THESIS

PRE-WEANED BEEF CALF MORTALITY ON HIGH ALTITUDE RANCHES IN COLORADO

Submitted by

Joseph Michael Neary

Department of Clinical Sciences

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Master's Committee:

Advisor: Franklyn Garry

David Dargatz
Daniel Gould
Anthony Knight

ABSTRACT

PRE-WEANED BEEF CALF MORTALITY ON HIGH ALTITUDE RANCHES IN COLOARDO

Nationwide Colorado has the 14th largest beef cow population. The sale of cattle and calves is Colorado's highest grossing agricultural product generating over $3 billion annually. Many of the 11,600 cow-calf operations are located at high elevations in the Rocky Mountains. Producer reports suggest that a large number of these ranches experience high pre-weaned calf death loss, as high as 20% of the calf crop, between branding in the spring and weaning in the autumn. During this time many cow-calf operations graze their animals on high altitude mountainous pastures. Due to the extensive, inaccessible nature of the mountainous terrain there have been limited prior investigations of the death loss problem. For the present study physiological, pathological and epidemiological investigations were performed to characterize the problem. A survey of Colorado's beef producers found that the odds of a producer having a greater than average calf death loss increased with increasing altitude. This greater death loss was mainly due to respiratory problems and pulmonary hypertension (also known as high altitude disease or brisket disease). Postmortem examination of calves revealed that approximately equal numbers of calves died from bronchopneumonia and pulmonary hypertension even on ranches that have selected for low pulmonary artery pressure herd sires for over 20 years. The postmortem lesions obtained from calves with pulmonary hypertension suggest that our current understanding of the disease is insufficient. Overlap of clinical signs makes appropriate treatment decisions by producers difficult. Typically, cases of bronchopneumonia occurred earlier in the summer

grazing season than cases of pulmonary hypertension, which occurred in the late summer-early fall period. Blood biochemical values for healthy calves at altitude (9,000 ft. or 2,743 m) were strikingly different from literature reports of similarly aged calves sampled at lower altitudes. This reflects the extreme physiological challenges associated with life at high altitude. Arterial blood-gas analysis determined that calves at high altitude have lower oxygen tensions than expected despite hyperventilation. Hematocrit and hemoglobin levels were not significantly increased over age-matched calves at sea level despite chronic hypoxia. In summary, this project determined that high levels of pre-weaned calf death loss occur on high altitude cow-calf operations due to bronchopneumonia and pulmonary hypertension. Why these diseases are particularly problematic is discussed. Further studies are necessary if the problem is to be addressed.

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INTRODUCTION

In spring 2009, several cow-calf beef producers from Gunnison, Colorado sought help from the food animal clinical group at Colorado State University. They reported significant calf death losses between turnout onto summer grazing and weaning. Based on herd records, 10-20% of calves would die between branding in May and weaning in October. These losses have occurred over several decades, but as the profit margins of cow-calf operations have decreased in recent years, these losses now threaten to put these ranches out of business [3]. A producer losing 20% of his 600 calves equates to $78,864 of lost potential income between summer turnout and weaning alone. This does not account for lost investment. This is based on current market prices ($1.24/lb. live weight, November 7th 2011) and the herd average weaning weight in 2009 (29.8 ± 72.4lbs), which, was the same as the national average (530lbs [4]). Besides being an animal welfare concern this high level of calf mortality is both distressing to the producer and financially unsustainable.

Many of the producers attribute their pre-weaned calf mortality to 'summer pneumonia' [5], an ill-defined disease syndrome of pre-weaned beef calves. Although well recognized by ranchers, there has been virtually no scientific pursuit of the problem in the published literature. This lack of information can be attributed to several factors: the problem occurs when calves are pastured at high altitude where there are no effective handling facilities or points of access; to clinically evaluate calves in the extensive and rugged terrain that the calves are located requires travel by horseback, limiting access by veterinarians; and, finding sick and recently deceased calves is extremely challenging and time consuming. Indeed, it is common for producers to be unaware of

any calf loss until weaning in the autumn when calves are brought down off the mountains and counted.

Producer descriptions of calves affected with summer pneumonia include: rapid breathing rate, dull expression, fever, roughened hair coats, drooped ears, and a stiff-gaited walk, usually followed by death. Open-mouth breathing occurs intermittently accompanied by a dry cough. Distended jugular veins and brisket edema occur in less than half of affected animals. Many of these clinical signs overlap with high altitude disease, also known as brisket disease. This disease is thought to result from an exuberant vasoconstrictive response of the pulmonary arteries to hypoxia culminating in congestive heart failure [6]. Approximately, 75% of cases occur in cattle less than two years of age [7] which means that pre-weaned calves are at an increased risk. Plausibly, this could explain the high prevalence of pre-weaned calf mortality since Gunnison is situated at and above 2,438m (8,000ft).

Altitude was first identified to be the chief causative factor for high altitude disease by Glover and Newsom almost a century ago [8]. Their epidemiologic approach provided valuable insight and ultimately information on measures that ranchers could take to protect their herds. One such measure considered essential for profitability at high elevation is pulmonary arterial pressure (PAP) testing [6]; currently the only available screening tool for selecting against the disease. Since PAP is moderately heritable (0.34) [9] ranchers of high altitude herds breeding only low PAP sires, and in some cases low PAP cows, should theoretically, significantly reduce the incidence of high altitude disease within their calf crop [10]. Producer reports suggest this to be true but peer-reviewed studies are lacking. However, reports from five Gunnison producers

suggest that the mortality of pre-weaned beef calves may be substantially higher than the national average (6.4%, [4]) despite the selection of low PAP scoring breeding animals for over 20 years. This suggests that either selective breeding of low PAP scoring sires is an insufficient management tool in reducing the occurrence of high altitude disease and/or pre-weaned calves are dying from other diseases, such as summer pneumonia, that may be unrelated to PAP testing *per se*. If calves are not dying from high altitude disease then it is likely that pneumonia is a large contributing factor since pneumonia is the biggest cause of mortality in pre-weaned calves over 3 weeks old [11].

PAP testing provides an indirect measure of resistance to blood flow through the pulmonary arteries but does not provide information regarding impedance to the flow of blood [12]. However, impedance to flow due to stiffening of the large elastic arteries may play a substantial role in the initiation and progression of pulmonary hypertension [12]. Changes in the small-medium sized pulmonary arteries may reflect a more advanced stage of the disease. It is these downstream changes that influence PAP (figure 1.1).

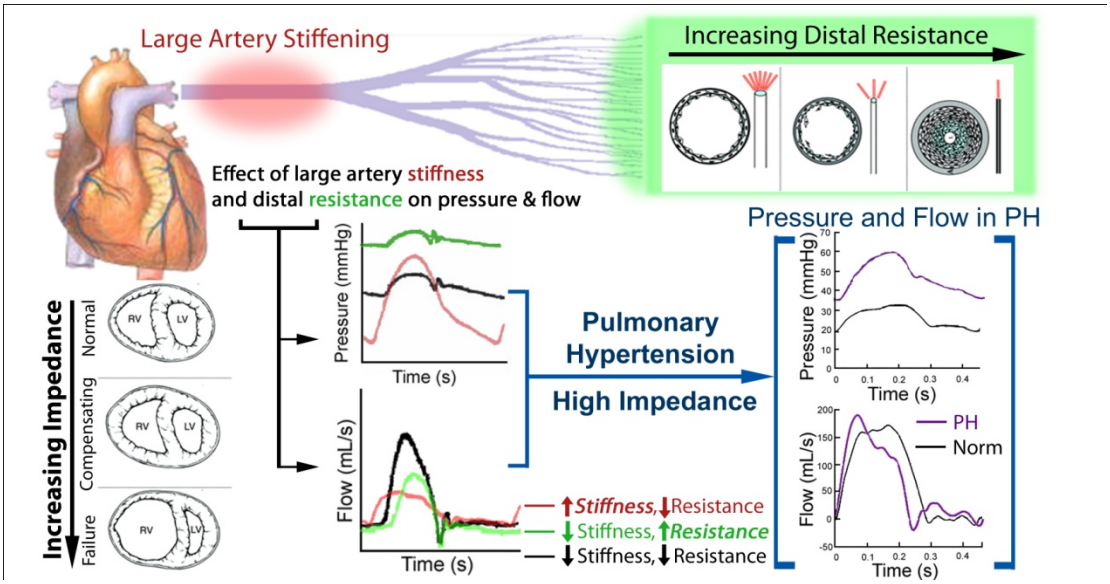

Figure 1.1: Diagram demonstrating the relationship between ventricular work, impedance to flow due to large pulmonary artery stiffening and resistance to flow due to narrowing of the distal pulmonary vessels. Vascular stiffness, due to structure-function changes in vessel wall elastin, and distal resistance, due to medial hypertrophy, do not act independently but instead form a coupled system, which determines the overall hemodynamic changes associated with pulmonary hypertension. (Diagram courtesy of Kurt Stenmark, UC Denver. Plot data obtained from [1, 2])

The similarity of summer pneumonia disease signs to those of high altitude disease has facilitated an assumption that the problem was high altitude disease, followed by the assumption that the problem would diminish in response to ongoing high altitude disease management efforts. However, affected calves have not been rigorously studied and most observations of the problem have been clinical anecdotes reported by ranchers and range riders. Because some signs of disease are similar to those of other common conditions, treatment and prevention measures have been implemented based on presumptive diagnoses, only to find later that the problem is unabated.

The rapid respiration rate, increased body temperature and depressed attitude of affected calves suggested to some ranchers and veterinarians that the problem was caused by viral or bacterial pneumonia. However, no postmortem examinations were performed to verify this assumption. Changes in vaccination protocols and antibiotic treatment have proven unsuccessful in mitigating the problem. Serologic testing to evaluate viral exposure has yielded no consistent results thus failing to demonstrate a common viral etiology. Other clinicians have presumed that copper deficiency could be responsible but modified copper supplementation has failed to decrease the problem. The producers have become frustrated by the 'try it and see' approach to solving the problem.

The goal of this project was firstly, to determine if producers from Gunnison experience a greater mortality of pre-weaned beef calves between turnout onto summer grazing and weaning relative to other regions of Colorado and the national average and secondly, to characterize the nature of the problem. This goal was met by answering the following specific questions:

1. What is the prevalence of pre-weaned beef calf mortality on cow-calf operations throughout Colorado?
2. What are typical blood and cardio-pulmonary physiological parameters for healthy calves at this altitude?
3. What are the pathophysiologic and pathologic features of sick and deceased calves, respectively?

The above questions are explored in chapters 2-4, respectively. An overall discussion of the findings and their implications for the beef industry can be found in the discussion section.

MORBIDITY AND MORTALITY OF PRE-WEANED BEEF CALVES: A RETROSPECTIVE SURVEY OF 142 BEEF PRODUCERS IN COLORADO

INTRODUCTION

Life at high altitude is associated with unique physiological stressors such as extreme temperature fluctuations, low oxygen tension and solar radiation. Animals native to such environments have evolved specific adaptations to these and other stressors. For example, to meet tissue oxygen demand under the hypobaric hypoxic conditions of the Andes, high-altitude adapted native humans have enhanced pulmonary O_2 diffusion capacity by evolving larger lungs than lowlanders [13].

Lowland species, more accustomed to higher oxygen tensions than high-altitude species, typically demonstrate an insufficient or maladaptive physiological response to hypoxia. A disease syndrome of domestic cattle familiar to high altitude cattle ranchers is pulmonary hypertension with right-sided heart failure known colloquially as high altitude disease or brisket disease. High altitude disease is thought to be due to an exuberant vasoconstrictive response of the pulmonary arteries to hypoxia culminating in congestive heart failure [6]. A blunted pulmonary pressor response is therefore beneficial for mammals at high altitude [14]. Studies suggest the pressor response to be highly heritable [9]. This means that producers of high altitude herds that select for low pulmonary arterial pressure (PAP) scoring sires, typically ≤ 45 mmHg [6], should theoretically be able to reduce the incidence of high altitude disease within their herds [10]. Producer reports suggest this to be true but peer-reviewed studies are lacking.

However, producer reports from ranches where cattle are grazed at altitudes over 8,000ft (2,438m) in southwest Colorado suggest that the mortality of pre-weaned beef calves may be substantially higher than the national average (6.4%, [15]) despite the selection of low PAP scoring breeding animals for over 30 years. This suggests that either selective breeding of low PAP scoring sires is an insufficient management tool in reducing the occurrence of high altitude disease and/or calves are dying from other diseases unrelated to PAP testing *per se*.

One plausible but ill-defined condition to which producers attribute pre-weaned calf mortality is 'summer pneumonia' [5]. Producer descriptions of this syndrome typically include panting, lethargy, dropped ears, rough hair coat and a stiff-gaited walk. Summer pneumonia, if it truly is a pneumonia and is unrelated to pulmonary hypertension, could explain why PAP testing has had limited success in reducing calf mortality.

The objective of this study was to determine the incidence of, and risk factors for, morbidity and mortality (including those attributed to summer pneumonia) of pre-weaned beef calves between turnout onto summer grazing and weaning in the fall. A survey, enquiring about the health of the 2009 calf crop, was sent out to cow-calf producers in Colorado.

MATERIALS AND METHODS

Selection of the respondent pool

The source population for potential survey respondents consisted of all members of the Colorado Cattlemen's Association (CCA). The CCA is managed cooperatively by, and consists of, beef producers from cow-calf production to feedlot. They work closely with the state and national legislators, agencies, media and consumers to promote the beef industry. In 2009 there were 1,870 members of the CCA and 11,600 beef cow-calf producers in Colorado [16]. The survey was pilot tested on 10 producers at the CCA convention in Pueblo, Colorado (June 14th-16th, 2010). The final survey was sent out all 1,870 members of the CCA.

The survey (a copy is available via the corresponding author) was made available in two formats depending on how members chose to be contacted by the CCA. Of the 1,870 members: 1,500 members received both a paper copy of the survey via mail and a notification of the survey via electronic mail containing a link to the online version of the survey [17], 300 members received only the paper copy and 70 members received only the email notification of the survey. The survey was sent out to CCA members in July 2010. Survey recipients were contacted only once. Respondents were required to provide their own return envelope and postage.

Data collection

Survey questions focused on animal demographics, animal management at key periods (calving, branding, weaning) pulmonary arterial pressure (PAP) testing, and calf health between movement to summer pastures and weaning for the 2009 calf crop only. Specifically, producers were asked to apportion the genetic makeup of their 2009 calf crop to various breeds and breed types; report the average altitude where calves were born and pastured on the August 1st 2009; the date for the onset of the calving season and the duration; the number of calves born alive, stillborn, died within one month and died between one and three months of age; and, the number of calves moved to summer pastures. Producers were asked for the dates and numbers of calves branded and weaned. Since movement onto summer grazing is often a period rather than an event the date of branding served as the date to reflect the start of summer grazing. For the 10 ranches that either did not brand or branded in the fall only, a turnout date was imputed based on data from herds with similar demographics. All of the above were evaluated as potential risk factors for pre-weaned calf mortality post-turnout.

Producers were asked to provide a count of calves affected by various disease syndromes (below), both fatally and non-fatally, between turnout to summer pastures and weaning. These counts were mutually exclusive i.e. a calf was either: sick but then recovered or, it was sick and died. Specific etiological diagnoses were not given in order to reduce responder bias. For example, producers at *lower altitudes* may not consider *high altitude* disease as a potential cause of calf morbidity. Instead, diseases were categorized according to the following syndromes:

i. Diarrhea only,

ii. Panting, lethargic, rough hair coat, dropped ears, stiff-walk, prefer not to move,

iii. Jugular vein distension, swollen brisket and belly, bulging eyes,

iv. Stumbling gait,

v. Lameness,

vi. Coughing and discharge from the nose,

vii. Other (specify).

Categories ii., iii. and vi. are potentially consistent with 'summer pneumonia', high altitude disease and infectious pneumonia respectively. Producers were asked whether they select sires for their herd based on pulmonary arterial pressure score and if so, for how many years have they done so.

Producer surveys were assigned numeric codes, so researchers had no access to the names of or contact information for survey participants. A separate page at the end of the survey allowed producers to provide their contact details through a separate mailing if they were interested in participating in a follow-up survey and/or receiving a summary of the results. The response period closed on 1st October 2010.

Statistical analysis

Survey responses were entered into a spreadsheet (Microsoft Excel) and checked for discrepancies. Respondents who had provided contact details were contacted for verification of

the information provided as necessary before the information was subsequently made anonymous.

Calving, branding, weaning and calf health statistics were calculated for each ranch. Depending on the data distribution either the mean and standard deviation, or median and quartiles were used to describe the data. Where appropriate the analysis was divided according to herd size: smaller herds (<100 calvings), medium sized herds (100-199 calvings) and larger herds (≥200 calvings).

Multivariable methods

Two multivariable analyses were performed. In the first analysis the outcome variable, herd weaning percentage, was dichotomized according to whether the herd value was greater or less than the median herd weaning percentage. In this study, herd weaning percentage is the proportion of calves turned out on to summer pasture that were subsequently weaned. Risk factors for herd weaning percentage were determined by logistic regression.

In the second analysis, risk factors for the six disease syndromes described above were determined. Producers indicated the number of calves affected by the various disease syndromes between turnout and weaning. The inter-herd variance of frequency counts for all of the disease syndrome categories was greater than twice the mean count; hence a Poisson model was not considered appropriate since this model assumes the mean and variance to be approximately the same. Extra-Poisson variation present in the frequency count data was accounted for using a

negative binomial model (STATA 12, Stata Corporation, College Station, Texas, USA). The number of calf-days at risk was calculated as the number of calves turned out onto summer grazing multiplied by the length in days from branding to weaning. Calves that died were assigned half of the risk period. Zero inflated negative binomial models were considered but they did not improve any of the models.

A backward elimination procedure was used for model building. During each step, the least significant variable was determined using the likelihood ratio χ^2 test and removed. For the variable herd size the overall significance of the entire set of the three herd sizes was determined by the likelihood ratio test. The variable "altitude of calves on the 1st August" was correlated with "altitude at birth" (r=0.72) and "difference in altitude between grazing on the 1st August and at birth" (r=0.62). The latter two variables were weakly correlated (r=-0.09). Therefore, to prevent co-linearity in the multivariable model, either the variable "altitude on the 1st August" was included or one, or both, of the other two altitude variables were included.

RESULTS

A total of 148 producers responded (8.0% overall response rate): 127 of the possible 1,800 producers responded by mail (7% response rate) and 21 of the possible 1,570 producers completed the survey online (1.3% response rate). Six surveys were not included in the analysis: 2 because the ranch did not have cattle in 2009, 3 because of extensive missing data and 1 survey was illegible. Data from 142 herds were included in the data analysis. This is 1.2% (142/11,600) of all beef cow-calf producers in Colorado. Survey responses came from 53 of the 64 counties in

Colorado. Delta County had the most replies with 11 producer responses. There was poor correlation between the total number of beef cows in the county [18] and the number of beef cows representing each county in the survey (r=0.21).

