

UNDERSTANDING CALF MORTALITY IN SOUTH FLORIDA: A UNIQUE APPROACH  
TO CONSERVATION

By

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To Rob, my parents, and Mowglie

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## TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS .....	4
LIST OF TABLES .....	8
LIST OF FIGURES .....	11
ABSTRACT.....	13
CHAPTER	
1 INTRODUCTION .....	15
2 VAGINAL BIRTHING SENSORS AS A TOOL TO STUDY CALF LOSSES ON LARGE SCALE APPLICATIONS.....	19
Background.....	19
Materials and Methods .....	22
Study Area .....	22
Birthing Sensor Technology.....	23
Birthing Sensor Insertion.....	24
Birthing Sensor Monitoring.....	25
Birthing Sensor Data .....	25
Data Analysis.....	26
Results.....	27
Sensor Success and Failure .....	27
Ranch and Time Effects on Sensor Failures.....	28
Discussion.....	28
Implications .....	33
Figures and Tables.....	34
3 CALF MORTALITY IN SOUTH FLORIDA.....	40
Background.....	40
Materials and Methods .....	44
Study Area .....	44
Cattle Monitoring and Data Collection .....	45
Postmortem Investigation.....	47
Data Analysis.....	47
Results.....	49
Calf Loss.....	49
Contributing Factors and Cause of Death .....	50
Discussion.....	51
Bacteria and Calf Mortality .....	52
Dystocia Related Calf Mortality.....	54

Mineral Imbalances, Toxicities, and Deficiencies .....	55
Depredations .....	57
Implications .....	58
Figures and Tables .....	60
<b>4 A NOVEL APPROACH TO PANTHER DEPREDATIONS: RARAMURI CRIOLLO CATTLE AS LIVESTOCK GUARDIANS .....</b>	<b>68</b>
Background.....	68
Materials and Methods .....	72
Study Site and Animals .....	72
Data Collection.....	73
Image Processing.....	74
Data Analysis.....	75
Occupancy Modeling.....	75
Cattle Behavior.....	76
Results.....	78
Camera Traps and Predator Occupancy .....	78
Cattle Response .....	79
Discussion.....	80
Figures and Tables.....	87
<b>5 CONCLUSIONS .....</b>	<b>97</b>
<b>APPENDIX</b>	
<b>A SUPPORTING FIGURES AND GRAPHS.....</b>	<b>101</b>
Figures .....	101
Tables.....	106
<b>B NECROPSY, CONTRIBUTING FACTORS TO DEATH, AND ASSUMED MORTALITY SUMMARIES .....</b>	<b>107</b>
Section 1A: BC Necropsy Reports .....	107
Calf 2 .....	107
Calf 8 .....	109
Calf 109 .....	111
Calf 122_2 .....	112
Calf 124 (twins).....	113
Calf 159 (twins).....	115
Calf 161 .....	117
Section 1B: BC Assumed Mortality Reports.....	119
Calf 11 .....	119
Calf 88 .....	119
Calf 198 .....	119
Calf 203 .....	120
Section 2A:BR Necropsy Reports .....	121

Calf 240 .....	121
Calf 549 .....	123
Calf 690 .....	126
Calf 1179 .....	127
Calf 1186 .....	129
Calf 3759 .....	131
Calf 5122 .....	134
Calf 5237 .....	136
Calf 5239 .....	138
Calf 6179 .....	141
Calf 6279 .....	144
Calf 7909 .....	146
Calf 11449 .....	149
Calf 21041 .....	152
Calf 091693 .....	154
Section 2A:BC Assumed Mortality Reports.....	156
Calf 6279_2 .....	156
Calf 6828 .....	157
Calf 7105 .....	157
Calf 09940 .....	157
Calf 032910 .....	158
Calf 091577 .....	158
Section 3: LR Necropsy Reports .....	159
Calf 149_2 .....	159
Calf 252_2 .....	161
Calf 304_3 .....	163
Calf 383_3 .....	165
Calf 427_3 .....	167
Calf 457_3 .....	170
LIST OF REFERENCES .....	172
BIOGRAPHICAL SKETCH .....	182

## LIST OF TABLES

<u>Table</u>	<u>page</u>
2-1	Sensor alerts, definitions, and initiation requirements for both the original and modified .....34
2-2	Failed sensors categorized by results from power and coverage tests. Not all sensors were tested for coverage. (Excludes sensors never found or expelled prior to testing protocol implementation).....34
2-3	Coefficient estimates with standard errors of binomial logistic regression models in logarithmic form, with AIC and McFadden’s R <sup>2</sup> . Model 1: null model- intercept only. Model 2: success~time. Model 3: success~ranch.....35
3-1	Total mortality statistics are based on 302 calves born at LR, BR, and BC from August 2017 to June 2018. Losses are calculated as a percentage of the mortality grouping to understand how losses occurred in each natal period.....60
3-2	Total calves examined in each area of post-mortem investigations at BADDL from August 2017 to June 2018 categorized by natal period and ranch. Percentages are the number of calves examined in each investigative area from the number sent for. ....60
3-3	Total calves diagnosed with each contributing factor to death on LR, BR, and BC from August 2017 to June 2018. Percentage of total ranch or natal cases are given for individual diagnoses.....61
3-4	Dystocia cases from LR, BR, and BC from August 2017 to June 2018 broken down by possible causative agent. Dystocia elements are not exclusive and each dystocia case could have more than one associated causative agent .....62
3-5	Most common bacteria associated with septicemia/bacteremia from LR, BR, and BC from August 2017 to June 2018. Note this list only includes bacteria that were isolated from more than one case associated with the CFD septicemia .....63
3-6	Trace minerals responsible for toxicities, deficiencies, and imbalances that possibly contributed to mortality at LR, BR, and BC from August 2017 to June 2018. Note trace mineral categorizations are not exclusive .....64
4-1	Site use estimates and detection probabilities from single-species modeling from 212 occasions at JB Ranch from September 2019 to April 2020 .....87
4-2	Results from binomial logistic regression of cattle integration with 95% CI.....87
4-3	Results from best fitted linear effects mixed model with 95% confidence intervals for model log(Act/Dist)~Breed*Time*Predator + random effects. Results are log-transformed .....88

4-4	AIC values from models of competing fixed effects.....	89
A-1	Random effects used in the Linear Mixed Effects Model with justification for inclusion in the model.....	106
B-1	Calf 2 contributing factors summary at BC.....	107
B-2	Calf 8 contributing factors summary at BC.....	109
B-3	Calf 109 contributing factors summary at BC.....	111
B-4	Calf 122_2 contributing factors summary at BC.....	112
B-5	Calf 124 (twins) contributing factors summary at BC.....	113
B-6	Calf 159 (twins) contributing factors summary at BC.....	115
B-7	Calf 161 contributing factors summary at BC.....	117
B-8	Calf 11 assumed mortality summary at BC.....	119
B-9	Calf 88 assumed mortality summary at BC.....	119
B-10	Calf 198 assumed mortality summary at BC.....	119
B-11	Calf 203 assumed mortality summary at BC.....	120
B-12	Calf 240 contributing factors summary at BR.....	121
B-13	Calf 549 contributing factors summary at BR.....	123
B-14	Calf 690 contributing factors summary at BR.....	126
B-15	Calf 1179 contributing factors summary at BR.....	127
B-16	Calf 1186 contributing factors summary at BR.....	129
B-17	Calf 3759 contributing factors summary at BR.....	131
B-18	Calf 5122 contributing factors summary at BR.....	134
B-19	Calf 5237 contributing factors summary at BR.....	136
B-20	Calf 5239 contributing factors summary at BR.....	138
B-21	Calf 6179 contributing factors summary at BR.....	141
B-22	Calf 6279 contributing factors summary at BR.....	144
B-23	Calf 7909 contributing factors summary at BR.....	146

B-24	Calf 11449 contributing factors summary at BR .....	149
B-25	Calf 21041 contributing factors summary at BR .....	152
B-26	Calf 091693 contributing factors summary at BR .....	154
B-27	Calf 6279_2 assumed mortality summary at BR .....	156
B-28	Calf 6828 assumed mortality summary at BR .....	157
B-29	Calf 7105 assumed mortality summary at BR .....	157
B-30	Calf 09940 assumed mortality summary at BR .....	157
B-31	Calf 032910 assumed mortality summary at BR .....	158
B-32	Calf 09940 assumed mortality summary at BR .....	158
B-33	Calf 149_2 contributing factors summary at LR .....	159
B-34	Calf 252_2 contributing factors summary at LR .....	161
B-35	Calf 304_3 contributing factors summary at LR .....	163
B-36	Calf 383_3 contributing factors summary at LR .....	165
B-37	Calf 427_3 contributing factors summary at LR .....	167
B-38	Calf 457_3 contributing factors summary at LR .....	170

## LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1	The birthing sensor from JMB North America. The orange tubular component houses the radio frequency transmitter and battery while the two blue appendages retain the sensor within the vaginal canal. ....36
2-2	Generalized schematic illustrating the different components necessary for the tower build in each of the three study sites. The components were typically housed on a large pole that extended up to 20m high, with the birthing sensor antenna attached as. ...36
2-3	Multi Proportion of successful sensor expulsions (N=297) by ranch over time. Individual observations are colored by ranch and coded so that 0 = sensor failure and 1 = sensor success. ....37
2-4	Binomial logistic regression predicting the probability of sensor success over time with 95% confidence intervals. Actual data points are plotted on the graph for reference.....38
2-5	Expelled sensor with a hole drilled in top to drain liquid. Residue and bacteria growth from moisture intrusion can be seen inside the cap of the sensor. Sensors without liquid intrusion would be the same orange color inside the cap rather than .....39
3-1	Location of ranches in south Florida participating in the calf mortality study. ....65
3-2	Visualization of overlapping CFDs. The number of total cases assigned to 9 of the 11 CFDs are displayed in the left corner histogram. Colored bar graph shows the natal period and frequency by which the CFDs with black dots overlap .....66
3-3	Dam with large mass on left jaw line whose month-old calf died of septicemia attributed to <i>Trueperella pyogenes</i> . ....67
4-1	Multi study pastures overlaid with camera grid. Each cell is 375m <sup>2</sup> and centerpoints are indicated by the numbers. ....89
4-2	<i>Puma concolor coryi</i> detection history at each of the camera traps from Sept 2019 to April 2020 .....90
4-3	<i>Canis latrans</i> detection history at each of the camera traps from Sept 2019 to April 2020.....91
4-4	<i>Ursus americanus floridanus</i> detection history at each of the camera traps from Sept 2019 to April 2020. ....92
4-5.	<i>Lynx rufus</i> detection history at each of the camera traps from Sept 2019 to April 2020.....93

4-6	Weekly predator frequencies at the study site. ....	94
4-7	Proportion of Brangus closest neighbors to Raramuri closest neighbors over time. ....	95
4-8	Cattle response of Brangus and Raramuri to different predator events. Nonevents that served as the control to predator events are graphed as the solid line. Time zero corresponds to the beginning of a predator event .....	96
A-1	BC study pastures utilized in birthing sensor deployments and calf mortality study .....	101
A-2	BR study pastures utilized in birthing sensor deployments and calf mortality study .....	102
A-3	LR study pastures utilized in birthing sensor deployment and calf mortality study .....	103
A-4	Bacterial species isolated during post-mortem investigations in all locations and from all specimens at LR, BR, and BC in 2017-2018. ....	104
A-4	Locations of isolated bacterial species during post-mortem investigations at LR, BR, and BC in 2017-2018. ....	105

Abstract of Thesis Presented to the Graduate School  
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Calf (*Bos taurus*) mortality is an important issue in Florida representing a major economic loss for cattle producers. Our aim was to evaluate an intravaginal birthing sensor developed for large-scale cow-calf operations to monitor calving, understand causes and rates of calf mortality from mid- to late gestation to weaning, and assess Raramuri Criollo as a potential same species livestock guardian for Brangus herds against the Florida panther (*Puma concolor coryi*). We deployed 297 birthing sensors in Brangus dams on three ranches in south Florida from September 2017 to August 2018. We identified power and battery issues of birthing sensors that led to failure of 47.1% of units. Despite failings from sensors, we were able to capture the majority (99.6%) of calving events. Total documented calf mortality was 13.24% and ranged from 5.4% to 18.9%. Through post-mortem investigations, we identified 11 factors that contributed to calf mortality. Bacterial infections, dystocia-related problems, and mineral issues were the major underlying factors associated with calf mortality. Although depredations were not a major cause of calf mortality in this study, we recognize its importance to producers and conservation efforts of carnivores. The Florida panther has been implicated in bovine calf depredations and to investigate a potential nonlethal strategy for Brangus herd protection against panthers, we examined Raramuri Criollo cattle responses to predator presence. We utilized

camera traps to capture predator presence over 37.64km<sup>2</sup> and deployed 20 GPS collars to monitor cattle behavior from September 2019 to April 2020. We did not find significant differences of cattle response between Raramuri Criollo and Brangus to panther presence; however, Raramuri Criollo did consistently exhibit different activity behaviors overall.

## CHAPTER 1 INTRODUCTION

Calf (*Bos taurus*) mortality in cow-calf operations throughout the world represents a major economic loss (USDA 2015) and may be used as an indication of animal welfare on such operations (Ortiz-Palaez et al. 2008; de Vries et al. 2011). Rates of calf mortality vary and depend on region of study, parity of dam, methodology, and definitions of natal period (Cuttance and Laven 2019). Even so, mortality rates range from 4.3% (Mee et al. 2004) to 9.8% (Cuttance et al. 2017) of total calf crop. Many of these same studies have also examined risk factors of calf mortality and describe dystocia (Lombard et al. 2007), increased severity of dystocia (Meyer et al. 2001; Johanson & Berger 2003; Bleul 2011; Motus et al. 2018), twinning compared to singleton births (Silva del Río et al. 2007; Lombard et al. 2007; Mee et al. 2008), and primiparous dams versus multiparous dams (Meyer et al. 2001; Lombard et al. 2007; Mee et al. 2008) as having increased risk of calf mortality. Other studies have described losses caused by infectious agents (Anderson 2007; Kirkbride 1992; Khodakaram-Tafti & Ikede; 2005), congenital malformation (Khodakaram-Tafti & Ikede 2005), and enteritis (Waldner et al. 2010).

Calf loss rates on cow-calf operations in the United States are estimated at 6% annually and equals approximately 2.14 million calves (USDA 2015). The loss of these calves equates to an estimated USD\$1 billion of economic losses for producers annually (USDA 2015). Similar to global trends, calf mortality rates vary by region and natal period within the United States. In one study, overall calf mortality was reported at 4.5% in the state of Colorado (Wittum et al. 1993); whereas a study in Minnesota reported perinatal losses, defined as losses of full-term calves within 48 hours of birth, at 8.0% (Silva del Río et al. 2007). As with other countries, causes of calf mortality in the United States are varied and can include a wide range of causes, such as digestive issues, respiratory issues, depredations, and weather-related problems (USDA 2015).

In Florida cow-calf operations represent a major industry that produces approximately 800,000 calves a year (USDA 2015). An estimated 4.4% of total calf crop are lost annually (USDA 2015). In a contrasting survey, 48% of cow-calf producers reported calf losses greater than the national average of 6% and some of these producers reported ~20% calf mortality rates (UF/IFAS unpublished report; Jacobs et al. 2015). Comprehensive research has not been conducted to describe rates of calf mortality nor the underlying causes of calf death in Florida. It is important for both researchers and producers to understand the discrepancies between producer perceived losses and the reported national calf losses. Awareness of the main drivers of calf loss in Florida could lead to better management, increased profit, and improved welfare conditions for calves.

To accurately estimate rates and understand causes of calf mortality, all losses must be quantified and investigated. According to the USDA (2015), calving related problems account for ~20% of all calf mortalities. Wittum et al. (1993) and Bellows et al. (1987) both recorded higher rates of losses at calving at 29.9% and 50.9%, respectively. Due to the large proportion of losses that occur at calving, it is crucial for researchers to monitor birthing events and accurately capture these losses. In general, Florida ranching operations are largely pasture-based, where pregnant dams are put out to pasture to graze over large acreage areas. Due to this style of management, tracking and monitoring birthing events and any losses associated with calving is particularly difficult. Birthing sensors to monitor calving have provided a solution for this problem but are mostly restricted to calving operations that utilize small areas where dams are confined to calving pens. Recently, the intravaginal birthing sensor, has been modified to monitor calving over large areas, but has yet to be critically evaluated in large-scale, pasture-

based applications. With this new tool, researchers could efficiently and accurately examine causes and rates of all calf mortality on Florida cow-calf ranches.

In order to effectively tackle calf mortality and increase rates of calf survival, every aspect of mortality must be addressed. One important cause of calf mortality in Florida that has wide-reaching effects beyond calf loss are depredations. In the survey of Florida ranchers, 49% of participants reported concerns regarding predator use on their ranches (UF/IFAS unpublished report). In Florida, the USDA estimates depredations accounts for a ~1/4 of total calf mortalities (2015). It is important to note this is producer reported and may be biased towards producers past experiences, thereby overestimating the impact of depredations. Regardless, rancher perceptions of predators can impact management and conservation of carnivores responsible for livestock depredations (Loveridge et al. 2010). Some predators responsible for these depredations in Florida are coyotes (*Canis latrans*), black bears (*Ursus americanus floridanus*), and panthers (*Puma concolor coryi*).

The Florida panther has been implicated in livestock depredations occurring in south Florida in its breeding range (FWC 2019). A portion of the supporting habitat required for panther recovery and expansion is held by cow-calf operations (Kautz et al. 2006). Ranchers have expressed growing concern as the panther population increases because some ranches will likely experience increasing rates of panther depredations (Pienaar et al. 2015). Current estimates of panther depredations range from 0.5-5.3% (Jacobs and Main 2015). Because of its endangered status, lethal mitigation is not an option; therefore, only nonlethal tools are available to mitigate some of these depredations.

Livestock guardians have been implemented as an effective nonlethal tool used to mitigate livestock losses in other regions (McManus et al. 2015). Livestock guardians are usually

different species than the stock they are protecting and include donkeys and dogs; however, novel methods for cattle use same species guardians and make use of different breeds with aggressive dispositions to protect the main herd. In particular, San Martinero cattle are documented as defensive of calves and anecdotal evidence suggests mixed cattle herds integrated with San Martinero experience decreased depredations by jaguars (*Panther onca*; Hoogesteijn & Hoogesteijn 2014). The Raramuri Criollo are close relatives to the San Martinero that display similar behavior. Therefore, its hypothesized that Raramuri Criollo integration into a beef herd may decrease panther depredations, thereby increasing calf survival in cow-calf operations in panther-use areas.

The purpose of this thesis is to 1.) critically test an intravaginal birthing sensor as a potential tool to study calf losses on large ranching operations, 2.) document and describe calf mortality and underlying causes of losses on Florida cow-calf operations, and 3.) investigate Raramuri Criollo's potential to serve as a same species livestock guardian for Brangus herds against the Florida panther.

## CHAPTER 2 VAGINAL BIRTHING SENSORS AS A TOOL TO STUDY CALF LOSSES ON LARGE SCALE APPLICATIONS

### **Background**

Remote sensors to monitor parturition of cattle have largely been developed to combat losses on dairy farms attributed to difficult birthing events, leading to dystocia (Ouellet et al. 2015; Saint-Dizier & Chastant-Maillard 2015). The goal of such sensors is to accurately predict and detect calving events in pregnant cows so that herd managers can assist with difficult births when necessary. Reported production gains from real-time sensor monitoring include decreases in both calf and cow mortalities and future postpartum health issues of the cow, as well as decreasing time and labor requirements of farm personnel (Mee 2004; Palombi et al. 2013).

Rapid development of technology combined with the need to monitor calving has led to multiple solutions that utilizes a variety of birthing indicators. Prior to calving, changes occur in both the behavior and the physiology of the dam that can be tracked and ultimately used to predict the onset of calving (Ouellet et al. 2015; Saint-Dizier & Chastant-Maillard 2015). Common behavioral indicators for calving include changes in rumination time, lying time, and distance from herd (Jensen 2012; Titler et al. 2015). Physiological indicators are dictated by biological changes and include changes in vaginal temperature, rectal temperatures, and hormone levels (Sakatani et al. 2018; Shah et al. 2006). Large differences exist between birthing sensors, varying greatly in their method of detection and applications. Algorithms predicting calving have been both univariate and multivariate while their application both external and internal on the dam. Saint-Dizier & Chastant-Maillard provide more detailed descriptions of the various kinds of birthing sensors (2015).

Despite advancements in sensor technology and dystocia research in other cow-calf operations (Laster et al. 1973; Meijering 1984; Zaborski et al. 2009), birthing sensors are

predominantly utilized in dairy farming. Undoubtedly, the use of accurate birthing sensors would benefit beef ranching operations similarly to what is reported for dairy farms and decrease losses associated with dystocia. Unlike dairy farms in the United States where dams are moved to calving pens (Dargatz et al. 2004; USDA 2016), multiparous dams in beef operations remain in pastures, often large, with greatly reduced observation (USDA 2009). This difference in management ability may lead to overlooked newborn calves in trouble and also introduces the inherent risk for increased depredation of said calves. According to the USDA (2015), an estimated 15.7% of total calf mortality is due to depredation in beef operations compared to 1.2% in dairy operations. Although beef calf losses are wide ranging, calving related problems and depredation account for approximately 33% of total calf losses (USDA 2015). The use of birthing sensors in the beef industry could alleviate some of these calving-related losses and losses attributed to depredation events by focusing effort towards newborn, weak calves. Furthermore, deploying birthing sensors in a ranching operation creates a unique opportunity to increase our understanding of the causes of calf loss by allowing researchers and producers rapid response capability in order to arrive in time to differentiate between various causes of mortality.

As is common with new applications of technology, there are major obstacles concerning scaling and retrofitting the current technology that must first be addressed. The use of birthing sensors within ranching operations will pose challenges for power supply, large area of application, communication between technology pieces, battery life, and sensor retention in/on the cow. Most birthing sensors require communication between a powered base receiver and the birthing sensors. Unlike dairy farms, calving pastures of ranch operations are often much larger in size and lack a constant source of power (USDA 2009). Accordingly, receivers and sensors must be able to work and communicate effectively on independent power sources throughout the

enlarged area associated with ranching operations. In addition, pregnant cows in dairy operations are moved to smaller pens (USDA 2016) and farmers have easy daily access to cows for up to 3 weeks prior to calving. In contrast, ranchers generally only have direct access to pregnant cows once prior to calving, which is during pregnancy checks and often months before the expected date of calving. This presents a problem for outfitting birthing sensors on/in the cows.

Consequently, birthing sensors must be retained by the cow for extended periods of time (~4 months) with no harmful side effects and the sensor battery must last the duration of the calving season. Furthermore, to ensure broad application, sensors must be cost-effective, which can be achieved by reusing technology across multiple calving seasons. Of the currently developed birthing sensors, the intravaginal temperature driven sensor is one that was considered most likely to overcome these concerns and be used as a research tool to monitor calving and calf loss and possibly be successfully used commercially. Notably, vaginal implant transmitters (VITs), a vaginal birthing sensor that relies on very high frequency (VHF) and temperature monitoring, has been developed for use in wild ungulate populations in large-scale environments. However, researchers have described a range of problems with their use, including inappropriate alerts, early expulsions, high equipment costs, large labor and monitoring costs, and most importantly, the tool is not conducive to immediate response to birthing events, rendering the sensor ineffective for monitoring calving and dystocia (Barbknecht et al. 2010; Johnstone-Yellin et al. 2006; Bishop et al. 2007). Cattle intravaginal birthing sensors have been effectively utilized in controlled environments (e.g., dairies), but have yet to be deployed and assessed in large-scale environments like ranching operations.

The main objectives of this study were to assess the performance of an intravaginal birthing sensor under large-scale ranching conditions and diagnose sensor failures when

applicable. Vaginal temperature sensors have previously been tested as an accurate tool to predict calving (Palombi et al. 2013; Ricci et al. 2018; Sakatani et al. 2018); therefore, our goal was not to test the accuracy of predicting calving but rather to test the ability of the technology to perform under said field conditions.

## **Materials and Methods**

### **Study Area**

The study was conducted in southern Florida on three separate ranches, BR, LR, and BC as part of an ongoing examination of calf loss. BR is Archbold Research Station's Buck Island, which manages a cattle herd of 3000 head. The study site is west of Lake Okeechobee with varied habitats including improved pasture, semi-native range, forests, and wetlands. BC is located in the Big Cypress reservation and is part of the Seminole beef cooperative that manages 5000 head of cattle between 67 tribal members. The habitats include wetlands, cypress domes, and wet hammocks and is the wettest of the three locations. LR is located near Myakka State Park and manages a total of 1500 to 2000 head of cattle. The ranch is ~8000 acres in size and consists of improved pasture, semi-native range, wetlands, and upland forests. Each study site included 3 to 4 individual pastures that ranged from 0.36 to 0.55 km<sup>2</sup> in area. The herd had access to a single pasture during any one time period and were moved through the pastures periodically, according to the management of the ranch. The pasture on all study sites were improved pastures with occasional patches of mesic oak hammocks. Average monthly temperature from August through May ranged from 15°C to 29°C, with daily minimum temperature < 0°C in December and daily maximum temperature > 34°C in August. The annual average precipitation was 132.5cm, with a defined wet season from May through October. All cows were multiparous and mixed breed of Angus and Brahman. All procedures involving

animals were approved by the Institutional Animal Care and Use Committee and complied with NIH guide for the care and use of lab animals.

### **Birth Sensor Technology**

The Vel'Phone® birthing sensor, which had been adapted to North American conditions by JMB North America (product # JMB-10005) was used in this study (Figure 2-1). The birthing sensor was originally developed by Medria Solutions®, France for use in dairy farms. The birthing sensor monitors and records temperature and sends all the data through a LoRaWan® gateway (LinkLabs® Symphony Link™ Gateway: product # LL-BST-8; outdoor version) to a host server. The design of the original sensor constantly monitored temperature to create a temperature profile of each cow, with access to the data made available to the client. In addition, the original design alerted researchers and farmers in three situations: when the sensor was activated after insertion in the cow, when the vaginal temperature increased indicating calving was probable within 24 to 48 hours, and when the sensor was expelled from the cow with the amniotic sac indicating parturition (Table 2-1; Medria Solutions 2002).

For the purposes of this study, the Vel'Phone was modified to extend battery life by removing constant temperature reporting (i.e., sensors neither recorded nor pushed temperature data through the gateway to the server to create temperature profiles for the client) and instead passively monitored temperature for changes associated with sensor expulsion from the vaginal canal, the third situation described above. The modified sensors did not send alerts for sensor activation or probable calving. Upon expulsion, the sensor detects a change in temperature and emits an alert data packet using LoRa radio wave frequency protocols that is received by the gateway (Tad & Floch 2017). Importantly, this step requires two-way communication between the gateway and the birthing sensor. Following initial communication from the sensor, the

gateway does two things: sends a confirmation message back to the sensor and distributes the sensor information, in our case via cellular network, to a server and subsequently creates an expulsion alert for the client. Alerts can be received directly on the host's server and may also be sent as SMS and/or emails. The birthing sensor will remain in expulsion mode and will continue to send expulsion alerts to the gateway until either the sensor receives gateway confirmation or 3-hours pass without gateway communication, at which time the sensor switches off. In such cases, no alerts would be sent and calving would be missed entirely. With gateway confirmation, the birthing sensor is able to move into beacon mode where the sensor emits a red flashing light and a unique ID number via Bluetooth (BLE) every 12 seconds and to help locate the sensor in the field. The first manufactured sensors also sent alerts to the gateway while in beacon mode every three hours until the sensor was found and powered off or consumed all power. This protocol has since been adjusted to send alerts for 48hrs after which it should turn-off.

Prior to sensor deployment, a tower was built that housed all pertinent communication components, including the LinkLabs gateway (Figure 2-2). The tower was placed in the center of each set of study pastures on three different ranches. Communication components were powered by 100-watt solar panels and a 12-volt deep cycle battery. The received signal strength indicator (RSSI) between the gateway and the sensor was tested around the perimeter of the study sites to ensure sensors could effectively communicate with the gateway within each area. When a perimeter was beyond the appropriate signal strength, a repeater was placed between the perimeter and the gateway to improve signal capture.

### **Birthing Sensor Insertion**

Sensors were connected to the gateway and prepared for insertion between 10 minutes to 2 hours prior to insertion. The web interface and flashing indicators on the sensors were used to confirm sensors were in the appropriate "sensor ready" mode and then disinfected with diluted

chlorohexidine before rinsing with fresh water just before insertion. Cows were confirmed pregnant and fetal aged using ultrasound by a skilled veterinarian, selected for the third trimester, and then fitted with a birthing sensor, with up to 110 cows per study site.

### **Birthing Sensor Monitoring**

Sensors were monitored by text message notifications and a website interface. The website was checked multiple times each day for new alerts and all alerts were downloaded weekly. The website also displayed a timestamp for the last time the gateway successfully connected to the server and could be used as a proxy indicator for an active gateway. When the last connection was greater than 3-hours, the communication tower was checked for power as it tended to be the most common cause of non-functioning gateway. Expulsion alerts were responded to as soon as possible, as well as all other alerts from sensors that had not yet been expelled. Study herds were checked every 2 days if no alerts were received. At the beginning of the study only alerts (i.e. sensor ready alerts, expulsion alerts, and beacon alerts) were recorded. As the study progressed and we noted more sensor failures, it was decided to also record all pertinent information relating to why a sensor may have failed to communicate an expulsion as well. This information included signal strength at the exact location and arrangement of sensor on the ground, whether the sensor was able to be restarted and connected to the gateway, or if it was completely non-functional. Furthermore, we also used a known working sensor to test gateway connectivity by resetting the working sensor at the failed sensors site of expulsion. Sensors were located in the field by sight or BLE beacon monitored by cellphones. The BLE capability was approximately 20m distance.

### **Birthing Sensor Data**

Sensor success was based on whether an expulsion alert or beacon mode alert was received via text messages or on the web interface. Conversely, we considered a failed sensor as

one that did not send either of these alerts on the server or by text. Sensors that did not work were then categorized by suspected failure type into two categories, power and coverage. The former was determined by reset capabilities and sensors were considered drained of power when the sensor would not turn on. Coverage failures were diagnosed according to signal strength of the area as recorded by the network tester and test sensors. Sensors were categorized under coverage failures when the received signal strength was  $\leq -120$  or the test sensor would not connect to the gateway. By these definitions, sensors can be categorized under both coverage and power failures. Sensors that did not fall into either of these categories were not diagnosable and categorized as unknown.

### **Data Analysis**

Overall sensor success was quantified using the above definition of success and failure in section 2.5. The success of sensors at each of the three ranches were compared using a Pearson's chi-squared test statistic. Descriptive statistics were employed to categorize causes of sensor failures. In addition, in the framework of generalized linear models (GLM), we used a binomial logistic regression to explore sensor success as a function of time (model 2), ranch (model 3), time and ranch (model 4), and an interaction between time and ranch (model 5). The logit link function was used in the GLM. Although we expect there are other predictors of failure, these two variables could be ascertained from our data collection of all sensors. We used Akaike's Information Criterion and McFadden's  $R^2$  to determine model ranking (Burnham & Anderson, 2002; McFadden, 1974). We conducted model comparisons among the model with the lowest AIC and models that were within  $2 \Delta AIC$ . We followed recommendations from Arnold (2010) when selecting the best supported model and considered the addition of a variable as unsupported if the  $\Delta AIC$  was  $< 2$ . We do not expect that our model will fully explain or predict

failures; rather, we were interested in how much variation is explained by these two independent variables and understanding their effect on overall sensor success. Lastly, we modeled success as a binomial logistic regression in order to predict probability of failure over time. All statistical analyses were completed in R, version 3.5.2 (R Core Team 2018) and employing the packages rcompanion (Salvatore 2020), dplyr (Wickham et al. 2019), and ggplot2 (Wickham 2016).

## **Results**

A total of 297 sensors were deployed between August 2017 and May 2018, with 110, 108, and 79 sensor deployments at BR, LR, and BC, respectively. Sensor deployments ranged from 0.5 – 146 days (mean $\pm$ SD; 72.1 $\pm$ 43.6) over all three ranches. Average sensor deployments on each ranch were 98.8, 75.9, and 29.7 days at BR, LR, BC, respectively. The large variation in sensor deployment days was a direct result of when sensors were fitted relative to gestation time. One-hundred percent of the sensors were expelled, and only five of the functioning sensors were expelled early or greater than 24-hours prior to calving (~2%).

### **Sensor Success and Failure**

A total of 140 (47.1%) sensors failed to send any type of alert upon expulsion. Total failures at BR, LR, and BC were 60.9%, 41.7%, and 35.4%, respectively. Sensor failures varied significantly by ranch (Pearson's chi-squared,  $X^2=14.005$ ,  $df=2$ ,  $p\text{-value}<0.001$ ) and subsequent chi-squared post-hoc pairwise comparison results revealed that the higher occurrence failures at BR was statistically different to both BC and LR ( $p\text{-value}_{BC-BR}=0.003$ ;  $p\text{-value}_{LR-BR}=0.01$ ).

Of the 140 sensors that failed, 41 (29.3%) were never found in the field; thus, further data was not collected and these sensors were excluded from further analysis. Twenty-two sensors were not tested because they were expelled prior to the time that stringent testing began. Therefore, a total of 77 sensors were tested in some capacity but not all tests were performed on all sensors (Table 2-2). All 77 sensors were tested for power but only 42 sensors were tested for

gateway coverage. Fifty sensors of the 77 failed sensors were identified to be drained of power and a total of six sensors from the 42 tested were out of range. Three of the sensors that were out of range were also out of power. The potential cause of failure was not identified in 24 sensors, as they were found with power. Of these unknown failures, 14 were in range and the other 10 were not tested for coverage.

### **Ranch and Time Effects on Sensor Failures**

Success of sensors declined at all three ranches as time sensors deployed increased (Figure 2-3, Table 2-3). Comparing binomial logistic regression models, model 4 had the lowest AIC value at 370.82; however, model 2 and model 5 were both within  $\Delta AIC$  2. According to Arnold (2010), the addition of variables is considered “unsupported embellishments” when the  $\Delta AIC$  is  $\leq 2$ . The McFadden’s  $R^2$  values demonstrate that very little variation was explained by the addition of either ranch or the interaction variables and the ANOVA comparing all three models was not statistically significant. In our case, model 2 demonstrates the strongest support and the addition of variables in model 4 and 5 are uninformative. Therefore, we selected model 2 to predict the probability of sensor success with 95% confidence intervals (Figure 2- 4). Our equation from this model is  $\log(P/(1-P)) = 1.490-0.019x$ , where P is the probability of sensor success and x is time in days.

