CARDIORESPIRATORY DISEASE DIAGNOSIS IN HOLSTEIN CALVES

by

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Abstract

Minimal research is available pertaining to the suspected acute cardiac injury of cattle experiencing the early signs of respiratory disease. One of the limitations of cardiac disease diagnosis in cattle is the lack of accurate measurement of cardiac disease. Blood biomoarkers such as cardiac troponin I could be used. Cardiac troponin I is a serum biomarker for cardiac injury in humans and many veterinary species. The goal of this thesis is to evaluate biophysical changes including cardiac injury that holstien calves experience during acute pneumonia. The first objective of this research was to evaluate a point of care cardiac troponin I assay for use in the bovine. Purified bovine cardiac troponin I was used to validate the accuracy of the assay over a wide range of concentrations in an *in vitro* experiment. This point of care assay was capable of accurately identifying bovine cardiac troponin I. An *in vivo* experiment was conducted to evaluate concentrations of cardiac troponin I in healthy Holstein calves. Concentrations evaluated with the point of care assay were similar to concentrations of a previously validated immunoassay. The second objective was to evaluate biophysical changes including serum biochemistry, complete blood count, cardiac troponin I, and high resolution digital thermography in Holstein calves during the first fourteen days after induction of pneumonia. Fibrinogen concentration increased with lung disease severity. The changes observed were considered clinically significant. Cardiac troponin I increased as pneumonia progressed during the study in this population.

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Chapter 1: Thesis Introduction

Cardiac disease in cattle remains a challenging diagnosis and may represent a poor to guarded prognosis. Heart failure is the result of cardiac disease that cannot be compensated with cardiac and neurohormonal mechanisms. Cardiac disease may occur as a primary disease or may be secondary to another etiology in cattle. Diagnostic tests that establish accurate identification of cardiac disease may allow more prompt treatment or salvage of affected cattle.

Traditional methods of evaluating BRDC in cattle such as behavior scores and rectal temperature may be less than ideal for an accurate diagnosis. (Griffin et al 2010) Thoracic auscultation is another commonly utilized method of evaluating cattle pneumonia. Further diagnostic tests including ultrasonography and radiology are not always available or practical for every animal suspected of having BRDC. Biophysical profiling of calves experiencing respiratory disease may identify biomarkers which may provide a more accurate diagnosis, disease severity and prognosis.

Cardiac disease in cattle may present due to a variety of disease conditions. In Chapter 2 a review of literature is presented. Chapter 2 offers a review of cardiac disease etiologies devided among anatomical locations with a focus on cardiac disease associated with respiratory disease known as cor pulmonale. Cor pulmonale in cattle is typically associated with two main etiologies of respiratory compromise. These two main etiologies include bovine high mountain disease and chronic pneumonia. Bovine high mountain disease results in cor pulmonale from a low atmospheric oxygen pressure leading to vasoconstriction of the pulmonary vasculature which ultimately results in right sided heart failure. Chronic pneumonia results in cor pulmonale due to loss of functioning lung parenchyma and changes in vascular flow leading to an increased workload on the right heart, ultimately leading to heart failure. There is no available literature regarding cattle with acute respiratory disease and cardiac injury.

Cor pulmonale associated with chronic respiratory disease respresents a gaurded prognosis for cattle. With increased knowledge of potential cardiac insults associated with early respiratory disease veterinarians may be able to offer a more accurate prognosis for respiratory disesase. Evaluation of mild cardiac disease in cattle is often not readily accomplished because many veteriarians due not have access or are financially limited by advanced diagnostic tests

while evaluating cattle. These diagnostic tests may include echocardiaography, electrocradiography, or thoracic radiographs. An inexpensive, portable diagnostic test may be valuable to veterinarians to evaluate cardiac disease in cattle.

A study in this thesis (Chapter 3) evaluated cardiac troponin I in, in vivo and in vitro models. Cardiac troponin I (cTnI) has been established as the gold standard for evaluating myocardial disease in humans because of rapid results, and specificity for myocardium. (O'Brien 2008 and Hasić et al. 2003) This diagnostic test has gained wide use in veterinary species. In both humans and veterinary patients, cTnI has been shown to increase with cardiac and extra cardiac thoracic disease including pneumonia. Cardiac troponin has been shown to be increased in several disease conditions of cattle. These studies suggest cardiac injury of cattle from direct cardiac insult and systemic diseases.

Diagnosis and providing an accurate prognosis of subclinical bovine respiratory disease in difficult. Evaluation of specific multiple measurements at once may aid in increasing the ability to differentiate calves that are experienceing severe or mild pneumonia. Measurements including components of a complete blood count and biochemistry in addition to high resolution digital thermography, and cardiac troponin I were evaluated as a part of this thesis. These combined individual measurements may be stated as a biophysical profile.

In this thesis (Chapter 4) Holstein calves were evaluated for biophysical changes experiencing induced *Mycoplasma bovis* pneumonia. The components of a biophysical profile were analyzed to identify differences between calves that experienced severe and mild pneumonia. These compenents were evaluated the day of induction in the middle of the respiratory disease and at the conclusion of the study. At the conclusion the calves were euthanized and each lung received a visual grade for the percent of diseased lung. This scoreing of the lungs was used to determine if the calves would be classified as experiencing mild or severe disease for data analysis.

Chapter 2 - Review of Literature

Introduction

Bovine respiratory disease is a multifactorial disease process that involves several etiologic agents resulting in disease of the bovine respiratory system. (Confer 2009, Apley 2006, and Allen et al. 1992) The United States cattle industry experiences a significant financial and death loss impact from BRDC. Despite continued research, more treatment options, and improved knowledge of pathophysiology, treatment of BRDC is not always successful. Cardiac injury as a result of BRDC may be a significant component of the disease syndrome.

Bovine Respiratory Disease Complex

Diagnosis and treatment of bovine respiratory disease complex remains challenging. Diagnosis of respiratory disease is most often made from subjective evaluation of cattle with respiratory disease. (Griffin et al 2010) Several studies have evaluated more objective measurements to improve the accuracy of diagnosis of bovine respiratory disease complex.