Table 2.1: The frequency of herds (and percent of all herds) according to the genetic makeup of the calf crop in 2009. One herd was a 100% brahman and another had 50% brahman and 50% british breed calves.

		Percentage of calf crop composed of *Continental* breeds				
		0%	25%	50%	75%	100%
Percentage of calf crop composed of *British* breeds	100%	70 (49.3%)	-	-	-	-
	75%	0	23 (16.2%)	-	-	-
	50%	1 (0.7%)	0	33 (23.2%)	-	-
	25%	0	0	0	6 (4.2%)	-
	0%	1 (0.7%)	0	0	0	8 (5.6%)

Across all herds, British breeds were the most prevalent with 49.3% (70/142) of herds having a calf crop consisting entirely of one or more British breeds (table 2.1).

Calving and mortality of calves less than 3 months old

Survey respondents reported 26,232 calvings in total. Of these, 98.2% (25,761/26,232) of calves were born live. The median number of calvings per herd and calves born live per herd was 117 and 115 (98.6%), respectively (table 2.2). This is a greater than the mean number of beef cows per herd reported for Colorado (62.4) [16]. Herds of different sizes were equally distributed amongst the various altitudes at which calving and summer grazing (altitude on August 1st) occurred (p=0.88). Five ranches (3.5%) calved between June 10th and September 15th. The start date of the calving season was not associated with: an increased risk of stillbirth (p=0.17),

mortality of calves less than one month old (p=0.40) or mortality of calves between one and three months old (p=0.08).

Table 2.2: Herd level descriptive statistics for calving. n = number of herds SD = standard deviation from the mean (95%).

Variable	n	Mean	SD	Min	Q_1	Median	Q_3	Max
Number of calvings	142	-	-	6	44	117	250	1500
Date of first calving	142	-	-	Jan 4th	Feb 10th	March 1st	March 27th	Sept 15th
Live births (%)	142	-	-	80	97.33	98.62	100	100
Live births that died < 1 month old (%)	142	-	-	0	0	1.06	2.39	36.36
Dead 1-3 months old (%)	142	-	-	0	0	0.85	2.08	19.05
Turned out (of those born alive) (%)	142	-	-	71.43	95.11	97.65	99.63	100
Altitude born	142	6,387	1,361	3,500	5,500	6,500	7,500	9,500
Altitude August	142	7,144	1,731	3,500	5,500	7,500	8,500	10,500
Difference in altitude between calving and location on August 1st	142	-	-	-1,000	0	0	1,000	4,500

Smaller herds (< 100 calvings) were 2.0 times more likely to have one or more stillbirths (mean= 3%) than larger herds (≥ 100 calvings) (mean= 1.5%) (p=0.002) (table 2.3). The odds that a herd has a greater percentage of live births than the average herd (98.6%) increases by 28% for every 1000 foot increase in the altitude at which the herd calves (p=0.05). There was no difference in the percentage of calves that died within 1 month of age or, from 1 to 3 months of age, among herds of different sizes (p=0.43 and p=0.14, respectively) or with altitude of calving (p=0.35 and p=0.70, respectively).

Table 2.3: Descriptive statistics for calving at the herd level according to herd size (<100 calvings, 100-199 and ≥200). n= number of herds. CI = 95% confidence interval of the mean.

Variable	Herd size	n	Min	Q_1	Median	Q_3	Max	Mean % (95% CI)	*p*-value
Number of calvings	<100	61	6	22	40	74	99	-	-
	100-199	34	100	115	136	165	196	-	-
	≥ 200	47	200	250	290	464	1500	-	-
Date of first calving	<100	61	Jan 15th	Feb 15th	March 1st	April 1st	Sept 15th	-	-
	100-199	34	Jan 4th	Feb 14th	March 4th	April 1st	May 1st	-	-
	≥ 200	47	Jan 5th	Feb 10th	March 1st	March 25th	Aug 1st	-	-
Live births (%)	<100	61	80	95.6	97.8	100	100	-	-
	100-199	34	93.1	97.9	98.6	99.5	100	-	-
	≥ 200	47	93.1	97.87	98.64	99.50	100	-	-
Stillborn (%)	<100	61	0	0	2.2	4.4	20	3.0 (2.0, 4.0)	Ref.
	100-199	34	0	0	1.3	2.0	4.2	1.3 (0.9, 1.8)	0.02
	≥ 200	47	0	0.5	1.4	2.1	6.9	1.6 (1.2, 2.1)	0.03
Live births that died < 1 month old (%)	<100	61	0	0	0	2.7	36.4	2.7 (0.99, 4.36)	Ref.
	100-199	34	0	0	1.4	2.1	9.7	1.8 (1.06, 2.60)	0.47
	≥ 200	47	0	0.63	1.1	2.1	7.6	2.3 (1.13, 2.01)	0.28
Dead 1-3 months old (%)	<100	61	0	0	0	2.8	19.1	2.3 (1.3, 3.2)	Ref.
	100-199	34	0	0	0.9	1.7	9.7	1.3 (0.7, 2.0)	0.17
	≥ 200	47	0	0.3	1.0	1.5	6.8	1.3 (0.9, 1.8)	0.10

Branding

Of all calves born alive, 96.1% (22,483/24,685) survived long enough to be branded. Six producers (4.2%) did not brand any of their calves. Of all the calves turned out onto summer grazing 99.0% (22,483/24,952) were branded. On average, the oldest calf branded at the first branding event of the year was 2 months old (66 days). Half of all producers branded between February 28th and May 10th. Four herds (3%) branded at weaning time.

Weaning

Half of all producers weaned calves between September 5th and November 12th (table 2.4). Weaning weights were recorded on only 49.3% (70/142) of all ranches. This includes both group and individual animal weights. The largest herds (≥ 200 calvings) were 4.1 times more likely to record weaning weights than the smallest herds (< 100 calvings) (p=0.002). Medium sized herds were 3.6 times more likely to record weaning weights than the smallest herds (p=0.002). The largest herds were no more likely to record weaning weights than medium sized herds (p=0.78). Average weaning weight did not differ among herd sizes (p=0.38). The average weaning weight for a herd that was predominantly British-based was 17.0 lbs. lighter than a herd with a calf crop that was at least 50% Continental breed composition but this was not statistically significant (p=0.33).

Table 2.4: Weaning statistics at the herd level. n= number of herds. SD = standard deviation from the mean (95%).

Variable	n	Mean	SD	Min	Q_1	Median	Q_3	Max
Date of weaning	142	-	-	July 4th	Sept 5th	Oct 27th	Nov 12th	April 30th 2010
Calves turned out that were weaned (%)	142	-	-	87.34	90	99.4	100	100
Days from start of calving to weaning	142	-	-	139	171	234.5	257	298
Weaning weight (kg)	76	255.3 (561.7 lbs.)	33.6 (74.0 lbs.)	-	-	-	-	-

The date of the first day of calving was a better predictor of weaning weight than the duration of the birth-weaning period. On average, herds that started to calve on January 4th had a herd average weaning weight of 631 lbs. One quarter of producers started calving between January 4th

and February 10th (table 2.2). For every one day after January 4th that the start of calving was delayed the average herd weaning weight decreased on average by 1.2 lbs. per day ($p<0.001$) (table 2.5). For every one-day increase in the calving-weaning period the average estimated herd weaning weight increased by 0.9 lbs. ($p=0.001$). However, when controlling for the start date of calving, the calving-weaning period was not significantly associated with the herd average weaning weight, which increased by only 0.2 lbs. per day ($p=0.55$). When controlling for the duration of the calving-weaning period, for every day that the start of calving was delayed past January 4th the average herd weaning weight decreased by 1.1 lbs. per day on average ($p<0.001$). The calving to weaning interval did not differ among herd sizes ($p=0.99$).

Table 2.5: Linear univariable regression of the variables: lag from January 4th to the start of herd calving (days) and, calving-weaning period (days), on the outcome, average herd weaning weight. SE = standard error of the mean (95%)

Variable (days)	Coefficient (lbs./day)	SE	*p*-value
Lag from Jan 4th to start of herd calving period	-1.21	0.21	<0.001
	Intercept: 630.95	14.23	<0.001
Calving-weaning period	0.88	0.26	0.001
	Intercept: 354.52	60.84	<0.001

Calf health

On the average ranch, 99.4% of all calves that were turned out onto summer grazing were weaned but this dropped to as low as 87.3% on one ranch (table 2.4). One quarter of all producers weaned between 87.3% and 90% of all calves that were turned out for summer grazing. When controlling for altitude on August 1st, herds with 100-199 and ≥ 200 calves born were, 71% and 80% more likely to have a herd weaning percentage less than the median (99.3%) when compared to herds with fewer than 100 calves born (table 2.6). When controlling for herd size, herds with calves located over 8,000 ft. on August 1st were 66% more likely to have a herd

weaning percentage less than the median (98.6%) than herds with calves located at or below 6,000 ft. (p=0.02). Herds with calves located at or below 6,000 ft. on August 1st did not have a greater weaning percentage than herds with calves located between 6,000 and 8,000 ft. (p=0.6). The county in which calves were located was not a confounder variable. This means that herds located in Gunnison County do not experience a significantly higher calf death loss than herds located within another Colorado county situated at a similar altitude.

Table 2.6: Logistic regression of the outcome, herd weaning percentage relative to the median, with risk factors herd size and the altitude of calves on August 1st.
Herd weaning percentage was dichotomized relative to the median herd weaning percentage (1= herd weaning percentage ≥ median; 0= herd weaning percentage < median). CI = 95% confidence interval of the mean.

Variable	Level	Odds Ratio (95% CI)	p value
Herd size			<0.001
	< 100	1.0	Reference
	100-199	0.29 (0.12, 0.71)	0.007
	≥ 200	0.20 (0.08, 0.47)	<0.001
Altitude of calves on August 1st			0.05
	≤ 6,000 ft.	1.0	Reference
	> 6,000 ft. ≤ 8,000 ft.	0.77 (0.31, 1.91)	0.6
	> 8,000ft	0.34 (0.13, 0.87)	0.02

On average, almost 1% of calves turned out to summer pastures were either missing or dead at weaning time in herds that had over 100 calves born (table 2.8). On average, herds with calves located over 8,000 ft. on August 1st had 1.5% of calves either missing or dead at weaning time irrespective of herd size. The average herd located at an altitude less than 8,000 ft. had 0% of calves either missing or dead at weaning time.

Table 2.7: The percentage of calves turned out to summer pastures that were known to have died and/or were unaccounted for at weaning according to the statistically significant predictor variables herd size and the altitude of calves on August 1st.

Variable	Level	Min	Q_1	Median	Q_3	Max
Herd size	< 100	0	0	0	1.7	12.7
	100-199	0	0	0.9	1.9	4.7
	≥ 200	0	0.4	0.8	1.9	10
Altitude of calves on August 1st	≤ 6,000 ft.	0	0	0	1.1	10
	> 6,000 ft. ≤ 8,000 ft.	0	0	0	1.6	7.1
	> 8,000 ft.	0	0.5	1.5	3.2	12.7

Of the 79 producers (56%) that had at least one calf die over the summer grazing period from turnout to weaning, 62.0% (49/79) of those 79 producers did see the ante-mortem signs for at least one calf. Whether a producer was likely to observe ante-mortem signs was dependent on herd size ($p<0.001$). The largest herds were on average 10.2 times more likely to have not observed the ante-mortem signs in a least one calf that died compared to the smallest herds (table 2.8). Across all herds that had at least one calf die, 39.2% (31/79) of producers did not see the ante-mortem signs in *any* of their calves that died. Producers with the largest herds were, on average, 9.7 times more likely to have not observed the ante-mortem signs in any of the calves that died compared to the smallest herds.

Table 2.8: Observation of ante-mortem signs according to herd size. OR = odds ratio, CI = 95% confidence interval of the mean.

Herd size (calvings)	Producers unaware of the ante-mortem signs for:					
	At least 1 calf			All calves that died		
	Producers within category (%)	OR (95% CI)	p value	Producers within category (%)	OR (95% CI)	p value
<100	14.8	1	Reference	6.6	1	Reference
100-199	29.4	2.4 (0.9, 6.7)	0.09	23.5	4.4 (1.2, 15.9)	0.02
≥ 200	64.0	10.2 (4.0, 25.7)	<0.001	40.4	9.7 (3.0, 31.1)	<0.001

The likelihood of a producer not observing ante-mortem signs in at least one calf that died or in all calves that died did not increase with the altitude at which the calves were located on August 1st (p=0.27 and p=0.41 respectively). Disease syndrome case fatality (% of calves affected that died) was highest for calves showing signs of high altitude disease and lowest for calves showing diarrhea (figure 2.1).

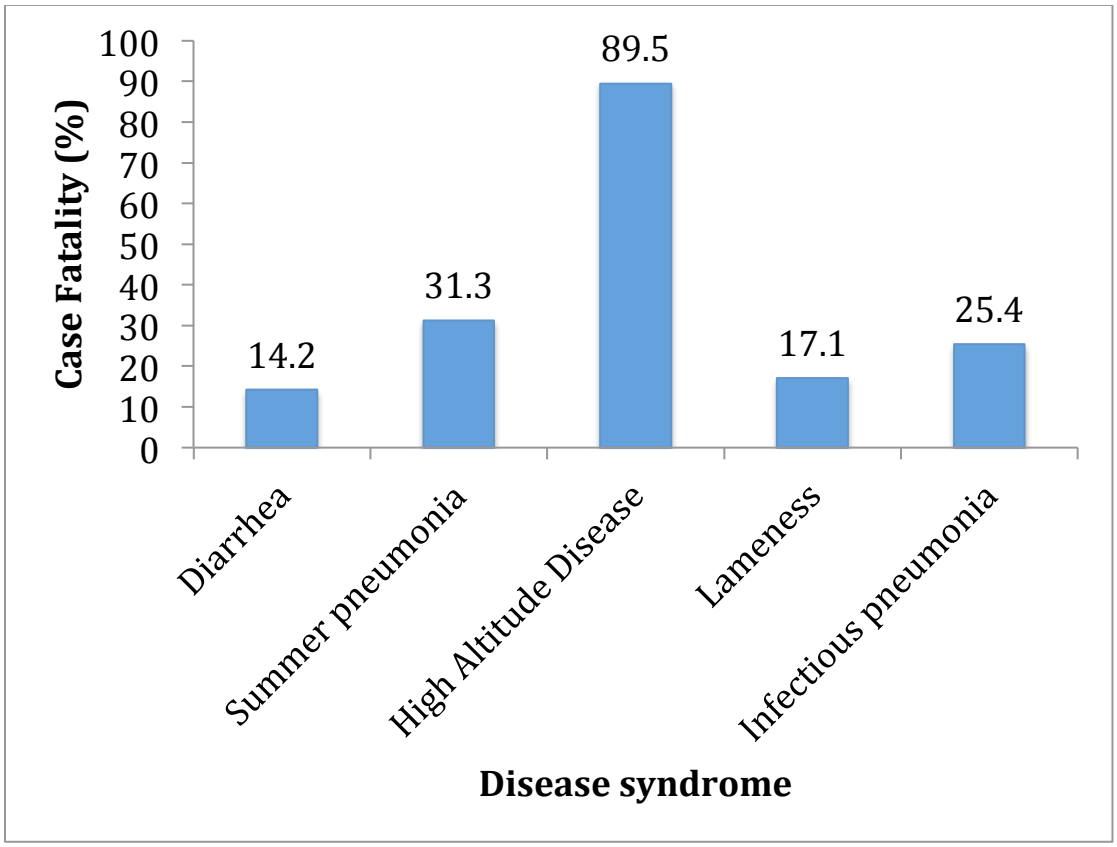

Figure 2.1: Bar chart showing disease syndrome case fatality across all herds that experienced the disease. Case fatality (%) is provided above each respective bar.

The prevalence of fatal disease at the herd level was highest for high altitude disease and summer pneumonia (figure 2.2). Infectious pneumonia was the most prevalent fatal disease amongst all calves. Diarrhea was the most prevalent non-fatal disease at the herd and calf level. Adverse weather was the most prevalent fatal and non-fatal disease of the 'other' disease syndromes. One

producer reported 160 calves were affected: 80 fatally and 80 non-fatally by a blizzard. No other producers reported that adverse weather had affected their calves.

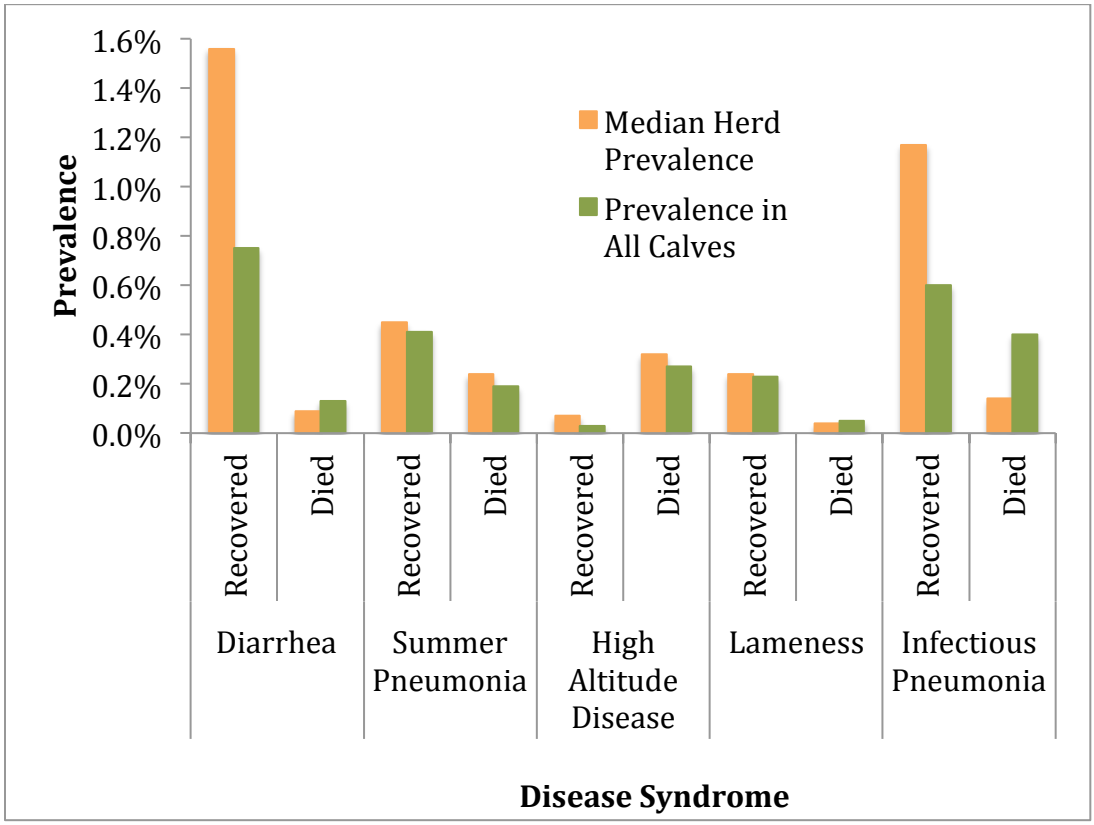

Figure 2.2: Median herd prevalence and prevalence among all calves according to disease syndrome.

