THE DEVELOPMENT AND ASSESSMENT OF A LUNG BIOPSY TECHNIQUE FOR EARLY BRD DETECTION

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By

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ABSTRACT

THE DEVELOPMENT AND ASSESSMENT OF A LUNG BIOPSY TECHNIQUE FOR EARLY BRD DETECTION.

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The objectives of this project were: 1) to determine if live animal lung biopsy could be used to characterize early pathologic changes in the bovine lung associated with bovine respiratory disease (BRD), 2) determine if specific infectious respiratory pathogens can be identified in association with early pathological changes, and 3) determine whether pulmonary pathology characterized by live animal lung biopsy at arrival and at the time of initial BRD diagnosis was associated with health and production outcomes of feedlot steers in a commercial feedlot.

A live animal percutaneous lung biopsy technique was developed to obtain a lung sample from the right middle lung lobe in intercostal space (ICS) 4 using a Bard® Magnum® reusable biopsy instrument and a modified 4-mm (8g) biopsy needle. The lung biopsy procedure was limited to 2 attempts per biopsy time. In the technique development, 34 animals chronically affected with BRD were utilized, 20 animals in the preliminary development followed by 14 additional animals in a commercial feedlot setting. The technique resulted in 1 fatality of 34 steers (2.9%) and lung parenchyma was harvested in 19 of 34 steers (55.9%) chronically affected with BRD. In addition, in the commercial feedlot setting this procedure was determined to take about 20 minutes per animal.

The final study was performed on one hundred feedlot steers considered at high risk of developing BRD from twenty pens within a commercial feedlot. Study animals were enrolled in

three different groups: sick on arrival (ARR-SA) consisting of 27 study animals and 13 matched control animals; pen pulls with no fever (PP-NF) consisting of 14 study animals and matched 7 controls; and pen pulls with an undifferentiated fever (PP-UF) consisting of 26 study animals and 13 matched controls. Live animal percutaneous lung biopsies were collected from the right middle lung lobe at 3 different times within the first 30 days of the feeding period, about 2 weeks apart. All samples were histopathologically evaluated and were assessed for the presence of *Mycoplasma bovis*, *Mannheimia haemolytica*, *Histophilus somni* and bovine viral diarrhea virus with immunohistochemistry.

A total of 295 lung biopsies were performed yielding 210 (71.2%) lung samples that were sufficient for histopathological evaluation. A histopathology score was awarded to each biopsy based on certain histopathological lesions being present. Only 20 lung biopsy samples from 19 animals received a histopathology score (ie, pulmonary lesions were present) with the most common score being a 1 (maximum score is 20). There were too few lung biopsy samples with a histopathology score to reveal any association with subsequent health events.

Immunohistochemistry (IHC) was performed on all lung biopsies recovered yielding one lung sample to be positive for both *Mannheimia haemolytica* and *Mycoplasma bovis* from the PP-UF group. There were too few positive samples to reveal any association between IHC and histopathology score.

A post mortem evaluation was performed by a study veterinarian on all study animals who died or were humanely euthanized due to poor treatment response. In this study only 4 steers died or were euthanized due to poor treatment response and 3 control steers were humanely euthanized. There were too few animals to reveal any association between histopathology score and post mortem diagnosis.

On entry into the feedlot, weights between ARR-SA and the PP-UF and PP-NF groups were significantly different ($p<0.05$). This is likely an effect of the different processing groups of cattle. At study allocation, the body weights of ARR-SA and PP-UF, PP-UF and their matched controls, and PP-NF and their matched controls were also significantly different ($p<0.05$). This is likely due to the PP-UF and PP-NF groups experiencing illness for a longer period of time resulting in greater weight loss than the ARR-SA animals as well as the control animals, who were not clinically sick.

The live lung biopsy procedure utilized in this study did not appear to cause any long lasting adverse effects as the BRD case fatality rates from the study animals were comparable to the overall case fatality rates reported by the feedlot for fall placed calves. In fact, the study animals experienced a decreased fatality rate compared to the feedlots overall fatality rate. This may be due to the study animals inadvertently being monitored more closely as the pen checkers were aware of and participating in the study. On post mortem evaluation there was no evidence of adhesions at the biopsy site. This procedure was performed on 134 feedlot steers resulting in only 2 acute deaths as a direct result of the live animal percutaneous lung biopsy procedure.

The results of this study indicate that live animal, percutaneous lung biopsy can be performed safely on feedlot steers in a commercial feedlot with few clinical side effects. In this study there were only 2 fatalities in 134 steers (1.5%) due to the biopsy procedure or 2 fatalities per 349 sampling times (0.6%) This technique did not prove useful either as a diagnostic tool for the determination of early lung pathology in BRD or as prognostic indicator for health and production outcomes. However, this lung biopsy technique may be a useful diagnostic tool for chronic pneumonia assessment.

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DECLARATION OF WORK PERFORMED

I, Brandy Ann Burgess, declare that this thesis is my own original work, conducted under the supervision of Dr. Steve Hendrick. It is submitted for the degree of a Master of Science at the University of Saskatchewan. The work performed for this thesis was carried out in Veterinary Biomedical Science department at the Western College of Veterinary Medicine, University of Saskatchewan between July 2006 and June 2009. All experimental results were obtained by me alone and to the best of my knowledge have not been published previously by any other person. No part of this research has been submitted in the past, or is being submitted, for a degree or examination at any other university.

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LIST OF ABBREVIATIONS

AAM – activated alveolar macrophages

ABP – Alberta Beef Producers

AF – alveolar fibrosis

AIP – acute interstitial pneumonia

AIR – alveolar interstitial reaction

ANI – alveolar neutrophilic infiltration

ARR-CT – sick on arrival control group

ARR-SA – sick on arrival group

BAL – bronchoalveolar lavage

BALT – bronchoalveolar associated lymphoid tissue

BB – Bard® Magnum® reusable biopsy instrument

BNI – bronchiolar neutrophilic infiltration

BO – bronchiolitis obliterans

BoHV-1 – bovine herpesvirus-1

BRD – bovine respiratory disease

BRSV – bovine respiratory syncytial virus

BVDV – bovine viral diarrhea virus

Bx – biopsy

CFIA – Canadian Food Inspection Agency

CP – chronic pneumonia

DOF – days on feed

Dx – diagnosis

ELISA – enzyme linked immunosorbent assay

FNA – fine needle aspirate

ga – gauge

H. somni – Histophilus somni

IBR – infectious bovine rhinotracheitis

ICS – intercostal space

IFAT – immunofluorescent antibody test

IM – intramuscular

LPS – lipopolysaccharide

M – middle lung lobe

M. bovis – Mycoplasma bovis

M. haemolytica – Mannheimia haemolytica

mm – millimeters

N – no

N/A – no lung tissue collected or unable to make a diagnosis

NL – no lung

No. – number

NPS – nasal pharyngeal swab

PAMs – pulmonary alveolar macrophages

PCR – polymerase chain reaction

PDS – Prairie Diagnostic Services

PI – persistently infected

PI-3 – parainfluenza virus-3

PM – post mortem

PNI – pleural neutrophilic infilatration

PP-NF – pen pulls with no fever

PP-CT – pen pull controls

PP-UF – pen pulls with undifferentiated fever

SB – suppurative bronchopneumonia

SC – subcutaneous

SD – standard deviation

TC – Tru-cut biopsy instrument

TTW – transtracheal wash

U.S. – United States

WCVM – Western College of Veterinary Medicine

Y – yes

CHAPTER 1

GENERAL INTRODUCTION

Bovine respiratory disease (BRD) is an important disease of feedlot cattle accounting for 10-61% of mortality in western Canadian feedlots (Church and Radostits 1981; Ribble, Meek et al. 1995) and 58.7% of morbidity (Church and Radostits 1981). BRD or shipping fever refers to the development of infectious bronchopneumonia secondary to the stress of shipping (Andrews and Kennedy 1997). Lesions usually develop in the cranioventral lung lobes and will likely be bilateral in distribution (Andrews and Kennedy 1997). The most common bacterial isolates from fibrinosuppurative bronchopneumonia are *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* (Andrews and Kennedy 1997; Gagea, Bateman et al. 2006). Bovine respiratory disease will clinically be seen 7 to 14 days after a stressful event such as transportation, passing through auction markets and comingling (Wikse 1985; Harland, Jim et al. 1991; Ribble, Meek et al. 1995).

Cattle seem to be prone to the development of BRD. They have complete interlobular septa resulting in no collateral ventilation with impaired cellular movement and debris removal. Feedlot steers are put through many stressful events at an early age including weaning, minor surgical procedures such as castration and dehorning, as well as transportation and mixing at auction markets. Often times these events are occurring closely in time resulting in stress which compromises the immune system. Additionally, these calves are comingled with cattle from many different sources resulting in exposure to more potential pathogens as well as novel microorganisms.

Currently, no blanket treatment or prevention strategy has been found to be completely effective for BRD prevention. Many studies have been done to determine if preconditioning programs are effective at BRD prevention however have resulted in mixed reviews (Woods, Mansfield et al. 1972; Woods, Pickard et al. 1973; Pritchard and Mendez 1990; Macartney, Bateman et al. 2003). Studies evaluating prophylaxis or metaphylaxis, however have consistently shown this practice to reduce BRD associated morbidity and mortality in the feedlot (Lofgreen 1983; Schumann, Janzen et al. 1990; Harland, Jim et al. 1991; Schumann, Janzen et al. 1991; Van Donkersgoed 1992; Morck, Merrill et al. 1993; Galyean, Gunter et al. 1995; Duff, Walker et al. 2000; Schunicht, Booker et al. 2002; Cusack 2004; Booker, Schunicht et al. 2006; Booker, Abutarbush et al. 2007; Rice 2008).

There currently is limited knowledge about BRD pathogenesis with few studies having been performed to determine what is occurring in the acute stages of disease. Experimentally, *M. haemolytica* results in pulmonary lesions within 6 hours of inoculation resulting in a fibrinopurulent alveolitis, bronchitis, alveolar septal necrosis, interlobular edema, and intravascular thrombosis (Breider, Walker et al. 1988).

Diagnostic tools used for respiratory disease diagnosis include auscultation, rectal temperature, bronchoalveolar lavage, transtracheal wash, thoracic radiography, thoracic ultrasound, and lung biopsy. Many of these modalities are not practical in a commercial feedlot setting. Lung biopsy has been demonstrated to be a safe procedure in cattle (Dungworth and Hoare 1970; Raphel and Gunson 1981; Braun, Estermann et al. 1999; Sydler, Braun et al. 2004) as well as in horses (Dungworth and Hoare 1970; Reif 1974; Schatzmann, Straub et al. 1974; Raphel and Gunson 1981; Venner, Schmidbauer et al. 2006). Tru-Cut® Soft Tissue Biopsy instruments (14-ga) have been successfully used to obtain lung biopsy specimens (1 to 20 mm)

in cattle and horses (Raphel and Gunson 1981; Braun, Estermann et al. 1999; Braun, Estermann et al. 2000; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006) as well as a 14-ga spring loaded biopsy systems such as the Bard Biopty-system® (1 to 18 mm) (Braun, Estermann et al. 1999; Braun, Estermann et al. 2000; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006). These lung biopsies were found to be useful for histopathologic evaluation and may be useful in assessing the early pathogenesis and associated infectious respiratory pathogens in BRD of feedlot cattle.

A better understanding of the early pathogenesis and associated infectious respiratory pathogens is needed in order to institute effective treatment and preventive measures for BRD. The objectives of this project were: 1) to determine if live animal lung biopsy could be used to characterize early pathologic changes in the bovine lung associated with BRD, 2) determine if there are specific infectious respiratory pathogens that can be identified in association with early pathological changes, and 3) determine whether pulmonary pathology characterized by live animal lung biopsy at arrival and at the time of initial BRD diagnosis was associated with health and production outcomes of feedlot steers in a commercial feedlot.

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CHAPTER 2

GENERAL LITERATURE REVIEW

2.1 INTRODUCTION

Bovine respiratory disease (BRD) or "shipping fever" is an important disease complex of feedlot cattle that has many factors contributing to its development. Respiratory disease has been reported to account for 6%-58.7% of feedlot morbidity (Church and Radostits 1981; Alexander, MacVean et al. 1989; Harland, Jim et al. 1991) as well as 10-61% of mortality in western Canadian feedlots (Church and Radostits 1981; Ribble, Meek et al. 1995), 86.8% of mortality in Ontario feedlots (Gagea, Bateman et al. 2006) and 44-67% of mortality in U.S. feedlots (Andrews and Kennedy 1997; Gagea, Bateman et al. 2006). BRD or shipping fever classically refers to the development of bronchopneumonia secondary to the stress of shipping or transportation (Andrews and Kennedy 1997). It typically occurs 7 to 14 days after transport, auction markets, comingling or other causes of stress (Wikse 1985). Lung lesions are usually found in the cranioventral lung lobes and will likely be bilateral in distribution (Andrews and Kennedy 1997). The most common bacterial isolates from fibrinosuppurative bronchopneumonia are *Mannheimia haemolytica*, *Mycoplasma bovis*, *Pasteurella multocida* and *Histophilus somni* (Andrews and Kennedy 1997; Gagea, Bateman et al. 2006; Booker, Abutarbush et al. 2008).

Early detection and recognition of BRD is necessary for effective treatment and prevention. Typically BRD detection is reliant on observing for signs of depression, coughing, decreased feed intake and standing away from the group as well as an increased rectal temperature. These criteria are very subjective and lend themselves to individual variation and

misdiagnosis. In South African feedlots it was found that 42.8% of all cattle had lung lesions at slaughter with 69.5% of these having never been treated for BRD (Thompson, Stone et al. 2006). Another study found that overall 35% of steers from a U.S. feedlot were treated for respiratory disease, however at slaughter 72% had pulmonary lesions (Wittum, Woollen et al. 1996). In that study, 78% of those treated actually had pulmonary lesions at slaughter while 68% of steers that had never been treated had pulmonary lesions at slaughter. These findings suggest that the selection criteria for BRD is non-specific, that treatment may be inappropriate in regards to drug choice or duration of therapy, and that there is an opportunity to improve individual animal health. By determining what is occurring early in the disease process, new treatment and prevention strategies may be implemented.

2.2 EPIDEMIOLOGY OF BOVINE RESPIRATORY DISEASE

Bovine respiratory disease in feedlot cattle is a multi-factorial disease complex that is typically associated with stressful events. For a feedlot calves these events include weaning, castration, auction markets, comingling, transportation, inclement weather and sudden changes in ration. Stress is thought to predispose to the development of respiratory disease due to its association with decreased pulmonary defense mechanisms and immunosuppression which allows microorganisms to establish infections within the lungs (Lillie 1974). The major effect of this stress is seen in the first 30 days after entering the feedlot (Johnson 1985). This translates into newly arriving cattle at the feedlot having a greater risk for the development of respiratory disease (Loneragan, Dargatz et al. 2001). In fact, most BRD outbreaks occur from October to April (Saunders, Thiessen et al. 1980) which correlates with calves entering the feedlot as well as probable inclement weather.

Fibrinous pneumonia has been reported to be the most common post mortem diagnosis of feedlot steers (Ribble, Meek et al. 1995). In southwestern Alberta, from 1985 to 1987, fatal fibrinous pneumonia accounted for 30% to 57% of all feedlot mortalities (Ribble, Meek et al. 1995). In western Canadian feedlots, clinical fatal fibrinous pneumonia peaks in November, 2 to 4 weeks after peak auction market sales (Ribble, Meek et al. 1995). The peak mortality from fibrinous pneumonia occurs 14 to 16 days after feedlot arrival (Ribble, Meek et al. 1995; Snowder, Van Vleck et al. 2006) with 50% of mortalities occurring within the first 3 weeks (Ribble, Meek et al. 1995). There are similar findings in the U.S. with the incidence of respiratory disease being the greatest in the first week of arrival with cattle from multiple sources and increased shipping distance having an increased morbidity (Sanderson, Dargatz et al. 2008). All of these studies suggest that the stress of transportation and auction markets predispose cattle to BRD within the first few weeks of the feeding period.

2.2.1 RISK FACTORS FOR BRD

There are many risk factors for the development of BRD including timing of weaning, dehorning and castration, transportation, comingling, passing through auction markets, inclement weather, and diet. All of these risk factors equate to events in a calf's life which are stressful.

The timing of weaning has been shown to have an effect on BRD in a feedlot. In general, calves weaned at transport tend to be more affected by respiratory disease with a morbidity of 30 to 50% (Johnson 1985) while calves who are weaned and remain on the home ranch for 45 days prior to feedlot entry are less likely to be treated for BRD (Step, Krehbiel et al. 2008). The timing of dehorning and castration also is reported to affect the occurrence of BRD in feedlots with a reduced mortality when dehorning occurred prior to feedlot entry (Martin, Meek et al.

1980). In addition, castration on feedlot arrival has been associated with a 92% greater incidence of BRD when compared to those castrated before arrival (Duff and Galyean 2007). These associations are most likely due to increased stress on entry in the feedlot but could also be due to minor surgical procedures predisposing calves to infection via wound contamination. These suggest that by separating in time the stressful events in a calf's life there may be an associated decrease in the occurrence of BRD.

Transportation can be stressful for cattle with transit times in excess of 24 hours increasing respiratory disease incidence (Johnson 1985). Alberta feedlot managers reported that cattle from greater than 480km (288 miles) away seemed more susceptible to disease than those transported a shorter distance (Church and Radostits 1981). Transportation has been associated with increased blood cortisol concentration in 6 month old Hereford-cross calves (Aich, Jalal et al. 2007) suggesting this to be a time of stress for calves. In addition, systemic dehydration and diesel fume inhalation can decrease mucociliary escalator function predisposing to the development of respiratory disease (Hjerpe 1983; Johnson 1985).

Comingling of cattle groups has been shown to increase feedlot calf morbidity (Martin, Meek et al. 1981; O'Connor, Sorden et al. 2005; Sanderson, Dargatz et al. 2008). While comingling increases an animal's exposure to different infectious microorganisms, it also increases animal stress as a new hierarchy has to be established and the available food and water sources must be located. Reducing the amount of comingling by purchasing and maintaining small groups should decrease calf mortality in the feedlot (Martin, Meek et al. 1980; Martin and Meek 1986). Unmixed groups have also been reported to have fewer health events than mixed groups within the first five weeks of the feeding period (Martin and Meek 1986). Ranch derived calves are less likely to be treated for BRD than auction market derived calves (Step, Krehbiel et

al. 2008) and have been found to have a lower overall morbidity than auction market derived calves (Thorlakson, Martin et al. 1990). These findings suggest that increased exposure to novel infectious microorganisms at a time of stress predisposes cattle to the development of BRD.

Inclement weather and rapid fluctuations in temperature have been shown to influence the incidence of feedlot respiratory disease with more cases occurring in the fall and winter months (Johnson 1985). It is suggested that this pattern is not a weather effect but rather reflects the time when animals are entering the feedlots (Johnson 1985). Indeed, in Canada the coldest temperatures typically occur from December to February with peak BRD incidence occurring in the fall months of October and November (Ribble, Meek et al. 1995). However, prolonged exposure to cold air can reduce mucociliary escalator function (Hjerpe 1983; Diesel, Lebel et al. 1991) as well as inhibit pulmonary alveolar macrophage function predisposing animals to respiratory disease (Hjerpe 1983). At a temperature of 2 to 4°C, nasal mucous velocity is decreased by 24% (Diesel, Lebel et al. 1991). Additionally, cold air induces a change in respiratory pattern to slow, deep respirations allowing particles to deposit within the lungs (Diesel, Lebel et al. 1991). Cold exposure has been shown to increase bacterial deposition within the lung by 66% with the apical lobes being most affected (Diesel, Lebel et al. 1991). A study which took place from 1985 to 1987 found that the greatest overall risk of fatal fibrinous pneumonia occurred in the year with the highest snowfall and coldest temperatures as well as in the year having the lowest snowfall and highest temperatures, both of which represent weather extremes (Ribble, Meek et al. 1995).

A sudden diet change can also predispose to the development of respiratory disease. Feeding high energy rations without allowing time for ruminal microbe adaptation can result in systemic acidosis which depresses pulmonary alveolar macrophage function (Hjerpe 1983).

In conclusion, BRD is a multifactorial disease which is affected by host, environment and microorganisms. Any disruption in the respiratory defense mechanisms can predispose to the development of bronchopneumonia, even from normal nasopharyngeal inhabitants. Defense mechanisms can be compromised by respiratory pathogens, such as bovine respiratory syncytial virus (BRSV) and bovine herpesvirus-1 (BoHV-1), by the environment, such as cold weather, by inhalation of diesel fumes during transportation or by the depressed immune system due to stressful events. All of these factors interplay to result in the development of respiratory disease.

2.3 ANATOMY OF THE BOVINE THORAX

Bovine respiratory disease (BRD) may result when a potential respiratory pathogen is able to invade a susceptible host. The respiratory system is vulnerable to infection due to its intake of environmental dust, infectious agents and noxious gases as well as the blood flow through an extensive vascular system. Pulmonary defense mechanisms that are overcome in pulmonary disease include pulmonary alveolar macrophages (PAMs), secretory defenses such as immunoglobulins, interferons and lysozyme, as well as mucous and the mucociliary escalator. Coughing and mucociliary clearance are the major mechanisms for clearing particles from the lung, however mucociliary clearance can take up to 6 hours, but during this time innate defense proteins inhibit bacterial growth (Caswell 2007).