Routine laboratory variables are not reliable indicators of lung involvement or the progression of pneumonia in cattle. (Hanzlicek et al 2010) Behavioral data may aid in identifying cattle affected with bronchopneumonia. (Hanzlicek et al 2010) Prompt identification of sick cattle is of great concern and signs may be masked from caretakers for a considerable amount of time due to the prey behavior that cattle present when ill. (Griffin et al. 2010) Onset of clinical signs of respiratory disease are typically not identified in cattle with respiratory disease until 7-10 days and up to 27 days after a stressful event. (Duff et al. 2007) Clinical observations are commonly used by cattle caregivers to identify the individuals suffering from pneumonia or other disease. These observations alone have questionable accuracy and may not be consistent between health care providers. Clinical observations have been shown to have limitations for accurate detection of BRDC calves with estimated sensitivity of 62% and specificity of 63%. (White et al. 2009) Rectal temperature, heart rate and respiratory rate may yield conflicting results, and may vary with time of day. (Hanzlicek et al. 2010) Considerable disagreement among veterinarians is present when evaluating calves for respiratory disease. (Amrine et. al. 2013)

The majority of the available literature focuses on the effect of BRDC on the respiratory system. Involvement of the cardiovascular system receives little attention in BRDC research. There are specific bovine respiratory disease pathogens that cause disease to the cardiac system. *Histophilus somni* is well known to cause myocarditis and myocardial infarction in addition to its contribution to BRDC. (Orr 1992) *M. bovis* has been isolated from the bovine heart alone and in conjunction with *H. somni* (Maunsell et al 2011), though does not typically cause gross or histological changes which may indicate a commensal role of *M.bovis*. Specific respiratory pathogens may have result in direct injury to the cardiac system in addition to more general insult to the heart from increased vascular resistance and increased right heart work.

Diagnosis of BRDC remains a chalenging endevor. Evaluation of more objective measurement of clinical illness scores and behavior have not yielded a highly accurate diagnosis of BRDC. More objective evaluation of laboratory components have also not resulted in an accurate diagnosis of BRDC. More accurate diagnosis and prognosis may continue to further the ability to identify and treat BRDC. Knowledge of cardiac injury as a result of respiratory disease in cattle may offer a more accurate prognosis.

Cardiac Disease Etiologies

Various components of the bovine heart may become injured through different disease states. The three main structures of the bovine heart that are commonly affected by disease include: the pericardium, the myocardium, and the endocardium including valvular disease. Disease of the conductive tissues are infrequent in the bovid. (Machida et al. 2005)

Traumatic reticulopericarditis is the most commonly reported pericardial disease of cattle. (Braun et al 2007, Roth et al. 1991) Other pericardial diseases of cattle described in one report in descending order of number of reported cases include; pericarditis secondary to pleural or lung infection, neoplastic effusion secondary to a lymphoma or mesothelioma and idiopathic aseptic pericarditis. (Buczinski et al. 2010)

In one study, dilated cardiomyopathy is the most common primary myocardial disorder of cattle. (Nart et al. 2004) Myocarditis, a secondary myocardial disease, may result from viral, bacterial, and parasitic infection. (Gunes et al. 2005, Uzal et al., 2003, and Otter et al. 1995) Nutritional cardiomyopathy may be caused by deficiencies of vitamin E, selenium, or copper, (Orr et al. 1996, and Leigh 1975) in addition to toxicity of ionophores. (Van Vleet et al 1983)

Cor pulmonale, secondary to pulmonary hypertension, as a result of reflex pulmonary arteriolar vasoconstriction, fibrosis and pulmonary injury is associated with right heart failure in cattle. Pulmonary hypertension may be caused by decreased oxygen partial pressure from pulmonary disease or high altitude. (Holt et al 2007)

Bacterial endocarditis is the most common endocardial disease of bovids. (Buczinski et al. 2010) The main etiologies include *Truperella pyogenes* and *streptococci* with the right atrioventricular valve being the most commonly affected. (Buczinski et al. 2010) Endocarditis may also be a result of turbulent blood flow striking the endocardium causing fibrosis and roughening known as jet lesions due to valvular dysplasia (Watson et al. 1991) or cardiac implantation during research scenerios. (Fossum et al. 2001)

Numerous congenital cardiac abnormalities have been reported in the bovid. Ventricular septal defects are the most commonly reported. (Buczinski et al. 2010) Other reported defects include atrial septal defects, patent ductus arteriosis, tetralogy or pentology of fallot, and Eisenmenger's complex.

Cardiac disease in cattle is represented by a variety of pathophysiology. The veterinarian must evaluate the cardiac system to identify what anatomical components are affected to form differential diagnosis's. Each disease etiology is not exclusive and the individual animal may have multiple etiologies present.

Cardiac Injury in Cattle

Diagnosis of cardiac injury prior to heart failure is challenging. Initially, cardiac auscultation is used to assess the heart for problems such as murmurs, arrhythmias, and other abnormal heart sounds. Arrhythmias may be further evaluated utilizing electrocardiography. The most common dysrhythmia of cattle is atrial fibrillation, and can only be confirmed with electrocardiography. (Buczinski et al. 2010) Physiologic and pathologic murmurs may be differentiated with aid of echocardiography. Thoracic radiographs are utilized to identify metallic foreign bodies associated with traumatic reticulopericarditis. (Buczinski et al. 2010) One study noted that thoracic radiographs are useful in cases of congenital heart disease in calves. (Farrow et al. 2000) Measurement of the caudal vena cava with thoracic radiographs has been noted as a reliable tool for detecting heart disease in cattle. (Jilintai et al. 2006) Thoracic

radiographs have shown to be of minimum utility for evaluating an increase of cardiac silhouette with bovid heart disease. (Buczinski et al. 2006a and Buczinski et al. 2006b)

Several blood biomarkers may be increased in association with cardiac disease to include: creatine phosphokinase, lactate dehydrogenase, cardiac troponin T, and cardiac troponin I. The gold standard biomarker for myocardial injury in humans is cardiac troponin I. (O'Brien 2008 and Hasić et al. 2003) Cardiac troponin T and cardiac troponin I have received more evaluation in bovine research that has been recently published. (Varga et al. 2009a, Varga et al. 2009b, Mellanby et al. 2009, Gunes et al. 2008, Peek et al. 2008, Mellanby RJ et al. 2007, and Gunes et al. 2005) A variety of assays have been implemented in studies evaluating bovine cTnI. The only validated assessment of bovine cardiac troponin I is a bench top immunoassay. (Varga et al. 2009a)

Heart failure is a condition when the heart can not pump an adequate blood supply to the rest of the body. The neurohormanal system attempts to compenstate through myocardium hypertrophy, tachycardia and systemic vasoconstriction. Clinical findings in one report of heart failure in the bovid include in descending order: tachycardia, abnormal heart sounds, jugular distention, peripheral edema, jugular pulse, ascites, cough, and syncope. (Buczinski et al. 2006b)

Cor Pulmonale in Cattle

Cor pulmonale in cattle can be a result of chronic respiratory disease. (Angel et al. 1992, and Jubb et al. 1992) Respiratory disease continues to be a concern representing a significant cause of economic and animal loss. Early recognition of BRDC can result in more rapid therapeutic implementation. (Apley, 2006) There is currently not a highly accurate method available for establishing an ante-mortem diagnosis of bovine pneumonia and case definitions can vary significantly. (Apley 2006) Ante-mortem diagnosis of BRDC is typically made from observations. The most common observations used for diagnosis are depression, appetite loss, respiratory character change, and temperate elevation. (Griffin et al. 2010) A hypothesis is that improved accuracy of prognosis may be obtained through evaluation of concurrent cardiac injury. Continued research efforts are needed to improve early diagnosis and establish improved prognosis of cattle suffering from BRDC.