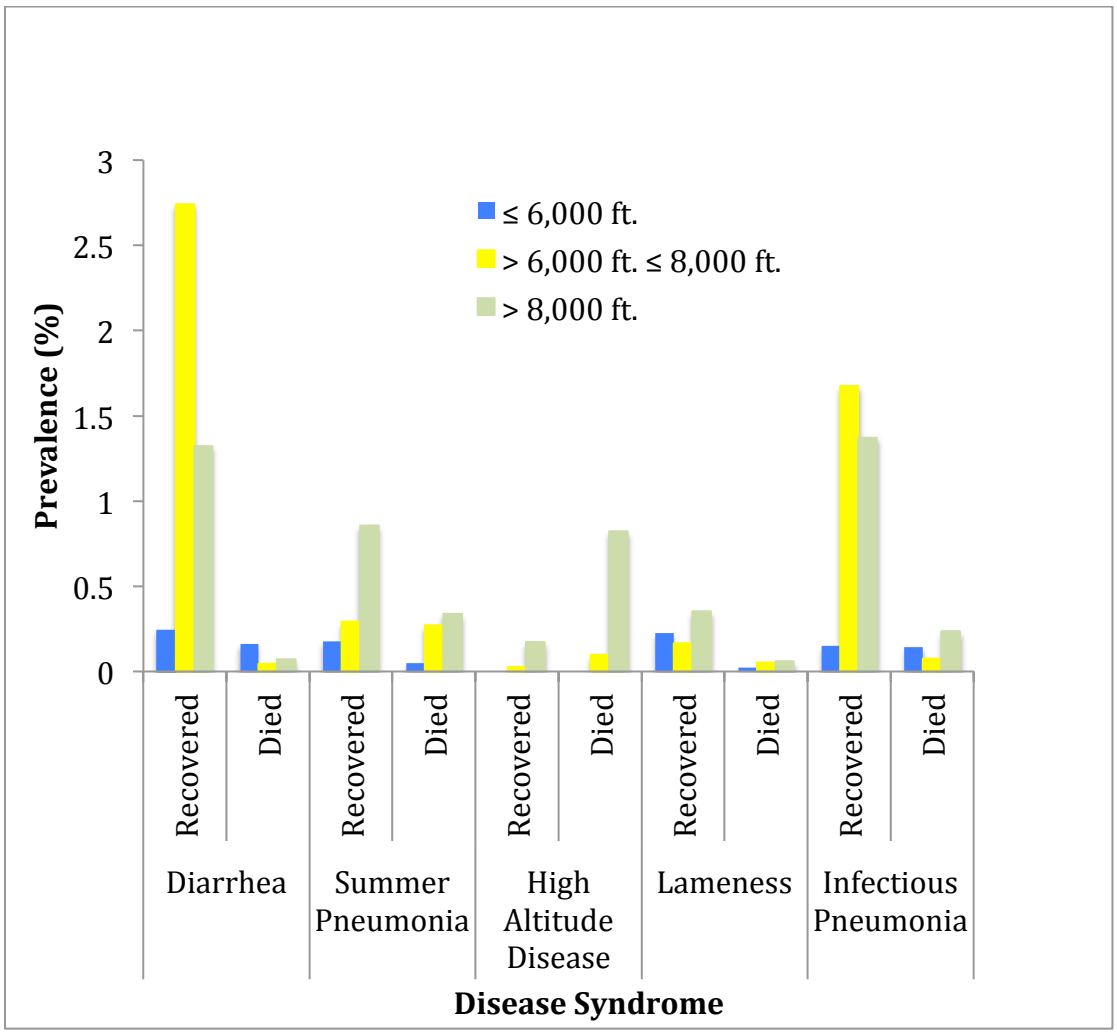

Figure 2.3: Mean herd prevalence of disease syndromes by altitude on calves on August 1st: ≤ 6,000 ft., > 6,000-8,000 ft. and > 8.000 ft.

The incidence of diarrhea, summer pneumonia and high altitude disease was significantly associated with the altitude at which calves were located on August 1st (table 2.9). The mean herd prevalence of the various disease syndromes according to altitude is shown in figure 2.3.

Table 2.9: Risk factors and incidence rate ratios for disease syndromes according to disease outcome. SE = standard error of the mean (95%).

Disease syndrome	Outcome	Risk Factor	Level	Incidence rate ratio	SE	p value
Diarrhea	Recovered					0.002
		Herd size	< 100			Reference
			100-199	0.30	0.20	0.07
			≥ 200	0.17	0.10	0.004
		Altitude on August 1st	≤ 6,000 ft.			Reference
			> 6,000 ft. ≤ 8,000 ft.	3.84	2.67	0.05
			> 8,000 ft.	4.19	2.59	0.02
	Died					0.07
		Difference in altitude August-Calving	Per 1,000 ft. difference	0.63	0.17	0.08
Summer pneumonia	Recovered					0.04
		Altitude on t August 1s	Per 1,000 ft.	1.41	0.22	0.03
	Died					0.004
		Altitude born	≤ 6,000 ft.			Reference
			> 6,000 ft. ≤ 8,000 ft.	2.84	1.54	0.05
			> 8,000 ft.	8.41	5.43	0.001
High Altitude Disease	Recovered					0.03
		Altitude on August 1st	Per 1,000 ft.	2.54	1.38	0.09
	Died					<0.001
		Altitude on August 1st	Per 1,000 ft.	2.94	0.63	<0.001
		Mortality of calves from 1-3 months (%)		1.23	0.12	0.04
Lameness	Recovered					0.36
		Average weaning weight	Per 100 lbs.	1.98	1.48	0.36
Infectious pneumonia	Recovered					0.03
		Herd size	< 100			Reference
			100-199	0.22	0.15	0.03
			≥ 200	0.22	0.14	0.02
	Died					0.23
		Altitude on August 1st	Per 1,000 ft.	1.35	0.33	0.22

Herd size and the altitude at which calves were located on August 1st were significant predictors of non-fatal diarrhea in calves. As the herd size increased from small (< 100 calves born), to

medium (100-199 calves born) to large (≥ 200 calves born) the incidence of diarrhea decreased by 60% (p=0.07) and 71% (p=0.03), respectively. As the altitude at which the calves were located on August 1st increased from ≤ 6,000 ft. to 6,001-8,000 ft. and > 8,000 ft. the incidence rate of diarrhea increased by 2.7 and 7.5 times, respectively. An increase in altitude from calving to the location of calves on August 1st by 1000 ft. decreases the likelihood of death from diarrhea by 37% (p=0.07).

The incidence of non-fatal summer pneumonia increased, on average, by 41% for every 1,000 ft. increase in the altitude at which calves were located on August 1st. The average herd prevalence for calves at 9,500 ft. was 1.1%. The prevalence of fatal summer pneumonia was similar across altitudes (figure 2.3). However, the risk period for disease (from turnout to weaning) is significantly shorter for calves born at higher altitudes (p=0.04, table 2.10). Therefore, the incidence of fatal summer pneumonia increases significantly with the altitude at which calves are born (p=0.004). A herd that calves at an altitude over 8,000 ft. is 8 times more likely to have calves die from signs consistent with summer pneumonia than a herd that has calves born < 6,000 ft.

Table 2.10: The risk period for disease (time from branding to weaning) decreases with increasing altitude of birth.

Altitude born	Herd average branding-weaning period (days)
≤ 6,000 ft.	173 (± 6.1)
> 6,000 ft. to ≤ 8,000 ft.	164 (± 5.6)
> 8,000 ft.	153 (± 6.0)

The incidence of non-fatal high altitude disease was most significantly associated with the altitude at which calves were located on August 1st (p=0.09). The incidence of fatal high altitude disease cases increased with both the altitude at which calves were located on August 1st

($p<0.001$) and the prevalence of herd calf mortality when calves are between one and three months old ($p=0.04$).

Non-fatal lameness was most closely associated with weaning weight but this was not significant statistically ($p=0.36$). For every 100 lb. increase in the average herd weaning weight the incidence of non-fatal lameness doubled. Fatal lameness was not associated with any of the risk factors analyzed in this survey.

The incidence of non-fatal infectious pneumonia was highest in herds with less than 100 calves born. In herds with 100 or more calves born the incidence decreased by 78% ($p=0.03$). Fatal cases of infectious pneumonia were most closely associated with the altitude of calves on August 1st ($p=0.22$).

DISCUSSION

Between branding in the spring and weaning in the fall beef calves in Colorado are seldom observed for two main reasons: firstly, the summer is a busy time for the cutting and storage of hay, which usually involves the majority of ranch employees; secondly, the extensive and rugged terrain over which cattle are often located during this summer period may require several hours of travelling on horseback before animals are reached. This makes the health of pre-weaned beef calves a particularly challenging subject to study.

The objective of this study was to determine the incidence of, and risk factors for, morbidity and mortality of pre-weaned beef calves between turnout onto summer grazing and weaning in the fall. The study population included all 1,870 members of the Colorado Cattlemen's Association. Of the 148 producers that responded, 142 were included in the final analysis.

Of all the calves born in 2009, 93.5% were born alive and survived until weaning; 1.9% were born dead or died within 2 hours of birth; 1.7% died within a month of age; 1.6% died between 1 and 3 months of age; 1.3% died between 3 months of age and weaning. Statistics from the most recent national survey of the cow-calf industry [15] indicate that of all the calves born during 2007, a similar percentage (93.6%) were born alive and survived until weaning across the USA. However, nationally, a greater proportion of calves were born dead (2.9%) and a smaller proportion of calves were born alive but died or were lost to other causes, such as theft, before weaning (3.5%). This difference in the distribution of calf losses may be related to Colorado's higher average altitude or large average herd size relative to other US states. With increasing herd size a greater percentage of calves over 3 weeks old die prior to weaning in the national survey [11]. For every one thousand foot increase in the altitude at which calving takes place the odds of a herd having a higher than average percentage of live births increases by 28%. This may be due to differences in calving management such as closer observation of cows due to calve.

Herds with < 100 calving animals were twice as likely to have a stillborn calf as herds with > 100 calving animals. The national data shows an approximately equal likelihood of stillbirth amongst herds of differing sizes [15]. In this study it is plausible that smaller herds tend to be

owned by hobby farmers who are perhaps less experienced in calving management than owners of larger herds.

Of calves born alive in 2009, 96.9% were turned out to summer grazing. Of all calves turned out to summer grazing, 98.2% survived to weaning. Of those calves that died between turnout and weaning, 54% were known to have died and 46% were unaccounted for at weaning. Diarrhea and infectious pneumonia were the most prevalent diseases affecting 0.88% and 0.80% of all calves between turnout and weaning respectively. Of those calves turned out that died, 45.6% died of respiratory problems (summer pneumonia and infectious pneumonia) and 10.4% died of digestive problems (diarrhea). In the national survey, of those calves older than 3 weeks that died, 31.4% died of respiratory problems and 22.6% died of digestive problems. The difference between studies may be related to the slightly different age range of calves studied and the geographic areas covered with their associated pathogens. Digestive problems tend to affect younger calves whereas respiratory problems are more prevalent in older animals [11].

Nationally, 12.2% of losses among calves 3 weeks and older are due to weather-related problems [11]. Only one rancher in this study reported calf mortality to adverse weather. This particular rancher lost 80 calves or 6% of the calves turned out in a blizzard. Due to the large number of calves that died this accounts for 25.2% of all calf losses between turnout and weaning in this survey.

Of particular interest in this study is the importance of altitude on the incidence of disease. The incidence of non-fatal diarrhea, summer pneumonia and high altitude disease increased with the

altitude at which calves were located on August 1st. Likewise, the incidence of fatal high altitude disease also increased with the altitude at which calves were located on August 1st. The incidence of infectious pneumonia increased with the altitude at which calves were located on August 1st but not with statistical significance (p=0.36). The increased incidence may be related to management factors changing with increasing altitude rather than the altitude per se. For example, the rugged terrain associated with increasing altitude may mean that finding and treating sick calves is more difficult than at lower altitudes. However, the likelihood of a producer not seeing the ante-mortem signs in at least one calf did not increase with the altitude at which calves were located on August 1st (p=0.27). More likely, the changing environmental conditions associated with altitude are responsible.

Calves at high altitude have higher baseline respiratory rates than calves at lower altitudes. A calf's respiratory rate at high altitude may be sufficient for a producer to consider it to have pneumonia. The same calf with the same degree of lung pathology but at a lower altitude may not show a sufficiently elevated respiratory rate to be considered sick. This could explain a higher reported incidence rate of respiratory problems. Calves residing at higher elevations have a lower threshold for pulmonary disease than calves residing at lower elevations since cattle have poor respiratory reserves. This is because the genetic selection of domesticated cattle has resulted in animals with small lungs relative to body size and metabolic demands [19]. Bovine lungs have approximately 25% of the lung volume per unit of body weight as compared to the mammalian average [20]. In other words, the alveolar surface area of the bovine lung expressed as a proportion of body weight or per unit of basal oxygen consumption is less than half of the mammalian average. In fact, in the resting bovine, tidal volume and pulmonary airflow are

approximately twice that of the mammalian average [20]. This means that an unusually high demand is placed on the bovine pulmonary system. Lung pathology is therefore more detrimental in the bovine than the 'average' mammalian species particularly with increasing altitude.

Higher tidal volumes at increasing altitudes may increase the risk of acquiring airborne respiratory pathogens. Firstly, for a given amount of oxygen a greater volume of air must be breathed at higher altitudes increasing the likelihood that airborne pathogens are inhaled and subsequently shed. Secondly, with increasing altitude humidity falls, resulting in drying of the tracheal cilia and subsequent impairment of mucociliary clearance of potential pathogens.

Hypoxia within the conducting airways can decrease mucociliary clearance [21] and impair macrophage phagocyctosis of bacteria in the lower respiratory tract [22, 23]. In other species, recent studies from the human literature suggest that immune function may be impaired at high altitude resulting in an increased risk of respiratory tract infections, particularly in children [24, 25]. These observations are supported by both in vitro [26] and animal model studies [27] that suggest hypoxia impairs immune function. Other physiological changes at altitude, such as elevated levels of catecholamines and cortisol, may also suppress immune function [28].

Unfortunately, there were not enough respondents to investigate breed of cattle as a potential risk factor for calf health but this should be investigated further. One might surmise that British breeds of cattle, which all originate from low altitude, are more predisposed to cardio-pulmonary diseases than breeds of cattle originating from mountainous areas of Europe such as Salers.

Producers were asked to recall only the previous year's calf crop when answering the survey since 79% of cow-calf operations nationally keep only hand-written records that may consist of notebooks and diaries [15]. Respondents likely answered questions based on memory recall rather than documented events which means that recall bias, such as the underestimation of the actual number of calf deaths that occurred, and inaccuracy may be an issue [29].

Summary

Herd size and the altitude at which pre-weaned beef calves were located on August 1st were significant risk factors for herd weaning percentage; the proportion of calves weaned that were turned out onto summer pastures. Increasing herd size and increasing the altitude at which calves were located on August 1st significantly reduced the likelihood of a herd having a higher than average weaning percentage. The latter association was due to the increased incidence of diseases involving the cardio-pulmonary system with increasing altitude such as high altitude disease and summer pneumonia. This may be because calves at higher altitudes have a lower threshold for pulmonary disease than calves residing at lower elevations since cattle have poor respiratory reserves when compared to the mammalian average. Lung pathology is therefore more detrimental in the bovine than the 'average' mammalian species particularly with increasing altitude.

PULMONARY PHYSIOLOGY AND BLOOD BIOCHEMICAL VALUES IN BEEF CALVES AT 1, 3 AND 6 MONTHS OF AGE AT ALTITUDES OVER 2000 METERS

INTRODUCTION

Organisms living in high altitude environments experience extreme physiological stressors including: low absolute and large fluctuating temperatures, low partial pressure of oxygen, high precipitation, rugged terrain and solar radiation. Species that have inhabited high altitude environments over thousands of years have evolved to cope with these stressors. For example, Himalayan yak (*Bos grunniens*), in the same genus as domestic European cattle (*Bos taurus*), have, amongst other adaptations to high altitude, a thick hair coat, a blunted hypoxic pulmonary vasoconstrictive response and thin-walled pulmonary vessels [14]. Indian cattle (*Bos indicus*, the zebu) tolerate the high altitude regions of the Himalayas and Ethiopia without problems. *Bos taurus* on the other hand is highly susceptible to developing pulmonary hypertension at high altitude [6].

British breeds of cattle, such as Hereford, were the first cattle to be introduced to the Rocky Mountain region in the mid-nineteenth century. According to the U.S. Geological Survey there are 6 U.S. states with a mean altitude of 1,500m (5,000ft) or higher: CO, ID, NV, NM, UT and WY. On January 1st 2010, these 6 states were home to 2.9 million beef cows and primiparous heifers [30]. This recent introduction of lowland cattle breeds, more acclimated to altitudes less than 1,219m (4,000 ft.), has provided little evolutionary time to adapt to the stressors of high altitude. Consequently, there are drawbacks to the production of cattle at high altitude. Cattle,

like certain other domesticated species such as swine, are particularly prone to the adverse effects of high altitude [10].

Despite the important health and economic livelihood implications for calves and their production there has been little attention paid to the effects of high altitude environments on calf physiology. In this study serum biochemistry values, hematocrit, hemoglobin concentration and PAPs were determined for beef calves at one, three and six months of age at altitudes ranging from 2,410m to 2,730m.

MATERIALS AND METHODS

Study site and herd management

The study was conducted on one ranch in Gunnison County, Colorado. The calves studied were a composite breed of British (50-75%) and Continental (25-50%) genetics. Calves were tagged at birth (March 20th to April 25th) and a Clostridial vaccine (One Shot Ultra® 8) plus growth hormone implant (Synovex® C, Pfizer) given at branding to both heifers and steers (May 15th-17th). Cows and heifers were given a pre-breeding modified live respiratory disease vaccine against IBR, BVD Types I and II, PI3 and BRSV (BoviShield® Gold 5, Pfizer). Calves were not given a respiratory disease vaccine until 2 weeks prior to weaning (October 5th). Ear notch samples are routinely taken every year from all calves kept as replacement heifers for BVD ELISA testing. No calves persistently infected with BVD have been detected in these herds to date.

Only bulls with a mean pulmonary arterial pressure (PAP) ≤ 42 mmHg when measured at 2,410m are allowed to breed the resident females. This selection criterion has been used on all potential herd sires since 1981 when PAP testing was first performed. Each year 250-350 female calves are retained as replacement heifers that is, females for future breeding. No females are brought in from an outside herd. Therefore, all females within the herd are progeny of low PAP bulls and have genetics predisposing to low PAP. Females are not PAP tested. Low PAP (≤ 42 mmHg) composite bulls (50-75% British and 25-50% Continental genetics) were bred to the dams used in this study: 1 bull for every 15 heifers, on average. The bulls were removed after one month of exposure with the heifers. All bulls are kept at an elevation of at least 2,410m year round.