The bovine lung consists of a right lung divided into cranial, middle, caudal and accessory lobes and a left lung divided into a cranial lobe with a cranial and caudal segment and a caudal lobe. The airways are lined by ciliated epithelium whose numbers decrease in the more distal airways. As air travels down the airways its velocity slows allowing suspended particles to settle out and land on the airway mucosa. Particles greater than 20 μm settle out in the nares,

pharynx and trachea, particles between 5 μm and 20 μm settle out in the bronchi and bronchioles and particles less than 5 μm can enter alveoli (Baskerville 1981). Particles tend to settle in the cranial and ventral portions of the lungs. These particles are then "swept" up and out of the lungs by a mucociliary escalator at about 15mm per minute (Veit and Farrell 1978; Breeze 1985). The upper airway contributes to this process by raising the relative humidity of inspired air to 95% to keep respiratory secretions from drying (Breeze 1985). The mucociliary escalator can be disrupted by the loss of cilia (e.g. BRSV and BoHV-1 infection) or changes in the secretions resulting in a "stagnant" flow allowing bacteria to become established in the airways (Breeze 1985). As animals begin to mouth breath, some of the defense mechanisms are bypassed.

In the bovine lung, primary lobules are separated by intact interlobular septa resulting in increased compartmentalization and less collateral air flow between lobules. In addition, the intact septa do not allow for much cellular movement between lobules resulting in the localization of inflammation and infection within a lobule leading to bronchopneumonia. The lack of collateral ventilation is thought to be the cause of interlobular emphysema commonly found on post mortem in cattle (Caswell 2007).

Bacterial pneumonia can be a primary condition or secondary to viral infections which allows the normal flora from the upper airway, such as *M. haemolytica* and *P. multocida*, to colonize the lower airway (Hjerpe 1983; Johnson 1985; Andrews and Kennedy 1997). Viral infections are site specific based on cell type and usually infect respiratory epithelium (Baskerville 1981). The site of initial infection may be the upper airway, however virus in an aerosol can reach the lower respiratory tract (Baskerville 1981). Viruses can cause cell death and inflammation resulting in exudates and blocked airways. Roughly 6 to 9 days post viral

infection, PAMs, the main lung defenders, are depressed, leaving the lung susceptible to bacterial infection and colonization (Liggitt 1985). Cattle also have fewer PAMs within the alveoli than do other mammals (Veit and Farrell 1978). Bacteria can colonize respiratory epithelium if they are not cleared away by normal defense mechanisms such as airflow, competition by normal resident flora, mucociliary clearance or phagocytosis. Bacteria have many mechanisms for attachment including fimbriae (Gram negative bacteria), molecular attachments (hemagglutinins and adhesions such as M proteins) or by physical means (electrostatic charge) (Baskerville 1981). The bacteria may attach to surface cilia, microvilli or to other bacteria. A common site for infection initiation is the junction of the alveolar ducts and the gas exchange region (Caswell 2007). The distal airways are more susceptible as there are fewer mucous cells and cilia. Additionally, bronchioles are more susceptible to disease as they have a smaller diameter, are thin walled, and collapsible (Caswell 2007).

2.4 ETIOLOGIC AGENTS

Bovine respiratory disease may result from infection of the respiratory tract with many etiologic agents including *Histophilus somni (H. somni)*, *Mannheimia haemolytica (M. haemolytica)*, *Mycoplasma bovis (M. bovis)*, BoHV-1, BRSV, and bovine viral diarrhea virus (BVDV). Some of these microorganisms are considered primary pathogens, such as BRSV and BoHV-1 (Liggitt 1985; Andrews and Kennedy 1997), while others are considered secondary invaders, such as *M. haemolytica* and *M. bovis* (Thomas, Ball et al. 2002; Rice 2008), or result in immunocompromise, such as BVDV (Campbell 2004), predisposing to the development of BRD.

2.4.1 *HISTOPHILUS SOMNI*

Histophilus somni (*H. somni*), previously named *Haemophilus somnus*, is a Gram negative aerobic bacteria from the family *Pasteurellaceae*. It is a normal inhabitant of the genital tract and respiratory tract of healthy bovine and can be carried on the respiratory and reproductive mucosa in an asymptomatic carrier state making it difficult to confirm as the cause for pneumonia (Corbeil 2007). A virulence factor displayed by *H. somni* is lipooligosaccharide (analogous to lipopolysaccharide) which aids in immune evasion. *H. somni* binds endothelial cells inducing apoptosis *in vitro*.

Clinical syndromes associated with *H. somni* infection include septicemia, thrombotic meningoencephalitis, reproduction problems such as endometritis, abortion and infertility, as well as pneumonia, pleuritis, laryngitis, myocarditis, and polyarthritis. *H. somni* is an important pathogen in shipping fever and has also been found to be the most common pathogen associated with myocarditis in feedlot calves (Haines, Moline et al. 2004). The median onset of fibrinous pneumonia is 12 days on feed (DOF) (Van Donkersgoed, Janzen et al. 1990). Death usually occurs within the first 5 weeks of the feeding period (Van Donkersgoed, Janzen et al. 1990; Corbeil 2007).

Post mortem findings with *H. somni* pneumonia include fibrinous pleuritis or pleuropneumonia that has a cranioventral distribution with thickened interlobular septae (Andrews, Anderson et al. 1985; Andrews and Kennedy 1997). Sheets of fibrin on the pleural surface (Andrews, Anderson et al. 1985) with straw colored pleural fluid may also be noted. Some of these animals will have myocarditis and signs of heart failure such as pulmonary edema and congestion (Andrews and Kennedy 1997). *H. somni* pneumonia is histopathologically characterized by neutrophilic vasculitis, pulmonary alveolar macrophage degeneration,

necrotizing bronchiolitis, suppurative bronchitis, lobular necrosis, and lymphatic dilation and thrombosis (Andrews, Anderson et al. 1985; Corbeil 2007). The caudodorsal lung is usually not consolidated but has a "meaty" consistency with some emphysematous bulla (Andrews, Anderson et al. 1985). *H. somni* is sensitive to antibiotic therapy (Tegtmeier, Angen et al. 2000) which may make diagnosis more difficult as diagnostic samples are usually obtained after the institution of antimicrobial therapy. Feedlot steers with clinical evidence of chronic pneumonia and arthritis have been found by immunohistochemistry (IHC) to be positive for *H. somni* in only 14% of post mortem lung samples (Haines, Martin et al. 2001). A study in 2008 found post mortem lung samples positive by IHC for *H. somni* to be correlated with suppurative bronchopneumonia and chronic suppurative bronchopneumonia as well as with bronchiolitis obliterans in cranioventral lung samples and fibrinous pleuritis in caudodorsal lung samples (Booker, Abutarbush et al. 2008). *H. somni* is more commonly isolated from bronchoalveolar lavage (BAL) fluid than from nasal swabs (Corbeil 2007).

2.4.2 *MANNHEIMIA HAEMOLYTICA*

Mannheimia haemolytica (*M. haemolytica*) is a Gram negative, non-motile, non-spore forming facultative anaerobe from the family *Pasteurellaceae*. It is a normal inhabitant of the nasopharynx with serotypes A1 and A2 colonizing the nasopharynx of healthy animals, but it is not a normal inhabitant of the bovine lung (Rice 2008). *M. haemolytica* has many virulence factors such as leukotoxin, lipopolysaccharide (LPS), neuraminidase and fimbriae. Leukotoxin is the main virulence factor that is associated with lung lesions (Jeyaseelan, Sreevatsan et al. 2002) and it is secreted by all *M. haemolytica* serotypes. A high concentration of leukotoxin results in lysis of platelets and leukocytes in cattle. LPS induces pulmonary alveolar

macrophages (PAMs) oxidative burst which may contribute to lung injury as well as the expression of tissue factor which mediates thrombosis. A polysaccharide-rich capsule is resistant to phagocytosis by neutrophils and macrophages. Neuraminidase increases the adhesion of the bacterium to the respiratory epithelium (Jeyaseelan, Sreevatsan et al. 2002). All of these virulence factors contribute to its pathogenicity.

Bovine pneumonia is associated mostly with *M. haemolytica* serotype A1 but also with serotype A6 and A2 (Fulton, Purdy et al. 2000; Jeyaseelan, Sreevatsan et al. 2002; Rice 2008). Of mid-western U.S. pulmonary lesion bacterial isolates, 60% were found to be serotype A1, 28% serotype A6, 7% serotype A2, and 7% were classified as other serotypes (Al-Ghamdi, Ames et al. 2000). Stress can result in nasopharyngeal overgrowth of bacteria, including *M. haemolytica*, which results in more bacteria reaching the lungs via inhalation of infected droplets. In addition, respiratory viruses such as BRSV and BoHV-1 can damage the ciliated epithelium (Liggitt 1985; Andrews and Kennedy 1997) thereby compromising the normal defense mechanisms allowing *M. haemolytica* to colonize the lung. Healthy cattle can clear 90% of inhaled *M. haemolytica* within 4 hours (Rice 2008). BoHV-1 infection has been shown to increases the severity of pneumonia due to *M. haemolytica* in experimental studies (Narita, Kimura et al. 2000). *M. haemolytica* pneumonia is common in recently weaned beef calves early in the feeding period (Rice 2008). It is the most common isolate from pneumonic lung of chronically affected cattle (Wikse 1985; Gagea, Bateman et al. 2006). Clinically, there are non-specific signs including fever, nasal discharge, cough, inappetence, and weight loss.

Post mortem findings include acute fibrinonecrotizing pleuropneumonia which begins in the cranioventral lung lobes (Andrews, Anderson et al. 1985; Jeyaseelan, Sreevatsan et al. 2002). Hallmarks of infection seen by histology include neutrophil influx with alveolar necrosis

(Jeyaseelan, Sreevatsan et al. 2002) and minimal bronchiolar necrosis (Andrews, Anderson et al. 1985). Alveoli in *M. haemolytica* pneumonia typically are filled with fibrinous exudates and "oat cells". Oat cells are degenerate leukocytes that are "streaming" or elongated in shape and are a characteristic feature of this infection (Andrews and Kennedy 1997). *M. haemolytica* causes areas of coagulative necrosis with alveolar capillary thrombosis and thickening of the interlobular septa due to fibrin. The pleural surface may or may not be covered with fibrin. Feedlot steers with clinical evidence of pneumonia have been found to be positive for *M. haemolytica* in 22.4% of post mortem lung samples when using IHC (Haines, Martin et al. 2001). A study in 2008 found a correlation between *M. haemolytica* and fibrinonecrotizing pneumonia as well as fibrinous pleuritis in all post mortem lung samples taken from cranioventral, midlateral, and caudodorsal lung lobes (Booker, Abutarbush et al. 2008). Animals that recover from infection or are vaccinated with a live bacterial vaccine appear to gain resistance to natural infection (Caswell 2007).

2.4.3 *MYCOPLASMA BOVIS*

Mycoplasma bovis (*M. bovis*) is a bacterium from the family *Mycoplasmataceae*. *M. bovis* lacks a cell wall and is therefore resistant to some antimicrobials, such as penicillin and cephalosporin, which act by inhibiting cell wall synthesis. *M. bovis* has variable surface lipoproteins allowing for bacterial adhesion and evasion of the immune system (Caswell 2008). It can induce apoptosis of lymphocytes in cattle and hinders neutrophil activation. *M. bovis* associated pneumonia and polyarthritis are typically seen in months 2 to 3 of the feeding period (Caswell 2007). These animals usually will have a prolonged course of treatment and are considered non-responders. Sources of infection for naïve cattle are secretions from the

respiratory tract, genital tract and mammary gland. Transmission is usually due to direct contact with respiratory secretions as well as aerosolization. Increased nasal shedding of *M. bovis* has been shown to be associated with stressful events (Caswell 2008). Infection may just result in respiratory disease however should bacteremia ensue joints may subsequently become infected.

Mycoplasma spp. are typically not found in healthy calves. In Belgium, BAL samples from healthy calves were culture negative for Mycoplasma spp. 84% of the time (Thomas, Ball et al. 2002). However, when healthy calves were culture positive for Mycoplasma spp. it was found to be *M. bovirhinis* (Thomas, Ball et al. 2002). When culturing BAL fluid, cattle with acute respiratory disease were culture positive 65% of the time and those with recurrent respiratory disease were culture positive 78% of the time for Mycoplasma spp. (Thomas, Ball et al. 2002). Post mortem samples were positive in 20% of pneumonic lungs (Thomas, Ball et al. 2002). This suggests that healthy calves typically are not harboring *M. bovis*.

Clinically, signs of infection are non-specific and include depression, anorexia, fever, lethargy, respiratory distress and weight loss. These animals may demonstrate lameness with swollen joints, most commonly the hip, stifle, hock, shoulder, elbow and carpal joints (Gagea, Bateman et al. 2006). Calves with pneumonia and arthritis demonstrate more chronic lung lesions associated with caseonecrotic bronchopneumonia typical of *M. bovis* infection when compared to calves with fibrinosuppurative bronchopneumonia which is more characteristic of *M. haemolytica* infection (Gagea, Bateman et al. 2006). A syndrome reported to occur due to infection with *M. bovis* includes respiratory disease with septic arthritis and tenosynovitis (Adegboye, Halbur et al. 1996). Affected animals develop respiratory disease within 2 weeks of exposure and then septic arthritis 5 days later. Clinical disease (respiratory disease and arthritis) is reported to occur within 8-10 days of exposure in naïve calves (Caswell 2008). Calves with

caseonecrotic bronchopneumonia reportedly die about 44 days after entry into the feedlot, significantly longer than those with fibrinosuppurative bronchopneumonia who die about 25 days into the feeding period (Gagea, Bateman et al. 2006). This coupled with more chronic lesions typically associated with caseonecrotic bronchopneumonia suggest *M. bovis* to be a more protracted infection in the feedlot.

M. bovis has been shown to have four distinct lung lesion patterns on post mortem evaluation; caseonecrotic bronchopneumonia, bronchopneumonia with coagulative necrotic foci, suppurative bronchopneumonia without necrosis and chronic bronchopneumonia with abscessation (Caswell 2008). Others have reported *M. bovis* to be characterized by a necrotizing bronchopneumonia with bronchiectasis and abscessation (Shahriar, Clark et al. 2002; Khodakaram-Tafti and Lopez 2004). Caseonecrotic bronchopneumonia is typically seen in the cranial and middle lung lobes but may affect up to 80% of the lung (Caswell 2008). *M. bovis* has been isolated from 98% of caseonecrotic lung lesions and from 75% of arthritis lesions from cattle with chronic pneumonia (Gagea, Bateman et al. 2006). Another study found feedlot steers with clinical evidence of chronic pneumonia and arthritis to be positive by IHC for *M. bovis* in 71% of lung samples and 41% of joint synovium samples (Haines, Martin et al. 2001). A study in 2008 found a correlation between *M. bovis* and bronchiectasis as well as suppurative bronchopneumonia, chronic suppurative pneumonia, fibrinonecrotizing pneumonia, fibrinous pleuritis and proliferative interstitial pneumonia in the cranioventral and midlateral lung regions when using IHC on post mortem samples as the method for detection (Booker, Abutarbush et al. 2008). Lung lesions progress from exudates within intact epithelium to a loss of bronchiolar epithelium to coagulative or liquifactive necrosis in more chronic lesions (Shahriar, Clark et al. 2002). *M. bovis* is typically found at the margins of the necrotic areas but is also found within

macrophages and bronchiolar exudates (Rodriguez, Bryson et al. 1996; Shahriar, Clark et al. 2002; Khodakaram-Tafti and Lopez 2004; Gagea, Bateman et al. 2006; Caswell 2008). Leukocytes within the alveoli and bronchioles undergo necrosis but retain a 'ghost-like' outline with hypereosinophilic cytoplasm and fragmented nuclei (Caswell 2008). Mycoplasma spp. are very sensitive microorganisms and should be cultured within 24hrs of sample collection (Caswell 2008).

2.4.4 BOVINE HERPES VIRUS-1

Infections bovine rhinotracheitis (IBR) is the result of a Bovine Herpes Virus-1 (BoHV-1) infection with genotype 1.1, an alpha herpesvirus. Most clinical disease from BoHV-1 is seen with new introductions into the herd or over-crowding. Clinically, it is seen in calves > 6 months of age (Kapil and Basaraba 1997). This virus demonstrates latency, persisting in the trigeminal and sacral ganglia, and can recrudesce at times of stress such as transportation or parturition (Caswell 2007).

BoHV-1 has been shown experimentally to increase susceptibility to bacterial pneumonia (Liggitt 1985; Andrews and Kennedy 1997). It results in a compromised ciliary clearance apparatus due to epithelial necrosis of the respiratory tract. This in turn allows bacteria to colonize the respiratory tract and ultimately results in the development of bronchopneumonia. BoHV-1 also impairs pulmonary alveolar macrophages, neutrophils and lymphocytes which contribute to the development of respiratory tract infections. A study in 2000 found *M. haemolytica* pneumonia to be more severe with concurrent BoHV-1 infection (Narita, Kimura et al. 2000).

Sources of BoHV-1 include clinical and subclinical infected cattle as well as reactivation of latent infections (Caswell 2007). Transmission occurs via aerosolization and direct or indirect contact with nasal secretions. Infected cattle may shed virus in nasal secretions for 10 to 16 days (Caswell 2007). Its incubation period is 2 to 6 days (Caswell 2007) and will typically run through the herd within 2 to 4 weeks (Kapil and Basaraba 1997). Clinically, IBR may go un-noticed or may be seen as high temperatures (105°F-107°F) [40.6°C-41.7°C], inflammation of the nasal mucosal with erosions, lacrimation, and conjunctivitis (Kapil and Basaraba 1997).

Typically, IBR lesions are seen as ulcerations of the upper respiratory tract however it may progress to a necrotizing bronchitis or bronchiolitis. Lesions include focal erosions, ulcerations, and diphtheritic membranes on upper airway mucosa. In addition, there are usually petechiae of the respiratory tract mucosa and conjunctivitis. Histological lesions include rhinitis, laryngotracheitis, and bronchitis characterized by a fibrinopurulent exudate and epithelial necrosis with erosions. Infected epithelial cells will have eosinophilic intra-nuclear inclusion bodies within 3 days of infection (Andrews and Kennedy 1997), however they are usually absent at the time of diagnosis. Virus isolation from nasal swabs as well as nasal mucosa, trachea and lung is useful in diagnosis (Caswell 2007). Rapid diagnosis can be made using immunohistochemistry or the use of fluorescent antibodies. Seroconversion is typically associated with clinical disease.

2.4.5 BOVINE VIRAL DIARRHEA VIRUS

Bovine viral diarrhea virus (BVDV) is a *Pestivirus* of the family *Flaviviridae*. There are two biotypes, non-cytopathic and cytopathic (based on visual effects in cell culture) and two genotypes, type I and type II. Clinically, BVDV infection can result in asymptomatic to acute

disease seen as respiratory disease, mucosal disease or fetal disease. The virus is able to cross the placenta in non-immune animals. Should this occur before immunological competence, the fetus will be persistently infected (PI). PI animals are the main source of virus for naïve animals, shedding large amounts of virus particles. PI animals do not seroconvert and may remain asymptomatic, can be "poor-doers" or develop acute mucosal disease (Campbell 2004). If an animal is infected after immune competence they will have a transient viremia, shedding only small amounts of the virus. These animals will seroconvert by 3 weeks and life-long immunity will develop against similar BVDVs.

BVDV has not been established as a primary respiratory pathogen. However, BVDV can be recovered from pneumonic lung and PI calves commonly die of respiratory disease suggesting that BVDV may play a role in the development of bovine respiratory disease (Potgieter 1997). Indeed, calves experimentally inoculated with only BVDV develop minor interstitial pneumonia (Potgieter 1997) with BVDV antigen being minimally detected in the respiratory tract (Spagnuolo-Weaver, Allan et al. 1997). BVDV causes immunosuppression seen as a leukopenia, lymphoid depletion and pulmonary alveolar macrophage destruction thereby predisposing to secondary infections and respiratory disease (Potgieter 1997; Campbell 2004). A study in 2005 demonstrated that 15.9% of initial BRD treatments were attributed to exposure to a PI calf (Loneragan, Thomson et al. 2005). Experimental infection of beef calves with BVDV demonstrated decreased phagocytic activity and decreased microbicidal activity of PAMs as well as a decrease in neutrophil chemotactic factor production (Welsh, Adair et al. 1995). Calves positive for BVDV have been shown to die earlier in the feeding period (median 21 days) than those who were BVDV negative (median 42 days) (Gagea, Bateman et al. 2006). BVDV seroconversion after arrival has been shown to be associated with an increased risk of BRD

treatment (Campbell 2004) and positive BVDV titers at arrival have been shown to decrease the risk of BRD treatment (Booker, Guichon et al. 1999; Campbell 2004). BVDV is more commonly found in calves with bacterial bronchopneumonia than in those without pneumonia (Gagea, Bateman et al. 2006). In addition, calves exposed to a PI animal have a 43% greater risk for BRD treatment than those not exposed to a PI animal (Loneragan, Thomson et al. 2005). Exposure in that study was defined as calves in a pen with a PI calf or in a neighboring pen, thus having fence line contact with a pen containing a PI calf.

Some studies have shown no difference in morbidity when animals are exposed to a PI calf. In a study in 2005, comingling was shown to be the risk factor associated with the development of BRD rather than the presence of a PI calf (O'Connor, Sorden et al. 2005). In that study, those comingled pens containing a PI calf had a morbidity of 29.3% and those without a PI calf had a morbidity of 28.6% (O'Connor, Sorden et al. 2005). The pens from a single source with a PI calf had a morbidity of 9.0% and those without a PI calf had a morbidity of 11.9% (O'Connor, Sorden et al. 2005). Another study in 2008 had similar findings with there being no difference in morbidity or overall mortality for pens with PI calves and those without (Booker, Abutarbush et al. 2008).