### **Discussion**

Calving alert systems have multiple technological components and failures may stem from any one of the components or interaction between components. Failures may derive from power losses to the gateway, battery issues within the sensors, moisture in the sensors, poor signal strength (sensors out of gateway range), and server issues, to name a few. Notably, the sensors we deployed did not have a 100% success rate, even at the beginning of the sensor

deployments. At best, the predicted the probability of success was 81.8% (95% CI [73.4%, 88.4%]) at day zero when modelled using logistic regression. After 50 days of deployment, the probability of sensor success was reduced to 63.4% and by day 80 of sensor deployments, the probability of success was predicted as 50%. While the likelihood of a successful alert could be increased by deploying sensors closer to the estimated date of birth, this solution is not feasible in most working ranches as it would require large changes in management, such as more defined birthing seasons and/or later palpations of herds.

A major source of failure we encountered throughout the study was sensors drained of power. Because birthing sensors were supposed to turn off after three hours if there was no communication with the gateway following expulsion, sensors should not continue to expend their battery at locations that occur out of range; thus, we were able to eliminate sensor-gateway connectivity issues at expulsion as a source of dead sensors. Furthermore, to ensure this 3-hour protocol was functioning correctly and not the source of our power failures, we checked the signal strength of the expulsion location to the gateway receiver. Relatively few sensors that were tested were out of range (14.2%) and only half of these were drained of power suggesting this protocol was working correctly. Other explanations of sensor power failures include moisture intrusion into the battery portion of the sensor or simply a much shorter battery life than initially promised (5-7years). Moisture can corrode electrical components over time and liquid was seen inside the lids of many of the sensors (Figure 5) but data on this factor was not collected during the field season. After discussions with the production company and in subsequent testing of new sensors in the lab, 100% of the new sensors had moisture in the cap following an 8-week test (unpublished data), but these lab-tested sensors did not fail at a similar rate to the ones in the field, suggesting that perhaps either environmental conditions, or the

communication between components contributing to sensor failures was fixed in this new batch of sensors. It should be noted that the electrical components of the sensor are embedded in a resin which is supposed to provide 100% protection from moisture intrusion independent of the outer packaging plastic shell. Besides corrosion of electrical components, the liquid in the sensor lids was particularly concerning for cow health as it could potentially transfer bacteria and result in infections when sensors are used across different cows as originally intended.

JMB North America and Medria Solutions, postulated either power outages, communication protocols, or undulating temperature during sensor activation caused drained sensor batteries. According to the companies, if a sensor encountered problems during activation, the sensor should move back into sensor ready mode and continue temperature sensing until the sensor has been activated once again. Due to a supposed error in the firmware, sensors did not follow this protocol. Instead, sensors that were interrupted or fluctuated above then below the threshold temperature during the activation period moved into beacon mode and would subsequently expend battery life within 21 days. Unknown power outages that would interrupt communication with the gateway during activation are unlikely the cause because sensor insertions occurred in chutes with a constant source of power. Nevertheless, if connection with the gateway was interrupted, sensors deployed nearer the interruption would have a higher likelihood of failure as they would have less time to reach the minimum temperature threshold for activation. This should result in sensor failures to occur in groups instead of randomly as we observed in this study. The undulating temperature issue may explain some sensor failures but does not explain dead sensors that were expelled during the first 21 days, which happened in 4 known cases and potentially another 11 cases. Additionally, we'd expect that failure rates would

remain constant over time. Instead we see decreased odds of success with time which supports the idea that either battery life or corrosion was the major cause of failed sensors.

Sensor failures were first discovered after the initial insertion at BC, the first ranch where we deployed sensors. Calves were found with no alerts, and sensors were found on the ground in off mode but could be turned on and connected to gateway. In this particular situation at BC, more than half of the sensors did not connect to the gateway during the activation period of sensor deployments and therefore would not have worked upon expulsion. Accordingly, approximately two months after the initial insertions, study cows came back through the chutes and disconnected sensors had to be removed, reset, and reinserted. If the problem were associated with an interruption of sensor-gateway communication during activation, we'd expect that the majority of these pulled sensors would be dead and that was not the case. The reason for failure between sensor and gateway communication is still unknown. Because days of insertion negatively affected the sensor success and BC sensors were deployed significantly fewer days due to the issue described, we would expect that the overall failure at BC would be less than LR and BR. This supports our findings and the large variation between failures at BC and BR. Regardless, results from the binomial logistic regression indicate that BC followed similar trends to BR and LR, and if sensors had remained in cows for the same duration, failure rates would likely be similar across all three ranches.

With the implementation of technology for a new application on a commercial scale, we expected to forgive approximately 10% failure rates with decreasing failures at new sites of sensor deployments as unseen issues were solved. Therefore, we theorized that BC, our first study site would have the highest number of failures and BR, our last study site would have the lowest sensor failures. Although we observed differences in sensor failures between the three

ranches, it was the opposite of our predictions, with failures increasing with each sensor deployment, possibly related to age of battery. BR had the highest rates of failures despite our attempts to rectify technological issues through eliminating potential sources of sensor failures, increasing checks and balances, and monitoring gateway connectivity and power.

Despite efforts to understand the source of sensor failures, we were unable to diagnose 31% of failed, tested sensors. All of these sensors were able to turn on and still had power but were not in beacon mode. This suggests that the sensors may have never connected to the gateway during the initial deployment, similar to the problem described at BC. In order to combat some of the ambiguity involved with initial sensor activation, sensors should send alerts when they are successfully activated and monitoring temperature. This feature was removed with the development of the new sensors but should be reinstated in some minimal way in the future versions of the birthing sensors. Another possibility for unknown sensor failures is an issue with the expulsion protocol. Expulsion alerts are only sent when the temperature decreases below 36.0°C. In our study system, air temperatures can reach >32°C in the shade during warm months. As is common with VITs, it is possible that the temperature of the sensor in direct sunlight does not meet the minimum temperature requirement for an expulsion alert but we would expect a delayed expulsion response later that evening as the weather cooled (Barbknecht et al. 2010; Bishop et al. 2010; Johnstone-Yellin et al. 2006). Although it is possible that some sensors were unable to reach the minimum temperature threshold because they were expelled during the hottest part of the day and then found within hours, it is highly unlikely to explain all 24 unknown failures. We know that at least eight of these 24 sensors either occurred at night or early morning before the temperature rose or were expelled in shaded areas that did not exceed the minimum required temperature.

It is possible that some sensors without power are due in part to initial gateway-sensor interruption during activation; however, it does not account for the entirety of the dead sensors. New sensors that were tested in the lab did not fail at the same rate as in the field. These new sensors had new replacement hardware component that may have fixed one of the issues causing dead, failing sensors. Ultimately, we believe a combination of hardware protocols, internal sensor components, and short battery life caused for the majority of sensor failures.

### **Implications**

With an improved success rate, birthing sensors could decrease calf and cow losses due to dystocia but at the current level of performance these vaginal birthing sensors are not an effective tool for ranchers or large-scale beef producers. Further development of other forms of birthing sensors may be beneficial for future research and commercial usage. Vaginal temperature is a reliable indicator to predict calving but the current vaginal birthing sensors are not an effective tool for a calving alert system in a large-scale environment. Commercial usage in large scale environments is unfeasible because half of the sensors would likely not be recovered in the field and would equate to a large financial loss totaling \$150 per sensor. Studies are currently in progress that are assessing an updated version of the JMB intravaginal sensor that may provide improved answers to questions regarding failed expected performance.

## Figures and Tables

Table 2-1. Sensor alerts, definitions, and initiation requirements for both the original and modified

Alert Type	Initiation Requirements		Supposed Meaning	
Activation	Temperature increases to or above 36.4°C	Sensor is now monitoring temperature and will send alerts as they occur	✓	*
Predicted Calving	Temperature decreases following an increase in temperature <sup>a</sup>	Calving may occur in 24 to 48 hours- sensor status has not changed	✓	
Expulsion	Temperature decreases to or below 36.0°C	Sensor is expelled with the amniotic sac indicating a birth event	✓	✓
Beacon	Receives expulsion confirmation from gateway	Sensor is in the field and has yet to be recovered	✓	✓
Error	Unknown <sup>b</sup>	Unknown	Unknown	✓

\*Modified sensors still require temperature to increase to 36.4°C but do not send alert when this occurs.

<sup>a</sup>The algorithms for temperature increases and decreases are described in more detail in Medria Solutions, 2002. p. 36. <sup>b</sup>Believed an issue with capacitors within the internal hardware of the sensors was responsible for the error messages. Note: Original sensors did send alerts in other circumstances related to daily cow monitoring that were omitted in this table for brevity and relevance.

Table 2-2. Failed sensors categorized by results from power and coverage tests. Not all sensors were tested for coverage. (Excludes sensors never found or expelled prior to testing protocol implementation).

Power Status	Test Outcomes			
	Out of Coverage	In Coverage	Not Tested for Coverage	Total
Drained Battery	3	22	25	50
Full Power	3	14	10	27
Total	6	36	35	

Table 2-3. Coefficient estimates with standard errors of binomial logistic regression models in logarithmic form, with AIC and McFadden's  $R^2$ . Model 1: null model- intercept only. Model 2: success~time. Model 3: success~ranch. Model 4: success~time+ranch. Model 5: success~time\*ranch.

Model	Constant	Time (days)	Sensor Success			Reporting Estimates					
			BC	BR	LR	Time*BR	Time*LR	Obs. Akaike Inf. Crit.	$\Delta$ AIC	McFadden's $R^2$	
(1)	0.115 (0.116)							297	412.76	41.94	0
(2)	1.490*** (0.261)	- 0.019*** (0.003)						297	371.73	0.92	0.105
(3)			0.600* (0.235)	-0.444* (0.195)	0.337 (0.195)			297	402.64	31.83	0.034
(4)		- 0.022*** (0.004)	1.281*** (0.276)	1.672*** (0.438)	2.064*** (0.394)			297	370.82	0	0.117
(5)		-0.017 (0.010)	1.137*** (0.407)	0.999 (0.633)	2.728*** (0.600)	0.003 (0.012)	-0.012 (0.012)	297	371.82	1.00	0.124

Note: \*  $p < 0.1$ ; \*\*  $p < 0.05$ ; \*\*\*  $p < 0.01$



Figure 2-1. The birthing sensor from JMB North America. The orange tubular component houses the radio frequency transmitter and battery while the two blue appendages retain the sensor within the vaginal canal.

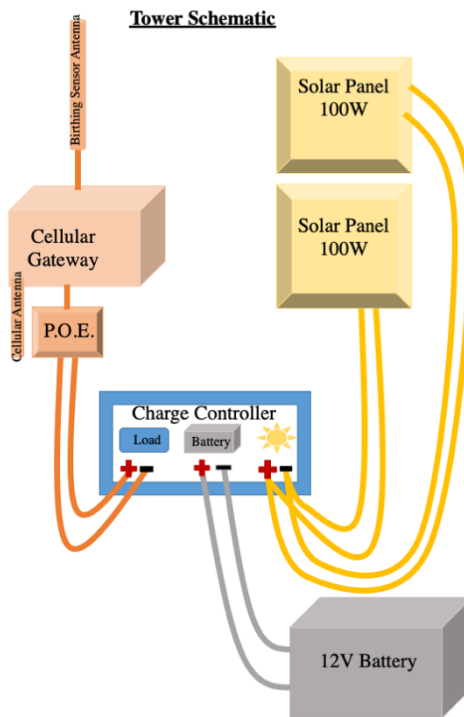


Figure 2-2. Generalized schematic illustrating the different components necessary for the tower build in each of the three study sites. The components were typically housed on a large pole that extended up to 20m high, with the birthing sensor antenna attached as high as possible and other components in reach for maintenance. P.O.E. is power of ethernet.

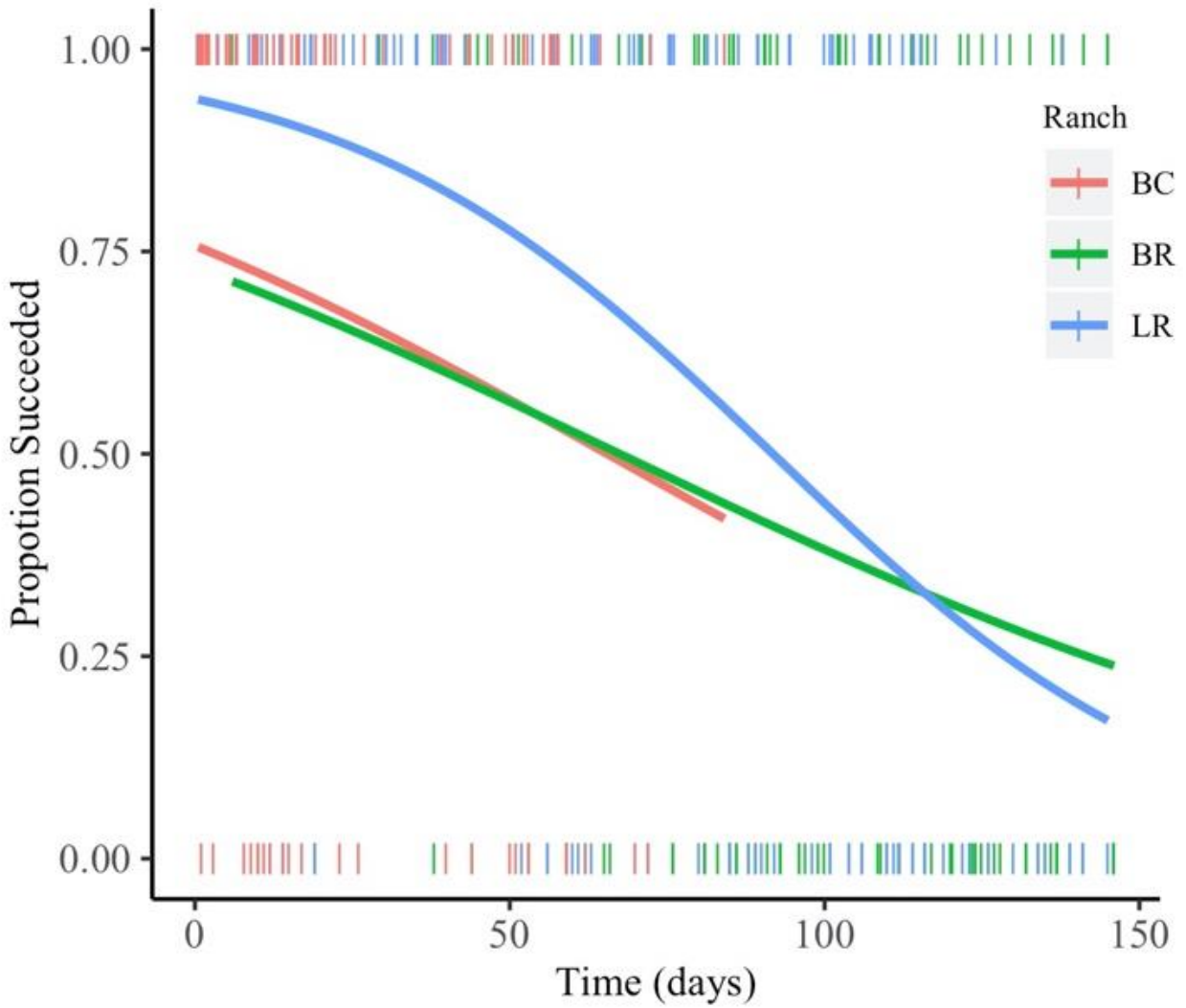


Figure 2-3. Multi Proportion of successful sensor expulsions (N=297) by ranch over time. Individual observations are colored by ranch and coded so that 0 = sensor failure and 1 = sensor success.

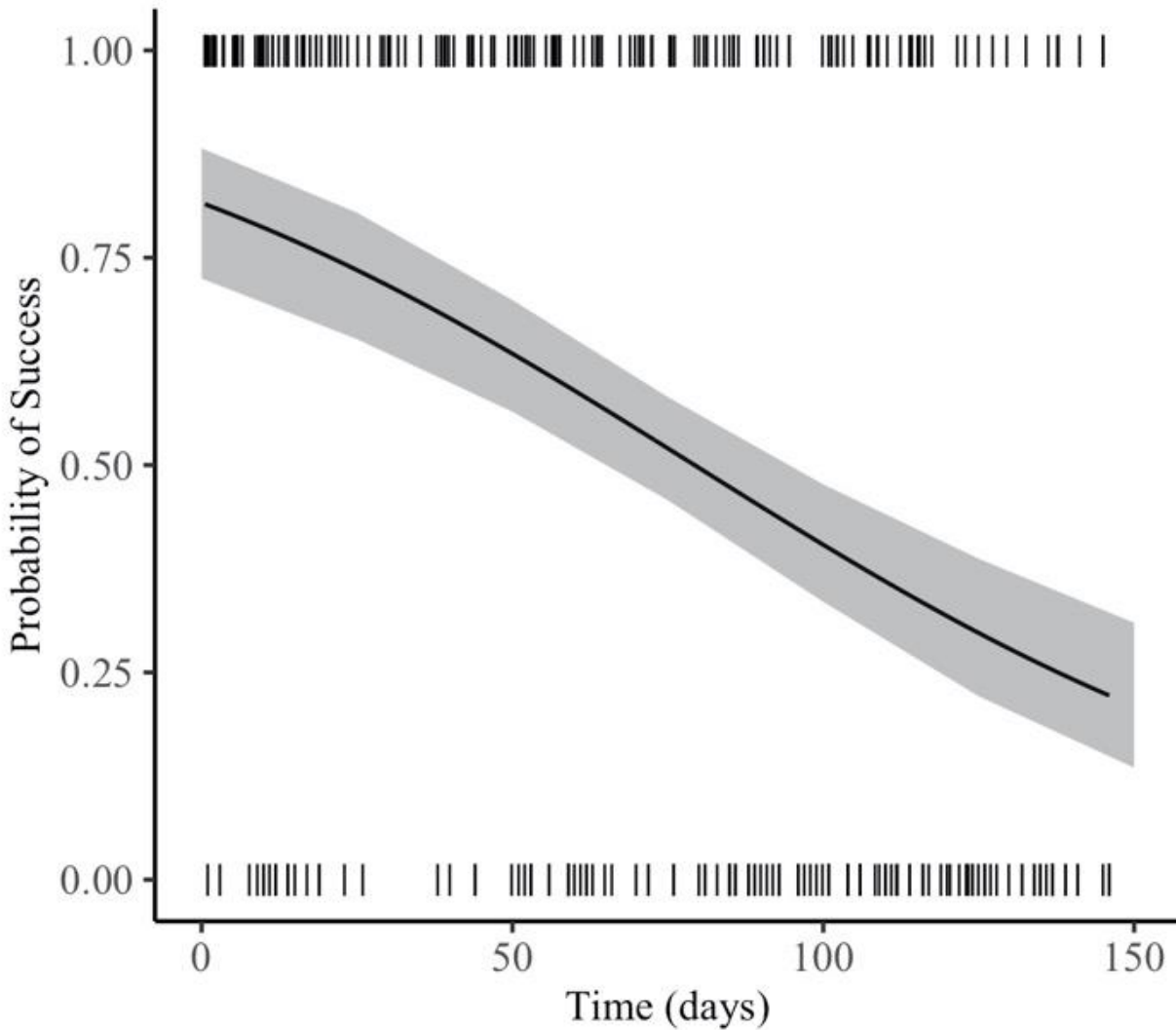


Figure 2-4. Binomial logistic regression predicting the probability of sensor success over time with 95% confidence intervals. Actual data points are plotted on the graph for reference.



Figure 2-5. Expelled sensor with a hole drilled in top to drain liquid. Residue and bacteria growth from moisture intrusion can be seen inside the cap of the sensor. Sensors without liquid intrusion would be the same orange color inside the cap rather than the dark color.

## CHAPTER 3 CALF MORTALITY IN SOUTH FLORIDA

### **Background**

Calf mortality has been a main focus for both producers and researchers in the cattle livestock industry because it represents a major economic loss for producers (USDA 2015) and can be used as an indication of animal husbandry and welfare on farms (Ortiz-Pelaez et al. 2008; de Vries et al. 2011). Studies conducted previously have reported total calf mortality rates from 4.3% (Mee et al. 2008) to 9.8% (Cuttance et al. 2017). However, these rates are dependent on definitions of natal period, the region of ranching, parity of dam, and methodology followed and have led to mortality rates that may be incomparable between studies (Cuttance & Laven 2019). When mortality has been observed most studies also evaluated the risk factors associated with calf mortality on both beef and dairy farms. Dystocia, parity, and twinning are well documented risk factors of calf mortality, where increased severity of dystocia (Meijering 1984; Meyer et al. 2001; Johanson & Berger 2003; Bleul 2011; Motus et al. 2018), primiparous versus multiparous dams (Meyer et al. 2001; Lombard et al. 2007; Mee et al. 2008), and twins versus singletons (Silva del Río et al. 2007; Lombard et al. 2007; Mee et al. 2008; Bleul 2011; Elghafghuf et al. 2014) are all associated with increased risk of calf mortality. Even so, there are risk factors that are less agreed upon in the literature, such as calf sex and season (Cuttance and Laven 2019).

While large, retrospective data sets commonly used in these studies are appropriate for calculating calf mortality and associated risk factors, they lack robust information surrounding circumstances of death. In some cases, broad categorizations of observed symptoms at the time of death may be derived from these datasets, such as respiratory or digestive issues, but these data are not appropriate for diagnosing underlying cause(s) of death. While understanding risk factors is an important aspect of calf mortality, understanding the various causes of death would

provide a more focused, comprehensive, and effective approach to calf mortality management. Producers and researchers could target the main causative agents of mortality rather than simply manage the symptoms. Moreover, the majority of these studies rely on producer participation and observation of calf death, which can be biased towards the producer's education and experience and underreporting. Despite data validation, incidents of calf mortality still may be biased because the validation process often requires large amounts of data be removed from the final dataset.

Underlying cause(s) of death is often complex, multifactorial, and requires increased time and expense to accurately diagnose. Furthermore, not all cases are diagnosable despite post-mortem investigations, hence the possible reason few studies have attempted to investigate cause of death. In fact, less than 50% of all submitted post-mortem aborted calves were diagnosed with an etiological cause in Kirkbride (1993) and Khodakaram-Tafti & Ikede (2005). Waldner et al. (2010) had the highest diagnostic success, with more than 75% of cases diagnosed for stillborn, neonatal, and older calves. Importantly, calf mortality diagnoses benefit from observational data by providing details of dam and calf behavior that may indicate and support clinical diagnoses. Despite the efforts of researchers, observations of new born calves and retrieval of carcasses in the event of mortality near the time of birth presents a challenge that essentially requires 24-hour surveillance of the calving herd. Additionally, the ability to observe and find dams, calves and carcasses becomes increasingly challenging as the scale of the pasture increases to hundreds or thousands of acres. For example, Waldner et al. (2010) only submitted 35% and 72% of aborted and stillborn calf carcasses compared to 94% of carcasses of older calves, suggesting aborted and stillborn calves were more difficult to retrieve in a timely manner. The inability to capture these

deaths could lead to biases or completely overlook causes of death that account for a large proportion of mortalities at birth.

In addition, depredation of calves is usually not included in these studies but has been investigated independently. These studies have found that depredation can range greatly between herds and year but can be as high as 85% of the total calf mortality in some instances (Breck et al. 2011). It may be of interest for producers to understand any relationships between weak calves, underlying illnesses, or nutritional deficiencies and depredations if they exist; thus, post-mortem examinations should be conducted on these calves, when possible. At the very least, observational data can provide insights on observed calf health and depredations.

In the United States, the national calf loss rate in cow-calf operations averages approximately 6% annually, equaling roughly 2.14 million calves (USDA 2015). The loss of these calves equates to approximately \$1 billion of economic losses in a single year (USDA 2015). By these estimates, a mere 1% reduction in calf losses would result in \$10 million in savings for producers. A breakdown of causes of calf mortalities is given in the same USDA report (2015) but categories are vague and describe broad, observable conditions related to health issues (i.e. respiratory issues, calving-related problems, digestive). The USDA report although useful, may not accurately describe calf loss as it is based on producer participation through survey responses. Particularly, differentiation between depredations and scavenging events is difficult and because of this, estimates by producers may overestimate the impact of depredations on their ranches.

Studies in the United States reported perinatal losses, defined as losses of full-term calves born at birth or within 48 hours, at 8.0% (Silva del Río et al. 2007), 8.2% of singleton births (Lombard et al. 2007), 11.1% and 5.7% for primiparous and multiparous dams, respectively,

(Meyer et al. 2001). Although perinatal rates often represent the majority of calf mortality, losses still occur in the prenatal and postnatal periods (Patterson et al. 1987; Bunter et al. 2014; Raboisson et al. 2013); thus, these figures represent an underestimation of total calf mortality and demonstrate the variability in calf loss rates throughout the country. Of the peer-reviewed studies conducted in the United States, only two attempted to diagnose all causes of calf death. Wittum et al. (1993) categorized cause of death but diagnoses were based solely on observational data recorded by farm personnel and relayed to veterinarians for final diagnoses. Bellows et al. (1987) investigated calf mortality in Montana through post-mortem investigations and found that dystocia was a leading cause of calf mortality. Thirty years of technological advancements have led to improved veterinary science and herd management of cattle; therefore, causes of calf mortality today could be vastly different than causes found in these previous studies. Additionally, these findings may not be representative of losses in cow-calf operations in other regions of the United States because of variations in climate and management practices.

In Florida, cow-calf operations produce around 800,000 calves a year and according to the USDA (2015) Florida cow-calf operations suffer a 4.4% calf loss. In a contrasting survey conducted solely in Florida, producers estimated calf loss at varying rates but 48% of producers estimated calf loss was >6%, and shockingly some producers estimated calf loss at ~20% of their calf crop (unpublished report). To the author's knowledge there have not been any studies that have investigated Florida calf loss nor potential reasons for this discrepancy. Although, one study conducted in Florida suggests that depredations may account for a portion of these additional losses on some ranches (Jacobs & Main 2015), it is unlikely to account for the 14% difference between some Florida estimates and the national calf loss average.

If calf losses are in fact higher in Florida, understanding why calf losses occur at these increased rates is of importance that would have significant implications. Awareness of the main drivers of calf loss in Florida could lead to better management, increased profit, and improved welfare conditions for calves; therefore, it is important to quantify and address all causes of calf loss. With advances in technology that permitted constant monitoring of parturition and calves, we attempted to conduct the most comprehensive study of pre-, peri- and postnatal calf loss to date in Florida. Our main objectives were to 1.) quantify all calf losses, including depredations, occurring on three Florida commercial cow-calf beef operations from final pregnancy checks to calf weaning in multiparous dams and 2.) diagnose contributing factors of death and when possible, diagnose cause(s) of calf mortalities through post-mortem investigation coupled with observational data.

## **Materials and Methods**

### **Study Area**

The study was undertaken in south Florida on three separate ranches, LR, BR, and BC (Figure 3-1). All study sites are located in the subtropical region of Florida, with a defined rainy season from May through October. LR, located near Myakka State Park, consists of approximately 8,000 acres and is a blend of improved pasture, semi-native range, wetlands and upland forest. The cattle herd ranges from 1500 to 2000 head. BR is similar in acreage to LR, with about 10,300 acres of land. BR runs approximately 3000 head of cattle and is known as Archbold's Buck Island Ranch. BR also consists of a mosaic of habitats including improved pasture, semi-native range, forests, and wetlands. BC is located in the Big Cypress Reservation and is part of the Seminole beef cooperative that's comprised of approximately 5000 head of cattle that are individually managed by 67 tribal members. This site is at the boundary of the everglades and the wettest of the three locations, with pastures surrounding cypress domes and

wet hammocks. All ranches are ditched to drain water from the landscape during the wet season but can also be used to flood irrigate through pumping in the dry season.

### **Cattle Monitoring and Data Collection**

Parturition was remotely monitored by JMB North America's intravaginal birthing sensor (# JMB-10005, JMB North America, Amsterdam, NY USA). Details of vaginal birthing sensor and system are described in Chapter 2. Cows were palpated transrectally 3 months before the expected first calf based on bull exposure records, to determine pregnancy status. Up to 110 pregnant cows in the last trimester at each ranch were fitted with a birthing sensor that was placed in the vaginal canal against the cervix. Sensors were disinfected with chlorohexidine and soaked and rinsed three times in water prior to insertion. Blood was also attained from all cows. The first 25 bloodwork samples from each site were sent to the Bronson Animal Disease Diagnostic Laboratory (BADDL) for anaplasmosis screening and a large animal blood chemistry profile for a herd summary of general health status. In addition, blood samples from dams who lost calves were sent at the end of the calving season to allow comparison among dams that produced a calf and those that did not. Birthing sensors sent alerts to researchers upon expulsion from the vagina and coincides with the appearance of the amniotic sac at the vulva. After this point, a normal birth should occur within ~ two to four hours. We monitored birthing sensors via SMS and a website interface provided by JMB North America. We responded to all expulsion alerts (i.e. calving alerts) as quickly as possible. Response time varied but we aimed to locate the calving cow as quickly as possible and no later than six hours after an alert. Each study herd was rotated through multiple pastures ranging from 50-200 acres and remained within a 600acre footprint of the birthing sensor monitoring system (Appendix A).

Upon successfully locating the dam, personnel would observe the dam for the newborn calf or indications of calving. For instances where neither were obvious, the dam was observed

for signs of labor and when present, the dam was given more space and either observed from afar until birth and/or rechecked within 2 hours. When feasible, the technician collected the placental afterbirth. Dams do on occasion expel sensors early but show no indication of labor or calving, these cows were checked daily to ensure calving was not missed. All monitoring was conducted by designated technicians at each study site and when unavailable ranch personnel would respond and monitor dams.

Upon responses to alerts and finding cow-calf pair, technicians observed and recorded calf behavior, including head movement, standing, and suckling. Newborn calves were identified with a numbered ear tag in one ear that corresponded to dam ear tag identification. In the other ear a unique frequency (between 164.001mhz to 166.999mhz) very high frequency (VHF) ear tag (#M3400 mammal ear tag, Advanced Telemetry Systems, Isanti MN USA) was placed in the middle third of the ear between the rises in the auricular cartilage. The VHF ear tags emitted two different pulse rates (24hz and 48hz) and would change to 48hz when no ear tag movement was recorded for two hours, indicating mortality. A VHF receiver (#R4500SD receiver datalogger, Advanced Telemetry Systems, Isanti MN USA) was stationed within the middle of each study site that monitored and recorded all calf VHF signals approximately every two hours. This data was downloaded every other day by technicians and checked for a recorded mortality signal or missing VHF data from the previous 48 hours. In these situations, individual calves were tracked using a yagi antenna and scanning receiver (#R410 receiver, Advanced Telemetry Systems, Isanti MN USA) in order to verify calf status. Using the VHF monitoring we were able to pinpoint the time of death within two hours for older calves with ear tags.

Initially, the majority of monitoring was supposed to be done remotely and technicians present at study sites only when responding to a calving alert or gathering and checking VHF

data, but failures associated with the vaginal sensor technology required that technicians be present at the study site either daily or every other day to check for missed births. Between investigating causes of failed equipment and searching for cow/calf pairs undetected by failed birthing sensors, our time spent within the pasture likely doubled from the beginning of the study. This increased human activity is an important consideration that may have an influence upon predator behavior.

In cases of calf mortality, the calf was collected, bagged, put on ice to slow necrosis and degradation, and delivered to Bronson Animal Disease Diagnostic Laboratory (BADDL) in Kissimmee, Florida for postmortem investigation within 24hrs if possible and all were conducted within 48 hours of death. We intended to sample dams that were associated with a calf mortality but due to infeasibility of capture and roping in the open pasture system and the need for experienced cowboy labor requirements, we were unable to attain samples from these dams.

### **Postmortem Investigation**

Postmortem investigations were all conducted by experienced professionals, and included gross necropsy and when necropsy results warranted further investigation, specific tests were conducted in bacteriology/mycology, histopathology, immunochemistry, parasitology and virology. When possible, liver tissue also was collected and sent for mineral analyses. Minerals examined in liver analyses were selenium, iron, copper, zinc, molybdenum, manganese, cobalt, and lead. Summaries from each investigation specific results are available. (Appendix B).

### **Data Analysis**

Descriptive statistics were calculated for total, prenatal, perinatal, and postnatal calf loss. Prenatal period was defined as an aborted calf that was less than 8 months of development and therefore not considered full-term. Perinatal period was defined as a full-term calf either stillborn

or born alive and died within 24h. The calf was deemed full term at the discretion of the veterinarian at the time of the necropsy. Postnatal period included mortalities >24h after birth.

The purpose of the study was to document mortality and on occasion study activities and observations identified sick calves that ranch management would likely have missed. It was at the discretion of each ranch what response occurred for said calves. We had to account for intervention that affected calf survival and was prompted solely because of the study actions. To do this we assigned a calf to assumed mortality, when the lead researcher, technician, rancher and veterinarian aware of the event agreed that mortality would have been inevitable had intervention not occurred. For instance, a calf had landed awkwardly after birth with its head underneath its body and could not right itself. An hour later, increased labored breathing and gasping indicated the calf was struggling to breathe and the technician took direct action to right the calf. In this circumstance, the calf would have died without technician intervention and also been overlooked or missed by normal management. We considered a calf that was orphaned before 90 days of age as an assumed mortality because calves are unlikely to survive without cow's milk and protection during this early life stage. Summaries of individual assumed mortalities are included in the Appendix B for reference. Assumed mortalities are only included in descriptive statistics of calf loss and excluded from further analyses as there was no post-mortem investigation.

Both observed calf history and full necropsy reports were used to identify probable contributing factors to death (CFD) and diagnose cause(s) of major CFDs when possible. A contributing factor of death was defined as the symptom or a category for which multiple symptoms fit. For example, dystocia is defined as difficult birthing and was considered a CFD. Symptoms of dystocia can be wide-ranging, from clinical signs on the calf (swelling of tongue,

face, jaw) to prolonged labor. The underlying cause could be incorrect position, bacterial infection, or any number of other issues. For each CFD, we provided supporting evidence for the claim in individual calf death summaries (Appendix B). We further explored the underlying causes of the major CFDs and summarized potential cause(s) when possible. Diagnoses were verified by veterinarians. In cases where no obvious CFDs were found through clinical or observational history, the mortality was categorized as unknown. We also conducted a binomial logistic regression to examine the potential negative affect of birthing sensors and the duration of insertion on calf mortality. All data was handled and analyzed in R using packages UpsetR (Gehlenborg 2019), dplyr (Wickham et al. 2019), and tidyr (Wickam & Henry 2020).