Chronic respiratory disease can lead to a syndrome known as cor pulmonale. (Angel et al. 1992, and Jubb et al. 1992) The increased cardiac workload in humans is responsible for the

right ventricular hypertrophy and eventual congestive heart failure. (Summer et al. 1978) Alveolar hypoxia, acidosis, increased blood viscosity, increased pulmonary blood flow or an anatomic reduction of the pulmonary vasculature bed to include empthsema and pulmonary emboli may cause contraction of the precapillary pulmonary vessels leading to increased vascular resistance. Pulmonary hypertension occurs as a result of increased vascular resistance and thus increases the cardiac workload. This increased cardiac workload can lead to cardiac injury. Cor pulmonale as a result of respiratory disease is often not diagnosed until the individual is experiencing heart failure. Little research is available which describes the effects of respiratory disease on the bovid cardiac system. Bovine high mountain disease is another mechanism that can result in cor pulmonale in cattle. The individual experiences lower atmospheric pressures at high altitude leading to pulmonary hypertension. Pulmonary artery pressure testing can aid in these diagnoses. (Holt et al 2007) Bovine high mountain disease results from hypoxia leading to pulmonary vasoconstriction, pulmonary remodeling, pulmonary hypertension, and finally right heart failure resulting clinically in brisket edema.

Conditions that may induce alveolar hypoxia include; exposure to high altitude, pneumonia, respiratory impairment secondary to chest wall abnormalities, airway obstruction, pulmonary edema, emphysema or increased pulmonary vascular disease. Lower ambient temperatures will also increase pulmonary artery pressures in cattle. (Will et al. 1978) This may exacerbate concurent respiratory disease. Polycythemia resulting in increased blood viscosity may exacerbate pulmonary hypertension and lead to a continued injury to the cardiac system. The cardiopulmonary system of cattle may be insulted in various methods or a combination of methods resulting in fulmonant cor pulmonale.

Pneumonia is a common condition in cattle leading to significant respiratory impairment and failure. In a chronic state this will lead to a clinical condition of right sided heart failure known as cor pulmonale. (Angel et al. 1992, and Jubb et al. 1992) Pulmonary tissue fibrosis and necrosis may cause pulmonary blood shunting and release of vasoactive facotrs such as atrial natriuretic peptide. The alveolar hypoxia that pneumonia induces leads to pulmonary artery hypertension. This extra work load on the heart does predictably induce cardiac injury. The heart is continually injured and must remodel and compensate for the increased work load due to hypertension. Cardiac hypertrophy occurs as the result of continued pulmonary hypertension from chronic alveolar impairment due to respiratory disease.

Limited literature is available concerning the contribution of respiratory disease to cor pulmonale in cattle. (Angel et al. 1992, and Jubb et al. 1992) The literature of respiratroy disease induced cor pulmonale is retrospective in nature. (Angel et al. 1992, and Jubb et al. 1992) No literature is availible concerning the injury the bovine heart may experience during early respiratory disease. Increased cardiac work load may result in cardiac injury during respiratory disease. The utility of cardiac biomarkers may aid in the classification of cardiac injury during the early stages of BRDC.

Bovine High Mountain Disease

High mountain disease of cattle is a common condition in cattle living at high altitudes greater than 5000 feet above sea level that results in cor pulmonale. (Holt et al. 2007) The disease process of bovine high mountain disease may allow a greater understanding of the less documented process of chronic respiratory disease resulting in cor pulmonale. The high altitude results in low atmospheric pressures of oxygen. This results in an alveolar hypoxia which leads to continued pulmonary vessel contraction and develops into pulmonary hypertension. The fulminant disease classically results in cattle with distended pulsating jugular veins and brisket edema. Disease is typically evaluated with pulmonary artery pressure testing, objectively measuring the degree of hypertension. Cattle at risk of developing bovine high mountain disease will have a pulmonary artery pressure greater than 45 millimeters of mercury. The common recommendation is for the movement of the animal to a lower altitude. (Holt et al. 2007)

Any concurrent illness that causes a temporary or permanent pulmonary hypoxia can lead to an increased pulmonary artery pressure. (Holt et al. 2007, Angel et al. 1992, and Jubb et al. 1992) Thus high mountain disease can be exacerbated with concurrent illness. Noxious plants known as locoweeds increase the risk of heart failure in cattle susceptible to the effects of high altitudes. (Knight et al. 2001) A genetic component likely exists that predisposes cattle to the development of high mountain disease. (Holt et al. 2007) Selection of cattle to remain or enter a herd may be aided with pulmonary artery pressure testing.

Cardiac Troponin I in Veterinary Medicine

Injury to the cardiovascular system can be evaluated with various biomarkers. Cardiac troponin I has been extensively utilized in human and veterinary medicine as a biomarker for cardiac injury. (Blass et al. 2011, Payne et al. 2011, Varga et al. 2009a, O'Brien 2008, O'Brien PJ et al 2006, Slack et al. 2005, and O'Brien et al 1997) This biomarker is a likely candidate for the evaluation of myocardial injury from pneumonia induced pulmonary hypertension in cattle. Pulmonary hypertension will increase the workload experienced by the right heart inducing mycardial injury and remodeling.

Cardiac troponin I has been used extensively in veterinary medicine as a biomarker for cardiac injury. Cardiac troponin I (cTnI), a myofibril protein that regulates contraction of the myocardium, is currently considered the gold standard diagnostic test for cardiac injury in humans. (O'Brien 2008 and Hasić et al. 2003) Cardiac injury of humans has been indentifed by increases in cardiac troponin in a variety of non cardiac diseases to include; chronic obstructive pulmonary disease, community-acquired pneumonia, acute lung injury or the acute respiratory distress syndrome after subarachnoid hemorrhage, and septic shock. (Monaghan et al. 2012, Moammar et al. 2010, Naidech, et al. 2009, and Oliveira et al 2008)

Cardiac troponin has been used as a biomarker of cardiac injury in a variety of diseases in a variety of species. In dogs it has been shown to be useful in differentiating cardiac and non-cardiac disease as a cause of acute respiratory distress. (Payne et. al. 2011) Specifically dogs with cardiac conditions including right ventricular cardiomyopathy have demonstrated increases in serum cTnI. (Baumwart et al 2007) Dogs with extra thoracic conditions that increase cardiac workload such as gastric dilatation-volvulus have been shown to have an increase in cTnI concentrations. (Schober et al. 2002) Cardiac troponin I has also been demonstrated to be an accurate indicator of cardiac injury with feline hypertrophic cardiomyopathy. (Herndon et al. 2002) Cardiac troponin has been shown to be a useful biomarker for cardiac injury in several veterinary species.