Sampling procedure

All participant calves in this study were born to primiparous 2 year old Aberdeen Angus replacement heifers between March 25th and April 22nd, 2010. All dams were healthy at calving time and during the testing period. All calves were born with minimal or no assistance. Calves were born at 2,410m and transported to summer grazing at 2,730m on June 25th. The cow-calf pairs were grazed at 2,730m in June to over 3,500m in late September. In early October all animals were moved from the grazing permits to private land at a lower elevation of 2,730m.

On May 15th, calves were separated from their dams in preparation for branding. Of these calves 16 were randomly selected for the study (table 3.1). Repeat measures were performed on calves at 3 and 6 months of age unless they were excluded from the study due to ill health or death

(table 3.1). If excluded, all values associated with that individual were excluded from the dataset. On July 2nd 2010 the second test was performed after the calves had been at 2,730m with their dams for at least 3 weeks. An additional 10 animals were randomly selected for testing (n total = 24). The dams of these calves were also tested. This was the only occasion that cows were sampled. The third and final testing was performed on October 5th. A total of 50 animals were tested. Of these 19 had been previously tested; 11 tested in May and July, 8 tested in July only and 31 had not been previously tested. A cattle chute provided calf restraint during sampling.

Table 3.1: The environmental pressure and temperature and the numbers of animals sampled in total, included in the study and reasons for their exclusion according to the date of sampling.
n= total number of animals sampled, n^1= total number of animals included in the analysis

Month of testing	Animal tested	Average age (months)	n	n^1	Reason for exclusion	Number of calves previously tested	Atmospheric pressure (mmHg)	Environmental Temperature (F)
May	Calves	1	16	12	1. Septic joint (n=1) 2. Died (n=3)	-	560	62
July	Calves	3	24	21	1. Died (n=3)	12 from May	551	73
	Cows	24+	23	23	-	-		
October	Calves	6	50	48	1. High altitude disease signs 2. Died (n=1)	11 tested in May and July. 8 tested in July only.	552	59

Pulmonary arterial pressure (PAP) testing

PAP testing is used as a screening test in cattle for pulmonary arterial hypertension so that appropriate management and breeding decisions can be made. Flexible catheter tubing is passed through a large bore needle inserted into the jugular vein down through the right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connecting the catheter to an oscilloscope provides a reading of the mean, systolic and diastolic pulmonary artery pressures. A full description of the equipment, materials and facilities required for PAP testing is provided by Holt and Callan [6].

Alveolar-arterial oxygen gradient

Blood gas analysis was performed using a handheld analyzer (VetScan® i-STAT 1®, Abaxis). Sample analysis takes 2 minutes and the results are automatically stored under the animal identification number.

Arterial blood was collected from the coccygeal artery using a 22 gauge, 1 inch hypodermic needle. The coccygeal artery is a suitable source of blood from bovines for blood-gas analysis [31, 32]. Arterial blood unlike venous blood can fill a pre-heparinized syringe without applying suction. Therefore, minimal, if any, negative pressure was applied to the plunger when obtaining a sample. Approximately, 1-2 ml of blood was collected in a 3 ml syringe. The sample was discarded if during collection the flow of arterial blood was interrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before 1-3 drops of blood (95 µl required) were placed on an i-STAT cartridge. The blood was verified to be arterial against the auricular artery in a sample of 10 calves (r=0.87). Blood-gas tensions were corrected according to rectal temperature.

The alveolar-arterial oxygen gradient (δA-a O_2) is a measure of gaseous exchange efficiency. An increase in the δA-a O_2 gradient (> 10mmHg) is an indicator of poor oxygen exchange efficiency due to ventilation-perfusion mismatching, diffusion impairment or right-to-left vascular shunt [33]. The δA-a O_2 gradient is calculated using the following formula:

$$Alveolar - arterial\ oxygen\ gradient\ (mmHg) = P_AO_2 - P_aO_2$$

$$P_AO_2\ (mmHg) = FiO_2(BP - pH_20) - \frac{PaCO_2}{R}$$

P_AO_2 = Alveolar oxygen tension; PaO_2 = Arterial oxygen tension; R= Respiratory exchange ratio; FiO_2 = Percentage of inspired oxygen; BP= Barometric pressure; pH_20= Water vapor pressure at body temperature (52.4 mmHg at 39°C).

The respiratory exchange ratio, determined by dividing VCO_2 exhaled by VO_2 inhaled, varies according to energy source (pure carbohydrate R =1, pure fat R=0.7). A study of 7 cows at least 2 years old reported a mean respiratory exchange ratio of 0.91 (±0.05) [34]. A respiratory exchange ratio for similarly aged pre-weaned calves could not be found in the peer-reviewed literature therefore, a value of 0.9 was used in this study. Previous studies [35, 36] have used a respiratory exchange ratio of 1.0, which increases their estimates of δA-a O_2 relative to the present study.

Biochemistry and hematocrit

Approximately 8mls of venous blood was acquired with a 12 ml syringe through the large bore needle used in PAP testing. Of that blood, 3-4mls was placed into 5ml EDTA tubes and inverted several times. The remaining blood was placed into a 10ml glass collection tube. After allowing for clotting (30-60 minutes) the blood, in 10 ml glass collection tubes, was spun at 1,200 rpm for 15 minutes (Clay Adams®, Dynac® centrifuge). Serum was pipetted into 10 ml glass collection tubes and placed in a portable refrigerator (5^{o}C). A biochemistry panel was run on serum samples (Hitachi 917, Roche) and a complete blood count performed on the calcium-chelated whole blood samples (Advia 120, Siemens Healthcare Diagnostics Inc.) the following morning.

Oxygen supplementation

At 6 months of age 5 calves were randomly selected and a rubber sealed facemask was placed over the nares and jaw for 5 minutes (100% oxygen, 10L/min). PAP and arterial blood-gas measurements were taken both before and after supplemental oxygen.

Physiological parameters associated with mean pulmonary arterial pressure

Associations between blood-gas, hematocrit and biochemical measures with the dependent variable mean pulmonary arterial pressure were assessed. Blood-gas measures included: p_aO2, sO2%, p_aCO2, alveolar-arterial oxygen gradient. Biochemical measures included: sodium, potassium, chloride, magnesium, iron, lactate, ionized calcium, total calcium, glucose, albumin, globulin, aspartate aminotransferase, sorbitol dehydrogenase, gamma glutamyl-transferase, creatine kinase and creatinine. Hematocrit and hemoglobin concentration were also included. The month of testing was forced into the regression analysis to control for the altitude and age at which calves were tested. A backward elimination procedure was used for model building.

Statistical analysis

All statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Type 1 error rate of 5% was used. Since a proportion of calves were tested on more than one occasion generalized estimate equations (GEE) were performed to determine significant differences in dependent variables according to the month of testing. GEE accounts

for repeat measures taken from the same calves tested in different months and also accounts for calves not tested on more than one occasion. Regression analysis for the dependent variable, mean pulmonary arterial pressure, was also performed using GEE.

RESULTS

Pulmonary arterial pressure

The average pulmonary arterial pressure for all calves trended upwards from 1 month of age to 6 months of age ($p<0.001$).

Table 3.2: Mean pulmonary arterial pressures by age (month of year) and sex. n= number of animals tested and SE = standard error of the mean (95%)

Age in months (Month of year)	Sex	n	Mean PAP (mmHg)	SE
1 (May)	Heifer	4	34.3	2.1
	Steer	8	34.9	2.7
3 (July)	Heifer	8	41.9	1.9
	Steer	13	42.6	2.5
6 (October)	Heifer	25	50.5	2.1
	Steer	23	54.4	2.2
24+ (July)	Cow	23	45.6	2.0

Steers had higher mean PAPs than heifers at all ages (table 3.2, figure 3.1) but this was not statistically significant ($p=0.16$).

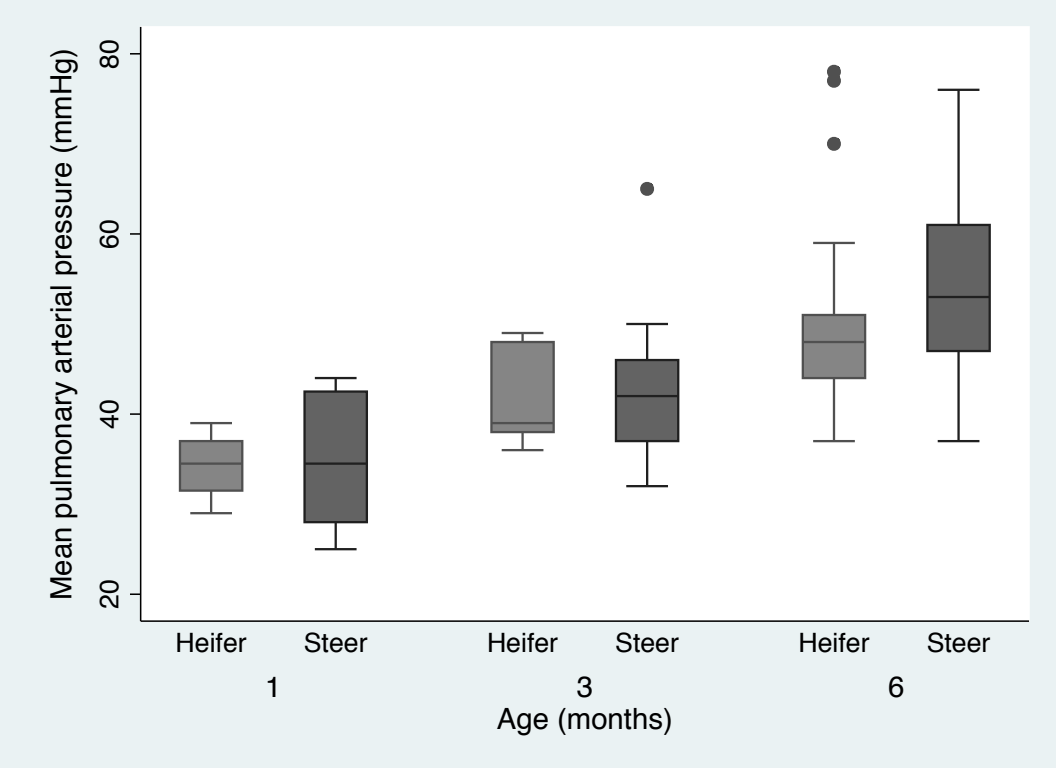

Figure 3.1: Median and quartiles of pulmonary arterial pressures by age (1, 3 and 6 months) and sex (heifer and steers).

There was no significant difference between PAP scores of the 3 month-old calves and their dams (p=0.78). The dam's PAP measure was not predictive of calf's PAP measurement at 3 months old (p=0.66).

Pulmonary Gaseous Diffusion and Hematocrit

Table 3.3 summarizes measures of pulmonary gaseous diffusion for calves and cows. Both calves and cows showed respiratory alkalosis. The pH of arterial blood from calves increased significantly from 1 month to 6 months of age ($p<0.001$). Mean arterial CO_2 tension (p_aCO_2) decreased from 1 month to 3 months ($p<0.001$) and from 1 month to 6 months of age ($p=0.006$) indicating more pronounced hyperventilation, and therefore respiratory alkalosis, with increasing

age. Mean arterial oxygen tension (p_aO_2) did not differ significantly amongst calves of different ages or between calves and their dams.

The diffusion of oxygen from the alveoli into the pulmonary blood, as determined by the alveolar-arterial oxygen diffusion gradient (δA-a O_2) did not differ significantly between calves of different ages (table 3.3). The high mean gradient (>10 mmHg) indicates poor oxygen exchange efficiency. Cows had a significantly lower gradient than their calves ($p=0.02$). Lactate, an indicator of anaerobic respiration, was higher than expected (< 1.5 mmol/L [37]) for both calves and cows with no significant difference in values.

Table 3.3: Blood variables associated with pulmonary function and acid-base status. Statistically significant differences between calves at different ages are indicated alphabetically by superscripts when p<0.05. Statistically significant differences between cows and calves (3 months old) are indicated with an asterisk. n=number of calves. SE = standard error of the mean (95%).

Variable	Cow/ Calf	Age (months)	n	Mean	SE	Reference interval
p_aCO_2 (mmHg)	Calf	1	11	40.9 [a]	1.2	42.9 ± 0.8 (sea level, n=11, [38] 45.8 ± 3.0 (Slovak Pied calves, n=6, sea level, [35]) 42.8 ±3.3 [36]
		3	13	34.5[b] (p<0.001)	0.9	45.8 ± 2.3 [35]
		6	37	37.0 [b] (p=0.006)	0.7	47.3 ± 3.0 [35]
	Cow	24+	11	34.6	1.9	
pH	Calf	1	11	7.41 [a]	0.02	7.36 ± 0.01 [35]
		3	13	7.45	0.01	7.39 ± 0.01 [35]
		6	37	7.48 [b] (p<0.001)	0.01	7.41 ± 0.03 [35]
	Cow	24+	11	7.47	0.04	
p_aO_2 (mmHg)	Calf	1	11	52.6	2.2	100 ± 2.3 [38] 87.0 ± 5.3 [35] 93.6 ± 7.7 [36]
		3	13	56.6	2.0	105.8 ± 5.3 [35]
		6	37	53.9	1.8	103.5 ± 2.3 [35]
	Cow	24+	11	59.4	2.7	
δA-a O_2 gradient (mmHg)	Calf	1	11	8.5	1.7	15.8 ± 5.3 [35] 17.3 ± 1.5 (Dutch Friesian, 17 days old, sea level, n=5 [38]
		3	13	11.7	1.5	1.5 ± 3.0 [35] 11.3 ± 0.8 (135 days, n=11, [38]
		6	37	11.6	1.5	7.5 ± 0.8 (228 days, n=5, [38] 0.8 ± 0.8 [35]
	Cow	24+	11	6.1* (p=0.02)	1.5	1.5 ± 0.4 (> 1 year old, n=19) [38] 18.0 ± 3.8 (HF cows, elevation=? n=7 [39])
sO_2%	Calf	1	11	80.3 [a]	3.1	95.0 ± 0.4 [35]
		3	13	91.3 [b] (p=0.005)	0.9	96.9 ± 0.4 [35]
		6	37	89.2[b] (p=0.007)	1.5	96.3 ± 0.3 [35]
	Cow	24+	11	92.9	0.8	
Lactate (mmol/L)	Calf	1	12	3.2 Median = 1.9	0.8	<1.3 (3-4 days old) (n=16) [40]
		3	5	2.7 Median = 1.42	1.1	
		6	18	3.4 Median = 3.0	0.7	
	Cow	24+	9	6.5 Median = 3.42	2.1	0.54 (median) (n=34) [41] <1.5 [37]
Packed Cell Volume (%)	Calf	1	0	-	-	34.8 ±7.3 (HF calves, n=20, sea level [36])
		3	9	35.7	0.9	
		6	26	35.6	0.7	33±4.6 (n= 41, 2 weeks-6 months old)[42]
	Cow	24+	5	31.4* (p=0.05)	1.5	38 ± 4.1 [43] 38 ± 5 [44]
Hemoglobin (g/L)	Calf	1	0	-	-	110 ± 22.1 [36]
		3	9	121.2	2.9	
		6	25	121.1	2.3	113 ± 14 (n= 41, 2 weeks-6 months old)[42]
	Cow	24+	5	107.0*(p=0.05)	4.9	128.0 ± 12.8 (n=59, 1.5-8 years old, Aberdeen Angus. elevation 520m [43] 138.0 ±21.0 [44]

Both packed cell volume and hemoglobin concentrations were, on average, significantly lower for cows (31.4%, 107.0 g/L) than calves (35.7%, 121.2 g/L) ($p=0.05$). Oxy-hemoglobin saturation was significantly higher for calves at 3 ($p=0.005$) and 6 ($p=0.007$) months of age than calves at 1 month of age.

Biochemistry

Blood biochemical parameters are summarized in table 3.4. Serum total calcium concentrations in calves (2.68 mmol/L) were, on average, significantly higher than in cows (2.53 mmol/L) ($p<0.001$). Total calcium concentrations in calves were, on average, significantly higher at 1 month (2.76 mmol/L) ($p=0.04$) and 6 months of age (2.76 mmol/L) ($p=0.007$) than at 3 months of age (2.68 mmol/L). Serum phosphorous was, on average, significantly higher in calves (3.16 mmol/L) than in cows (2.40 mmol/L) ($p<0.001$). In calves, average phosphorous concentrations increased significantly from 1 month (2.86 mmol/L) to 3 months of age (3.16 mmol/L) before dropping to a lower average at 6 months of age (2.23 mmol/L). Average creatinine concentrations were similar between calves at 3 months old (93.1 μmol/L) and cows (94.8 μmol/L). In calves, average creatinine concentrations were significantly higher at 6 months of age (137.6 μmol/L) than at either 1 month (110.2 μmol/L) ($p<0.001$), or 3 months, of age (93.1 μmol/L) ($p<0.001$). Sodium, chloride and magnesium concentrations agree with prior studies [42, 45].

Table 3.4: Mean blood biochemical values for calves (one, three and six months old) and cows. Statistically significant differences between calves at different ages are indicated alphabetically by superscripts when p<0.05. Statistically significant differences between cows and calves (3 months old) are indicated with an asterisk. n=number of calves or cows. SE = standard error of the mean.