## **Results**

Birthing sensors only worked for ~50% of the cases (see chapter 2). Due to these issues, visitations to the study sites increased in both frequency and duration, possibly influencing depredations. Of the working birthing sensors, ~2% were expelled early. Despite, technological issues, all newborn calves were found and there were no missing calves at the end of calving season. Only five calving events with non-functioning sensors were noted as older than one day at the time of calf tagging and the remainder of the calves were found and tagged within 24 hours of birth. One failed sensor resulted in an overlooked calf mortality and was ineligible for post-mortem investigations due to severe decomposition. The binomial logistic regression to examine the effect of duration of birthing sensors on calf mortality showed no trend and was not significant ( $p=0.644$ ,  $df=299$ ).

### **Calf Loss**

Total calf loss was 13.24% including assumed mortalities. Excluding assumed mortalities, the average calf loss was 9.93% for the three ranches. Breakdown of mortality by ranch and natal period is displayed in Table 3-1. Only a single suspected depredation occurred in

our study at LR and represented 3.3% of observed calf mortality. The depredation rate at all three ranches was only 0.3% of the total calf crop. There were no prenatal losses in the study herds. One cow retained a sensor at the end of the study and during re-examination was found not to have a calf but a fluid filled uterus. Of the postnatal mortalities, assumed mortalities accounted for 44.4% of these losses and when excluded the majority (86.6%) of calf mortality occurred in the first 10 days. All calf mortality occurred in the first 90 days.

Assumed mortalities were excluded from further analyses as there were no post-mortem investigations undertaken. Post-mortem investigations were conducted on 26 (86.7%) of the remaining 30 calf mortalities. Areas of post-mortem investigation were at the discretion of the veterinarian and not all necropsies included tests in each category (Table 3-2).

### **Contributing Factors and Cause of Death**

There were a total of 11 factors that were identified as contributing to calf mortality (Table 3-3). CFDs are not exclusive, therefore, one calf mortality could be attributed to multiple CFDs (Figure 3-2). The most common CFDs were dystocia, mineral imbalances, septicemia, and poor dam health. Dystocia, septicemia, mineral imbalances were investigated further.

Dystocia was a contributing factor of death in 15 of the 30 calf mortalities (Table 3-4). In all but two cases, more than one possible causative agent played a role in the difficult birth. All but one case occurred during the perinatal period. The post-natal case was a 3-day old calf that had obvious signs of pneumonia caused by meconium aspiration attributed to dystocia at the time of birth. Calf weight was an underlying cause of at least half of the dystocia mortalities. These calves all weighed  $\geq 41$  kg, with 4 cases between 43-46kg and one calf weighing 54.5kg. One calf was noted as large-framed and a contributing cause of dystocia by the attending veterinarian and was included in this group. All bacterial species identified in the table were infections of either the calf or placenta, excluding *Leptospira spp.* which was isolated from the dam's kidneys.

A total of fourteen cases had mineral imbalances outside the normal reference range but considered not critical by the toxicology lab, therefore, were not included in the table. The mineral deficiencies included in table were deemed critical by the lab and occurred in 5 of the 15 cases. In addition to mineral imbalances, four of these calves also had lead present in the liver samples.

Septicemia/bacteremia was another major CFD and was identified in 18 of the thirty post-mortem investigations. Table 3-5 describes the most common bacteria isolated from the calf, placental, and vaginal samples during post-mortem investigations. In both instances, some bacteria were only identified to the genus and in these cases the species is listed as spp. Sixteen (88.9%) of the 18 cases also had mineral imbalances that were outside the normal reference ranges. Four of these cases also had critical mineral values, two considered toxic and two considered deficient. In one of these toxic cases at BR (calf 1179), urinalysis suggested the calf was suffering from nitrite poisoning. Dystocia was also considered a CFD in 11 of the 18 septicemia cases.

Although all 22 investigations that conducted mineral analyses revealed mineral imbalances outside of the normal reference range, only 8 calves had critical mineral values that were deemed toxic or deficient by the toxicology lab. All 8 of these calves revealed other mineral imbalances that were also present but not considered critical. Trace minerals that were responsible for the mineral imbalances are described in Table 3-6. A single case (ranch BR, calf 1179) where mineral toxicity was considered a CFD was not included in table 6 because the finding was a result of a urinalysis and not liver mineral analyses.

### **Discussion**

The average calf loss in our study was considerably higher than the national average of 6% described by the USDA (2015). Our findings of 13.24% calf mortality are close to three

times higher than the Florida calf mortality estimates of 4.4% (USDA 2015). Notably, BC and BR had higher than 10% calf mortality and when assumed mortalities were added, the rates increased above 15%. These figures agree with the producer survey undertaken in Florida that reported higher than national calf mortality rates in ~1/2 of the respondents (unpublished data). Although we did not see rates in excess of 20% as some producers reported, this may be due to a number of reasons such as, reporting from smaller herds where losses of just a few individuals could equate to high percentage calf loss. Also, our human presence was much greater than initially anticipated and ranches that might have incurred calf losses due to depredation may have seen decreased losses in relation to our study activities. Even so, on LR we did observe suspected depredation event of a weak calf, although not on the ranch that experiences high predator use, BC. This observed depredation is much less than observed in a previous study conducted in a high panther use area (Jacobs and Main 2015).

### **Bacteria and Calf Mortality**

This study highlighted the importance of dystocia, bacterial infections, and mineral issues pertaining to calf loss in Florida. Our study was unique in that post-mortem investigations were undertaken; therefore, in some circumstances, the underlying cause(s) of CFDs could be deduced and at minimum, we were able to examine trends with associated underlying issues. A major finding of our study was the role of bacterial infections linked to mortality events. Infections with mixed bacterial species were often found in cases of septicemia; therefore, understanding the bacterial species responsible for initial infection and ultimately death is near impossible. *Trueperella pyogenes*, formerly, *Arcanobacterium pyogenes*, is an opportunistic bacterium causing a wide range of issues in cattle, including bovine mastitis, umbilical infections, sporadic abortions, pneumonia, and abscesses in organs (Radostits et al. 2007). This particular bacterium was present in at least 8 of the 18 bacteremia cases and often afflicted the placenta. *Eshcherichia*

*coli* was present in 8 of our infections and co-occurred with *T. pyogenes* in four cases. Notably, co-infection from these bacterial species is known to affect the reproductive tract of post-partum dams (Bicalho et al. 2012). Bicalho et al. (2010) found that presence of *E. coli* in the uterus post-partum was affected by status of the calf, particularly, stillborn outcomes had higher odds of uterus infection. Our findings suggest that the bacterial infections were present in calves and may be a source of continuing post-partum infections. However, it seems likely bacterial issues occurred in utero because advanced infections were seen in major organs, such as mineralized concretions in the brain of the fetus. Further investigation of in-utero infection, calf status, cow infection, and interactions are necessary to understand the significance of these findings.

Despite evidence that some infections occurred in utero, there is also evidence that other bacterial infections originated from exogenous sources. For instance, a sudden death of an otherwise healthy appearing, 1-month old calf revealed major infection of *T. pyogenes* throughout the umbilicus, bladder, and abdomen. The origin of infection likely ascended the umbilical cord after birth from the environment. Notably, in this case the dam had a large softball sized mass on her jaw (Figure 3-3) that resembled a mandibular osteomyelitis caused by *T. pyogenes* described by Caffaro et al.. (2014), therefore this was a cow to calf infection or environmental exposure to both. Environmental conditions of Florida may provide a favorable setting for the persistence of bacterial species because of its humid and warm conditions. *E. coli* is known to persist in environmental conditions with high moisture and a variety of temperatures (Williams et al. 2005). Although studies examining persistence under these conditions is not described for *T. pyogenes*, it likely can survive and persist in the environment (Rzewuska et al. 2019). *T. pyogenes* is ubiquitous and found as part of microflora of healthy cattle but little is known about the cause of pathogenic tendencies. Future studies should focus on prevalence of *T.*

*pyogenes* and bacterial species found in co-infections and the conditions leading to virulence of *T. pyogenes* in calves.

### **Dystocia Related Calf Mortality**

As with many calf mortality studies, dystocia was a significant contributing factor to death. Our perinatal losses accounted for 20 observed mortalities and 15 of those were associated with dystocia, which was higher than in some other studies (Bellows et al. 1987; Johanson & Berger 2003; Bleul 2011). Bleul (2011) and Johanson & Berger (2003) found ~20% of perinatal losses were associated with dystocia which is considerably less than our findings of 75%. It is important to note that both studies are associated with dairy cattle and have differing management surrounding birthing events. Additionally, Johanson & Berger (2003) did not include mal-presented calves or twins, which are both known to increase the risk of dystocia and were included in our results. Bellows et al. (1987) attributed 50.9% of calf mortality to dystocia and 32.4% of these were presented abnormally. We did observe abnormal position in two (13%) of the dystocia cases but in other circumstances the birthing event and death had already occurred. Thus, it is possible that incorrect birthing position played a role in more of the losses associated with dystocia. In half of our dystocia cases, calf weight likely contributed in the prolonged and difficult birth. Johanson & Berger (2003) described a higher risk of mortality for calves that weighed >42kg and increased risk as the ratio of calf birth weight to cow weight increased over 7.2%. At least five of the calves were larger than 42kg, with the ratio of calf birth weight to cow weight >8.0%. Often inappropriate sire selection is attributed to feto-pelvic incompatibility (Meijering 1984). All heavy calves occurred at BR and are likely due to sire selection that produced incompatible calves given cow size and emphasizes the importance of these selections even with multiparous dams.

Dystocia and poor health may in part be driven by mineral deficiencies. Trace mineral issues in Florida have been described as early as 1965 (Becker et al. 1965). Subsequent studies have evaluated the soil and forage type associated with grazing cattle and found that both do not contain adequate trace minerals for healthy cattle development (Salih et al. 1983; Arthington 2012). Deficiencies in trace minerals can interrupt enzyme processes and retard proper development. Moreover, many of these trace minerals have a complex, interrelationships that impact the absorption and function of others and have repercussions associated with reproduction (Arthington 2012). For instance, molybdenum is often referred to as a trace mineral antagonist and has been associated with decreased copper absorption, which is essential for immune functioning (Corah 1996; McDowell & Arthington 2005; Arthington 2012). Likewise, high iron levels are often concerning because iron affects the absorption of copper (Corah 1996).

### **Mineral Imbalances, Toxicities, and Deficiencies**

Major deficiencies occurred in five of the 22 mineral analyses. A set of twins at BC accounted for two of these cases and had critically low zinc, manganese, and cobalt. However, liver samples were pooled and as such interpretation is limited. No other cases revealed cobalt or zinc deficiencies and conclusions from these calves may be erroneous. There was a calf at BR that had hair loss and skin lesions, similar to described symptoms of zinc deficiency but the calf survived and a mineral analysis in this case was not conducted. The other instance of manganese deficiency also occurred at BC. Some evidence suggests manganese deficiencies can cause abortion and deformities (Rojas 1965) and it is suspect that the single anatomical abnormality noted in the study occurred at BC. Surprisingly, selenium deficiencies were rarely an issue in necropsied calves despite previous studies describing selenium deficiencies as a problem in many Florida grazing cattle (McDowell et al. 1982). This may be a result of increased education and proper selenium supplementation by producers. Contrary to the literature, we consistently

found low levels of molybdenum, one of which was critically low. According to McDowell (1985) and to the author's knowledge, molybdenum deficiencies have not been described in cattle. Similar to other trace minerals, molybdenum is important for enzyme function and metabolism but unlike other minerals, molybdenum is usually found in adequate levels in forage and soil (Salih et al. 1983; McDowell & Arthington 2005). In seven of these cases molybdenum imbalances were associated with other mineral imbalances but no obvious correlations were present.

Toxic levels of trace minerals occurred less often and appeared in three cases. High iron levels were found in numerous cases and one was considered toxic; however, results did not indicate high iron impacted copper levels as described in literature (Arthington 2012). In addition, copper is often noted in the literature as deficient in cattle yet results from our mineral analyses revealed a critically high copper concentration. In one study, dams exhibiting copper toxicosis from excessive copper supplementation had calf mortality rates at ~50% (Perrin et al. 1990). Toxic levels of zinc were found at BR in a single case and are known to exacerbate deficiencies of iron and copper but this interaction was not evident in our findings (McDowell & Arthington 2005). Mineral supplementation occurred as part of liquid molasses and molasses block on all three ranches, and BR and LR recorded toxic concentrations of minerals, which potentially resulted from over supplementation of these individuals. Although toxicities only accounted for a small portion of mineral findings toxicosis and over supplementation can result in serious consequences for cattle and may indicate closer monitoring and supplementation evaluation at individual ranches.

Notably, lead was present in five of the mortality events and occurred at all three study sites. Four of the cases were perinatal losses suggesting the lead crossed the placenta and was

passed from the dam to the fetus. Despite laboratory categorization as high but likely not significant, the findings are intriguing. As part of a study examining lead exposure in cow- calf herds, one of eight pregnant cows with high blood lead concentrations calved a stillborn calf (Waldner et al. 2002). The remaining seven calves had lead present in their blood in low levels (Waldner et al. 2002). In another study, the rate of lambing was 18% for sheep exposed to sub-lethal lead concentrations as compared to 100% lambing rate for unexposed sheep (Sharma & Buck 1976). Although both studies are small sample sizes, they demonstrate the possible connection to lead poisoning and calf mortality. In addition, lead can remain in the blood for extended time after the exposure event and potentially cause birthing issues months after initial exposure. Soil, batteries, flaking lead paint, old machinery, and contaminated feed have all been implicated as sources of lead in pasture-based systems. Lead could be a significant finding from this study that warrants further investigation particularly since it was found on all three ranches.

### **Depredations**

Depredations in this study were lower than anticipated. The rate of depredation was only 0.3% and accounted for 3.3% of documented calf mortality. In contrast to our findings, Florida ranchers report higher rates of depredation losses in Florida (USDA 2015) and perceive predators as a risk to their livestock (Jacobs et al. 2015; Pienaar et al. 2015). As noted earlier, it is difficult to differentiate between depredations and scavenging events. We likely recorded losses of calves that would have normally been overlooked for multiple days, scavenged, and severely decomposed. In these instances, producers may incorrectly attribute those losses to predators, thereby inflating the impact of depredations. In addition, rancher's negative perceptions of predators could influence their reporting whereby they are overestimating the impact of these predators on their livestock.

We would have expected to see more depredations at BC because that area has high use of panthers, black bears, and coyotes. However, our only suspected depredation occurred at LR. It is important to note that surrounding pastures and neighboring ranches to BC had at least 4 confirmed depredations during our study period. Due to the sensor failures, we were in the study sites for extended periods of time, searching for overlooked calving events and missing birthing sensors. Although, rarely in the site at night our presence throughout the day and in the entirety of the study site could have influenced the predator behavior and impacted depredation rates. Additionally, depredations vary by year and ranch and our study could have occurred during a year with low depredations on each of the ranches.

### **Implications**

The aim of our study was to understand the major issues underlying calf mortality in Florida and was not designed to infer cause and effect relationships between CFDs. However, associations between CFDs highlight the negative and potentially synergistic interactions contributing to calf mortality. Dystocia and septicemia were CFDs in eleven of the 30 cases. It is unclear if bacterial infections caused dystocia but it potentially exacerbated difficult birthing events. A study by Jadon et al. (2005) supports this idea and found increased bacteria loads, particularly of *T. pyogenes*, at the time of delivery in the uterus of buffalo suffering dystocia compared to normal calvings. Similarly, trace mineral imbalances were seen in the majority of septicemia (88.9%) and dystocia (93.3%) cases. It is not a stretch to think that imbalanced trace minerals could decrease immune responses and lead to infections of opportunistic pathogens. Although the mineral results highlight issues with both deficiencies and toxicities, it is important to consider our small sample size and the lack of a control herd. Further investigation of trace mineral concentrations in calf mortality is warranted because of their vital role in enzyme

function, development, and immune response. In addition, future studies should focus on the role of bacterial infections in utero as it pertains to dystocia events.

Our research was unique in that we were the first comprehensive study in Florida to examine all calf mortality losses from pregnancy checks to calf weaning. Although the birthing equipment did not perform as well as expected, we were able to capture 96.7% of all losses, even the early losses that have been missed in other studies (Waldner et al. 2010). Therefore, for a more extensive and holistic understanding of calf mortality in Florida, additional studies should be undertaken using this system in other regions of Florida and with differing management practices. In addition, more attention could be focused on understanding mineral imbalances and bacterial loads associated with successful calf rearing to compare to calf mortalities.

## Figures and Tables

Table 3-1. Total mortality statistics are based on 302 calves born at LR, BR, and BC from August 2017 to June 2018. Losses are calculated as a percentage of the mortality grouping to understand how losses occurred in each natal period. Losses at the ranch are calculated as a percentage of total losses at individual ranch. Perinatal losses are losses of a full-term calf stillborn or within 24-hours. Postnatal losses are >24hours.

Mortality Group	Overall	Natal Period			Ranch	
		Perinatal	Postnatal	BC	BR	LR
Born	302	-	-	80	111	111
Overall Mortality	40 (13.25%)	22 (55%)	18 (45%)	13 (32.5%)	21 (52.5%)	6 (15%)
Assumed Mortality	10 (3.31%)	2 (20%)	8 (80%)	4 (40%)	6 (60%)	0 (0%)
Observed Mortality	30 (9.93%)	20 (66.67%)	10 (33.33%)	9 (30%)	15 (50%)	6 (20%)

Table 3-2. Total calves examined in each area of post-mortem investigations at BADDL from August 2017 to June 2018 categorized by natal period and ranch. Percentages are the number of calves examined in each investigative area from the number sent for necropsy in each natal period and ranch. Perinatal losses are losses of a full-term calf stillborn or within 24-hours. Postnatal losses are >24hours.

Post-Mortem Investigations	Overall	Natal Period			Ranch	
		Perinatal	Postnatal	BC	BR	LR
No. Total Calf Losses	30	20	10	9	15	6
Sent for Necropsy	26 (86.67%)	19 (95%)	7 (70%)	7 (77.78%)	14 (93.33%)	5 (83.33%)
Bacteriology/ Mycology	20 (76.92%)	14 (73.68%)	6 (85.71%)	5 (71.43%)	10 (71.43%)	5 (100%)
Clinical Pathology	5 (19.23%)	2 (10.53%)	3 (42.86%)	0 (0%)	4 (28.57%)	1 (20%)
Histopathology	24 (92.31%)	18 (94.74%)	6 (85.71%)	6 (85.71%)	14 (100%)	4 (80%)
Immunohistochem.	8 (30.77%)	6 (31.58%)	2 (28.57%)	0 (0%)	7 (50%)	1 (20%)
Serology	3 (11.54%)	1 (5.26%)	2 (28.57%)	0 (0%)	3 (21.43%)	0 (0%)
Molecular Biology	12 (46.15%)	6 (31.58%)	6 (85.71%)	3 (42.86%)	6 (42.86%)	3 (60%)
Parasitology	11 (42.31%)	6 (31.58%)	5 (71.43%)	4 (57.14%)	3 (21.43%)	4 (80%)
Virology	22 (84.62%)	17 (89.47%)	5 (71.43%)	4 (57.14%)	14 (100%)	4 (80%)
Mineral Analyses	21 (80.77%)	16 (84.21%)	5 (71.43%)	7 (100%)	10 (71.43%)	4 (80%)

Table 3-3. Total calves diagnosed with each contributing factor to death on LR, BR, and BC from August 2017 to June 2018. Percentage of total ranch or natal cases are given for individual diagnoses. Perinatal losses are losses of a full-term calf stillborn or within 24-hours. Postnatal losses are >24hours. Note CFDs are not exclusive and each case could be categorized by more than one CFD.

Diagnoses	Overall Cases	Natal Period			Ranch	
		Perinatal	Postnatal	BC	BR	LR
Anatomical abnormality	1 (3.33%)	-	1 (10%)	1 (11.11%)	-	-
Depredation	1 (3.33%)	-	1 (10%)	-	-	1 (16.67%)
Dystocia-fetal distress/anoxia	15 (50%)	14 (70%)	1 (10%)	5 (55.56%)	9 (60%)	1 (16.67%)
Mineral Deficiency	5 (16.67%)	5 (25%)	-	3 (33.33%)	1 (6.67%)	1 (16.67%)
Mineral Imbalances	22 (73.33%)	17 (85%)	5 (50%)	7 (77.78%)	11 (73.33%)	4 (66.67%)
Mineral Toxicity	4 (13.33%)	3 (15%)	1 (10%)	-	2 (13.33%)	2 (33.33%)
Parasitic Infection	1 (3.33%)	1 (5%)	-	-	1 (6.67%)	-
Poor dam health	12 (40%)	7 (35%)	5 (50%)	1 (11.11%)	9 (60%)	2 (33.33%)
Septicemia/Bacteremia	18 (60%)	12 (60%)	6 (60%)	4 (44.44%)	11 (73.33%)	3 (50%)
Twinning	6 (20%)	5 (25%)	1 (10%)	5 (55.56%)	1 (6.67%)	-
Unknown	5 (16.67%)	2 (10%)	3 (30%)	2 (22.22%)	3 (20%)	-
Viral Infection	3 (10%)	2 (10%)	1 (10%)	1 (11.11%)	1 (6.67%)	1 (16.67%)
<b>Total Cases</b>	<b>30</b>	<b>20</b>	<b>10</b>	<b>9</b>	<b>15</b>	<b>6</b>

Table 3-4. Dystocia cases from LR, BR, and BC from August 2017 to June 2018 broken down by possible causative agent. Dystocia elements are not exclusive and each dystocia case could have more than one associated causative agent.

Possible Associated Elements	No. of Associated Dystocia Cases	Ranch		
		BC	BR	LR
<b>Physical Elements</b>				
Calf Weight $\geq$ 90 lbs.	7	-	7	-
Cow Physical Inability	1	-	1	-
Posterior Position	2	-	2	-
Twin	4	4	-	-
<b>Critical Mineral Deficiencies</b>				
Cobalt	2	2	-	-
Manganese	3	3	-	-
Molybdenum	1	-	1	-
Selenium	1	-	-	1
Zinc	2	2	-	-
<b>Bacterial Species</b>				
<i>Clostridium sporogenes</i>	1	-	1	-
<i>E. coli</i>	3	2	1	-
<i>E. coli Beta hemolytic</i>	1	-	1	-
<i>Leptospira sp.</i>	1	-	1	-
<i>Trueperella pyogenes</i>	7	2	4	1
<i>Mannheimia haemolytica</i>	2	2	-	-
<i>Mannheimia spp.</i>	2	-	1	1
<i>Streptococcus alpha haemolytic</i>	1	-	1	-

Note that natal period was not included in this table because dystocia only contributed to a single postnatal case (pneumonia from meconium aspiration).

Table 3-5. Most common bacteria associated with septicemia/bacteremia from LR, BR, and BC from August 2017 to June 2018. Note this list only includes bacteria that were isolated from more than one case associated with the CFD septicemia. All bacterial species isolated from all post-mortem investigations are summarized in the Appendix A. Perinatal losses are losses of a full-term calf stillborn or within 24-hours. Postnatal losses are >24hours.

Species Present	Overall Septicemia Cases	Natal Period		BC	Ranch	
		Perinatal	Postnatal		BR	LR
<i>Escherichia coli</i>	8	4	4	3	5	1
<i>Klebsiella pneumoniae</i>	2		1	2	-	-
<i>Mannheimia haemolytica</i>	2	1	1	2	-	-
<i>Mannheimia spp.</i>	3	2	1	1	1	1
<i>Pseudomonas spp.</i>	4	3	1	-	3	1
<i>Staphylococcus aureus</i>	2	1	1	-	2	-
<i>Streptococcus lutetiensis</i>	2		1	-	2	-
<i>Streptococcus plurimalium</i>	3	3		-	3	-
<i>Trueperella pyogenes</i>	8	6	2	2	4	2

Table 3-6. Trace minerals responsible for toxicities, deficiencies, and imbalances that possibly contributed to mortality at LR, BR, and BC from August 2017 to June 2018. Note trace mineral categorizations are not exclusive and cases could have more than one mineral imbalance, toxicity, or deficiency. Perinatal losses are losses of a full-term calf stillborn or within 24-hours. Postnatal losses are >24hours

Trace Mineral	Overall Mineral Cases	Natal Period			Ranch	
		Perinatal	Postnatal	BC	BR	LR
<b>Cobalt</b>						
Deficient	2*	2	-	2	-	-
Imbalances	2	-	2	1	1	-
<b>Copper</b>						
Toxic	1	-	1	-	-	1
Imbalances	6	5	1	1	5	-
<b>Iron</b>						
Toxic	1	1	-	-	-	1
Imbalances	5	3	2	3	2	-
<b>Lead</b>						
Imbalances	5	4	1	2	2	1
<b>Magnesium</b>						
Imbalances	1	1	-	1	-	-
<b>Manganese</b>						
Deficient	3*	3	-	3	-	-
Imbalances	3	3	-	-	3	-
<b>Molybdenum</b>						
Deficient	1	1	-	-	1	-
Imbalances	10	10	-	2	5	3
<b>Phosphorous</b>						
Imbalances	1	1	-	1	-	-
<b>Selenium</b>						
Deficient	1	1	-	-	-	1
Imbalances	4	1	3	-	3	1
<b>Zinc</b>						
Deficient	2*	2	-	2	-	-
Toxic	1	1	-	-	1	-
Imbalances	5	2	3	3	2	-

\*Denotes inclusion of set of twins where a pooled liver sample was used.

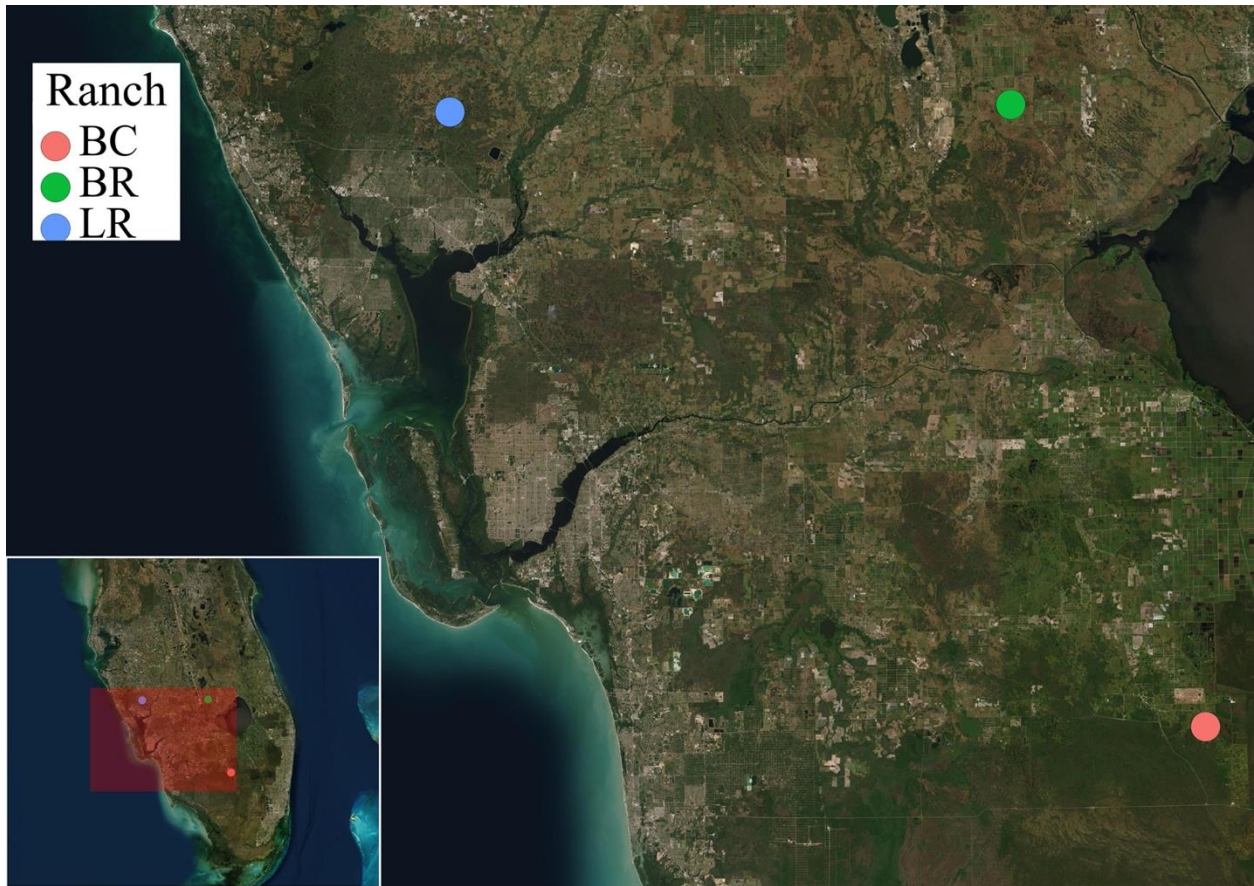


Figure 3-1. Location of ranches in south Florida participating in the calf mortality study.

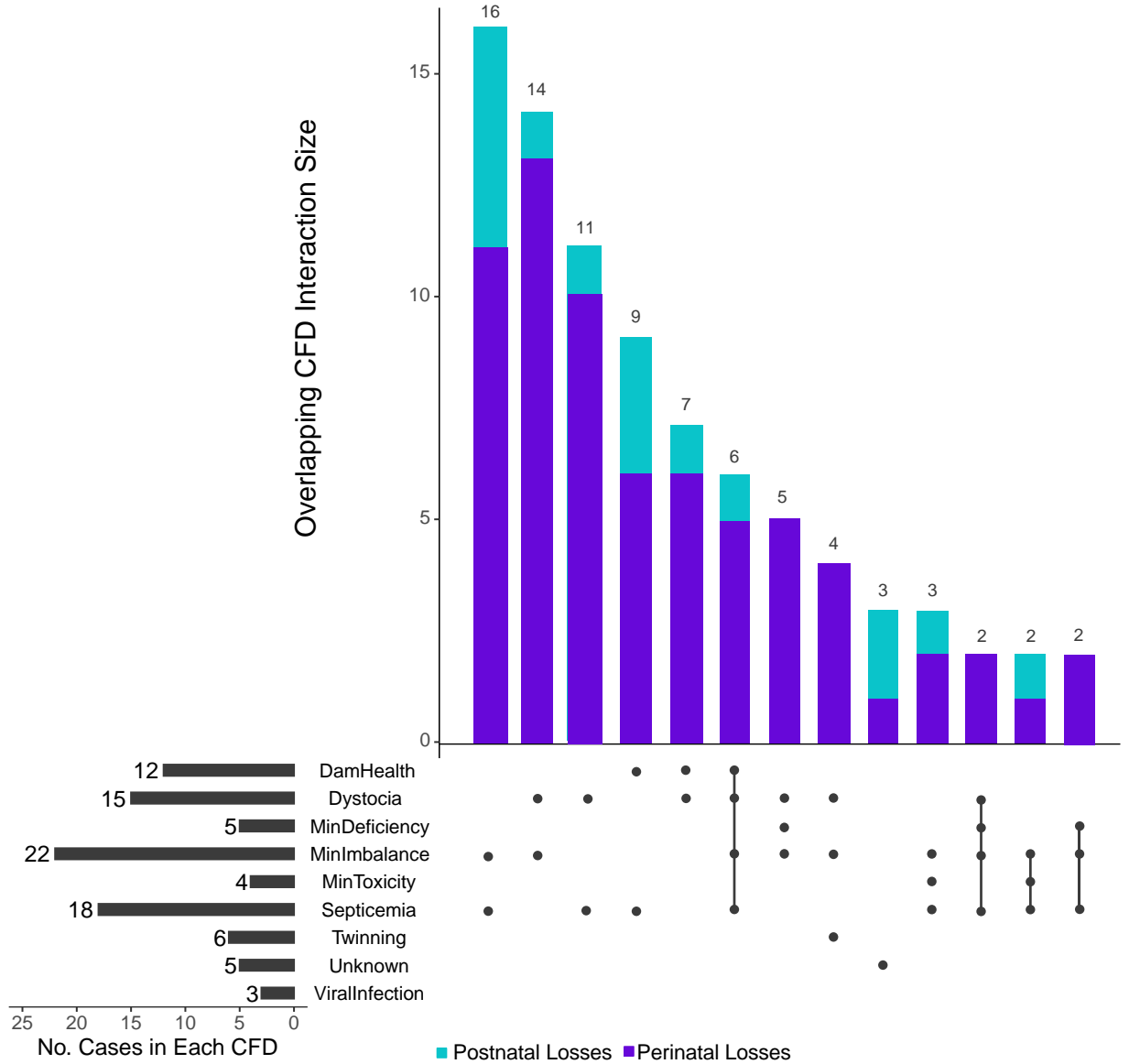


Figure 3-2. Visualization of overlapping CFDs. The number of total cases assigned to 9 of the 11 CFDs are displayed in the left corner histogram. Colored bar graph shows the natal period and frequency by which the CFDs with black dots overlap. CFDs connected by a black solid line indicate that these overlaps are mutually exclusive and the cases are not categorized in other CFDs. Black dots not connected indicate that these CFDs overlapped but are not mutually exclusive and could be diagnosed with other CFDs. Note CFDs depreddation and parasitic infection are not included in the figure because they were only diagnosed in one case.



Figure 3-3. Dam with large mass on left jaw line whose month-old calf died of septicemia attributed to *Trueperella pyogenes*.

CHAPTER 4  
A NOVEL APPROACH TO PANTHER DEPREDACTIONS: RARAMURI CRIOLLO CATTLE  
AS LIVESTOCK GUARDIANS

**Background**

The cattle industry has focused on decreasing calf mortality rates because it represents a major economic loss to producers (USDA 2015), can increase production efficiency, and increase animal welfare conditions (Ortiz-Pelaez et al. 2008). As reported in the chapter 3, calf mortality is difficult to diagnose but encompasses a broad range of causes, including bacterial infections, calving-related issues, and viral infections (USDA 2015; Waldner et al. 2010; Khodakaram-Tafti & Ikede 2005). A component of these losses can also stem from depredations in areas where cattle and predators overlap (USDA 2015; Breck et al. 2011; Loveridge et al. 2010).

In the United States, depredations of calves on both dairy and beef ranches accounts for 11.1% of total calf mortality according to reported USDA figures (2015). Predators responsible for depredations are varied but coyotes (*Canis latrans*) and puma (*Puma concolor*) account for more than half of all reported calf depredations (USDA 2015). Research has shown that depredation rates are highly sporadic, varying from year to year and ranch to ranch (Breck et al. 2011; Jacobs & Main 2015; Michalski et al. 2006; Muhly & Musiani 2009) and in some specific cases can represent a major portion of the overall calf losses (Breck et al. 2011). In one study, calf mortality was 6.5% where 85% of the losses were attributed to depredations (Breck et al. 2011).