Cardiac troponin has been proven to be an accurate biomarker for cardiac injury in large animals as well. A study demonstrated increased cardiac troponin protein in an equid as a response to feed contaminated with ionophore due to myocardial injury. (Schwarzwald et al 2003) Cardiac troponin has also been shown to be higher in septic foals when compared to non-septic foals. (Slack et al. 2005) This may have been form vascular changes or direct bacterial or

toxin effects on the heart representing that cTnI may detect indirect cardiac injury. A human cardiac troponin assay recently was able to successfully calculate a concentration of cardiac troponin I in healthy alpacas. (Blass et al 2011)

Cardiac troponin I has been used extensively in research and clinical cases within the literature regarding cattle representing a variety of disease states. Foot and mouth disease has caused cardiac injury based upon an increase of cTnI as compared to controls. (Gunes et al. 2005) Cardiac troponin may lack specificity for direct cardiac injury and may increase with systemic disease that causes changes in vascular dynamics that result in increased cardiac work and injury. Diseases known to cause cardiac injury of bovids have also been shown to result in an increase cTnI. These conditions include monensin toxicosis (Varga et al. 2009b), idiopathic pericarditis (Jesty et al. 2005), traumatic reticulopericarditis (Gunes et al. 2008 and Mellanby et al 2009), and endotoxemia. (Peek et al 2008) Cattle that suffer from chronic suppurative pneumonia have been shown to have higher cTnI concentrations as compared to controls. (Mellanby et al. 2009) Cardiac troponin changes have not been reported in acute bovine respiratory disease. All of these studies utilized various human cardiac troponin diagnostic testing devices. There is over 96% homology of human and bovine cardiac troponin I. (O'Brien et al 1997) The similar protein structure of cardiac troponin demonstrates that human assays for cTnI should be able to successfully identify cardiac troponin in cattle plasma. Each assay should be evaluated for the intended species of application. A variety of diseases and conditions in cattle have resulted in an increased cTnI concentration. This represents a wide range of disease where cardiac injury is present. Cattle with pneumonia likely will have pulmonary hypertension and thus experience cardiac injury. Cardiac troponin I may be a sensitive and accurate biomarker to identify cardiac injury in cattle.

Chapter 3 - Assessment of a commercially available point-of-care assay for the measurement of bovine cardiac troponin I

As accepted by the American Journal of Veterinary Research:

Abstract

Objective—To assess a commercially available point-of-care assay for measurement of bovine cardiac troponin I (cTnI) in blood and plasma samples.

Sample—Prepared bovine plasma standard samples with known concentrations (0 to 1.0 ng/mL) of cTnI and blood and plasma samples obtained from 28 healthy 2.5-month-old Holstein calves.

Procedures—Coefficients of variation were calculated for concentrations of cTnI in prepared standards determined with the point-of-care assay and values were compared with the known concentrations. The cTnI concentrations in blood samples obtained from calves determined with the point-of-care assay were compared with cTnI concentrations in plasma samples obtained from those animals determined by use of a validated immunoassay.

Results—The coefficients of variation of cTnI concentrations determined for prepared standards by use of the point-of-care assay were low (< 20%) for standards with cTnI concentrations of ≥ 0.025 ng/mL. The blood cTnI concentrations determined with the point-of-care assay were not significantly different from the plasma cTnI concentrations determined with the validated immunoassay.

Conclusions and Clinical Relevance—Results of this study indicated the point-of-care assay had high precision for determination of cTnI concentrations in most evaluated prepared bovine plasma standard samples. The point-of-care assay may be useful for determination of circulating concentrations of cTnI for cattle.

Introduction

Measurement of concentrations of cTnI, a myofibril protein that regulates contraction of myocardium, is considered the gold standard diagnostic test for detection of cardiac injury in humans. (O'Brien et al. 2008 & Hasic et al. 2003) There is > 96% homology between cTnI of humans and that of cattle (O'Brien et al. 1997); therefore, antibodies against human cTnI in commercially available assays are expected to cross-react with bovine cTnI. Cardiac troponin has been used as a biomarker of cardiac injury attributable to various diseases in dogs, cats, and horses. (Serra et al. 2010, O'Brien et al. 2006, Payne et al. 2011, Schober et al. 2002, Baumwart et al. 2007, Herndon et al. 2002, Schwarzwald et al. 2003, and Slack et al. 2005) Also, cTnI concentration increases during various diseases of cows, including idiopathic pericarditis, (Jesty et al. 2005) traumatic reticulopericarditis, (Gunes et al. 2008, and Mellanby et al. 2007) foot and mouth disease, (Gunes et al. 2005) monensin toxicosis, (Varga et al. 2009b) and experimentally induced endotoxemia. (Peek et al. 2008) Results of another study (Mellanby et al. 2009) indicate that cattle with various cardiac diseases or non-cardiac intrathoracic diseases have high serum cTnI concentrations, compared with concentrations for healthy control animals.

Recently, a benchtop immunoassay has been validated for measurement of bovine cTnI concentrations. (Varga et al 2009a) Although that assay is not portable, it could be used to evaluate the accuracy of a point-of-care diagnostic assay for measurement of bovine cTnI concentrations in field settings. A commercially available point-of-care device and disposable cartridge have been used to measure plasma cTnI concentrations for animals including dogs, (Payne et al. 2011) alpacas, (Blass et al. 2011) and horses. (Kraus 2009) The objective of the study reported here was to determine the repeatability of results of a point-of-care diagnostic test for determination of cTnI concentrations in plasma samples obtained from calves and to compare results with those determined by use of a previously validated diagnostic test. (Varga et al 2009a) We hypothesized that the point-of-care diagnostic test would detect bovine cTnI and the test would be precise for measurement of cTnI concentrations.

Materials and Methods

Samples— Various concentration For determination of point-of-care cTnI assay precision, standards with various concentrations of cTnI were prepared with bovine plasma obtained from a university owned, healthy mature cross bred cow, for determination of point-of-care cTnI assay precision,. In addition, blood and plasma samples were obtained from 28 healthy Holstein calves (approx age, 2.5 months) for cTnI testing. ; whole blood sample cTnI concentrations determined by use of a point-of-care assay were compared with plasma cTnI concentrations determined by use of a validated immunoassay. Calves were comingled from multiple sources in Iowa and purchased as a group. Calves were housed together in a pen (25 X 25 m) outdoors during the study. The study protocol was approved by the Kansas State University animal care and use committee.

Point-of-care cTnI assay precision—The precision of a commercially available point-of-care device (i-STAT 1 portable clinical analyzer, Abaxis North America, Union City, CA) and disposable cartridge (i-STAT cTnI cartridge, Abaxis North America, Union City, CA) was determined via measurement of various cTnI concentrations in standards prepared with bovine plasma containing lithium heparin and purified bovine cTnI. (Purified bovine cTnI: Life Diagnostics, West Chester Pennsylvania) The assay was repeated 3 times for standards without cTnI (cTnI concentration, 0 ng/mL [negative control sample]) and 5 times for standards with various concentrations of cTnI. (1, 0.5, 0.25, 0.1, 0.05, 0.025, and .01 ng/mL) Precision of the assay for measurement of cTnI concentrations in bovine plasma was determined via calculation of coefficient of variations (CV).