On post mortem evaluation, vascular lesions associated with BVDV antigen occurred in 54-56% of lungs affected with chronic pneumonia (Shahriar, Clark et al. 2002). Lesions include necrotizing vasculitis and lymphocytic infiltration with BVDV antigen being located within the damaged vessel walls (Shahriar, Clark et al. 2002). Not all histological lesions will contain vascular lesions or BVDV antigen in animals that are infected with BVDV (Shahriar, Clark et al. 2002). A study in 2008 found IHC staining of post mortem lung samples for BVDV to be

correlated with fibrinous pleuritis and bronchiolar necrosis in the midlateral lobe, and bronchiectasis in the caudodorsal lung lobe (Booker, Abutarbush et al. 2008).

Virus neutralization is the standard reference test for BVDV serology (Sandvik 1999). PI calves are diagnosed by repetitive isolation of BVDV from blood without seroconversion with samples taken 3 to 4 weeks apart (Sandvik 1999). Virus titers are low in acutely infected animals and therefore these animals should be diagnosed based on paired serology 3 to 4 weeks apart demonstrating seroconversion (Sandvik 1999). Diagnostic samples that should be obtained post mortem include thymus, spleen, lung, kidney and liver as well as small intestine, mesenteric lymph nodes and Peyer's patches. Freezing does not harm BVDV in samples however it is sensitive to freeze-thawing and should be kept frozen or refrigerated for transport. Refrigeration for a few days will not affect the laboratory's ability to isolate the virus (Saliki and Dubovi 2004). Virus isolation or culture and identification of BVDV in clinical samples is the gold standard diagnostic in regards to BVDV testing (Saliki and Dubovi 2004). The best live animal samples for BVDV isolation is the buffy coat from a whole blood sample (Saliki and Dubovi 2004). When sampling suspected PI animals, any secretion, excretion, or tissue sample will work as the amount of BVDV present within the animal is very high. Antigen detection of BVDV is of two methods, antigen capture ELISA or immunologic staining of fresh formalin-fixed tissue via immunohistochemistry. ELISA is useful for PI screening, but not for acute disease. It can be performed on serum or skin biopsy samples ("ear notches"). IHC of skin biopsies is useful not only for PI animal detection but also in acute disease detection. Nucleic acid detection can be performed directly or via PCR amplification however this type of testing is not performed routinely by diagnostic laboratories. Reverse-transcription PCR can be used as a screening tool for PI animals on pooled milk or serum samples. Serology can be used to

determine BVDV exposure status using an ELISA or a serum neutralization assay and is useful to assess vaccine efficacy and vaccination compliance, or associating exposure status with clinical signs (Saliki and Dubovi 2004).

2.4.6 BOVINE RESPIRATORY SYNCYTIAL VIRUS

Bovine respiratory syncytial virus (BRSV) is a *Pneumovirus* in the family *Paramyxoviridae*. BRSV was common in cattle prior to vaccine availability with a reported seroprevalence of 65% to 81% (Baker, Ellis et al. 1997). Stressful situations such as transportation and comingling contribute to BRSV outbreaks with the peak occurrence being in the fall and winter months (Baker, Ellis et al. 1997) at the time of feedlot filling. BRSV may predispose to secondary bacterial infections by impairing pulmonary defense mechanisms such as pulmonary alveolar macrophage function and destruction of ciliated epithelium (Caswell 2007). It is transmitted via aerosolization of respiratory secretions and has an incubation period of 3 to 5 days (Baker, Ellis et al. 1997). BRSV has a high morbidity of 60% to 80% with a mortality of 20% (Baker, Ellis et al. 1997). Viral shedding occurs from day 2 to 8 after infection (Caswell 2007).

Clinical signs of BRSV infection include inappetence, depression and mild respiratory signs seen as oculonasal discharge and tachypnea which may progress to dyspnea, high fevers of 104°F to 107.6°F (40°C to 42°C) (Baker, Ellis et al. 1997) and open mouthed breathing. Many will have a cough, bronchovesicular sounds and crackles. If emphysematous bullae rupture, subcutaneous emphysema may be present over the shoulder. The duration of disease is typically 1 to 2 weeks (Baker, Ellis et al. 1997).

BRSV can cause interstitial pneumonia that grossly appears as lungs that fail to collapse and are very heavy on post mortem examination (Andrews and Kennedy 1997). Lesions are typically seen in the cranioventral lung lobes as firm, consolidated lung, and in the caudodorsal lung lobes as interstitial emphysema, edema and emphysematous bullae (Andrews and Kennedy 1997; West, Bogdan et al. 1998). Viral antigen is most commonly found in the bronchiolar epithelium of the cranioventral lung lobes (Viuff, Uttenthal et al. 1996; Caswell 2007) as well as alveolar cells (Viuff, Uttenthal et al. 1996). Histological findings are characterized by bronchointerstitial pneumonia with necrotizing bronchiolitis of the cranioventral lung lobes (Baker, Ellis et al. 1997). Histological changes include respiratory epithelial cell degeneration, syncytial cell formation, eosinophilic cytoplasmic inclusions within mononuclear and syncytial cells as well as neutrophilic infiltration. Syncytial cells are multinucleated cells formed from cell fusion of bronchiolar epithelium and are a hallmark of BRSV infection. Chronic changes include bronchiolar epithelial hyperplasia with increased fibrous tissue within the bronchiolar walls, bronchiolitis obliterans and mononuclear cell infiltration (Andrews and Kennedy 1997).

BRSV is difficult to isolate and is sensitive to freeze-thawing during transport to a diagnostic laboratory making it challenging to diagnose in a feedlot setting (West, Bogdan et al. 1998). However, antigen immunoassays are commercially available for Human RSV that can be used for BRSV diagnosis as well as a BRSV ELISA (Quinting, Robert et al. 2007). In addition, IHC, IFA and PCR are available. Seroprevalence in adults is reported to be 40 to 95% and correlate well with disease protection (Caswell 2007).

2.5 BRD PREVENTION

Historically, many strategies have been employed to reduce BRD such as preconditioning, prophylaxis, metaphylaxis and vaccination. Pre-conditioned calves are calves that have been weaned for 30 to 45 days, vaccinated with clostridial and viral vaccines, treated with anthelmintics, castrated, dehorned and introduced to feed bunks and water troughs prior to transport to the feedlot. The purpose of a preconditioning program is to separate stresses and allow calves which are unstressed to properly respond to the vaccinations prior to pathogen challenge. Some producers believe preconditioning to be beneficial to lighter weight calves (less than 318kg or 700lbs) (Duff and Galyean 2007) as heavier cattle are less likely to be treated for BRD (Wieringa, Curtis et al. 1976; Step, Krehbiel et al. 2008). This finding is likely due to weight being a marker for age with older cattle being less susceptible to the development of BRD.

A study in 2003 assessed the risk of BRD treatment for feeder calves based on cattle sale type (conventional, vaccinated, or conditioned) within the first 28 days of feedlot entry (Macartney, Bateman et al. 2003). This study found a significant difference in BRD treatment rates between conventional sales and vaccinated or preconditioned sales with cattle from preconditioned sales having the lowest risk for BRD treatment. Feeder calves in the vaccination sales had been vaccinated with a killed-virus vaccine against IBR, PI-3, BVDV, and BRSV as well as vaccinated against *H. somni* and *M. haemolytica*. A booster was required 2 to 4 weeks prior to sale. Feeder calves in preconditioned sales not only were required to be vaccinated as above, they additionally had to have been castrated 2 months prior to the sale, dehorned, and weaned 4 weeks prior to the sale. A study in 1973 assessed a preconditioning program which included weaning 30 days prior to shipping, transitioning calves to eating grain from a bunk and

using water troughs, as well as vaccinating against blackleg, malignant edema, IBR and PI3 (Woods, Pickard et al. 1973). This study found an increase in BRD treatment for preconditioned calves in year 1, similar treatment rates in year 2, and a decrease in treatment for BRD of preconditioned calves in year 3. The authors suggest the yearly differences to be due to ranch factors. Others have found no advantage in preconditioning calves prior to marketing (Woods, Mansfield et al. 1972; Pritchard and Mendez 1990). A study in 1972 found no advantage to vaccinating 5 days before shipping with bovine antiserum containing IBR, PI-3 and BVDV antibodies in regards to BRD (Woods, Mansfield et al. 1972). Another study in 1972 had similar findings when vaccinating 30 days before shipping with IBR, BVDV, PI-3, and clostridia.

Prophylaxis is a treatment instituted prior to the occurrence of challenge. For example, the treatment with antibiotics or vaccinations prior to shipping calves to auction markets or feedlots. Metaphylaxis is a treatment instituted at the time of challenge. For example, the treatment with antibiotics at the time of exposure, but prior to clinical signs, in regards to antimicrobial administration on entry into feedlots. Currently, ceftiofur, tilmicosin, florfenicol, and tulathromycin are labeled for use in BRD (Rice 2008). Parenteral administration of tilmicosin (Galyean, Gunter et al. 1995; Duff, Walker et al. 2000; Cusack 2004; Rice 2008), long-acting oxytetracycline (Lofgreen 1983; Schunicht, Booker et al. 2002; Rice 2008), sustained release sulfadimethoxine (Lofgreen 1983), ceftiofur crystalline free acid (Booker, Schunicht et al. 2006), or tulathromycin (Booker, Abutarbush et al. 2007) administered on arrival have been shown to reduce morbidity from BRD in the feedlot. A study in 1993 demonstrated that administration of long-acting oxytetracycline (20 mg/kg, IM, once) or tilmicosin (10 mg/kg, SC, once) on entry into the feedlot significantly reduced morbidity attributed to BRD (Morck, Merrill et al. 1993). Tulathromycin (2.5mg/kg, SC) has been shown to reduce re-treatment when

compared to tilmicosin (10mg/kg, SC) and oxytetracycline (30mg/kg, IM) administered on entry into the feedlot (Booker, Abutarbush et al. 2007). One study using a long-acting oxytetracycline and sustained-released sulfadimethoxine combination showed an additive decrease in morbidity compared to using each drug alone (Lofgreen 1983). Tilmicosin (10 mg/kg, SC, once) has been shown to significantly reduce the rate of treatment for BRD within the first 30 days of the feeding period when given either on entry (Schumann, Janzen et al. 1990; Schumann, Janzen et al. 1991) or by 72 hours of entering the feedlot (Schumann, Janzen et al. 1991). A study in 2003 found a significant difference in BRD treatment rates of feeder calves which had received metaphylaxis on feedlot arrival when compared to those that had received no metaphylaxis (Macartney, Bateman et al. 2003). These studies suggest prophylaxis or metaphylaxis with antimicrobials may be a useful way to reduce BRD in feedlots.

Vaccination has been employed for years in BRD prevention strategies. Vaccination serves to decrease pathogen transmission through decreased shedding as well as by increasing immunity. In general, vaccines should be given prior to disease risk to healthy, unstressed cattle to have the maximum effect. Vaccination has been associated with an increased risk of death in some studies (Martin, Meek et al. 1980; Martin, Meek et al. 1981) which in a later study was decreased by delaying vaccination until two weeks after arrival (Martin, Meek et al. 1982). Other studies have shown a decreased feed consumption upon receiving a second vaccination with a 7-way clostridial vaccine, however this did not equate to decrease weight gain (Stokka, Edwards et al. 1994). Fibrinous pneumonia was significantly reduced by vaccinating within 24 hours of feedlot arrival with a *M. haemolytica* vaccine which contained outer membrane proteins and attenuated leukotoxin when compared to a modified live vaccine (Harland, Potter et al. 1992). Another study found no difference in BRD morbidity when auction market derived

calves as well as ranch derived calves were vaccinated using a *M. haemolytica* bacterial extract vaccine 3 weeks before feedlot arrival, at feedlot arrival, both prior and on arrival, or not at all (Thorlakson, Martin et al. 1990). Both auction market derived calves and ranch derived calves have been shown to have lower BRD relapse rates when vaccinated for *M. haemolytica* (Thorlakson, Martin et al. 1990). Vaccinating auction market derived calves with a *H. somni* bacterin has been shown to decrease the incidence of fatal disease and mortality within the first 60 days of the feeding period (Ribble, Jim et al. 1988). Vaccinating against BRSV has been shown to decrease clinical signs of respiratory disease in dairy calves with a naturally acquired infection (Verhoeff and van Nieuwstadt 1984). Vaccinating with a *M. haemolytica* toxoid on arrival has also been shown to reduce BRD morbidity and mortality of feedlot cattle (Jim 1988). A study in 2008 showed that combined vaccination with a *M. haemolytica* toxoid and tilmicosin decreased calf mortality when compared to treating with tilmicosin alone (Bryant 2008).

These studies suggest the use of vaccination, prophylaxis, and metaphylaxis may be effective at reducing the occurrence of BRD, however even when applying these practices some feedlots may experience significant BRD in a given year. This demonstrates the limit of what is known about the risk factors for BRD and what is occurring in the acute stages of BRD development.

2.6 CHRONIC BOVINE RESPIRATORY DISEASE

Chronic suppurative bronchopneumonia develops when an infection is not resolved and remains active. Pulmonary lesions may be colonized by secondary invaders, such as *Arcanobacterium pyogenes*, making it difficult to determine the primary causative agent.

Chronic BRD is associated with multiple etiologic agents and many pathological processes which are not necessarily distinct from one another. Chronics or "non-responders" are calves who are not responding to antimicrobial therapy. These calves typically will have weight loss, chronic pyrexia, and/or lameness. In a 1998 study, it was found that "chronics" contributed to 40% of calf mortalities in a western Canadian feedlot with 21% having only polyarthritis and 40% having chronic bronchopneumonia and polyarthritis (Pollock 2000). A study in 2008 found *M. bovis* and *M. haemolytica* to be the most common agents isolated from animals with chronic BRD by IHC on postmortem samples (Booker, Abutarbush et al. 2008). In that study, 96% of BVDV positive animals were also positive for *M. haemolytica* and 80% of animals positive for *H. somni* were also positive for *M. bovis*. Interestingly, all *H. somni* positive animals were negative for *M. haemolytica* as well as BVDV. That study demonstrates that multiple etiologic agents are at work in animals with chronic BRD.

2.7 EFFECT OF BRD ON PERFORMANCE

BRD has been shown to affect the ADG as well as carcass quality. At slaughter, carcasses with pulmonary lesions have an associated decreased gain of 0.07 to 0.20 kg/animal-day and have been shown to have a decreased carcass quality (Wittum, Woollen et al. 1996; Loneragan, Dargatz et al. 2001; Cusack, McMeniman et al. 2007). Auction market derived calves have been shown to have increased BRD morbidity and mortality, increased BRD relapse rates and lower rates of gain than ranch derived calves (Thorlakson, Martin et al. 1990). In the early feeding period, less than 35 DOF, pleural adhesions have been associated with a reduced ADG and in the late finishing period, greater than 35 DOF, bronchopneumonia has been

associated with a decrease in ADG (Thompson, Stone et al. 2006). All of these studies suggest BRD to result in significant economic losses to feedlots.

2.8 GENERAL PULMONARY PATHOLOGY

Atelectasis is the incomplete expansion of the lungs and can be classified as congenital or acquired (Caswell 2007). Congenital atelectasis is seen in fetal lungs which have not been aerated. Acquired atelectasis can be the result of complete airway obstruction or compression secondary to pleural or intrapulmonary lesions. Obstructive atelectasis can result if the small bronchi are completely occluded as they have very little collateral ventilation. Grossly atelectic lungs will be dark-red in color with a fleshy texture. Histologically, the alveolar walls will be congested with edema and there will be PAMs within the alveoli. Atelectasis can be the result of hypoxic damage, hypoxic vasoconstriction, reduced surfactant or an artifact from processing.

Tissue expansion due to gas accumulation is called emphysema and can be classified as alveolar or interstitial (Caswell 2007). Alveolar or vesicular emphysema refers to alveolar expansion with accompanying damage to the alveolar walls, but without fibrosis. Grossly, emphysematous lungs are pale in color and have a puffy texture and may have rib imprints on the pleural lung surface. Air-filled bullae may form from coalescence of small gas filled vesicles. Emphysematous lung has decreased alveolar surface area equating to decreased gas exchange, loss of elastic recoil, and thus having decreased function. Histologically, the alveoli will be expanded with the alveolar septae being thickened with fibrous tissue. Interstitial emphysema is common in cattle likely due to the interlobular septae and lack of collateral ventilation. This finding in cattle does not equate to primary lung damage and may occur

secondarily to interstitial pneumonia due to BRSV or 3-methylindole toxicity. It is thought to result from increased expiratory effort and high intra-alveolar pressures.

In cattle, pulmonary vasculitis can be due to bacterial emboli or damage to the vessel walls by BVDV or *H. somni* resulting in neutrophil infiltration into the vessel walls and possible thrombi within the vessel lumen.

The inflammatory response in the lung varies with the cause, route of infection and duration resulting in different patterns of lung injury. Bronchial necrosis can be caused by many things including bacterial or viral infections, parasitism as well as exposure to allergens and toxic gases. Ciliated epithelium is very sensitive to injury and will usually become necrotic and slough early on in the disease process. Fibrinonecrotic bronchitis is seen with viral infections and results in epithelial necrosis and adherent fibrinous exudates. With the removal of the stimulus, bronchial necrosis can be repaired by epithelial regeneration. However, if the injury is severe or prolonged, fibrosis may occur as well as epithelial hyperplasia or metaplasia. Chronic infection and airway damage may ultimately result in bronchiectasis. Bronchiectasis is the chronic, irreversible dilation of bronchi due to chronic bronchial obstruction or infection. In this process, neutrophil proteases and free radicals damage the bronchiolar wall epithelium and connective tissue leading to a weakened wall and mucociliary clearance failure. Thus, it is a self propagating cycle of exudate accumulation within the lumen, airway obstruction, and bronchiolar wall destruction and dilation. In cattle, it is typically seen as a complication of bronchopneumonia or bacterial bronchitis. The complete lobular septae and lack of collateral ventilation in cattle impairs the resolution of bronchopneumonia and leads to widespread atelectasis of the lobule (Caswell 2007).

Bronchiolitis obliterans occurs secondarily to chronic bronchiolar damage and represents inappropriate wound healing. Damaged epithelium results in fibrin formation. If this fibrin is not removed promptly, it can be reorganized within 7 days resulting in a fibrous polyp occluding the airway (Caswell 2007). In cattle, it is commonly seen with chronic bronchopneumonia due to viral or neutrophil mediated damage to the epithelium.

Bronchopneumonia is the result of the inhalation of bacteria resulting in an exudative lesion at the junction of the bronchioles and alveoli. Most commonly it will be seen in the cranioventral lung lobes. Neutrophils will be found filling the bronchi and alveoli. Terminal bronchioles appear predisposed as they have limited protection from both mucociliary clearance as well as PAMs. Typically, bronchopneumonia is caused by opportunistic invaders as a result of increased exposure or impaired defenses. In cattle, impaired defenses can occur via viral or *Mycoplasma* infections, exposure to cold or toxic gases and stress (Caswell 2007). Impaired mucociliary clearance can be due to BoHV-1, BRSV, or parainfluenza-3 (PI3) infections and reduced PAM function can be due to BoHV-1 infections (Caswell 2007). Decreased neutrophil function and numbers can be seen with BVDV infection as well as stress, acidosis, or uremia (Caswell 2007). Grossly, bronchopneumonia appears as cranioventral consolidation which is firm on palpation and may appear gray to dark red in color. On cut surface there will be edema and purulent exudate from the airways. If it is acute fibrinous bronchopneumonia, there will be no exudate and the lung will be firm and discolored. Histologically, the nidus of infection occurs at the bronchiolar-alveolar junction with neutrophils filling bronchioles and alveoli as well as infiltrating the bronchiolar walls. Bronchiolar necrosis can be the result of viral infections and neutrophil mediated damage. Resolution of bronchopneumonia occurs by clearance through the airways via coughing and collateral ventilation. However, cattle have poor collateral ventilation

which hinders the clearance of exudates and predisposes to the development of chronic suppurative bronchopneumonia.

Chronic suppurative bronchopneumonia develops when an infection is not resolved and remains active. Pulmonary lesions are usually colonized by secondary invaders, such as *Arcanobacterium pyogenes*, making it difficult to determine the primary causative agent. It may present as continued neutrophil recruitment and exudate production or as pulmonary fibrosis, abscessation or sequestration. A sequestrum is an area of necrotic lung parenchyma and represents a permanent, non-functional region that will serve as a nidus of infection. Pleural adhesions may also develop with the organization of fibrin containing exudates.

Interstitial pneumonia is damage or inflammation to the alveolar and/or the interlobular septae. Diffuse alveolar damage results in acute exudation, then proliferation of type II pneumocytes, followed by interstitial fibrosis. It is typically seen in caudodorsal lung lobes and may result from infectious microorganisms as well as thermal or chemical injuries to the lung. Bronchointerstitial pneumonia is seen as bronchiolar necrosis and alveolar damage which can result from viral infections, such as BRSV, or toxic gases.

The pleura is a contiguous layer of mesothelial cells with an underlying basal lamina. It is divided into the parietal pleura, covering the thoracic wall, diaphragm, and mediastinum, and the visceral pleura, covering the lungs. Normally there is a small amount of pleural fluid within the thoracic cavity. The pleura has very limited defense mechanisms. Pleuritis in cattle may result by extension of bronchopneumonia or abscessation of the underlying lung parenchyma as well as hematogenously. Fibrinous exudates form adherent sheets on the pleural surface and there may be excessive fluid within the thoracic cavity. In cattle, pleuritis can result from *H. somni* infection.