Historically, these conflicts lead to decreased tolerance by the negatively affected stakeholders and can result in rising tensions between wildlife managers and said stakeholders (Woodroffe et al. 2000). Decreased tolerance can result in negative impacts, such as retaliatory killings, and hamper conservation efforts (Inskip & Zimmerman 2009). Often, lethal control is

used to manage depredations (Loveridge et al. 2010; Moreira-Acre et al. 2018), despite issues surrounding efficacy of such measures (Peebles et al. 2013; McManus et al. 2015; Allen 2015). The removal of animals can open a niche that may be filled by a different individual more apt to kill livestock and depredations may actually increase (Knowlton et al. 1999; Peebles et al. 2013; Wielgus & Peebles 2014). Moreover, lethal control is not always selective and problem animals may avoid lethal means (Loveridge et al. 2010). In order to prevent extinction and promote conservation of carnivores where needed, mitigation must incorporate the needs of both humans and felids (Inskip & Zimmerman 2009); thus, lethal controls should be minimized and where possible supplemented or replaced by non-lethal control methods.

A range of non-lethal techniques exists and typically fall into two categories, primary repellents and secondary repellents (Shivik et al. 2003). Primary repellents focus on disrupting predator's actions, often by the use of auditory, visual, or chemical stimuli. Whereas, secondary repellents focus on the predator's learned behavior by establishing a negative link with the undesired behavior of hunting or attacking livestock. The use of livestock guardian animals is commonly implemented and could potentially serve as both a primary and secondary repellent (Inskip & Zimmerman 2009). Typically, guardian animals are a different species than the livestock they are protecting; however, a new technique for cattle makes use of more aggressive breeds to protect the main cow herd. Few management changes are required to integrate same species guardians into existing herds and could be beneficial for easy implementation. In particular the San Martinero, a type of Criollo/Creole cattle, is described by Hoogesteijn & Hoogesteijn (2014) as very defensive of their calves and anecdotal evidence suggests that incorporation of San Martinero in beef herds experience decreased numbers of depredations by jaguars (*Panther onca*).

South Florida is home to three overlapping carnivore species that have all been reported in calf depredations: the panther (*Puma concolor coryi*), coyote (*Canis latrans*), and black bear (*Ursus americanus floridanus*). The coyote is not native to Florida but populations have expanded throughout the state in the past 50 years as a result of open niches with the loss of large predators in combination with their adaptive behavior (Boughton et al. 2001). Typical prey includes small rodents and fruit but occasionally coyotes opportunistically depredate livestock (Boughton et al. 2001). Reports of coyote depredations have increased in the past decades and are largely viewed as a nuisance by ranchers (Boughton et al. 2001). The Florida black bear is a unique subspecies of *Ursus americanus* and has suffered population declines statewide largely from habitat loss and fragmentation (Annis et al. 2007). Black bears are omnivores and only about 5% of their diet consists of meat (Pieenar 2014). As with coyotes, bears will sometimes opportunistically depredate livestock (Jacobs & Main 2015).

The Florida panther is a subspecies of the *Puma concolor* and until recently the only breeding population existed south of the Caloosahatchee River in south Florida (Onorato et al. 2010; Frakes et al. 2015). In 2018-2019 multiple observations of female panthers with kittens were observed just north of the river confirming an expanding population (FWC 2019). Panthers expanding north face a myriad of threats and managers have warned to be cautiously optimistic about their prospects north of the river (FWC 2019). The panther was driven close to extinction in the 1990's largely due to habitat losses and human persecution (USFWS 2008; Onorato et al. 2010). A genetic restoration project involving the release of female pumas from Texas (*Puma concolor stanleyana*) in 1995 led to an increase in the population size (Johnson et al. 2010). Despite the success, the panther still remains classified as endangered by the USFWS and federally protected by the Endangered Species Act.

The panther prefers a mosaic of forested habitat (Kautz et al. 2006; Land et al. 2008; Oronato et al. 2011) and much of that habitat supporting the existing breeding population and that is necessary for population expansion and further recovery is in private holdings (Kautz et al. 2006), with cow-calf operations throughout a portion of said lands. The increase in panther population in its current breeding habitat has resulted in an increase in documented depredations (FWC 2019). As the panther population continues to expand and disperse in these ranch lands, some cow-calf operations are likely to experience increased negative interactions and varying degrees of depredations. Jacobs & Main (2015) reported panther depredations on two south Florida ranches ranging from 0.5-5.3% of total calf mortality in a single year. Due to federal protection of the panther, lethal mitigation is not an option. Interviews with Florida ranchers have demonstrated a large concern surrounding panther depredations as the panther population increases (Pienaar et al. 2015). Currently, the Livestock Indemnity Program is used as a mitigation strategy for ranchers and compensates them for incurred cattle losses by the Florida panther. However, this program does not deter future depredations. Given that the land in cow-calf operations is critical for the future recovery of the panther and tolerance of stakeholders can drive conservation, other nonlethal mitigation is necessary and must focus on both human and panther needs. That said, ineffective tools for mitigation may contribute to increased frustrations by stakeholders, thereby decreasing their tolerance to panthers that they may perceive as a burden to their way of life or source of income. Therefore, nonlethal mitigation techniques should be experimentally examined for hypothesized affects prior to widespread implementation.

Our main goals were two-fold. First, we sought to understand predator communities that use calving pastures and neighboring lands by examining occupancy and interactions of four predator species in Florida during calving season, panther (*Puma concolor coryi*), coyote (*Canis*

*latrans*), bobcat (*Lynx rufus*), and black bear (*Ursus americanus floridanus*). Our second goal was to assess the potential of Raramuri Criollo as a same species livestock guardian for Brangus cattle against panthers on a cow-calf ranch because of their close relation to San Martinero Criollo and similar defensive behavior. Specifically, we examined Raramuri Criollo integration with Brangus herds and compared behavioral responses of Raramuri Criollo and Brangus dams to predator presence in calving pastures. According to the anecdotal evidence, San Martinero stand their ground during predator attacks by jaguars unlike Brangus that flee in different directions (Hoogesteijn & Hoogesteijn 2014); thus, we hypothesized that Raramuri Criollo would display different behavior in response to predators when compared to behavior of non-predator nights and compared to Brangus response. Additionally, on the basis that Raramuri are of close relation to San Martinero, and San Martinero supposedly defend calves from jaguars, we hypothesized that Raramuri would show similar behavior towards the panther and would differ from Brangus response.

## **Materials and Methods**

### **Study Site and Animals**

The study took place on a cow/calf ranch in Collier County, FL that consists mainly of Brangus cattle (*Bos taurus* mixed with *Bos indicus*) for beef production with a small number of Cracker cattle maintained on separate pastures on 37.64 km<sup>2</sup> of land. The ranch is situated between the Panther National Wildlife Refuge, the Bear Island unit of Big Cypress National Preserve, and the town of Immokalee. The climate is subtropical and there is a defined rainy season from May through October. Monthly rainfall averages are 3.7cm and 16.5cm during the dry season and wet season, respectively. Total yearly rainfall averages ~120cm. The dominant habitats of the ranch are improved pastures, semi-native range, mesic forests dominated by pines, palms, and oaks, and wetlands. In 2017, a small herd of Raramuri Criollo cattle were purchased

by JB Ranch and the Naples Zoo that consists of eleven females and one bull. The Raramuri cattle were bred one time prior to the study by JB Ranch. Cattle were checked for pregnancy status in June 2019 and confirmed as pregnant by attending veterinarian for inclusion in the study herd. The final study herd consisted of 80 Brangus and 11 Raramuri Criollo dams. The two breeds were maintained in the same herd beginning in September 2019 to February 2020 and were rotated between two neighboring study pastures of 0.98km<sup>2</sup> and 0.51km<sup>2</sup> area for the duration of calving.

### **Data Collection**

We monitored the study pastures and surrounding area from September 2019 to April 2020. We deployed a total of 27 game cameras (Reconyx HyperFire #HC550, Holmen, WI, USA). Cameras were activated by motion, had white LED flash for nighttime and were set to 5-image bursts with 1-minute quiet time to limit the number of cattle images. We used a grid consisting of 375 m<sup>2</sup> cells over an area 3.59 km<sup>2</sup> that contained the study pastures (Figure 4-1) and deployed cameras in each of the 27 cells in areas with the highest likelihood of panther detection. In some circumstances, the cells on the boundary of the calving pastures consisted of heavy, thick vegetation that had low probability of panther use. Instead of placing cameras in areas with zero to low probability of panther use, we chose sites outside of the predefined cells that had higher likelihood of panther use and served as corridors to the calving pastures. Cameras were checked every month to collect photos, replace batteries, and maintain sites.

A total of 20 cattle, 11 Raramuri and 9 Brangus dams, were outfitted with GPS collars in September 2019 (Advanced Telemetry Systems, #G5-2D Iridium/GPS Collars, Isanti, MN USA). Collars were programmed with 10-minute fix schedule and recorded activity, location, and temperature that was then uploaded to ATS web server. Activity measurements were recorded as the percent of active seconds during the 10-minutes. A second was considered active

when a difference of 78mg was detected on any axis of a 3-way axis accelerometer built in the collars.

## **Image Processing**

We created a data pipeline for easy management, storage, processing, and identification of camera imagery utilizing open source programs. Images were processed using a machine-learning model developed in R (MLWIC, for details see Tabak et al. 2018; Tabak 2020) that classifies species within images. Each classification or guess the model provides is also associated with a model confidence. Following classification from MLWIC, ExifTool (Harvey 2016), an application that reads, writes, and edits photographic metadata, was used to write the species identification from MLWIC to individual photos. For our purposes, we only wrote metadata to images when model confidence was >95% for all species except cattle, in which case we used 80% model confidence as the minimum threshold. Once metadata were written on the photos, they were then opened using digiKam, an open-source photo management program, which allows photo filtering based on metadata information. We were able to then check automated identification, identify remaining photos that were not classified, and update metadata tags for those images. Finally, metadata tags could then be read back to camtrapR for detection history of each species, among other features (Niedbella et al. 2016).

In order to ensure we were not losing data by our selected model confidence cutoff, we followed the above protocol for a test subset consisting of 71600 photos and manually validated all photos processed by the machine learning model. Overall, 62.8% of these photos were not identified because model confidence was below the decided thresholds, 34.4% were correctly identified, and 2.7% were incorrectly identified. Predators of interest (bobcats, black bears, coyotes, and panthers) accounted for a total of 631 photos that were identified correctly by the model. Of interest were the 2.7% incorrect identifications and the majority (~40%) were empty

photos misidentified as a species. A total of 18 predator photos were misidentified, only one of which resulted in complete misidentification as a racoon, *Procyon lotor* (i.e. all five photos from the burst misidentified). Subsequently, racoon identifications by MLWIC were checked for the duration of the study to ensure predator images were not misidentified.

## **Data Analysis**

We discarded GPS records that had horizontal dilution of precision  $>3$ ,  $<5$  satellites used for acquiring position, location recorded outside of the pasture boundaries, biologically impossible distances traveled between points, and wrong fix time. The approach was conservative but only resulted in 811 discarded records of  $\sim 350,000$  ( $>0.01\%$ ). All data was managed and analyzed in R (Rstudio Core Team 2019), digikam, ExifTool (Harvey 2016), using packages exifr (Dunnington & Harvey 2019), camtrapR (Niedballa 2016), unmarked (Fiske & Chandler 2011), dplyr (Wickham et al. 2019), ggplot2 (Wickham 2016), lme4 (Bates et al. 2015), MLWIC (Tabak 2020).

## **Occupancy Modeling**

Occupancy is a common metric of interest for ecologists and is defined as the proportion of sites occupied by a species. Naïve occupancy utilizes the proportion of the sites where a species was detected and assumes any time a species is not detected it is therefore, absent. Absence can also occur when a species is present but not detected. Occupancy modeling set forth by MacKenzie et al. (2002) accounts for this imperfect detection of species by using detection histories and presence to estimate detection probability and occupancy by a species. We used occupancy modeling (MacKenzie et al. 2002) to estimate the predator use and detection probability of four predator species, bobcats, panthers, coyotes, and black bears, within the study area. Bobcats are very rarely implicated in calf depredations and therefore were not of direct interest for the cattle behavior but are known to interact with panthers on different scales,

sometimes as competition and others as prey (Harveson et al. 2000; Hass 2009). Thus, we included bobcats in occupancy estimates. Sampling occasions were defined as a 24-hour period of camera monitoring beginning at 1200 hours. Due to the high density of cameras throughout the study area, it is possible that individuals used more than a single site during a sampling occasion, thus, we defined our measure of interest as species use rather than occupancy (MacKenzie & Royle 2005). We tested broad habitat types and cattle occurrence as covariates in the models. In addition to single species use, we examined interactions between predators using a multispecies occupancy model following Rota et al. (2016).

### **Cattle Behavior**

We assessed Raramuri integration with mixed Brangus in the framework of binomial logistic regression as a function of breed, week since integration, and time of day. If cattle were completely integrated then any individual's closest neighbor no matter the breed has the same likelihood of being a Brangus or Raramuri. Using this logic, the response variable was binomial, where closest neighbor was either Raramuri (0) or Brangus (1). The null hypothesis was breed, time of day, and time since integration did not influence breed of nearest neighbor. Model selection was undertaken by Akaike's Criterion Information (AIC), where the most parsimonious model with the lowest AIC was selected as the most explanatory model. If AIC did not improve by  $\Delta 2$  with the addition of new fixed effects, the addition was not included in the model (Burnham and Anderson 2002). We used area under curve (AUC) to examine fit of the binomial logistic regression (Hanley & McNeil 1982).

To understand the behavioral response of both Brangus and Raramuri to predator events we examined activity per meter traveled in the context of linear mixed effects models (LMEM). Defensive behavior includes lowering the head, pawing the ground, and stomping, all of which

would increase our activity metric. In addition, cattle that display defensive behavior typically stand their ground and we would see a decrease in their distance traveled. LMEMs are able to deal with nested or hierarchal data and are flexible when it comes to repeated measures on the same individuals, as they can account for differences in individual response as a random factor. Explanatory variables of interest were time from the predator/control event, type of predator present, and breed of cattle. Following a similar study, we defined predator events as predator presence that occurred inside the pasture with cattle (Laporte et al. 2010). Predator presence was determined by camera traps and records were considered independent when separated by an hour regardless of capture location. That is when a species was captured inside the cattle pasture, detections of the same species following the initial detection were considered the same predator event if they occurred within the hour. Unlike occupancy, there was no occasion period; therefore, more than one predator event could have occurred in a single night. While the main interest was cattle behavior in the presence of panthers, we also examined cattle response to black bears and coyotes, as they are known to depredate calves in Florida and also represent a threat to calves. We compared cattle response to predator visitations to cattle behavior on nights without known predator activity. To limit behavioral variation throughout the calving season, we randomly selected a non-predator night for each predator event that was  $\pm 2$  weeks from said event. In order to account for the variation in night length and corresponding cattle patterns, the responses were compared at the same percentage of the night rather than the same time of night. We were interested in the immediate response and the effect of time on cattle behavior following an event; therefore, GPS data were taken from 1 hour prior and 3 hours following all events.

Despite flexibility in LMEM, some assumptions of linear mixed models must be met, such as a normal distribution. Our log-transformed data followed a normal distribution

(Anderson Darling Normality Test  $p$ -value $<0.001$ ). For the LMEM, we built the random effects model that was maximal but reasonable given study design. The random effects accounted for variation of individual cattle behavior, distance from the initial predator event, and night of the predator event. All random effects used in the models are described in Appendix A. Fixed effects of interest were time from event, breed, and predator treatment, which included the control. We used event as a random effect to account for the matched sampling described above. We fit the full model with interactions between all three fixed effects and then performed a backward step-wise regression to select the most parsimonious significant model.

## **Results**

### **Camera Traps and Predator Occupancy**

Twenty-seven cameras were deployed for a total of 212 days (5724 trap days) and collected ~425,000 images. The detection probability and site use of four predator species of interest are displayed in Table 4-1. Mapped detection histories and weekly predator frequencies are displayed in Figures 4-2 through 4-5 and Figure 4-6, respectively. Habitat and proportion of time cows used the pasture were not significant covariates affecting either predator use or detection probability for any species. Therefore, they were not included in the table nor in multispecies occupancy modeling. Due to the low occurrence and high standard error of black bear estimates, black bears were not included in the multispecies occupancy modeling. We examined the influence of coyote, bobcat, and panther interactions on species use of the site and found no support that either of these three species were influencing use or detection probabilities of others ( $p_{\text{coyote:panther}}>0.9$ ;  $p_{\text{bobcat:panther}}>0.9$ ;  $p_{\text{bobcat:coyote}}>0.9$ ).

## **Cattle Response**

Brangus and Raramuri were monitored during calving season for 148 days, for a total of 2696 collar days. During this time three collars stopped functioning. They all experienced similar issues where the fix time suddenly changed, fixes became erratic, and collars stopped functioning shortly after the initial issue. Excluding these days of inconsistent monitoring, overall collar fix success was very high and ranged from 95.11% to 99.98% among remaining collars.

Results of the binomial logistic regression examining cattle integration are displayed in Table 4-2 and Figure 4-7. The lowest AIC was in model 3 that included all three fixed effects, breed, time of day (TOD), and week and showed a significant interaction of all three effects. Area under the curve measure to test goodness of fit was 0.67. The model demonstrates as time progresses and during different times of the day, Raramuri and Brangus have differing odds of closest neighbor being Brangus. The proportion of Brangus to Raramuri closest neighbors converges over time between the breeds. All other effects being equal, Brangus have a much higher odds than Raramuri of having a Brangus nearest neighbor. Conversely, Raramuri are much more likely to have a Raramuri neighbor.

A total of 28 panther, 10 bear, and 33 coyote independent events occurred inside calving pastures during the 148 days of monitoring and were used in LMEM. Note that that these records differ from occupancy occurrence records for three reasons: predator presence for breed response must have been inside the same pasture as cattle while occupancy monitored the whole study site, occurrences were not limited to 24-hour occasions like occupancy records, and study duration differed because cattle were separated at the end of calving in February while camera traps were deployed until April. A total of 62,633 GPS points occurred during all of the four-hour monitoring periods (1-hour before and 3-hour) surrounding all the predator/control events

and were used in LMEM. All 71 predator events observed on a camera in the pasture occurred within 1.2 km of the furthest collared individual. The average distance to predator events was  $467.60\text{m} \pm 237.63\text{m}$  (SD) for each collared individual, with a minimum of 5.5m. The best fit model from backwards step regression included a three-way interaction of the fixed effects, time since event, breed, and predator treatment (Table 4-3, Figure 4-8). AIC values from other models are displayed in Table 4-4 for comparison. Breed was a significant factor affecting activity patterns, where Raramuri consistently had a lower activity per distance traveled. Responses varied to different predators. Cattle showed a significantly different response to coyotes and panthers than control events. However, the direction and magnitude of response differed between breed. Response was not significantly different in the presence of a panther between breeds. Neither Brangus nor Raramuri showed consistent change in magnitude nor direction in response to predator treatments.

### **Discussion**

Monitoring of the predator community in a sub-tropical ranching environment of Florida showed high predator use by three species throughout the ranch and that panthers, bobcats and coyotes seemingly co-exist in these habitats during the winter/spring calving season. The fourth species of interest, Florida black bear, was recorded much less frequently and mostly during late October and November. Our occupancy use models suggest that panthers do not affect occupancy or detection probabilities of bobcats or coyotes. This is particularly interesting as other studies in the United States have shown that bobcats modified behavior on fine spatial and temporal scales in response to pumas (Lewis et al 2015). In our study, the camera traps were placed specifically in areas we thought would have a high panther probability; thus, there may be biases in monitoring that impacted occupancy use estimates of both bobcats and coyotes.

Specifically, because of preferential camera placement towards panthers, other species may have been captured less often than were truly present at the site, thereby decreasing overall occupancy estimates, potentially skewing detection probability, and influencing results of multispecies interactions. In addition, it is possible and likely that in at least a few incidences a single individual occupied more than one site during a 24-hour occasion. Therefore, we do not use our results to infer abundance of any species, rather we describe them to demonstrate a coexisting predator community and reveal areas of high predator use throughout the ranch. We know from previous studies that local habitat covariates influence the probability of panther presence such as forest edge and forest cover (Frakes et al. 2015). We examined habitat influence on predator detection and use and found no significant impact but future analyses could incorporate other micro-habitat variables to understand driving factors of predator use and detection.

In addition, the camera traps demonstrated the importance of this ranch habitat for predator communities, particularly the endangered panther. In order to maintain a vital panther population, critical habitat for conservation and expansion must include areas that can support breeding and rearing of young, which is very clearly represented by this ranch (Kautz et al. 2006). A female panther with two kittens was regularly captured during the study. Through camera footage, we know another male and female were using the ranch during the study period. Video observation suggests the two individuals may have mated, as the female can be heard caterwauling and a scrape, which indicates estrous in females, can be seen in the background of the image as the male passes. At minimum the ranch supports 3 adult panthers and two young. These occurrences highlight the overall importance of this habitat and its significant contribution to the current population.

A single depredation confirmed to be a panther occurred on the ranch during the study period. The night of the depredation there was evidence that the male panther responsible may have also been searching for a female who was heard caterwauling in non-study videos, described above. Although we cannot say with 100% confidence that the kill did not occur within the study herd it is highly unlikely. The panther thought to be responsible was captured on camera by other non-study monitoring devices the night of the depredation outside of our study pasture and was traveling south and located east of our study site. It is possible that our camera traps did not detect the panther; however, entrance into the study pasture from the east requires traveling through agricultural fields with large perimeter canals. Camera traps were placed on land bridges over said canals that connected forested areas to agricultural fields and represented the most likely path into the study pasture from the east. Therefore, missed detection of the panther entrance was unlikely. Furthermore, only two calf losses were documented in the study herd during counts at weaning, one loss in the Brangus herd and one loss in the Raramuri herd. The losses from other herds from the ranch were higher during the same period (~21% calf mortality), increasing the odds of the depredation occurring outside the study herd.

It is strongly evident that Raramuri and Brangus were much more likely to be nearer to individuals of their own breed type at the beginning of the study. Over the course of the study period both cattle had increased nearest neighbors of the opposite breed, suggesting that increased integration occurred with time. This pattern of integration between breeds over time could also be a response to calving behavior. As more dams calve, cattle may form tighter herds for increased protection of calves. The supposed protection incurred by Raramuri Criollo is hypothesized to stem from their defensive behavior towards predators. Without integration, Raramuri may not extend this protection to other members of the Brangus herd and potentially

render any deterring behavioral differences ineffective. The slow integration we observed over the breeding season of this study could have significant implications for implementation of such mitigation methods since protection of Brangus herds may not occur immediately. The delayed intermingling of the cattle breeds should also be considered for future research on guarding cattle breeds.

Unfortunately, due to limitations of monitoring 9 of the 80 Brangus cattle, nearest neighbor comparisons between herds were not meaningful. A collared individual Brangus, who are more likely to be closer to the same breed according to our results of breed integration, could be clustered with individuals without collars. Subsequently, nearest neighbor estimates from this data would suggest that the individual was further from the “collared” herd. Comparing these metrics to Raramuri who we have shown to have higher odds of being close to each other and were all collared would consistently report significantly tighter herding behavior of Raramuri compared to Brangus and conclusions would be erroneous.

The results from the linear mixed models support a significant three-way interaction among the fixed effects of breed, time since event, and predator treatment. This indicates that the interaction of breed and predator type are changing over time. Specifically, the difference between Raramuri response to coyotes over time is changing compared to Brangus response towards control treatments. In general, both breeds of cattle did not show consistent behavior in response to predator presence. An explanation to this seemingly erratic behavior could be that cattle behavior is highly variable and may be a direct result of the loss of anti-predator behaviors through domestication. Erratic cattle behavior was also reported in cattle responses of sinuosity and nearest neighbor metrics to wolf treatments (Laporte et al. 2010). Muhly et al. (2010) reported a lag in cattle response to wolf presence in Alberta, Canada. Authors from both studies

suggested these behaviors could indicate a decrease or loss of anti-predator behavior. However, the difference in response to the panthers versus coyotes is not completely surprising as panthers are ambush predators, used to moving quietly, and coyotes are chase and harass predators. Coyote hunting style could induce larger responses, particularly if cattle have previous negative experiences with coyotes.

Another important finding from the analysis is that Raramuri Criollo consistently had lower activity per meter traveled compared to Brangus. Perhaps more importantly, behavior response to panthers did not differ between breed; however, panther treatment did affect overall cattle response compared to control events. Both breeds showed a decrease in activity/meter traveled with panther presence. Different from these results, Kluever et al. (2009) found cattle did not change behavior when presented with puma stimuli in contrast to wolf stimuli.

Surprisingly, significant findings from predator treatments seem largely driven by Brangus responses, except maybe black bear. This suggests that Brangus modified their behavior and showed a stronger reaction to predators while Raramuri Criollo did not change their behavior. These results could indicate that Raramuri are unaware of predators, which seems unlikely, or that they do not react. However, reactions could be measured multiple other ways like path sinuosity or vigilant behavior. Raramuri Criollo could have other defense strategies not investigated or observed in this study which they rely upon for protection from predators.

In this study activity measured by GPS collars seemed particularly sensitive, ~30% of records showed  $\geq 90\%$  activity. This suggests even small movements were  $> 78\text{mg}$  on any of the three axes. Defensive behavior for cattle includes behaviors like lowered head and kicking. Therefore, we would expect that activity would be high when displaying defensive behavior. In addition, we would expect that defensive behavior would decrease distance traveled since

individuals would stand their ground. In combination, activity would remain steady or increase and distance traveled would decrease, thereby increasing the response variable when defensive behavior was exhibited. Our prediction that Raramuri would show defensive behavior and result in an increase in the measured response to panther presence was not correct. Instead, the results suggest both Raramuri Criollo and Brangus decreased activity per meter traveled in panther treatments. However, we do see an increase in the response variable towards bear treatments by both Raramuri and Brangus. Only ten bear events occurred during the study and many of the estimates including bear treatment show large variability and generalizations may not be meaningful. Surprisingly, we do see an increased response from Brangus towards coyote treatments. This could stem from different behavior that mimic similar activity over distance traveled patterns. For instance, an increased sinuosity of cattle which has been reported in response to predator presence in other studies could result in decreased distance traveled and increased activity (Laporte et al. 2010).

This study was not robust enough to understand the efficacy of Raramuri as a livestock guardian, rather we examined differences in behavior that may demonstrate their ability to defend Brangus herds. Although it is hypothesized that defensive behavior is responsible for protection and decreased depredations, we do not know this is true as it is based on anecdotal evidence. Therefore, any reasonable difference in behavior between Raramuri and Brangus may be worth further investigation. In this study we demonstrated that Raramuri do behave differently than Brangus; thus, it is reasonable to hypothesize that other behaviors differ between the two breeds and one these may serve as protection for Brangus herds against panthers. Other metrics could be investigated with the current dataset and future analyses should include sinuosity of cattle path, habitat selection analyses, and predator avoidance of Raramuri and Brangus. A more

thorough calf mortality study similar to the one conducted in chapter 3 but with a focus on depredations would provide a better understanding of Raramuri's efficacy as a livestock guardian.

## Figures and Tables

Table 4-1. Site use estimates and detection probabilities from single-species modeling from 212 occasions at JB Ranch from September 2019 to April 2020

	Occurrences at Study Site	Occupancy (Use) Estimate ( $\psi$ )	Lower 95% CI	Upper 95% CI	Detection Probability (p)	Lower 95% CI	Upper 95% CI
<i>Canis latrans</i>	204	0.96	0.78	0.99	0.037	0.032	0.042
<i>Lynx rufus</i>	385	0.93	0.75	0.98	0.073	0.066	0.080
<i>Puma concolor coryi</i>	83	0.56	0.40	0.76	0.025	0.020	0.031
<i>Ursus americanus floridanus</i>	24	0.485	0.27	0.71	0.009	0.005	0.014

Note that occurrences are based on 24-hour sampling period from the hours of 1200 to 1200 and are different than predator visitations. Multiple visitations can occur during the 24-hour period at a single camera and only equate to one occurrence.

Table 4-2. Results from binomial logistic regression of cattle integration with 95% CI.

Predictors	M1			M2			M3		
	Odds Ratio	CI	p	Odds Ratio	CI	p	Odds Ratio	CI	p
(Intercept)	0.66	0.65-0.67	<0.001*						
Week	1.01	1.00-1.01	<0.001*	0.98	0.98-0.98	<0.001*	0.98	0.98-0.98	<0.001*
Breed [Brangus]				1.68	1.65-1.71	<0.001*	1.94	1.89-2.00	<0.001*
Breed [Raramuri]				0.27	0.26-0.28	<0.001*	0.27	0.26-0.28	<0.001*
Week*Breed				1.06	1.06-1.06	<0.001*	1.06	1.05-1.06	<0.001*
TOD [Night]							0.76	0.74-0.79	<0.001*
Week*TOD							1.00	1.00-1.01	0.185
Breed * TOD							1.29	1.22-1.36	<0.001*
Week*Breed *TOD							1.01	1.00-1.01	0.001*
Observations		399354			399354			399354	
AIC		543205.3			507326.0			506527.0	

\*p-value is significant. M1=ClosestNeighbor~Week; M2=ClosestNeighbor~Week\*Breed; M3=ClosestNeighbor~Week\*Breed\*TOD.

Table 4-3. Results from best fitted linear effects mixed model with 95% confidence intervals for model  $\log(\text{Act}/\text{Dist}) \sim \text{Breed} * \text{Time} * \text{Predator} + \text{random effects}$ . Results are log-transformed

Predictors	Model			
	Estimates	CI	Statistic	p
(Intercept)	1.3726	0.9925 – 1.7527	7.0772	<0.001*
Brangus	<i>Reference</i>			
Raramuri	-0.2079	-0.3785 – -0.0374	-2.3896	0.017*
Raramuri:ΔTime	-0.0003	-0.0007 – 0.0000	-1.7492	0.080
Raramuri:Panther	-0.0317	-0.0940 – 0.0306	-0.9974	0.319
Raramuri:Panther: ΔTime	0.0004	-0.0002 – 0.0011	1.3209	0.187
Raramuri: Bear	-0.0178	-0.1095 – 0.0738	-0.3814	0.703
Raramuri: Bear: ΔTime	-0.0004	-0.0013 – 0.0005	-0.8550	0.393
Raramuri: Coyote	0.0691	0.0089 – 0.1293	2.2489	0.025*
Raramuri: Coyote: ΔTime	-0.0006	-0.0013 – -0.0000	-2.0264	0.043*
ΔTime	-0.0002	-0.0004 – 0.0001	-1.3294	0.184
Predator Control	<i>Reference</i>			
Panther: ΔTime	-0.0001	-0.0006 – 0.0003	-0.6016	0.547
Panther	-0.0842	-0.1630 – -0.0055	-2.0956	0.036*
Bear: ΔTime	0.0014	0.0007 – 0.0021	3.9773	<0.001*
Bear	0.0054	-0.1231 – 0.1340	0.0830	0.934
Coyote: ΔTime	0.0010	0.0005 – 0.0014	4.0576	<0.001*
Coyote	-0.1523	-0.2116 – -0.0930	-5.0334	<0.001*
Observations	62633			
AIC	192013.5			

\*P-value is significant.

Table 4-4. AIC values from models of competing fixed effects.

	M1	M2	M3	M4	M5	M6
AIC	192065.8	192053.6	192066.0	192051.5	192061.5	192053.5

Note: M1= log(Act/Dist)~random effects (null); M2= log(Act/Dist)~Predator + random effects; M3=log(Act/Dist)~Time random effects; M5= log(Act/Dist)~Breed + random effect; M6= log(Act/Dist)~Breed\*Time + random effects; M4=log(Act/Dist)~Breed\*Predator + random effects; M4= log(Act/Dist)~Breed\*Predator + random effects

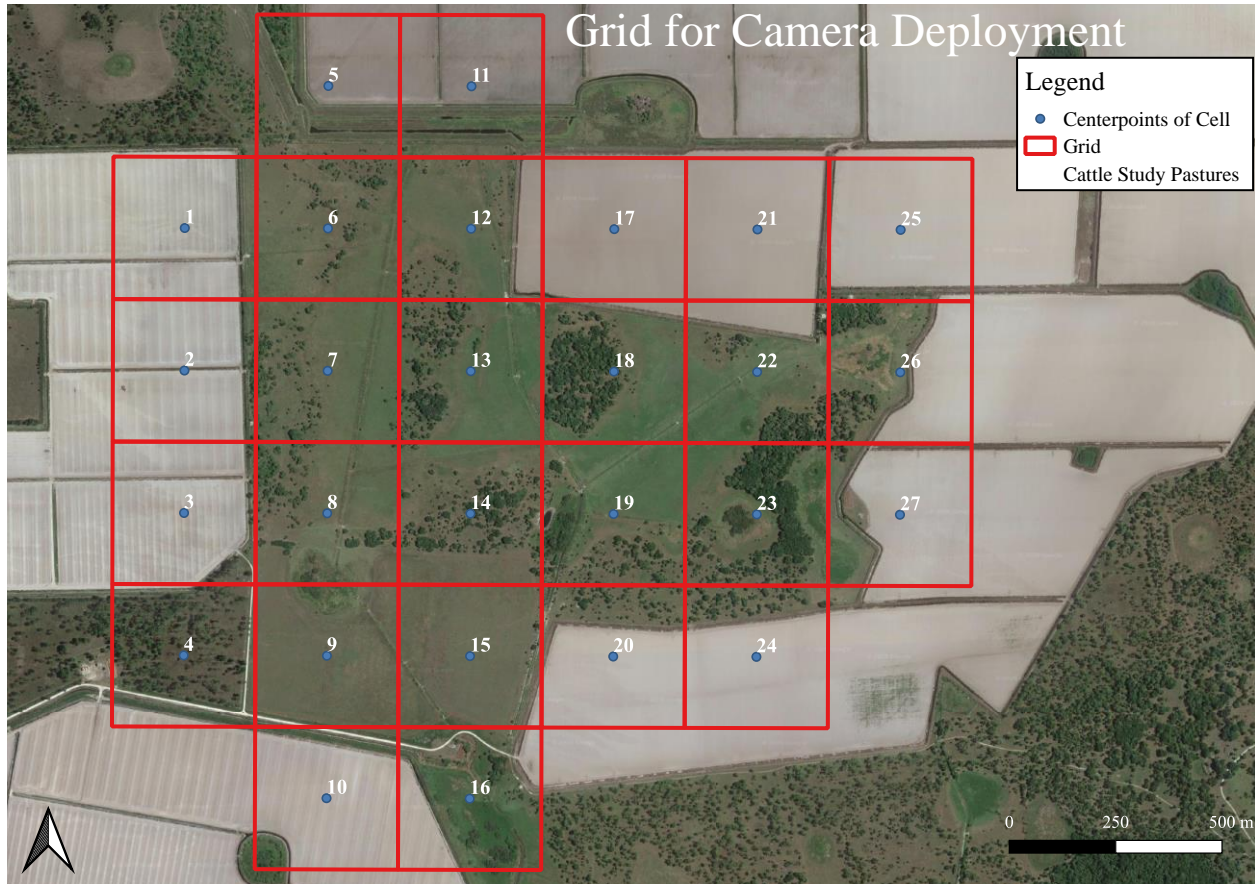


Figure 4-1. Multi study pastures overlaid with camera grid. Each cell is 375m<sup>2</sup> and centerpoints are indicated by the numbers.