Variable	Cow/ Calf	Age (months)	n	Mean	SE	Reference (Mean ±SD, n)
Total Calcium (mmol/L)	Calf	1	14	2.76[b] (p(b=a)=0.04)	0.03	2.60 ± 0.22 (n=43)[46]
		3	17	2.68[a]	0.02	2.62 ± 0.21 (n=53)[46] 2.27 (n=20) [47] Holstein 2.8 ± 0.3 (n=46) [44] Shorthorn
		6	44	2.76[b] (p(b=a)=0.007)	0.01	2.66 ± 0.18 (n=39)[46] 2.54 ± 0.10 (n= 42, 2 weeks-6 months old)[42] Holstein 2.5 ± 0.2 (n=46) [44] Shorthorn
	Cow	24+	25	2.53* (p<0.001)	0.02	2.0 ± 0.2 [43] 2.3 ± 0.2 [44] 2.3 ± 0.1 [48]
Ionized Calcium (mmol/L)	Calf	1	-	-	-	1.34 ± 0.08 (n=43) [46]
		3	10	1.34	0.01	1.33 ± 0.08 (Italian Friesian calves, elevation≈ 120m, n=53)[46]
		6	25	1.33	0.02	1.27 ± 0.05 (n=39)[46]
	Cow	24+	5	1.20* (p<0.001)	0.01	1.18 ± 0.06 (n=141, Holstein cows, ≥ 2 years old [48])
Phosphorous (mmol/L)	Calf	1	14	2.86[a]	0.07	
		3	17	3.16[b] (p(b=a)=0.006)	0.05	2.13 (n=20)[47] 3.0 ± 0.4 (n=46) [44]
		6	44	2.23[c] (p(c=a)<0.001) (p(c=b)<0.001)	0.05	2.61 ± 0.32 (n= 42, 2 weeks-6 months old)[42] 2.6 ± 0.2 (n=46) [44]
	Cow	24+	25	2.40* (p<0.001)	0.09	1.4 ± 0.4 [43] 1.6 ± 0.3 [44]
Creatinine (µmol/L)	Calf	1	13	110.2[a]	5.2	
		3	17	93.1[b] p(b=a)=0.02)	3.6	81 (n=20) [47] 97.2 ± 12.3 (n=46) [44]
		6	44	137.6[c] (p(c=a)<0.001) (p(c=b)<0.001)	3.3	71 ± 9 (n= 41, 2 weeks-6 months old)[42] 92.3 ± 9.4 (n=46) [44]
	Cow	24+	25	94.8	7.3	120 ± 27.7 [43] 103.2 ± 17.9 [44]
Sodium (mmol/L)	Calf	1	18	138.1[a]	0.5	
		3	20	143.6[b] (p(b=a) <0.001)	0.5	142 (n=20) [47]
		6	47	140.0[c] (p(c=a)<0.001) (p(c=b)<0.001)	0.3	141 ± 2.3 (n= 42, 2 weeks-6 months old)[42]
	Cow	24+	25	141.3* (p=0.001)	0.4	144 ± 17.1 [43]
Potassium (mmol/L)	Calf	1	18	5.1[a]	0.08	
		3	20	5.0[a]	0.10	
		6	47	4.4[b] (p(b=a)<0.001)	0.08	4.2 ± 0.4 (n= 42, 2 weeks-6 months old)[42]
	Cow	24+	25	4.8	0.08	5.1 ± 0.8 [43]
Chloride (mmol/L)	Calf	1	18	101.2[a]	0.6	
		3	20	101.2[b]	0.7	103.6 (n=20) [47]
		6	47	99.5[c] (p(c=a)=0.004) (p(c=b)=0.005)	0.3	99 ± 2.6 (n= 42, 2 weeks-6 months old)[42]
	Cow	24+	25	98.6* (p=0.004)	0.4	105 ± 13.9 [43]
Magnesium (mmol/L)	Calf	1	18	0.78[a]	0.02	
		3	20	0.89[b] (p(b=a)<0.001)	0.02	
		6	47	0.97[c] (p(c=a)<0.001) (p(c=b)<0.001)	0.01	0.90 ± 0.08 (n= 42, 2 weeks to 6 months old)[42]
	Cow	24+	25	1.15* (p<0.001)	0.02	1.2 ± 0.3 [43]

Oxygen supplementation

After 5 minutes of supplemental oxygen (100% O_2) mean PAP fell by 17 mmHg in one of the 5 calves (table 3.5, calf number 5) but did not change in the remaining 4 calves. P_aCO_2 increased in all calves up to 12 mmHg. P_aO_2 increased by at least 45 mmHg in all calves. The greatest change occurred in calf number 2 (+102 mmHg). Before supplementation, oxy-hemoglobin saturation was similar for all calves (82-93%). After supplementation, oxy-hemoglobin saturation (sO_2%) increased to over 97% for all calves. The oxygen index (P_aO_2/F_iO_2) for all calves was low irrespective of oxygen supplementation status. This is most likely due to the high altitude at which the calves were tested [49]. However, oxygen index did not improve when calves were given pure oxygen (F_iO_2 approximately 0.5). This may be due to low ventilation: perfusion mismatching and/or shunting of blood away from regions of gaseous exchange.

Table 3.5: Pulmonary gaseous diffusion measurements for six calves pre and post oxygen supplementation provided by a rubber-sealed facemask (100% O_2 at 10 L/min).

Variable	± O_2	Calf (Heifer/Steer)				
		1	2	3	4	5
		Heifer	Heifer	Heifer	Steer	Steer
Mean PAP mmHg (Sys/Dias)	-	43 (71/23)	57 (77/34)	48 (67/30)	48 (64/30)	74 (99/50)
	+	43 (63/19)	51 (76/21)	49 (69/32)	47 (64/31)	57 (76/41)
pCO_2 (mmHg)	-	32	36.3	40.2	37.4	41.8
	+	44	39.5	42.3	37.9	48.5
pO_2 (mmHg)	-	55	34	44.8	58	51.1
	+	100	136	105	109	132.9
sO2%	-	93	82	85	92	90
	+	98	99	98	99	99
O_2 index	-	262	162	213	233	243.3
	+	200	272	210	218	238
A-a gradient (mmHg)	-	16.2	26.9	22.3	7.37	9.2

Physiological parameters associated with mean pulmonary arterial pressure

Three measures were significantly associated with mean PAP: serum iron (p=0.003), gamma-glutamyl transferase (p=0.02) and total bilirubin (p=0.03). On average, when controlling for other variables, an increase in iron by 1 μg/dL was associated with an decrease in mean PAP of 0.05 mmHg; an increase in total bilirubin by 1mg/dL was associated with an increase in mean PAP of 42.3 mmHg; an increase in GGT by 1 IU/L was associated with an increase in mean PAP of 0.24 mmHg.

DISCUSSION

High altitude environments offer unique physiological stressors to all animals but particularly lowland breeds of livestock such as British breeds of cattle. Therefore, physiological parameters of healthy calves born and raised at an altitude over 2,438m (8,000 ft.) may differ from healthy calves raised at a lower altitude. For this reason, we have documented the changes in the pulmonary physiology and the blood biochemical and cellular changes occurring in healthy calves born and raised on one ranch in southwest Colorado at 1, 3 and 6 months of age.

Pulmonary arterial pressure (PAP) trended upwards with age supporting the observation that the risk of high altitude disease in calves is greatest in the autumn months [50]. However, from this study we cannot separate the seasonal effects, such as temperature fluctuations in the autumn, from the effect of age alone on pulmonary artery pressures. This same trend was demonstrated by Will et al. [51] who also showed that this increase in PAP with age was even greater in cattle native to sea level when compared to cattle raised at higher altitudes.

PAP was moderately autocorrelated; calves with the highest PAPs at 1 month of age tended to have the highest PAPs at 6 months of age. This may be because the effects of lung pathology on PAP in early life persist, or a genetic susceptibility to higher PAP exists from birth since PAP is moderately heritable (0.34 ± 0.05)[9]. However, maternal PAP was not predictive of her calf's PAP at 3 months old suggesting that the heritability of PAP is not phenotypically expressed until after 3 months of age. Levalley [52] found that the phenotypic expression of PAP heritability increases from birth to weaning. Steers had higher PAPs than heifers at all ages but the difference became more pronounced with increasing age. A phenomenon known as "fat steer disease" has been reported in feedlot steers with similar manifestations to high altitude disease and this may reflect the greater tendency of steers to show elevated PAP relative to heifers found in this study.

Both calves and cows exhibited respiratory alkalosis due to hyperventilation, a classic response of lowland natives to high altitude [53]. The high alveolar-arterial (δA-a O_2) oxygen gradients in calves (>10 mmHg) indicate that the pulmonary circulation is not efficiently extracting alveolar oxygen. A high δA-a O_2 gradient is expected in young calves but this should fall with increasing age as gas-exchange efficiency improves [35, 38]. The resultant systemic hypoxia may contribute to the elevated arterial lactate levels, an indicator of anaerobic respiration. Despite this hypoxic state, hematocrit and hemoglobin levels were only up to 5% and 10g/L, respectively, higher than age-matched calves at sea level [42, 45, 54]. The lower than expected hematocrit and the higher than expected potassium, phosphorous and creatinine concentrations suggest that renal function may be compromised, perhaps due to the high susceptibility of renal tissue to hypoxaemia [55].

Cows had higher oxy-hemoglobin saturation (sO_2%) than calves but a significantly lower hematocrit and hemoglobin concentration. This is important because sO_2% is predictive of pulmonary hypertension in cattle [56] and broilers [57]. One might anticipate calves to have higher sO_2% than cows since fetal hemoglobin which has high affinity for oxygen is not completely cleared from the blood until 6-7 months after birth [58].

Alexander et al. [59] found that 100% supplemental oxygen transiently reduced mean PAP but to levels still substantially higher than that of cattle measured at lower altitudes. In the present study, 100% supplemental oxygen did not reduce PAP. This suggests that the PAP, when measured at 6 months of age, is not a result of an acute hypoxia-induced vasopressor response but instead is due to more permanent changes within the vasculature. Classically, this is thought to be due to the medial smooth muscle hypertrophy in the small pulmonary arteries in response to chronic hypoxia [60].

The oxygen diffusing capacity of the lung, measured by the oxygen index, did not improve with supplemental oxygen. Since the hypoxia was not oxygen responsive this suggests that neither diffusion impairment, such as pulmonary edema, nor a high V/Q mismatch was responsible for the poor gaseous diffusion. Poor response to supplemental oxygen indicates low ventilation to perfusion (V/Q) mismatch. Hypoxemia with a low V/Q mismatch is normally due to intrapulmonary disease, heart failure or an intrapulmonary shunt. Ventilation (V), as previously discussed, was not low, which leaves high pulmonary blood flow (Q). A previous study by Will et al. [56] showed that the cardiac output of yearling Hereford steers did not increase with

increasing altitude. This suggests that it is unlikely that the low V/Q mismatch is due to high pulmonary blood flow. However, in the same study, the rate of body weight gain in the low altitude steers (1,500m, n=10) was more than double that of the high altitude steers (3,000m, n=10) over a 6-month period. Therefore, cardiac output measurements at altitude were confounded by metabolic oxygen demand. Calves born and maintained at a simulated altitude of 3,353m (11,000ft) show an underdeveloped pulmonary arterial system and an over developed bronchial arterial system relative to age-matched controls born and maintained at 305m (1,000ft) [61]. Davie et al. [62] showed that the vasa vasorum of the pulmonary vasculature significantly increases in neonatal hypoxic calves. The possibility that perhaps this provides a conduit between the pulmonary and bronchial circulations resulting in an intrapulmonary shunt cannot be ruled out. Studies in healthy humans have shown that hypoxia opens intrapulmonary arteriovenous anastomoses [63]. It has been suggested that these vessels are fetal remnants that shunt blood away from the gas-exchange capillaries when the lung is not in use [64] and may decrease in number as an animal matures, as demonstrated in lambs [65]. Perhaps, like broiler chickens, the intrapulmonary shunt fraction is correlated with rapid growth [66].

The concentrations of serum calcium (Ca) and phosphorous (P) in calves were higher than cows reflecting increased turnover of skeletal mineral during growth. The concentrations of Ca and P in calves reported here are typically higher than most prior studies [42, 44-47]. This may be related to rate of growth. The high Ca and P concentrations may predispose calves to develop soft tissue mineralization under certain pathologic states, such as high altitude disease [67], since alkaline blood conditions, tissue injury and hypoxia are all predisposing factors for mineralization of tissues [68].

Summary

Residence at high altitude is associated with unique physiology. The most dramatic serum biochemical differences when compared to calves resident at lower altitude included: higher total calcium, phosphorous, lactate and creatinine. Arterial blood-gas differences included: lower p_aCO_2, p_aO_2, sO_2% and higher pH and alveolar-arterial oxygen gradient. Strikingly, hematocrit and hemoglobin levels do not appear to compensate for the dramatic reduction in p_aO_2 associated with the reduced level of atmospheric oxygen (pO_2 = 110 mmHg at 3,000m). Mean pulmonary arterial pressures increase with age from 1 to 6 months and are not reduced by oxygen supplementation at 6 months of age suggesting that chronic changes in the pulmonary vasculature are primarily responsible for the mean pulmonary arterial pressure in this age group. The oxygen uptake capacity of the lung did not improve with supplemental oxygen, which suggests that, the ventilation: perfusion ratio of the calf pulmonary system at altitude is low. The reason for this is unclear.

BRONCHOPNEUMONIA AND HIGH MOUNTAIN DISEASE ARE THE MAIN CONTRIBUTORS TO PRE-WEANED BEEF CALF MORTALITY ON RANCHES OVER 2,000M IN COLORADO

INTRODUCTION

A cohort of 612 pre-weaned calves on one ranch in SW Colorado was monitored extensively from July 10th to October 15th 2010 during turnout onto mountainous pastures over 2,730m (9,000ft) in altitude. Clinical signs of morbid calves were recorded and whole blood, serum and arterial blood-gas samples were collected. From these were analyzed: the whole blood leukogram, serum biochemistry, seroprevalence to respiratory viruses (BVD, PI3, IBR and BRSV) and gasesous exchange efficiency. Field necropsies were performed and tissue samples taken for histopathology and bacterial culture.

MATERIALS AND METHODS

Study site

Post mortem examinations of calves were performed on 5 ranches in southwest Colorado that have selected low pulmonary pressure bulls for at least 20 years. Calves on these 5 ranches consisted of predominantly British-based breeds and were pastured at or above 8,000 ft. (2,438 m) during the summer months.

Calves on one ranch, the principle study ranch, were extensively monitored in order to maximize the number of calves observed. Calves were tracked from horseback for 6 days a week from July 10th to October 5th over which time period the calves had access to 80,000 acres. Calves were tagged at birth (March 20th to April 25th 2010) and a Clostridial vaccine containing *Mannheimia haemolytica* bacterin (One Shot Ultra® 8) plus growth hormone implant (Synovex® C, Pfizer) were given at branding (May 15th-17th). Cows were given a pre-breeding modified live respiratory disease vaccine according to the manufacturers instructions (BoviShield® Gold 5, Pfizer: IBR, BVD, BRSV and PI-3). Salt and mineral blocks (Colorado Agri-Feed 12% block with copper) were provided free choice year round. Calves were not given a respiratory disease vaccine until pre-weaning. Ear notch samples are routinely taken every year from all calves kept as replacement heifers for BVD ELISA testing. No BVD positives have been detected.

Calves were born at an altitude of 2,410m and 612 calves were transported to summer grazing at an altitude of 2,730m on June 25th. The cow-calf pairs were grazed at an altitude of 2,730m in June to over 3,500m in late September. In early October all animals were moved from the federal grazing permits to private land at a lower elevation of 2,730m. Calves were monitored from horseback for 6 days a week from 10th to October 5th over which time period the calves had access to 80,000 acres of land. To maximize both the area of land covered and the number of calves observed in a week the land was split into 3 areas of approximately equal size. A different area was covered on consecutive days so that each of the 3 areas was searched for sick and dead calves twice per week for 8-10 hours each day. One day every seven was spent transporting blood and tissue samples to the diagnostic laboratory at Colorado State University, Fort Collins.

Clinical signs evaluation

On finding a sick calf clinical signs were recorded. A standardized clinical signs (CS) form was used so that observations were systematically recorded (appendix 1). Observations included: calf identity, date, altitude, body condition score, estimated weight and signs of respiratory, cardiovascular and/or gastrointestinal disease. Signs were recorded from the first observation of ill health to recovery or death. A separate CS form was used for every subsequent day that the calf was observed. This was typically 1-3 days after the previous observation. Data from the CS forms were transferred into an Excel worksheet at least once per week.

Blood sampling

After clinical signs were recorded calves were roped or, whenever possible, restrained in a nearby chute or corral for blood sampling. In many situations calf roping is the only feasible way of restraining, sampling and treating sick calves on open rangeland. Briefly, two riders each with one 15' polyester calf rope were necessary to rope, restrain, and take a blood sample. The first rope was cast over the calf's head and the other end tied, or dallied, to the saddle horn. The calf was then walked into a position that enables a second rope to be cast around the two hind limbs of the calf by the second roper, or heeler, and dallied. Tension placed on both ropes would unbalance the calf and prevent it from standing. After the calf had been secured, a blood sample was obtained from the jugular vein using a 6ml syringe and 1.5" 16 gauge hypodermic needle. Approximately, 2mls of blood was placed in an EDTA tube and 4mls in a plain glass 'red top' tube. After allowing sufficient time (30-60 minutes) for the blood to clot at body temperature the

blood samples were placed in the nearest running stream under shade or inside a plastic re-sealable zipper storage bag containing cool stream water (6-10 ^{o}C), which was replaced every few hours. The clotted blood was spun at 3000 rpm for 10 minutes in a portable centrifuge machine (Mobilespin, Vulcon Technologies, Inc.) no later than 10 hours from the time of collection. Serum was pipetted into a plain glass blood tube and stored with the EDTA blood sample at 5^{o}C if analysis was planned to be within 2 days of collection. If analysis was not feasible within 2 days of collection the serum was frozen for future serological analysis (BVD, PI3, IBR and BRSV) and the EDTA blood discarded.

When a chute was available for calf restraint arterial blood-gas analysis was performed. Calves were given 15-20 minutes to recover from the exertion involved in chute restraint. After sufficient time had elapsed for the calf's resting respiratory rate to be reached an arterial blood sample was collected from the coccygeal artery using a 20 gauge, 1 inch heparinized hypodermic needle. Arterial blood unlike venous blood can fill a pre-heparinized syringe without applying suction. Therefore, minimal negative pressure was applied to the plunger when obtaining a sample. Approximately, 1-2 ml of blood was collected in a 3 ml syringe. The sample was discarded if during collection the flow of arterial blood was interrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before 1-3 drops of blood (95 µl) were placed on an i-STAT cartridge.

Blood gas analysis was performed using a handheld analyzer (VetScan® i-STAT 1®, Abaxis). Both CG4+ and CG8+ cartridges (Abaxis) were used during the study. Both cartridges measure: pH, pCO_2, HCO_3, TCO_2, Base Excess, PO_2 and sO_2. The CG8+ cartridge also measures:

hematocrit, hemoglobin, ionized calcium, glucose, sodium and potassium. The CG4+ cartridge also measures lactate. Sample analysis takes 2 minutes and the results are automatically stored under the animal identification number.

Healthy calves approximately 3 months old from the same herd served as controls. The control calves were sampled on July 2nd 2010 for arterial blood-gas (n=17), biochemistry (n=20) and serology (n=8) at 2,730m (9,000ft). Calcium-chelated whole blood was attained from healthy calves (n=27) on October 11th 2010 when the calves were approximately 6 months old.

Postmortem examination and tissue sample collection

A standardized postmortem (PM) evaluation form was used to record gross pathological observations (appendix 2). PMs were performed on calves found within approximately 48 hours of death. The producer euthanized moribund calves with a single .22 caliber pistol shot to the brain using landmarks previously described [69, 70]. Three sections of the right lung were taken for histopathology: the apex of the cranioventral lobe, the base of the middle lobe and the dorsal aspect of the diaphragmatic lobe. Sections, <2cm wide, were immediately placed in a plastic re-sealable zipper storage bag containing 5-10mls of formalin (10%); sufficient to coat the tissue surfaces. Samples were later immersed in a 10:1 volume ratio of formalin (10%) to tissue no later than the night of the PM. All PMs were performed or supervised by the lead investigator (Neary).

All lung lesions were sampled for aerobic culture. Tissue samples, with margins of at least 4cm, were collected at junctions adjacent to normal tissue using sterile technique. Tissues were refrigerated (5^{o}C) and submitted for culture within 24 hours if possible. After 24 hours tissues were discarded.

