THE USE OF DRUGS IN CAPTIVE WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*): AN EVALUATION OF TWO COMMON EXTRA-LABEL DRUG USES IN THE DEER BREEDING INDUSTRY

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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December 2019

Major Subject: Biomedical Sciences

ABSTRACT

The white-tailed deer (WTD) breeding and hunting industry is a large and growing industry with an economic impact of over a billion dollars in Texas alone. The increasing number of deer in captive breeding facilities results in increased veterinary drug use on deer. However, drugs can act differently even among similar species so further information on drug use in deer would help managers and veterinarians make more sound decisions when using drugs in deer. Two examples of novel extra-label drug use in WTD are studied, evaluated, and explained. Tulathromycin is a macrolide antibiotic used commonly to treat livestock, including deer, with bacterial respiratory infections, often delivered subcutaneous (SQ) or intramuscular (IM). Since it is logistically difficult to put hands on a WTD, even in most captive breeding situations, a study was conducted to evaluated tulathromycin as a candidate for remote-delivery dart (RDD) in WTD. Twelve WTD were darted with CO2 powered RDD projectors to administer 2.5 mg/kg of tulathromycin IM. Blood was then collected nine times over 30 days and the serum concentration for each sample was quantified in order to determine the pharmacokinetics of tulathromycin in each deer. Overall tulathromycin was poorly absorbed, reached low mean peak concentrations, had a high bioavailability, and an extremely long elimination half-life. The results indicated that darting with tulathromycin is an unpredictable means of administration and may not reach therapeutic concentrations. Flunixin meglumine (FM), a non-steroidal anti-inflammatory drug (NSAID), was also evaluated for extra-label use in WTD in a case study with 72 WTD

in South Texas. Texas Parks and Wildlife has seen anecdotal evidence of FM effectively reducing the body temperature of hyperthermic animals during game captures. Three of the deer in the case study became severely hyperthermic and the FM was tested in conjunction with cold water enemas. With over half of the 72 deer being hyperthermic, and three severely hyperthermic, a 100% post 30-day survival is strong evidence of the effect of FM.

DEDICATION

I would like to dedicate my thesis to my wife Madilyn Holmes Nunez for always being by my side and helping push me through. I could not have done it without her.

ACKNOWLEDGEMENTS

I would like to start by thanking my committee chair, scientific mentor, and friend, Dr. Cook for believing in me and giving me the chance to accomplish what I have. There are few people that are as caring and passionate as he, and I can honestly say that his guidance and friendship have bettered me as a person and scientist. I would also like to thank my committee members Dr. Fajt and Dr. Blue-McLendon for being extremely patient, helpful, and knowledgeable, as well as working to share their knowledge with me. They have been more than anyone could ask for in committee members and I owe them a great deal of gratitude for what they've done and what they've taught me. Two professors that absolutely deserve recognition as well but were not on my committee were Drs. Jeffrey Musser and Jim Derr. They were very influential in helping me decide on graduate school and have been phenomenal friends and mentors since. If there are ever two people I can talk about hunting and fishing with besides Dr. Cook, it is these two gentlemen and the conversations never disappoint.

The two people that I owe most to are my parents. They have been there to support me in everything I do and every {smart} decision I have made. I cannot begin to tell them how thankful I am for their love and encouragement, but it is certain that the values and work ethic they instilled in me have undoubtedly contributed to my success. My entire family has been behind me and deserve recognition as well but more specifically is my little brother. He has been my best friend since the day he was born and always will be. It means a lot to have someone looking up to you and I know, that

thought has helped push me to be better. Last but absolutely not least is my wife Madilyn. She is my biggest supporter and the most wonderful and charismatic person in the world. If there is anybody, I truly owe everything too, it is her. She is the one who is there for me every day and night; beside me through the most incredible experiences and the biggest disappointments graduate school has had to offer. I love her and thank her with all my heart for putting up with me over the years.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professor Walter Cook [advisor] of the Department of Veterinary Pathobiology and Professors Virginia Fajt and Alice Blue-McLendon of the Department of Veterinary Physiology and Pharmacology.

The overseeing veterinarian during the case study in Chapter 2 was Dr. Michael Vickers from the Las Palmas Veterinary Hospital in Falfurrias, Texas. The delivery of injections and monitoring for Chapter 2 were conducted by Katherine Trotter (an undergraduate student) and Logan Thomas (lab-mate/graduate student). The analytical chemistry work used to quantify the serum concentrations of tulathromycin for each deer serum sample in Chapter 3 was conducted at the Texas Veterinary Medical Diagnostic Lab on campus by Dr. Travis Mays. In Chapter 3, blood collection from deer via jugular venipuncture was conducted by multiple undergraduate, graduate, and veterinary students either employed by the Winnie Carter Wildlife Center at Texas A&M University or volunteers including Gabriella Quintana, Katlin Stone, Maddie Sacula, Taylor Williams, Cynthia Colbert, Cheyenne Green, Jessie Meibaum, Jessica Stephenson, Jacob Ross, Garrett Meurer, Brianna Stofas, Ashley Dane-Gresman, and Logan Thomas.

Funding Sources

Graduate study was supported by a graduate research assistantship (RA) in the Cook Wildlife Lab and in part by a graduate teaching assistantship provided by the Department of Veterinary Pathobiology at the Texas A&M University College of Veterinary Medicine and Biomedical Sciences.

NOMENCLATURE

AT	Ambient Temperature
AUC	Area Under the Curve
AUC_{obs}	The Observed AUC
AUB_{inf}	The AUC Extrapolated to Infinity
BAM	Butorphanol-Azaperone-Medetomidine
BBB	Blood-Brain Barrier
BT	Body Temperature
CLEN	Clenbuterol-d9
CM	Capture Myopathy
C_{max}	Peak Concentration (Maximum Concentration)
CWD	Chronic Wasting Disease
e-CFR	electronic Code of Federal Regulations
FDA	United States Food and Drug Administration
FM	Flunixin Meglumine
IAC	Interalveolar Concentration
IACUC	Institutional Animal Care Use Committee
IL8	Interleukin-8
IM	Intramuscular
IV	Intravenous
λ	Clearance

LC	Liquid Chromatography
LTB4	Leukotriene B4
LX4	Lipoxin 4
MIC	Minimum Inhibitory Concentration
MOA	Mechanism of Action
MS	Mass Spectrometry
NSAID	Non-Steroidal Anti-Inflammatory Drug
PD	Pharmacodynamics
PGE2	Prostaglandin-E2
PK	Pharmacokinetics
PLA2	Phospholipase A2
PLD	Phospholipase D
PO	Oral (Per Oz)
QDMA	Quality Deer Management Association
RDD	Remote-Delivery Dart
ROA	Route of Administration
SQ	Subcutaneous
TAMU	Texas A&M University
$t_{1/2}$	Elimination (Terminal) Half Life
t_{max}	Time of C_{max}
TPWD	Texas Parks and Wildlife Department
TUL	Tulathromycin

V_d	Volume of Distribution
WMA	Wildlife Management Area
WMS	White-Muscle Syndrome
WP	Withdrawal Period
WTD	White-Tailed Deer

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CHAPTER I

INTRODUCTION

The White-Tailed Deer Breeding Industry

The white-tailed deer (WTD) (*Odocoileus virginianus*) breeding and hunting industry is a rapidly growing economic portion of the agriculture industry in Texas and the United States (Anderson et al., 2008; Earle, 2016; NADeFA, 2019). This is most likely correlated to the fact that the WTD are the number one sought after big game animal in North America, with over 5.6 million WTD harvested across the U.S. and over 722,000 harvested in Texas in the 2016-17 hunting season alone (QDMA, 2018). This is part of the $380 million dollar industry in Texas that contributes over a billion dollars of economic impacts within the state (Anderson et al., 2008). The increasing number of captive WTD means increased handling and an increased use of veterinary drug on WTD. This is nearly always done extra-label by utilizing information about a drug from similar species in order to make assumptions on dosing. When handling free range or captive game animals like WTD, there is an increased chance of trauma, stress, and related pathologies (ie. CM, hyperthermia) (Berringer et al., 1996; Kreeger and Arnemo, 2018). It is also well understood that increased population density, as in the case of WTD breeding pens, amplifies the potential for the spread of contagious infections. Coincidentally, Haigh et al. (2005) found that trauma and bacterial infections (necrobacillosis and pneumonia) are the leading causes of morbidity and mortality in captive WTD.

Stress-Induced Hyperthermia

When capturing wildlife, two of the biggest concerns are arguably stress-induced hyperthermia and capture myopathy (CM). Hyperthermia is a major concern when capturing and immobilizing wildlife because of the potential neurological effects and associated morbidity and mortality. When body temperature increases, so does metabolic oxygen demand of the animal, which can lead to hypoxemia if ventilation does not sufficiently increase (Caulkett and Arnemo, 2014). When mammals become severely hyperthermic, the blood-brain barrier can break down, causing subsequent cephalic edema (Sharma and Hoopes, 2003). Heat-damaged cells also begin leaking potassium and damaged proteins into the interstitial space which can overwork the liver, clog glomeruli in the kidneys, and cause hyperkalemia resulting in irreversible damage (Cooper, 1996). Temperatures greater than 2°C (3.6°F) above normal body temperatures (BT) are considered hyperthermic, which in ruminants is assumed to be approximately 38.5°C (101.4°F); 40.6°C (105°F) is considered the threshold for hyperthermia in cervids (Kreeger and Arnermo, 2018; Wolf et al., 2004).

Contributing Factors

Factors frequently cited as contributors to hyperthermia when capturing wildlife include, but are not limited to, the drug combination used for immobilization, high ambient temperatures (AT) during the capture, the method of capture used, the amount of energy physically exerted by the animal during the capture event, the stress placed on the animal, and the onset of CM.

Certain immobilizing drugs can inhibit or alter thermoregulation and cause respiratory depression, which increases the potential for hypoxia and exacerbates thermoregulation issues in animals that rely on panting as ungulates do (Kock and Burroughs, 2012; Kreeger and Arnemo, 2018; Young, 1979). The effect of AT on capture-induced hyperthermia is relatively insignificant at mild AT's (<24°C/<75°F) (Beringer et al. 1996). However, as AT's increase, capture-induced hyperthermia and CM occurs more frequently so it is recommended that capture events take place in temperatures less than 25°C (77°F) to reduce the risk (Beringer et al., 1996; Ko and Krimins, 2014; Kock and Burroughs, 2012; Kreeger and Arnemo, 2018; Paterson, 2014). The method of capture goes more along with the amount of physical exertion and stress that the animal is put through during the capture. However, chemical immobilization as a method of capture (as stated earlier) can also inhibit normal physiological thermoregulation (Kreeger and Arnemo, 2018).

The stress and physical exertion an animal endures during a capture event are most likely to impact the rate of hyperthermia and CM (Beringer et al., 1996; Kreeger and Arnemo, 2018). When muscle fibers contract and energy is expended, heat is produced. If a wildlife capture event induces a large amount of physical exertion, long chases, or prolonged struggling, BT will increase (Beringer et al., 1996; Meyer et al., 2008). The link between hyperthermia and CM is incompletely understood (Williams and Thorne, 1996). However, anecdotal evidence suggests that CM exacerbates hyperthermia and vice versa (Kreeger and Arnemo, 2018; Paterson, 2014; Williams and Thorne, 1996).

Capture Myopathy and the Relation to Hyperthermia

CM is a non-infectious disease induced by prolonged psychological and physiological stress and extended periods of physical exertion that results in muscle necrosis and occasionally organ failure (Kreeger and Arnemo, 2018; Paterson, 2014). Other names for the condition are: exertional myopathy, exertional rhabdomyolysis, transport myopathy, capture disease, cramp, overstraining disease, myodegeneration and spastic paresis due to the nature of the disease and the situations in which it is most commonly associated. CM can occur in most animal species but ungulates, especially WTD, seem to be more susceptible than other mammals (Beringer et al., 1996; Paterson, 2014; Williams and Thorne, 1996). Deaths from CM can occur within hours of a capture event and as far out as a month. The pathogenesis of the disease is not well understood according to Kreeger and Arnemo (2018). However, there are certain commonalities in CM cases including myoglobinuria from skeletal muscle lysis and subsequent myoglobin release, extremely elevated levels of creatine kinase, and occasionally organ failure in chronic cases (Paterson, 2014). Skeletal muscle degradation is most likely due to sympathetically-induced overexertion during extreme stress, which increases BT and subsequently causes further harm to the organs and muscles. There is no cure for CM and often, the damages that result from of severe hyperthermia and CM are irreversible.

White muscle syndrome (WMS) is another non-infectious disease that appears similar to capture myopathy during post-mortem analyses because of the pathological changes to the muscles in the diseased animals (Kreeger and Arnemo, 2018; Paterson, 2014). However, the source of the diseased state is nutritional rather than stress related.

WMS occurs because of a deficiency of the trace element selenium, but it is more common in avian species than ruminants (Williams and Thorne, 1996; Ohlendorf, 1996).

Treatment

Since both CM and hyperthermia are associated with elevated BT, constant temperature monitoring is important and thresholds should be established before the capture so proper treatment can take place. Treatment for hyperthermia and CM are essentially palliative and restorative with a focus on keeping the animal calm and comfortable while restoring physiological norm.

Hyperthermia can occur for many reasons, but the commonality in cases is the thermoregulatory center of the brain, the hypothalamus. Prostaglandins are released during cellular damage, regardless of cause, which cross the blood-brain barrier (BBB) and bind to the hypothalamus. This triggers a fever-like immunological response and allows the BT to rise. When stress and injury occur during capture, prostaglandins are released, potentially exacerbating capture-induced hyperthermia.

Flunixin meglumine (FM) (Banamine®) is a nonsteroidal anti-inflammatory drug (NSAID) associated with the inhibition of prostaglandin synthesis. There is some physiological argument that stress hyperthermia works similar to that of a fever; where BT increases through a negative feed-back system to a higher set-point by thermogenesis, thermo-conservation, and decreased heat dissipation (Briese and Cabanac, 1991). A study in dairy cattle conducted by Soto et al. (2003) showed that inhibition of prostaglandin synthesis with FM did not actually reduce BT. Furthermore, a similar study in broilers found that there was no statistical difference in blood

concentration of prostaglandin when treated with FM (Oliver and Birrenkott 1982). The broiler study did, however, find that treatment of FM significantly increased survival of hyperthermia in broilers compared to those that did not receive treatment. Little is known about the exact physiological pathways in which FM reduces BT, but anecdotal evidence supports its use in treating hyperthermia and increasing survival. Texas Parks and Wildlife Department (TPWD) found that the use of FM for pronghorn capture in the Texas Panhandle for relocation to the Trans-Pecos Region of Texas not only reduced BT, but also had a much better survival rate than the pronghorn released without the treatment (B. Dittmar, pers. comm). He further explained that FM is administered to bighorn sheep during capture events in the Black Gap WMA in western Texas for effective BT reduction.

Drug Delivery Methods

When the label of an approved drug is created, it includes a specified route of administration (ROA). The ROA of a drug is important because different routes and delivery methods can significantly impact the pharmacokinetics (PK) of a drug. PK is how a drug moves through the body in terms of absorption, distribution, metabolism, and elimination. The ROA can also sometimes alter the pharmacodynamics (PD) of a drug in a body. PD is the effect the drug on a body; there must be the appropriate receptors in order for a drug to bring about an effect in a body (Sherwood and Ward, 2019).

There are numerous routes in which a drug can be administered to a body. These include, but are not limited to: oral or per oz (PO), intravenous (IV), intramuscular (IM),

subcutaneous (SQ), transdermal (TD), inhalation, etc. (Sherwood and Ward, 2019). When administering drugs to wildlife, IM, SQ, and IV are the most common methods. IV is normally only given when a rapid response is desired because the absorption rate of IV is essentially 100% and IV requires a hand injection to insure the placement of the needle. IM is frequently the ROA when delivering chemical immobilization drugs such as those discussed previously and it is often done so via remote-delivered dart (RDD) IM injection (Kreeger and Arnemo, 2018). Antibiotics can, in some cases, be given orally over feed but are often administered with a hand injection SQ or IM, with animals rarely dosed via RDD projector.

Remote-Delivered Dart Injection

Often times, captive deer breeding ranches do not have handling facilities to work deer and rely on chemical immobilization to conduct "hands-on" work. These chemical immobilants are often delivered IM via RDD from a dart gun. Chemical immobilization drugs are not the only drugs given with IM darts though; it is possible to give vaccines, antibiotics, and other drugs as well. The equipment used to project these flying syringes varies, as well as the darts, are available on the market (Dan Inject, 2016; Pneu Dart, 2019).

Dart projectors can be complex rifle or pistol models powered by .22 caliber rifle blanks or CO_2 cartages, or even as simple as blow guns powered by a deep forceful breath (Dan Inject, 2016; Kreeger and Arnemo, 2018; Pneu-Dart, 2019). All models have their pros and cons. Rifle models powered by .22 caliber blank shells with modified barrels are normally louder than CO_2 powered, and though blanks with varying amounts

of gun-powder exist for different range shots, close shots are difficult and prone to bruising and even fractured bone from high dart impact velocities (Coetsee et al., 2018; Jessup, 2001; Kreeger and Arnemo, 2018; Rivera et al., 2019). This does however mean longer shots are possible, with some veterinarians comfortably shooting at 120+ m distances (D. Pretorius, pers. comm.) Dart projectors such as the X-Caliber from PneuDart cannot accurately shoot as far but have bleed valves in order to set the air pressure exact for each shot based on dart size and distance to the target. These CO_2 powered dart guns are ideal for close range shooting such as shots taken in deer breeding pens. They are also generally quieter which is highly desirable when darting around multiple animals in small spaces to reduce stress and panic.

There are also a multitude of various syringe darts with different volume capacities, needle gages and lengths, barbed or barbless needles, end- or side-port, inertia driven injection or charged injection, etc. (Dan Inject, 2016; Kreeger and Arnemo, 2018; Pneu-Dart, 2019). Different darts are ideal for different situations. If a slow delivery is desired, a side port needle with an inertia driven delivery mechanism would be favorable to an end-port needle with a charge aided delivery (Cattet et al., 2006). If longer shots are to be taken, smaller mass darts are favorable because they are more accurate and precise than larger darts (Cattet et al., 2006; Jessup, 2001; D. Pretorius, pers. comm.). The barbed or collared darts on the market may be selected to ensure a dart stays in the animal once it hits (for marking purposed when darting large numbers of animals and for validation of dose). The dart volume and needle size should be chosen based on the animal and drug that is being delivered. The volume of the dart

should be completely or nearly completely filled so there is no leakage from the dart or altered flight from air-spaces (Kreeger and Arnemo, 2016; Walter Cook, personal communication).

Effect of Darting on Pharmacokinetics

The injury that is accompanied by a RDD IM injection is dependent on the projector and dart used, and can alter the PK of a drug (Cattet et al., 2006; Coetzee et al., 2018; Rivera et al. 2019). It has been scientifically shown that dart wounds can be 2x to 3x the length of the dart needle, especially with rapid-injection dart with end-port needles (Cattet et al., 2006). For this reason, needle length is important and varies across species. Larger darts tend to cause more traumatic injury, even fracturing bone, when compared to smaller darts, which can likely be attributed to the kinetic energy traveling with an item of larger mass (Cattet et al., 2006; Jessup, 2001; D. Pretorius, pers. comm.). The introduction of foreign contaminated material is also consistently seen in dart wounds of bigger gauge needles which can result in infection and even mortality (Cattet et al., 2006). Barbed darts seem to cause more tearing when removed than barbless darts, however the barb opening up the dart wound to the external environment decreases the potential for anaerobic infections that are commonly associated with puncture wounds (W. Cook, pers. comm.).

The trauma that is caused from the impact and injection from the dart generally results in bruising and localized inflammation (Coetzee et al., 2018). The swelling is caused by a slight increase in blood flow to the area with high densities of neutrophils coping with the damaged tissue and cells. This congregation of blood in the injured area

will in theory slow the absorption of the drug into circulation. If the absorption rate of the drug is altered enough and the body begins to metabolize and eliminate the drug at a fast-enough rate, the drug could begin to be cleared before fully absorbed (Coetzee et al., 2018). This could potentially decrease the drug peak concentration (C_{max}) and possibly result in plasma or tissue concentrations that are below the minimum inhibitory concentration (MIC) required to combat pathogens, in the case of antimicrobials.

Dart placement on an animal can also drastically affect the PK of a drug (Kreeger and Arnemo, 2018). The ideal shot location for an IM RDD is in a major muscle group such as the shoulder, rump, or neck for rapid absorption (Kreeger and Arnemo, 2018). If a dart is injected into a really fatty area such as the tail-head, absorption can be dramatically reduced. If a dart ends up in coming in contact with bone, it could result in a plugged needle and either an incomplete or non-existent dose of drug, prolonging any effects. There are also obvious issues that accompany extremely poorly placed shots (head, distal limb, etc.), however the trauma that results in these situations is normally of greater concern that drug dose (or lack there-of).

There is also the possibility that a RDD fails on impact either by bouncing of the animal, breaking on impact, or getting clogged before delivering a complete dose. This dart failure was shown by Coetzee et al. (2018) and Rivera et al. (2019) when 4 and 3 of 15 RDD darts from an air-powered projector failed to deliver a full dose. This can be problematic in that it is sometimes difficult to tell when dart failure occurs. Furthermore, though the altered concentrations are a misrepresentation of a fully functioning IM darts

on a drug's PK, the lower systemic concentrations of drugs that result from incomplete dosing depict the true efficacy of the RDD's as a delivery method.

Tulathromycin (Draxxin)

Tulathromycin (TUL) is a semi-synthetic, macrolide antibiotic from the subclass triamilide (Evans, 2005; Papich, 2016). The trade name is Draxxin, produced by Zoetis (Kalamazoo MI) mostly for the FDA approved treatment of cattle and domesticated pigs infected with respiratory infection, cattle with interdigital necrobacillosis, and cattle with keratoconjunctivitis (Pfizer, 2005; Villarino et al., 2013). Macrolides are particularly useful at treating lower respiratory infections because of their affinity for pleural tissue and tendencies to accumulate in such tissue (Evans, 2005; Frank et al., 1992; Papich, 2016; Villarino et al., 2013). However, TUL is often used off-label for other species and treatment protocols foreign to those described on the approved label use. Through experimental trials, TUL has been shown to be efficacious in the approved livestock (swine and cattle) against pathogens such as *M. haemolytica, P. multocida, T. pyogenes, F. necrophorum, Histophilus somni, Actinobacillus pleuropneumoniae, Bordetella bronchisepticum,* and *Mycoplasma spp.* (Pfizer, 2005). TUL has also been shown to be efficacious in vitro and in several other animal species against dozens of various pathogens (Carlson et al., 2010; Silva et al., 2018; Venner et al., 2007; Villarino et al., 2015).

Many of the pathogens significant in livestock respiratory diseases are also important causative agents of respiratory disease in cervids such as WTD (Dyer et al., 2004; Haigh et al., 2005; Palmer, 1999; Tell et al., 2011). This means that many of the

drugs used to combat these pathogens as well as information about effective treatments can also be extrapolated to use in WTD.

Bronchopneumonia is, as explained prior, a serious source of morbidity and mortality of captive WTD in North America (Haigh et al., 2005; Hattel et al., 2004). *M. haemolytica, T. pyogenes,* and *P. multocida*, three of the most commonly identified pathogens in WTD pneumonia, are all susceptible to and frequently treated by veterinarians with TUL (Haigh et al., 2005; Palmer, 1999; Tell et al., 2011). There has been some emergence of antimicrobial resistance to macrolides, including TUL; though it seems to be minimal in most cases, there are increasing reports (Alexander et al., 2013; Desmolaize et al., 2011; Hariharan et al., 2016; Kadlec et al., 2011; Olsen et al., 2013; Rajamanickam et al., 2019; Timsit et al., 2017; Woolems et al., 2018).

Methods of Delivering Tulathromycin

TUL's approved label ROA include IM in domesticated swine and SQ in cattle via hand injection (Pfizer, 2005). However, alternative routes and delivery methods can be utilized extra-label while still providing at least some degree of protection against foreign microbials. Some proposed extra-label alternatives to the approved ROA's include oral via gavage, IV hand injection, and IM via RDD injection and the approved ROA's in alternative species (Abo-El-Sooud et al., Alexander et al., 2018; 2012; Amera et al., 2012; Angen et al., 2008; Bachtold et al., 2015a,b; Clothier et al., 2010; Coetzee et al., 2018; Cook et al., 2016; Gáler et al., 2004; Grismer et al., 2013; Huang et al., 2012; Mackay et al., 2019; Rivera et al., 2019; Romanet et al., 2012; Scheuch et al., 2007; Tohamy et al., 2011; Wang et al., 2011; Yang et al., 2013; Venner et al., 2010;

Zhao et al., 2018). Although, technically the use of a drug in any species other than those approved, for any reason besides the intended approved purposes, and by other ROA's is considered extra-label (e-CFR, 2019).

The serum C_{max} following an IM dose of TUL ranges from as low as 240 ng/mL and 330 ng/mL in foals and Holstein calves, respectively, with a 2.5 mg/kg dose, to as high as 1080 ng/mL in guinea pigs dosed with only 1 mg/kg (Venner et al., 2010; Zhao et al., 2018). IM TUL serum C_{max} values tended to be as good or better than the FDA approved SQ cattle results (Abo-El-Sooud et al., 2012; Amer et al., 2012; Huang et al. 2012; Venner et al., 2010; Zhao et al., 2018). Even when the IM injection comes from a RDD, the PK was relatively unaffected in cattle (Coetzee et al., 2018; Rivera et al., 2019).

There were, however, indications of bruising, stress, and inflammation following a dart injection of TUL such as increases in creatine kinase in response to damaged tissues compared to other ROA's (Coetzee et al., 2018; Rivera et al., 2019). There was also a notable increase in cortisol shortly after drug administration with RDD compared to SQ injections indicating acute stress (Coetzee et al., 2018). Even with alterations in the PK and slight decreases in the concentration of tulathromycin, a comparative study showed the C_{max} following administration with RDD to be very comparable to, and even slightly higher than on average, administration via SQ (Rivera et al., 2019).

Regardless of the ROA, TUL has a very long half-life ($t_{1/2}$) compared to other antimicrobials as well as other macrolides (Foster et al., 2016; Villarino et al., 2013). In its approved label uses, the $t_{1/2}$ of TUL in bovine and swine is 54-80 hrs and 90-158 hrs,

respectively (Gáler et al., 2004; Huang et al., 2012; Papich, 2016). Two wild ruminants observed had longer serum $t_{1/2}$ of TUL than the studies over domesticated animals (Bachtold et al., 2015a,b). WTD resulted in a terminal $t_{1/2}$ of TUL ranging from 151-454 hrs; the average was approximately 281 hrs per $t_{1/2}$ (Bachtold et al., 2015a).

Besides dose, time, and ROA, other factors can influence the PK of a drug. Mzyk et al. (2018) demonstrated that age can be an impactful variable in the PK of a drug. This is likely due to the direct relationship between age and the increase in body fat content. Continuously, a similar argument could likely be made to explain why certain animal species, even between ruminants, metabolize and eliminate TUL at different rates. Pregnancy did not seem to dramatically affect the $t_{1/2}$ of TUL considering sheep pregnant during treatment displayed $t_{1/2}$ consistent with other ruminants (110.8 hrs) (Mackay et al., 2019).

The $t_{1/2}$ of a drug in tissue, especially in the case of macrolides such as TUL in lung tissue, can often be substantially greater than the plasma $t_{1/2}$, lasting upwards of 6 to 8 days (Papich, 2016). This is due to a reduced amount of TUL in the blood steam; the same blood stream that is responsible for transporting chemicals to the liver for metabolization and transporting metabolites to the kidney to be filtered as urine. The delayed $t_{1/2}$ requires longer established withdrawal periods (WP) to insure the elimination of all drug residues before human consumption.

The area under the serum or tissue concentration-time curve (AUC) of TUL is what is used to identify the bioavailability of the drug following a particular ROA. Bioavailability is defined as the ratio of active drug in the system to the amount of drug

delivered in the dose (Trepanier, 2013). TUL generally has an extremely high bioavailability (Abo-El-Sooud et al., 2012; Amera et al., 2012; Pfizer, 2005). WTD seemed to struggle with bioavailability of TUL following a SQ injection, harboring a low mean serum AUC (Bachtold et al., 2015a). Furthermore, Wang et al. (2011) found that bioavailability of TUL given by oral dose via gavage in swine was significantly lower (51.1±10.2%) than other IV and IM studies.

Mechanisms of Action (MOA)

TUL, being a triamilide macrolide, is slightly lipophilic and tends to have a higher affinity toward lipid-based tissues including body fat and lungs especially (Carbon, 1998; Evans, 2005; Papich, 2016; Villarino et al., 2013). Since the drug also contains a slight positive charge, it is most efficacious in an environment that is neutral to very slightly basic in pH (~7.4–8.0) (Evans, 2005). These characteristics of TUL enable it to be very effective against gram-negative bacteria (Hariharam et al., 2016; Papich, 2016; Song et al., 2016). TUL is considered to be a bacteriostatic antimicrobial, meaning it inhibits further growth of bacteria in order for the natural immune system to catch up rather than killing the bacteria (Maglio et al., 2003). That does not mean, however that TUL cannot have bactericidal ("bacteria killing") effect; in fact, at high enough doses, TUL can act as a bactericidal antimicrobial (Maglio et al., 2003). Like many other macrolides, TUL binds to the bacteria's ribosomal subunits, generally at the ribosomal 50s subunit, which inhibits the production of bacterial proteins and retards bacterial growth (Papich, 2016). TUL is a bit unique in that its' efficacy is considered both time-dependent and concentration-dependent (Frank et al., 1992; Maglio et al.,

2003). This means that the longer the drug interacts with bacteria the more effective it is and the higher the concentration of the drug the more effective it is. There is also some speculation that the macrolides, including TUL as well as other drugs with similar MOA's, interact with the ribosomal 23S subunit, which is why mutations in genes associated with the 23S ribosomal binding site mutations in bacteria are correlated multi-drug resistance (MDR) (Olsen et al., 2014).

There has also been shown in multiple studies, the anti-inflammatory effect TUL has on an infection (Fischer et al., 2010, 2014; Rajamanickam et al., 2019). TUL is absorbed into the blood stream and makes its way into neutrophils and macrophages, essentially using these as transport vessels to the infection site (Frank et al., 1992; Kadlec et al., 2011). Once at the infection site, aside from its antimicrobial effects, TUL reduces inflammation through increased leukocyte apoptosis and a reduction in two key proinflammatory precursors, leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) (Fischer et al., 2010, 2014). Both work by inducing the expression of multiple cytokines, including interleukin-8 (IL8) which is a strong chemoattractant for white-blood cells. The inhibition of LTB4 is at least in part, caused by TUL's effect on phospholipases A2 (PLA2) and D (PLD), which were correlatively reduced with the introduction of TUL (Fischer et al., 2014). PLA2 plays an important role in the release of arachidonic acid that lead to the production of immunomodulators such as leukotrienes, prostaglandins, and lipoxins; of which, a notable increase in lipoxin 4 (LX4) was also seen in association with TUL (Fischer et al., 2014). The apoptotic reaction is enzyme-dependent on caspase-3, which induces apoptosis in cells through an extrinsic death receptor *Fas* pathway or

intrinsic mitochondrial pathway; the exact pathway activated by TUL is unknown (Fischer et al., 2010). There was also in vitro evidence of TUL dependent apoptosis of neutrophils, however there was no apoptotic effect in fibroblasts, epithelial, or endothelial cells (Fischer et al., 2010). Drugs used for treatments similar to TUL, such as penicillin G, oxytetracycline, and ceftiofur, did not induce any apoptosis in neutrophils (Fischer et al., 2010). Apoptosis is intentionally programmed cell death while necrosis is cellular death caused by a diseased state (Poon et al., 2014). Prolonged inflammation can be damaging to tissues so the ability for the body to induce cellular death is pertinent in alleviating inflammation and restoring the body to homeostasis (Poon et al., 2014).

Maximum Residue Limits and Withdrawal Periods

A residue limit is the maximum amount of chemical measured in parts per million (ppm) that is safe for human consumption (Cattet, 2003). For TUL, the residue limit is 5.5 ppm (Cook et al., 2016). Using the label recommended dose and ROA in cattle and swine, the WP established were 18 days in cattle and 5 days in pigs in order for the injection site to drop below the legal residue limit (Pfizer, 2005). When measured in WTD, Cook et al. (2016) found that although TUL was detectable at 31 days post-injection following IM injection, the residue level was below the legal limit at the first blood draw on day 11 post-injection. SQ injections of TUL in WTD resulted in lung tissue residues 56 days post injection (Bachtold et al., 2015a).

Conclusion of Literature Review

With the steadily increasing number of WTD breeders in Texas and the United Stated, there is a corelated increase in the use of veterinary drugs on deer in order to comply with animal husbandry laws and in attempt to protect economic investments (Earle, 2016; Anderson et al., 2008; QDMA, 2018). Unfortunately, it is often logistically difficult to administer hand-delivered injections, especially to WTD on ranches that lack restraint facilities (Coetzee et al., 2018). Capture of the animals, frequently by using chemical immobilizing drugs from a RDD, must otherwise take place to handle them which adds unnecessary stress, exacerbates the potential for injury, and is significantly more expensive. These cons can be minimized through monitoring and often the issues stemming from the immobilization cocktail and/or capturing event can be alleviated with other pharmaceuticals and precautionary techniques, however the risks are still much greater when an animal is captured. This leads WTD managers and veterinarians to deliver drugs extra-label legally without knowing the true consequences of RDD on the PK of the drug in the animals. Based on information from several studies, it is understood that there is some alteration in the absorption and subsequent PK of drugs following RDD administration, however it varies from drug to drug (Cattet et al., 2006; Coetzee et al., 2018; Rivera et al., 2019; V. Fajt, pers. comm.). Being as TUL is a frequently used veterinary drug to treat bacterial pneumonia, the fact that pneumonia is one of the leading causes of morbidity and mortality among captive WTD, and that TUL has been shown to be efficacious when administered via IM RDD in other ruminants, the

validation of RDD as a viable means of delivery of TUL for WTD seems to be a starting point to fill the knowledge gap.

CHAPTER II

TREATMENT OF SEVERE HYPERTHERMIA IN CAPTIVE WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) WITH FLUNIXIN MEGLUMINE

Introduction

Hyperthermia is a major concern when capturing and immobilizing wildlife because of the potential neurological effects and associated morbidity and mortality. When BT increases, so does metabolic oxygen demand of the animal, which can lead to hypoxemia if ventilation does not sufficiently increase (Caulkett and Arnemo, 2013; Seal and Bush. 1987). When mammals become severely hyperthermic, the BBB can break down, causing subsequent cephalic edema (Sharma and Hoopes, 2003). Heat-damaged cells also begin leaking potassium and damaged proteins into the interstitial space which can overwork the liver, clog glomeruli in the kidneys, and cause hyperkalemia resulting in irreversible damage (Cooper, 1996). BT greater than 2°C (3.6°F) above normal BT are considered hyperthermic, which mean approximately 40.6°C (105°F) is considered the threshold for hyperthermia in cervids (Kreeger and Arnemo, 2018; Wolfe et al, 2004). Factors frequently cited as contributors to hyperthermia when capturing wildlife include, but are not limited to the drugs used during immobilization, the AT during captures, the methods of capture used and the subsequent stress and exertion caused on the animals, the onset of exertional myopathy (CM), and the species captured (Beringer et al., 1996; Ko and Krimins, 2014; Kock et al., 1987; Kock and Burroughs, 2012; Kreeger and Arnemo, 2018; Meyer et al., 2008;

Patterson, 2014; Williams and Thorne, 1986; Young, 1979). The link between capture myopathy and capture induced hyperthermia is incompletely known, however there is clear evidence that one exacerbates the other and vice versa, with no cure known for resulting damage.

When immobilizing wildlife, constant BT monitoring is important and thresholds should be established before the capture so proper treatment can take place when BT rise to unacceptable levels. Treatment for hyperthermia and CM are essentially palliative and restorative with a focus on keeping the animal calm and comfortable while restoring physiological norm. The case report that follows describes an apparently effective treatment protocol for the control of capture-induced hyperthermia in three WTD on the Texas-Mexico Border.

Case Reports

On the 14th and 15th of May 2019 near Zepata, Texas, 72 captive WTD were captured for regulatory ante-mortem Chronic Wasting Disease (CWD) surveillance. The capture event took place between the hours of 7:00pm (5/14/19) and 4:00am (5/15/19) using chemical immobilization via remote delivered dart. Weather conditions during the capture were partly cloudy, no precipitation, muggy (humidity greater than 70%), and AT was in the range of 22.2°C to 31.6°C (72°F to 89°F) (WeatherSpark.com, 2019). The WTD surveyed consisted of bucks and does, all intact adults (>16 months), which were darted from the ground in their respective breeding pens using a Dan-Inject .22 blank powered rifle, modified to accept Pnue-Dart darts. The bucks and does were darted with 2.0mL and 1.5mL of BAM® (Wildlife Pharmaceuticals, Inc., Windsor CO) respectively;

a premixed immobilization cocktail of butorphanol at 27.3 mg/mL (a mild opioid), azaperone at 9.1 mg/mL (a neuroleptic tranquilizer), and medetomidine at 10.9 mg/mL (an alpha-2 agonist). Animals were immobilized in groups of three to seven (regardless of sex and age), loaded on a flat-bed trailer and transported approximately 400m to the sampling station.

Because these deer were being sampled for CWD surveillance to meet regulatory requirements, TPWD staff assisted by checking official unique identification numbers, tattoos, and owner ear tags to verify that 100% of the age-eligible WTD were tested. Tonsil biopsies were taken from every animal. WTD were immobilized for longer than ideal periods of time, sometimes upwards of 50 minutes, due to the nature of the surveillance program.

Immediately upon arriving at the surveillance station, BT and respiration rate were evaluated and monitored approximately every 10 minutes until returned to pens. BT was monitored using an AmerisourceBergen® thermometer (Amerisource Bergen, Chesterbrook PA) inserted in the rectum. Regardless of BT, animals were intermittently sprayed with water. All 72 of the WTD that arrived to the survey station received FM (Bayer Corporation, Whippany NJ); if the rectal temperature was less than 40.56°C (105°F), 1.5mL of FM at 50 mg/mL was administered via IM injection in the rump, if the rectal temperature was greater than 40.56° (105°F), the same dose was administered via IV injection in the jugular vein. If rectal temperatures were over 41.67°C (107°F), a single cold-water enema was given in conjunction with the dose of FM as explained previously.

After samples were collected from the last deer in their respective immobilization group, all of the WTD received a SQ injection in the shoulder of Excede® (Zoetis, Parsippany NJ) at 200 mg/mL and dosed at 1 mL/33 lbs and the group was transported back to their pen and reversed simultaneously with a dose 2 mL of Atipamezole with a concentration of 25 mg/mL (alpha-2 antagonist) per 1 mL of BAM® and 0.5 mL of Naltrexone with a concentration of 50 mg/mL (opioid antagonist). Over 50% of WTD had elevated BT (40.5°C+ or 105°F+) and required treatment to decrease BT; some required very aggressive treatments.

Three WTD does arrived to the CWD sampling station with severe hyperthermia. The deer were all from different immobilization groups and were the only individuals in their respective group with such extreme BT. The BT of each of the three individuals were 42.78°C (109.0°F), 43.00°C (109.4°F), and 43.28°C (109.9°F). These animals were the first of their groups to be handled by the sampling team due to the severity of their BT. Reversal of the immobilizing agents was not an option due to the regulatory requirements that needed to be fulfilled. They were immediately given an IV dose of 1.5 mL of FM in the jugular vein followed by two cold-water enemas and continuous external dousing with water on the axillary region, groin, and head. The enemas were 16.9 fl oz refrigerated water bottles given one immediately after the other. The tail was held down tight against the anus for several minutes (2–4 mins) to ensure that most of the water given stayed in the animal rather than leaking out. BT could no longer be read with a rectal thermometer because the results would be skewed from the introduction of

cold water to via the rectum. However, all three WTD BT decreased subjectively (head and thoracic palpation) within 2–3 minutes.

All 72 of the WTD surveyed responded quickly and positively to the treatment and survived more than 30 days and appear healthy; all of the WTD tested negative for CWD. The repercussions of the physiological damage associated with capture-induced stress and hyperthermia typically occur within a month (Paterson, 2014; Williams and Thorne, 1996). Therefore, it is reasonable to assume that any detrimental effects of the capture event would have occurred within 30 days and that any morbidity or mortality thereafter was most likely unrelated.

Discussion

Hyperthermia can occur for many reasons, but the thermoregulatory center of the brain, the hypothalamus, is always involved. Briese and Cabanac (1991) even assert that the manner in which hyperthermia works is physiologically similar to that of a fever. Prostaglandins are released during cellular damage, which cross the BBB and bind to the hypothalamus. This triggers a fever-like immunological response and allows the BT to rise. When stress and injury occur during capture, prostaglandins are released, potentially exacerbating capture-induced hyperthermia.

FM, a common NSAID, is associated with the inhibition of prostaglandin synthesis. A study in dairy cattle conducted by Soto et al. (2003) indicated that inhibition of prostaglandin synthesis with FM did not actually reduce BT. Furthermore, a similar study in broiler chickens found that there was no statistical difference in blood concentration of prostaglandin when treated with FM (Oliver and Birrenkott, 1982).

They did, however, find that treatment with FM significantly increased survival of post hyperthermia in broilers compared to those that did not receive treatment. Little is known about the exact physiological pathways in which FM reduces BT, but anecdotal evidence supports its use in treating hyperthermia and increasing survival. TPWD found that the use of FM during pronghorn capture in the Texas Panhandle for relocation to the Trans-Pecos Region of Texas not only reduced body temperatures, but also resulted in a better survival rate than in the pronghorn released without the treatment (B. Dittmar, pers. comm.). TPWD routinely administers FM to bighorn sheep during capture events in the Black Gap WMA in western Texas for effective BT reduction (Bob Dittmar, pers. comm.).

[A proposed mechanism for hyperthermia-mitigating effects of FM in wildlife capture events follows.] Capture events are typically associated with some degree of physical exertion, trauma, and tissue damage (Brivio et al, 2015). Evidence suggests that both damage to tissue and stress from capturing can independently drive a febrile response by increasing thermogenesis and decreasing heat loss.

The stress response is associated with tachycardia, increased metabolic rate, and increased muscle tone which serve to increase heat production while adrenergic vasoconstriction serves to reduce heat loss to the environment (Sherwood and Ward, 2019). Additionally, adrenergic agonism at the hypothalamus stimulates non-shivering thermogenesis by brown adipose tissue (Bray, 2000). It is hypothesized by the authors that both handling-induced stress and direct adrenergic agonism by medetomidine would stimulate these mechanisms of heat production and inhibit of heat loss via decreased

dermal circulation. Though physiological changes resulting from stress and/or chemicals contribute to hyperthermia, it is likely that tissue damage and subsequent prostaglandins also play a major role.

Prior to capture, animals often experience a period of heavy exertion, and sometimes trauma while trying to avoid capture (Seal and Bush, 1987). Regardless of the nature of damage, damaged cells lyse their products and agonize Toll-like receptors of immune cells, which then produce pro-inflammatory cytokines including Interleukin-1 beta and Interleukin-6 (Lukens et al., 2012; Rani et al., 2017; Rock et al., 2010). These inflammatory signals interact with the hypothalamus and induce prostaglandin synthesis – specifically PGE2 (Coceani et al., 1986; Eskilsson et al., 2014). PGE2 then signals the hypothalamus to induce purposeful thermogenesis through increased muscle tension, non-shivering thermogenesis by brown adipose tissue, dermal and peripheral vasoconstriction, and increased metabolic rate (García-Alonso et al., 2016; Morrison, 2016; Takahashi et al., 2013). FM stops the activation of PGE2 by inhibiting the enzyme cyclooxygenase-2 (Clark, 1979; Dannhardt and Kiefer, 2001; Samad et al., 2001). With decreased PGE2 levels, the thermogenic stimuli at the hypothalamus may decrease and subsequent heat production decreases while heat loss is allowed through dermal vasodilation. After FM has taken effect, other measures of cooling such as water enemas and physical wetting of the animal may be enhanced due to peripheral vasodilation at the gastrointestinal tract and skin respectively.

BT of >41°C (>106°F) are considered by most veterinarians to be a medical emergency and are often associated with both immediate and delayed fatality (Kreeger

and Arnemo, 2018). Furthermore, the experience of the senior author is that animals with BT greater than 42.2°C (108°F), prior to the use of FM, seldom survived (W. Cook, pers. comm.).

Conclusion

This case report serves as further anecdotal evidence of: 1) the efficacy of FM for BT reduction in wild ruminants, most likely due to the immunologically-induced febrile response, and 2) an effective protocol for the treatment of severe hyperthermia in captive WTD capture events. The recommendations for the treatment of severe hyperthermia in WTD, which can most likely be extrapolated to other exotic and wild hoof-stock, is administration of FM, external cooling through direct water application, and delivery of cold water via rectal enema.

Acknowledgements

We thank Cuatro Whitetails (Jaime Delgado) and their staff for their hospitality during the sampling process as well as their continued cooperation and aid that allowed the creation of this publication. The Texas Parks and Wildlife Department, Jim Hogg County lead game warden Carlos Maldonado III and his team of respective game wardens involved in this project deserve a great deal of gratitude for donating their time to aid in the surveillance progress (as well as protecting us and the natural resources we all enjoy.) We also wanted to thank the capture crew for a job well done, immobilizing all necessary animals in a rapid, efficient, and professional manner that allowed us to complete the surveillance in one night's time.

CHAPTER III

TULATHROMYCIN AS A CANDIDATE FOR REMOTE DELIVERY INTRAMUSCULAR DART INJECTION IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

Introduction

WTD are an iconic North American big game species and are the most heavily sought-after big game species in the United States (QDMA, 2018). The WTD breeding and hunting industry is a $380 million industry in Texas alone, and over $1.2 billion across the United States (Anderson et al., 2008; Earle, 2016). One of the major issues faced by WTD breeders is death loss with bacterial pneumonia being one of the primary contributors in the United States and Canada (Haigh et al., 2005; Hattet et al., 2004). The pathogens often associated with the bronchopneumonia in ruminants, and WTD especially, are *Trueperella pyogenes, Pasteurella multocida, Mannheimia haemolytica, Fusobacterium necrophorum* and several other bacteria (Hattet et al., 2004; Palmer et al., 1999). TUL is a macrolide antibiotic frequently utilized by veterinarians to treat respiratory infections caused by these pathogens in livestock such as cattle and swine (Alexander et al., 2013; Evans, 2005; Kilgore et al., 2005). It is also used “extra-label” in other ruminants such as sheep, goats, and WTD (Bachtold et al., 2015; Clothier et al., 2010; Cook et al., 2016; Washburn et al., 2007). This drug is generally administered via hand injection SQ or IM, although studies indicate efficacious treatment with RDD in cattle (Coetzee et al., 2018; Pfizer, 2005; Rivera et al., 2019).

Often times, WTD breeders do not have working facilities and rely on dart guns to chemically immobilize deer for restraint. If an antibiotic could be delivered via dart gun and RDD injection, it would save ranchers money on immobilization drugs and reduce the risk of capture related injury and stress. However, there is concern is that the impact of the dart on the animal could affect the absorption of the drug and result in reduced therapeutic effects (Cattet et al., 2006; Coetzee et al., 2018). Though there is a wealth of information about delivering drugs with a remote delivered dart, there is little in the literature about the administration of antibiotics with RDD, especially in deer.

Bachtold et al. (2015) has shown that TUL reaches therapeutic concentrations in lung tissue of WTD following SQ hand-injection. Furthermore, another study conducted by Cook et al. (2016) exhibited that the drug reaches significant concentrations in muscle and liver following IM hand injected administration. These are consistent with other findings that show TUL reaches therapeutic concentrations in various ruminants following multiple ROA (Alexander et al. 2013; Bachtold et al., 2015; Clothier et al., 2010; Cook et al., 2016; Kilgore et al., 2005; Washburn et al., 2007). Data showed some changes in PK following an IM injection in cattle via RDD, however it did not seem dramatic in most cases (Coetzee et al., 2018; Fajt, pers. comm.; Rivera et al., 2019). Rivera et al. (2019) showed that RDD TUL resulted in C_{max} values in cattle similar to those following SQ injections. However, Coetzee et al. (2018) illustrated issues with drug absorption and distribution in cattle TUL PK following RDD injection as well as indication of increased stress and inflammation to injection sight when using RDD. The

use of RDD for the administration of TUL has conceptually be proven, but never validated in WTD.

It was hypothesized that TUL could be a viable candidate for IM dart injection for the treatment of pneumonia in WTD. It was further hypothesized that there would likely be a notable, but insignificant, decrease in absorption of the drug due to bruising in the muscle from the impact of the RDD. This study was conducted to determine the PK of TUL administered via RDD IM injection.

Materials & Methods

This study was conducted by darting captive WTD with an IM dose of TUL, delivered via RDD, and measuring the serum concentration of TUL in each of the WTD at various time points over the course of approximately one month in order to gauge the therapeutic potential of the drug as well as decipher the effects of the delivery method on the PK in WTD. Twelve captive WTD, consisting of two bucks and ten does, were utilized as sample subjects in the study. The WTD were housed at the Winnie Carter Wildlife Center on the Texas A&M University (TAMU) campus, housed under the Animal Use Permit (AUP) 2018-0106 issued by the TAMU Institutional Animal Care and Use Committee (IACUC). Ages ranged from 1.5–10.5 years. The animals were broken into groups as follows: 3 does (Tag #'s 11, 17, and 25), 3 does (Tag #'s 21, 24, and NT), 4 does (Tag #'s 2, 66, 70, and 73), 1 buck (Tag #22), and 1 buck (Tag #72). The groups were created in order to minimize the number of WTD in a holding pen during the darting events as well as to reduce any aggression between deer because many of the deer came from different permanent housing pens. The division of does was

based on normal pen-mates while bucks were singled out to ensure no undesired mating and potential aggression that accompanies sexual drive. The study took place from January to February so AT would be below 77°F (as recommended by Beringer et al., 1996, and Kreeger and Arnemo, 2018) in order to reduce the potential for capture-induced hyperthermia or CM while handling deer.

Initially, the WTD were run through a working pen system created specifically for working and moving cervids and fed into a squeeze chute, designed by Priefert Ranch Equipment, for WTD breeding facilities. Once WTD were in the squeeze cradle, they were blind folded with a size small deer blind-fold from the Texas Deer Association and manually restrained by four people so blood could be collected (Figure 1). One of the restrainers held the head with the nose pointed up at about a 60° angle from the ground while blood was collected from the jugular vein. Jugular venipuncture was done with an 18 ga needle and a 10 mL syringe used to draw approximately 10 mL of blood from each animal prior to administration of the drug to act as a negative control and baseline measurement for the WTD serum as well as to insure there was no TUL on board any of the animals in the study. The blind-fold was removed and the WTD were then released from the squeeze and directed to another chute with a scale. The weight of each WTD was collected in order to calculate the dose of TUL needed for each WTD based on a target dose of 2.5 mg/kg. They were then returned to their housing pens. The five groups were moved into their respective holding pens and darted with a dose of TUL (2.5 mg/kg) from a CO_2 powered dart gun. The darting began with a Pneu Dart X-Caliber CO_2 rifle using either 1 cc, 1.5 cc, or 2 cc gel-collared IM dart with a ¾" end-

port needle depending on the volume of TUL being administered. Darts were fired from a catwalk above the holding pens (approximately 12 feet above the ground) (Figure 2) with the desired dart site being in the gluteal muscles of the rump. Consequently, shot distances were close; ranging from 5 to 8 yards at an extreme angle (>45°).

Five of the first seven Pneu darts shot resulted in partial or complete dart failure either by bursting on impact with the animal or failure due to darts bouncing off, both of which potentially resulting in incomplete doses or no dose at all. A projector switch was made and the remaining dart injections were delivered with a Dan Inject CO_2 Injection Pistol shooting pressurized IM darts with 1.5" side-port needles. For the WTD that experience some form of dart failure, visual examination of the dart wound was conducted and one of the five deer with dart failure was re-dosed because there appeared to be drug on his fur, indicating an incomplete dose. The other four darts had no drug left in them and the charge was fired so the deer were assumed to have gotten a dose. Including re-dosing one buck WTD (Tag #22), a total of six Dan Inject darts were used. The time of administration was recorded for each dart fired as Time Zero of the study; the second dart time was Time Zero for the deer administered a second dose.

Each deer was run through the chute system and into a Priefert squeeze chute in the same order in which they were darted for blood collections as previously described. Blood was collected in order to measure the concentration of the drug in each deer's blood serum over time. This process was repeated for a total of nine blood draws from each WTD; one negative control (that took place previous to RDD injection as explained prior), and then one at 2h, 4h, 12h, 1d, 2d, 5d, 10d, 20d, and 30d (Table 1). Each WTD

was restrained for an average of 3 mins from start to finish when collecting blood so the restraint times were quick and stress on the animals was minimal. When released from the squeeze cradle, most deer walked away, seemingly unphased with few occasionally trotting down the alley back to their perspective group pen.

During the course of the study, the twelve WTD were fed their usual diet and housed in their permanent housing pens. Approximately one hour prior to the scheduled blood drawing events, the WTD were moved into their respective group holding pens and prepared to be run through the working chutes. The entire process generally took about 2 hours to work and bleed all the deer, meaning most days in the holding pens were relatively short; except the first day of blood sampling post-darting, when four blood collections were scheduled in a 24-hour time period.

After blood was collected, it was allowed to sit for about an hour before it was then spun in a centrifuge to separate the serum from the rest of the blood. The serum was then pipetted into cryo-vials for transportation to, and frozen storage in, the lab until the analysis using liquid chromatography (LC) tandem mass spectrometry (MS).

In order to quantify the deer blood serum concentration of TUL, each sample underwent a multistep chemical process to clean, purify, and condition the sample for analysis using LC followed by tandem MS. The LC step is used to remove the drug from its background for a pure sample that can be quantitatively analyzed by the mass spectrometer.

The LC process is initiated by conditioning the column with ~1 mL of methanol and ~1 mL of water. The column is comprised of a cotton filter than contains

$SiC_3H_6SO_2OH$, which is slightly acidic (~pH 5). By first washing the column with methanol and water, the hydrogen is pulled off of the sulfonic acid group by hydrolysis, "conditioning" the column by creating a negative charge. Clenbuterol-d hydrochloride (CLEN) was added at a concentration of 50 ng/mL to the serum sample and mixed using a test tube vortex mixer for ~2 seconds. The CLEN works as the internal reference standard (IRS) for the quantitative analysis. Since the TUL administered to each WTD was at a concentration of 100 ng/mL, the observed ratio of CLEN to TUL in each sample can be compared to the base ratio (50:100 ng/mL) positive control in order to quantify the approximate TUL serum concentration of each sample. This solution is then applied to the conditioned column, along with another ~1 mL of water, ~0.4 mL of 1N acetic acid, and ~1 mL of methanol. The acetic acid is used to bring the sample solution pH to acidic conditions (~pH 5) by hydrogenating an amine group of the drugs (creating a positive charge). This allows for a cation exchange between the negatively charged sulfonic group in the cotton column and the positively charged amine group of each drug compound's residues; fixing the tulathromycin and clenbuterol to the column and thus extracting them from the serum sample.

Air is then passed through the column for 10 minutes to completely dry it before the column is washed. The column was washed with a solution of methanol, ethyl acetate, and ammonium hydroxide at a ratio of 50:50:4, respectively. The wash cleaves the ionic bond that was used to extract the TUL and CLEN from the WTD serum by bringing the pH back up to slightly basic conditions and releasing the drug residues into a drop. An addition of 0.1% formic acid is added to the sample drop in order to cleave

the residues into hydrolytic fragments that can be easily detected by the mass spectrometer.

The mass-spectrometer is used to detect charges; with spikes indicating mass-charges (m/z) that are specific to particular compounds or fragments of compounds. A positive peak around 403 m/z and 806 m/z is indicative of TUL fragments or the parent TUL molecule, respectively. Peaks around 158 m/z and 116 m/z point towards CLEN fragments and a peak around 276 m/z for CLEN. The height of the spike details the abundance of the compound associated with the spike. The abundance of each residue can be used to calculate the observed ratio of CLEN to TUL and compared to the base ratio for quantification of the concentration of TUL.

The MS analysis determines the serum concentration at the exact time point that each blood sample was collected. A PK curve can then be created for each deer to illustrate the absorption and elimination of tulathromycin following the IM dart injection (Figure 3). The averages can then be calculated for the, C_{max}, time of C_{max} (t_{max}), $t_{1/2}$, volume of distribution (V_d), and serum AUC. This will help the researchers determine: 1) if tulathromycin reaches therapeutic concentrations following RDD IM injection and 2) how long the drug is protective if it is.

Results

The PK of TUL had some consequential differences when delivered via RDD. Though all twelve of the WTD in the study developed at least a detectable level of TUL in their serum, the C_{max} reached in the group, as an average and within individuals, was significantly lower than the C_{max} of other in the same and similar species (Table 2). The

C_{max} among the WTD in the study ranged from 32 ng/mL to 242 ng/mL with a mean serum C_{max} of 113.75 ng/mL (Table 3). Four individuals (Tag #'s 2, 22, 24, and 25) in the study that never developed significant TUL concentrations (Cmax ≤ 70 ng/mL) were all among the first seven darted with Pneu Dart products and all four experienced partial dart failure. This could account for the lower C_{max} experienced for these four cases.

When the four lowest C_{max} values are removed, the mean C_{max} increases to 151.25 ng/mL which is likely more indicative of the PK of TUL administered via RDD in WTD. The two animals that had the most comparable C_{max} values to other ROA in same and similar species (Tag #17 = 229 ng/mL; Tag #72 = 242 ng/mL) are the most likely, if any, to reach therapeutic levels of TUL in lung or other tissues.

The t_{max}, was calculated to occur around 4.03 ± 2.72 hours post dart injection, ranging from 1.5–11.9 hrs (Table 3). Based on the nature of the PK curves, there is a sharp rise to the C_{max} as the drug is absorbed into the system. From the C_{max}, metabolism of the drug begins to occur at a faster rate than absorption of the bolus, initiating the elimination process of the drug. This is indicated in the PK curve (Figure 4) by a sharp drop off that seems to asymptote out at a concentration of 0 ng/mL, modeling a sort of exponential decay. This is based on the idea of the $t_{1/2}$ of the drug. The resulting PK analysis identified the $t_{1/2}$ of TUL to be approximately 296.33 ± 224.16 hrs with an average clearance (λ) of about 0.003 ± 0.001 mL/hr. The $t_{1/2}$ explains how long the animal takes to eliminate TUL to half of its initial concentration. Based on this idea, after the time period of five elimination half-lives, the drug concentration is essentially reduced by ~98%.

The bioavailability of TUL following IM administration via RDD can be interpreted by looking at the AUC. To calculated the true bioavailability of IM administered TUL as a percentage, one would need the AUC following an IV injection to compare that of the IM injection to, since IV is considered to be 100% bioavailable. However, since only the one ROA was used to administer TUL for PK monitoring, there is no area to compare with and the AUC must suffice to provide a relative means of assessing bioavailability. The AUC can be calculated as the true AUC observed AUC_{obs} for the time points collected or as the AUC if the concentration trend line was extrapolated to infinity AUC_{inf}. The mean AUC_{obs} of TUL in WTD was calculated to be 12,846 ± 10,354 ng·hr/mL from Time 0 until the last measured time point at day 30 (~720 hrs). The AUC_{inf} = 14,518 ± 10,473 ng·hr/mL, which was calculated with 16.8% ± 14.2% extrapolation from the AUC_{obs}. A trend of the elimination of the drug is used to estimate the continuation of PK curve until the drug is completely undetectable; this extrapolated PK curve is used calculate the AUC_{inf} and the difference between the two AUC's divided by the AUC_{inf}, then multiplied by 100 is the percent of extrapolation.

Discussion

The mean C_{max} of TUL (Cmax = 114 ng/mL) observed in WTD serum following an IM dose via RDD was significantly lower than other PK studies focusing on TUL. In other similar ruminants, the C_{max} of serum following a hand delivered IM injection was 330 ng/mL in Holstein calves and 730 ng/mL in goats (Amera et al., 2012; Tohamy et al., 2011). When bovids and caprids were administered similar doses via SQ hand injection, their blood C_{max}'s were 377 ng/mL and 633 ng/mL, respectively (Nowakowski

et al., 2004). Bachtold et al. (2015) indicated WTD to reach adequate protective concentrations of systemic TUL following a hand injected bolus SQ, with the mean C_{max} of the study WTD peaking around 359 ng/mL. Since the TUL SQ hand injection blood concentration results were comparable to their IM counterparts in other ruminants similar to WTD, it was expected that an IM injection in WTD would yield similar PK values in bioavailability and the mean C_{max}. Furthermore, PK studies observing cattle following administration of 2.5 mg/kg dose of TUL via RDD showed little to no indication of altered PK, with a mean C_{max} of 498 ng/mL (V. Fajt, pers. comm.). Rivera et al. (2019) had similar results with mean C_{max} reaching around 755 ng/mL. However, the mean C_{max} of TUL post IM injection via RDD in WTD serum peaked at less than a third of the SQ resultant C_{max} in WTD serum in the study by Bachtold et al. (2015). Even the WTD that was re-dosed, which can be done safely with TUL because of its high therapeutic index, had a low C_{max}.

It is unclear what exactly caused the altered PK of TUL. However, there is reason to believe the use of an IM dart was a contributing factor considering Cook et al. (2016) determined the adequate absorption of TUL IM delivered via hand injection and Coetzee et al. (2018) showed altered PK of TUL in darted cattle. Cook et al. (2016) did not perform a PK study so there is no literature that discusses the serum concentration over time following IM hand injected TUL. They did measure drug residues in WTD blood and found 2.09 ppm (~2090 ng/mL) at 11 days, however this was in liver tissue and the distribution of TUL to certain tissues results in accumulation of the drug that are higher concentration than the plasma concentration (Cook et al., 2016).

The proximity of the darting distance is suspected to have been a contributing factor for both dart failure and poor serum C_{max} among the WTD darted with RDD injected TUL. Partial or complete dart failure occurred in 5 of the first 7 dart fired from the Pneu Dart X-Caliber, which is highly uncharacteristic for this dart projector. The lowest distance setting the gun has is for a 5-yard shot and several shots were taken at this distance or slightly closer and at extreme angles. The shots had to be taken so closely because the holding pens were the only location the animals could be separated to reduce stress and fear when darting occurred. The pens were completely walled in 10 ft high and were approximately 10' x 10' with a cat-walk running above the inside edge of each holding pen. The proximity of the target animals when darting could have simply been too close and beyond the minimum capabilities of the X-Caliber.

The Dan Inject Remote Tranquilizer Pistol worked as an effective backup at such close distance shots. However, there was still significant force from the dart when it impacted the deer, similar to that of the X-Caliber but slightly less and with longer needles on the darts. The reduced mean WTD serum C_{max} could have been a result of trauma and bruising from the force of the dart hitting the animal. Bruising, if significant enough, would result in an increased blood flow to the area resulting in redness and inflammation to heal the damaged tissue, but decreasing the absorption and distribution of the drug throughout the body. Close shots with an inability to decrease the CO2 pressure past a certain minimum threshold, coupled with the extreme downward angles shots which exacerbate the dart velocity issue from the acceleration due to gravity is a recipe for higher dart impact velocities and increased dart site trauma. The dart failure

observed in which several Pneu Dart IM darts shattered on impact as well as the gel collar failure when the darts bounced off are also indicative of high dart impact velocities. The shot placement of all RDD's was in the desired gluteal muscle group so it is unlikely that the poor absorption of the drug was a result of poor shot placement in fat, bone, or connective tissue. The shots conducted in the cattle darting study with TUL were taken around 25 ft which is further than all 12 darting events conducted in the present study in WTD (V. Fajt, pers. comm.).

Regardless of the low average WTD serum C_{max} (114 ng/mL), these results do not definitively indicate that TUL did not reach therapeutic levels in the lungs or other tissues. Since TUL, like other similar macrolides, tends to have a higher affinity for pleural and other fatty tissue, the drug accumulates the highest concentrations in the lungs (Carbon, 1998; Evans, 2005; Villarino et al., 2013). The MIC of TUL against most primary targets is around 1,000 ng/mL (for pathogens including *P. multocida* and *M. haemolytica*) while *A. pyogenes* is upwards of 4,000 ng/mL (Pfizer, 2005). Serum concentrations rarely get that high after a single dose, regardless of ROA, although tissues do. Cattle administered a hand delivered SQ labeled dose of TUL had a mean serum C_{max} of about 377 ng/mL while the lung tissue samples revealed a mean C_{max} of 4,100 ng/mL (Nowakowski et al. 2004). Similarly, when Bachtold et al. (2015) hand delivered TUL SQ to WTD, the serum C_{max} was 359 while the lung tissue C_{max} was 2,225 ng/mL. Respectively, WTD and cattle lung peak TUL concentrations were 6.2-fold and 10.9-fold higher than their respective C_{max} (Bachtold et al., 2015; Nowakowski et al., 2004). Although total lung tissue concentration does not truly indicate the

interalveolar concentration (IAC) and is where the drug concentration would need to be above the MIC threshold the be therapeutic against respiratory infections, it is a useful estimation of the IAC. Because of the variability and therefore lack of predictability, one cannot extrapolate the expected lung concentration based on the serum concentration. It is safe to assume that the lung C_{max} is substantially greater than the serum C_{max} based on the movement of macrolide through the body.

Another possible contributor the comparatively low C_{max} was the delay in blood sample collections after the darting events were completed. Time delays were created by the dart failures and equipment shifts mid-study as well as the difficulty involved in the learning curve of working hand raised deer through the chute system. These time delays resulted in the first blood drawing event, scheduled to take place one-hour post-injection, to actually take place around two-hours post-injection. The literature often describes the C_{max} of serum, regardless of species to peak around the one-hour mark (Pfizer, 2005) So, it is possible that the first blood draw actually missed the peak of the concentration-time curve and caught a point in the rapid concentration decline associated with the beginning of the negative exponential curve depicting the drug metabolization and elimination.

Overall the PK curve of TUL appears relatively normal, harboring what seems to be some “white noise” likely depicting the minor error in the analysis. The shape of the concentration-time curve of the drug illustrated the rapid absorption of TUL to the observed serum C_{max}, where the drug is then shown to be metabolized an eliminated at a more rapid rate by the shift to rapid exponentially decline that seems to asymptote out at zero. The minor concentration spikes seen in the mean WTD serum TUL PK curve at the

48 hr (2 d) and 120 hr (5 d) time point are likely caused by the slight degree of error in the quantitative detection and/or analysis. The average concentration dip to 0 ng/mL at the 240 hr (10 d) time point is highly likely an inaccurate measurement since there were detectable levels of TUL of all samples following the 10 d blood draw (Figure 4). The serum samples collected were on day 10 were misplaced in a normal freezer for over a month, allowing the drug time to degrade beyond detectable levels due to improper storage and slightly negatively skewing the AUC. For this reason, the samples from this time point were eliminated in the PK analysis to increase the accuracy of the analysis.

The prolonged elimination portion of the PK curve is indicative of the long $t_{1/2}$ of macrolides, especially TUL which is notoriously long (Bachtold et al., 2015; Foster et al., 2016; Nowakowski et al., 2004; Papich, 2016; Villarino et al., 2013). The $t_{1/2}$ of TUL in WTD serum (~281 hrs) was even longer than that seen in cattle, domestic pigs, and goats (~50–160 hrs) with little variation between SQ and IM injection (Bachtold et al., 2015; Gáler et al., 2004; Huang et al., 2012; Papich, 2016). The $t_{1/2}$ of TUL in deer serum following RDD IM injection (296 hrs) was quite similar to the SQ injection $t_{1/2}$ of TUL in deer serum. This alludes to the idea that the metabolism and elimination of TUL by WTD is not altered by IM injection or remote delivery there-of. The long $t_{1/2}$ of TUL is likely in part due to the large V_d that is frequently recorded in PK analyses of TUL. The substantially longer $t_{1/2}$ seen in both the present study and the study conducted by Bachtold et al. (2015) does not mean for certain, but rather is indication of, a larger V_d and can be used to assume little or no impact on the distribution of TUL in WTD.

To calculate the bioavailability of TUL in WTD following an IM injected administered via RDD, the AUC_{inf} would be needed for an IV injection of the same drug and dose. The AUC_{inf} of the IM RDD injection could be divided by the AUC_{inf} of the IV injection in order to determine the percentage of the drug that was bioavailable to the WTD following the particular ROA. Since an IV PK curve was not obtained, the true bioavailability cannot be determined. However, it is reasonable to compare the AUC_{inf} of TUL from other studies in species such as cattle, goats, and WTD using the same and alternative ROA in order to determine the relative bioavailability. When comparing the serum AUC of TUL in WTD from Bachtold et al. (2015a) and cattle serum AUC from Nowakowski et al. (2004) following SQ injection, WTD have a relative bioavailability of about 40.7%. The relative bioavailability of TUL in cattle after a dose from RDD injection compared to that of a SQ injection is about 156.6%. Similarly, in WTD when comparing the same two ROA's, the relative bioavailability of TUL in deer serum is about 303.5% for RDD compared to SQ hand injection. Although there are apparent similarities in the changes in PK of TUL caused by darting in cattle and WTD, the bioavailability reached in deer is still lower with a relative bioavailability of only 77.2% compared to the bioavailability in cattle. This moderate similarity in the bioavailability of TUL between cattle and deer does not mean that deer reach moderately similar protective tissue concentrations because the C_{max} for deer following RDD is only 15.1% of the C_{max} reached in darted cattle. The similarity in serum AUC's following darting in cattle and WTD can most likely be explained by the longer $t_{1/2}$ noticed in WTD which means longer clearance of the drug from the animal and in turn more AUC.

The analytical methods used to determine and measure the serum of WTD for TUL was used similarly by Cook et al. (2016). This study serves to further validate the use of LC-MS/MS for the identification and quantification of macrolide drugs in WTD tissues, especially serum.

Conclusion

The use of RDD systems to administer drugs to livestock and wildlife has been an effective means for many drugs, including TUL in cattle (Coetzee et al., 2018; Rivera et al., 2019). TUL is a commonly used macrolide antibiotic utilized frequently by deer breeders to treat bacterial pneumonia. Using a RDD allows for ease of drug administration and cuts down on time and money for the ranch as well as stress and injury to the deer by eliminating the need for hands-on interaction. This study sought to determine whether or not the PK of TUL would be altered, and if so to what extent, when delivering the drug IM via RDD. It was hypothesized that TUL PK would be minimally affected by the darts, however, there was significant decline in the C_{max} noticed. It is likely that there is some misnomer in the data, likely caused in part by the proximity of darting distances. However, the data indicates that darting is an unpredictable and can potentially result in dosing that results in sub-therapeutic tissue concentrations which can lead to antimicrobial resistance and increased death loss. There were several individuals that had comparable serum C_{max} to that of WTD administered TUL via SQ injection, but it was infrequent.

Acknowledgements

The authors would like to take a moment to thank all of those people that helped make the research possible. The employees and student workers of the Winnie Carter Wildlife Center at Texas A&M University deserve immense gratitude for the early mornings, late nights, and occasional weekends they spent working deer during the project as well as caring for and monitoring the animals outside of the project. Several students in particular, including Gabriella Quintana, Katlin Stone, Maddie Sacula, Taylor Williams, Cynthia Colbert, Cheyenne Green, Jessie Meibaum, Jessica Stephenson, Jacob Ross, Garrett Meurer, Brianna Stofas and Ashley Dane-Gresman were continuously involved and helping in the project. Kim Green from the Texas A&M University Institutional Animal Care and Use Committee (IACUC) is also owed a huge debt of gratitude for aiding in and facilitating the rapid processing of the Animal Use Permit (AUP) for the tulathromycin study. Finally, the Texas Deer Association is to thank; without their support and their generous donation to the advancement of science in the field of wildlife medicine, this project could not have been conducted and for that, the authors are greatly appreciative.

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APPENDIX A

Figures

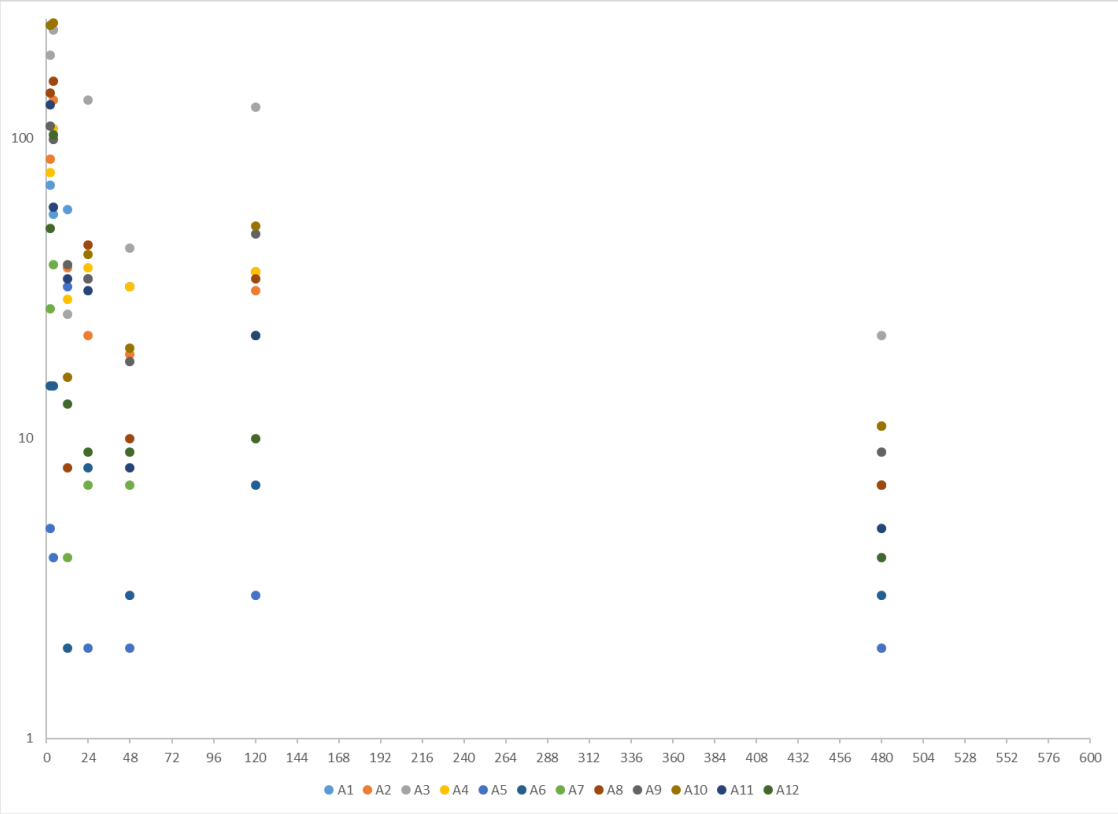
Figure 1: Deer Restraint Chute and Blood Collection

The squeeze chute designed by Priefert Ranch and Farm Equipment with a white-tailed deer does being manually restrained by several volunteers from the Winnie Carter Wildlife Center at Texas A&M University. Shown is Jake Ross restraining the head of the deer while Chase Nunez collects blood from the jugular vein.

Figure 2: Visualization of Darting from the Catwalk and One of the Darted White-Tailed Deer Buck Subjects

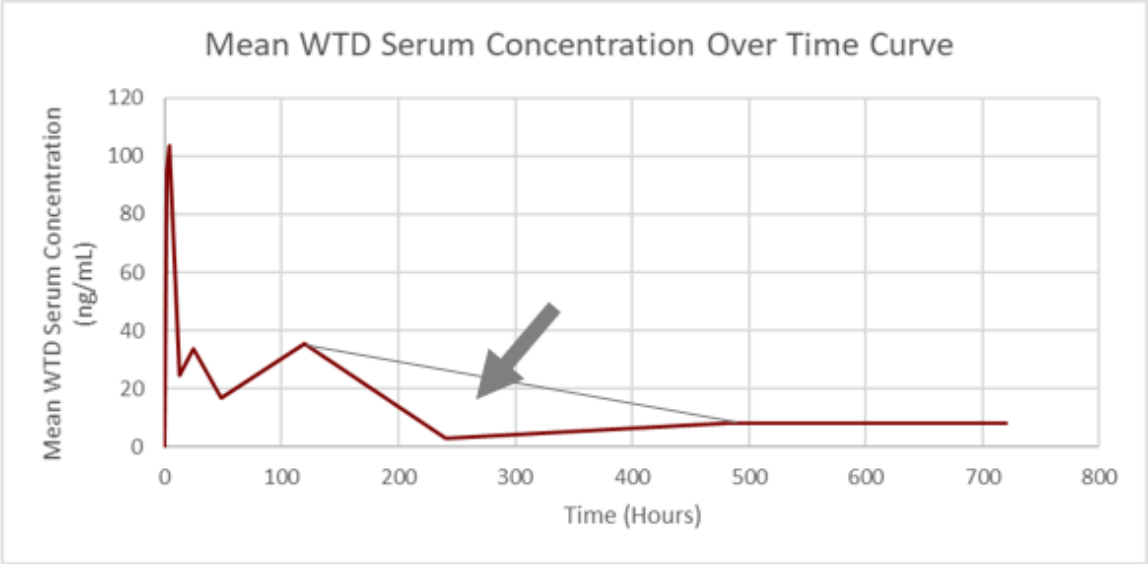
Chase Nunez preparing to dart using the Pneu Dart X-Caliber CO_2 powered dart gun from a catwalk that is 10 feet from the ground. Deer are being held in the 10'x10' holding pens below and to the right and left of the walk. Directly below the catwalk is an alley that leads to the squeeze chute (partially visible behind Chase) where the blood collections took place before returning the deer to their respective pens. The right picture shows one of the bucks (Tag #72) with a Dan Inject dart in his left rump. The downward view on the buck in the photograph also shows the extreme angle from the catwalk to the target animal. This picture of the buck was taken almost one year after the study but darted with the same equipment in the same manner. He was de-antlered before the study began.

Figure 3: The Distribution of White-Tailed Deer Serum Concentrations of Tulathromycin Over 30 Days



The serum concentration of each deer at each time point is represented by the deer respective dot color. The y-axis (vertical) describes the serum concentration in ng/mL while the x-axis (horizontal) represents the time lapse in hours. The sharp spike in concentration is representative of the raid absorption of tulathromycin followed by the gradual exponential decline as the drug is metabolized and eliminated. The spread of the concentrations at each time point shows how unpredictable remote-delivery darts can be.

Figure 4: The Mean Pharmacokinetic Curve of Tulathromycin in Deer Serum



The figure above illustrates the mean pharmacokinetic curve of tulathromycin in white-tailed deer serum following intramuscular injection via remote-delivered dart. The spikes in the curve after the initial peak concentration are likely "noise" in the analysis caused by the minor error in the analytical methods. The 240-hour time mark had an overall significant and likely unrepresentative dip that was a result of improper storage of the serum before analysis. When the results from the 240-hour blood draws are removed, there is a marked increase in the area under the serum concentration-time curve, indicated by the arrow.

APPENDIX B

Tables

Table 1: Serum Concentrations of Each White-Tailed Deer Blood Draw

Time (hrs)	Tag #11	Tag #17	Tag #21	Tag #24	Tag #25	No Tag	Tag #2	Tag #66	Tag #70	Tag #73	Tag #22	Tag #72	Average
0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	85	189	77	15	27	50	70	142	110	129	5	238	95
4	134	229	108	15	38	103	56	155	99	59	4	242	104
12	37	26	29	2	4	13	58	8	38	34	32	16	25
24	22	134	37	8	7	9	34	44	34	31	2	41	34
48	19	43	32	3	7	9	32	10	18	8	2	20	17
120	31	127	36	7	22	10	36	34	48	22	3	51	36
240	4	6	2	2	2	0	3	6	7	3	0	0	3
480	11	22	7	3	7	4	7	7	9	5	2	11	8
720	6	10	4	2	5	3	46	5	5	3	2	5	8

This table depicts the actual observed concentration of each deer for each blood draw. Notice the low serum concentrations across all twelve deer on the 240-hour time draw as well as the abnormal spike in Tag #2 at the 720-hour time draw. These outliers were excluded from the PK analysis to better the accuracy of the results.

Table 2: Comparing the Pharmacokinetics of Tulathromycin in Other Studies

	Cattle SQ	Cattle RDD1	Cattle RDD2	WTD SQ	WTD RDD	Goat SQ
AUCinf (ng·hr/mL)	12000	8433	18796	4883	14518	12500.00
Cmax (ng/mL)	377	270	756	359	114	633.00
t1/2 (hrs)	54	66	185	281	296	110.00

The table above compares three pharmacokinetic value to other studies with tulathromycin in cattle subcutaneously (Nowakowski et al., 2004), cattle with remote delivery dart (Cattle RDD1- Coetzee et al., 2018), cattle with remote delivery dart (Cattle RDD2- Rivera et al., 2019), and goats subcutaneous (Goat SQ- Young et al., 2010), and white-tailed deer subcutaneous (Bachtold et al., 2015a).

Table 3: The Mean Pharmacokinetic Values of Tulathromycin in White-Tailed Deer Serum and Their Respective Standard Deviation

	Average	St. Deviation
T_{max} (hrs)	4.03	2.72
C_{max} (ng/mL)	113.75	71.56
λ (mL/hr	0.00	0.00
$t_{1/2}$ (hrs)	296.3	224.3
AUC_{obs} (ng·hr/mL)	12846	10354
AUC_{inf} (ng·hr/mL)	14518	10473
AUC % Extrapolated	16.91	14.79

The values above are the mean pharmacokinetic values of tulathromycin measured and calculated from the serum of the twelve deer in the study (excluding the data from the 240-hour blood draw.)