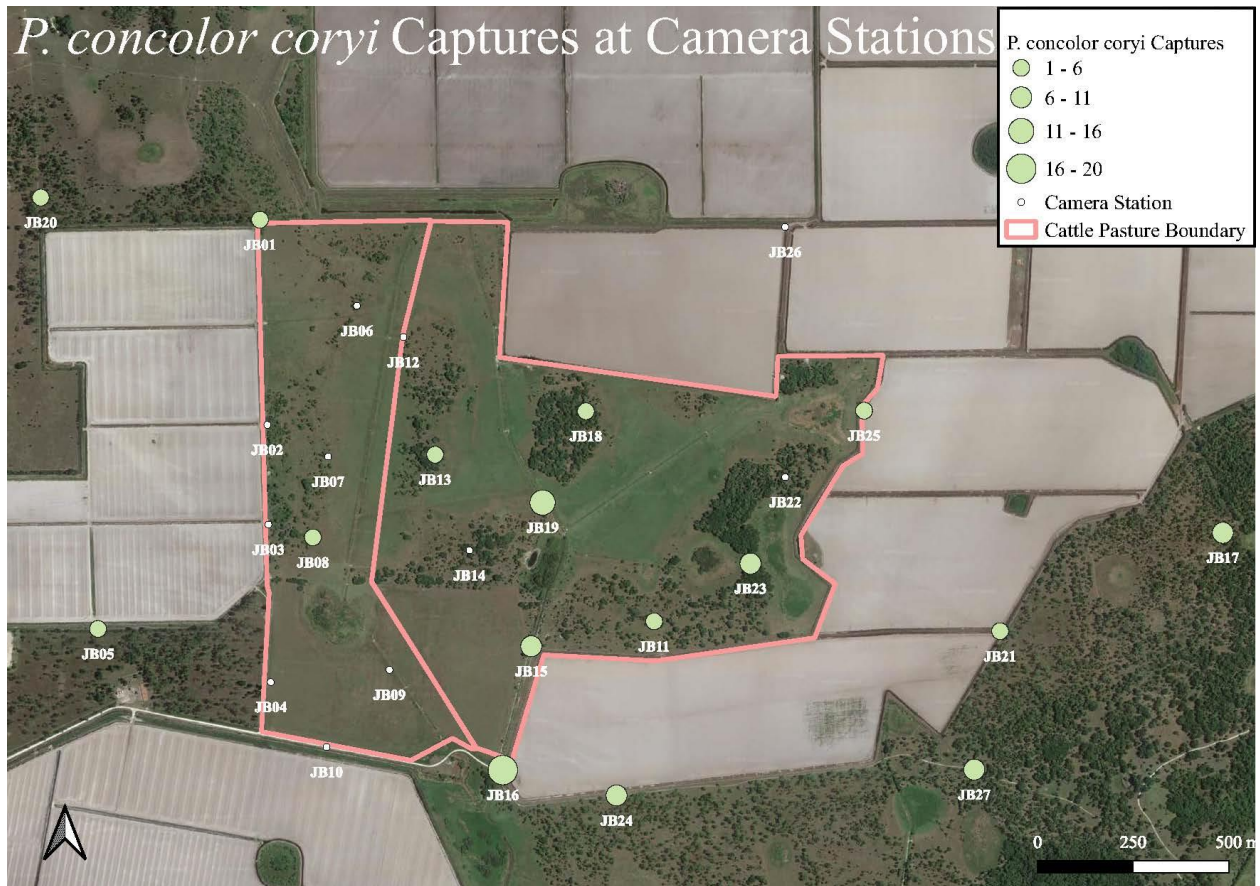


Figure 4-2. *Puma concolor coryi* detection history at each of the camera traps from Sept 2019 to April 2020

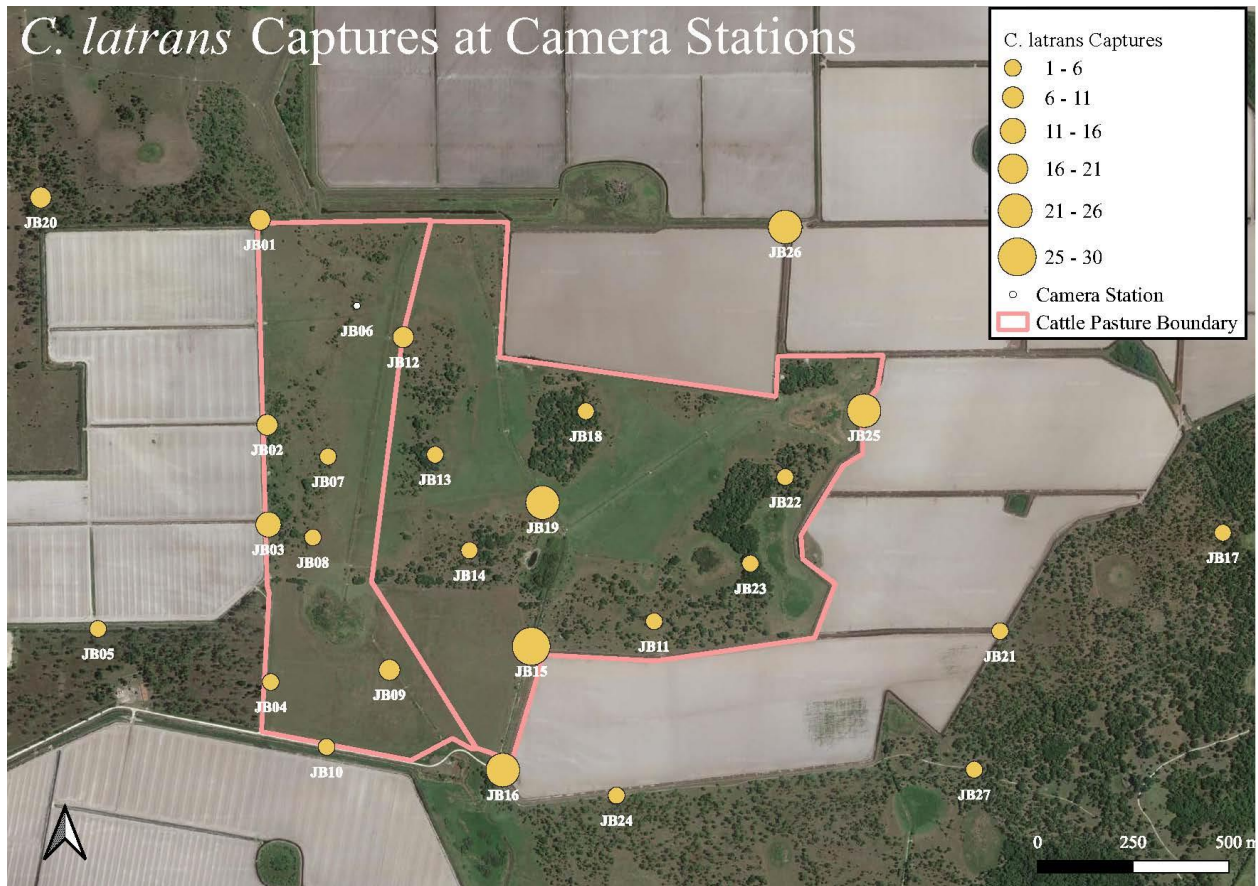


Figure 4-3. *Canis latrans* detection history at each of the camera traps from Sept 2019 to April 2020.

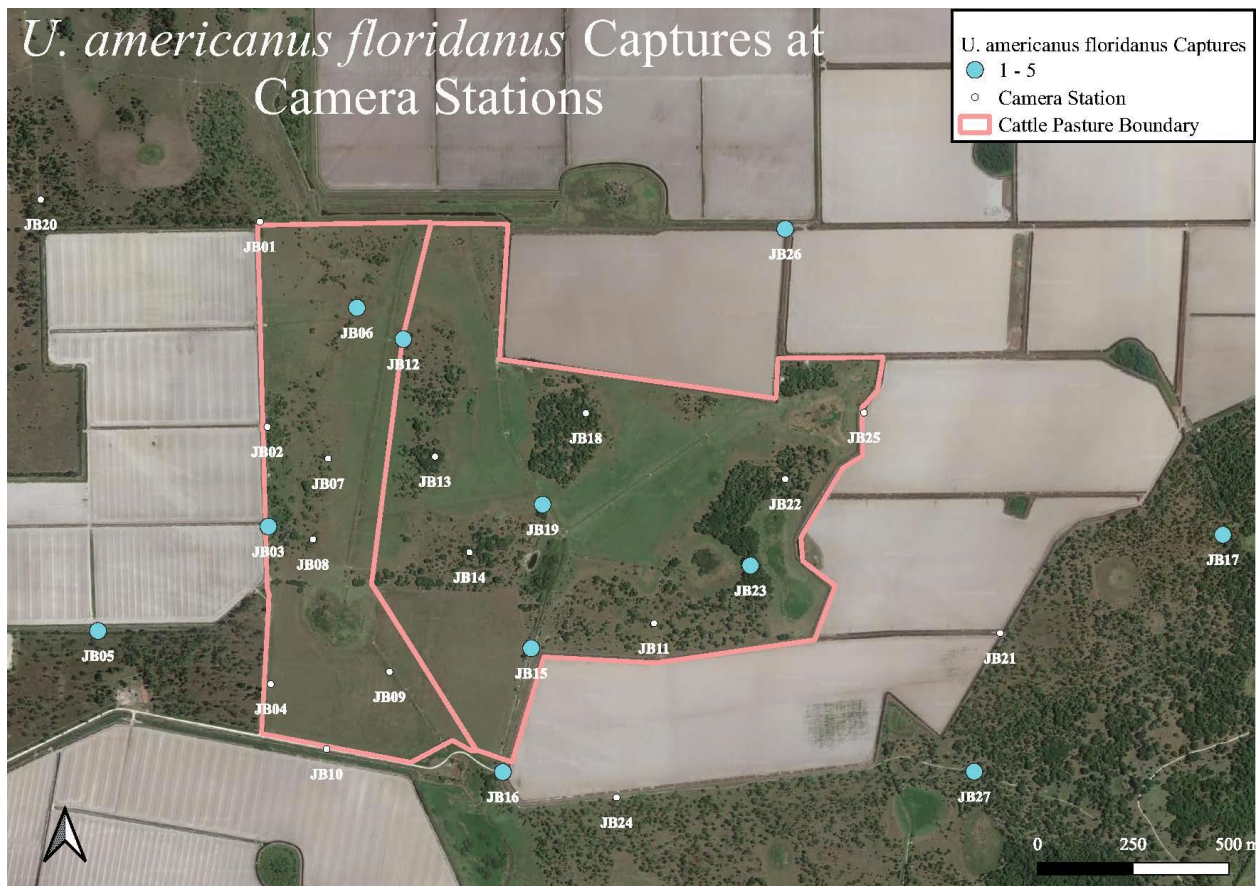


Figure 4-4. *Ursus americanus floridanus* detection history at each of the camera traps from Sept 2019 to April 2020.

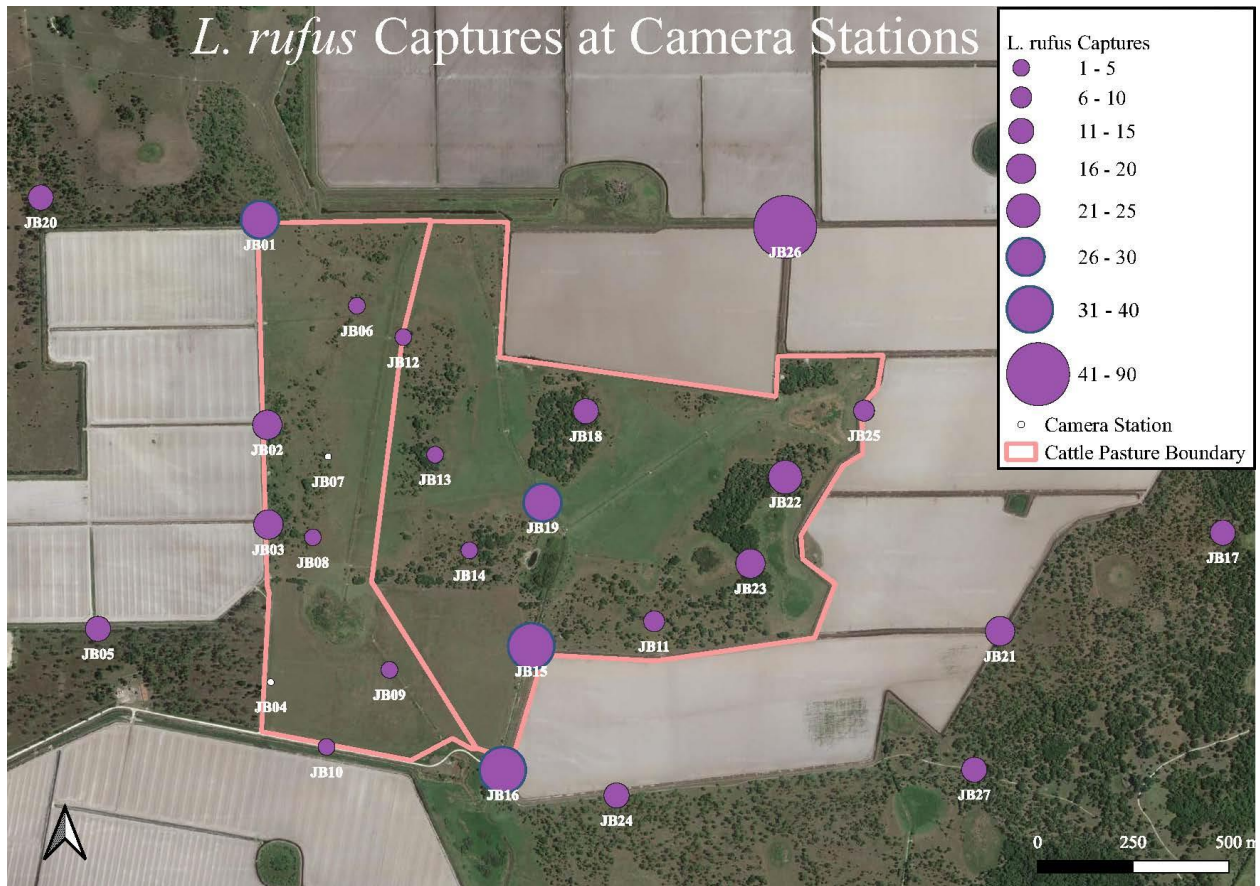


Figure 4-5. *Lynx rufus* detection history at each of the camera traps from Sept 2019 to April 2020.

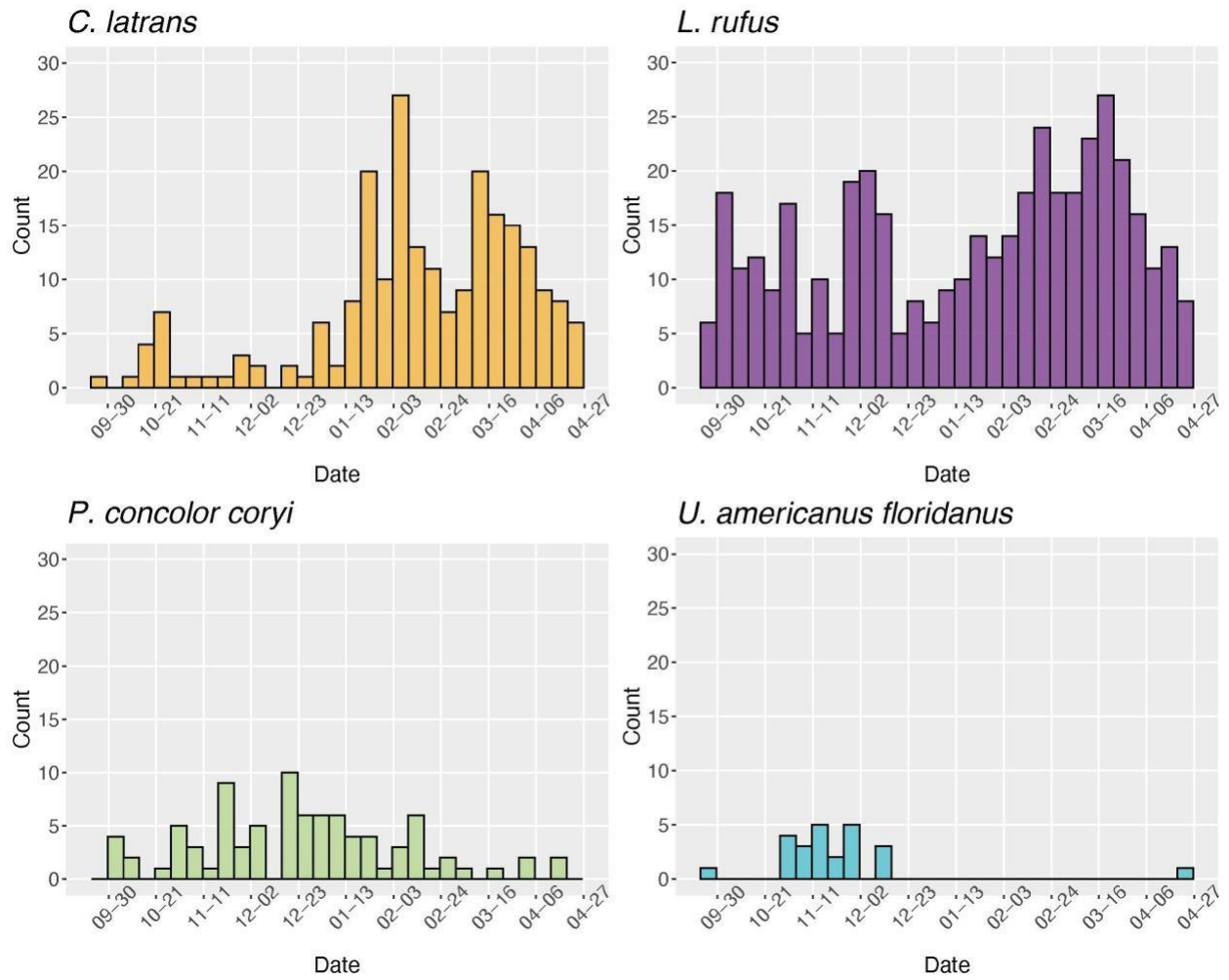


Figure 4-6. Weekly predator frequencies at the study site.

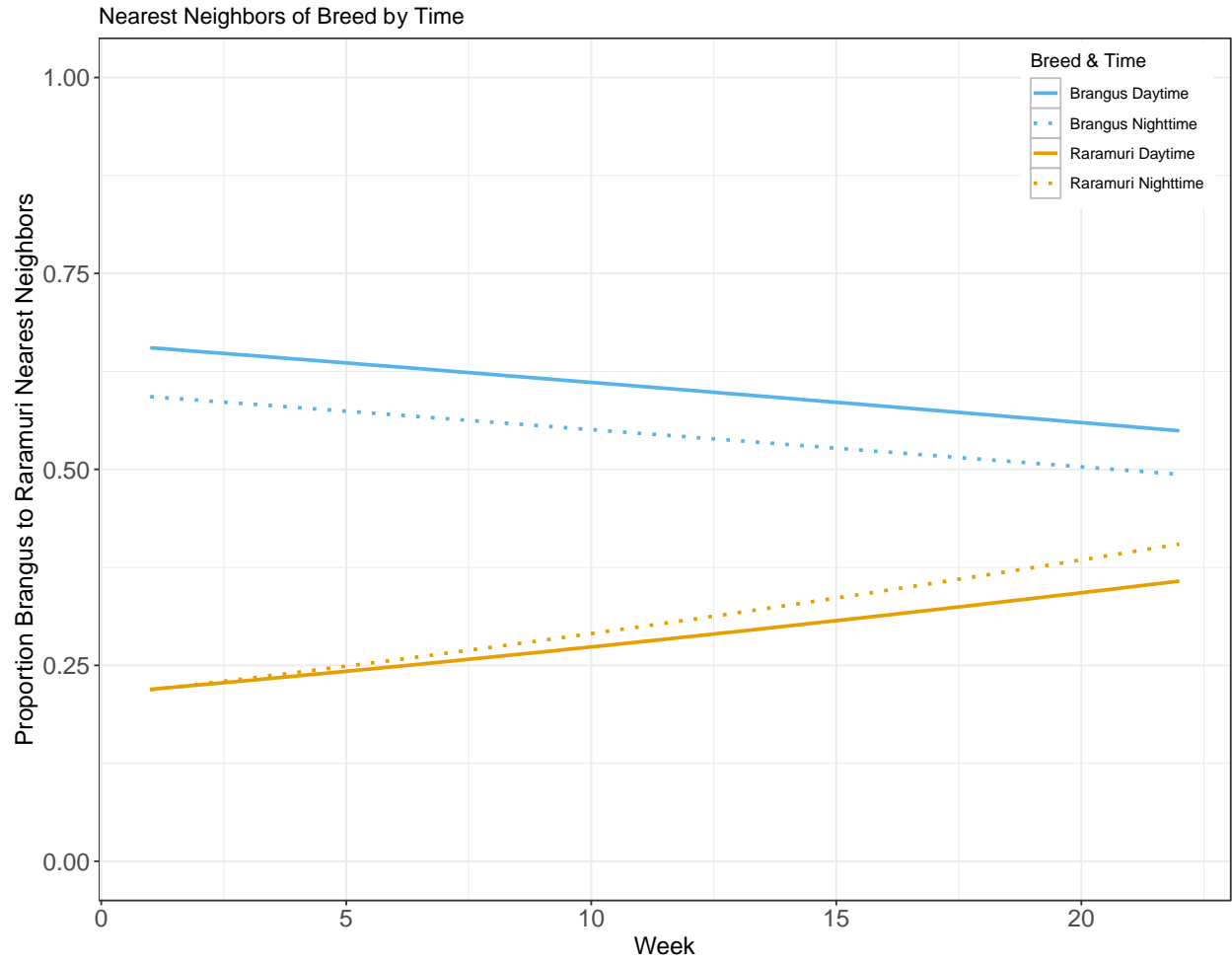


Figure 4-7. Proportion of Brangus closest neighbors to Raramuri closest neighbors over time.

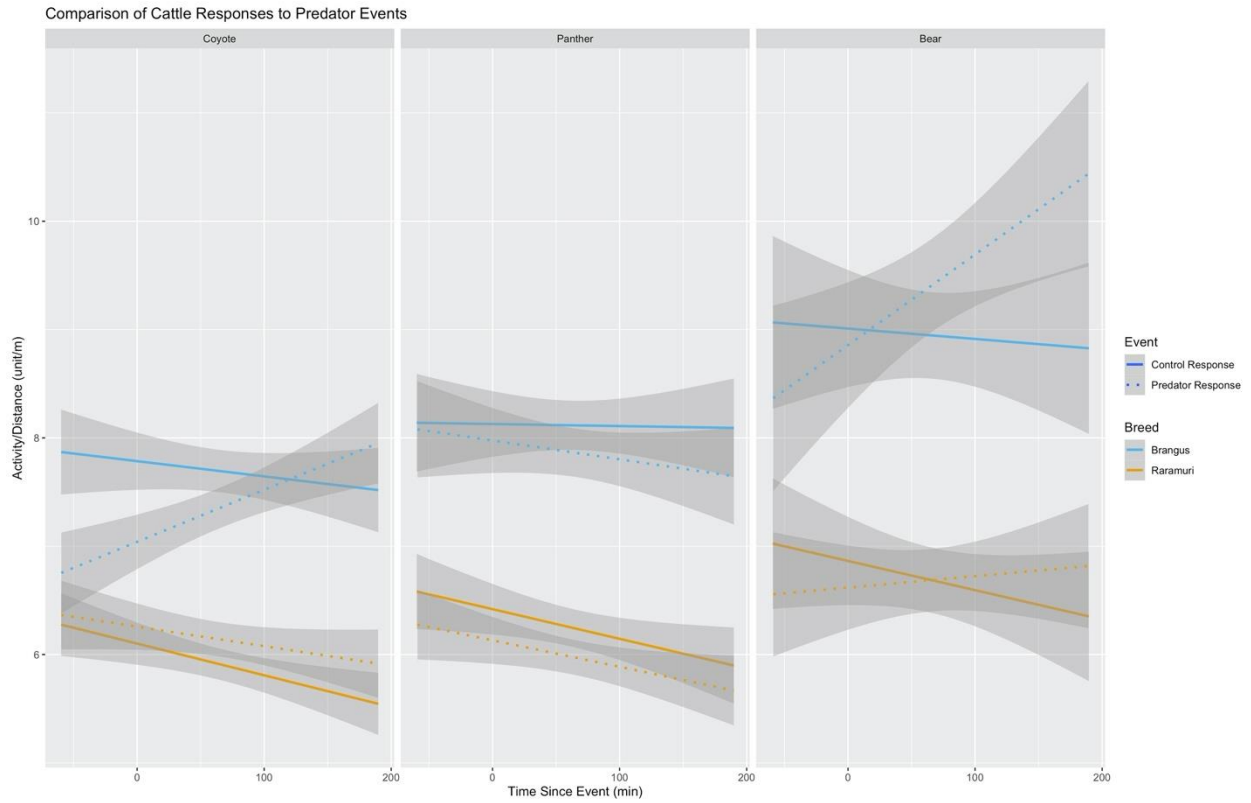


Figure 4-8. Cattle response of Brangus and Raramuri to different predator events. Nonevents that served as the control to predator events are graphed as the solid line. Time zero corresponds to the beginning of a predator event (i.e. time when predator was first captured by camera traps in calving pasture with study cattle).

## CHAPTER 5 CONCLUSIONS

In this study we achieved three major objectives. First, we evaluated a tool to monitor calving in large-scale ranching applications for future use by researchers and producers. Second, we described overall calf mortality rates of beef cow-calf operations in Florida and evaluated the major underlying causes contributing to calf mortality. Lastly, we examined Raramuri Criollo as a possible nonlethal mitigation technique for Brangus protection in high panther use areas.

Monitoring calving and parturition has important implications for researchers and producers that can mitigate a portion of calving related losses and help us better understand calving related problems. To address these needs a modified intravaginal birthing sensor was developed. We found that 47% of the sensors did not function properly and testing revealed failures were in large part due to issues with battery power, software protocols, and hardware components. At the current development stage, the birthing sensors are not an effective tool for monitoring calving. However, with a clear understanding of current limitations and development addressing said limitations, vaginal birthing sensors could fill a gap in calving management and research on large-scale operations.

We concurrently examined calf mortality rates and despite sensor failures we were able to capture 99.6% of all calving events. This is the most comprehensive calf mortality study undertaken in Florida and examined calf loss from late gestation to weaning. Additionally, to date it is the only study in Florida that investigated underlying causes of all calf mortalities through post-mortem investigation. We found that Florida calf mortality was more than two times higher than the national average at 13.24% (USDA 2015). The major contributing factors of death were bacterial infections, mineral toxicities and deficiencies, and dystocia-related issues. Many of these underlying problems overlapped and further research should be conducted to

understand the effects and interactions between these factors. Bacterial species isolated through postmortem investigations revealed *Trueperella pyogenes* and *Escherichia coli* in a large number of mortalities. Infections originated both in utero and from exogenous sources. Additionally, co-infection of multiple bacterial species was common. More directed research is needed to understand the effects of Florida's subtropical environmental conditions on bacterial infections associated with calf mortality. Dystocia related mortalities included a range of potential causes. Feto-pelvic incompatibility was implicated in half of the dystocia issues and highlights the importance of sire selection during breeding. Deficiencies and toxicities were reported in all three ranches and indicate supplementation should be more closely monitored by producers. In the short-term, cow-calf operations could potentially decrease calf mortalities through improved management strategies of supplementation programs, sire selection, and removal of known sources of lead on the pastures. Only a single depredation occurred during our study and accounted for 3.3% of the documented mortalities. Due to our increased human presence in the pastures, we may have impacted predator behavior thereby underestimating depredation rates. Although depredations did not occur on the ranch with the highest suspected predator community, there were documented depredations in neighboring pastures and ranches to that study site. That said, depredations due vary by year and ranch; therefore, we may have observed a year with low depredations.

Decreased tolerance of stakeholders towards the Florida panther (*Puma concolor coryi*) as a result of calf depredations can greatly impact the conservation of the species. We examined predator use of a cattle ranch and explored the potential ability of Raramuri Criollo to serve as a livestock guardian for Brangus against panthers. A diverse predator community was observed in this study and described the ranch use of four predator species, the coyote (*Canis latrans*),

bobcat (*Lynx rufus*), Florida black bear (*Ursus americanus floridanus*), and Florida panther (*Puma concolor coryi*). Three of the predators, the coyote, bobcat, and panther, all had high visitations and high use of the ranch area. There was no evidence that these species were influencing each other's use or detection at the ranch, which demonstrates a coexisting predator community throughout the ranch landscape. The high use of the ranch highlights the importance of this habitat for predator communities, particularly the endangered Florida panther.

The Raramuri Criollo herd was distinct from the Brangus herd when first placed together in a pasture; however, over time, the two breeds integrated. Raramuri may only protect the Brangus herd if they are integrated as a single herd; therefore, this delayed intermingling should be accounted for in future studies on guarding cattle breeds. From the metrics assessed, our findings suggest Raramuri Criollo did not display different behavioral responses towards panthers compared to Brangus. However, we demonstrated that Raramuri consistently behave differently than Brangus regardless of treatment. Although Raramuri's defensive behavior is hypothesized to protect the main herd, we do not know if that behavior is the responsible mechanism of protection. Any reasonable difference in behavior of Raramuri may protect the Brangus herd from panthers. That said, our findings show a difference in behavior between the breeds. Additionally, because of this difference it is reasonable to hypothesize that other behaviors differ between Raramuri and Brangus. Any one of these differences may protect Brangus herds against panther depredations; therefore, Raramuri Criollo may be an appropriate livestock guardian for Brangus in south Florida. Further investigations are warranted and necessary to understand other behavioral differences of the two breeds and the mechanism of protection from Raramuri Criollo.

As improvements of birthing sensors are developed, methodologies from this thesis could be combined to undertake a variety of different studies. Intravaginal birthing sensors could be deployed on herds with and without Raramuri to understand if their integration decreased depredations by panthers. In addition, more longitudinal studies could improve our understanding of overall calf mortality rates and the true impact of depredations on Florida cow-calf operations.