Of the calves that had a post-mortem examination the following ante-mortem samples were also obtained:

- Serum for biochemistry and viral serology collected within 5 days of death,
- Calcium-chelated whole blood for leukograms collected within 2 days of death,
- Arterial blood-gas performed within 53 days (bronchopneumonia) and 7 days (pulmonary arterial hypertension) of death respectively.

Statistical analysis

All statistics were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Type 1 error rate of 5% was used. Kruskal-Wallis rank tests were used for group average comparisons as sample sizes were small. Bonferroni's method was then used to adjust the comparison-wise error rate to match the study-wise error rate (5%).

RESULTS

On the principal study ranch 612 calves were turned out with their dams onto summer grazing. Of those, 59 (9.6%) died before weaning: 33 (66%) were found dead or were euthanized and 26

(44%) were not found. The corpses of 10 calves were too deteriorated to perform a postmortem. In total, 28 necropsies were performed: 23 on the principal study ranch and 5 on the other 4 ranches. Of the 28 necropsies: 13 calves (46%) had lesions consistent with pulmonary hypertension and right-sided heart failure and 15 calves (54%) died from bronchopneumonia.

Clinical signs and post mortem lesions

Lethargy, sporadic coughing, panting, drooped ears and a rough hair coat were early onset signs shared by calves with pneumonia and pulmonary hypertension (figure 4.1, table 4.1).

Figure 4.1: A morbid calf showing signs consistent with both bronchopneumonia and the onset of pulmonary hypertension: lethargy, drooped ears and a rough hair coat.

Calves with chronic pneumonia or pulmonary hypertension also showed distension and pulsation of the jugular vein and scours. Both occurred primarily in late summer to fall. Only calves with pulmonary hypertension showed brisket edema, exopthalmia, ascites and ataxia. Calves with acute or chronic pneumonia showed more pronounced open-mouth breathing than calves with pulmonary hypertension, particularly on exertion. Calves with chronic pneumonia had the lowest body condition scores. Cases of acute pneumonia occurred from mid to late summer.

Gross lesions showed 2 basic patterns. The first pattern with lesions consistent with pulmonary hypertension included: jugular distension, right cardiac ventricular hypertrophy, pericardial, pleural and/or peritoneal effusion, chronic passive liver congestion and edema of mesenteric lymph nodes, colonic mucosa and subcutis of the brisket area (figure 4.2). The lungs of these calves had the normal spongy texture and were homogeneously pink or mottled red and pink. Occasional thrombi were present in medium sized pulmonary arteries. Mucosal and sub-mucosal abomasal ulcers were present in several animals. A large thrombus was found in the hepatic portion of the caudal vena cava in one animal.

The second pattern consisted of lesions associated with bronchopneumonia. Cranio-ventral consolidation affecting over 80% of the lung was common to both acute and chronic pneumonia (figure 4.3). Acute bronchopneumonia was often associated with fibrinous pleuritis. The affected cut surfaces were diffusely red-black or mottled red and pink. Calves with chronic pneumonia had parenchymal consolidation, pleuritis with fibrous adhesions and abscessation.

Table 4.1: Pre-weaned beef calf mortality: causes of death, clinical signs, post-mortem lesions and seasonality.

Disease syndrome	Clinical signs				Gross lesions				Season
	Common			Distinguishing	Common			Distinguishing	
Pulmonary hypertension (n= 13)	• Lethargy • Cough • Panting • Dropped ears • Rough hair coat	• Jugular swelling and pulsation • Scours		• Brisket edema • Dry sporadic cough • Exopthalmia • Ascites • Ataxia • BCS ≥ 6/9		• RV dilation • Pleural effusion • Micro-ulcers in abomasum. • Muscle and fat atrophy		• Pulmonary and systemic thrombi • Roughening of PA and aortic intima • Chronic passive congestion of the liver • Pericardial and peritoneal effusion • Enlarged mesenteric lymph nodes • Edema of intestinal mucosa	Late-summer to fall
Chronic bronchopneumonia (n=2)			• Open-mouth breathing	• BCS ≤ 3/9			• >80% of lung affected.	• Fibrous suppurative pleuropneumonia	Late-summer to fall
Acute bronchopneumonia (n= 13)				• Moist cough • BCS ≥ 4/9				• Good muscle and fat content • Fibrinous pleural adhesions	Mid to late Summer

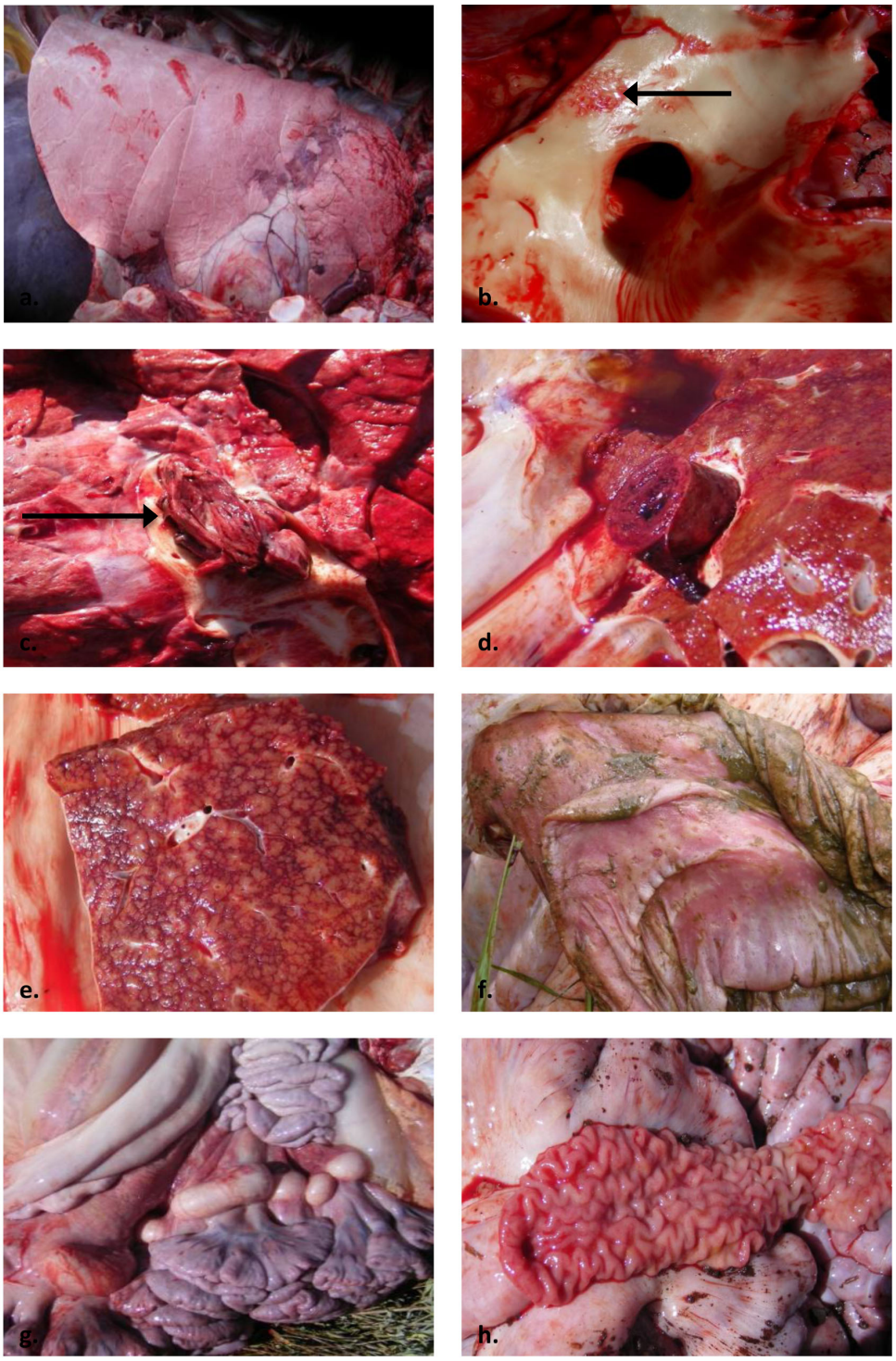

Figure 4.2: Pulmonary hypertension in pre-weaned calves post-mortem. a) Enlarged heart with spongy, pink lungs b) Roughening of the pulmonary artery intima at the level of the first bifurcation (arrow), c) Thrombi within a medium sized pulmonary artery (arrow), d) A large thrombus within the hepatic portion of the caudal vena cava, e) Chronic passive congestion of the liver f) Multiple mucosal and sub-mucosal ulcers within the abomasum, g) Enlargement of the mesenteric lymph nodes, h) Edematous folds of the colonic mucosa.

The calves with chronic, but not acute, pneumonia showed gross evidence of lesions consistent with right-sided heart failure such as dilation of the right ventricle and several clinical signs consistent with pulmonary arterial hypertension (PAH) (table 4.1). The two calves with chronic pneumonia lived for 27 and 53 days beyond the first observation of clinical signs. Of the calves necropsied, the average time until death from the first observation of clinical signs was 5.3 (± 1.2) days for acute pneumonia and 8.6 (± 5.0) days for PAH (mean ± s.d.).

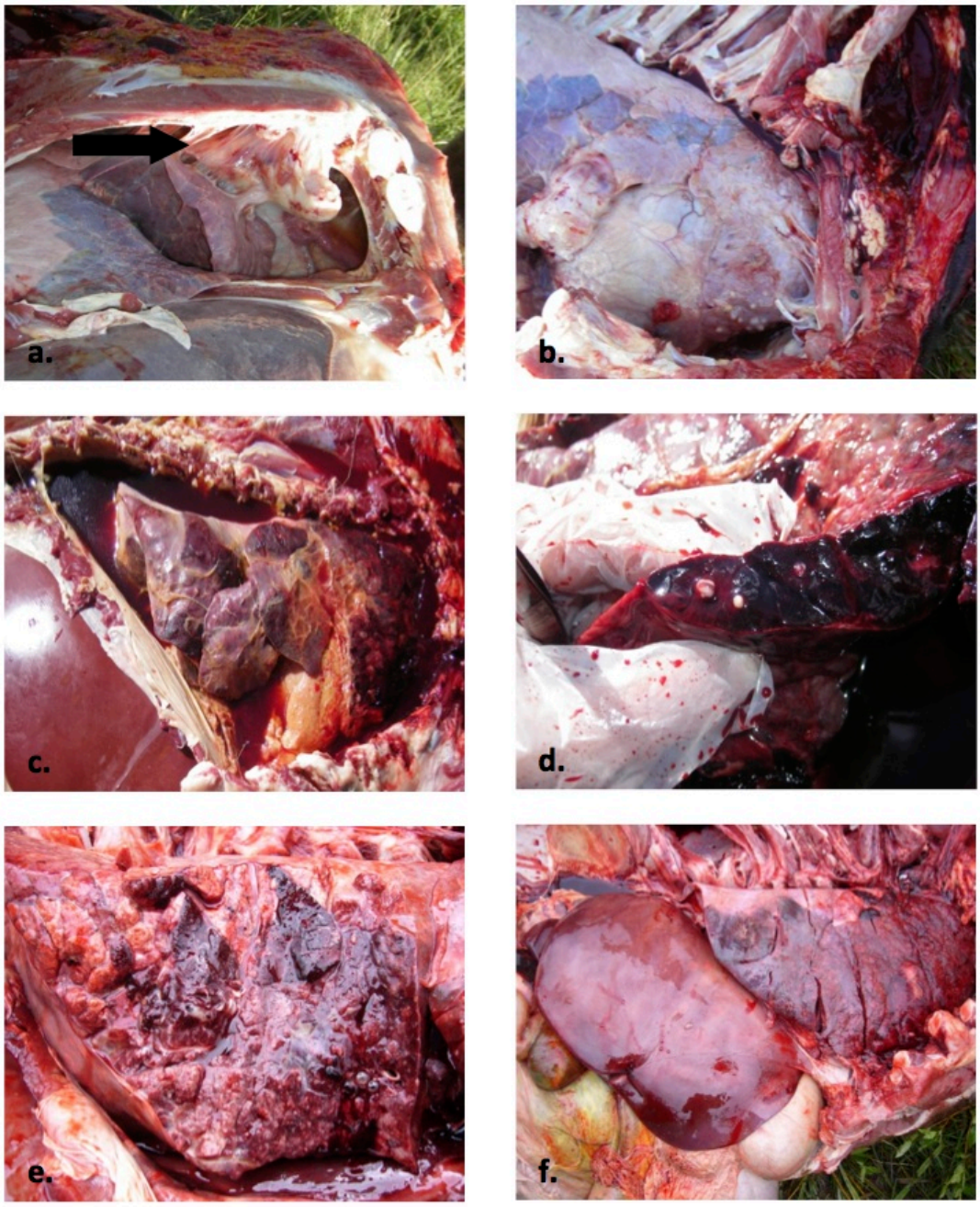

Figure 4.3: Bronchopneumonia in pre-weaned beef calves post mortem. Chronic bronchopneumonia showing: a) fibrous pleural adhesions (arrow), b) enlarged globoid heart consistent with pulmonary hypertension, c) fibrinous pleuritis, d) abcessation, e.) cross-section of consolidated lung lobules containing an airway with suppurative exudate. f) Acute bronchopneumonia.

Of the 28 calves necropsied the following were also measured:

- Serum biochemistry (n=20, 71.4%),
- Blood leukogram (n=19, 67.9%),
- Arterial blood-gas (n=14, 50%).

Serology was performed on 11 of the 15 calves (73%) that died of bronchopneumonia.

Blood gas

Arterial blood-gas analysis was performed on 5 calves that subsequently died of pulmonary hypertension 5.2 ± 1.8 days (mean ± s.d.) later and 9 calves that subsequently died of bronchopneumonia9.7 ± 5.6 days (mean ± s.d.) later. The results are compared to 17 healthy calves from the same ranch sampled at 3 months of age and located at 2,730m (table 4.2).

Table 4.2: Arterial blood-gas results from healthy 3 month old control calves and calves that subsequently died of pulmonary arterial hypertension (PAH) and bronchopneumonia. (* = p(PAH=Controls) <0.05; φ = p(PAH=bronchopneumonia) <0.05; • = p (bronchopneumonia=Controls) <0.05)

Variable	PAH			Bronchopneumonia			Controls (3 months old)		
	n	Mean	95% CI	n	Mean	95% CI	n	Mean	95% CI
Temp (°C)	8	39.6	(39.0, 40.1)	11	40.3	(39.6, 41.0)	17	39.6	(39.4, 39.7)
pH	5	7.37 φ	(7.32, 7.41)	9	7.49	(7.45, 7.54)	17	7.48	(7.46, 7.50)
pCO_2 (mmHg)	5	36.6	(31.8, 41.5)	9	30.2	(26.7, 33.7)	17	32.3	(30.5, 34.1)
pO_2 (mmHg)	5	36.6* φ	(29.6, 43.6)	9	52.7	(48.7, 56.6)	17	50.2	(45.9, 54.5)
sO_2%	5	64* φ	(50.2, 70.8)	9	89.3	(86.4, 91.8)	17	87.2	(84.3, 90.1)
Aa gradient (mmHg)	5	25.9* φ	(21.6, 30.2)	9	17.5	(11.6, 23.4)	17	18.3	(15.2, 21.4)
Oxygen index (p_aO_2/F_iO_2)	5	174.3* φ	(141.0, 207.6)	9	250.8	(232.1, 269.5)	17	239.2	(219.0, 259.4)
Lactate (mmol/L)	5	5.4	(4.2, 6.52)	9	3.4	(1.9, 5.0)	17	2.7	(0.5, 4.9)
Base excess	5	-5.8	(-9.4, 2.2)	9	-0.5	(-5.9, 4.9)	17	0.59	(-5.3, 6.5)

Although bronchopneumonia calves had higher average body temperatures there was no difference among all 3 groups ($p=0.37$). The pH of arterial blood was lower for calves with PAH than calves with bronchopneumonia ($p=0.004$) and lower than controls ($p=0.07$). For calves with PAH the P_aO_2 was, on average, 16.1mmHg and 12.7mmHg lower than calves with bronchopneumonia ($p=0.004$) and healthy calves ($p=0.01$), respectively. Similarly, the oxy-hemoglobin saturation of PAH calves was lower than bronchopneumonia calves ($p=0.001$) and control calves ($p=0.002$). The alveolar-arterial oxygen gradient was higher for PAH calves than bronchopneumonia ($p<0.001$) or control calves ($p<0.001$).

Biochemistry

Both sodium and chloride were different among all 3 groups ($p<0.001$) (table 4.3). In descending order they were highest for controls, bronchopneumonia calves and finally PAH calves. Potassium did not differ among groups ($p=0.13$). Bicarbonate was higher in controls than both bronchopneumonia and PAH calves ($p<0.001$). Phosphorous was higher for PAH calves than bronchopneumonia ($p<0.001$) or control ($p=0.02$) calves. Total calcium levels were lower for PAH calves than bronchopneumonia ($p=0.01$) or control ($p<0.001$) calves. Control calves had higher iron levels that both PAH and bronchopneumonia calves ($p<0.001$). Magnesium levels did differ among groups ($p=0.3$). Calves with bronchopneumonia had, on average, glucose levels 108.6 mg/dL and 97.4 mg/dL higher than calves with PAH ($p=0.001$) and controls ($p<0.001$) respectively.

Albumin levels were, on average, 0.7 g/dL lower for PAH calves than controls ($p<0.001$). However, globulin levels were, on average, 0.8 g/dL higher for both PAH and bronchopneumonia calves than controls ($p=0.01$). Total bilirubin was higher for PAH calves than both bronchopneumonia and control calves ($p<0.001$). The anion gap was, on average, 6.9 units higher for calves with bronchopneumonia than controls ($p=0.008$). Creatinine levels in bronchopneumonia and PAH calves were 36.7 μmol/L ($p=0.05$) and 73.8 μmol/L ($p<0.001$) higher than control calves respectively. AST levels were, on average, 76.6 IU/L higher for PAH calves than controls ($p=0.03$). GGT levels for PAH calves were, on average, 186.1 and 242.7 IU/L higher than both bronchopneumonia ($p=0.002$) and control ($p<0.001$) calves. There were no significant differences in SDH levels among groups ($p=0.29$).