Comparison of cTnI point-of-care assay results with immunoassay results—Results obtained with a point-of-care assay (i-STAT 1 portable clinical analyzer, Abaxis North America, Union City, CA) and disposable cartridge (i-STAT cTnI cartridge, Abaxis North America, Union City, CA) were compared with those obtained by use of a validated (Varga et al 2009a) immunoassay (ADVIA Centaur TnI-Ultra, Siemens Medical Solutions Diagnostics, NY) for measurement of cTnI in whole blood and plasma samples obtained from calves. A venous blood sample (2 mL) was collected from a jugular vein of each calf into a tube containing lithium heparin by use of a 1.5-inch X 20-gauge needle. Whole blood samples were analyzed for determination of whole blood cTnI concentrations once with the point-of-care assay (i-STAT 1 portable clinical analyzer, Abaxis North America, Union City, CA) and disposable cartridge (i-

STAT cTnI cartridge, Abaxis North America, Union City, CA) on the same day of sample collection. (i-STAT 1 portable clinical analyzer, Abaxis North America, Union City, CA) Then, each sample was centrifuged (2,350 X *g*) for 10 minutes at 22°C, plasma was harvested, and plasma samples were stored at –80°C for up to 14 days until determination of cTnI concentrations by use of a validated (Varga et al 2009a) immunoassay (ADVIA Centaur TnI-Ultra, Siemens Medical Solutions Diagnostics, NY); results of another study (Varga et al 2009a) indicate cTnI concentrations do not decrease in bovine plasma samples stored at –80°C for 14 days.

Data and Statistical Analysis

Precision of the point-of-care assay for determination of cTnI concentrations in prepared bovine plasma standards was determined via calculation of CVs. A 1-sample *t* test was used to identify differences between known and measured concentration means. Outlier values were defined as values > the 99th percentile value of the mean value for each known cTnI concentration. (Abbott point of care website. http://www.abbottpointofcare.com/ Cardiac troponin /(cTnI) Cartridge and Test Information Sheets, 2012); such data (1 value each for 0.01 and 0.05 ng/mL cTnI concentration standards) were attributed to faulty cartridges and were not included in the statistical analysis. The whole blood cTnI concentration values determined by use of the point-of-care assay for calves were compared with plasma cTnI concentrations determined by use of the validated immunoassay for those animals via a Student *t* test for paired data. For statistical analysis, plasma cTnI concentration values determined by use of the immunoassay that were < 0.01 ng/mL were considered to be 0 ng/mL. Values of $P < 0.05$ were considered significant. All analyses were performed with commercially available computer software. (Excel for Mac. 2008. Microsoft Corporation, Redmond Washington)

Results

Point-of-care cTnI assay precision—The cTnI concentrations determined by use of the point-of-care assay were typically higher than the known concentrations of cTnI in prepared bovine plasma standards (Table 1); values were significantly different for all cTnI concentration standards except the 0.01 ng/mL standard. However, the CV for the point-of-care assay was < 20% for all prepared bovine plasma cTnI standards except for the lowest cTnI concentration.

(0.01 ng/mL; CV, 38%) Concentrations of cTnI in negative control samples determined by use of the point-of-care assay were 0 ng/mL for all 3 repeated tests; therefore, use of the bovine plasma as a diluent for the cTnI concentration standards was considered appropriate.

Comparison of cTnI point-of-care assay results with immunoassay results—The cTnI concentrations in whole blood samples determined by use of the point-of-care assay mean ± SD, 0.01 ± 0.01 ng/mL; median, 0 ng/mL; range, 0 to 0.04 ng/mL) were similar to plasma concentrations determined by use of the validated immunoassay. (mean ± SD, 0.006 ± 0.006 ng/mL; median, 0.01 ng/mL; range, 0 to 0.02 ng/mL) (mean ± SD, 0.01 ± 0.01 ng/mL; median, 0 ng/mL; range, 0 to 0.04 ng/mL) Results of statistical analysis performed with a Student *t* test for paired data indicated concentrations determined via the 2 assays were not significantly different. Of the 28 whole blood and harvested plasma samples, 12 (43%) had identical cTnI concentration test results, 13 (46%) had a concentration difference of 0.01 ng/mL between the 2 tests, 2 (7%) had a concentration difference of 0.02 ng/mL between the 2 tests, and 1 (4%) had a concentration difference of 0.03 ng/mL between the 2 tests.

Discussion

The results of this study suggested that the point-of-care assay may be appropriate for determination of concentrations of cTnI in blood samples obtained from cattle. This point-of-care assay has been used to measure circulating cTnI concentrations for healthy alpacas[20]; however, investigators of that study could not validate the test for that species because purified alpaca cTnI was not available. The point-of-care assay was validated for measurement of bovine cTnI in the present study via testing of standards prepared with bovine plasma and purified bovine cTnI. To obtain accurate results with this assay, whole blood samples must be collected into tubes containing lithium heparin. Initially, we attempted to prepare cTnI standards with bovine plasma containing acid-citrate-dextrose solution, but results had high variability and cTnI concentrations were markedly lower than the known concentrations in standard samples. Conclusions of this study may have been stronger if high cTnI concentrations had been detected in blood and plasma samples; this would have necessitated obtaining blood samples from cattle with myocardial disease. Cattle with myocardial disease were not available for use in this study, and induction of cardiac injury in calves was not feasible. Future research is warranted to determine the clinical usefulness of the point-of-care assay for measurement of high

concentrations of cTnI in blood samples obtained from cattle. On the basis of results of this study, we concluded that clinicians would be unlikely to misdiagnose myocardial injury for calves with low cTnI concentrations. Further research is warranted to determine the likelihood that cattle with myocardial injury would have clinically normal cTnI concentrations as determined by use of the point-of-care assay.

Results of other studies (O'Brien et al. 1997 and Varga et al 2009a) indicate cTnI assays have minimal cross-reactivity with proteins in noncardiac bovine muscle homogenates; these results suggest that assays for measurement of bovine cTnI are likely specific for myocardial proteins. In the present study, we used an immunoassay and a point-of-care analyzer that were designed for detection of cTnI in blood and plasma samples of humans. These assays were expected to be useful for measurement of cTnI in bovine blood and plasma samples because bovine and human cTnI are > 96% homologous. (O'Brien et al. 1997) This assumption was supported by results of another study (Varga et al. 2009) in which known concentrations of bovine cTnI were analyzed by use of an assay designed for measurement of human cTnI.