APPENDIX A  
SUPPORTING FIGURES AND GRAPHS

Figures

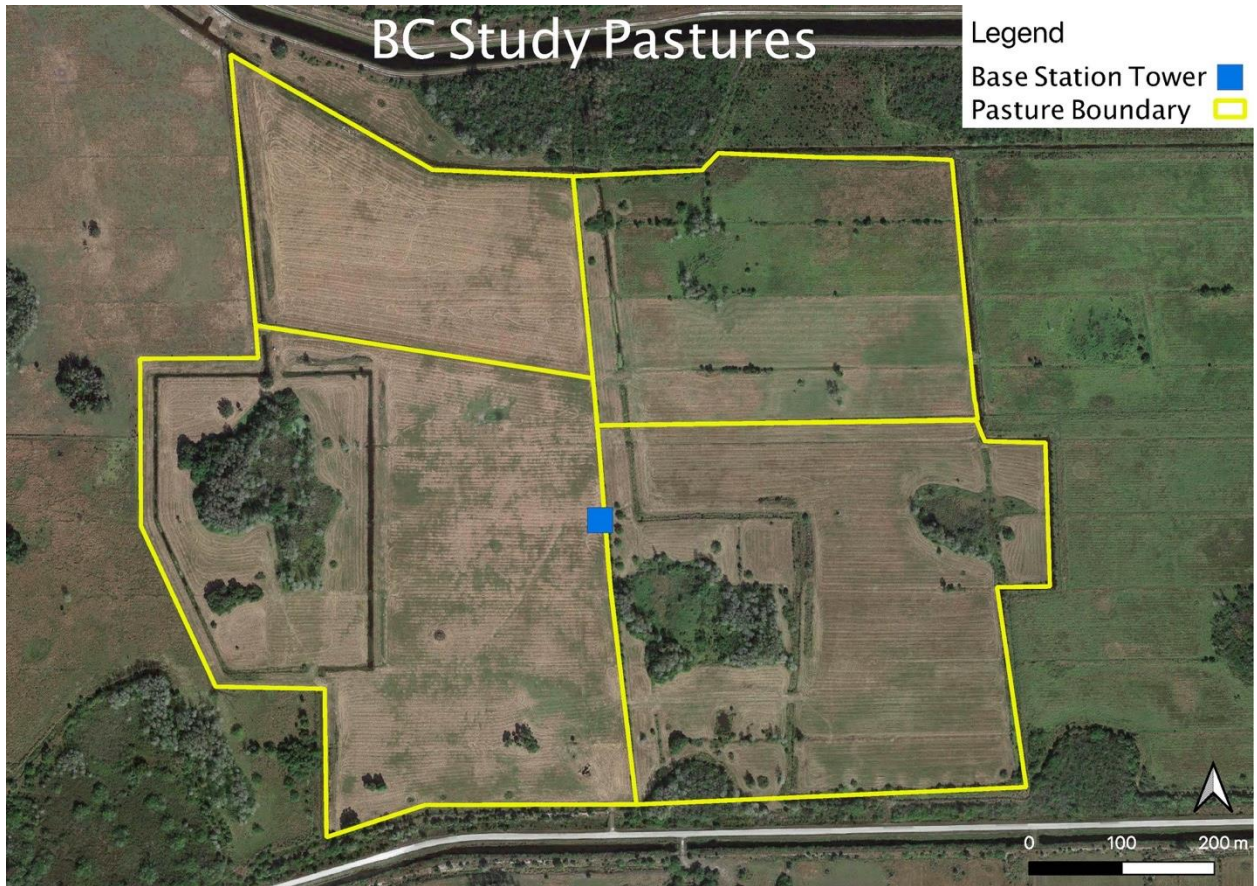


Figure A-1. BC study pastures utilized in birthing sensor deployments and calf mortality study

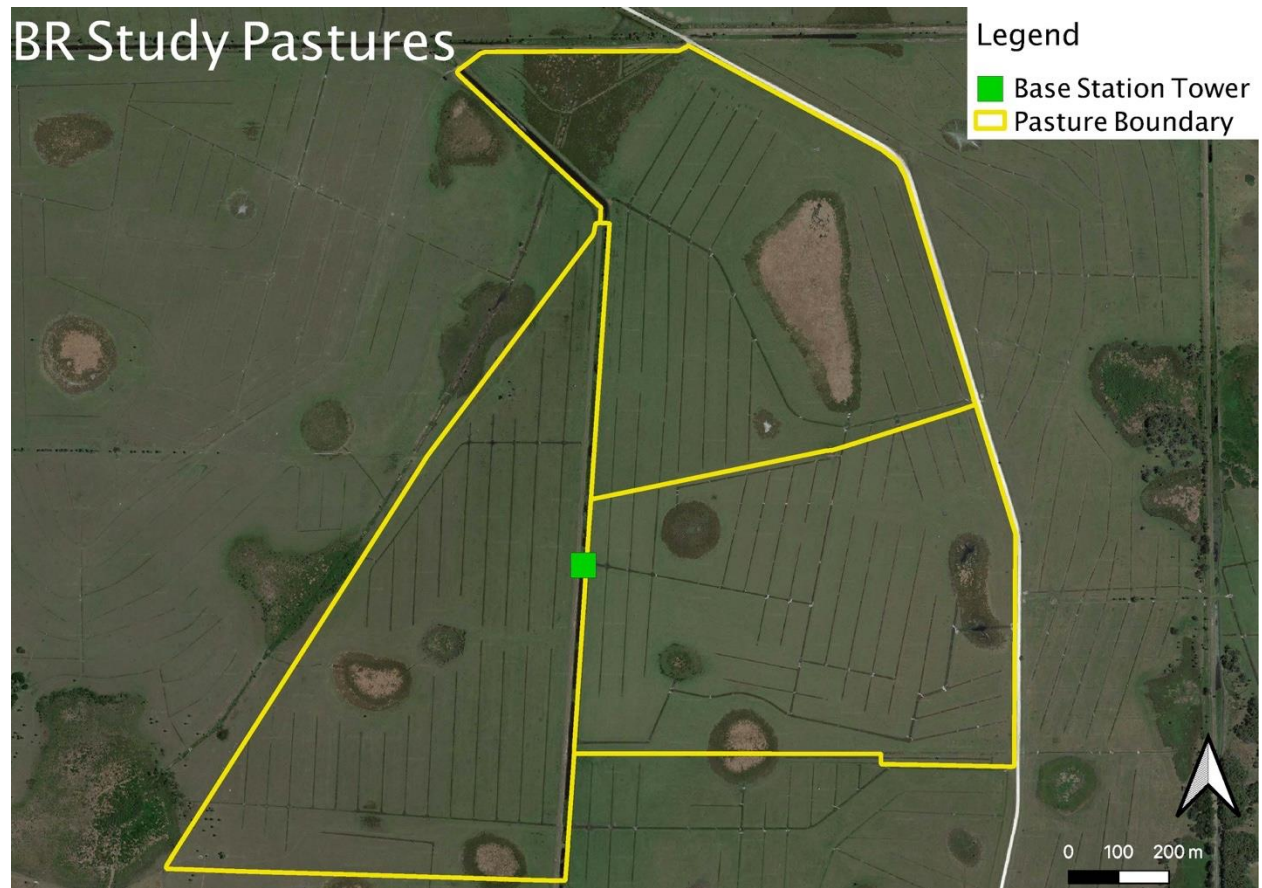


Figure A-2. BR study pastures utilized in birthing sensor deployments and calf mortality study.

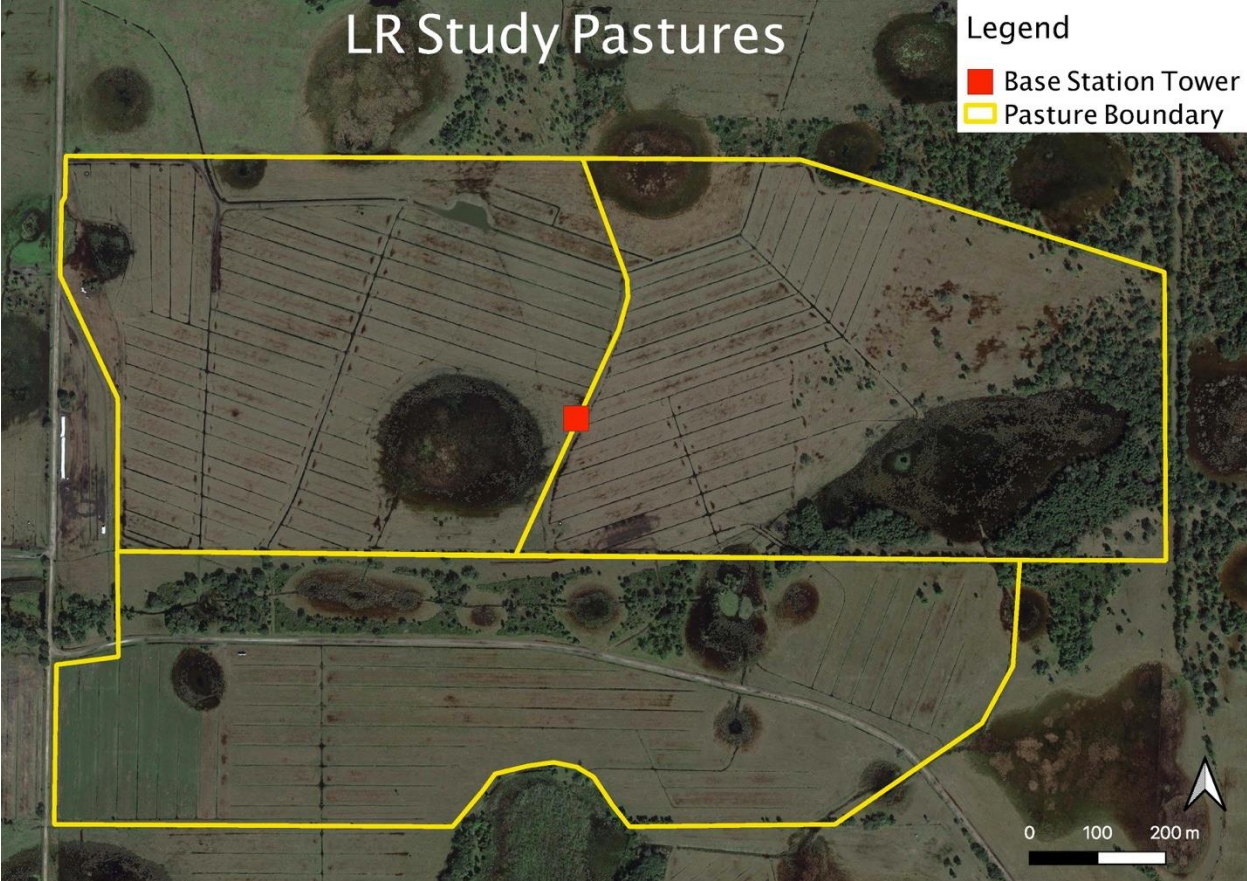


Figure A-3. LR study pastures utilized in birthing sensor deployment and calf mortality study.

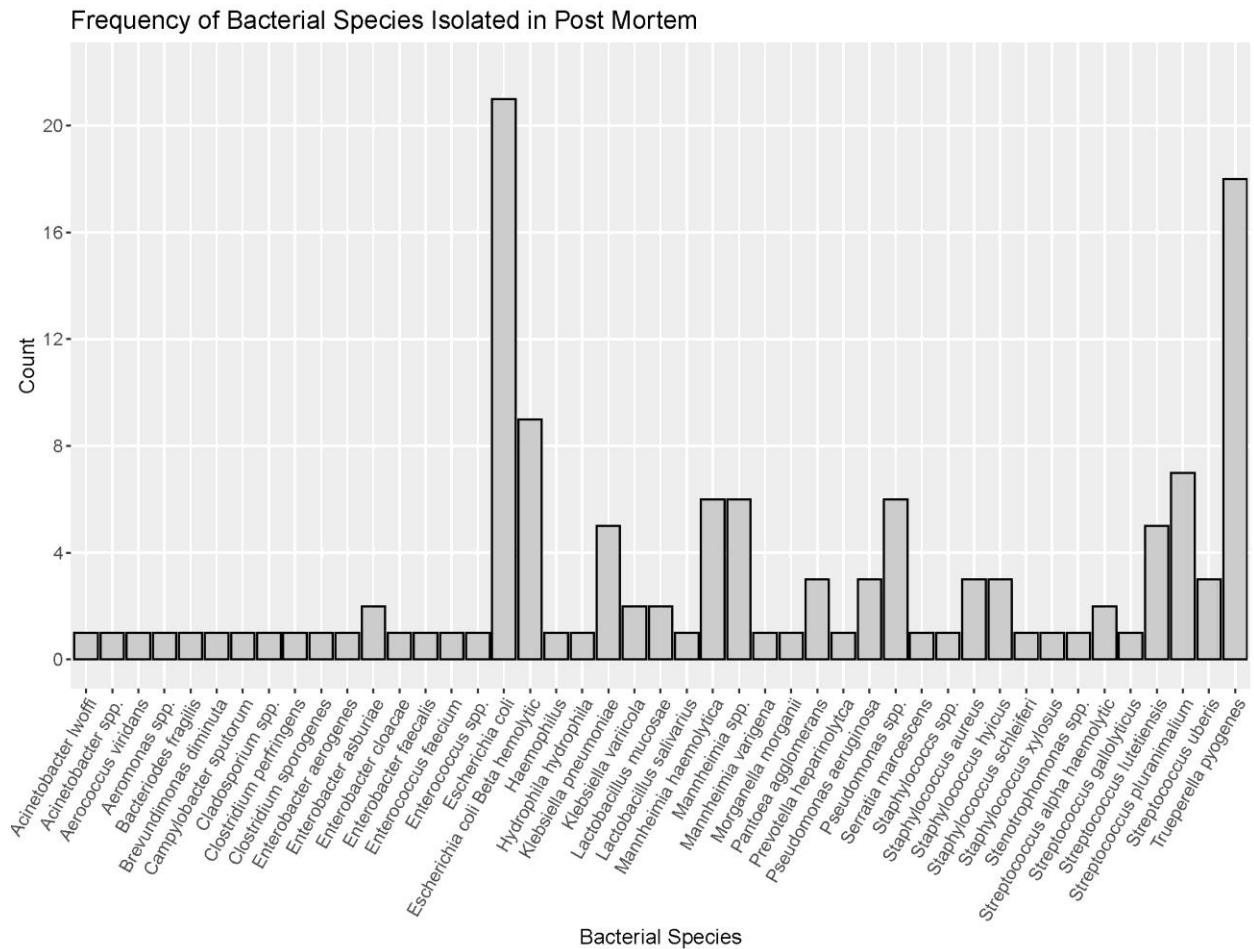


Figure A-4. Bacterial species isolated during post-mortem investigations in all locations and from all specimens at LR, BR, and BC in 2017-2018.

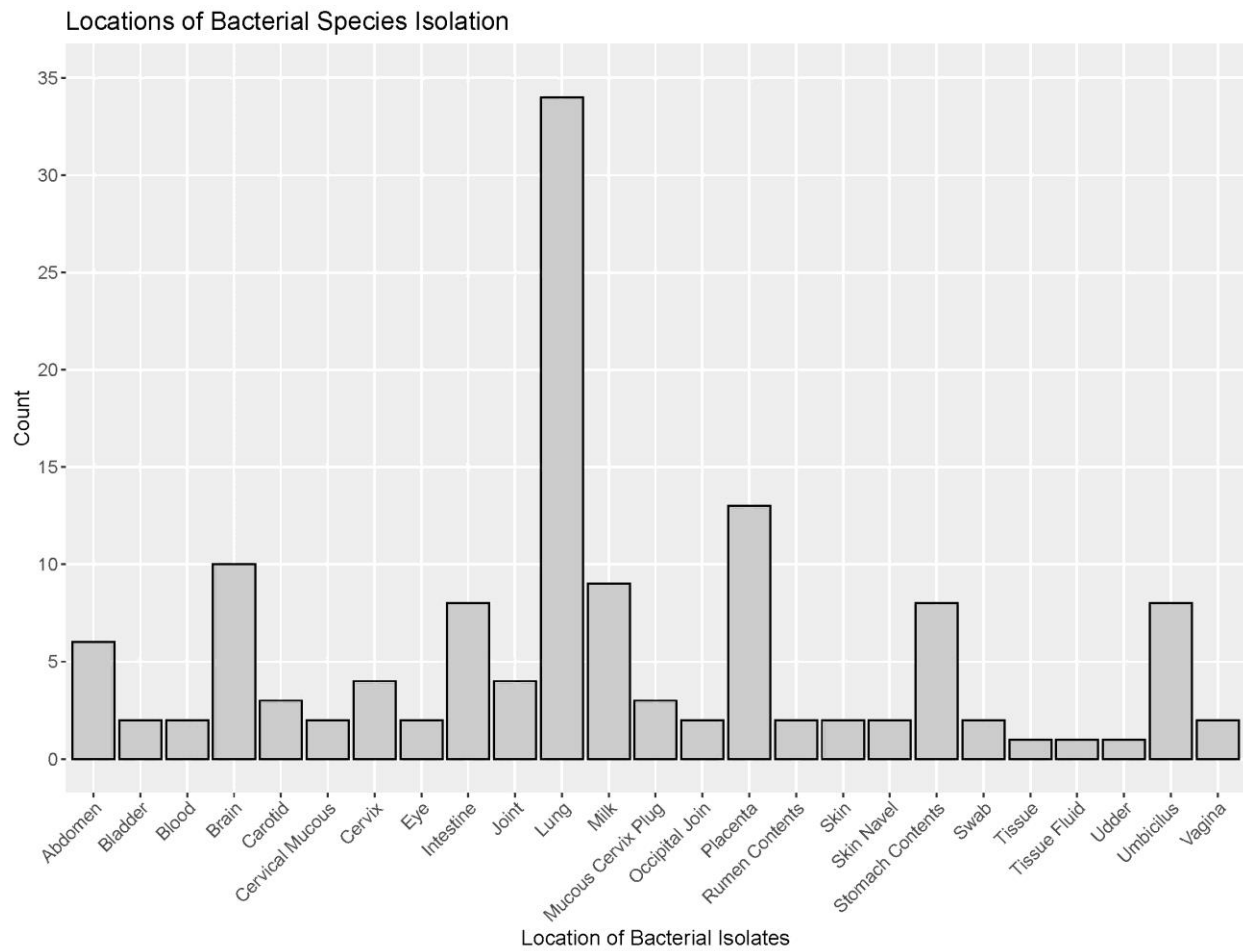


Figure A-4. Locations of isolated bacterial species during post-mortem investigations at LR, BR, and BC in 2017-2018.

## Tables

Table A-1. Random effects used in the Linear Mixed Effects Model with justification for inclusion in the model.

Random Effect	Justification
Individual Cow ID	Individual cows may have different underlying behavior that we were not interested in describing.
Paired Comparison Event	Predator events were compared to non-predator events within $\pm 2$ weeks; therefore, we needed to account for variations associated with the paired comparison.
Day	Response may have varied depending on the day, specifically as the number of predators visited the study site.
Precipitation Category	Rain may have decreased cow's ability to smell or hear predator; thus, influencing the response of cattle.
Distance from Initial Event Category	Proximity to predator event would likely lead to differing responses by individuals. We categorized distance into 50m increments for varying levels of proximity.

APPENDIX B  
 NECROPSY, CONTRIBUTING FACTORS TO DEATH, AND ASSUMED MORTALITY  
 SUMMARIES

**Section 1A: BC Necropsy Reports**

**Calf 2**

Table B-1. Calf 2 contributing factors summary at BC

3 days old	
Contributing factors to death	Support for the claim
Septicemia/bacteremia	<ul style="list-style-type: none"> <li>• Lesions likely caused by bacteremia on umbilical cord and lesions affecting joints</li> <li>• <i>Mannheimia spp.</i> found in eye, lung, and occipital joint</li> <li>• <i>Trueperella pyogenes</i> isolated from lung and occipital joint.</li> <li>• <i>Klebsiella pneumoniae</i> found in eye, brain, lung, and umbilicus</li> </ul>
Mineral imbalances	<ul style="list-style-type: none"> <li>• Low iron at 116.35 ug/g (min. ref. value 165 ug/g)</li> <li>• High zinc at 946.42 ug/g (max. ref. value 820 ug/g)</li> </ul>

<b>Calf Loss Summary</b>
<p>Calf had multiple bacteria isolated throughout body, consistent with septicemia. Lesions found in the joints and lungs corroborated widespread infection. <i>Mannheimia spp.</i>, <i>Klebsiella pneumoniae</i>, and <i>Trueperella pyogenes</i> were the likely culprits of infection as they were isolated in multiple organs of the calf. Calf had lesions consistent with secondary opportunistic bacterial pneumonia. Origin of initial infection was thought to be from umbilical cord acquired post-partum that spread throughout the body.</p>
<b>Timeline</b>
<ul style="list-style-type: none"> <li>• Birthing sensor did not send message upon expulsion.</li> <li>• Calf was found and tagged on 11/7/2017 <ul style="list-style-type: none"> <li>○ Calf was standing and walking with dam.</li> <li>○ Calf appeared to be at least a day old at the time of tagging.</li> </ul> </li> <li>• Calf was found dead on 11/10/2017.</li> <li>• Calf was put on ice and taken to Bronson Laboratory on 11/10/2017.</li> </ul>
<b>Necropsy and/or Laboratory Test Results</b>
<ul style="list-style-type: none"> <li>• <i>Mannheimia species</i> found in the eye, lung, and occipital joint.</li> <li>• <i>Klebsiella pneumoniae</i> found in the eye, brain, lung, and umbilicus.</li> <li>• <i>Trueperella pyogenes</i> found in the lung and occipital joint.</li> <li>• <i>Corynebacterium spp.</i> isolated in the brain.</li> <li>• Swelling of joints caused from pus containing fibrin (protein involved in clotting blood)</li> <li>• Umbilicus congested, heavily infiltrated by neutrophils, and tissues exhibit liquefactive necrosis with mats of mixed bacteria.</li> <li>• Lungs were marbled with dark purple, red areas alternating with pinkish red zones indicating bronchopneumonia.</li> <li>• Cause of death likely attributed to septicemia/bacteremia as a result of omphalophlebitis.</li> <li>• Mineral results show <ul style="list-style-type: none"> <li>○ Low level of iron 111.63 (ref 165-1600 ug/g)</li> <li>○ High level of zinc 946.42 (ref 150-820 ug/g)</li> </ul> </li> </ul>
<p><i>Concerns</i> None</p>

## Calf 8

Table B-2. Calf 8 contributing factors summary at BC

---

Stillborn	
Contributing factors to death	Support for the claim
Dystocia- fetal distress/anoxia	<ul style="list-style-type: none"><li>• Swelling of thorax, jaw, and abdomen</li><li>• Scattered meconium in the lungs</li><li>• Sensor expelled and labor not progressed for 24 hours</li></ul>
Mineral deficiencies	<ul style="list-style-type: none"><li>• Deficient level of manganese at 0.48 ppm (min ref value 0.9 ppm)</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• Low level of copper at 44.640 ppm (min. ref. value 65ppm)</li><li>• Low level of magnesium at 93.139 ppm (min. ref. value 100 ppm)</li><li>• Low levels of phosphorous at 1301.573 ppm (min. ref. value 2000 ppm)</li></ul>

---

### **Calf Loss Summary**

Sensor was expelled and cow had not given birth within 24 hours, suggesting a difficult birth. Post-partum investigation showed, swelling of the mandible, thorax, and abdomen, consistent with dystocia or difficult and prolonged labor. Meconium found in lungs of calf and lungs never expanded, supporting anoxia as cause of death.

### **Timeline**

- Birthing sensor expelled on 10/6/2017 13:40
- Cow was found approximately 24 later on 10/7/2018
  - Cow had not given birth and was in labor.
  - Dead calf was pulled from the cow
- The carcass was put on ice and taken to Bronson Laboratory

### **Necropsy and/or Laboratory Test Results**

- Lungs were collapsed and swollen with hemorrhage and scattered meconium in the alveolar spaces.
- Lungs were dark red to purple, rubbery, and did not float.
- Subcutaneous tissues of jaw, thorax, and abdomen swollen.
- Both thoracic cavity and abdominal cavity contain small amount of thin, watery, dark red fluid.
- Mineral results show
  - Low level of copper 44.640 (ref 65-150 ppm)
  - Deficient level of manganese 0.480 (ref 0.9-4.5 ppm)
  - Low level of magnesium 93.139 (ref 100-250 ppm)
  - Low level of phosphorous 1301.573 (ref 2000-4500ppm)

### *Concerns*

Didn't give calf weight

**Calf 109**

Table B-3. Calf 109 contributing factors summary at BC

~2 days

Contributing factors to death	Support for the claim
Unknown	<ul style="list-style-type: none"> <li>• Post-partum investigation was not conducted because calf was found three days after death.</li> <li>• Calf was scavenged and carcass was too autolyzed for submission</li> </ul>
Anatomical Abnormality	<ul style="list-style-type: none"> <li>• Field notes state there was a possible anatomical abnormality</li> </ul>

<b>Calf Loss Summary</b>
Unknown cause of death. Possible anatomical abnormality that contributed to mortality.
<b>Timeline</b>
<ul style="list-style-type: none"> <li>• Birthing sensor did not send expulsion messages               <ul style="list-style-type: none"> <li>○ Sensor was expelled prior to calving (no estimate on days?)</li> </ul> </li> <li>• Calves were last checked 10/16/2019</li> <li>• Calf 109 was found dead on 10/19/2017.               <ul style="list-style-type: none"> <li>○ Calf likely was between 1 to 3 days old</li> <li>○ Calf found dead under pepper bush on side of cypress head, likely calving location.</li> <li>○ Torn ear, tail, anus and mouth eaten through.</li> <li>○ Possible deformity.</li> <li>○ Scavenged by buzzards when found</li> </ul> </li> <li>• Calf was not sent to the Bronson Laboratory for necropsy</li> </ul>
<b>Necropsy and/or Laboratory Test Results</b>
None. Carcass was not sent to Bronson Laboratory for necropsy.

## Calf 122\_2

Table B-4. Calf 122\_2 contributing factors summary at BC

Stillborn	
Contributing factors to death	Support for the claim
Unknown	<ul style="list-style-type: none"><li>• Calf was born in the pens during sensor re-insertion</li><li>• Second twin of cow 122, first twin lived.</li><li>• No post-mortem investigation undertaken</li></ul>
Twining	<ul style="list-style-type: none"><li>• Calf was suspected twin</li></ul>

<b>Calf Loss Summary</b>
Unknown cause of calf mortality and calf was not sent for post-partum investigation.
<b>Timeline</b>
<ul style="list-style-type: none"><li>• Cow 122 gave birth in the pens during spider/sensor re-insertion on 10/25/2017.</li><li>• Cow 122 had a newborn calf with her, calf 122.</li><li>• Calf 122_2 was found dead in the pens.<ul style="list-style-type: none"><li>○ Believed that cow 122 gave birth to twins while in the pens</li></ul></li><li>• Calf was not sent to the lab</li></ul>
<b>Necropsy and/or Laboratory Test Results</b>
None. Carcass was not sent to Bronson Laboratory for necropsy.

## Calf 124 (twins)

Table B-5. Calf 124 (twins) contributing factors summary at BC

---

Stillborn

Contributing factors to death	Support for the claim
Dystocia- fetal distress/anoxia	<ul style="list-style-type: none"><li>• Meconium found in lungs</li><li>• Lungs never expanded and were swollen</li></ul>
Mineral deficiencies	<ul style="list-style-type: none"><li>• Critically low levels of zinc at 52.17 ug/g (min. ref .value 90 ug/g)</li><li>• Critically low levels of manganese at 2.51 ug/g (min. ref .value 5.5 ug/g)</li><li>• Critically low levels of cobalt at &lt;0.05 ug/g (min. ref, value 0.10 ug/g)</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• Low level of molybdenum at 0.75 ug/g (min. ref. value 1.8 ug/g)</li><li>• Iron levels in pooled liver samples were at high level of 1213.29 ug/g (max. ref. value 750 ug/g)</li></ul>
Twining	

---

Note: Both calves are included in this table because the necropsy and findings did not differ between individuals. If supporting claim was only found for one calf it is noted.

### **Calf Loss Summary**

Stillborn twin calves had high levels of iron, which can impact the absorption of other minerals. In addition, mineral analysis reported critical levels of Zinc, manganese, and cobalt and a low level of molybdenum, supporting poor mineral absorption perhaps from increased iron. Calves lungs contained meconium and never expanded suggesting fetal stress and possible anoxia. Other causes were not conclusive.

### **Timeline**

- Birthing sensor expelled on 12/7/2017 15:30
  - Dead twin calves were found later that evening
  - Calves did not appear to have been alive or stood.
- Calves were put on ice and sent to Bronson Laboratory.

### **Necropsy and/or Laboratory Test Results**

#### **Calf #1**

- *Aeromonas hydrophila hydrophila* found in intestine.
- Lung does not float and pulmonary parenchyma collapsed. Alveolar spaces contain meconium, fibrin, edema, and rare inflammatory cells.
- Liver is swollen and dark
- Brain and kidneys congested.
- Bloody fluid in body cavities (serous cavities).

#### **Calf #2**

- See report for calf twin #1 above.
- Mineral results show (all units in ug/g dry)
  - High level of Iron 1213.29 (ref 170-750)
  - Critical level of Zinc 52.17 (ref 90-500)
  - Low level Molybdenum 0.75 (ref 1.8-4.7)
  - Critical level of Manganese 2.51 (ref 5.5-15)
  - Critical level of Cobalt <0.05 (ref 0.10-0.40)

#### *Concerns*

Only appears to have one liver sample sent for testing. Pooled the liver samples from both twins. Problematic for interpretation of results.

Also states that the Zinc levels are optimum in the liver samples in interpretation from BADDL despite the Zinc levels being critical as indicated by the lab.

## Calf 159 (twins)

Table B-6. Calf 159 (twins) contributing factors summary at BC

---

Stillborn\*

Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"><li>• Calves pulled from cow</li><li>• Labor &gt;12 hours</li><li>• Swollen tongue (twin calf #1)</li></ul>
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• <i>Mannheimia haemolytica</i> found in stomach contents, lung, and umbilicus (twin calf #1)</li><li>• <i>E.coli</i> isolated from stomach, intestine, and umbilicus (twin calf #1)</li><li>• <i>Trueperella pyogenes</i> isolated from placenta (possibly contaminated) (twin calf #1)</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• Calves had lead present in liver sample</li><li>• High levels of Zinc at 508.77 ug/g (ref. max. value 500 ug/g)**</li></ul>
Twinning	

---

Note: Both calves are included in this table because the necropsy and findings did not differ between individuals. If supporting claim was only found for one calf it is noted. \* Both calves were pulled from cow by personnel \*\*Only the liver samples for mineral analyses differed slightly. Calf liver 1 had high Zinc and Calf 2 did not. See necropsy report for further details.

### **Calf Loss Summary**

Swollen tongue of calf #1 indicates dystocia or difficult labor, which is supported by field observation of labor that was at least 12 hours in duration before intervention. Infection in placenta likely caused by *Trueperella pyogenes*, which is known to cause endometritis and sporadic abortions at any stage in pregnancy and could have contributed to dystocia. *Mannheimia haemolytica* was isolated from calves and is associated with respiratory illnesses of cattle. Lead was also found in the liver samples.

### **Timeline**

- Cow was observed 10/29/2017 and noted as close to calving.
- Birthing sensor was expelled 10/31/2017 16:04
  - Cow was observed in labor that afternoon following birthing sensor expulsion
- Cow was checked again on 11/1/2017 9:30
  - Labor had not progressed and assistance was provided
  - Dead twin calves were pulled from the cow approximately 30 minutes later.
- Calves were put on ice and transported to Bronson Laboratory on 11/2/2017

### **Necropsy and/or Laboratory Test Results**

#### **Calf Twin #1**

- *Mannheimia haemolytica* found in stomach contents, lung, and umbilicus.
- *Trueperella pyogenes* found in the placenta.
- Lungs did not float, were dark red, and congested.
- Brain congested and blood capillaries depict perivascular edema.
- Large intestine contained large mats of bacteria in core of all blood capillaries.

#### **Calf Twin #2**

- Intestines pale in color.
- Liver is pale and very light in color.
- See report above for calf twin #1
  
- Mineral results show
  - Liver 1
    - High level of zinc 508.77 (ref 140-500 ug/g)
    - Lead level was 0.11 ug/g (no reference range given but indicated level was outside adequate range)
  - Liver 2
    - Lead level was 0.11 ug/g (no reference range given but indicated level was outside adequate range)

#### *Concerns:*

No reference given for lead levels of mineral testing by TVMDL.

Liver sample does not have twin ID associated, simply labeled liver 1 and liver 2.

Only calf #1 appears to have bacteriology/mycology cultures, histopathology, and virology conducted.

State this is both an abortion and a stillbirth which are defined as two different things in cattle literature (stillbirth=full-term, abortion= greater than or equal to 1-month prior to full-term)

*Trueperella pyogenes* indicated as the cause of abortion but the bacterium was only found in the placenta. Placental culture stated “Also present mixed pathogens, probable contamination.”

## Calf 161

Table B-7. Calf 161 contributing factors summary at BC

---

3 days old

Contributing factors to death	Support for the claim
Septicemia	<ul style="list-style-type: none"><li>• Field observations noted a weak calf unable to stand</li><li>• Calf never observed nursing</li><li>• Umbilical cord was necrotic with yellow, green pus</li><li>• <i>Mannheimia haemolytica</i> found in the abdomen, umbilicus, and lung</li><li>• <i>Klebsiella pneumonia</i> and <i>E. coli</i> isolated from abdomen</li></ul>
Viral infection	<ul style="list-style-type: none"><li>• Lungs tested positive for IBRV by DFA test.</li><li>• Lesions associated with lungs were indicative of viral-bacterial infection</li></ul>
Poor dam health	<ul style="list-style-type: none"><li>• <i>Serratia marcescens</i> isolated from umbilicus (associated with mastitis in dams)</li></ul>
Mineral imbalance	<ul style="list-style-type: none"><li>• High levels of zinc at 1089.25 ug/g (max ref. value 820 ug/g)</li><li>• High levels of cobalt at 0.36 ug/g (max ref. value at 0.25 ug/g)</li></ul>

---

### Calf Loss Summary

Field notes observed the calf was weak and unable to stand, indicative of a calf righting infection or mineral deficient. Calf was septic, likely stemming from navel infection of *Mannheimia haemolytica* acquired post-partum. Lesions found during histopathology are supportive of this finding. Pulmonary lesions were indicative of concomitant viral-bacterial infection of *Mannheimia haemolytica* and IBRV. In addition, calf tested positive for IBR by DFA test. *Serratia marcescens* was also isolated from the umbilical cord and is often associated with mastitis which could suggest calf did not receive sufficient immunity due to mastitis

### Timeline

- Birthing sensor did not send expulsion message.
- Calf was found on 11/9/2017 and believed to be born that day.
  - Unable to stand or stay upright.
  - Was not observed nursing.
- Calf died 11/12/2017.
- Calf was put on ice and sent to Bronson Laboratory the following day 11/13/2017

### Necropsy and/or Laboratory Test Results

- *Mannheimia haemolytica* found in abdomen, umbilicus, and lung.
- *Klebsiella pneumoniae* isolated in the abdomen.
- *Serratia marcescens* found in umbilicus.
- Lungs tested positive for IBRV, were non-collapsing but segments sank in formaldehyde, and had thick mats of bacteria present.
- Liver was pale red-tan, some cells contained bacteria and pus, and parenchyma (connective tissue) exhibited lytic necrosis and hemorrhage.
- Navel had small forming bacteria colonies.
- Umbilicus tissues exhibits liquefactive necrosis and mats of mixed bacteria.
- Umbilical artery was dark red-purple with large emboli in left umbilical artery.
- Spleen was pale and exhibited coagulative necrosis surrounding mats of mixed bacteria.
- Forestomachs full with runny, whitish-yellow, milky ingesta.
- Mineral results show
  - High level of zinc 1089.25 (ref 150-820 ug/g)
  - High level of Cobalt 0.36 (ref 0.04-0.25 ug/g)

Concerns: None

## Section 1B: BC Assumed Mortality Reports

### Calf 11

Table B-8. Calf 11 assumed mortality summary at BC

---

83 days old when removed

---

- Sensor did not send an alert.
  - Calf was found and tagged on 11/11/2017 and appeared to be a day old.
  - Mom died on 2/2/2018 – no reason given to cow death
  - Calf was taken to barn on 2/2/2018 to be bottle fed and was subsequently removed from study.
- 

### Calf 88

Table B-9. Calf 88 assumed mortality summary at BC

---

31 days old when removed

---

- Sensor was expelled 12/16/2017 at 7:17am
  - Calf was found and tagged on 12/17/2017.
  - Mom died on 1/16/2018– no reason given for cow death
  - Calf was taken to barn on 1/16/2018 to be bottle fed and subsequently removed from study. .
- 

### Calf 198

Table B-10. Calf 198 assumed mortality summary at BC

---

78 days old when removed

---

- Sensor was expelled 10/30/2017 at 11:51am
  - Calf was found and tagged 10/31/2017.
  - Calf was healthy and nursing the following days
  - Cow stopped producing milk (no date specified) and calf was noted as failing to thrive.
  - Calf was taken to the barn on 1/16/2018 to be bottle fed and subsequently removed from study.
-

## Calf 203

Table B-11. Calf 203 assumed mortality summary at BC

---

60 days old when removed

---

- Sensor was expelled 11/5/2017 at 12:59am
  - Calf was tagged 11/6/2017
  - Calf was noted as struggling to stand after birth.
  - After observation it appeared the rear legs/hips were fused together as calf could not walk without moving both legs.
  - Calf was nursing and began to stand.
  - Calf was able to walk around and cow was diligent to remain with calf.
  - Calf was thriving but was separated from herd, therefore was removed from study on 1/5/2018
-

## Section 2A:BR Necropsy Reports

### Calf 240

Table B-12. Calf 240 contributing factors summary at BR

Contributing factors to death	Support for the claim
Stillborn*	
Poor dam health	<ul style="list-style-type: none"> <li>• Kidneys of cow tested positive for <i>Leptospira sp.</i> (can cause bovine abortion)</li> </ul>
Septicemia/bacteremia	<ul style="list-style-type: none"> <li>• <i>Trueperella pyogenes</i> and <i>Clostridium sporogenes</i> isolated from placenta</li> <li>• <i>Pseudomonas aeruginosa</i> and <i>Streptococcus uberis</i> found in lungs</li> <li>• Kidneys of cow tested positive for <i>Leptospira sp.</i> and can cause bovine abortion</li> <li>• Areas of calf's kidneys were suppurative and necrotizing with presence of bacteria</li> <li>• Cholestasis noted of liver possibly resulting from <i>Clostridium</i> infection</li> </ul>
Fetal distress/anoxia	<ul style="list-style-type: none"> <li>• Meconium aspiration indicated in lungs</li> </ul>
Mineral imbalance	<ul style="list-style-type: none"> <li>• Low level of molybdenum at 0.45 ug/g (min. ref. value 0.80 ug/g)</li> </ul>

\*Calf died with cow

### **Calf Loss Summary**

Cow kidney tested positive for *Leptospira* sp. which is one of the infectious organisms commonly associated with bovine abortion. Calf lungs showed meconium aspiration indicative of fetal distress and anoxia. Calf lungs also had two different bacteria isolated from samples. Bile buildup was present and could have been a result of clostridial disease which was isolated from placenta. Additionally, the placenta contained *Trueperella pyogenes* which can cause sporadic bovine abortion at any stage of pregnancy.