2.9 DIAGNOSTIC METHOD IN BRD DIAGNOSIS

2.9.1 LUNG BIOPSY

Percutaneous trans-thoracic lung biopsy and fine needle aspirate (FNA) have been reported to be successfully used in human medicine to aid in the diagnosis of pneumonia and malignancy (Youmans 1968; Klein 1969; Grode, Faurschou et al. 1993; Milman 1993; Milman 1995; Niden and Salem 1997). A FNA is useful for cytological evaluation which typically will identify benign or malignant lesions, but it usually requires a core biopsy sample to correctly identify the type of tumor as this sample will provide structure (Milman 1993). In 1968, a Franklin Silverman biopsy needle was used successfully in human medicine to obtain lung samples in 90% of cases with diffuse disease and 100% of those with solitary masses, however radiograph guidance was utilized and there were up to 5 attempts made to obtain lung tissue (Youmans 1968). In 1993, a spring loaded biopsy needle (1.2 mm diameter) was proven effective (Milman 1993) and in 1995 a vacu-cut needle (1.2 mm diameter; 200 mm length) with a drill technique was shown to be an effective method (Milman 1995). Others have described using a semi-automatic lung biopsy instrument (diameter 0.95 or 1.2 mm) to successfully retrieve lung samples (2 to 30 mm in length) under fluoroscopic or CT guidance that were suitable for histopathology (Milman 1993).

Percutaneous lung biopsy has also been used successfully in veterinary medicine in many species, including dogs (Izumi, Oyama et al. 2004), swine (Izumi, Oyama et al. 2004), sheep (Braun, Estermann et al. 2000), cattle (Dungworth and Hoare 1970; Raphel and Gunson 1981; Braun, Estermann et al. 1999; Sydler, Braun et al. 2004) and horses (Dungworth and Hoare

1970; Reif 1974; Schatzmann, Straub et al. 1974; Raphel and Gunson 1981). Initially, the use of a trephine and electric drill were used for percutaneous lung sample collection. A technique reported in 1970 used a 6.4mm trephine with an electric hand drill to obtain lung samples from the caudal lung lobes of both cattle and horses in the 8th or 9th ICS to a depth of 5 cm (Dungworth and Hoare 1970). With this method, lung samples were obtained in 70% of cattle (14 of 20) and 100% of horses (2 of 2). The biopsies were 10mm to 30 mm in length with firm lungs yielding larger lung cores. In 1974, again a trephine with a pneumatic drill was used in horses, some healthy and some with heaves, under general anesthesia (Schatzmann, Straub et al. 1974). Samples were collected from the caudal lung lobes via the 7th or 8th ICS, using a 6mm trephine. Samples were obtained in 76% of attempts (19 of 25) with biopsy lengths ranging from 3mm to 30mm in length.

A study in 1981 using a Tru-cut biopsy instrument (14-ga; 15 cm length) was used successfully to obtain lung samples from 20 horses, 3 with heaves, 1 with pneumonia, and 16 healthy horses (Raphel and Gunson 1981). The sample was taken while the horse was standing through the 7th or 8th ICS at the level of the elbow, to a depth of 2 cm, yielding lung tissue in 100% (20 of 20) of the horses. The lengths of the biopsies ranged from 5mm to 20mm with all containing alveoli, bronchioles and blood vessels. In 1999, a method was described to sample the caudal lung lobes of standing, healthy adult cows inserting the biopsy needle in the 9th ICS, 5 cm to 10 cm dorsal to the ventral lung border, to a depth of 8 cm, with ultrasound guidance (Braun, Estermann et al. 1999). This study compared three different biopsy instruments, an 18-ga Autovac biopsy cannula, a 14-ga Tru-Cut biopsy instrument (142mm length) and a 14-ga Bard Biopty instrument (160mm length). Lung samples were obtained in 9% (1 of 11) of the Autovac biopsy cannula attempts, 96% (48 of 50) of the Tru-Cut biopsy instrument attempts

ranging in length from 1mm to 12 mm, and in 97% (38 of 39) of the Bard Biopty system attempts ranging in length from 1mm to 18mm. The same research group, in 2000, assessed the utility of two different biopsy instruments, a 14-ga Tru-Cut biopsy instrument (152mm length) and a 14-ga Bard Biopty-System (160mm length), on 20 healthy sheep using ultrasound guidance (Braun, Estermann et al. 2000). Samples were obtained from the 9th ICS in 100% of attempts (20 of 20) with 90% (18 of 20) being useful for histopathology. The biopsy length ranged from 6mm to 17mm with the Tru-Cut biopsy instrument and 2mm to 20mm with the Bard Biopty-System.

Another research group, in 2004, performed percutaneous lung biopsies on pigs and dogs to assess the utility of a larger bore biopsy (Izumi, Oyama et al. 2004). They performed biopsies successfully using a 12-ga automated cutting needle (240mm length) in the 5th ICS to a depth of 4 cm with minimal side effects. Finally, in 2006, Venner, etal, evaluated two biopsy instruments, a 14-ga Tru-Cut biopsy instrument (152mm length) and a 14-ga automated biopsy instrument (100mm length) on 50 standing horses (Venner, Schmidbauer et al. 2006). The biopsy was taken in the 8th ICS, mid-thorax, to a depth of 6cm yielding lung in 98% (97 of 99) of biopsies obtained. All of these studies suggest that percutaneous lung biopsy is a viable diagnostic tool to assess lung parenchyma in many animal species, including cattle. However, this diagnostic is not routinely performed in a clinical setting.

In summary, lung tissue sample lengths varied in size with the Tru-Cut® Soft Tissue Biopsy instruments (14-ga) obtaining samples 1mm to 20mm in length from cattle and horses (Raphel and Gunson 1981; Braun, Estermann et al. 1999; Braun, Estermann et al. 2000; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006) and a 14-ga spring loaded biopsy systems such as the Bard Biopty-system® obtaining samples ranging in length from 1mm to 18 mm

(Braun, Estermann et al. 1999; Braun, Estermann et al. 2000; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006). With all of these biopsy instruments, a stylet with a sample notch is advanced into the tissue, a cannula slides over the stylet, cutting a piece of tissue which is retained in the sample notch.

Many of the previously mentioned studies have demonstrated lung biopsy techniques which recovered lung tissue which was suitable for histopathology. Lung biopsy has been demonstrated to yield samples suitable for histological evaluation when using a pneumatic drill (Schatzmann, Straub et al. 1974), Tru-Cut® needle or a Bard Biopty-System® (Raphel and Gunson 1981; Braun, Estermann et al. 1999; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006). In horses, Raphel, et al (1981) found lung biopsy samples from the caudal lung lobe, taken with a 14-ga Tru-cut® biopsy, were appropriate for histological evaluation, containing pulmonary parenchyma and airways, and ranging in length from 5 to 20 mm (Raphel and Gunson 1981). Ultrasound guidance has been used to obtain lung biopsy samples with a 14-ga Tru-cut® biopsy and a 14-ga Bard Biopty®-System yielding a tissue sample 71% of the time (Sydler, Braun et al. 2004). In this study, the average length with a Tru-cut biopsy was 4.4±2.6mm and with a Bard Biopty-System was 5.7±4.4mm. Biopsies that were >4mm were found to be of excellent quality containing over 100 alveoli with several bronchioles and arterioles. Biopsies between 2 to 4 mm were found to be of good quality with > 50 alveoli with few bronchioles. Biopsies of 1 to 2 mm were found to be 'usable' containing 30 or more alveoli. Biopsies of < 1 mm were found to be unusable. The only artifact noted on histopathological evaluation was collapse of the alveoli. This study found no difference between the Tru-cut® biopsy and the Bard Biopty®-system. A study in 1998 determined that there is no gain in

diagnostic information with greater than 2 biopsies and that larger biopsies was more likely to be diagnostic (Curley, Johal et al. 1998).

Lung can be a difficult tissue to sample due to its compliant nature. Additionally, introduction of an instrument through the thoracic wall may induce pneumothorax which will push the lung away from the thoracic wall as well as the biopsy needle. Logically, one would expect to obtain a more diagnostic sample from a diffusely affected consolidated lung. Consolidated lung is not as compliant and would allow for a better core sample (Dungworth and Hoare 1970).

The "float sign" or the floatation of lung samples in 10% buffered formalin may indicate the presence of air within the biopsy sample implying the biopsy was of better diagnostic quality (Sydler, Braun et al. 2004). In a study performed by Curley, *et al*, (1998) there was no affect on sinking or floating in regards to size or number of alveoli obtained in a lung biopsy sample. If a sample floated it was determined to be due to either alveoli or abnormal tissue. The "float sign" did not appear to improve the diagnostic ability of percutaneous lung biopsies.

Percutaneous lung biopsy is indicated when a histological diagnosis of a diffuse lung disease or thoracic mass is required or when a bacteriological sample is needed. It is typically used when other less invasive diagnostic procedures have been exhausted. Contraindications include those animals with a hemorrhagic diathesis or those with forceful respiration or severe coughing. Coughing may result in a lung laceration or air embolism. Patients in whom emphysematous bullae are suspected should also not be biopsied as the potential for the development of a pneumothorax would be greater.

Possible side effects of percutaneous lung biopsy reported in humans include pleural hemorrhage, parenchymal hemorrhage, pneumothorax (Milman 1995; Niden and Salem 1997),

epistaxis, hemoptysis (Niden and Salem 1997), arterial air embolism (Aberle, Gamsu et al. 1987), and death. Air embolism is thought to occur when intra-alveolar or intra-bronchial air is introduced into the pulmonary circulation (Aberle, Gamsu et al. 1987). This can occur if the biopsy needle creates a broncho-venous fistula. For an air embolism to form, the perforated vessel must remain open in addition to a pressure gradient for air passage from the alveoli or bronchiole into the vessel. A favorable pressure gradient can occur with forceful respiration, coughing or ventilation. Reported side effects in veterinary medicine include pneumothorax in 30-44% (Dungworth and Hoare 1970; Braun, Estermann et al. 1999; Venner, Schmidbauer et al. 2006), hemoptysis in 10% (Raphel and Gunson 1981), epistaxis in 10%-12% (Braun, Estermann et al. 2000; Venner, Schmidbauer et al. 2006), coughing in 20% (Venner, Schmidbauer et al. 2006), collapse (Braun, Estermann et al. 1999), and sudden death (Reif 1974).

In 2006, endoscopy was performed in horses after lung biopsy was performed (Venner, Schmidbauer et al. 2006). Endoscopy revealed bleeding in the main stem bronchus in 32% of cases when using the Tru-cut® biopsy instrument and only 10% when using the automatic gun (Venner, Schmidbauer et al. 2006). In human medicine it is thought that using a needle greater in diameter than 18-g is associated with hemorrhage (Laurent, Montaudon et al. 2003). Hematoma formation was imaged via ultrasound in 14% with the Tru-cut instrument and 6% with automatic gun. This difference in hematoma formation was significant with the hematoma size being significantly smaller via the automatic gun (Venner, Schmidbauer et al. 2006). Long term effects were found to be minimal in a 1990 study on cows with the animals maintaining a normal appetite and respiratory rate as well as having normal white blood cell counts and fibrinogen concentrations for 10 days after the lung biopsy procedure (Braun, Estermann et al. 1999).

Lung biopsy sites on post mortem examination have revealed minimal hemorrhage with less than 10% of the lesions being severe and the animals not displaying any clinical signs (Braun, Estermann et al. 1999). In addition, biopsy sites had healed without complications within 10 days of the biopsy procedure (Braun, Estermann et al. 1999). No pleural adhesions were noted at post mortem examination (Dungworth and Hoare 1970; Braun, Estermann et al. 1999; Braun, Estermann et al. 2000) and there was only a small scar present at the biopsy sites (Braun, Estermann et al. 2000). In a 1999 study, cows were biopsied using both a Bard Biopty®-System and a Tru-cut® Needle on opposite sides of the thorax with similar lesions seen on post mortem evaluation (Braun, Estermann et al. 1999). These studies indicate that there are minimal long term effects associated with percutaneous lung biopsy and suggest that, in general, it is a safe procedure.

2.9.2 POLYMERASE CHAIN REACTION

Polymerase chain reaction (PCR) is a method used for the *in vitro* amplification of a specific DNA sequence. Amplification allows for the identification of very few copies of DNA in a sample and only requires intact DNA, not viable microorganisms. PCR has been found to be a very sensitive method for *H. somni* detection when compared to bacterial culture and immunohistochemistry (Tegtmeier, Angen et al. 2000). Real time reverse transcription PCR (RT-PCR) has also been show to be a more sensitive test for BRSV detection when compared to IHC or immunofluorescence antibody test (IFAT) (Boxus, Letellier et al. 2005; Willoughby, Thomson et al. 2008). Real time RT-PCR has been shown to be effective for BVDV identification in fresh and formalin fixed tissue samples (Bhudevi and Weinstock 2003). The formalin fixed tissues were stored for up to 7 years, all of which demonstrated BVDV with some

having decreased signal strength. This study suggests that real time RT-PCR may be useful for retrospective identification of BVDV. Overall, PCR seems to be a diagnostic method that would be useful for pathogen detection in lung samples from a feedlot animal. The microorganism does not need to be alive and it can be performed on fixed tissue samples.

2.9.3 IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) is a technique using specific antibodies to visualize the distribution of immunogenic epitopes in tissue sections. It is a direct method of antigen detection. IHC is most commonly used for infectious disease diagnosis in veterinary medicine. It is a technique that can rapidly identify many infectious agents including bacteria, viruses, and protozoa. It can be used for those organisms that prove to be difficult to isolate and culture as well as nonviable organisms. IHC can be performed on fixed tissue or on the same tissue sample as that used for histological evaluation (Haines and West 2005).

Although using IHC also allows one to assess the histopathology present, it can be difficult to obtain positive samples, even on animals with chronic pneumonia. In a study performed on feedlot cattle with chronic pneumonia, 10% (5 of 49) were IHC negative for *M. bovis*, *H. somni*, *M. haemolytica*, and BVDV, of which 60% (3 of 5) had no visible lesions (Haines, Martin et al. 2001). *H. somni* was demonstrated in 14.3% (7 of 49), *M. haemolytica* in 22.4% (11 of 49), *M. bovis* in 82% (40 of 49) and BVDV in 41% (20 of 49) using IHC. That study demonstrates that the ability to detect certain infectious agents using IHC is difficult even with the benefit of post mortem samples taken from chronically affected animals (Haines, Martin et al. 2001).

IHC is a diagnostic tool that can provide not only information about the microorganisms present in the tissue but also histopathology. Specimens should be no more than 1cm thick with a formalin to tissue ratio of 10:1 (Andrews and Kennedy 1997). To perform IHC the antigenic epitope must be intact, but it does not need to be viable.

2.9.4 BACTERIAL CULTURE

Bacterial culture of a nasal swab or wash is not necessarily indicative of microorganisms within the lung (Thomas, Dizier et al. 2002; Caswell 2008). Bacterial culture was found to have the lowest sensitivity when compared to IHC or PCR techniques (Tegtmeier, Angen et al. 2000). One reason for this difference may be that to culture bacteria the presence of viable bacteria is a requirement. This may be difficult in a feedlot setting as the best results are to culture soon after collection and to obtain samples prior to antimicrobial therapy. Bacterial culture of treated feedlot cattle were positive 31.7% of the time and those not treated were positive 35.5% of the time (Saunders, Thiessen et al. 1980). Bacterial culture of a nasopharyngeal swab (NPS) did not predict results from BAL cytology in individual animals however at the group level NPS and BAL yielded similar results (Allen, Viel et al. 1991). *P. multocida* cultured from NPS and BAL was associated with morbidity in one study of naturally occurring respiratory disease (Allen, Viel et al. 1991). BAL and nasal swabs compared to post mortem lung cultures demonstrated BAL samples to be representative of post mortem lung culture results while nasal swabs were not (Thomas, Dizier et al. 2002). Similarly, BAL compared to nasal swabs in living animals showed nasal swabs not be to representative of the Mycoplasma spp. found in BAL cultures (Thomas, Dizier et al. 2002). These findings suggest that BAL samples or lung samples are more diagnostic samples for determining microbiological agents in regards to bovine pneumonia.

Another study in 2000, comparing *Pasteurella* isolates (*P. haemolytica* and *P. multocida*), found both nasal swabs and transtracheal swabs from acutely infected cattle to be positive 96% of the time for the same bacterial species with 70% being genetically identical based on serotype and antimicrobial sensitivity (DeRosa, Mechor et al. 2000). These findings suggest nasal swabs to be a useful tool for pathogen identification and antimicrobial susceptibility in acute cases.

2.9.5 VIRUS ISOLATION

Virus isolation is a direct method of antigen detection. Virus' require a host cell for survival and therefore require a cell culture that may take multiple days for identification. Some viruses display cytopathic effects that are usually apparent within 2-10 days. If there are only a few virus particles in the sample serial passage may be required to allow for virus amplification and identification. Once a virus is isolated, further testing may be required to differentiate vaccine strains from field strains. Some viruses are very sensitive making virus isolation a challenging diagnostic test. Samples tested by VI for BRSV were found to be hindered due to the sample transportation environment affecting virus viability when compared to ELISA or PCR (West, Bogdan et al. 1998). Indeed, this diagnostic method may be more useful for herd incidence or prevalence or possibly in an outbreak situation where virus identification is essential.

2.9.6 TRANSTRACHEAL WASH AND BRONCHOALVEOLAR LAVAGE

Transtracheal wash (TTW) and bronchoalveolar lavage (BAL) are useful diagnostics for diseases of the airway or alveoli, but may not be as useful for interstitial disease. BAL is

indicated in suspected cases of diffuse disease and TTW is more useful for localized disease and sample collection for bacterial culture. BAL samples have been demonstrated to show similar inflammatory changes in feedlot calves with and without signs of pneumonia with 35% of cases and 40% of controls having normal differential cell counts (Allen, Viel et al. 1992). Cases did, however, show a higher proportion of neutrophils and controls showed a higher proportion of macrophages and lymphocytes (Allen, Viel et al. 1992). Another study found cases to be associated with the presence of neutrophils and *P. multocida* in BAL fluid (Allen, Viel et al. 1992). TTW samples have been demonstrated to be an effective means of determining respiratory bacterial pathogens in calves (Espinasse, Alzieu et al. 1991). However, these diagnostic samples are not easily obtained in a feedlot setting and require relatively rapid processing to ensure proper interpretations.

2.9.7 THORACIC ULTRASOUND

Lung has high air content and as such a high attenuation of ultrasound waves making it difficult to utilize ultrasound as a diagnostic tool (Pedersen and Ozcan 1986). Normal aerated lung has a hyperechoic line, indicating the pleura, and reverberation artifacts resulting in an inability to assess the lung parenchyma of normal lung (Braun, Sicher et al. 1996). Pneumonic lung will appear like liver parenchyma and have comet tails arising from a roughened pleural surface and limited reverberation lines (Reef, Boy et al. 1991; Braun, Pusterla et al. 1997; Scott 1998). Using ultrasound, one is able to demonstrate altered lung parenchyma penetration due to pulmonary congestion, pneumonia, edema and embolism (Pedersen and Ozcan 1986; Flock 2004) as ultrasound penetration in lung tissue is proportional to pulmonary consolidation (Pedersen and Ozcan 1986).

Ultrasound can also be utilized in detecting pleural effusion, guiding FNA or biopsies, assessing the pleural surface or locating superficial abscessation or embolism (Pedersen and Ozcan 1986; Flock 2004). Physical examination and ultrasound findings have been determined to correlate well with post mortem findings in adult sheep (Scott and Gessert 1998), calves (Rabeling, Rehage et al. 1998; Flock 2004), young cattle (Flock 2004) and cows (Scott 1998; Flock 2004). Thoracic studies have found ultrasound to be superior to radiography in the detection of small volumes of pleural fluid (Reef, Boy et al. 1991; Braun, Pusterla et al. 1997). A study in 1997 used ultrasound to accurately determine thoracic lesion severity and thoracocentesis site (Braun, Pusterla et al. 1997; Scott and Gessert 1998). In characterizing pulmonary lesions, the lung periphery must be affected to utilize ultrasonography.

2.9.8 ACUTE PHASE PROTEINS

Acute phase proteins, including serum amyloid A, haptoglobin, fibrinogen, and α_1-acid glycoprotein are produced and secreted by the liver as a part of the acute phase response to tissue injury. Acute phase proteins indicate inflammation, either chronic or acute. Acute phase proteins were evaluated in clinically ill cattle to differentiate between acute and chronic inflammation (Horadagoda, Knox et al. 1999). Acute inflammation was determined to be present if the case was sudden in onset with a fever, depression, and signs consistent with inflammation of a specific organ system such as swollen joints, mastitis, or acute pneumonia. Chronic inflammation was determined to be present if the case was of a longer duration, an absence of fever and an absence of pain such as with chronic respiratory disease, hepatic abscessation, and amyloidosis. In that study, a high serum concentration of serum amyloid A was found in 100% of acute cases and 54% of chronic cases; high concentrations of serum

haptoglobin were found in 68% of acute cases and 24% of chronic cases; and high concentrations of α_1-acid glycoprotein was found in 89% of acute cases in 72% of chronic cases (Horadagoda, Knox et al. 1999). All of these findings were significantly different ($p<0.05$) between acute and chronic cases. They also found acute phase proteins to be a more sensitive method for differentiation between acute and chronic inflammation than neutrophil counts, however these changes were not specific for a particular cause. These findings were mirrored in another study evaluating healthy cattle and cattle with acute or chronic inflammation in relation to haptoglobin and serum amyloid-A concentrations (Alsemgeest, Kalsbeek et al. 1994). Fibrinogen is the circulating precursor for fibrin. It is a non-specific marker for inflammation. It has been shown to be elevated with experimental injection of turpentine or endotoxin and clinically with pericarditis and peritonitis (Eckersall and Conner 1988). It has also been found to be elevated in calves after transportation (Arthington, Eichert et al. 2003).