Table 4.3: Biochemical values for calves with pulmonary arterial hypertension (PAH), bronchopneumoniaand 3 month old healthy controls. (a=Pulmonary hypertension b=Bronchopneumonia c=Controls)

	PAH n=10		**Bronchopneumonia n=10**		**Controls n=20**	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Sodium (mmol/L)	130.2 P(a=b)<0.001 P(a=c)<0.001	(127.1, 133.3)	138.8 P(b=c)=0.002	(135.8, 141.8)	143.6	(139.5, 147.7)
Potassium (mmol/L)	6.0	(4.7, 7.4)	5.6	(4.5, 6.7)	5.0	(4.0, 6.0)
Chloride (mmol/L)	90.3 P(a=b)<0.001 P(a=c)<0.001	(87.0, 93.6)	97.2 P(b=c)=0.04	(94.1, 100.2)	101.2	(94.9, 107.5)
HCO_3^- (mmol/L)	17.5 P(a=c)=0.004	(15.2, 19.9)	16.5 P(b=c)=0.001	(11.0, 22.0)	24.2	(19.1, 29.3)
Phosphorous (mmol/L)	3.87 P(a=b)<0.001 P(a=c)=0.02	(3.02, 4.71)	2.57	(2.15, 2.98)	3.16	(2.73, 3.59)
Total calcium (mmol/L)	2.36 P(a=b)=0.01 P(a=c) <0.001	(2.24, 2.49)	2.58	(2.41, 2.75)	2.68	(2.52, 2.84)
Albumin (g/dL)	2.9 P(a=c) <0.001	(2.67, 3.13)	3.26	(2.92, 3.60)	3.58	(3.05, 4.11)
Globulin (g/dL)	3.24 P(a=c) =0.01	(2.79, 3.68)	3.3 P(b=c)=0.009	(2.78, 3.76)	2.46	(1.17, 3.75)
Anion Gap	28.3	(26.1, 30.5)	30.7 P(b=c)=0.008	(24.8, 36.6)	23.8	(14.4, 33.2)
Creatinine (µmol/L)	164.4 P(a=c) <0.001	(125.9, 203.0)	127.3 P(b=c)=0.05	(92.0, 162.6)	93.1	(64.3, 121.9)
AST (IU/L)	150.2 P(a=c) =0.03	(95.9, 204.5)	131.2	(43.1, 219.3)	73.7	(66.8, 80.5)
GGT (IU/L)	255.1 P(a=b)=0.002 P(a=c) <0.001	(103.6, 406.6)	69.0	(15.8, 122.2)	12.5	(10.5, 14.4)
SDH (IU/L)	46.1	(8.1, 84.1)	56.3	(0, 123.7)	17.9	(15.5, 20.3)
Total bilirubin (mg/dL)	1.08 P(a=b)=0.04 P(a=c) <0.001	(0.54, 1.62)	0.51	(0.08, 0.94)	0.19	(0.16, 0.21)
Iron (µg/dL)	84.4 P(a=c) <0.001	(38.0, 130.8)	83.2 P(b=c)<0.001	(53.7, 112.7)	201	(168.5, 233.5)
CK (IU/L)	1504.6 P(a=c) =0.04	(64.5, 2944.6)	414.4	(88.7, 740.1)	282.7	(230.3, 335.1)
Magnesium (mmol/L)	0.95	(0.87, 1.04)	0.86	(0.62, 1.09)	0.83	(0.79, 0.86)
Glucose (mg/dL)	75.3 P(a=b)=0.001	(59.5, 91.1)	183.9 P(b=c)<0.001	(100.9, 266.9)	86.6	(79.6, 93.5)

Leukogram

Besides mean cell hemoglobin concentration (MCHC) there were no other statistically significant differences in the cellular characteristics of whole blood (table 4.4). MCHC was, on average, 2.2 g/dL and 1.9 g/dL higher for control calves than bronchopneumonia ($p<0.001$) and PAH ($p=0.008$) calves respectively.

Table 4.4: Whole blood cell counts for calves with pulmonary arterial hypertension (PAH), bronchopneumonia and 3 month old healthy controls. (a=Pulmonary hypertension b=Bronchopneumonia c=Controls)

	PAH n=8		**Bronchopneumonia n=10**		**Controls n=27**	
	Mean (Median)	95% CI (Q1, Q3)	Mean	95% CI	Mean	95% CI
RBC ($x10^6$µL)	10.8 (10.4)	(9.4, 12.9) (9.4, 11.9)	11.3 (11.4)	(10.8, 12.2)	11.2	(10.8, 11.5)
Hb (g/dL)	13.1 (12.8)	(12.4, 15.4) (12.0, 14.6)	13.7 (14)	(12.8, 14.5)	14.1	(13.8, 14.5)
PCV (%)	35.6 (36)	(34.0, 40.6) (32, 39)	38.6 (40.0)	(35, 42)	37.5	(36.4, 38.6)
MCV (fl)	34.3 (35.0)	(31.3, 36.1) (32.5, 36.0)	33.9 (34)	(32, 36)	33.7	(32.8, 34.6)
MCHC (g/dL)	36.9 (37.0) p(a=c)=0.008	(35.9, 38.4) (35.5, 38.0)	35.5 (35) p(b=c)<0.001	(34, 37)	37.8	(37.5, 38.1)
Fibrinogen	200 (200)	(146.6, 253.4) (200, 200)	718 (600)	(400, 1000)	489	(445, 533)
Band Neutrophils ($x10^3$µL)	0.01 (0)	(0, 0.05) (0, 0)	0.07 (0)	(0, 0)	0	
Segmented Neutrophils ($x10^3$µL)	3.7 (3.05)	(1.1, 6.6) (2.05, 4.65)	5.3 (5.1)	(2.6, 7.8)	4.5	(3.8, 5.1)
Lymphocytes ($x10^3$µL)	4.1 (3.8)	(2.0, 6.4) (3.3, 5.5)	7.3 (7.6)	(5.3, 10.5)	7.6	(7.1, 8.2)
Monocytes ($x10^3$µL)	0.3 (0.3)	(0.09, 0.60) (0.2, 0.4)	1.7 (0.6)	(0.4, 1.0)	0.4	(0.3, 0.6
Eosinophils ($x10^3$µL)	0.1 (0.1)	(0, 0.2) (0, 0.1)	1.3 (0.1)	(0, 0.2)	0.3	(0.2, 0.4)
Myelocytes	0	0	0.4 (0)	(0, 0)	0	
Blastocytes	0	0	0.07 (0)	(0, 0)	0	
Metamyelocytes	0	0	0.02 (0)	(0, 0)	0	

Sero-conversion and bacterial culture

Sero-conversion to any of the respiratory viruses (BRSV, BVD, IBR and PI-3) at antibody titer levels ≥1:256 was found in 3 of the 11 calves (27%) that died of bronchopneumonia (table 4.5). Two calves (18%) showed elevated titers to BRSV (≥1:256) and one other calf (11%) showed elevated titers to PI-3. These titers were not vaccine induced since no vaccines had ever been administered to the calves. Therefore, titers were determined from only one sample per calf. Titers from 8 healthy control calves were <1:256 for all 4 viruses.

Table 4.5: The number and percentage of calves with antibody titers equal to or greater than 1:256 according to each virus.

Virus	Bronchopneumonia n=11	Controls n=8
BRSV	2 (18%)	0
BVD-I	0	0
BVD-II	0	0
IBR	0	0
PI-3	1 (11%)	0

Aerobic culture of lung tissue from 3 calves that died of bronchopneumonia was performed. All 3 calves showed a light-moderate growth of *Pasteurella multocida* and moderate growth of *Mannheimia haemolytica.* In addition, one of the three calves showed moderate growth of *Arcanobacterium pyogenes*. The latter calf had suppurative pleuropneumonia consistent with chronic pneumonia. Samples were not submitted for *Mycoplasma* or *Histophilus* culture both of which require special culture conditions.

DISCUSSION

Producer reports from ranches over 2,400m (8,000ft) in southwest Colorado suggest that the mortality of pre-weaned beef calves may be substantially higher than the national average (6.4%, [15]). However, there have been limited field investigations of pre-weaned beef calf mortality particularly in association with high altitude. Calves summer-pastured at high altitude are scattered over a large area of rugged mountainous terrain and therefore, are seldom observed by producers or veterinarians. This study tracked a cohort of calves belonging to one ranch in southwest CO from turnout onto summer grazing at 2730m (9,000ft) until weaning, a period of 14 weeks. The objectives were to determine: the risk of calf mortality, causes of death and the associated physiological changes.

Of those calves turned out onto summer pastures 9.6% were known to have died prior to weaning or were unaccounted for at weaning. This equates to $38,800 of lost potential income between summer turnout and weaning from calf mortality alone. This is based on current (November 7th 2011) market prices ($1.24/lb live weight) and the herd average weaning weight in 2009 (529.8 ±72.4lbs) which was the same as the national average (530lbs [15]).

All of the 28 calves examined by post-mortem died of cardio-pulmonary disease: either bronchopneumonia (15/28, 54%) or pulmonary arterial hypertension (PAH) (13/28, 46%). Despite screening sires for high pulmonary arterial pressure (PAP) this producer still experiences substantial calf loss from PAH. The only currently available screening method detects PAP but not resistance to pulmonary blood flow, an attribute of vessel compliance (for a review of PAH

see Schermuly et al. [71]). This highlights a need for a greater understanding of the pathogenesis of bovine PAH so that ultimately, a more sensitive screening test for susceptibility to PAH can be developed.

The remainder of the calves (15/28) died of bronchopneumonia most likely due to members of the *Pasteurellaceae* family. Primary infections with respiratory viruses often predispose to proliferation of the opportunist residents *Pasteurella* and *Mannheimia,* which are found in the upper respiratory tract. However, the majority of the cases occurred in July, two weeks after being driven for 0.5 miles from one pasture to another along a dusty dirt road. This non-infectious trigger of inflammation could explain why only 27% of calves with bronchopneumonia seroconverted to any of the respiratory viruses. It's also possible that the duration of the disease from onset of clinical signs to death was too short for a substantial humoral immune response to develop. Calves at high altitude have less pulmonary reserve and are therefore at greater risk of death for a given degree of lung pathology than an equivalent calf with the same degree of lung pathology located at a lower altitude. In addition, hypoxia may impair immune function (for a review see [72]). The cell mediated T cell response is impaired more so than the humoral response [73]. This is possibly because immunosuppressive glucocorticoids are increased by hypoxia [74] which mainly suppress T cell function [75]. This may also explain the occurrence of bacterial pneumonia in the apparent absence of a viral infection.

Since bronchopneumonia and PAH share overlapping clinical signs particularly early in the course of the disease determining the appropriate management strategy for a sick calf may not be

very apparent to a producer or veterinarian. In fact, it may be true that many cases of PAH are falsely attributed to bronchopneumonia or 'summer pneumonia' so that the true incidence of PAH in pre-weaned calves at high altitude is currently underestimated. It is only later in the course of the disease that signs truly indicative of PAH, such as ascites, brisket edema and exopthalmos, more clearly distinguish PAH from pneumonia. Of course, it is also true that chronic pneumonia predisposes a calf to PAH [6] further blurring the disease boundaries.

The alveolar-arterial gas exchange of calves with PAH was significantly poorer than healthy calves or calves with bronchopneumonia. This was most likely due to the diffusion impairment created by thickening of the terminal branches of the pulmonary artery and a reduction in alveolar perfusion due to pulmonary thrombi. The gaseous exchange efficiency (GEE) of calves with bronchopneumonia was, on average, no different than healthy control calves. This is surprising as the inflammation and edema induced by active infection should theoretically impair gaseous diffusion [76]. However, it may be that since GEE was assessed early in the course of the disease calves had sufficient pulmonary reserve to counter the bacterial proliferation higher in the respiratory tract. Interestingly, the low oxygen index and high alveolar-arterial oxygen gradient of the control group implies that the GEE of even healthy calves was poor. This may, in part, be because optimal GEE in cattle is not reached until at least one year of age [38].

Aspects of the blood biochemical and cellular changes associated with PAH have been previously described [77, 78]. Hyponatraemia and hypochloraemia result from sequestration of fluid into extravascular spaces such as the pleura and peritoneum due to increased capillary hydrostatic pressure resulting from venous congestion. This also manifests as an alveolar-arterial

diffusion impairment of oxygen. In order to maintain blood volume and pressure calves with PAH drink water with greater frequency than apparently healthy calves. This further dilutes blood constituents such as albumin and circulating blood cells.

Dilution of blood means that a given volume of blood carries less oxygen. Therefore, the failing heart must increase cardiac output to maintain adequate tissue perfusion; this is unsustainable. The resultant ischaemia affects those organs that are most metabolically active, such as the brain, liver, kidney and heart. Poor oxygenation of the brain manifests as ataxia and mental stupor. Venous congestion of the liver results in elevation of the enzymes GGT and AST. Sorbitol dehydrogenase (SDH) is not a stable enzyme. This means that levels reported in this study may be artificially low since the refrigerated serum was not analyzed until as long as 2 days after collection. Poor renal perfusion manifests as elevated serum creatinine and phosphorus. There is currently no marker available for determining cardiac damage in the bovine but cardiac troponin is a potential candidate currently under investigation (Neary, Holt, Anderson).

Summary

Pre-weaned beef calves turned-out onto mountainous pastures over 2,730m (9,000ft) on one ranch in southwest Colorado were at a higher risk of mortality than the national average. All calf mortality was attributed to cardio-pulmonary diseases: bronchopneumonia and pulmonary arterial hypertension. It is concluded that the high-altitude environment is a significant risk factor for cardio-pulmonary diseases in calves.

DISCUSSION

Review of pre-weaning calf mortality

The two disease syndromes responsible for the greatest proportion of morbidity and mortality in pre-weaned beef calves are diarrhea (chapter 2) and respiratory tract disorders (chapters 2 and 4) [11]. Both disease syndromes have a significant positive association with altitude (chapter 2). The peak incidence of pneumonia occurs between 2-6 months of age [79], which is when calves raised in the Rocky Mountains are out on mountainous terrain in the summer months. This factor has limited previous studies of the disease syndrome commonly described by producers as 'summer pneumonia'. In this study field necropsies determined that producer descriptions of the disease syndrome 'summer pneumonia' were consistent with pulmonary hypertension, bronchopneumonia or both (chapter 4).

Unfortunately, perhaps due to prior unawareness of the problem amongst producers and veterinarians, there has been little research over recent decades to further our understanding of the pathogenesis and epidemiology of pulmonary hypertension in cattle. However, anecdotal reports suggest that pulmonary hypertension is still rife in the beef industry despite current screening methods and it is being seen at increasingly lower altitudes than ever before witnessed [80]. The reasons why 'high mountain disease' (an alternate name for pulmonary hypertension in cattle) is, 'coming down off the mountain', will be discussed later in this chapter since there are no explanations available in the literature. There are however several good reviews available discussing the known risk factors for pneumonia, or bovine respiratory disease, in beef calves

[81-83]. Bovine respiratory disease is the most devastating disease condition affecting the beef industry and with increasing incidence. In this summary chapter I will discuss plausible risk factors for both pulmonary hypertension and bovine respiratory disease not previously discussed in the published literature that this study of pre-weaned beef calf mortality has brought to light.

Mortality trends in the beef industry

There are few studies describing mortality trends in the US beef industry. A feedlot mortality study involving over 21 million cattle in 121 feedlots spread throughout the United States found that the yearly mortality ratio increased by 38% over a 5 year period: from 10.3 deaths per 1000 cattle entering the feedlot in 1994 to 14.2 per 1000 cattle entering the feedlot in 1999 [84]. Averaged over the 5 year period, 12.6 per 1000 cattle entering the feedlot died. Of those, 57.1% (7.2/12.6) died of respiratory tract disorders and 23.0% (2.9/12.6) died of digestive tract disorders. In other words, for every animal that dies of a digestive tract disorder, on average, 2.5 animals die of a respiratory tract disorder. The relative risk of dying from a respiratory disease was significantly greater in 1999 than 1994 (RR=1.46, $p<0.01$). Deaths from gastrointestinal tract disorders and other disorders did not vary statistically over time ($p=0.11$ and $p=0.52$, respectively) [84]. In summary, this study showed that the majority of feedlot deaths occurred from respiratory disorders and, unlike other disorders, respiratory disorders increased significantly over the 5 year period.

Similarly, amongst pre-weaned beef calves over 3 weeks of age, respiratory disorders account for the greatest proportion calf mortality. Of all the pre-weaned calves at least 3 weeks old that

died, or were lost, prior to weaning in the latest national survey of the cow-calf industry 37.0% (± 5.6%) died from respiratory problems and 17.7% (± 3.2%) died from digestive problems [11]. In other words, for every animal that dies of a digestive tract problem, on average, 2.1 animals die of a respiratory problem. This suggests that young cattle are more likely to die from respiratory disease over other diseases whether they are in a feedlot operations or cow-calf operation. This begs the question: why are beef cattle over 3 weeks old relatively more susceptible to dying from respiratory diseases over other disease conditions?

Sadly, it appears that the dairy industry has an even more troublesome mortality trend [85]. In the 2002 survey of the US dairy industry, it was determined that 4.2% of dairy cows die on-farm each year [86], which is much greater than the equivalent statistic in the beef industry: 1.0% of breeding cattle die per year, on average [11]. In a feedlot environment, dairy cattle have a greater relative risk of dying from respiratory tract disorders compared with beef steers (RR= 1.99, 95% CI: 1.23, 3.21) but this may be confounded by time at risk since dairy cattle typically spend longer in the feedlot environment [84]. An alternative explanation is that there may be anatomical and physiological differences between dairy and beef cattle such as, aerobic capacity relative to oxygen consumption, that predispose the former, even more so than the latter, to respiratory disease. An excellent discussion of the relevance of bovine anatomy and physiology to bovine respiratory disease susceptibility is provided by Veit and Farrell [20].

In summary, despite modern advances in vaccine technology, antimicrobial efficacy and anti-inflammatory therapeutics the incidence of bovine respiratory disease remains unabated. In order

to explore why, we must consider each of the following components of the epidemiological triad in our discussion: the environment, agent and host.

ENVIRONMENT

Animal care and husbandry practices within cow-calf and feedlot operations are well known risk factors for the occurrence of respiratory disease [87]. Dr. Temple Grandin of Colorado State University has spearheaded a movement towards low stress handling of cattle. One of the many benefits of minimizing stress is to decrease the incidence of respiratory disease. As low stress handling facilities, housing and husbandry becomes more widely accepted in the cattle industry we should see an associated fall in mortality trends.

Climate change will, if it hasn't already, alter the spread and prevalence of both native and foreign infectious diseases within the US cattle industry. The most recent example of the effect of climate change on livestock disease is the emergence of Blue Tongue Virus in Great Britain. For an excellent review of the implications of climate change on livestock disease see Gale et al. [88]. Although climate change will have an increasingly important impact on livestock morbidity and mortality it does not explain the upward trend in cattle mortality that has occurred over the past several decades.

AGENT

Cattle are exposed to a large variety of potential pathogens during their lifetime. Some, such as the *Pasteurellaceae* family of bacteria, are opportunist bovine respiratory disease pathogens of the upper respiratory tract [89]. Bovine respiratory disease is most commonly caused by: *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis*. However, these bacteria are not capable of causing disease without some other predisposing risk factor that results in an immunosuppressive event. This may be environmental, physiological or a concurrent viral infection, most commonly: IBR, BVDV, PI-3 or BRSV [90].

Immunosuppression due to stress and/or infection commonly occurs following transportation or shipping, hence 'shipping fever' as an alternate name for bovine respiratory disease. Since calves are being weaned at a younger age and at heavier weights (207 days and 530 lbs., respectively [16]) they are being shipped to feedlots at increasingly younger ages when their lungs, and immune systems are still maturing. It is also likely that the composition of breeds within the US beef industry has changed over time due to consumer demand for 'Angus beef', for example. This may have influenced trends in bovine respiratory disease incidence and mortality since breeds may differ in their susceptibility [79]. Therefore, it seems that changes in both the cattle population and bovine respiratory disease pathogen population are likely to be occurring. Whether the agents of bovine respiratory disease are becoming more pathogenic and/or virulent is currently a matter of speculation. Nevertheless, furthering our understanding of the pathogenesis of bovine respiratory disease and the virulence mechanisms of infectious agents is necessary if we are to improve our pro- and metaphylactic treatment of bovine respiratory disease [90].

HOST

The third corner of the epidemiological triangle is the bovine host (*Bos taurus*). After approximately 10,000 years of selective breeding, the modern bovine has become a paragon of agricultural efficiency and production performance [91]. Initially, cattle were selected based on temperament and use as a multi-purpose animal but, since the advent of quantitative genetics in the mid-20th century, it has been possible to dramatically improve specific production traits resulting in greater milk production in dairy cattle and faster growth, yielding heavier weaning beef cattle [92]. In 2007, the average weaning age and weight for beef calves was 207 days, 530 lbs. [93]: 60 lbs. heavier than similar calves in 1965 [94].

However, genetic selection for one trait can occur at the expense of other traits [95]. This may include increased susceptibility to cardiopulmonary disease [84]. In 1978, Veit and Farrell [20] concluded that,

> "Genetic selection in cattle for greater digestive capacity, muscle mass, milk production or growth rates is collectively increasing total body metabolic O_2 requirements relative to the anatomical pulmonary gaseous exchange capability…The relatively small bovine gaseous exchange capacity may make the ox more susceptible to high altitude disease".

Oxygen consumption of a fast growing beef calf at rest is twice that of a horse and yet they possess less than 30% of a horse's lung volume [20]. The total alveolar surface area for gas exchange expressed as a proportion of basal oxygen consumption is just 43.9% of the mammalian average [20]. This means that the bovine lung has limited, if any, reserve and works close to maximal capacity under 'normal' physiologic conditions.

Similar changes are notable in the production of broiler chickens. In 1923 it took 16 weeks to produce a broiler chicken: 70 years later it took only 6.5 weeks [95]. Selection solely for growth and feed efficiency neglected to maintain a physiologic balance between oxygen supply and demand. Fast-growing feed efficient birds have a high metabolic demand for oxygen. A broiler's risk of developing pulmonary hypertension is proportional to its oxygen demand or metabolic rate [96]. Two conflicting mechanisms have been proposed: broilers may have an inadequate pulmonary vascular capacity to accommodate the high cardiac output (CO) necessary for high metabolic demand [97]; A broiler's pulmonary vascular capacity is sufficient to accommodate blood flow in fast-growing birds [98], but these birds cannot achieve the CO necessary to meet the oxygen demand and so develop hypoxemia [66]. The result is that high-performing broilers are predisposed to pulmonary hypertension, heart failure and death. Pulmonary hypertension now costs the broiler industry $1 billion per year [99]. A New York Times report recently estimated that over 20,000 cattle die from pulmonary hypertension each year [100] but this is likely a gross underestimate as most animals die from pulmonary hypertension unobserved while out on remote, inaccessible mountainous pastures in the summer.

New thoughts on pulmonary hypertension

Bovine pulmonary hypertension has been a burden to producers in the Rocky Mountain States, killing up to 20% of a producer's calf crop [10]. Historically, bovine pulmonary hypertension has been considered a disease of increased pulmonary vascular resistance. In 1959, Alexander and Jensen reported the thickening of the distal pulmonary artery walls in association with pulmonary hypertension [101]. This led to the idea that chronic alveolar hypoxia directly resulted

in medial hypertrophy of the pulmonary arterioles [10]. The resultant narrowing of pulmonary arterioles increases pulmonary vascular resistance to the forth power (figure 5.1).

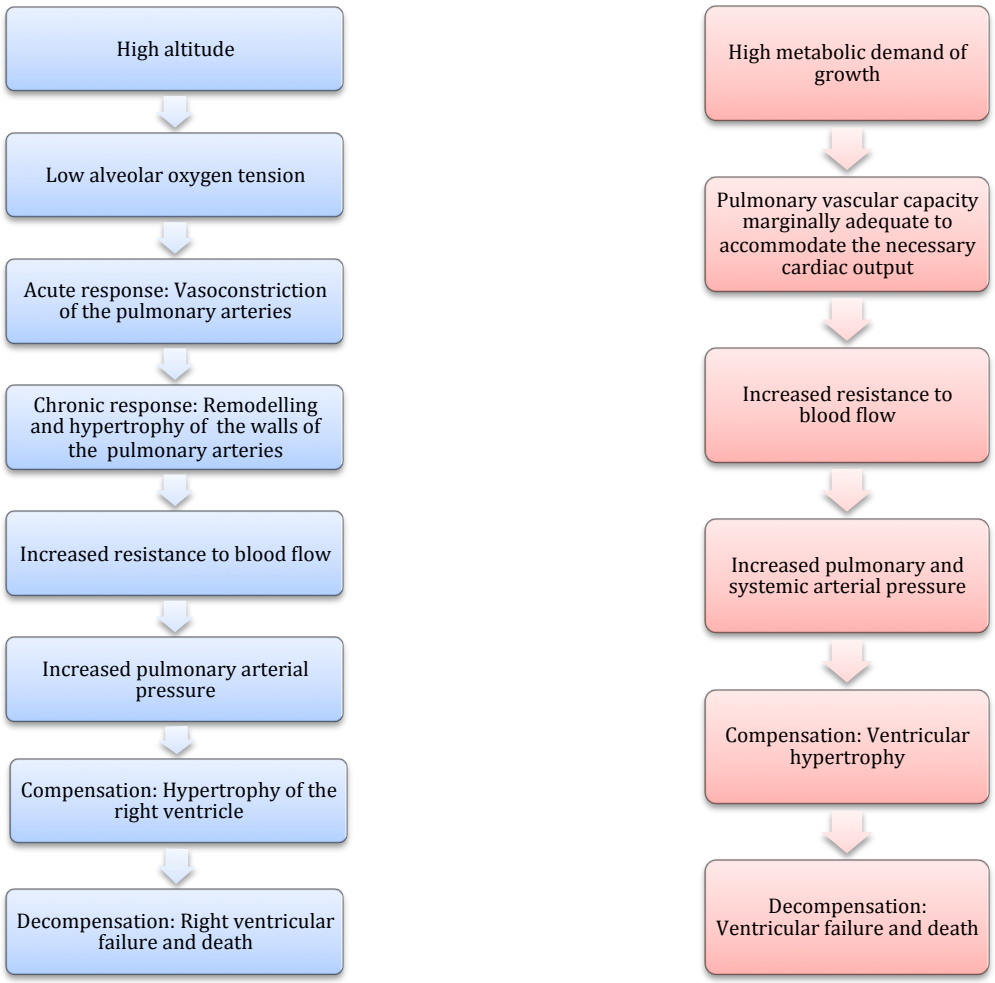

Figure 5.1: The pathogenesis of pulmonary hypertension in cattle (left) and broilers (right) as it is currently understood.

This pathway may be somewhat true but it is lacking in considerable detail. It is now known that there are several different forms of pulmonary hypertension in humans [71] and it is likely that the same is true in cattle. Histopathological examination of lesions from the proximal pulmonary artery of pre-weaned calves that died of pulmonary hypertension (unpublished, Neary) show prominent intimal proliferation and a reduced elastin: collagen ratio and areas of tissue mineralization within the tunica media. Pulmonary intimal plexiform lesions have been recorded in an experimentally induced systemic-pulmonary shunt model of pulmonary hypertension in sheep [102]. The authors of this study believe the lesions represent an inability to accommodate the increased pulmonary blood flow created by the shunt. It has been previously observed that in response to chronic hypoxia calves activate the cardiovascular system rather than the erythropoetic system [103]. These findings suggest that high cardiac output may be in part responsible for the development of pulmonary hypertension in calves as it is in broilers. The pathogenesis of pulmonary hypertension in cattle and broilers is likely to share many similarities (table 5.1).

Table 5.1: Similarities in the pathogenesis of pulmonary hypertension in cattle and broilers.

Risk Factor for pulmonary hypertension	Broilers	Cattle
Low oxygen environment • Condition first discovered at high altitude • Now affects animals at low altitude	[104] [96]	[8] Now occurs in feedlots at 4,000ft [80]
High rate of growth	[96]	[9] 4.5 mmHg/1 lb. average daily gain (Neary, unpublished)
Poor pulmonary vascular capacity	[97]	[105] Further reduced by chronic hypoxia [106, 107]
Insufficient cardiac output	[66]	Poor cardiac and respiratory reserve in double-muscled breeds [108]
Loss of pulmonary vessel wall elasticity	[109]	[12]
Ventilation: Perfusion mismatch	[110]	(Chapter 3)
Small lung volume: body surface area	[97, 111]	[20]

Since pulmonary hypertension is thought to be a heritable trait [112-115] the selective breeding of cattle determined to be pulmonary hypertension resistant [6] for over 30 years should have virtually eliminated the disease. Instead, pulmonary hypertension remains prevalent at altitudes over 8,000 ft. (chapter 2) and appears to be occurring with increasing incidence at lower altitudes where pulmonary hypertension has not previously been found [80]. Pulmonary artery pressure testing is only a modestly useful screening tool as it only evaluates pulmonary vascular resistance, the static component of pulmonary hemodynamics [116]. It cannot assess stiffening of the proximal pulmonary arteries [12], chronic inflammation [117, 118] or alterations in pulmonary blood flow [119] all of which can contribute to pulmonary hypertension.

Studies investigating the genetics of bovine respiratory disease have become more numerous. The genetic heritability of pre-weaning bovine respiratory disease has been estimated to be low (0.08 ± 0.01 [120], 0.10 ± 0.02 [121]). However, genetic differences in susceptibility to the clinical expression of bovine respiratory disease are difficult to interpret as comparisons occur across different locations, year-year variations in bovine respiratory disease risk and year-year variations in the presence or absence of various breeds at the feedlot [79, 121]. Most importantly, prior studies have not taken subclinical pulmonary lesions into consideration and so they have really only estimated the phenotypic heritability of bovine respiratory disease in weaned calves and not the true genetic heritability of bovine respiratory disease. In order to estimate the latter, the extent, type and severity of pulmonary lesions should be included in all genetic studies of bovine respiratory disease heritability. Since pulmonary lesions are almost as prevalent in apparently healthy calves as they are in calves expressing signs of bovine respiratory disease [122] failure to account for them may explain why some studies, such as Martin and Bohac [123], did not find a negative association between bovine respiratory disease and weight gain. In fact, when accounting for pulmonary lesions, the association between bovine respiratory disease and weight gain becomes statistically non-significant [122].

Mugglicockett et al. [121] suggest that their heritability estimates of bovine respiratory disease resistance in both pre-weaned and weaned calves were low because fitness traits have low heritability. This may be particularly true in populations that have been selectively bred by humans, such as the domestic dog and livestock. Domestic cattle have been subject to selective breeding by humans primarily based on production and performance traits rather than fitness traits (reproductive success). In nature, an animal balances its energy between survival and

reproductive success. It cannot maximize both since it has limited energy resources to do so [124, 125]. This may explain why the modern beef calf can outperform its ancestors in production measures such as, rate of growth and muscling, but is more likely to succumb to disease. The selective breeding of energetically costly high performance traits in cattle has occurred at the expense of fitness traits. In other words, high performance traits are a handicap to survival. The result is that the expected longevity of an animal has been steadily decreasing over time and will continue to do so, meaning that beef calf mortality will continue to trend upwards. Modern advances in antimicrobials, vaccines and husbandry can do little to correct the balance between survival and performance.

Growth implants, resistance to infectious disease and pulmonary hypertension

In the process of collecting data for this project an observation was made: of the five producers in the Gunnison valley involved with this project, only one producer used hormone growth-promoting implants in his pre-weaned calves. This producer consistently has the highest prevalence of pre-weaned calf mortality between turn out, shortly after branding when the implant is given, and weaning. This may coincidental, but a review of the literature suggests other plausible explanations.

The most common steroidal growth implants used alone or in combination in the beef industry are: estradiol, progesterone, testosterone, zeranol, melengesterol acetate and trenbolone acetate. They can be more broadly categorized as estrogens, progestins and androgens. Growth-promoting hormones were first approved for use in the United States beef industry in the 1950s and have been used extensively since the 1970s [126]. On average they increase weaning weight

by 10-20 lbs. when given to a calf at approximately 2 months of age [127]. There are numerous studies documenting the benefit of implants on beef production productivity (for a review see [127]) but few studies have documented the impact of these sex hormone implants on the health of animals particularly at environmental extremes. Estradiol benzoate/progesterone implants were first approved for use in steers in 1956 [126]. At this time, calves 200-219 days old weighed, on average, 472 lbs. (n=577 [94]). In 2007, the average weaning age was 206.7 days (± 1.1) and weaned calves weighed, on average, 530 lbs. (± 2) [93]: a 60 lb. increase. Is it reasonable to assume that implants are just as safe in these two very different populations of animals? Some of this increase in weaning weight can be attributed to the 9.8% (± 0.7%) [16] of producers that implant some calves prior to weaning but the majority appears to be genetics.

Evidence exists that use of estrogenic implants may be adverse to health during periods of high heat stress (30-40°C) as estrogen seems to impair body heat loss through an unknown mechanism [128]. This may be an issue for dark colored cattle at high altitude where solar radiation is substantial and may explain the relatively high rectal temperatures of both healthy (chapter 3) and sick calves (chapter 4) in this study when compared to age-matched animals at lower altitudes.

By increasing an animal's rate of weight gain by on average 0.23 kg/d and, total body muscling [127], implants consequently increase an animal's basal metabolic energy requirements or oxygen demand. Unfortunately, there are few studies documenting the change in oxygen demand in association with growth promoting implants. Under the hypoxic conditions of high altitude any increase in oxygen consumption for a calf means a disproportional increase in the effort of

breathing than a calf at a lower altitude, which also increases the metabolic demand for oxygen. Rapid, shallow breathing increases basal metabolic rate by 7% and deep, open-mouthed breathing increases basal metabolic rate by 18% [129].

The implant used by the cow-calf producer in Gunnison is specifically designed for use on pre-weaned calves and contains 100mg progesterone and 10mg estradiol benzoate per implantation, which is released over a period of 4-8 weeks. These hormones have been found to suppress both innate and adaptive immune responses in the female reproductive tract to pathogens [130]. It is thought that susceptibility to infection from viruses, such as HIV, could be increased by use of hormonal contraceptives, which contain sex hormones [131]. One influence of progesterone during the metestrus and diestrus or secretory stage of the menstrual cycle is to suppress CD8+ T cell activity in the female reproductive tract even though the number of T cells remains the same [132]. It has also been shown that mucosal responses to intravaginal immunization with herpes simplex virus are suppressed by progesterone [133]. What effect does a super-physiological dose of estradiol and progesterone have on the developing acquired immune system of a pre-weaned calf or the mucosal immunity of the lung? The lung is directly connected to the external environment and is therefore, exposed to large variety of pathogenic and innocuous particles. The threshold at which an immune response is initiated must be high enough that it doesn't initiate a dangerous inflammatory event in response to an innocuous particle [134] but not so high that it cannot maintain control of opportunistic pathogens of the upper airways. Is it safe to assume that steroidal growth implants have no impact on this critical balance?

Growth implants and pulmonary hypertension

The influence of the sex steroids estrogen and progesterone on cardiovascular disease is a contested issue in human medicine. The role of sex steroids in pulmonary hypertension remains unclear and even contradictory (for a review see [135, 136]). At the cellular level, estrogen and progesterone promote vascular smooth muscle cell motility [137], which could plausibly mean that they promote the vascular remodeling associated with pulmonary hypertension and other vascular diseases. Any of the supposed protective cardio-vascular benefits of estrogens may be opposed when supplementation is provided in combination with progestins [138]. It is apparent that calves could serve as a suitable human model for studies investigating the effect of sex steroid supplementation on pulmonary immune function and cardiovascular disease.

Summary

In summary, pre-weaned beef calves are highly susceptible to cardio-pulmonary diseases particularly with increasing altitude. This susceptibility continues post-weaning into the feedlot where the trend for cardiopulmonary diseases may be increasing. Our current understanding of pulmonary hypertension is insufficient to explain the increasing incidence of the disease despite selective breeding of animals with low pulmonary arterial pressures. It is suggested that the pathogenesis of pulmonary hypertension in cattle shares many similarities with pulmonary hypertension in broilers. Future studies should determine genetic markers for cardiopulmonary function so that future selection of performance traits can maintain a healthy physiological balance.

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APPENDIX 1

Ranch.....Calf ID .....

Date .....

Location .....Altitude (approx.).....ft.

Vegetation present; Larkspur Lupine Locoweed

Other.....

Previous treatment Yes ☐ .....

No ☐

BCS ...../9 Est. weight.....

Respiratory

A. Rate .....breaths per minute

B. Effort Mild ☐ Moderate ☐ Severe ☐

C. Nasal discharge Yes ☐ No ☐

D. Cough Yes ☐ Nov ☐

Cardiovascular

A. Lethargy Yes☐ No ☐

B. Distension; Under jaw ☐ Jugular vein☐ Brisket☐

Abdomen ☐

Gastrointestinal

A. Nursing/Eating Yes☐ No ☐

B. Diarrhea Yes ☐ No ☐

Other systems

A. Ocular discharge No☐ One eye ☐ Both eyes ☐

B. Ocular redness No☐ One eye ☐ Both eyes ☐

C. Hair coat appearance .....

COW BCS...../9 Write any other cow and calf observations and any treatments given on back of sheet.

APPENDIX 2

Ranch..... Calf ID ..... Date.....Time.....am/pm. Necropsy performed by.....BCS...../9

Location..... Altitude (approx.).....ft. Vegetation present *(circle)*; Larkspur Lupine Locoweed

Other..... Previous treatment Yes☐ No ☐ Est. weight..... Necropsy performed with animal on☐ left ☐ right side

Organ *(Check if abnormal)*

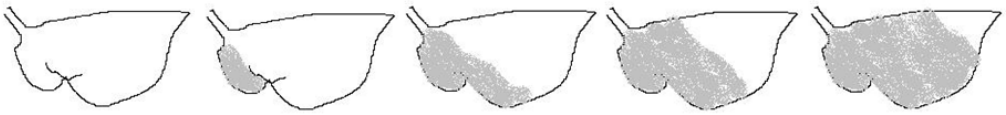
☐ Eyes.....	☐ Liver.....
☐ Mouth and tongue.....	☐ Kidneys.....
☐ Trachea.....	☐ Stomach.....
☐ Heart.....	☐ Intestine.....

Other observations.....

.....

Suspected cause of death ☐ Respiratory Disease ☐ Digestive☐ Other.....

Lung consolidation *(check appropriate score for each lung)* ▨ = lung consolidation



Right lung	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
Left lung	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4

Cow BCS ...../9 Please write any additional comments about necropsy or cow health on back of sheet.