### **Timeline**

- 3/22/2018 cow was observed away from herd, possibly close to calving
- 3/23/2018 in the morning, the cow was found on the ground with blood around her tail and swollen vulva.
  - The cow was roped and during that time the cow expelled her sensor.
  - The cow was vaginally examined and the bag, calf's head, and 2 front feet were felt.
  - It was difficult to get hand through the vagina.
- 3/24/2018 at 9:30 am the cow was found lying down and unable to get up.
  - The amniotic sac had still not popped so personnel intervened and popped amniotic sac.
  - The calf was impossible to pull out and would either have to deliver by c section or fetotomy.
  - The cow was euthanized and the calf was cut out.
  - The calf was dead but in the correct birthing position.
  - Kidneys of the cow appeared mottled and so were sent to the lab for testing.
- Calf was placed on ice and sent to Bronson Laboratory the following day.

### **Necropsy and/or Laboratory Test Results**

- Buildup and blockage bile (cholestasis) within the liver as well as periportal calcification of calf.
- Kidneys suppurative, necrotizing, and areas of bacteria colonies.
- Lungs showed congestion and meconium aspiration.
- *Trueperella pyogenes* and *Clostridium sporogenes* isolated from placenta.
- *Pseudomonas aeruginosa* and *Streptococcus uberis* isolated from placenta and lung of calf.
- Cow serum revealed high levels ALB, PHOS, MG.
- Kidney of the dam tested positive for *Leptospira* sp. (IHC)
- Mineral results show
  - Low levels of molybdenum 0.45 (ref. 0.80-2.2 ug/g)

#### *Concerns*

Gross necropsy has no findings

## Calf 549

Table B-13. Calf 549 contributing factors summary at BR

---

3 days old

Contributing factors to death	Support for the claim
Dystocia- fetal distress/hypoxia	<ul style="list-style-type: none"><li>• Meconium aspiration evident in lungs and alveoli</li><li>• Pulmonary lesions</li><li>• Weak calf syndrome- poor colostrum intake- filed observation</li></ul>
Bacteremia	<ul style="list-style-type: none"><li>• Bacteria in the lung, joint, carotid tissue, intestine.</li><li>• Lungs contained <i>Streptococcus gallolyticus</i>, <i>E. coli</i>, and <i>Haemophilus spp.</i></li></ul>
Poor dam health	<ul style="list-style-type: none"><li>• Serum from cow has <i>L. ictero</i> but could be from a vaccination</li><li>• Chronic phase of disease is associated with fetal infection in pregnant cows presenting abortion, stillbirth, or birth of premature</li></ul>
Mineral imbalances	<p>and weak infected calves.</p> <ul style="list-style-type: none"><li>• High levels of cobalt at 0.81 ug/g (max. ref. value 0.34 ug/g)</li><li>• Low levels of selenium at 1.25 ug/g (min. ref. value 1.65 ug/g)</li></ul>

---

\*Euthanized

### Calf Loss Summary

Pneumonia of the lungs was attributed to meconium aspiration during birth. Histopathology show severe lesions in the lungs and necropsy support these findings. In addition, multiple bacterial species were isolated from the calf lungs, including *E. coli*. Pneumonia caused by meconium aspiration can lead to a reduction in colostrum absorption, which is supported by field observations of a weak calf, unable to stand. In addition, calf had high levels of copper and low levels of selenium.

### Timeline

- Dam observed with swollen vulva, full udders, and distanced from herd on the afternoon 4/6/2018.
- Birthing sensor did not send a message upon expulsion.
- Cows were checked late afternoon 4/6/2018 and again the morning of 4/7/2018.
- Calf was found on 4/7/2018 at 7:30am.
  - Cow was cleaning calf and technician left the pair alone with intentions to recheck the pair in a few hours.
- 10:00am 4/7/2018, calf was approached and tagged.
  - Calf was weak, unable to stand, and dam was not in sight.
  - Calf was taken to the barn and given antibiotics in the afternoon and powdered colostrum at night.
  - Calf stayed in the barn overnight
- 4/8/2018, calf was given another bag of powdered colostrum and returned to pasture near dam that morning
  - Dam never returned to calf
  - When checked at 15:00 calf was breathing heavily, hot to the touch, and unable to hold its head up.
  - Calf was taken back to the barn and its condition never improved.
  - Calf would not nurse bottle of powdered colostrum that evening and its condition continued to deteriorate.
- Calf was euthanized on 4/9/2018 and taken to Bronson Laboratory that day.

### Necropsy and/or Laboratory Test Results

- Bloodwork prior to euthanasia show high levels of WBC, BUN, Neutrophils, lymphocytes, and ALP.
- Some alveoli in lungs had thick orange colored material consistent with traces of meconium.
- Bacterial isolates from the lung of include *Haemophilus spp.*, *E. coli*, and *Streptococcus gallolyticus*.
- Pulmonary lesions seen were moderate to severe.

- Bacteria and discoloration of subcutaneous tissue of the carotid tissue likely from IV treatment.
- Leptospira panel returned titer for *Lepto. ictero* for the cow (possible vaccination)
- Mineral results show
  - high levels of cobalt 0.81 (ref. range 0.08-0.34 ug/g)
  - low levels of selenium 1.25 (ref. range 1.65-6.60ug/g)

*Concerns:*

None

## Calf 690

Table B-14. Calf 690 contributing factors summary at BR

Stillborn	
Contributing factors to death	Support for the claim
Unknown	<ul style="list-style-type: none"><li>• Severely autolyzed</li></ul>

<b>Calf Loss Summary</b>
Unknown cause of the death because the calf was severely autolyzed when sent for necropsy.
<b>Timeline</b>
<ul style="list-style-type: none"><li>• Birthing sensor did not send message upon expulsion</li><li>• 3/8/2018<ul style="list-style-type: none"><li>○ Dam was found dead at 11am with sensor nearby</li><li>○ Vultures had pecked the eyes and anus</li><li>○ Dam was still pregnant and showed no indications of a birthing event.</li><li>○ Calf was pulled/cut out of cow</li><li>○ Calf skull was rotting and its hide was not present</li><li>○ Bottom portion of the calf was intact, just slightly swollen.</li></ul></li><li>• Calf was put on ice and taken to Bronson Laboratory that day.</li></ul>
<b>Necropsy and/or Laboratory Test Results</b>
<ul style="list-style-type: none"><li>• Calf was so severely autolyzed that only lung and ear notch samples were taken.</li><li>• Lungs and pulmonary tissues had large bacterial colonies present, possibly cause of post mortem.</li><li>• Lung air spaces were flattened and pulmonary tissues were poorly developed.</li></ul> <p><i>Concerns</i> Liver sample not collected during necropsy and no mineral testing conducted</p>

## Calf 1179

Table B-15. Calf 1179 contributing factors summary at BR

Stillborn\*

Contributing factors to death	Support for the claim
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• Lungs contained <i>E. coli</i>, <i>Streptococcus lutetiensis</i>, and <i>Enterobacter aerogenes</i>.</li><li>• Blood contained <i>Mannheimia varigena</i></li><li>• Suppurative meningitis of brain, mixed bacterial colonies throughout, and necrosis of neurons</li><li>• Bronchopneumonia</li><li>• Calf was twitching and unable to stand</li><li>• <i>L. pomona</i> titer returned value that could be indicative of infection but needs supporting evidence to confirm</li></ul>
Mineral toxicity	<ul style="list-style-type: none"><li>• Nitrite found in urine sample</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• Low levels of molybdenum at 0.86 ug/g (min. ref. value 1.80 ug/g)</li><li>• Low levels of manganese at 3.58 ug/g (min. ref. value 5.5 ug/g)</li></ul>

\*Calf born alive but died within a few hours of birth

### **Calf Loss Summary**

Gross necropsy described colostrum/milk present in calf stomach, indicating calf was up and walking after birth. Septic shock attributed to *E. coli* although other bacteria were present in blood, lung, and brain. Septic shock is also supported by field observation of a calf that was twitching and unable to stand. Meningitis of the brain and pneumonia of the lungs attributed to bacterial infection. *L. pomona* titer returned value of 800 which could be indicative of disease but requires supporting evidence to confirm. Urine contained nitrite which may indicate nitrite poisoning. Calf was alive at maximum 24 hours and because of the advanced infection of the brain, calf was likely infected in utero.

### **Timeline**

- Birthing sensor did not send a message upon expulsion.
- Dam was noted as distant from herd on 5/6/2018.
- 5/7/2018
  - Calf and cow were found at 8am.
  - Calf was in poor condition. Very weak, laying down, limbs sprawled, and eyes were swollen shut.
  - Calf was twitching and covered with flies.
  - Calf did not attempt to get up while technician was present.
- Calf was taken to Bronson Laboratory that morning.

### **Necropsy and/or Laboratory Test Results**

- Evidence of milk in calf's stomach and rumen contains thick, yellow, cheesy ingesta.
- Brain contains large areas of mixed bacteria colonies (bacilli and cocci), suppurative inflammation indicative of meningitis, and necrosis in neurons.
- Urinalysis tested positive for nitrite.
- *Escherichia coli* and *Enterobacter aerogenes* found in lung.
- *Mannheimia varigena* isolated from blood culture.
- Serum showed high levels of ALP, Ca, and GGT.
- *L. pomona* titer from serology returned value of 800, indicative of either disease or previous infection/vaccination
- Mineral results show
  - Low levels of molybdenum 0.86 (ref. range 1.80-4.70L)
  - Low levels of manganese 3.58 (ref. range 5.5-15 L)

#### *Concerns*

Brain contained large areas of mixed bacteria yet no bacteriology samples reported for the brain.

## Calf 1186

Table B-16. Calf 1186 contributing factors summary at BR

---

Stillborn\*

Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"><li>• Calf weighed 95 pounds</li><li>• Brain swollen</li><li>• Expulsion of sensor, which typically coincides with expulsion of amniotic sac, occurred ~24 hours prior to birth of calf</li><li>• Lungs indicated meconium aspiration</li><li>• Lesions in lungs and liver supportive of fetal anoxia and distress</li></ul>
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• <i>Trueperella pyogenes</i> isolated from the brain</li><li>• Mineralized concretions slightly indicative of meningitis or chronic infection</li></ul>
Poor dam health	<ul style="list-style-type: none"><li>• Chemistry panel of cow's had elevated levels of WBC, lymphocytes, and monocytes- possibly indicating cow was fighting infection</li><li>• Field observation after birth noted foul smell emanating from vaginal canal.</li><li>• Cow feces tested positive for presence of Trichostrongyle-like ova parasites</li></ul>
Mineral imbalance	<ul style="list-style-type: none"><li>• High levels of copper at 669.11 ug/g (max ref. value 600 ug/g)</li></ul>

---

\*Calf born alive and attempted to breathe but died within a few hours of birth.

### **Calf Loss Summary**

Calf weighed 95 pounds which may contribute to a difficult birthing. The lungs of the cow show meconium within the alveoli and mineralized concretions suggesting fetal distress and hypoxia. Further tests of lung samples reveal no growth of mycoplasma or ureaplasma. The brain bacteriology returned *Trueperella pyogenes* and histopathology showed mineralized concretions in the brain, suggesting meningitis of the brain from possible infection. Cow serology returned elevated levels of white blood cells, lymphocytes, and monocytes and field notes describe a foul odor emanating from vaginal canal, which may indicate the cow was fighting an infection. This could have occurred pre- or post- partum as the sample was taken after calving and further evidence needed to confirm dam health.

### **Timeline**

- Birthing sensor expelled 4/8/2018 7:56.
  - Cow was located within two hours of sensor expulsion but there was no indication of a birthing event.
  - Cows were observed again at 17:00 that evening and there was no sign of a newborn calf.
- Dead calf was found the morning of 4/9/2018 11:30.
  - Vulture activity on the carcass. Eye was completely pecked out.
  - Calf was completely cleaned on one side but a yellowish fluid remained on the other side of the carcass.
  - No cow nearby the carcass.
  - Cow 1186 was located without a calf by her side but had clear indications of calving.
  - Palpation revealed the cow was not pregnant with a calf and had a very foul odor emanating from vaginal canal.
- Calf was put on ice and taken to Bronson Laboratory on 4/9/2018.

### **Necropsy and/or Laboratory Test Results**

- *Trueperella pyogenes* and *E. coli* found in brain
- Multiple *Staphylococcus spp.* isolated from brain, skin, and milk. Species *aureus*, *schleiferi*, and *hyicus*.
- Umbilical cord area had large wound that extended to organs of peritoneal cavity, likely from scavenging.
- Histopathology revealed mineralized concretions on the brain lining and congestion of the meninges.
- Froth in tracheal lumen and bronchi indicates calf was born alive and attempted to breathe.
- Lungs have mineralized concretions
- Lungs were non-collapsed, congested, and contained meconium.
- Cow serology shows elevated WBC, Lymphocytes, and Monocytes.
- Mineral results show
  - High level of copper 669.11 (ref 165-600ug/g)

### **Concerns**

States no infectious agents identified yet *Trueperella pyogenes* was isolated from brain and is known to cause spontaneous abortion

**Calf 3759**

Table B-17. Calf 3759 contributing factors summary at BR

---

89 days	
Contributing factors to death	Support for the claim
Unknown	• Too autolyzed

---

### **Calf Loss Summary**

Too severely autolyzed to draw any conclusions. Field observation describes a calf that was unhealthy just two days after birth and remained in poor condition until death. Calf was visually observed 7/12/2018 with droopy ears and appeared unhealthy. Upon examination calf lymph nodes felt normal and energy level was normal. VHF datalogger recorded mortality signal morning of 7/14/2018 at 1:52am and was found at ~9:30am scavenged with a cow nearby, ~10m away that had been struck by lightning.

### **Timeline**

- Birthing sensor was expelled at 4:51am on 4/15/2018
- Calf was born and tagged on 4/15/2018
- 4/17/2018 the calf was noted as unhealthy.
  - Lungs sounded wet and the calf appeared weak.
  - Calf was nursing but mother did not stay near calf
  - Calf was laying down the majority of the days
- 4/27/2018 calf was noted as coughing
- 5/1/2018 calf had yellow mucus coming from the nose. Calf was noted as having energy and alert
- 5/2/2018 calf was roped, samples were taken, and medicated
  - EDTA, serum, blood cultures
  - 5 cc of nuflor
  - 5 cc of penicillin
- 5/17/2018 calf observed breathing out of mouth, head lowered.
- Calf was roped on 5/17/2018 to medicate with 5cc nuflor
  - Still had lots of energy
- From 5/17/2018 to 6/28/2018 calf's health deteriorated.
  - Coat looked dull
  - Mucus from nose turned to brownish, blood color
- 6/28/2018 cows and calves were worked up.
- Pulled samples and medicated the calf
  - Edta, serum
  - Nasal swab for the calf
  - Temperature was 103.5F
  - Calf was medicated with 5 cc of Excede
- 7/12/2018 calf was observed with droopy ears, head down, breathing out mouth. Lymph nodes did not feel swollen. Still had energy to run away from technician
- 7/14/2018 Calf died approximately at 1:52am
- The calf was found 7/14/2018 at ~9:30am.
- Calf was located 10 meters away from cow that appeared to have been struck by lightning
- Vultures had eaten the calf's organs, tongue, and one eye.
- Carcass was put on ice and transported to BADDL.

### **Necropsy and/or Laboratory Test Results**

- Lab testing prior to death:
  - Serum reveals high levels of ALP, Ca, GGT, and PHOS.

- EDTA reveals high levels of WBC, lymphocytes, and MCHC.
- Necropsy tests show all calf tissues severely autolyzed due to postmortem and as such tests are inconclusive.

*Concerns*

Do not understand the timeline of advanced autolysis and necrosis throughout the body when the calf was retrieved 12 hours after death and either kept on ice or in a storage freezer until necropsy.

## Calf 5122

Table B-18. Calf 5122 contributing factors summary at BR

---

Stillborn*	
Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"><li>• Calf was 120 pounds!</li><li>• Calf was not alive when cow/calf pair was found</li></ul>
Poor Dam Health	<ul style="list-style-type: none"><li>• Mild emphysema of the lungs</li><li>• Lungs had <i>E. coli</i> and <i>Streptococcus lutetiensis</i></li><li>• Myocarditis</li><li>• Sarcocysts found throughout the heart</li><li>• Rumen pH was 5.15 indicative of acidosis (bloat?)</li><li>• Cow was 13 years old</li></ul>
Mineral Imbalance	<ul style="list-style-type: none"><li>• Low copper at 39.85 ug/g (min. ref. value at 40 ug/g)</li></ul>

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\*Calf died with cow

### **Calf Loss Summary**

Calf weighed 120 pounds. There were no other statements suggesting calf attempted to breathe. Cow was in poor condition. Dam's lungs had mild emphysema and *E. coli* was extracted from samples. The rumen pH was 5.15 indicative of acidosis. Field observations and necropsy both state cow appeared bloated and had rumen contents coming out the cow's mouth, supporting acidosis. Dam was also aged 13. Dams older than 10 have higher risks of calf mortality. Despite many possible contributing factors to death clinical findings were inconclusive. In addition, little attention was given to calf during post-mortem investigations that could have provided a better understanding of calf mortality, such as histopathology or gross necropsy of calf lungs.

### **Timeline**

- Cow was observed 5/4/2018 away from the herd.
- Birthing sensor was expelled at 1:41am 5/7/2018.
  - Cow was found dead at ~9:30am on 5/7/2018.
  - Cow was still pregnant and had not given birth.
  - Rumen contents were coming out of cow's mouth.
  - The amniotic sac was not broken.
  - Blood was noted around sac and anus.
  - Cow appeared bloated.
- Pregnant cow was driven to Bronson Laboratory for necropsy.

### **Necropsy and/or Laboratory Test Results**

- Cow necropsy shows:
  - Calf was full-term, 120 lbs.
  - Mild emphysema of the lungs
  - Liver is mildly swollen and yellow-orange in color.
  - Myocarditis (inflamed heart) and sarcocysts scattered throughout muscular tissue of the heart.
  - Spleen shows an overload of iron (hemosiderosis) and an accumulation of histiocytes.
  - Rumen pH was 5.15
  - *E. coli* isolated from lung, intestine, and cervix.
- Mineral results for cow show:
  - Low levels of copper 39.85 (ref. range 40-650 L)

#### *Concerns:*

No mineral analysis of the calf.

Interpretive summary only states "Nonspecific pathological findings".

Very little attention given to the calf versus the cow on the report

Only did a swab of the fetus for bacteriology and not of internal organs

## Calf 5237

Table B-19. Calf 5237 contributing factors summary at BR

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Stillborn\*

Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"><li>• Calf was in posterior position</li><li>• Mild swelling of joints</li><li>• Lungs do not float (stillbirth)</li><li>• Hemorrhages on the neck area</li><li>• Large-framed calf as noted by attending veterinarian</li><li>• Sensor expulsion occurred ~12-24 hours prior to intervention, suggestion prolonged labor</li></ul>
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• Lungs, tissue fluid, intestine, placenta, and milk from cow all contained <i>E. coli Beta hemolytic</i></li><li>• Placentitis noted in the histopathology with colonies of bacteria throughout</li><li>• Milk and placenta also contained other bacteria</li></ul>
Poor dam health	<ul style="list-style-type: none"><li>• Body condition score of cow was 3-3.5</li><li>• Lepto panel from serum of cow had result of 1600 for <i>L. ictero</i> suggestive of recent infection</li></ul>
Mineral imbalance	<ul style="list-style-type: none"><li>• Low level of molybdenum at 0.64 ug/g (min. ref. value 0.80 ug/g)</li></ul>

---

\*Calf pulled from cow by ranch personnel

### **Calf Loss Summary**

Calf was in the posterior position in the birthing canal and likely contributed to a long labor and difficult birthing as was evident by field observations. Veterinarian on site stated calf was large-framed suggesting its size could have also contributed to dystocia. Swollen joints discovered in necropsy support difficult birthing. Placentitis and infection of the placenta from *E. coli* Beta hemolytic. Infection of *E. coli* beta hemolytic had also invaded the calf and may have amplified dystocia. In addition, the *Lepto* mat from the cow's serum revealed she may have just recently suffered infection from *L. ictero*.

### **Timeline**

- Birthing sensor was believed to be expelled at 10:49pm 4/9/2018
  - Sensor did not send proper message.
- Cow was found the following morning of 4/10/2018 with red bag and feet hanging out.
  - Calf was pulled by veterinarian around 1pm after an hour of intervention
  - The bull calf was coming out backwards (posterior presentation).
  - It was a medium to large framed calf.
- The veterinarian believed the calf was dead for at least 36 hours.
- Cow was a BCS of 3-3.5
- Calf was put on ice and taken to BADDL.

### **Necropsy and/or Laboratory Test Results**

- Mild autolysis seen on the liver, kidneys, and heart.
- Lungs do not float.
- Joints are mildly swollen.
- Placenta is inflamed (placentitis) and colonies of bacteria throughout.
- *E. coli Beta hemolytic* isolated from calf intestine, lung, and tissue fluid, as well as dam's milk and placenta.
- Cow serum and EDTA indicate high levels of MCH and CK.
- Cow *Lepto* mat serology result was 1600 for *L. ictero* indicative of a recent infection.
- Mineral results show
  - Low levels of molybdenum 0.64 (ref. range 0.80-2.20 ug/g)

#### *Concerns*

Calf weight not given in necropsy.

**Calf 5239**

Table B-20. Calf 5239 contributing factors summary at BR

Stillborn*	
Contributing factors to death	Support for the claim
Parasitic infection	<ul style="list-style-type: none"> <li>• Cow EDTA blood tube had count of 23.7 Anaplasma which indicates abundant Anaplasma in the sample.</li> <li>• Cow serum tested positive for Anaplasmosis in cELISA</li> <li>• Round worms found in intestines of cow</li> <li>• Cow tested positive for Johne's</li> <li>• Cow had lesions in lower intestine and colon that were textbook for mycobacterium paratuberculosis (causative agent of Johne's)</li> <li>• Fecal sample had over 50 trichostrongyle-like ova present</li> </ul>
Poor dam health	<ul style="list-style-type: none"> <li>• Dam tested positive for Bovine Leukemia Virus</li> <li>• Pneumonia in lungs</li> <li>• Positive for Anaplasma</li> <li>• Positive for Johne's by ELISA</li> </ul>
Viral Infection	<ul style="list-style-type: none"> <li>• Cow tested positive for Bovine Leukemia Virus by AGID</li> </ul>
Septicemia/bacteremia	<ul style="list-style-type: none"> <li>• <i>Trueperella pyogenes</i> isolated from swab</li> <li>• <i>Streptococcus uberis</i> isolated from swab</li> </ul>
Mineral toxicity	<ul style="list-style-type: none"> <li>• Critical levels of zinc at 1221.47 ug/g (max. ref. value 820 ug/g)</li> </ul>
Mineral imbalance	<ul style="list-style-type: none"> <li>• Low levels of molybdenum at 0.41 ug/g (min. ref. value 0.80 ug/g)</li> </ul>

\*Calf died with cow

### **Calf Loss Summary**

According to field observations cow was bloated and technician attempted to decompress but was unsuccessful. Dam was in very poor health and was suffering from parasitic, viral infections, and possibly bacterial infections. Bacterial infection was not completely elucidated despite isolation for *Trueperella pyogenes* and *E. coli* because swab was from unknown origin. Tested positive for Anaplasma, Johne's, and Bovine Leukemia Virus. Lesions seen throughout colon and lower intestine were "textbook" indication of *mycobacterium paratuberculosis*, the causative agent of Johne's. Initially tested positive for Infection Bovine Rhinotracheitis by DFA but further tests negated this finding as a false positive. Calf had critically high levels of zinc.

### **Timeline**

- Birthing sensor did not send message upon expulsion.
- Cow was found 3/29/2018 around 8am standing in the middle of the wetland.
  - She appeared close to calving but had not calved so was given space and left alone.
- Around noon on 3/29/2018 the cow was rechecked.
  - There was buzzard activity so the cow was approached.
  - The cow was found close to death. She was bloated and having a hard time breathing. Her head was pointing backwards.
  - Technician tried to decompress her on left paralumbar fossa.
  - Cow died within five minutes of being approached.
  - Samples were taken from the cow excluding a milk sample.
  - Vultures had picked around her anal area.
  - Vulva was swollen but she was not palpated to check for calf.
- The cow/calf was taken for necropsy that day at Bronson Laboratory.

### **Necropsy and/or Laboratory Test Results**

- Necropsy for cow show
  - Heart contains scattered sarcocysts.
  - Pneumonia present in the lungs
  - Small and large intestines reveal nematodes, mucosa hemorrhages, and the appearance of granulomatous enterocolitis resembling lesions associated with *mycobacterium paratuberculosis*, the causative agent of Johne's Disease.
  - Blood serum tested positive for Johne's, Bovine Leukemia Virus, and Anaplasmosis.
  - Tested positive for Infection Bovine Rhinotracheitis.
    - Subsequent testing of the lung returned negative results for Infectious Bovine Rhinotracheitis.
  - EDTA blood tested positive for Anaplasma.
  - Fecal tested positive for 50+ trichostrongyle-like ova.
  - *Trueperella pyogenes* and *E. coli* isolated from a swab and the intestine, respectively.
  - Serum shows high levels of AST and CK.
- Mineral results for the cow show
  - No abnormal results
- Mineral results for the fetus show

- Critical levels of zinc 1221.47 (ref 150-820 ug/g)
- Low levels of molybdenum 0.41 (ref 0.80-2.20 ug/g)

*Concerns*

Swab was used in bacteriology but does not state what was swabbed (not a sample given by study).

Unsure if veterinarian examined calf during necropsy as there is no mention of the calf in the necropsy section other than its presence in the cow uterus.

Not sure which animal intestine was sent to bacteriology as the specimen says “Cow and Calf Intestine”

## Calf 6179

Table B-21. Calf 6179 contributing factors summary at BR

---

Stillborn	
Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"><li>• Meconium aspiration</li><li>• 100-pound calf</li><li>• Lungs collapsed</li><li>• Field observation suggests she was in labor two days prior to cow euthanasia</li></ul>
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• Posterior position</li><li>• Field notes indicate cow had infection of uterus</li><li>• <i>Trueperella pyogenes</i> isolated from Milk, placenta and brain of cow</li><li>• 3 other bacteria species isolated from rumen, lung, and milk of cow.</li><li>• Calf heart, kidney, brain, and liver all invaded with mixed bacteria</li><li>• Placenta had abscesses present and attributed to <i>Trueperella pyogenes</i></li><li>• <i>Campylobacter spurtorum</i> isolated from placenta which has been occasionally associated with abortion.</li><li>• Placentitis noted during histopathology and supported by lesions in placenta and necrotic placentomes.</li></ul>

---

### **Calf Loss Summary**

The lesions affecting the placenta, the cervix and uterus were judged to be severe, mainly generalized and directly attributed to bacteria; *Truperella pyogenes* appears to be the culprit among all bacteria isolated. Calf was 100 pounds and in the posterior position which would contribute to dystocia. Field observations of the cow contracting two days prior to euthanasia support dystocia. Calf displayed autolysis suggesting calf had died in the cow at least 24 hours prior to cow euthanasia.

### **Timeline**

- 4/20/2018 cow was noted as being close to labor, large vulva and bag full.
- 4/25/2018 cow was again noted as being close to calving. Isolated from the herd
  - Observed contracting around ~2:30 pm
- Birthing sensor was expelled at 1:15am 4/26/2018.
  - When the cow was checked that morning there were no visible signs of the amniotic sac.
  - Cow was roped in the afternoon.
  - The cow was strong and had lots of energy.
  - The cow was palpated in the field. Observed yellow/white curds in vulva opening. Amniotic sac was not broken. Calf did not feel alive
  - Farm personnel broke the amniotic sac and clear, red fluid was expelled.
  - Chain was placed around leg of calf to pull it out but the hooves separated from the legs.
  - The cow was moved to the chutes.
  - Calf was not in the correct presentation. The right leg was pushing out first followed by the head. The left leg was behind the head about four inches.
  - The crew could not pull the calf out because of swelling and position of the calf.
- 4/27/2018 cow was given epidural and epinephrine
  - A veterinarian corrected the position of the calf and attempted to pull the calf from the cow but was unable. Cow was either too swollen or not fully dilated.
  - Veterinarian attempted a fetotomy but still was unable to pull the calf out.
  - Cow had infection in uterus so did not qualify for c-section
- Cow was euthanized 4/27/2018.
- Calf and cow were taken to the Bronson Lab for necropsy.

### **Necropsy and/or Laboratory Test Results**

- Calf necropsy shows
  - Fetus was full term and weighed 100 lbs.
  - Lungs collapsed and alveoli had moderate amounts of meconium.
  - Placentomes were upset, greenish-yellow in color, underlying tissue exhibited purple-red discoloration, and had a rough surface.
  - Postmortem autolysis.
  - Heart, kidneys, brain, and liver, all invaded by mixed bacteria.
- Cow necropsy shows
  - Placenta inflamed, suppurative, and necrotizing, with abscess formation and intralesional mats of small colony forming bacteria.

- Cervix and uterus are inflamed, suppurative, and necrotizing, with thick mats of small colony forming bacteria.
- *Trueperella pyogenes* was isolated from cow's milk, placenta, and brain.
- *Campylobacter sputorum* found in the placental tissue.

*Concerns*

No mineral analyses done.

Does not appear calf specimens were submitted to Bacteriology/Mycology for testing

## Calf 6279

Table B-22. Calf 6279 contributing factors summary at BR

---

3 days

Contributing factors to death	Support for the claim
Twinning	<ul style="list-style-type: none"><li>• Male and female born with male calf much smaller and weaker at time of birth.</li><li>• Male calf died three days after birth and female calf was much stronger at that time.</li></ul>
Poor dam health	<ul style="list-style-type: none"><li>• Dam was unable to stand following birth</li><li>• Dam died a few days following birth</li></ul>
Unknown	<ul style="list-style-type: none"><li>• No post-mortem investigation was undertaken so unknown causes could have contributed to calf mortality</li></ul>

---

### **Calf Loss Summary**

Calf was the smaller twin of the pair. Cow appeared to have physical injury following birth that eventually led to cow death. Despite being cared for by farm personnel, calf died two days after dam.

### **Timeline**

- Birthing sensor was expelled on 12/18/2017.
  - Cow did not appear to have calved. Cow did not look close to calving
  - Suspected early loss of sensor (failure to retain sensor)
- Cow checked on multiple days after 12/18/2017 but no calf found.
- Twin calves were initially found 12/24/2017.
  - Appeared healthy and around a day old.
- Tagged three days later on 12/27/2017.
  - Female calf was much larger in the size than male calf but both appeared healthy.
  - After calves were found and during tagging the cow was limping around and walking abnormally.
  - Her hind legs stretched behind her when trying to defend her calves
  - She seemed overly upset and agitated when we roped her calves to tag.
  - A few hours later she was found ~20-30 meters from the spot the calves were tagged.
- The following days she did not stand up or move around at all.
- She continued to nurse her calves but her health continued to deteriorate
- She never stood back up and died 5 days after the calves were initially tagged on 1/2/2018.
- Subsequently, the calves were taken to the barn to be bottle-fed.
- 1/4/2018 male calf died.
- The female calf is still alive and healthy.
- Neither the cow nor calf were not taken to the lab.

### **Necropsy and/or Laboratory Test Results**

Calf was not delivered to BADDL for post-mortem investigation.

**Calf 7909**

Table B-23. Calf 7909 contributing factors summary at BR

Stillborn*	
Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"> <li>• Meconium aspiration</li> <li>• Calf weighed 90 lbs.</li> </ul>
Septicemia/bacteremia	<ul style="list-style-type: none"> <li>• Calf brain tissue had growth of <i>Pseudomonas</i> and <i>Acinetobacter</i> species.</li> <li>• Lesions found in pattern of vaginal sensor</li> <li>• <i>Streptococcus alpha haemolytic (pluranimalium)</i> isolated from cervix plug, cervical mucous, and vaginal swab</li> <li>• Other bacteria isolated from mucous cervix plug, cervical mucosa and vagina were <i>E. coli</i>, <i>Pantoea agglomerans</i>, <i>Aerococcus viridans</i></li> <li>• Cow lung samples revealed <i>Morganella morganii</i> and <i>Brevundimonas diminuta</i></li> </ul>
Mineral imbalances	<ul style="list-style-type: none"> <li>• Low levels of copper 160.9 ug/g (min. ref. value 165 ug/g)</li> <li>• Low levels of manganese 3.34 ug/g (min. ref. value 4 ug/g)</li> <li>• Low levels of molybdenum 0.26 ug/g (min. ref. value 0.56 ug/g)</li> <li>• Low levels of selenium 1.04 ug/g (min. ref. value 1.65 ug/g)</li> <li>• Lead present in the mineral analysis of the calf at 0.10 ug/g</li> </ul>
Poor dam health	<ul style="list-style-type: none"> <li>• Rumens pH was 5.0 which indicated acidosis</li> <li>• 11-year-old dam</li> </ul>

\*Calf died with cow

### Calf Loss Summary

Field reports suggest cow was close to calving for approximately 8 days prior to death. This may suggest difficult labor but because we are unsure when the sensor was expelled other supporting evidence necessary to elucidate prolonged calving. Calf weight 90 pounds and could have contributed to difficult calving. Findings of fetal meconium aspiration supports fetal distress and difficult labor. Multiple bacteria were isolated from cervix plug, cervical mucous, and vagina. Lesions were noted in vagina in circular pattern that resembled the sensor attachments. Rumen pH was 5.0 and could indicate bloat of the cow. Calf had many mineral deficiencies and lead was present in liver samples. Lesions of the calf liver support ischemia, but not causative agent is given.