Several studies have suggested serum haptoglobin to be a useful marker for BRD in a feedlot setting. Serum haptoglobin concentration has been found to be greater in auction market derived calves than those that are weaned and maintained on the home ranch for 45 days prior to entering the feedlot (Step, Krehbiel et al. 2008). Studies have shown serum haptoglobin concentrations to be significantly greater in steers with BRD compared to those without (Godson, Campos et al. 1996) as well as in those steers requiring more than one treatment for BRD (Carter, Meredith et al. 2002; Berry, Confer et al. 2004). Additionally, serum haptoglobin concentration has been found to be associated with BRD mortalities (Godson, Campos et al. 1996; Carter, Meredith et al. 2002) as well as with experimental BoHV-1 infections (Aich, Jalal et al. 2007). In Finland, calves with respiratory disease were evaluated for specific pathogens and associated elevations in acute phase proteins (Nikunen, Hartel et al. 2007). This study found

that *P. multocida* was significantly ($p<0.05$) associated with an elevation of acute phase proteins including fibrinogen, haptoglobin, serum amyloid-A, and α_1-acid glycoprotein. There was no association noted with viral pathogens or with *M. dispar, Fusobacterium necrophorum* and *Actinomyces pyogenes*. That study did not isolate *M. haemolytica*.

There are, however, additional studies that have not found serum haptoglobin concentration to be a useful marker for BRD in a feedlot setting but rather a tool to assess treatment response. Serum haptoglobin concentration was found to be a useful marker for treatment response in those animals treated for BRD having significantly lower concentrations than those with BRD that were not treated (Wittum, Young et al. 1996). Additionally, this study found that those animals with elevated serum haptoglobin concentrations did not necessarily have the final outcome of BRD suggesting serum haptoglobin to be a non-specific indicator of disease. Certainly it has been found to increase with the experimental injection of turpentine or endotoxin as well as in cases of *M. haemolytica* infection, liver abscessation, pyometra and traumatic reticulopericarditis (Eckersall and Conner 1988). Additionally, mean serum haptoglobin concentrations did not differ in accordance with lung lesions at slaughter (Wittum, Young et al. 1996). This may be due to those animals not having respiratory disease or may indicate effective therapy with no residual lung lesions. Serum haptoglobin concentration does not appear to be a very specific method for BRD detection. It is likely a non-specific marker for inflammation and can possibly be a useful indicator for treatment response. However, it will not be useful during a hemolytic crisis as haptoglobin binds free hemoglobin and is removed from circulation by the liver (Eckersall and Conner 1988).

2.9.9 SEROLOGY

Serology is a technique that determines the antibody titer to specific microorganisms and is indicative of recent exposure. In a study in 1992, acute titers to PI-3, BVDV, BRSV, and BoHV-1 did not differ between cases and controls with seroconversion being similar between cases and controls with the exception of PI-3 which was more common in controls (Allen, Viel et al. 1992). In that study there was also no association between titers and BAL cytology. In a 1999 study, seroconversion to BoHV-1 and increased titers to H. somni on decreased the risk of an undifferentiated fever (Booker, Guichon et al. 1999). In that same study, seroconversion to BVDV was higher in cases than in controls and high BoHV-1 titers at arrival were associated with an increased mortality (Booker, Guichon et al. 1999). Serology may be better for determining *M. bovis* infection, especially in those animals with chronic disease and those that have been previously treated as *in vitro* growth may be impaired (Caswell 2008). Serology coupled with clinical signs may be indicative of infection with a specific microorganism and could be used in a feedlot setting.

2.9.10 POST MORTEM EVALUATION

Post mortem evaluations are an essential part of an effective feedlot management system. However, they yield information on the cause of death rather than on the inciting cause in regards to BRD. With today's technology it is now possible to utilized digital images for the determination of a diagnosis and have similar results as gross post mortem examination (Wildman, Schunicht et al. 2000). In addition, cultures from post mortem samples may yield secondary invaders and antimicrobial sensitivities may be altered due to previous therapy also clouding the determination of the inciting cause of BRD.

2.10 CONCLUSION

In the literature, there is ample information on chronic BRD and the microorganisms associated with it, but there is relatively little information on acute BRD. Many diagnostic tools are at our disposal including TTW, BAL, serology, ultrasound, etc., but to actually determine what is occurring early on in the disease process lung samples need to be obtained from feedlot steers on entry into the feedlot and on initial diagnosis.

In feedlots, there are many reports assessing post mortem findings and serology but this only gives information on chronic BRD and microorganism exposure both of which may not correlate with an animal's disease. Lung biopsy on live cattle has been shown to be a relatively safe procedure in many animal species including cattle. However, these studies were sampling the caudal lung fields which are not the typical location for BRD. It is reasonable to think that a lung biopsy technique could be developed to sample the cranioventral or middle lung lobes which are a more typical location for the development of BRD. Additionally, previous studies have reported that appropriate samples can be obtained via this method for histopathology.

To this end, the following study was undertaken. The objectives of this project were: 1) to determine if live animal lung biopsy could be used to characterize early pathologic changes in the bovine lung associated with BRD, 2) to determine if there are specific infectious respiratory pathogens that can be identified in association with early pathological changes, and 3) to determine whether pulmonary pathology characterized by live animal lung biopsy at arrival and at the time of initial BRD diagnosis was associated with health and production outcomes of feedlot steers in a commercial feedlot.

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CHAPTER 3

THE DEVELOPMENT OF A PERCUTANEOUS LUNG BIOPSY PROCEDURE FOR USE ON FEEDLOT STEERS IN A COMMERCIAL FEEDLOT SETTING

3.1 INTRODUCTION

Lung biopsy has historically been used as a diagnostic tool in human (Youmans 1968; Klein 1969; Grode, Faurschou et al. 1993; Milman 1993; Milman 1995; Niden and Salem 1997) and veterinary medicine (Dungworth and Hoare 1970; Reif 1974; Schatzmann, Straub et al. 1974; Raphel and Gunson 1981; Smallwood and Zenoble 1993; Braun, Estermann et al. 2000; Izumi, Oyama et al. 2004; Sydler, Braun et al. 2004). In veterinary medicine, many studies have shown percutaneous lung biopsy to be a relatively safe procedure (Dungworth and Hoare 1970; Braun, Estermann et al. 1999; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006). Post mortem evaluation of lung biopsy sites have revealed minimal hemorrhage with less than 10% of the lesions being severe and the animals not displaying any clinical signs (Braun, Estermann et al. 1999). In addition, biopsy sites have healed without complications within 10 days of the biopsy procedure (Braun, Estermann et al. 1999). Multiple studies have found no pleural adhesions on post mortem examination (Dungworth and Hoare 1970; Braun, Estermann et al. 1999; Braun, Estermann et al. 2000) and report only a small scar present at biopsy sites (Braun, Estermann et al. 2000).

Previous studies have demonstrated that adequate tissue can be obtained by percutaneous lung biopsy of the caudodorsal lung lobes via the 7^{th} to 9^{th} ICS in cattle, sheep, and horses for histopathology (Schatzmann, Straub et al. 1974; Raphel and Gunson 1981; Braun, Estermann et

al. 2000; Venner, Schmidbauer et al. 2006). Different instruments used include a pneumatic drill (Schatzmann, Straub et al. 1974) and a Tru-Cut® Soft Tissue Biopsy Needle or a Bard Biopty-System® (Raphel and Gunson 1981; Braun, Estermann et al. 1999; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006). Sample sizes range from 3 to 30 mm, depending on the instrument used for collection, with many of these reported to be adequate for histopathology.

Using a pneumatic drill to biopsy horses and cattle, samples collected ranged in length from 3 to 30 mm, with all being suitable for histological evaluation (Schatzmann, Straub et al. 1974). In 1981, a study in horses found lung biopsy samples taken with a 14-ga Tru-Cut® Soft Tissue Biopsy Needle were appropriate for histological evaluation, containing pulmonary parenchyma and airways, and ranging in length from 5 to 20 mm (Raphel and Gunson 1981). In 2000, healthy sheep were used to assess the utility of two different biopsy instruments, a Tru-Cut® Soft Tissue Biopsy Needle (14-ga) and a Bard Biopty-system® (14-ga) (Braun, Estermann et al. 2000). Sheep were biopsied on both sides of the thorax, up to 2 attempts per side, with 90% of samples being useful for histological evaluation. In 2006, another comparison study was done in horses using a 14-g Tru-Cut® Soft Tissue Biopsy Needle and a 14-ga automatic biopsy gun (Venner, Schmidbauer et al. 2006). This study found that samples obtained with either instrument were of similar quality with 98% yielding lung and 2% muscle (Venner, Schmidbauer et al. 2006).

Ultrasound guidance was used to obtain lung biopsy samples from healthy cows with a 14-ga Tru-Cut® Soft Tissue Biopsy Needle and a 14-ga Bard Biopty®-System yielding a tissue sample 71% of the time (Sydler, Braun et al. 2004). Biopsies that were >4mm were found to be of excellent quality containing over 100 alveoli with several bronchioles and arterioles. Biopsies between 2 to 4 mm were found to be of good quality with > 50 alveoli with few bronchioles.

Biopsies of 1 to 2 mm were found to be 'usable' containing 30 or more alveoli. Biopsies of < 1 mm were found to be unusable. The only artifact noted on histopathology was collapse of some of the alveoli. This study found no difference between the Tru-Cut® Soft Tissue Biopsy Needle and the Bard Biopty®-system.

Percutaneous lung biopsy has not been previously assessed in feedlot cattle in a commercial feedlot setting. The purpose of this project was to develop a percutaneous lung biopsy technique to be used on feedlot steers in a commercial feedlot setting. This technique is being developed for later use as a potential diagnostic tool for assessment of early BRD pathology in feedlot steers.

3.2 MATERIALS AND METHODS

3.2.1 STUDY ANIMALS

Part A: All study animals utilized in this portion of the study were auction market derived crossbred steer and heifer calves from a commercial feedlot in southern Alberta and were chronically affected with respiratory disease. A total of 20 animals were transported in accordance with Canadian Food Inspection Agency (CFIA) regulations 6 hours by tractor trailer to the Western College of Veterinary Medicine (WCVM). All animals were housed in an open-air paddock with a bedding pack, shelter, and free access to grass hay and water. Animals arrived in three different groups: group A (n=7), B (n=6), and C (n=7). The overall mean body weight was 468 lbs (213 kg), ranging from 332 lbs to 720 lbs (151 to 327 kg).

Part B: All animals utilized in this portion of the study also were auction market derived crossbred steer and heifer calves from the chronic pen of a commercial feedlot in Southern

Alberta. Animals in the chronic pen are those who have been removed from their home pen due to poor response to previous treatment for BRD or have had a reoccurrence of disease. A total of 14 animals were utilized in this portion of the study with a mean weight of 623 lbs (283 kg), ranging from 318 lbs to 783 lbs (145 to 356 kg). Animals were housed in side-by-side, open-air, dirt-floor pens with a central feed alley and 20% porosity wood-fence windbreaks.

3.2.2 PERCUTANEOUS LUNG BIOPSY OF THE RIGHT CRANIOVENTRAL LUNG LOBE OF FEEDLOT STEERS WITH CHRONIC BRD

Part A: Each study animal was restrained in a squeeze chute with a rope halter securing the head. Bars were placed in front of and behind the animal to prevent forward and backward movement. Sedation was given via the tail vein or in the epaxial muscles (Rompun®, Bayer, Inc., Toronto, ON; 0.05 to 0.1mg/kg, IM or IV). A rope was fastened below the fetlock to secure the forelimb in extension to gain access to intercostal space (ICS) 2. A wooden block was placed under the extended forelimb as well as beneath the sternum to facilitate the animal standing comfortably for the procedure. [Figure 3.1]

The sample site for cranioventral lung lobe sampling encompassed a 12 x 12 inch (30 x 30 cm) area from ICS1 to ICS4. Beginning at mid-scapular level and extending below the elbow on both right and left sides of the thorax. Intercostal spaces were counted and labeled prior to sampling. The site was prepared using a standard aseptic technique. Local anesthetic was infused (Lido-2 with epinephrine, Rafter Products, Calgary, AB; 0.5 mg/kg, IM and SC) in the musculature and subcutaneous tissues of the biopsy site in ICS2 half the distance between mid-scapula and elbow. A stab incision through the skin was made using a #15 blade. The biopsy instrument, either a 14-ga Tru®-cut biopsy needle (Cardinal Health, McGraw Park, IL) or a Bard® Magnum® reusable biopsy instrument (C. R. Bard, Inc., Covington, GA) with a 12-ga or

14-ga biopsy needle, was inserted off the front edge of rib 3 into ICS2 at a 45° angle to the body wall directing the tip cranial-dorsally to a depth of 3-5 cm. No more than two samples were taken per side. The stab incision was closed using tissue staples (Appose® ULC35, Tyco Healthcare, Norwalk, CT). The lung biopsy samples were removed from the biopsy instrument using a needle and placed in a 10% formalin solution. Samples were held at room temperature until submission to the diagnostic laboratory (Prairie Diagnostic Services, Saskatoon, SK) for processing.

Part B: The right cranioventral lung lobe was sampled in each animal using a Bard® Magnum® reusable biopsy instrument with a 12-ga biopsy needle as per the biopsy procedure stated above.

3.2.3 POST-BIOPSY MONITORING, EUTHANASIA AND POST MORTEM

The steers were monitored for any signs of distress, hemoptysis, epistaxis or depression for the hour after the procedure was performed. All animals were visually inspected on a daily basis until being humanely euthanized for post mortem evaluation. Post mortems were performed using a standard field post mortem technique (Booker 1991). A digital image was obtained of the opened right thoracic cavity as well as a cross section of the lung tissue. [Appendix A]

3.2.4 LUNG BIOPSY OF THE RIGHT CRANIOVENTRAL AND RIGHT MIDDLE LUNG LOBES OF PLUCKS FROM CALVES ACUTELY INFECTED WITH BRSV

In order to assess the utility of different biopsy needle gages, partial plucks from 76 Holstein-Friesian dairy calves (aged 2 to 4 months) were harvested from another study. Plucks, by definition, include the thoracic viscera (larynx, trachea, lungs, and heart) and liver. In this

study only the trachea and lungs were harvested. All of the calves had been experimentally infected with BRSV and were in the acute stages of disease. Each pluck was placed on a bench top in anatomic orientation. There were three different groups of plucks, group E, F and G. Group E (n=20) were atelectic plucks that were biopsied using the Bard® Magnum® reusable biopsy instrument with a 14-ga and 12-ga biopsy needle. Samples were taken from the right cranioventral and right middle lung lobes. Group F (n=26) plucks were all inflated to 1 psi. Inflation was accomplished by placing a 14-french cuffed endotracheal tube within the trachea and inflating the lungs with an air compressor to 1psi. Inflated plucks were biopsied with the Bard® Magnum® reusable biopsy instrument using a 14-ga and 12-ga biopsy needle of the right middle lung lobe. Group G (n=28) plucks were randomly assigned via coin toss to be inflated to 1 psi or remain atelectic. In this group, the right middle lung lobe was sampled using the Bard® Magnum® reusable biopsy instrument with a 12-ga, 14-ga, and a fabricated 4mm (8-ga) biopsy needle. Two samples were taken per pluck per biopsy needle gage within every group. The samples were removed from the biopsy channel using a needle and placed in a 10% formalin solution.

3.2.5 SAMPLE PROCESSING

Lung samples were placed into micro-centrifuge tubes of a 10% formalin solution. Live animal lung biopsy samples were evaluated via histopathology and IHC for BVDV, *M. bovis*, *H. somnus*, and *M. haemolytica*. Post mortem lung samples were also collected for histopathology as well as bacterial culture. Lung tissue samples for aerobic and anaerobic bacterial culture were submitted within 12 hours of collection. Lung biopsy samples from plucks were measured and evaluated for histopathology. All samples were submitted to Prairie Diagnostic Services (PDS)

(Saskatoon, SK) for processing and evaluation. All lung biopsy samples were evaluated by histopathology based on a standard form by the same board certified veterinary pathologist in a blinded manner. In part A, a histopathology score was assigned to all lung biopsy samples based on certain lesions being present (received a 1) or not (received a 0), with a maximum score of 11. Lung pathology assessed included suppurative bronchopneumonia, chronic suppurative bronchopneumonia, bronchiectasis, fibrinonecrotizing pneumonia, fibrinous pleuritis, bronchiolar necrosis, bronchiolitis obliterans, acute/subacute interstitial pneumonia, proliferative interstitial pneumonia, BALT hyperplasia, and vasculitis. [Appendix B] In part B and section 3.2.4, a pathology score was assigned to all lung biopsy samples based on certain lesions being present (received a 1) or not (received a 0), with a maximum score of 20. Lung pathology assessed in the bronchioles included neutrophilic infiltration, abscessation, bronchiectasis, bronchiolar necrosis, syncytial cells, and bronchiolitis obliterans. Lung pathology assessed in the alveoli included neutrophilic infiltration, abscessation, necrosis and fibrin, oat cells, interstitial reaction, syncytial cells, hyaline membranes and fibrosis. In addition, bronchoalveolar associated lymphoid tissue (BALT) was assessed for hyperplasia, the pleura were assessed for neutrophilic infiltration and fibrinous pleuritis and the vessels were assessed for neutrophilic infiltration and fibrinoid necrosis. [Appendix C]

3.3 DATA ANALYSIS

The lengths, in millimeters, of the lung biopsy samples collected from the plucks, were compared between the 14-ga, 12-ga, and 4-mm (8-ga) biopsy needle as well as between cranioventral and middle lung lobes within groups. The data was analyzed using a paired t-test as the differences were normally distributed based on the Shapiro-Wilks test for normality. A *p*

value of ≤0.05 was accepted as significant. Statistical analysis was performed with STATA/IC version 10 (Statacorp LP, College Station, TX).

3.4 RESULTS

Part A: Of the 20 study animals biopsied, one was fatally wounded during the biopsy procedure. On post mortem evaluation, the pulmonary artery had been inadvertently biopsied. All other study animals did not demonstrate any adverse effects. Lung tissue was obtained from 13/20 (65%) of the steers.

Group A (n=7) were all biopsied using a 14-ga Tru-Cut® Soft Tissue Biopsy Needle. Of the 7 animals sampled, lung tissue was successfully harvested from 6. In general, the lung biopsy samples received lower histopathology scores than post mortem samples. In addition, of the 6 animals successfully sampled, 4 had the same diagnosis made from the biopsy and post mortem sample including acute interstitial pneumonia and suppurative bronchopneumonia, while 2 did not, including acute interstitial pneumonia and BALT hyperplasia. Bacterial culture results from post mortem tissue samples resulted in 2 culture positive for only *M. bovis*; 2 culture positive for *M. bovis, H. somni*, and *P. multocida*; 2 culture positive for *M. bovis* and *P. multocida;* and 1 culture positive for *M. bovis*, *H. somni*, and *M. haemolytica*. All of the post mortem lung samples were negative by IHC for *M. bovis*, *H. somni*, *M. haemolytica*, and BVDV.

Group B (n=6) were biopsied using a 14-ga Tru-Cut® Soft Tissue Biopsy Needle and the Bard® Magnum reusable biopsy with a 14-ga biopsy needle. Of the 6 study animals, 3 had successful biopsies taken. The automatic gun was successful in all 3 animals and the Tru-Cut® Soft Tissue Biopsy Needle was successful in 2 animals. Only 1 study animal had the same diagnosis based on the lung biopsy sample and the post mortem sample, chronic suppurative

bronchopneumonia. The other 2 study animals only revealed hemorrhage on the lung biopsy sample, but were diagnosed with suppurative bronchopneumonia based on the post mortem evaluation. Bacterial culture results from post mortem lung tissue samples resulted in 4 culture positive for *M. bovis* only; 1 culture positive for M. *bovis* and *H. somni*,; and 1 culture negative. IHC performed on post mortem samples revealed 2 positive for *M. bovis* alone; 1 positive for *M. bovis* and *M. haemolytica*; and 3 were negative. IHC performed on lung biopsy samples revealed only 1 to be positive for *M. bovis*. This steer was IHC negative on the post mortem sample.

Group C (n=7) were biopsied using the Bard® Magnum® reusable biopsy with a 14-ga and 12-ga biopsy needle. Each steer was sampled using one biopsy needle gauge based on a coin toss. Of the 7 steers, 5 were biopsied using the 12-g biopsy needle and 2 were biopsied using the 14-g biopsy needle. The 14-ga biopsy needle did not result in any tissue recovery. The 12-ga biopsy needle recovered lung parenchyma in 4 of 5 steers with some similarities found between lung biopsy samples and post mortem samples in 3 of 4 steers. Histopathology scores ranged from 0 to 3 for lung biopsy samples and 1 to 6 for post mortem lung samples. (Table 3.1) In general, lung biopsy samples and post mortem samples had similar findings such as BALT hyperplasia, bronchiolitis obliterans and bronchiectasis. However, the diagnoses were not consistent with post mortem samples revealing suppurative bronchopneumonia in 4 steers and acute interstitial pneumonia in 1 steer. IHC performed on post mortem lung samples were all negative for *M. bovis*, *H. somni, M. haemolytica*, and BVDV.

Part B: This lung biopsy procedure took about 20 minutes an animal under field conditions. The lung biopsy samples from this portion of the study resulted in 6/14 (42.9%) samples containing lung tissue. Of those samples, 4 (28.6%) contained bronchioles and 6

(42.9%) contained alveoli with 4 samples containing both structures. Histopathology scores ranged from 0 to 6 for lung biopsy samples with the pathologist finding the lung biopsy tissue samples to be extremely small making it difficult for histological evaluation. IHC revealed 1 sample to be positive for *M. haemolytica*.