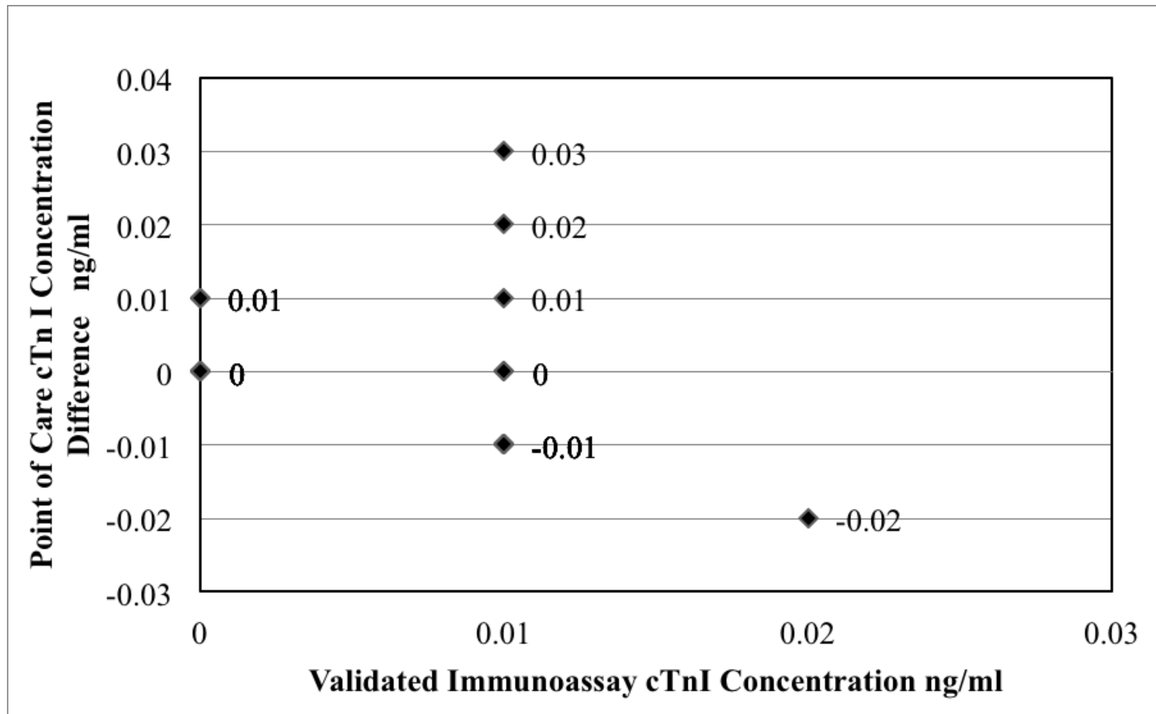
Results of the present study indicated that the precision of the point-of-care assay for measurement of cTnI concentrations > 0.01 ng/mL was good, because the CV of data for such concentrations was < 20%. The CV was high (38%) for measurement of a low cTnI concentration (0.01 ng/mL) in prepared plasma standards; this finding suggested that results for low cTnI concentrations were not accurate. Results of other studies (Varga et al. 2009a, Varga et al. 2009b, Peek et al. 2008) indicate the reference range for cTnI concentrations in blood samples of cattle is < 0.05 to < 0.08 ng/mL. Therefore, the point-of-care analyzer evaluated in the present study may be useful for identification of cattle with myocardial disease, although further research is warranted.

The point-of-care assay evaluated in the present study over estimated cTnI concentration, which may aid in data interpretation. (Abbott point of care website. Cardiac troponin /(cTnI) Cartridge and Test Information Sheets, 2012) Results of this study suggested the analytic sensitivity of the point-of-care device for measurement of cTnI concentrations in bovine blood samples was 0.02 ng/mL (the lowest value that could be distinguished from a concentration of 0 ng/mL); therefore, a cTnI concentration of 0.01 ng/mL could not be reliably differentiated from a concentration of 0 ng/mL. The estimated 20% and 10% functional sensitivities of the point-of-care assay determined via calculation of CV percentages for cTnI concentrations are 0.07 and

0.10 ng/mL, respectively. (Abbott point of care website. Cardiac troponin /(cTnI) Cartridge and Test Information Sheets, 2012) This information may aid in the understanding of a poor CV at a cTnI concentration of 0.01 ng/mL, and a dramatic decrease associated with values > 0.10 ng/mL. The point-of-care diagnostic test has improved accuracy in previously reported abnormal concentrations of cTnI, which are considered to be > 0.05 to > 0.08 ng/mL on the basis of previous reports. (Varga et al. 2009a, Varga et al. 2009b and Peek et al. 2008) Low concentration results may not be precise, though would rarely cause a misclassification of a concentration associated with disease.

The concentrations of cTnI determined with the point-of-care assay for blood samples obtained from calves in the present study were similar to those determined via a different method for cattle in another study. (Varga et al. 2009a) This assay may be useful for point-of-care determination of cTnI concentrations for cattle. Further research may be warranted to determine the usefulness of this assay for identification of cattle with cardiopulmonary disease. Use of the point-of-care assay evaluated in the present study may allow rapid determination of cTnI concentrations and aid management of cattle with cardiopulmonary disease.

Figure 3.1 Comparison of the difference of cTnI concentration as measured by the Point of Care method and the bench top method utilizing Bland Altman analysis.



Comparison of the difference of cTnI concentration as measured by the Point of Care method and the bench top method utilizing Bland Altman analysis. Concentration of cTnI was measured on 28 healthy, 2.5 month old Holstein calves. Data represented in the graph include: difference = 0.03, n = 1; difference = 0.02, n=1; difference = 0.01, n=5; difference = 0, n=12; difference = -0.01, n=8; difference = -0.02, n = 1. Differences of (+) value indicate overestimation of cTnI by the hand-held analyzer; differences of (-) value indicate underestimation by the hand-held analyzer as compared to the immunoassay method of analysis.

Table 3.1 Concentrations of cTnI in prepared bovine plasma standard samples determined by use of a point-of-care assay.

Known cTnI concentration in standard (ng/mL)	Mean ± SD measured concentration (ng/mL)	*P* value*	CV (%)
0	0 ± 0	—	—

0.01	0.015 ± 0.006	0.136	38
0.025	0.068 ± 0.010	0.001	19
0.05	0.078 ± 0.010	0.003	12
0.1	0.120 ± 0.020	0.03	16
0.25	0.296 ± 0.030	0.03	11
0.5	0.702 ± 0.050	0.001	7
1	1.502 ± 0.050	0.009	10

Chapter 4 - Biophysical Profiling of calves with experimentally induced Mycoplasma bovis pneumonia

Introduction

Bovine respiratory disease complex (BRDC) has a major impact on cattle health and economic performance in the cattle industry. The annual cost of BRDC to the United States cattle industry exceeds 3 billion dollars annually and is believed to be the most frequent cause of animal death. (Griffin et al. 1997) Bovine respiratory disease complex is a multifactorial disease involving multiple pathogenic agents. (Apley 2006, Allen et al. 1992, Confer 2009, Poulsen et al. 2009, Wiggins et al. 2011)