### Timeline

- First noted 4/25/2018 as being close to calving. Her bag was full and vulva was enlarged.
- Cow was found dead ~8am 5/3/2018.
  - The sensor was not expelled and did not send any messages. It was hanging half way out of cow when found.
  - The cow appeared to have bled from her anus.
  - There was no evidence of vulture or scavenging activity on the carcass.
- Cows were last checked ~12pm 5/2/2018.
- Cow was driven to BADDL for necropsy of cow and calf.

### Necropsy and/or Laboratory Test Results

- Calf necropsy show
  - Fetus was full term and weighed 90 lbs.
  - Lungs were collapsed and meconium broncho-aspiration.
  - Liver contains swollen cells and exhibit lesions consistent with ischemia.
  - Moderate to advanced postmortem changes in kidneys, liver, pancreas, adrenal glands, and brain.
- Cow necropsy show
  - Heart infiltrated by protozoa consistent with *Sarcocystis* sp.
  - Cervix and colon have areas of hemorrhage.
  - Lesions found in pattern of vaginal sensor.
  - No obvious lesions of the uterus or ovaries noted.
  - Bruising on the right flank, dorsum and right rib cage.
  - Ruminal pH was 5 indicating acidosis.
  - *Streptococcus alpha haemolytic (plurimalium)* isolated from the mucous cervix plug, cervical mucous, and vagina.
  - *Brevundimonas diminuta* isolated from lung and *Aerococcus viridans* isolated from vagina.
  - *E. coli* present in mucous cervix plug and cervical mucous.
- Mineral results for cow show
  - Low levels of iron 147.41 (ref. 180-1200 ug/g)
  - Low levels of manganese 9.46 (ref. 10-24 ug/g)
- Mineral results for fetus show
  - Low levels of copper 160.90 (ref. 165-600 ug/g)

- Low levels of manganese 3.34 (ref. 4-14 ug/g)
- Low levels of molybdenum 0.26 (ref. 0.56-5.6 ug/g)
- Low levels of selenium 1.04 (ref. 1.65-6.6 ug/g)
- Lead present 0.10 ug/g

*Concerns*

State lesions of the fetal liver are consistent with ischemia but doesn't explain what the means or why it would/could appear in necropsy

## Calf 11449

Table B-24. Calf 11449 contributing factors summary at BR

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49 days\*

Contributing factors to death	Support for the claim
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• Beta-hemolytic <i>Bacillus spp</i> isolated from blood culture</li><li>• Lesions found in the intestine and liver consistent with bacterial infection</li><li>• Suppurative enteritis revealed in histopathology</li><li>• Three different bacteria isolated from the brain</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• Low levels of selenium 1.17 ug/g (min. ref. value 1.65 ug/g)</li><li>• Low levels of copper 159.02 ug/g (min. ref. value 165 ug/g)</li><li>• Iron was high 2800.69 ug/g (max. ref. value 1200ug/g)</li><li>• Zinc levels were high at 886.70 ug/g (max. ref. value 500 ug/g)</li></ul>

---

\*Euthanized

### **Calf Loss Summary**

Calf was initially noted as low birthweight. At a month of age, field observation suggested a weak, calf barely able to stand due to stiff and swollen joints that may have been mineral deficient or fighting infection. Initial samples from the calf revealed elevated ALP, Ca, CK, and WBC, lymphocytes, and monocytes, *E. coli* in feces, and Beta-hemolytic *Bacillus spp.* in anaerobic blood culture. Despite treatment, calf did not improve in the following month and was subsequently euthanized. Post-mortem showed calf was underweight and had lesions on liver consistent with infection from bacteria that likely ascended from intestine. Although no bacteria were isolated from intestine, multiple bacteria were isolated from brain and lung tissue. Tests for *C. botulinum* were negative following further investigation. Iron levels of the calf were very high, which can impact the absorption of other minerals. Stiff and swollen joints as observed in the field

### **Timeline**

- Birthing sensor did not send message upon expulsion.
- Calf believed to be born 2/19/2018.
  - Smaller and had a low birth weight.
  - Calf was initially tagged with an incorrect ID and was later retagged (handled again) with the correct ID.
- 3/13/2018 calf was noticed away from the herd. When approached she was slow to get up and was limping.
  - Her hind right leg appeared swollen but had no obvious wounds or lesions.
  - Obtained blood samples from calf prior to medicating but did not obtain blood for blood culture.
  - Calf was given 6cc penicillin, 5 cc Vitamin B complex, and 8 cc Probios
- The following day 3/14/2018 (~16hours after medicating), blood was obtained for a blood culture sample.
  - The calf was still nursing and hydrated but would not move very far from her location.
  - Both hind legs appeared stiff and swollen. She was walking very slowly.
- Over the course of the next days, she remained in the same spot with her head up and still nursing but not moving.
- Herd was moved to northern pasture.
- The calf did not move to pasture and was moved by technicians.
- In the new pasture she was again still nursing, kept her head up, but all four limbs appeared to be bothering her.
- All four limbs were swollen and stiff and she was slow to move or get up.
- 4/9/2018 the calf had not improved and refused to move from her location.
  - Calf was subsequently euthanized.
- Calf was put on ice and driven to BADDL.

### **Necropsy and/or Laboratory Test Results**

- Lab testings prior to death:
  - Blood culture isolated Beta-hemolytic *Bacillus species*
  - *E. coli* isolated in the fecal testing.
- Liver was pale in color and inflamed with lesions.

- Small intestine was inflamed, suppurative, and somewhat hemorrhagic.
- Hemolysis in the intestine resembled *C. botulinum* but tested negative for botulism types A, B, and C in subsequent testing.
- *Pseudomonas species*, *Stenotrophomonas species*, and *Staphylococcus aureus* isolated from the brain.
- EDTA blood tests show high levels of MCH, MCHC, monocytes, and platelets.
- Serology panel shows high levels of ALP, Ca, and low levels of ALB, AST, GGT, TP, and GLOB.
- Mineral results show
  - Low levels of copper 159.02 (ref 165-600 ug/g)
  - High levels of iron 2800.68 (ref 400-1200ug/g)
  - Low levels of selenium 1.17 (ref 1.65-6.60 ug/g)
  - High levels of zinc 886.70 (ref 140-500 ug/g)

*Concerns*

Attribute lesions in liver and infection in intestine to bacteria but no bacteria from these areas was isolated.

## Calf 21041

Table B-25. Calf 21041 contributing factors summary at BR

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Stillborn	
Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"><li>• 99.8-pound calf</li><li>• Swollen head, neck, and tongue</li><li>• Meconium aspiration</li><li>• Lungs did not float (stillbirth)</li><li>• Location of birth appeared very disturbed and possibly indicated difficult, prolonged labor.</li></ul>
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• Lung samples contained <i>Enterobacter asburiae</i>, <i>Pantoea agglomerans</i>, and <i>Pseudomonas spp.</i></li><li>• <i>Streptococcus pluranimalium</i>, <i>Enterobacter asburiae</i>, and <i>Pantoea agglomerans</i> isolated from stomach contents</li></ul>
Mineral deficiency	<ul style="list-style-type: none"><li>• Critically low levels of molybdenum 0.47 ug/g (min. ref. value 1.8 ug/g)</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• High levels of iron 823.72 ug/g (max. ref. value 750 ug/g)</li><li>• Low levels of manganese 4.11 ug/g (min. ref. value 5.5 ug/g)</li></ul>

---

### Calf Loss Summary

Calf was 99.8lbs and had swelling of the neck, head, and tongue which all support dystocia and fetal distress/hypoxia. Meconium was found in the alveoli of the lungs. Field notes state sensor was expelled 5:53am and did not give birth until approximately 11:30am; thus, the dam could have possibly been in labor for ~5 hours supporting dystocia clinical findings. Bacteria was found in both the lungs and stomach contents of the calf but histopathology did not mention bacterial infections. Bacteria may have exacerbated dystocia but did not appear to cause difficult labor. Liver samples revealed high iron which can interfere with absorption of other minerals. In support, calf had critically low levels of molybdenum and low levels of manganese.

### Timeline

- Cow was observed and noted secreting white discharge on 4/27/2018.
- Birthing sensor expelled at 5:53am on 5/3/2018.
  - Cow was seen at 11:30 am in the middle of the wetland with a calf on the ground (unsure if the calf was alive or not)
  - Cow appeared to have just given birth so the cow was given space.
  - Calf was found again in the middle of the wetland on 5/3/2018 at ~6pm.
  - Cow was by the calf's side but ran away when approached.
  - Calf was dead with placental remains still on the back of the calf's head and neck.
  - The calf was in an awkward pose.
  - Calf was very large (estimates around 90-110 lbs.).
  - The area where the cow had calved was very trampled down. It appeared to be a large area and may point to cow struggling to give birth
- Calf was put on ice and taken to Bronson Laboratory the following day, 5/4/2018.

### Necropsy and/or Laboratory Test Results

- Calf weighed 99.8lbs.
- Lungs did not float and contained small amounts of meconium.
- Calf was stained, indicative of meconium.
- Intestines, liver, thymus, renal interstitium, and lymph nodes all hemorrhagic.
- Head, neck, and tongue moderately edematous.
- *Streptococcus pluranimalium*, *Enterobacter asburiae*, and *Pantoea agglomerans* isolated from stomach contents
- Lung contained *Pseudomonas spp.*, *Enterobacter asburiae*, and *Pantoea agglomerans*.
- No normal intestinal flora present in the bacteria culture.
- Mineral results show
  - High levels of iron 823.72 (ref 170-750 ug/g)
  - Critically low level of molybdenum 0.47 (ref 1.8-4.7 ug/g)
  - Low levels of manganese 4.11 (ref 5.5-15 ug/g)

### Concerns

States no normal intestinal flora present but does not state what that could possibly suggest, if anything.

**Calf 091693**

Table B-26. Calf 091693 contributing factors summary at BR

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Stillborn\*

Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"> <li>• 100-pound calf</li> </ul>
Poor dam health	<ul style="list-style-type: none"> <li>• Meconium aspiration</li> <li>• Muscle tears in internal aspect of rectus abdominis, oblique abdominal muscles, and latum uteri ligament.</li> <li>• Pneumonia noted in the lungs</li> <li>• Observed cow laying and barely able to stand on multiple says prior to death</li> </ul>
Septicemia/bacteremia	<ul style="list-style-type: none"> <li>• Peritonitis</li> <li>• Rumen pH 5.6 indicating acidosis</li> <li>• <i>Trueperella isolated</i> from abdomen, cervix, placenta of the cow</li> <li>• <i>Mannheimia species</i> isolated from placenta.</li> <li>• <i>E.coli</i> isolated from cervix, placenta, and udder</li> <li>• <i>Acinetobacter lwoffii</i> isolated from abdomen</li> <li>• <i>Clostridium perfringens</i> isolated from the intestine</li> </ul>
Mineral imbalances	<ul style="list-style-type: none"> <li>• Zinc was high. 941.06 ug/g (ref. max. value 500 ug/g)</li> <li>• Copper was high at 719.95 ug/g (ref. max. value 600 ug/g)</li> <li>• Lead was detected at 0.11 ug/g in liver sample</li> </ul>

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\*Calf died with cow

### **Calf Loss Summary**

Dam had tears in abdominal muscles and uteri ligaments which could have affected her ability to deliver. In addition, the calf was very large at 100 pounds and meconium aspiration indicates fetal distress/anoxia. The dam had low rumen pH at 5.6 suggesting acidosis, although it is possible this was caused by cow lying down on her side for an extended period of time. Bacteria was present in abdomen, cervix, placenta, udder, and intestine of cow. *Trueperella pyogenes* was isolated from placenta, abdomen, and cervix of cow and is known to cause abortion of calf at any stage of pregnancy. In addition, *Mannheimia spp.* was isolated from placenta which is known to cause pneumonia in cattle. The zinc levels of the calf were very high and there was also lead present in liver samples.

### **Timeline**

- Cow was first noted on 4/20/2018 as being close to calving with a full bag.
- 4/22/2018 cow was found in a ditch laying down and appeared bloated.
  - She had very thick white, yellow discharge
  - Abdomen was extremely hard when touched
  - The cow was pulled out of the ditch and got up within the next five minutes.
- 4/23/2018 at 8am cow was observed having difficulty standing but was able to get up.
  - In the afternoon the cow was found lying down and unable to get up.
- 4/24/2018 prior to 10am the cow was lying completely broad side but still alive.
  - Cow was to be euthanized but died prior to euthanasia.
  - Vultures had scavenged eyes, vulva, and teats.
  - Birthing sensor was “expelled” at 10:48am on 4/24/2018.
    - Cow died with sensor just hanging halfway out.
  - Blood and serum samples were obtained.
  - Cow was leaking fluid from teats after death.
  - Rest of the cows nearby were observed acting upset, flipping lips up. The bulls were digging at ground and bulls were bellowing.
- Cow was driven to Bronson Lab for cow and calf necropsy.

### **Necropsy and/or Laboratory Test Results**

- Calf necropsy shows
  - Full term and weighed 100 lbs.
  - Lungs full of thick, bright orange material indicative of meconium aspiration
  - Liver was pale, brownish-red in color, and fatty.
  - Kidneys were hemorrhagic.
- Cow necropsy shows
  - Lungs were marbled in appearance and depicted mild emphysema, likely airborne bronchopneumonia.
  - Muscle tears in the internal aspect of rectus abdominis (lower abdominal muscle), oblique abdominal muscles, and latum uteri ligament.
  - Rumen pH 5.6 indicating acidosis.
  - Heart muscles randomly infiltrated by *sarcocystis sp.* protozoa but no inflammatory response noted.
  - *Trueperella pyogenes* isolated from the abdomen, cervix, and placenta.
  - *Mannheimia spp.* isolated from the placenta.

- *E. coli* found in cervix and placenta, and *E. coli Beta hemolytic* found in the udder.
- *Eimeri spp.* isolated from fecal test.
- Mineral results for cow show
  - High levels of cobalt 0.42 (ref 0.08-0.34 ug/g)
- Mineral results for calf show
  - High levels of copper 719.95 (ref 165-600 ug/g)
  - High levels of zinc 941.06 (ref 140-500 ug/g)
  - Lead detected 0.11 ug/g

Concerns

None

### Section 2A:BC Assumed Mortality Reports

#### Calf 6279\_2

Table B-27. Calf 6279\_2 assumed mortality summary at BR

9 days old when removed

- Sensor was expelled on 12/18/2017.
- Cow did not appear to have calved and did not show any indication that she was in labor.
- Suspected early loss of sensor (failure to retain sensor)
- Cow checked on multiple days after 12/18/2017 but no calves found.
- Twin calves were initially found 12/24/2017.
- Appeared healthy and around a day old.
- Tagged three days later on 12/27/2017.
- Female calf was much larger in the size than male calf but both appeared healthy.
- After calves were found and during tagging the cow was limping around and walking abnormally.
- Cow seemed overly upset and agitated when roped in order to tag calves.
  - Dam's hind legs stretched behind her in unnatural position when trying to defend her calves
- A few hours later she was found ~20-30 meters from the spot the calves were tagged, unable to get up.
- The following days she did not stand up or move around at all.
- Dam continued to nurse her calves but health deteriorated.
- She never stood back up and died 5 days after the calves were initially tagged on 1/2/2018.
- Subsequently, the calves were taken to the barn to be bottle-fed.
- Two days after being taken the barn the male calf died as was considered a mortality event.
- The female calf is still alive and healthy.
- The cow and/or calf were not taken to the lab.

## **Calf 6828**

Table B-28. Calf 6828 assumed mortality summary at BR

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9 days old when removed

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- Sensor was expelled on 4/28/2018 at 7:07am
  - At approximately 8:30 am cow was found with amniotic sac visible.
  - Cow was contracting and lying down.
  - Cow did not seem to be progressing.
  - Ranch manager checked the cow at 11:30am.
  - Cow was brought to pens to assist in birthing.
  - Calf was easily pulled from cow alive at ~1pm
  - Cow and calf pair are healthy and alive.
- 

## **Calf 7105**

Table B-29. Calf 7105 assumed mortality summary at BR

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7 days old when removed from study

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- Calf was born 4/9/2018
  - Calf moved into the incorrect pasture on its own accord without the herd on 4/14/2018
  - Calf remained in the wrong pasture without mother until 4/16/2018
  - Calf was moved back with herd and mother on 4/16/2018.
  - Cow did not appear to be searching to calf during this time period.
  - Believe the calf had not received milk from the cow while in the wrong pasture and would not have returned to pasture without help of technician.
- 

## **Calf 09940**

Table B-30. Calf 09940 assumed mortality summary at BR

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2 days old when removed from study

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- Sensor did not send any expulsion messages
  - Calf was found on 4/21/2018 and believed to be born that day as well.
  - Calf was standing but seemed to have trouble nursing.
  - Cow's teats appeared full and large, giving the impression the calf had not suckled.
  - Calf was checked throughout the weekend but did not move much from original position.
  - 4/23/2018 the cow and calf pair were taken to the pens.
  - Calf appeared strong and had energy when caught.
  - Calf appeared to have trouble latching on to teats while in the pens but was eventually able to nurse.
-

### **Calf 032910**

Table B-31. Calf 032910 assumed mortality summary at BR

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90 days old when removed from study

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- Calf was born 1/20/2018
  - Was tagged 1/23/2018
  - Calf was not noted with any medical issues until 4/11/2018
  - Calf was noted as appearing sickly on 4/22/2018
  - Calf was given 10cc of Ia200 on 4/22/2018
- 

### **Calf 091577**

Table B-32. Calf 09940 assumed mortality summary at BR

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0 days old when removed from study

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- Calf was born 4/3/2018
  - Calf was obviously alive and moving chest up and down.
  - Cow and calf pair were left alone to bond.
  - An hour later the cow and calf pair were checked.
  - Cow and calf were in the exact same position and the calf had not lifted its head or moved. The chest could still be seen falling and rising.
  - When calf was approached its head was turned underneath its left shoulder and its nose was between its two hind legs. Very awkward position
  - The calf kept trying to free its head by kicking its hind legs but appeared stuck.
  - Over the next 15 minutes the calf's breathing became more labored and it started struggling for air (snorting, etc.)
  - The calf was then turned over by flipping to its right.
  - Within 10 minutes the calf was up and walking with cow
-

### Section 3: LR Necropsy Reports

#### Calf 149\_2

Table B-33. Calf 149\_2 contributing factors summary at LR

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3 days

Contributing factors to death	Support for the claim
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• <i>Klebsiella variicola</i> isolated from blood culture and lungs</li><li>• Possible enteritis noted during gross necropsy</li><li>• <i>Bacteriodes fragilis</i> isolated from intestine</li><li>• Weak and unable to stand for long periods</li><li>• White, cataract eyes and low birthweight</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• Low selenium levels found in the liver sample at 1.28 ug/g (min. ref. value 1.65 ug/g)</li><li>• Lead present in liver sample at 11 ug/g</li></ul>
Mineral toxicity	<ul style="list-style-type: none"><li>• Toxic levels of copper found in the liver sample at 1148.04 ug/g (max. ref. value 600ug/g)</li></ul>

---

### **Calf Loss Summary**

Multiple day observation of very small calf that was lethargic and did not nurse, suggested possible infection or deficiencies. Blood culture samples confirmed *Klebsiella variicola*, and infection suspected to have been in utero during development, causing small weight, pale eyes and systemic infection through-out calf organs and supported by necropsy. In addition, selenium deficiency may also be involved. Toxic levels of copper reported only occurred in this calf out of 21 calves sampled. Check management practices of ranch for cow supplementation

### **Timeline**

- Birthing sensor expelled at 10/31/2017 13:43
  - Calf was found with white cataract looking eyes, lethargic and sickly
  - Calf had low birthweight
  - Appeared weak, inability to stand for long periods and follow cow
- Calf's health deteriorated over the next three days.
- Calf not suckling, still unable to follow cow, eventually left for long periods unattended
- Calf collected and euthanized 11/2/2017
- The calf was placed on ice and sent to BADDL.

### **Necropsy and/or Laboratory Test Results**

- Lung lobes and intestines demarcated reddened inflamed areas. Intestine enteritis
- No evidence of milk consumption
- *K. variicola* from lungs, and whole blood EDTA, as well as a confirmation from blood culture sent to Texas A&M.
- Urine low specific gravity, probably associated with dehydration
- Low ALB, TP, GLOB in serum suggest no nursing also, and probably no absorption of colostrum. Supported by field observations
- Mineral results show
  - Toxic level of copper 1148.04 (ref range 165-600 ug/g)
  - Low levels of selenium 1.28 (ref range 1.65-6.6 ug/g)

#### *Concerns:*

No placenta submitted with this carcass. Comments on placenta do not relate to this necropsy. Calf not 2 years of age but 2-3 days.

## Calf 252\_2

Table B-34. Calf 252\_2 contributing factors summary at LR

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Stillborn	
Contributing factors to death	Support for the claim
Dystocia-fetal distress/anoxia	<ul style="list-style-type: none"><li>• Tongue, face, and neck were swollen</li><li>• Laryngeal and tracheal airway obstruction</li></ul>
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• <i>Trueperella pyogenes</i> isolated from stomach contents and joints</li><li>• <i>Mannheimia spp.</i> isolated from from lung and joint</li><li>• <i>Staphylococcus spp</i> isolated from lung</li><li>• <i>Pseudomonas spp</i> isolated from joint.</li><li>• <i>Prevotella heparinolytica</i> isolated from from stomach contents</li><li>• Umbilical cord and navel tissues necrotic and suppurative with intralesional mats of bacteria noted</li><li>• Bronchopneumonia of the lungs</li></ul>
Mineral deficiencies	<ul style="list-style-type: none"><li>• Critically low levels of selenium at 0.70 ug/g (min. ref. value 1.1 ug/g)</li></ul>
Mineral imbalances	<ul style="list-style-type: none"><li>• Low level of molybdenum at 0.67 ug/g (min. ref. value 0.8 ug/g)</li></ul>

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### **Calf Loss Summary**

Gross morphology of carcass confirms death due to dystocia. The cause of dystocia may have been amplified by infection of both *Mannheimia spp.* and *Trueperella pyogenes*, in a joint swab, lung sample and stomach sample. Liver enlarged and abnormal color. No evidence of calf being able to breath and umbilical cord and navel grossly infected with mats of mixed bacteria. In addition, critically low selenium levels.

### **Timeline**

- Birthing sensor expelled at 12/27/2017 at 8:21am.
  - Cow was checked around 9:30-10am and was not in labor
  - Checked again at 9pm same day, cow was still not in labor
- The next morning, 12/28/2017 ~9am the cow was found standing next to her newborn dead calf.
  - There was no buzzard activity near the deceased calf.
  - The calf was laying awkwardly as though it were stillborn and had not moved after birth.
- The carcass was immediately put on ice and sent to Bronson Laboratory

### **Necropsy and/or Laboratory Test Results**

- Chronic compression of face and neck supports dystocia findings
  - Face and neck edema, severe and locally extensive
  - Tongue edema, severe and subacute
- Congestion and edema of lungs associated with face and neck compression onto these tissues
- *Mannheimia spp.* and *Trueperella pyogenes* found in multiple sites, joints, stomach and lungs, and were likely causes of bronchopneumonia
- Mineral results show
  - critical low levels of selenium 0.70 (ref 1.1-5.9 ug/g)
  - low level of molybdenum 0.67 (ref 0.8-2.2 ug/g)

#### *Concerns*

Umbilical cord and navel bacterial infections described as severe after <24hrs since birth.

### Calf 304\_3

Table B-35. Calf 304\_3 contributing factors summary at LR

---

Stillborn

Contributing factors to death	Support for the claim
Mineral toxicity	<ul style="list-style-type: none"><li>• Toxic levels of iron found in the liver sample at 2111.13 ug/g (max. ref. value 750 ug/g)</li></ul>
Mineral imbalance	<ul style="list-style-type: none"><li>• Low level of molybdenum at 1.01 ug/g (min. ref. value 1.80 ug/g)</li></ul>

---

### **Calf Loss Summary**

Cause of death unable to be identified for full term calf. The fungus *Cladosporium* spp. was isolated from the lung. Lungs, brain and kidneys are hemorrhagic. Microscopic findings of meninges suggest mild inflammation, but no bacterial or fungal pathogens found. Tested for leptospirosis, toxoplasmosis, *Neospora caninum*, and IBR with all being negative. High levels of iron were recorded in liver.

### **Timeline**

- Birthing sensor expelled at 11:55pm on 2/3/2018.
- Cow was found standing by her calf at 7am on 2/4/2018
  - Cow was still cleaning calf upon arrival.
  - The calf had soft hooves and placental remains indicating the calf never stood.
- There was no vulture activity on calf carcass.
- The calf was placed on ice and transported to Bronson Laboratory for necropsy.

### **Necropsy and/or Laboratory Test Results**

- Lungs dark colored, only right lung floated
- Kidney hyperemic and brain moderately congested
- Liver mildly light in color
- Mineral results show
  - critical high levels of iron 2111.13 (ref 170-750 ug/g)
  - low level of molybdenum 1.01 (ref 1.8-4.7 ug/g)

*Concerns:*

None

### Calf 383\_3

Table B-36. Calf 383\_3 contributing factors summary at LR

---

39 days.

Contributing factors to death	Support for the claim
Septicemia/bacteremia	<ul style="list-style-type: none"><li>• <i>Trueperella pyogenes</i> found in abdomen, bladder, umbilicus.</li><li>• Tissue of navel, abdominal cavity, and bladder were necrotic</li></ul>
Poor dam health	<ul style="list-style-type: none"><li>• Calf was under weight for age</li><li>• Cow appeared underweight</li><li>• Possible infection of jaw on left side</li></ul>

---

### **Calf Loss Summary**

Calf seemingly healthy from birth 1/1/2018 until found dead on 2/9/2018. Calf weight 115lbs at necropsy. In the field calf likely alive morning of the 2/9/2018 based on VHF signals, and was dead by 11.30am. Eyes had been removed by vultures but tongue, ears and abdomen all intact. Subcutaneous infection was found beneath the umbilicus (navel area), necrotic tissue, and infection had spread deeply into muscular wall and peritoneum (lining of the abdominal cavity) and extending in to the pelvic region and bladder. Infectious agent was isolated as the bacteria, *Trueperella pyogenes*. Field notes also suggest cow was poorly and may have had infection as left side of jaw was swollen.

### **Timeline**

- Birthing sensor did not send message upon expulsion.
- Cows and calves were checked 12/31/2017.
- Calf was found on 1/1/2018 and believed to be born that day.
  - Calf was standing, nursing, appeared healthy.
- Tagged 1/2/2018
- Nothing out of ordinary on health status reported between birth and mortality event.
- Calf was found dead on 2/9/2018 at approximately 11:30am.
  - The joints were still soft and limber, had not stiffened.
  - Buzzards had just started to peck on the calf.
- Receiver download showed an alive signal from calf 383\_3 throughout the morning of 2/9/2018.
  - Calf was alive at 8:54am and 9:59am.
- If the VHF data is correct the calf died between 9:59am and 11:30am.
- There is a possibility of the VHF moving at 9:59am either from buzzard activity or wind as it was extremely windy that morning.
  - Gusts were a max of 17mph with average of 9mph (according to wunderground.com)
- Calf was put on ice and taken to the Bronson Laboratory on 2/9/2018.
- Cow was swollen on left side of jaw when calf died.

### **Necropsy and/or Laboratory Test Results**

- *Trueperella pyogenes* found in abdomen, bladder, and umbilicus.
- Cause of death likely attributed to septicemia/bacteremia as a result of exudative omphalophlebitis

#### *Concerns:*

Liver sample not collected during necropsy and no mineral analyses conducted

### Calf 427\_3

Table B-37. Calf 427\_3 contributing factors summary at LR

---

9 days

Contributing factors to death	Support for the claim
Depredation	<ul style="list-style-type: none"><li>• Field notes show last visual observation was at 15:15 on 1/01/2018</li><li>• VHF datalogger confirms calf at 14:19 on same day but is never logged again.</li><li>• Calf VHF tracked but unable to find signal until two days after calf was missing</li><li>• Traces of the calf not found but tag was found 1.1km northeast of last known location of calf</li></ul>
Poor dam health	<ul style="list-style-type: none"><li>• Cow was extremely thin prior to calving. So thin she did not appear pregnant in the weeks prior to calving.</li><li>• Calf was nursing but did not seem to be fattening up, possibly poor-quality colostrum</li></ul>

---

## Calf Loss Summary

Calf born 12/23/2017 small ~30lbs or less, but did stand and was nursing. Last observed visually on 1/01/2018 at 15:15 with calf calling for cow, observed walking south from approximately center of pasture. Calf poorly. VHF datalogger confirms calf presence at 14:19 same day. No further logging of calf via VHF occurred, and should have with the cycling of transmitter in about ~2hrs, between 16:00 and 17:00. Calf may have wandered out of range beyond pasture boundary, laying low in a ditch, or depredated and removed from pasture at some time. Over next 4 days calf was searched for and eventually VHF ear tag with no calf was found 1.1km from last visual to the NE.

## Timeline

12/23/2017

- Calved
- Very small calf. Low birth weight.
- Standing, nursing.
- Tagged- 164.074

1/01/2018

- Calf is very tiny- is it actually getting milk from mom?
- Could see bones of the calf and was not filling out at all. Looked like it wasn't receiving many nutrients
- Was walking but moving slowly.
- Downloaded receiver data at 15:00 on 1/01/2018.
- Calf was last seen by receiver at 1/01/2018 at 14:19.
- Visibly observed the calf around 15:15 on 1/01/2018. This was the last time the calf was seen. Both cow ID and VHF ear tag still securely in place. Calf was heading south towards the lone palm tree and was located just north of the center of the pasture.

1/02/2018

- Receiver data did not record a signal for calf 427\_3 since yesterday at 1/01/2018 at 14:19.
- Tried to track but could not get a signal. We drove the entire perimeter of the fence while listening and scanning for the calf's frequency. We never heard a signal. Searched for about an hour.
- The cow 427\_3 seemed to be searching for the calf as well. Calling and looking around.
- There was no buzzard activity all day.

1/03/2018

- Gate between Northwest pasture and northeast pasture was opened.
- Cows begin to move from northwest to northeast pasture.
- Drove through the northwest pasture where the calf was last seen for multiple hours while scanning. Searched all the ditches and covered all ground on the pasture. 99% sure I would have seen a calf carcass.
- No buzzard activity in either of the pastures.
- Searched the northeast, new pasture, as well.

1/04/2018

- Found a ribcage in the northeast pasture (new) that had been picked clean. A few days later found a pig skull approximately 100 meters away from the ribcage. Believe these are from the same animal as they are about the same size and similar decomposition rate.
- While observing a birthing event, saw a coyote in southwest side of the pasture of the new pasture.
- Picked up a mortality signal for 427\_3 approximately 700 m north east of the center of the old pasture.
- No buzzard activity in either of the pastures.

1/05/2018

- Found 427\_3 ear tag approximately 1.1km from the northeast of the center of the pasture between the edge of a large wetland and a densely vegetated fence line. The ear tag was located approximately 700 meters from the northern most fence line and 1.5 km from the southernmost fence line in the pasture.
- No remainders of a carcass close by.
- Did not see the other white ear tag.
- No prints of animals close by. No hair on the fence line close by.
- Ear tag was located approximately at 27°09'50.4"N 82°05'41.4"W (see map)

**Necropsy and/or Laboratory Test Results**

None. No carcass ever found

### Calf 457\_3

Table B-38. Calf 457\_3 contributing factors summary at LR

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Stillborn

Contributing factors to death	Support for the claim
Viral infection	<ul style="list-style-type: none"><li>• Infectious bovine rhinotracheitis detected by ELISA but no supportive lesions noted in liver or lungs</li><li>• Liver swollen and hemorrhagic</li><li>• Pleuritis noted in histopathology</li></ul>
Mineral imbalance	<ul style="list-style-type: none"><li>• Low levels of molybdenum at 0.8 ug/g (min. ref. value 1.8 ug/g)</li></ul>

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### **Calf Loss Summary**

Full term calf found with cow during the morning. Evidence of vulture activity. Lack of air in lungs suggest still born. Lungs showing signs of infection, inflammation and hemorrhaging. Lung tissue positive for IBR antigen using DFA, no other supporting evidence of IBR. Possible cause but not conclusive.

### **Timeline**

- Cows checked morning of 3/2/2018 calf not born
- Birthing sensor probably expelled at 3/2/2018 at 8:55pm but sent incorrect message, could have been earlier.
- Cows were not checked until the next morning 3/3/2018.
- Calf was found at ~9am 3/3/2018.
- Cow was with calf and had completely cleaned calf upon finding.
- Calf was dead upon arrival and did not show indication of being alive or any movements after birth.
- Signs of vulture activity on eyes and rectum
- Calf was put on ice and transported to Bronson Lab.

### **Necropsy and/or Laboratory Test Results**

- Tongue missing and rectum with large hole likely from scavenging
- Lungs did not float, still birth reported, liver swollen and autolysis
- Lung tested positive for Infectious Bovine Rhinotracheitis via an ELISA but no supportive lesion evidence from lungs or liver of calf.
- Lung: pleuritis has edema and hemorrhaging and some inflammation, liver normal
- Mineral Test
  - Low molybdenum 0.8ug (ref. range 1.8 -4.6ug/g)

#### *Concerns:*

Need more information on IBR ELISA test, false positive?

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## BIOGRAPHICAL SKETCH

Kelly Koriakin was raised in North Carolina and attended high school in Winston Salem, NC. She graduated from the University of North Carolina at Chapel Hill in 2013 with a Bachelor of Science in environmental sciences and minors in both Spanish and geology. Following graduation, she worked in many wildlife biology field jobs from the remote wilderness of Alaska to the swamps of Louisiana. She was able to work with a wide variety of species that gave her exposure to many different fields of ecology including disease ecology, plant ecology, population biology, and predator biology. As Kelly gained experience with wildlife procedures, protocol, and data collection, she developed a passion for wildlife conservation and human-carnivore conflict. Following her master's, she hopes to continue research in large carnivore ecology, particularly involving human dimensions. In her free time, Kelly loves to be outside hiking, backpacking, biking, competing in triathlons, and scuba diving.