Part C: The descriptive data for biopsy groups is represented in Table 3.2. Briefly, when sampling the right middle lung lobe of inflated lung, sample length ranged in size from 2 to 11 mm when using a 14-ga biopsy needle or a 12-ga biopsy needle, and from 2.5 to 10 mm when using a 4-mm (8-ga) biopsy needle. When sampling the right middle lung lobe of atelectic lung, sample length ranged in size from 2 to 12 mm with a 14-ga biopsy, from 1.5 to 15.5 with a 12-ga biopsy, and from 3 to 7.5 mm with a 4-mm (8-ga) biopsy needle. When sampling the cranioventral lung lobe of atelectic lung, sample length ranged in size from 2 to 11.5 mm when using a 12-ga biopsy needle and 3.5 to 11.5 mm when using a 14-ga biopsy needle. There was no significant difference in mean length of the paired biopsy samples taken from each pluck with each gauge biopsy needle. There was a significant difference in mean length ($P<0.05$) between the 14-ga and 12-ga biopsy needles within group F (inflated lung) when sampling the right middle lung lobe with the 12-ga biopsy sample being of greater length. There was a significant difference ($P=0.05$) between the 12-ga and 4-mm (8-ga) biopsy needle within the inflated lungs of group G with the 4-mm (8-ga) biopsy sample being of greater length. There was a significant difference in mean length ($P<0.05$) between the 12-ga and 4-mm (8-ga) biopsy needle within the atelectic lung of group G with the 12-ga biopsy sample being of greater length. There was a significant difference in mean length ($P<0.05$) between the 14-g and 4-mm (8-ga) biopsy needle within the atelectic lung of group G with the 14-ga biopsy sample being of greater length. There was also a significant difference ($P<0.05$) between the cranioventral lung lobe and middle lung

lobe when using the 14-ga biopsy needle with samples from the middle lung lobe being of greater length.

Histopathology was performed on 5 pluck biopsy samples taken from inflated lung which were randomly selected. The biopsy sample lengths were 2mm, 5mm, 7mm, 8mm, and 12mm. Of these samples only the 2mm length biopsy was determined to be an inadequate sample by the veterinary pathologist. The rest of the samples contained bronchioles and alveoli and demonstrated enough pathology for a diagnosis to be made. In addition, 2 of the samples also contained syncytial cells indicating BRSV infection.

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3.5 DISCUSSION

In this study, lung was obtained in 55.9% of lung biopsy samples from feedlot steers chronically affected with BRD which is lower than previous reports. In previous studies, recovery of adequate lung tissue occurred in 71% to 98% of lung biopsy samples from the caudodorsal lung lobes when using 14-ga Tru®-cut biopsy needle or the Bard Biopty® instrument on sheep and cattle (Braun, Estermann et al. 2000; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006). In our study, lung tissue was obtained in 70% (14/20) of lung samples from chronic animals in a hospital setting (part A) and 42.8% (6/14) of lung samples from chronic animals in a commercial feedlot setting (part B). The post mortem sample and percutaneous lung biopsy sample resulted in the same diagnosis in 61.5% (8/13) animals suggesting that percutaneous lung biopsy may be an effective tool for the diagnosis of chronic pneumonia in feedlot cattle.

In this study, calf plucks inflated to 1 psi and sampled with a 12-ga biopsy needle yielded a significantly ($p=0.0002$) longer lung biopsy sample from the middle lung lobe than a 14-ga

biopsy needle when using a Bard® Magnum® reusable biopsy instrument. Also, when sampling a pluck inflated to 1 psi, a modified 4-mm (8-ga) biopsy needle obtained a significantly ($p=0.05$) longer sample of lung from the middle lung lobe than did a 12-ga biopsy needle when using a Bard® Magnum® reusable biopsy needle. This suggests that the 4-mm (8-ga) biopsy needle may yield a better sample from the right middle lung lobe when using this technique. When sampling atelectic plucks, a 14-ga and 12-ga biopsy needle obtained a significantly longer piece of lung tissue from the middle lung lobe when compared to a 4-mm (8-ga) biopsy needle suggesting the smaller gauge biopsy needles may be better for atelectic lung. Finally, a 14-ga biopsy needle obtained a significantly ($p=0.04$) longer piece of lung tissue from the middle lung than from the cranioventral lung lobe of an atelectic pluck. This suggests that the middle lung lobe may be a better sampling site than the cranioventral lung lobe for this technique and should be evaluated in live animals (chapter 4). In addition, anatomically the right cranioventral lung lobe is relatively small and thin when compared to the right middle lung lobe likely making it more difficult to sample using this technique.

When sampling inflated lung, sample length ranged in size from 2 to 11 mm when using a 14-g biopsy needle or a 12-ga biopsy needle, and from 2.5 to 10 mm when using a 4-mm (8-ga) biopsy needle. The inflated lung samples obtained in this study were similar in size to those reportedly obtained from live animal biopsy (Raphel and Gunson 1981; Braun, Estermann et al. 1999; Braun, Estermann et al. 2000; Sydler, Braun et al. 2004; Venner, Schmidbauer et al. 2006). Additionally, in this study, lung samples from 5 to 12 mm in length were deemed adequate for histopathologic evaluation which is similar to a previous study in which lung biopsy samples > 4 mm in length were of excellent quality for histopathologic evaluation (Sydler, Braun et al. 2004). This indicates that this lung biopsy technique may be useful for this purpose.

In this study, IHC results were poor in comparison to bacterial culture results. This may be due to an inadequate sample obtained for IHC or post mortem bacterial growth may have resulted in the positive cultures. These findings suggest that IHC may not be useful for evaluating lung biopsies taken without the benefits of imaging guidance. Additionally, in this study 22.2% of lung biopsy samples were positive for at least one antigen (*M. bovis, M. haemolytica, H. somni,* BVDV) and post mortem lung samples were found to be positive 15% of the time for at least one of the above antigens with 33.3% being positive on both lung biopsy and post mortem sample. These findings indicate that the lung biopsy sample had similar low positive results on IHC as post mortem samples.

Overall, this preliminary work indicates that this percutaneous lung biopsy procedure of the right middle lung lobe may be a viable technique when used on feedlot steers with chronic pneumonia. These findings suggest that using a Bard® Magnum® reusable instrument with a 4-mm (8-ga) biopsy needle may yield lung tissue samples suitable for histopathological evaluation. However, further evaluation with this instrumentation in a field setting is needed to determine this procedures usefulness for elucidating early pulmonary histopathologic changes and associated infectious pathogens in steers with BRD.

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FIGURE 3.1: ANIMAL RESTRAINT FOR PERCUTANEOUS LUNG BIOPSY OF THE CRANIOVENTRAL LUNG LOBES

TABLE 3.1: HISTOPATHOLOGY SCORES OF LIVE ANIMAL LUNG BIOPSY SAMPLES OF THE CRANIOVENTRAL LUNG LOBE FROM FEEDLOT STEERS CHRONICALLY AFFECTED WITH BRD

Animal No.	Lung Bx Score	Lung Bx Dx	PM Score	PM Dx	Gage	Instrument
K1630	0	N/A	1	AIP	14	TC
G2690	1	N/A	2	AIP	14	TC
G2691	1	AIP	2	AIP	14	TC
R4004	1	SB	4	SB,CP	14	TC
R4360	1	SB	3	CP	14	TC
B4447	N/A	N/A	6	SB,CP	14	TC
Y6716	3	CP	5	CP	14	TC
U8369	N/A	N/A	6	SB,CP	14	TC, BB
U8415	N/A	N/A	3	CP	14	TC, BB
U8519	3	CP	2	CP	14	TC, BB
U8531	1	SB	4	SB,CP	14	TC, BB
U8573	0	N/A	5	SB,CP	14	TC, BB
U8571	N/A	N/A	4	CP	14	BB
G3864	N/A	N/A	6	CP,AIP	14	BB
O8587	N/A	N/A	6	SB,AIP	14	BB
Y24	3	SB	4	CP,AIP	12	BB
G3954	1	N/A	4	SB,AIP	12	BB
U8320	1	N/A	5	SB	12	BB
U8322	2	N/A	5	CP	12	BB
U8521	0	N/A	1	AIP	12	BB

AIP=acute interstitial pneumonia; BB=bard magnum reusable biopsy instrument; Bx=lung biopsy sample; CP=chronic pneumonia; Dx=final diagnosis; N/A=no lung tissue collected or unable to make a diagnosis; PM=post mortem lung sample; SB=suppurative bronchopneumonia; TC=tru-cut biopsy instrument

TABLE 3.2: LENGTHS OF LUNG BIOPSIES OF THE PLUCKS FROM CALVES ACUTELY INFECTED WITH BRSV

Group	Gage	Inflated	Lung Lobe	Mean Biopsy Length (mm)	SD	Minimum Length	Maximum Length
E	14	N	CV	7.1[a]	2.9	3.5	11.5
E	12	N	CV	6.5	2.8	2	11.5
E	14	N	M	8.5[a]	2.2	2	12
E	12	N	M	7.9	2.4	4.5	15.5
F	14	y	M	4.6[b]	2.8	1	11.5
F	12	y	M	7.3[b]	2.5	2	11.5
G	14	N	M	7.9[c]	2.4	5	11.5
G	12	N	M	6.8[d]	2.9	3	11
G	8	N	M	4.4[c,d]	1.3	3	7.5
G	14	Y	M	6.8	2.8	2	11
G	12	Y	M	5.6[e]	2.3	1.5	10
G	8	Y	M	7[e]	2.1	2.5	10

N=no; Y=yes; CV=cranioventral; M=middle; mm=millimeters; SD=standard deviation

a, b, c, d, e = significant difference ($p \leq 0.05$) between groups

CHAPTER 4

THE USE OF LUNG BIOPSY TO DETERMINE EARLY LUNG PATHOLOGY AND ITS ASSOCIATION WITH HEALTH AND PRODUCTION OUTCOMES IN HIGH-RISK FEEDLOT STEERS

4.1 INTRODUCTION

Bovine respiratory disease (BRD) is an important disease complex that has many factors contributing to its development. BRD reportedly accounts for 10-61% of mortality in western Canadian feedlots (Church and Radostits 1981; Ribble, Meek et al. 1995), 86.8% of mortality in Ontario feedlots (Gagea, Bateman et al. 2006) and 44-67% of mortality in US feedlots (Andrews and Kennedy 1997; Gagea, Bateman et al. 2006). The multifactorial nature of BRD results in a complex pathogenesis which is not well understood and additionally makes prevention difficult. Typical feedlot BRD refers to the development of bronchopneumonia secondary to the stress of shipping or transportation (Andrews and Kennedy 1997). Clinically, BRD usually occurs 7 to 14 days after a stressful event such as transportation, passing through auction markets and co-mingling (Wikse 1985). Lesions typically develop in the cranioventral lung lobes and will likely be bilateral in distribution (Andrews and Kennedy 1997). The most common bacterial isolates from fibrinosuppurative bronchopneumonia are *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* (Andrews and Kennedy 1997; Gagea, Bateman et al. 2006).

Early detection and recognition of BRD is necessary for effective treatment and prevention. Typically BRD detection is reliant on observation for signs of depression, coughing, decreased feed intake, standing away from the group and an increased rectal temperature. These

criteria are subjective and lend themselves to individual variation and misdiagnosis. In South African feedlots it was found that at slaughter there were 42.8% of all animals with lung lesions; with 69.5% having never been treated for BRD (Thompson, Stone et al. 2006). Another study found that overall 35% of steers from a U.S. feedlot were treated for respiratory disease, however at slaughter 72% had pulmonary lesions (Wittum, Woollen et al. 1996). In that study, 78% of those treated actually had pulmonary lesions at slaughter while 68% of steers that had never been treated had lung lesions at slaughter. These findings suggest that treatment criteria is non-specific, that treatment may be inappropriate in regards to drug choice or duration of therapy, and that there is an opportunity to improve individual animal health and productivity. By determining what occurs early on in the disease process we may be better able to institute preventive measures and have better treatment protocols.

The objectives of this project were: 1) to determine if live animal lung biopsy could be used to characterize early pathologic changes in the bovine lung associated with BRD, 2) to determine if specific infectious respiratory pathogens can be identified in association with early pathological changes, and 3) to determine whether pulmonary pathology characterized by live animal lung biopsy at arrival and at the time of initial BRD diagnosis was associated with health and production outcomes of feedlot steers in a commercial feedlot.

4.2 MATERIALS AND METHODS

4.2.1 STUDY FACILITIES

This study took place in a commercial feedlot with a 30,000 animal capacity in southern Alberta, Canada, in the fall of 2007. Animals were housed in side-by-side, open-air, dirt-floor

pens with a central feed alley and 20% porosity wood-fence windbreaks. Each pen housed approximately 220 animals. This feedlot has a processing facility and two hospital facilities.

4.2.2 STUDY ANIMALS

Animals included in the study were 100 auction market derived crossbred steer calves purchased from auction markets in western Canada. These animals were housed in 20 home pens of 5059 animals that were considered at high risk for developing bovine respiratory disease. The mean weight (±SD) of the study animals was 629 lbs (±60 lbs) [286 kg ± 27kg] with a minimum of 465 lbs (211 kg) to a maximum of 830 lbs (377 kg) at enrollment.

Within 18 hours of feedlot arrival, all cattle were vaccinated with a modified live virus vaccine (Pyramid®FP 5 Vaccine, Wyeth Animal Health, Guelph, ON) for bovine rhinotracheitis, bovine viral diarrhea virus (types I and II), bovine parainfluenza-3, and bovine respiratory syncytial virus. They were also vaccinated with a *Mannheimia haemolytica* toxoid (Presponse®SQ bacterin toxoid, Wyeth Animal Health, Guelph, ON) as well as a bacterin toxoid (Ultrabac® 7/ Somubac®, Pfizer Canada, Inc., Kirkland, QC) for *Clostridium chauvoei*, *Cl. septicum*, *Cl. novyi*, *Cl. sordelli*, *Cl. perfringens* types C and D and *Histophilus somnus*. All steers were also treated with an anthelminthic (Vetomectin pour-on, Vetoquinol N.A., Inc., Buena, NJ) and given a growth promoting implant (Synovex® Choice, Wyeth Animal Health, Guelph, ON). On arrival all steers were treated metaphylactically with oxytetracycline (Oxymycin LA, 30mg/kg, IM, once; Wyeth Animal Health, Guelph, ON) unless they were sick on arrival, eg. BRD, with a rectal temperature >104°F (40°C) at the time of processing then they were treated with tulathromycin (Draxxin™ Injectable Solution, 2.5mg/kg, SC, once; Pfizer Canada, Inc., Kirkland, QC).

4.2.3 EXPERIMENTAL DESIGN

Animals were enrolled in three different groups: sick on arrival (ARR-SA), pen pull with undifferentiated fever (PP-UF) and pen pull with no fever (PP-NF) with matched controls for each group. The sick on arrival group (ARR-SA) included steers that had just arrived at the feedlot, with a rectal temperature >104°F (40°C) at the time of processing, and no abnormal clinical signs referable to other body systems other than the respiratory system. The sick on arrival control group (ARR-CT) included steers that had just arrived at the feedlot, with a rectal temperature <102.9°F (39.4°C) and no clinical signs of illness at the time of processing. These animals were from the same processing groups as those steers enrolled in the ARR-SA group. Two of the groups were derived from animals that were pulled from the pens or pen-pulls. Pen-pulls were steers identified by feedlot personnel as being sick with BRD, i.e., showing clinical signs of depression, decreased feed intake, nasal discharge or coughing and having a lack of clinical signs referable to other body systems other than the respiratory system. These steers were moved to the hospital for determination of rectal temperature and previous treatment history. Only those animals that had not been previously treated and were between 3 and 30 days on feed (DOF) were enrolled in the study. Steers were enrolled in the pen pulls with undifferentiated fever (PP-UF) group if the rectal temperature was >105°F (40.5°C), or enrolled in the pen pulls with no fever (PP-NF) group if the rectal temperature was >102.9°F (39.4°C) and < 104.9°F (40.5°C). Pen pull controls (PP-CT) were steers from the same home pens as the pen pulls, with no clinical signs of illness, a rectal temperature <102.9°F (39.4°C), no previous treatment history, and between 3 and 30 DOF. All PP-NF were treated with oxytetracycline (Oxymycin LA, 20mg/kg, IM, once; Wyeth Animal Health, Guelph, ON) and PP-UF were

treated with tulathromycin (Draxxin™ Injectable Solution, 2.5mg/kg, SC, once; Pfizer Canada, Inc., Kirkland, QC) and returned to their home pens.

Steers were allocated into the study at the time of processing or first pen pull. ARR-SA and ARR-CT were allocated based on the processing group which is a group of animals that represents one buyer. More than one processing group may arrive on one trailer or be housed in the same home pen. Within a processing group, the first ARR-SA was allocated to the study with the next animal fitting the control criteria also being allocated to the study as a matched control (ARR-CT) for the ARR-SA. There were no more than 4 ARR-SA and 2 ARR-CT allocated per processing group. Animals were enrolled in groups of three with at least one ARR-SA matched with one ARR-CT within that group. The group of three may include another ARR-SA from a different processing group that does not have a matched control. Pen pulls were also enrolled in groups of 3 with 2 PP-UF with 1 PP-CT, or 2 PP-NF with 1 PP-CT. The PP-CT was a healthy animal from the same home pen as the PP-NF or PP-UF. At the time of study enrollment, every animal had an ear notch taken for BVDV testing as well as an ear tag placed to designate them as a study animal. All study animals were followed from enrollment until feedlot exit (death or shipment to slaughter).

4.2.4 PERCUTANEOUS LIVE ANIMAL LUNG BIOPSY OF THE RIGHT MIDDLE LUNG LOBE OF FEEDLOT STEERS IN A FIELD SETTING

A lung biopsy sample was taken within 24 hours of initial diagnosis of BRD and again on weeks 2 and 4. The study animal was placed in a squeeze chute and sedated with xylazine (Rompun®, Bayer, Inc., Toronto, ON; 0.05mg/kg, IM). (Figure 4.1) An area on the right side of the thorax extending from intercostals space 6 (ICS6) to ICS3 and from mid-scapula extending 1

inch (2.5 cm) below the elbow was aseptically prepared. (Figure 4.2) Lidocaine (Lido-2 with epinephrine, Rafter Products, Calgary, AB; 6mls of 20mg/ml, SC and IM) was used to locally block the biopsy site in ICS4 one inch proximal of the elbow. A stab incision was made using a #15 blade. The biopsy was taken with a spring-loaded biopsy instrument (Bard® Magnum® reusable biopsy instrument) with a modified 4mm biopsy needle. (Appendix D) The needle was inserted perpendicular to the body wall, angled slightly caudally to avoid the heart, and advanced to a depth of 7-10 cm for sample collection. As the animals gained weight the required depth for sample collection increased to > 10cm.

4.2.5 SAMPLE PROCESSING

Lung samples were removed from the biopsy instrument using a 20-gauge needle and placed into individual wells within a sample cartridge. Up to five samples were placed into a cartridge with a control tissue (BVDV positive bovine kidney). (Appendix E) The cartridge was placed in a 10% formalin solution and shipped daily to a diagnostic laboratory for processing, Prairie Diagnostic Services (PDS), Saskatoon, SK. Ear notches were placed in peptone buffered saline for weekly processing. A BVDV antigen capture ELISA (HerdChek® BVD Antigen ELISA Ear-Notch/Serum Test Kit, IDEXX Laboratories, Inc., Toronto, ON) was used in-house for BVDV testing. Immunohistochemistry (IHC) was performed at PDS for BVDV, *M. bovis*, *H. somnus*, and *M. haemolytica*. All lung biopsy samples were evaluated based on a standard form by the same board certified veterinary pathologist in a blinded manner. (Appendix C) A pathology score was assigned to all lung biopsy samples based on certain histopathologic lesions being present (received a 1) or not (received a 0), with a maximum score of 20. Lung pathology assessed in the bronchioles included neutrophilic infiltration, abscessation,

bronchiectasis, bronchiolar necrosis, syncytial cells, and bronchiolitis obliterans. Lung pathology assessed in the alveoli included neutrophilic infiltration, abscessation, necrosis and fibrin, oat cells, interstitial reaction, syncytial cells, hyaline membranes and fibrosis. In addition, bronchoalveolar associated lymphoid tissue (BALT) was assessed for hyperplasia, the pleura were assessed for neutrophilic infiltration and fibrinous pleuritis and the vessels were assessed for neutrophilic infiltration and fibrinoid necrosis. All study animals that died or were euthanized were weighed at the time of death. A study veterinarian performed a field post mortem (Booker 1991) and a cause of death was determined for each animal based on gross post mortem examination. A digital image was obtained of the opened right thoracic cavity as well as a cross section of the lung tissue. (Appendix A) Tissue samples from the cranioventral, middle, and caudal dorsal lung lobes were collected from each study animal that died and were submitted to PDS for IHC and histopathological scoring as described above.

4.2.6 DATA COLLECTION & ANALYSIS

Individual animal data was collected from the feedlot database (FHARM© software, Feedlot Health Management Services, Okotoks, AB) including treatment history, health events through-out the feeding period, and where applicable, cause of death.

The entry weight and allocation weight data were analyzed using a one-way ANOVA as the data was normally distributed based on the Shapiro-Wilks test for normality and the groups had equal variances using Scheffe's test for equal variances. The ADG, DOF and final weight data was not normally distributed and was therefore analyzed using the Kruskal-Wallis equality-of-populations rank test. A p value of ≤ 0.05 was accepted as significant. Statistical analysis was performed with STATA/IC version 10 (Statacorp LP, College Station, TX).

4.3 RESULTS

4.3.1 STUDY ANIMALS

There were a total of 100 steers enrolled in this study. The ARR-SA group consisted of 27 study animals and the ARR-CT group included 13 matched controls; the PP-UF group consisted of 26 study animals and the PP-CT group included 13 matched controls; and the PP-NF group consisted of 14 study animals and the PP-CT group included 7 matched controls.

During the study period (November 2007 to September 2008) 4 steers died or were euthanized for humane reasons and 1 steer was railed (ie., sold early for salvage slaughter) due to chronic bloat. (Table 4.1) Three of the 4 steers died or were euthanized early in the feeding period. On post mortem examination 1 steer was diagnosed with myocarditis (PP-UF), 1 with chronic fibrotic pleuritis (PP-UF), 1 with chronic pneumonia and arthritis (ARR-SA). In addition, 3 matched control steers were euthanized, 2 were PP-CT and 1 was AR-CT. No gross lesions of BRD were found on post mortem examination of the control animals. Later in the feeding period (after re-implantation at approximately 130 DOF) 1 more steer died with chronic pneumonia (PP-UF). As this death occurred much later in the feeding period, no matched control was euthanized. The case fatalities of our study animals were 3.7% (1 of 27) for ARR-SA, 3.8% (1 of 26) for PP-UF, and 0% for PP-NF. This differs slightly from the feedlots overall case fatalities for respiratory disease. From 2007 to 2008, the feedlot reported a case fatality rate of 2.86% for fall placed calves diagnosed as sick on arrival BRD cases, 12.92% for calves diagnosed as pen pull BRD cases with undifferentiated fever, and 9.86% for calves diagnosed as pen pull BRD cases with no fever.

4.3.2 LUNG BIOPSIES

Study animals were examined in the chute 3 times, at enrollment, week 2 and week 4, making it possible for all animals to have been sampled 3 times. Most of the animals were sampled once at each of these sampling times however a maximum of 2 attempts could be made at each sampling time. During the lung biopsy procedure, one steer sustained a fatal injury due to the procedure (cardiac tamponade) and subsequently died. In total, the steers were sampled 295 times yielding 283 samples. Of the 283 different biopsy samples, 210 (74%) contained lung tissue and 73 (34.8%) contained tissue other than lung, such as liver or skeletal muscle. Of the 210 lung biopsy samples obtained, only 5 (2.3%) contained all of the lung structures we were assessing, i.e., bronchioles, alveoli, BALT, pleura and vessels, and 137 (65%) contained bronchioles and alveoli.

Overall, lung lesions were identified in 12/67 (18%) of cases and 7/33 (21%) of controls. At study enrollment, lung lesions were identified in 12/67 (18%) cases and 4/33 (12%) controls; at the second biopsy time, lung lesions were identified in 1/66 (1.5%) cases and 2/33 (6%) controls; and at the third biopsy time, lung lesions were identified in 1/64 (1.6%) cases and 1/31 (3.2%) controls.

All study animals were negative for bovine viral diarrhea virus (BVDV) by capture ELISA performed on ear notches indicating that none of the study animals were persistently infected with BVDV. Immunohistochemistry (IHC) was performed on every lung biopsy for *Mycoplasma bovis*, *Histophilus somnus*, BVDV, and *Mannheimia haemolytica*. One lung biopsy sample, from a steer in the *PP-UF* group, was positive for both *M. bovis* and *M. haemolytica*. (Table 4.1) All the other samples were negative for all antigens tested.

4.3.3 PATHOLOGY SCORES

Of the 210 lung biopsy samples, 20 samples from 19 animals received a pathology score with the most common score being a 1. (Table 4.2) A pathology score of 1 was awarded to 13 biopsy samples (1 each from PP-C, ARR-SA; 2 from ARR-C, 5 from PP-UF, 4 PP-C), 4 received a score of 2 (2 from PP-NF, 1 from ARR-SA, 1 from PP-UF), 2 received a score of 3 (both from the same PP-UF steer), and 1 a score of 5 (PP-UF). Only one steer, from the PP-UF group, had more than one lung sample with a pathology score >0. This animal received a score of 3 on the first biopsy and on the third biopsy. The PP-NF received a score of 1 on the first biopsy and a 2 on the second biopsy. There were too few samples with lung pathology to reveal any association between lung biopsy pathology score and allocation group. However, a higher percentage of animals with lung lesions were identified in the PP-UF group compared to the other case and control groups.

Of the 100 steers enrolled in the study, 12 had at least 1 additional health event recorded through-out the feeding period, including no fever, undifferentiated fever, diphtheria, bloat, arthritis, and inappetence. One steer (PP-NF) was found to be sick and diagnosed with no fever 22 days after enrollment, 5 days after the second lung biopsy was performed. It was treated according to standard feedlot protocols and did not have a recurrence. This steer's first biopsy received a biopsy score of 1 and its second biopsy received a score of 2. A second steer (ARR-CT) was found to be sick and diagnosed with an undifferentiated fever 24 days after enrollment, 4 days after the third biopsy was performed. This steer's third biopsy sample received a pathology score of 1. This steer was euthanized as a matched control 13 days later and no visible

pulmonary lesions were found on post mortem. There were too few samples with lung pathology to reveal an association between pathology score and subsequent health events.

4.3.4 POST MORTEM FINDINGS

In this study we were unable to characterize lung pathology with a live animal lung biopsy. During the study period (November 2007 to September 2008) 4 steers died or were euthanized due to poor treatment response or humane reasons. The post mortem diagnoses included one each due to myocarditis, fibrotic pleuritis, chronic pneumonia, and pneumonia with arthritis.

The steer with myocarditis (PP-UF) had 1 biopsy performed prior to death, yielding no lung tissue. At the time of post mortem examination, lung tissue was collected, processed for histopathological examination and received a pathology score of 3. The steer with chronic fibrotic pleuritis (PP-UF) had 2 lung biopsies performed yielding no lung tissue. At the time of post mortem examination, lung tissue was collected and received a pathology score of 4. The steer with chronic pneumonia and arthritis (ARR-SA) had 3 lung biopsies performed yielding one sample of lung tissue (biopsy 1) with a biopsy pathology score of 0. On histopathology, this sample was of good quality containing normal bronchioles, alveoli, and vessels. This sample did not contain BALT or pleura. At the time of post mortem examination, lung tissue was collected and received a pathology score of 4. The steer with pneumonia (PP-UF) had 3 biopsies performed yielding 2 lung tissue samples (biopsy 2 and 3). Biopsy 2 was of good quality with normal bronchioles, alveoli, BALT, and vessels resulting in a score of 0. Biopsy 3 was also of good quality with normal bronchioles, alveoli and vessels. This sample did not contain BALT or

pleura and received a biopsy pathology score of 0. At the time of post mortem examination, lung tissue was collected and received a pathology score of 5.

Three steers were euthanized as matched controls, 2 PP-CT and 1 ARR-CT. One pen pull control had two successful biopsies performed before euthanasia, biopsy 1 and 2. Biopsy 1 was of good quality containing normal bronchioles, alveoli, pleura and vessels. Alveolar macrophages were present as well as some alveolar hemorrhage. This sample did not contain any BALT and received a biopsy pathology score of 0. The second biopsy was also of good quality containing normal bronchioles, alveoli, BALT, pleura and vessels resulting in a biopsy pathology score of 0. At the time of post mortem examination, lung tissue was collected and received a pathology score of 4. The second pen pull control animal euthanized had two biopsies taken with the first biopsy yielding no lung tissue. The second biopsy was of moderate quality with normal alveoli, and vessels and contained neutrophilic infiltration of the pleura resulting in a biopsy score of 1. There were no bronchioles or BALT in this sample. At the time of post mortem examination, lung tissue was collected and received a pathology score of 7. The third control animal euthanized was a sick on arrival control which had three successful biopsies taken prior to euthanasia. The first was of good quality having normal bronchioles, alveoli, and vessels. This sample did not contain BALT or pleura and received a biopsy pathology score of 0. The second biopsy was also of good quality containing normal bronchioles, alveoli, pleura and vessels resulting in a pathology score of 0. This sample did not contain any BALT. The third biopsy sample was also of good quality containing normal bronchioles, pleura and vessels with alveolar macrophages. There was no BALT in this sample however the alveoli had neutrophilic infiltrates resulting in a biopsy pathology score of 1. Lung tissue collected at the time of post mortem examination received a pathology score of 4.

4.3.5 INDIVIDUAL ANIMAL WEIGHT AND AVERAGE DAILY GAIN

Study animal weights on feedlot entry were significantly different between ARR-SA and both the PP-UF and PP-NF groups ($p<0.05$). The ARR-SA (n=27) had a mean weight (±SD) of 654 lbs (±73 lbs) [297 kg ± 33kg]. The ARR-CT (n=13) had a mean weight of 647 lbs (±60 lbs) [294 kg ± 27 kg]. The PP-UF group (n=26) had a mean weight of 597 lbs (±63 lbs) [271 kg ± 29 kg] and its matched control group PP-CT (n=13) had a mean weight of 623 lbs (±70 lbs) [283 kg ± 32 kg]. The PP-NF group (n=14) had a mean weight of 581 lbs (±79 lbs) and its matched control group PP-CT (n=7) had a mean weight of 634 lbs (±61 lbs) [288 kg ± 28 kg].

Study animal weights at allocation were significantly different between the ARR-SA group and the PP-UF, between the PP-UF and their matched controls and the PP-NF and their matched controls ($p<0.05$). The ARR-SA (n=27) had a mean weight (±SD) of 643 lbs (±59 lbs) [292 kg ± 27 kg]. The ARR-CT (n=13) had a mean weight of 633 lbs (±54 lbs) [288 kg ± 25 kg]. The PP-UF group (n=26) had a mean weight of 600 lbs (±48 lbs) [273 kg ± 22 kg] and its matched control group PP-CT (n=13) had a mean weight of 653 lbs (±53 lbs) [267 kg ± 24 kg]. The PP-NF group (n=14) had a mean weight of 606 lbs (±66 lbs) [275 kg ± 30 kg] and its matched control group PP-CT (n=7) had a mean weight of 672 lbs (±74 lbs) [305 kg ± 34 kg].

At the time of re-implantation (130 days into the feeding period) there was no significant difference ($p=0.69$) between treatment groups. The mean weight (±SD) at this time for the ARR-SA group (n=27) was 1146 lbs (±190 lbs) [521 kg ± 86 kg] and for its matched control group ARR-CT (n=13) it was 1143 lbs (±153 lbs) [520 kg ± 70 kg]. The PP-UF group (n=24) had a mean weight of 1124 lbs (±182 lbs) [511 kg ± 83 kg] and its matched control group PP-CT (n=11) had a mean weight of 1146 lbs (±191 lbs) [521 kg ± 87 kg]. The PP-NF group (n=14)

had a mean weight of 1164 lbs (±81 lbs) [529 kg ± 37 kg] and its matched control group PP-CT (n=7) had a mean weight of 1224 lbs (±68 lbs) [556 kg ± 31 kg].

The ADG at the time of re-implantation was not significantly different (p=0.35) between groups. The mean (±SD) average daily gain (ADG) of the PP-NF group (n=14) was 4.21 lbs (±0.56), and its matched PP-C group (n=7) was 4.28 lbs (±0.20). The ADG for ARR-SA group (n=27) was 3.43 lbs (±2.04), and its matched ARR-C group (n=13) was 3.97 lbs (±1.11). The ADG for PP-UF group (n=26) was 3.86 lbs (±1.23) and its matched PP-C group (n=13) was 4.09 lbs (±1.20). There was also no significant difference (p>0.05) in the ADG based on the pathology scores.

4.4 DISCUSSION

This percutaneous lung biopsy technique was effective at obtaining lung samples of variable quality from feedlot steers in a commercial feedlot. With the exception of one fatal complication and 1 steer with epistaxis, no abnormal clinical signs related to the biopsy procedure were observed in the remaining 98 steers. The attempts per biopsy sampling time were limited to 2 per steer. This was based on a previous finding in human medicine that there is no gain in diagnostic information with greater than 2 biopsies (Curley, Johal et al. 1998) as well as a previous study performed in mature dairy cattle demonstrating that on average only 1.2 samples were required when using a spring loaded biopsy instrument (Braun, Estermann et al. 1999). In our study, under field conditions, we obtained lung tissue 74% of the time from the right middle lung lobe which is lower than other reports of 97-98% when using the same instrument to sample the caudodorsal lung lobes of horses (Venner, Schmidbauer et al. 2006) and

cows (Braun, Estermann et al. 1999). This difference may be due to sampling location with the middle lung lobe being more difficult given its close proximity to the heart.

The study animals were steers considered to be at high-risk for the development of BRD. As such, we had expected to demonstrate lung pathology in more of the steers, especially in the PP-UF group and PP-NF group at the time of enrollment. Overall, lung lesions were demonstrated by lung biopsy in 12/67 (18%) cases and 7/33 (21%) controls. Most of the lung lesions where observed at the time of enrollment, 12/67 cases and 4/33 controls. The yield dropped considerably with the second and third biopsy. This may be due to: 1) the sampling method being inadequate for the purpose of elucidating lung pathology from these animals as early lung pathology is typically focal in nature, 2) it may demonstrate the effectiveness of metaphylaxis, 3) the size of the animals could have precluded our obtaining a sample or 4) it could be related to the case definition, and 5) observational skills of the pen-riders. The focal nature of BRD has been demonstrated in a previous study assessing lung biopsy as a diagnostic tool (Sydler, Braun et al. 2004) as well as in experimental infection with *M. haemolytica* serotype A1 (Allan, Gibbs et al. 1985). In our study, lung biopsies were obtained blind and therefore a lung lesion could very well have been missed upon sampling with a 4mm (8-ga) biopsy instrument. In our study we elected to biopsy the right middle lung lobe. This decision was based on previous work, see Chapter 3, performed in preparation for this study in which the right cranial lung lobe proved difficult to obtain a lung sample consistently. It is possible that the right middle lung lobe is not affected as early on in the disease process as the right cranial lung lobe making it less likely to obtain a lung biopsy with pathology. Additionally, all steers received metaphylaxis on arrival. This may cause a delay in the development of respiratory disease beyond the time of the biopsies in this study as has been shown in previous studies

(Schumann, Janzen et al. 1990; Morck, Merrill et al. 1993). Feedlot personnel are very skilled at determining which animals are becoming sick, however, the same personnel were not performing this task through-out the entire study. Some feedlot personnel may have been more adept at selection resulting in very acute cases while others may have required more obvious clinical signs. This may affect the severity of the lungs lesions if they were present. Some of the first signs noted by feedlot personnel are depression, standing away from the group, and not coming up to the feed bunk. These are relatively non-specific signs that we assume indicates possible respiratory disease when in fact the steer could be experiencing another disease such as gastrointestinal disease or lameness.

Pathology scores were assigned to lung biopsy samples containing lesions without regard to the significance of the histopathology. We did not deem this to be necessary as we harvested only 20 lung tissue samples with any pathology and had no significant findings with respect the pathology score and enrollment group. Only 19 steers received a pathology score, 2 of those being from the ARR-SA group and 2 of their matched controls, 8 from the PP-UF group and 2 from the PP-NF group as well as 5 of their matched controls (Table 4.2). There were too few samples with histopathologic lesions to reveal an association between pathology score and subsequent health events, average daily gain, enrollment group or post mortem findings. This may be a true finding or it may be due to the power of the study. However, when a steer received a biopsy pathology score and a post mortem pathology score, the post mortem pathology score was always greater suggesting the percutaneous lung biopsy technique to be an inadequate sampling method for our purposes. Interestingly, at study allocation, body weights were found to be significantly different between AAR-SA, PP-UF and PP-NF groups with the ARR-SA group having higher weights. This is likely due to pen pulls having been sick for a

longer duration resulting in greater weight loss. In addition, PP-UF, on entry into the feedlot, had lower weights than the ARR-SA group. This may simply be due to these animals being derived from different processing groups.

Immunohistochemistry (IHC) is a technique used to visualize the distribution of antigens in tissue sections. It can be utilized in the detection of microorganisms which prove difficult to isolate or culture as well as non-viable organisms. The utilization of IHC in this study did not prove to be useful resulting in 1 sample being positive, for both *M. bovis* and *M. haemolytica*, out of 210 lung samples (0.48%). Previous antibiotic therapy may have precluded our abilities to identify bacteria either due to the bacteria not being present or reducing the numbers below the level of detection. In addition, the distribution of lung lesions as well as microorganisms may have been focal in nature. Studies have shown that not all post mortem samples are equal (Haines, Martin et al. 2001; Shahriar, Clark et al. 2002). One study showed the vascular lesions associated with BVDV infection or BVDV antigens were not present in all post mortem sections evaluated (Shahriar, Clark et al. 2002). Even in chronic BRD it can be difficult to demonstrate antigen in tissue sections taken at post mortem (Haines, Martin et al. 2001). In this study, not only were we sampling animals with acute disease, but we were obtaining a very small sample and doing so blindly, resulting in increased difficulty in obtaining a sample with a lung lesion let alone one that would display the antigens of interest.

The live lung biopsy procedure utilized in this study did not appear to cause any long lasting adverse effects as the case fatality rates from the study animals were comparable to the overall case fatality rates reported by the feedlot for fall placed calves. In fact, the study animals experienced a decreased fatality rate compared to the feedlots overall fatality rate for fall placed

calves. This may be due to the study animals inadvertently being monitored more closely as the pen checkers were aware of and participating in the study.

The results of this study indicate that live animal, percutaneous lung biopsy can be performed safely on feedlot steers in a commercial feedlot with few clinical side effects. This technique did not prove useful either as a diagnostic tool or prognostic indicator. However, this lung biopsy technique may be a useful diagnostic tool on a case by case basis. To further elucidate the early pathogenesis of BRD, one should consider serially sacrificing feedlot animals at high risk for developing BRD, in order to evaluate lung pathology at the gross and microscopic levels and identify the pathogens present.

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FIGURE 4.1: RESTRAINT OF A FEEDLOT STEER FOR LIVE ANIMAL PERCUTANEOUS LUNG BIOPSY IN A FIELD SETTING

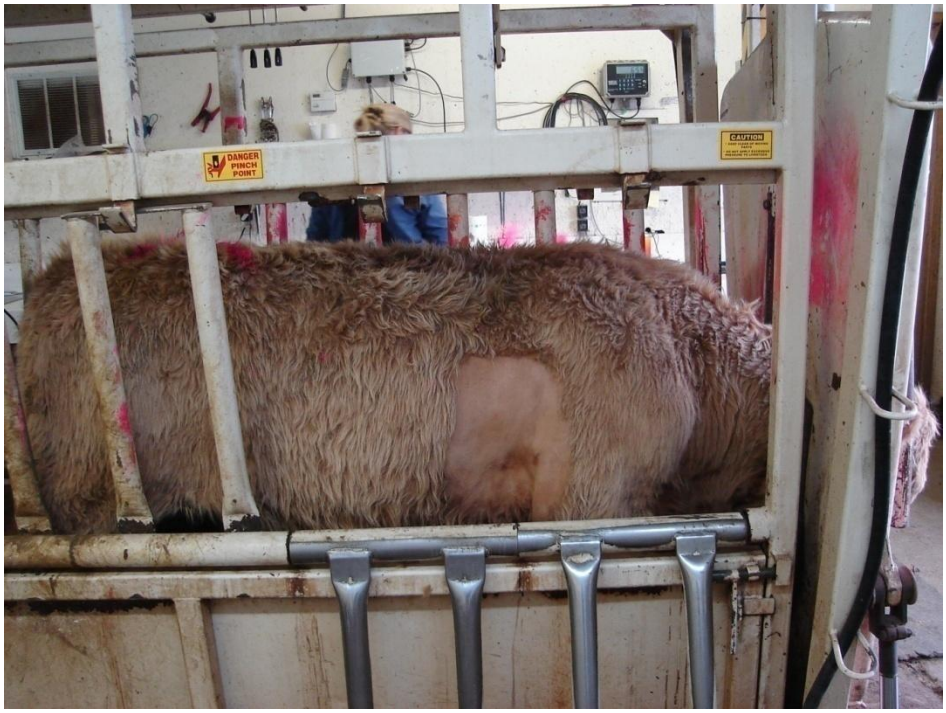

FIGURE 4.2: LIVE ANIMAL LUNG BIOPSY SITE FOR PERCUTANEOUS LUNG BIOPSY OF A FEEDLOT STEER IN A FIELD SETTING

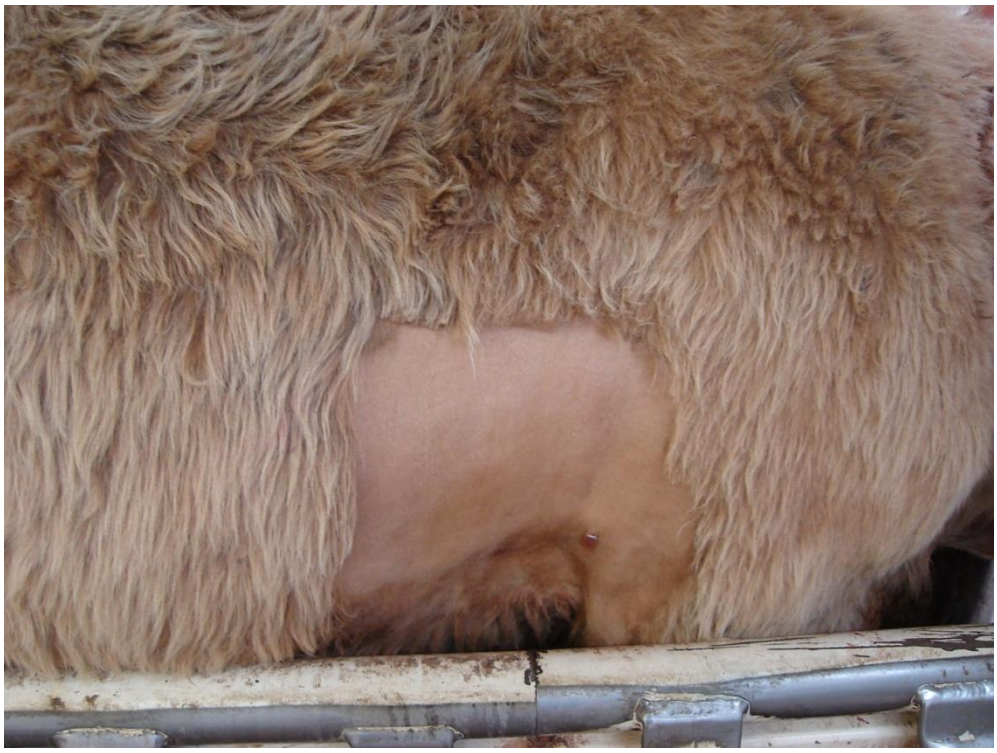

TABLE 4.1: STUDY FATALITIES DURING THE 2007-2008 FEEDING PERIOD

Steer No.	Group	PM Findings	PM score	Bx 1	Bx 2	Bx3
R4794	PP-UF	cardiomyopathy	3	NL	n/a	n/a
W5309	PP-CT	control	4	0	0	n/a
W6174	ARR-SA	cardiac tamponade	1	NL	NL	n/a
W5838*	PP-UF	pleuritis	4	NL	NL	n/a
W6695	PP-CT	control	7	NL	1	n/a
W6624	ARR-SA	pneumonia with arthritis	4	0	NL	NL
W6625	ARR-CT	control	4	0	0	1
O6790	PP-UF	chronic pneumonia	5	NL	0	0
W5276	PP-CT	bloat	railed	NL	0	NL

ARR-CT=sick on arrival control; ARR-SA=sick on arrival; n/a=no biopsy performed; No.= number; NL=no lung obtained; PM=post mortem; PP-CT=pen pull control; PP-UF=pen pull with an undifferentiated fever

*Positive IHC for *M. bovis* and *M. haemolytica*

TABLE 4.2: LIVE ANIMAL PERCUTANEOUS LUNG BIOPSY HISTOPATHOLOGY SCORES AND TREATMENT GROUPS

Animal No.	Allocation Biopsy	Allocation Histopath Findings	Week 2 Biopsy	Week 2 Histopath Findings	Week 4 Biopsy	Week 4 Histopath Findings	Treatment Group
O6162	3	ANI, AIR, AAM	n/a		3	ANI, AIR, AF	PP-UF
O6341	1	ANI	0		0		PP-C
O6395	5	BNI, BO, ANI, AIR, AAM	0		0		PP-UF
O7748	1	ANI	0		0		ARR-C
R3733	0		2	BNI, ANI	0		PP-NF
R3835	2	BNI, ANI	0		0		PP-NF
W5291	1	ANI	0		0		PP-UF
W5421	1	ANI	0		0		PP-UF
W5615	0		1	ANI	0		PP-C
W5731	1	BNI	0		0		PP-C
W5917	2	BNI, ANI	0		0		PP-UF
W5925	1	ANI	0		0		PP-UF
W6177	1	AIR	0		0		ARR-SA
W6606	1	ANI	0		0		PP-UF
W6625	0		0		2	ANI, AAM	ARR-C
W6654	1	ANI	0		0		PP-UF
W6694	1	ANI	0		0		PP-C
W6695	0		1	PNI	PM		PP-C
W6859	2	BNI, ANI	0		0		ARR-SA

ARR-C=sick on arrival control; ARR-SA=sick on arrival; Histopath=histopathology; n/a=no biopsy obtained; No.=number; PM=post mortem performed prior to week 4 biopsy; PP-C=pen pull control; PP-NF=pen pull no fever; PP-UF=pen pull with an undifferentiated fever.

AAM=alveolar activated macrophages; AF=alveolar fibrosis; AIR=alveolar interstitial reaction; ANI=alveolar neutrophilic infiltration; BNI=bronchiolar neutrophilic infiltration; BO=bronchiolitis obliterans; PNI=pleural neutrophilic infiltration

CHAPTER 5

GENERAL CONCLUSION

Bovine respiratory disease (BRD) is an important disease complex of feedlot cattle which develops due to a combination of host, agent and environmental factors. Much of what is known about BRD is based on post mortem evaluation of chronically affected cattle. Unfortunately, chronic cases are not necessarily representative of the acute disease process. A study in 2008 found *M. bovis* and *M. haemolytica* to be the most common agents isolated from animals with chronic BRD by IHC on postmortem samples (Booker, Abutarbush et al. 2008). However, there have been many etiologic agents identified, such as BRSV, BoHV-1, and BVDV, which may predispose cattle to the development of BRD but which may not be identified at the time of post mortem examination as the infection has resolved leaving behind a damaged, compromised respiratory system.

Historically, preconditioning programs, prophylaxis and metaphylaxis have been employed as preventive measures in a feedlot setting (Lofgreen 1983; Schumann, Janzen et al. 1990; Harland, Jim et al. 1991; Schumann, Janzen et al. 1991; Van Donkersgoed 1992; Galyean, Gunter et al. 1995; Duff, Walker et al. 2000; Schunicht, Booker et al. 2002; Macartney, Bateman et al. 2003; Cusack 2004; Booker, Schunicht et al. 2006; Booker, Abutarbush et al. 2007; Bryant 2008). Bovine respiratory disease is still a common cause of mortality in feedlots accounting for 44-67% of mortality in U.S. feedlots (Andrews and Kennedy 1997; Gagea, Bateman et al. 2006) and for 10-61% of mortality in western Canadian feedlots (Church and Radostits 1981; Ribble, Meek et al. 1995) even with the use of these preventive measures. This suggests that what is currently being done is inadequate in regards to individual animal health and that assessing

chronic BRD may not be providing the necessary information to adequately prevent this disease complex.

The purpose of this study was to use live animal lung biopsy to elucidate the early pathogenesis of BRD and the associated microorganisms. To this end a percutaneous lung biopsy technique was developed that can be used on feedlot steers in a field setting to obtain lung samples from the right middle lung lobe. Briefly, a lung biopsy was obtained from the right middle lung lobe in ICS4 using a Bard® Magnum® reusable biopsy instrument and a modified 4-mm (8g) biopsy needle. Other studies have used similar techniques to biopsy the caudodorsal lung lobes of cattle (Dungworth and Hoare 1970; Raphel and Gunson 1981; Braun, Estermann et al. 1999; Sydler, Braun et al. 2004) obtaining diagnostic samples with few side effects. In this study, we could obtain a lung biopsy sample suitable for histopathology however we were sampling the middle lung lobe, caudal to the heart. There were very few side effects in this study with only 2 deaths due to the biopsy procedure which was performed on 134 steers. Many of the steers were biopsied at three separate biopsy dates with those steers enrolled in the study not experiencing any greater incidence of respiratory disease or mortality as compared to the other fall placed calves in the feedlot. Our study found this live animal lung biopsy technique to be safe, 0.5% fatalities due to the procedure, and able to obtain a lung biopsy sample suitable for histopathology. However, very few of the samples from sick on arrivals as well as first time pen pulls actually contained pulmonary pathology resulting in an inability to comment on the early pathogenesis of BRD. Lung biopsy samples from the PP-UF group yielded the most pulmonary pathology, 9 of 20 (45%) lung biopsy samples. This was to be expected as these animals should be the most severely affected by BRD when compared to the ARR-SA, ARR-CT, PP-NF, and PP-CT groups.

This project used an invasive diagnostic tool, lung biopsy, to try and gain knowledge about the pathogenesis of acute BRD. This technique enabled us to collect lung tissue repeatedly in feedlot steers in a commercial feedlot setting however there was not much pathology present within the lung biopsy samples. This may be due to the lack of lung pathology during the acute stage of BRD, it may be due to lesions being localized or focal in nature, or this sampling technique may not have been adequate if there was pulmonary pathology present. Chronically, pulmonary pathology was obtained in 61.5% of lung biopsy samples suggesting this technique is adequate. However, in acute BRD pulmonary pathology was obtained in 9.5% of lung biopsy samples. This suggests that either there is no pathology seen in the acute stages or that it is localized or focal in nature making it very difficult to obtain a sample blindly.

If this project were to be repeated, one should consider using ultrasound guidance to target the lung biopsy to an area of affected lung or a CT scan to plan the biopsy site. Beyond this, one should consider humane euthanasia of feedlot steers on entry into the feedlot as well as pen pulls, given ethical and financial constraints, to elucidate the pathogenesis of acute BRD and the associated microorganisms.

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APPENDIX A

FIGURE A.1: OPENED RIGHT THORACIC CAVITY

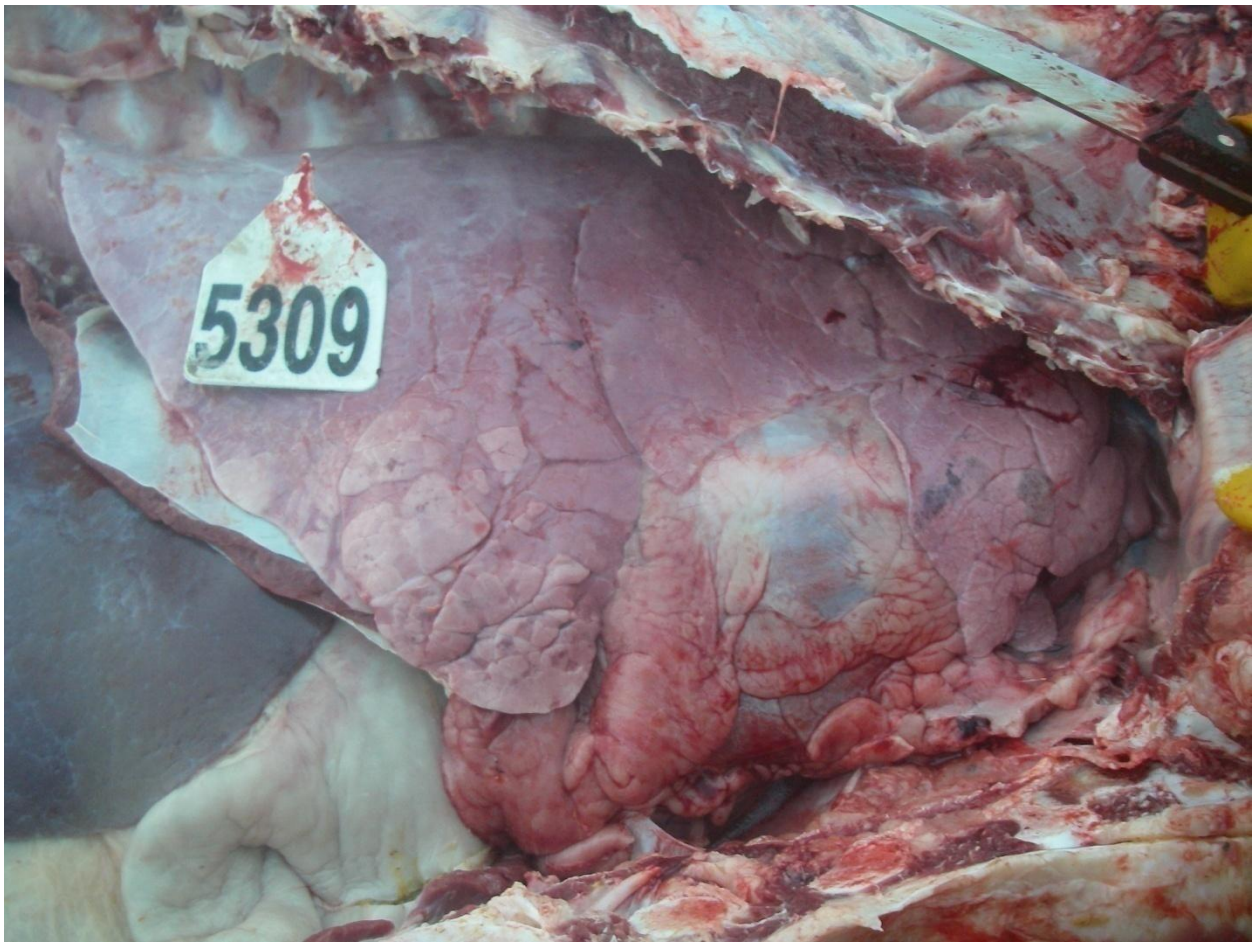
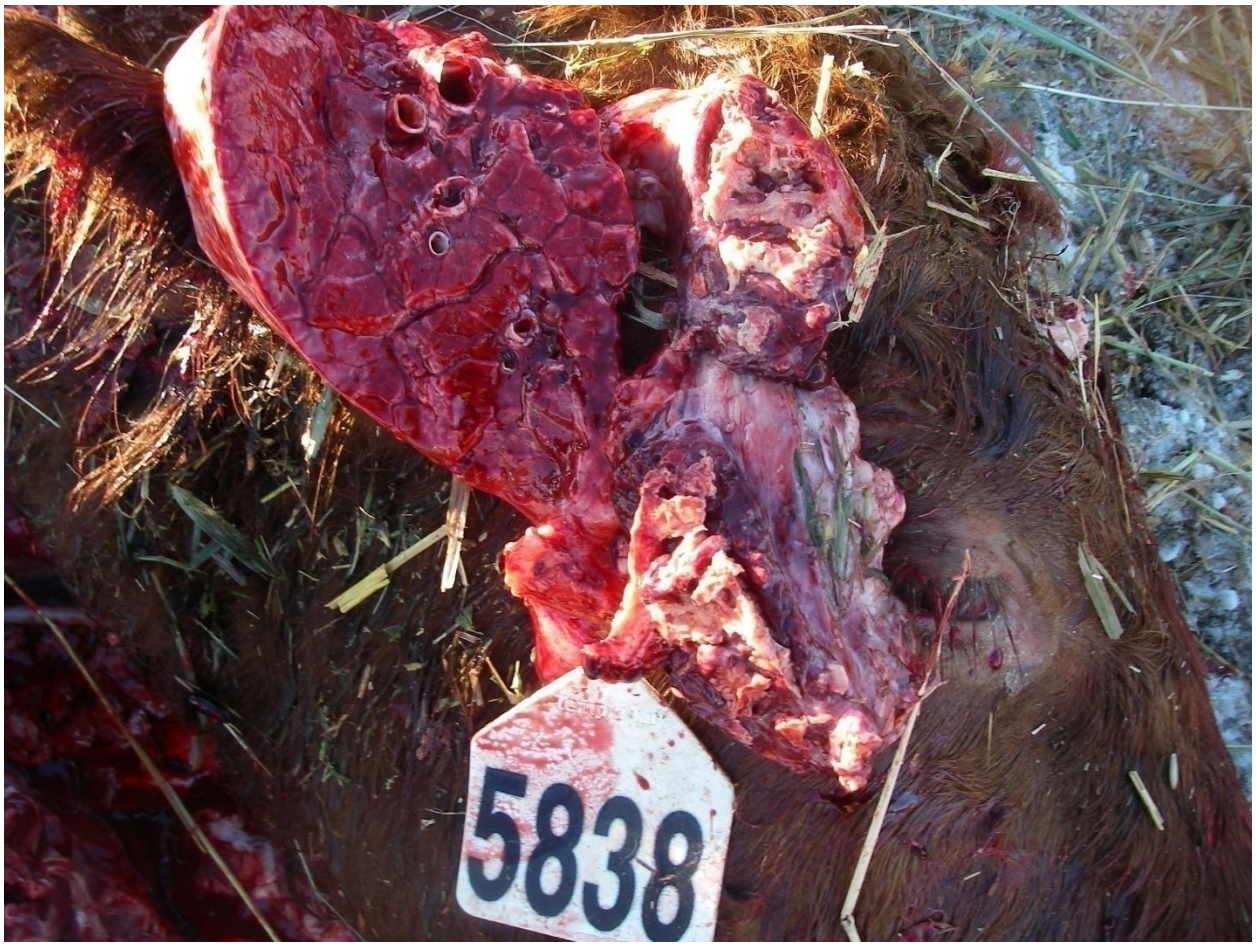


FIGURE A.2: CROSS-SECTION OF RIGHT LUNG



APPENDIX B

FORM B.1: HISTOPATHOLOGIC SCORE SHEET FOR CRANIOVENTRAL LUNG LOBE BIOPSY SAMPLES FROM STEERS WITH CHRONIC BRD

Histopathologic Lesion	Animal Number
Suppurative bronchopneumonia	
Chronic suppurative bronchopneumonia	
Bronchiectasis	
Finbrinonecrotizing pneumonia	
Fibrinous pleuritis	
Hemorrhage	
Bronchiolar necrosis	
Bronchiolitis obliterans	
Acute/ subacute interstitial pneumonia	
Proliferative interstitial pneumonia	
BALT hyperplasia	
Vasculitis	
Quality of biopsy	

BALT=bronchiolar associated lymphatic tissue

APPENDIX C

FHMS 516 Lung Biopsy Histopath Scoring

Case Number	1	**Date of histopath scoring:**	

Bronchioles Normal	☐	Bronchioles bronchiolar necrosis	☐
Bronchioles Absent	☐	Bronchioles syncytial cells	☐
Bronchioles neutrophilic infiltrate	☐	Bronchioles bronchiolitis obliterans	☐
Bronchioles abscesses	☐	Bronchioles fibrosis	☐
Bronchioles eosinophilic necrotic exudate (bronchiectasis)	☐		
Bronchioles other			

Alveoli Normal	☐	Alveoli interstitial reaction	☐
Alveoli Absent	☐	Alveoli activated macrophages	☐
Alveoli neutrophilic infiltrate	☐	Alveoli syncytial cells	☐
Alveoli abscesses	☐	Alveoli hyaline membranes	☐
Alveoli necrosis and fibrin; oat cells	☐	Alveoli type 2 pneumocytes	☐
Alveoli hemorrage	☐	Alveoli fibrosis	☐
Alveoli other			

BALT Normal	☐
BALT Absent	☐
BALT hyperplasia	☐
BALT other	

Pleura Normal	☐
Pleura Absent	☐
Pleura neutrophilic infiltrate	☐
Pleura fibrinous pleuritis	☐
Pleura hemorrhage	☐
Pleura other	

Vessels Normal	☐
Vessels Absent	☐
Vessels neutrophilic infiltrate	☐
Vessels fibrinoid necrosis	☐
Vessels hemorrhage	☐
Vessels other	

Other (fungi, granulamatous pneumonia, parasites)	

APPENDIX D

FIGURE D.1: BARD® MAGNUM® REUSABLE BIOPSY INSTRUMENT WITH 4-MM BIOPSY NEEDLE USED FOR LIVE ANIMAL PERCUTANEOUS LUNG BIOPSY OF FEEDLOT STEERS IN A FIELD SETTING

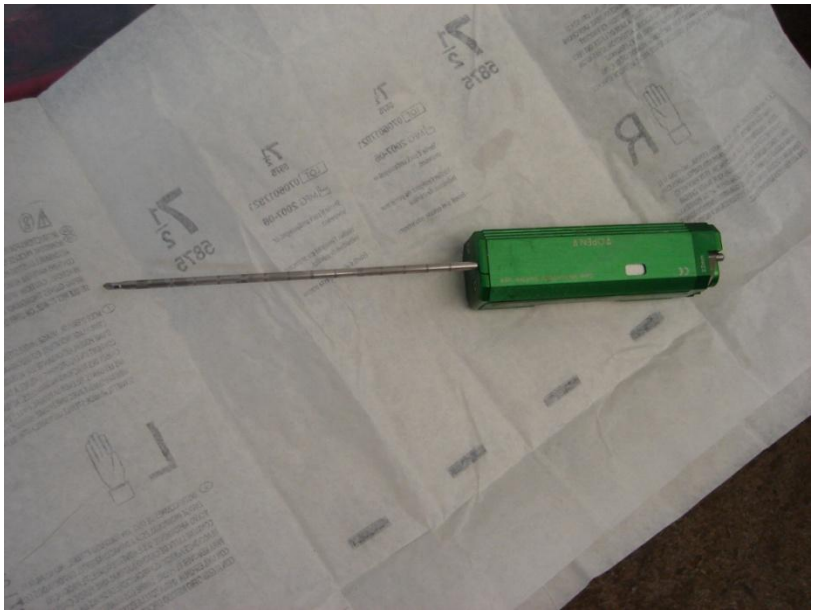
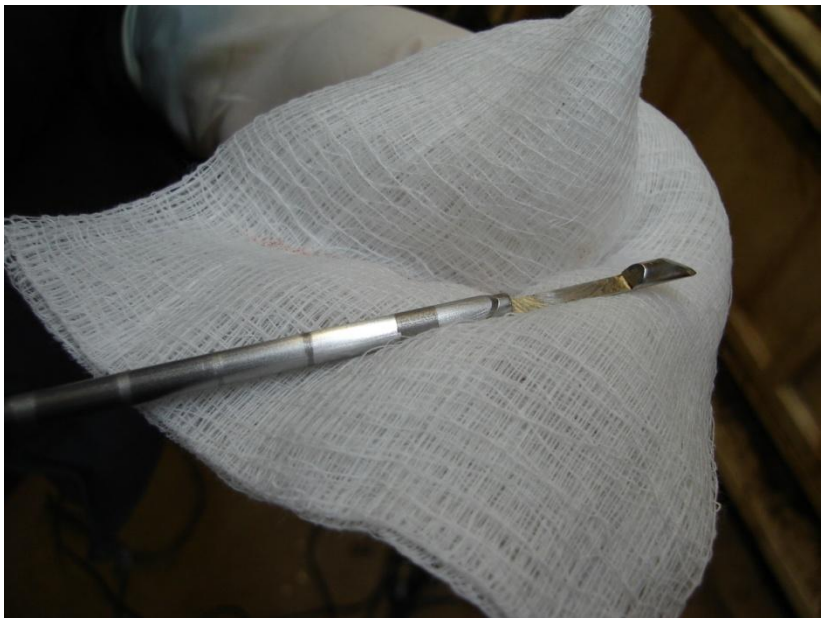


FIGURE D.2: MODIFIED 4-mm (8-g) BIOPSY NEEDLE USED WITH THE BARD® MAGNUM® REUSABLE BIOPSY INSTRUMENT



APPENDIX E

FIGURE E.1: LIVE ANIMAL PERCUTANEOUS LUNG BIOPSY SAMPLES FROM FEEDLOT STEERS IN A CARTRIDGE FOR PROCESSING