Despite improvements in research quality and quantity, therapy for BRDC is not always successful. A more complete understanding of the individual's response to BRDC may aid in prompt diagnosis and successful treatment of this disease. (Griffin et al. 2010) Diagnoses of BRDC most often is based on visual observation and follow up examination with rectal temperature. These diagnostic tests have questionable cost effectiveness for clinical management of the case. Clinical observations have been shown to have limitations for accurate detection of BRDC calves with estimated sensitivity of 62% and specificity of 63%. (White et al. 2009)

Mycoplasma bovis is an increasingly recognized pathogen in bovine respiratory disease complex. (Maunsell et al. 2011, Hanzlicek et al. 2011, Caswell et al. 2010, Maunsell et al. 2009, Fulton et al. 2009, Casewell et al. 2007, and Francoz et al. 2005) In normal cattle *M. bovis* has been recovered from up to 7% of healthy calves without recent transportation history. (Casewell et al. 2010) One study showed an increasing number of calves shed *M. bovis* after 42 days at a stocker unit, and the average daily gain is less in positively testing calves. (Hanzlicek et al. 2011) *M. bovis* may be a common bovine respiratory pathogen and may represent a significant component to BRDC.

Limited research has been aimed at evaluating the biophysical changes that weaned calves experience during development of BRDC. (Hanzlicek et al. 2010) This depth of knowledge can be attributed to the lack of temporal control in the development of BRDC with respect to collection of samples and acquisition of data. Continued research is needed to aid in

the early identification of BRDC and to assess the severity of disease. Biophysical profiling is an evaluation of multiple measurements including components of a complete blood count, biochemistry, cTnI and high resolution digital thermography. Diagnosis of BRDC is challenging and biophysical profiling may be an accurate tool to accomplish these goals.

Chronic respiratory disease results in right heart hypertrophy and cor pulmonale. (Angel et al. 1992, and Jubb et al. 1992) This is due to chronic increased work load to the right heart. Acute respiratory disease may increase cardiac workload, though it is unlikely the heart would compensate and hypertrophy resulting in histologic changes over a short period of time.

The objectives of this study were to identify changes in parameters during the early periods of development of pneumonia, evaluate those changes most closely associated with pneumonia, and to determine associations with severity of disease in Holstein calves. The hypothesis was that we could use biophysical profiling to identify calves suffering pneumonia and to associate these changes with severity of pneumonia. A secondary objective of this study was to evaluate gross changes and histologic changes of each calf's cardiac papillary muscle. Chronic respiratory disease is known to result in cardiac injury in cattle resulting in a syndrome of cor pulmonale. (Angel et al. 1992, and Jubb et al. 1992) We hypothesized this short duration of pneumonia would not result in gross or histologic changes of the calves hearts.

Materials and Methods

Animals and Management

Research included 38 Holstein bull calves and 1 Holstein heifer calf, single sourced and co-housed. The research was conducted at Kansas State University and all practices followed an approved protocol and guidelines set forth by the animal care and use committee. (# 2949)

All calves in this study originated from a private owner. These calves were purchased and co-housed in Iowa then transported to Kansas State University. Calves selected for inclusion were based on criterion of negative nasal swab culture and PCR of M. bovis, low M. bovis ELISA titers, and negative BVD RT PCR by ear notch. Calves selected for trial had the lowest *M. bovis* serum antibody levels of 39 tested. The calves included in the trial were 27 Holstien bull calves and 1 Holstein heifer calf.

At arrival to the facility, all calves received 6.6 mg/kg of ceftiofur crystalline free acid at the left ear base to treat any other major bovine bacterial respiratory pathogen. *M. bovis* is not

susceptible to this antibiotic. The feeding program for all calves consisted of free choice grass hay and a dairy calf complete starter grain ration (Land O Lakes, Herd Maker Supreme B90, Shoreview, MN) throughout the study period. The ration contained 18.0% crude protein, 3.2% crude fat, 1.0% calcium, and 0.5% phosphorus. For the first week after arrival, calves were fed 2.0 pounds of this starter ration per head per day and subsequently increased to 3.0 pounds per head per day for the remainder of the study.

After an acclimation period of 14 days, calves were randomly assigned using a random number generator into either a treatment (*M. bovis* challenge) group or a control (no challenge) group resulting in 24 challenged calves and 4 control caves. Challenge calves were randomly assigned to an inoculation method or inoculation dose with the aid of a commercial software program. (Excel, Microsoft Corp, Redmond WA) Details of sample collection procedures, laboratory protocols, and the trial procedures used are described elsewhere. (Amrine et al. 2013 and White et al. 2012)

All challenged calves were housed together in a dry lot pen measuring approximately 25 by 25 meters. All control calves were housed approximately two hundred meters away from the treatment calves for the duration of the study in a similar sized dry lot pen. All participants with interactions between the treatment and control group changed protective clothing between pens. The study concluded on trial day 14 when all calves were euthanized and necropsied. No calves were euthanized or died prior to the conclusion of the trial.

Body Weight and Rectal Temperature

Calves were each individually weighed on arrival to Kansas State University on day -14. Weights were obtained again on day 7 and day 14 of the trial. Rectal temperatures were obtained on day 7 and day 14 of the trial.

Clinical Illness Scores

Clinical Illness cores were performed to evaluate if the calves showed clinical signs of illness. One author (BCF) performed all CIS's independently on each calf twice daily. The CIS's were performed starting 3 days prior to challenge and continuing until the evening prior to necropsy. These scores were assigned based on classification system below.

Clinical Illness Score Classifications

1=Normal

2=Slight illness (i.e. mild depression and/or cough)

3=Moderate illness (i.e. severe depression, labored breathing and/or cough)

4=Severe illness (i.e. moribund, unresponsive)

Blood Collection and Analysis

Blood was collected via jugular venipuncture from each of the 28 calves on trial days 0, 7 and 14. Blood samples were transferred to serum vacutainers (10-ml Serum blood tube, BD, Franklin Lakes, NJ), lithium heparin tubes, (1.3-mL lithium tube, Sarstedt, Numbrecht, Germany) and EDTA vacutainers. (2-mL EDTA blood tube, BD, Franklin Lakes, NJ) Blood samples were submitted to the clinical pathology service for hematology (Cell-dyn 3700 hematology analyzer (Abbott diagnostics, Abbott Park, Illinois) and serum biochemistry (Cobas 6000 Roche diagnostics, west Sussex England) analysis. (Clinical Pathology Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS) Hematology analysis included differential cell counts performed (Geisma-Wright quick stain) under direct microscope examination. Total protein and fibrinogen were anaylized using a heat precipitation method.

Blood samples collected into lithium heparin tubes (i-STAT 1 portable clinical analyzer, Abbott Point of Care Inc., Princeton, NJ) were used for analysis of cardiac troponin I concentration. Whole blood samples were centrifuged at 2350 x g for ten minutes at 22C. Plasma was harvested and maintained at -80 Celsius until analyzed.

Infrared Thermography

Images were captured with HRDIRT (ThermaCAM S65, FLIR Systems, Wilsonville, OR) of the entire orbit and muzzle separately for calculation of maximal surface temperature of each anatomical location on days 0, 6 and 13. All HRDIRT images were obtained at the same time of day in a sheltered area to control for environmental influences. Each image was captured from a distance of one meter and the angle of the image was perpendicular to each respective anatomical location. All images were imported into an image analysis software program. (ThermaCAM Researcher 2.8 Professional, FLIR Systems, Wilsonville, OR) The orbit was traced and the maximum temperature determined each sample day. The muzzle was divided at the level of the ventral aspect of the nares in a horizontal fashion to form a dorsal and ventral area of measurement. This method has not been published previously, and was performed based on the author’s personal experience. The two areas of the muzzle were traced separately and

were considered the dorsal nose and the ventral nose for analysis. The maximal temperature of each section was used as the value for analysis.

Necropsy and Pulmonary Consolidation

On day 14 each calf was humanely euthanatized with a captive bolt and a gross necropsy performed. Samples of the cardiac papillary muscle and lung were obtained from each calf for histologic examination. Aerobic cultures and *M. bovis* cultures were performed on all calf lungs. On necropsy one calf had pericardial effusion. This was collected and aerobically cultured. The heart of each calf was evaluated grossly and the papillary muscle was collected for histologic evaluation. We chose this site because this is the most sensitive site for diagnosing myocardial injury based on the pathologist's experience.

Pulmonary Consolidation Evaluation Method

All lungs from all calves were assessed by one of the trained and experienced investigator (DM) using a previously validated lung scoring method. (Fajt et al. 2003) Using this method, total percentage lung consolidation was determined as follows: (0.053 · cranial segment of left cranial lobe %) + (0.049 · caudal segment of left cranial lobe %) + (0.319 · left caudal lobe %) + (0.043 · accessory lobe %) + (0.352 · right caudal lobe %) + (0.061 · right middle lobe %) + (0.060 · caudal segment of right cranial lobe %) + (0.063 · cranial segment of right cranial lobe %). Total lung consolidation was used to divide calves into two categories of lung lesions for statistical analysis: 1) mild lesion severity: included all calves with 10% lung consolidation or less, and 2) moderate lesion severity: included all calves with greater than 10% of the lung affected. A 10% cutoff was established based on behavior changes from previously published data. (White et al. 2012)

Statistics

Statistical analysis software programs (JMP 7.0, SAS Institute Inc., Cary NC, and Excel, Microsoft Corp, Redmond WA) were used for data analyses. The biophysical parameters were the primary outcomes of interest in this study and they were organized in accordance with sample day and pulmonary consolidation category. (mild, moderate) Treatment groups and control groups were evaluated together to better evaluate a variety of respiratory disease severity with the biophysical profile. Generalized linear models were used to determine potential

associations between outcomes of interest relative to study day (0,7,14), pulmonary consolidation category (< 10%, > 10%) and the potential interaction between these two variables. An effect was also included in each model to account for repeated sampling on individual calves over trial day. Significance was predetermined to be a p-value of 0.05 or less.

Results

All 39 calves tested for the study were found to be negative for bovine viral diarrhea virus and *M. bovis* by culture and PCR prior to the initiation of the trial. The 28 calves selected for this study had the lowest titers to M bovis of the total 39 calves qualified to enter the study. Calves selected for the trial had a 0 or +1 on the ELISA serum antibody test out of a maximum score of 4 These ELISA's were compared with a positive control sample. The calf with the highest percent of positive ELISA control was 29.6 with the remainder of the claves being lower. On day 14 of the study all calves were euthanized and received full gross necropsies.

Body Weight and Rectal Temperature

Body weights and rectal temperatures were not analyzed due to incomplete collection of data. The body weights were not obtained on day 0 of the trial. Accurate comparison with other variables would have not been possible. Body weights of the 4 control calves were not obtained on day 7. Rectal temperatures were not obtained on day 0. These results would be difficult to interpret while being incomplete. The body weight data is presented in table 5, while the rectal temperature data is presented in table 6. This is only the raw data and was not analyzed though provided for completeness.

Clinical Illness Scores

The number of calves receiving abnormal CIS's increased through the trial for the 24 study calves that received an inoculation of *M. bovis.* Initially all CIS's were normal, and 24 of the 28 calves received at least one abnormal CIS by the study's conclusion with the 4 non challenged calves not receiving abnormal CIS. (Chart 1) No calf received a CIS of 4, at any point during the trial. The four control calves had CIS's of 1 for the duration of the trial, and are not included in Chart 1.

Serum Biochemistry and Complete Blood Count

Serum biochemistry variables that were significantly associated with sample day and pulmonary consolidation category or had a significant interaction can be found in table 1. Variables that were significantly associated with sample day include: albumin, anion gap, blood urea nitrogen, chloride, creatinine, globulin, glucose, HCO3, potassium, and phosphorus. The variables that were significantly associated with pulmonary consolidation category include: albumin, anion gap, chloride, globulin, HCO3, phosphorus, and total protein. Biochemistry variables associated with significant interactions between trial day and lung consolidation category include: alb/globulin ratio, ALP, calcium and sodium.

Complete blood count variables that were significantly associated with sample day and pulmonary consolidation category or had a significant interaction can be found in table 2. Variables that were significantly associated with sample day include: band neutrophil concentration, basophil concentration, hematocrit, HGB, MCHC, and RBC. The variables that were significantly associated with pulmonary consolidation category include: lymphocyte concentration, MCH, MCHC, and RBC. The only CBC variable that had a significant interaction is fibrinogen.

Bovine Cardiac Troponin I

There was no apparent significant difference with pulmonary consolidation category and bovine cTnI. (Table 3) The concentration of bovine cTnI was significantly associated with sample day. No interaction between trial day and pulmonary consolidation category was observed.

Infrared Thermography

There was no effect of sample day or pulmonary consolidation category on orbit temperature as determined by thermography. (Table 3) An effect of sample day was not identified with temperatures of the dorsal and ventral portions of the nose as determined by thermography. A significant difference was found with pulmonary consolidation category on both the temperatures of both the dorsal and ventral portions of the nose.

Necropsy and Histology

Gross necropsies were performed on all calves and a single veterinary pathologist evaluated all lungs histologically. Individual calves may be represented with several abnormal finding associated with their lungs. Pneumonia had a wide range of findings to include varying degrees of consolidation, exudate on the cut surface, pus in varying airways, small nodules with pus and fibrinous pleuritis. No evidence of gross pneumonia with minimal airway disease was found histologically in 4 of the 28 calves. Lobular bronchopneumonia was identified histologically in 23 of the 28 calves. Bronchitis was identified in 21 of the 28 calves histologically.

The total percent of pulmonary consolidation ranged from 0% to 54% for calves in this study. