with a thermography camera. White and others (2013) used cutaneous thermography to demonstrate that administration of a clostridial vaccine caused an increase in surface temperature at the injection site compared with control animals. This finding is consistent with the results of the present report in which successful RDD was associated with greater mean temperatures at the injection

Table 3. Individual pharmacokinetic parameters adjusted for dose and biomarker measurements of four calves by remote drug delivery (RDD) sequence (1–15) over 24 h following failed RDD administration of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector (Pneu-Dart, Williamsport, PA)

Parameter	Unit	1	3	12	14	Mean	SEM
Dose	mg/kg	2.70	2.58	2.61	2.81	2.67	0.05
AUCall	h*ng/mL	248	217	212	183	214	13.33
Cl/F obs	mL/min/kg	128.3	85.1	173.5	118.8	122.5	18.21
Cmax	ng/mL	44.3	21	81.7	32.3	39.6	13.18
Half-life (T _{1/2})	H	59.5	76.7	26.4	97.7	58.6	15.09
Tmax	H	0.25	0.5	0.25	0.25	0.30	0.06
Vz/F obs	L/kg	660.1	565.3	395.8	1004.7	620.7	128.31
Mean cortisol	(nmol/L)	25.5	22.6	37.0	23.6	27.3	3.03
Mean creatine kinase (CK)	(IU/L)	72.3	134.9	657.5	196.0	262.5	89.5
Mean aspartate aminotransferase (AST)	(IU/L)	73.5	78.5	98.6	95.1	86.14	3.88
Mean Difference of mechanical nociception threshold (MNT)	(KgF)	1.4	1.2	1.1	0.6	1.1	0.39
Infrared thermography (IRT)	(°C)	32.6	33.5	32.4	33.3	32.9	0.79

AUC_{ALL} = area under the curve for all timepoints; Cl/F obs = the observed Cl per fraction of the dose absorbed; C_{MAX} = maximum plasma concentration; T_{1/2} = terminal half-life; T_{MAX} = time to C_{MAX}; Vz = volume of distribution, area method; Vz/F = Vz per fraction of the dose absorbed.

Table 4. Pharmacokinetic parameters adjusted for dose following administration of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector (Pneu-Dart, Williamsport, PA) (remote drug delivery [RDD]; n = 11) or subcutaneous injection (INJ; n = 8)

Parameter	Group	Mean	SEM	Lower 95%	Upper 95%	P-value
AUCall (h*ng/mL)	INJ	11485.79	451.20	10,418.87	12,552.71	0.005
	RDD	8433.53	811.10	6,626.29	10,240.78	
Cl/F obs (mL/min/kg)	INJ	3.56	0.12	3.26	3.85	0.025
	RDD	5.03	0.55	3.80	6.26	
Cmax (ng/mL)	INJ	153.49	28.67	85.69	221.30	0.3
	RDD	206.45	40.63	115.92	296.97	
Half-life (T _{1/2}) (h)	INJ	111.65	2.97	104.63	118.67	0.66
	RDD	103.95	16.68	66.79	141.10	
Tmax (h)	INJ	1.00	0.72	-0.69	2.69	0.33
	RDD	0.27	0.02	0.22	0.32	
Vz/F obs (L/kg)	INJ	34.37	1.46	30.92	37.82	0.43
	RDD	39.36	5.91	26.19	52.53	

AUC_{ALL} = area under the curve for all timepoints; Cl/F obs = the observed Cl per fraction of the dose absorbed; C_{MAX} = maximum plasma concentration; T_{MAX} = time to C_{MAX}; T_{1/2} = terminal half-life; Vz = volume of distribution, area method; Vz/F = Vz per fraction of the dose absorbed.

site than INJ control animals indicating increasing blood flow due to inflammation.

Mechanical Nociceptive Threshold

The difference in the MNT between the right noninjected neck and the left injected neck is presented in Table 5 and Figure 4. There was no treatment effect of RDD compared with subcutaneous injection ($P = 0.26$). However, there was an effect of time ($P < 0.0001$) and a treatment by time interaction

on the difference in MNT ($P = 0.046$). Specifically, the RDD calves demonstrated a greater difference in mean MNT at 12 h [2.44 KgF (95% CI: 1.71–3.17 KgF) vs. 0.98 KgF (0.13–1.84 KgF)] ($P = 0.0156$), 216 h [2.26 KgF (95% CI: 1.52–3.18 KgF) vs. 0.84 KgF (95% CI: -0.04–1.72 KgF)] ($P = 0.0237$) and 288 h [1.85 KgF (95% CI: 0.90–2.98 KgF) vs. 0.62 KgF (95% CI: -0.48–1.72 KgF)] ($P = 0.0494$).

The results of the MNT testing suggest that calves tolerated less force relative to the control site at the site where RDD was successful compared

Table 5. Mean least square estimates following administration of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector (Pneu-Dart, Williamsport, PA) (remote drug delivery [RDD]; n = 11) or subcutaneous injection (INJ; n = 8)

Outcome	Treatment group				P-values		
	RDD		INJ		Trt	Time	Trt*Time
Creatine kinase (CK) (IU/L)	Mean	SEM	Mean	SEM			
Aspartate aminotransferase (AST) (IU/L)	372.7	44.1	327.0	51.6	0.62	<0.0001	0.37
Cortisol (nmol/L)	89.7	1.94	85.1	2.28	0.14	<0.0001	0.03
Difference of mechanical nociception threshold (MNT) (KgF)	27.8	1.68	25.9	2.11	0.83	<0.0001	0.10
Infrared thermography (IRT) (°C)	1.5	0.18	1.2	0.20	0.26	<0.0001	0.046
	36.5	0.14	35.4	0.17	0.0002	<0.0001	0.0034

** Indicates statistical difference of $P \leq 0.05$.

* Indicates statistical difference of $P \leq 0.10$.

Figure 3. Mean ($\pm$ SEM) skin temperatures taken at the injection site following successful (dots with circles) remote drug delivery of 10 mL of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector (n = 11) or traditional hand-injection (triangles and solid line) subcutaneously (n = 8). Skin temperature was assessed using a commercial infrared thermography camera (ThermaCAM FLIR SC 660, FLIR Systems, Nashua, NH) with a thermal sensitivity of 0.05 Celsius, 320 x 240 pixel display, and precision > 98%.

with calves where tulathromycin was injected subcutaneously. Pressure algometry is an example of a mechanical nociception test that has been used to assess pain associated with dehorning, lameness, and vaccination (Millman, 2013). Pain thresholds are established based on the amount of force applied to a surface that results in a withdrawal response. White and others (2013) demonstrated that calves vaccinated with a clostridial vaccine tolerated less pressure at the injection site than non-vaccinated calves. This is consistent with the results of this report that show that the difference between the injection and the control site was greater in calves where RDD was successful compared with the calves where tulathromycin was injected subcutaneously. This difference reflects decreased pain tolerance in the RDD group compared with the injected group.

** Indicates statistical difference of $P \leq 0.05$.

Figure 4. Mean ($\pm$ SEM) difference in mechanical nociception threshold (MNT) (KgF) between the right (control) and left (darted) injection site after successful (dots with circles) remote drug delivery of 10 mL of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector (n = 11) or traditional hand-injection (triangles and solid line) subcutaneously (n = 8). MNT was assessed at 0, 2, 6, and 24 h at 4 sites located at 4 corners of the injection and control site in each animal using a Wagner Force Ten FDX 25 Compact Digital Force Gage, Wagner Instruments, CT, USA.

Creatine Kinase

The results of the CK analysis are presented in Table 5 and Figure 5. There was no treatment effect ($P = 0.1996$) or treatment by time interaction ($P = 0.3748$) for CK concentrations, but there was a significant time effect ($P < 0.0001$).

At 12 and 24 h after injection, 9 of 11 calves in the group where RDD was successful and 4 of 8 calves in the injection group had serum CK concentrations that were greater than 350 IU/L (the highest concentration in the reference interval) (Bender, 2003). CK is a cytosolic enzyme that occurs at high concentrations in the skeletal muscle where it is critical to muscle energy production (Bender, 2003). The plasma half-life of CK in the bovine is typically short at 4 h. Therefore, persistent elevations in CK typically indicate ongoing muscle damage (Bender, 2003). The extent of muscle damage from injections has been evaluated by measuring blood concentrations of CK (Pyörälä et al., 1999; Fajt et al., 2011).

Although RDD resulted in greater mean serum CK concentrations at 12 and 24 h after administration, these differences were not statistically significant.

Aspartate Aminotransferase

The results of the AST analysis are presented in Table 5 and Figure 6. There was no effect of treatment on serum AST concentrations ($P = 0.1336$). However, there was evidence of a time ($P < 0.0001$) and treatment by time interaction ($P = 0.0307$) on AST results. Calves in the RDD group had greater mean AST concentrations compared with the subcutaneous injected group at 24 h [95.18 IU/L (95% CI: 89.9–100.5 IU/L) vs. 84.88 IU/L (95% CI: 78.6–91.1 IU/L)] ($P = 0.0043$), 48 h [92.71 IU/L (95% CI: 85.0–97.9 IU/L) vs. 83.0 IU/L (95% CI: 76.2–89.8 IU/L)] ($P = 0.0147$), and 72 h [88.49 IU/L

(95% CI: 81.0–93.5 IU/L) vs. 79.88 IU/L (95% CI: 73.3–86.5 IU/L)] ($P = 0.00277$) after tulathromycin administration.

There were no samples in either group where serum AST concentrations were above 125 IU/L, which is the highest concentration in the reference interval (Bender, 2003). Serum AST is tissue non-specific but muscle and liver cells are considered the major sources of the enzyme (Bender, 2003). The serum elimination half-life of AST is typically longer than CK and concentrations increase more gradually and persist for longer. Taken together, the elevation of both CK and AST at 24-h post injection combined with the elevated skin temperature measured using IRT supports the conclusion that successful RDD potentially causes more muscle damage and inflammation than subcutaneous injection of tulathromycin.

Figure 5. Mean ($\pm$ SEM) serum creatine kinase (CK) concentrations (IU/L) after successful (dots with circles) remote drug delivery of 10 mL of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector ($n = 11$) or traditional hand-injection (triangles and solid line) subcutaneously ($n = 8$).

** Indicates statistical difference of $P \leq 0.05$.

Figure 6. Mean ($\pm$ SEM) serum aspartate aminotransferase (AST) concentrations (IU/L) after successful (dots with circles) remote drug delivery of 10 mL of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector ($n = 11$) or traditional hand-injection (triangles and solid line) subcutaneously ($n = 8$).

Cortisol

The results of the cortisol analysis are presented in Table 5 and Figure 7. There was no effect of treatment ($P = 0.83$), but there was evidence of a treatment by time interaction ($P = 0.10$) for cortisol concentrations between groups. Specifically, mean plasma cortisol concentrations were greater at 0.5 h after RDD [54.5 ng/mL (95% CI: 40.4–68.6 ng/mL) compared with INJ [38.2 ng/mL (95% CI: 22.2–54.7 ng/mL)] ($P = 0.02$). Although there was a large increase in cortisol for both groups from the 12- to 24-h time

Figure 7. Mean ($\pm$ SEM) plasma cortisol concentrations (nmol/L) after successful (dots with circles) remote drug delivery of 10 mL of tulathromycin (Draxxin 100 mg/mL, Zoetis, Kalamazoo, MI) injected using a Type U 10.0 cc $\frac{3}{4}$ inch 14 GA Needle pneumatic dart (Pneu-Dart, Williamsport, PA) administered with a Model 178B breech loading projector ($n = 11$) or traditional hand-injection (triangles and solid line) subcutaneously ($n = 8$).

point, this was likely due to stress associated with the collection procedures.

Cortisol has been widely used to assess distress since the response magnitude, as indicated by peak height, response duration, and/or integrated response, usually correlates with the anticipated noxiousness of different procedures (Broom, 2000; Mellor et al., 2000). An increase in salivary cortisol above baseline concentrations was observed at 20 and 30 min after immunization in preterm infants (Grunau et al., 2010). Although we did not assess salivary cortisol in the present report, this increase is consistent with the elevation in plasma cortisol concentrations observed in the present report.

In conclusion, RDD of tulathromycin was unsuccessful in 4 of 15 calves as evidenced by greater MNT measurements, lower plasma cortisol, serum CK and AST concentrations, and the absence of significant injection site swelling during the 24-h period postadministration. Furthermore, it was observed that darts recovered from calves without significant injection site swelling weighed 24 g compared with 13.5 g. Calves in which RDD was successful tended to have lower MNT measurements, greater skin temperatures, and increased AST concentrations compared with subcutaneous-injected calves suggesting that RDD caused more tissue damage and inflammation than INJ.

Unlike RDD of compounds intended to provide chemical restraint, pneumatic dart delivery of antimicrobials produces no observable changes in animal behavior or demeanor that would indicate successful drug delivery as observed in this group of calves. Furthermore, the only observed difference in the outward appearance of darts recovered following successful RDD was an increased opacity of the clear plastic cylinder connecting the shaft of the dart to the flight. The risk of dart failure described in this report, combined with the reduced likelihood of dart recovery in field settings due to the prolonged attachment time after RDD, creates significant challenges for an operator to confirm successful drug delivery to sick animals. Therefore, the failure of high-capacity pneumatic darts to consistently deliver antimicrobial therapy could have a negative impact on the welfare of sick animals treated with RDD technologies.

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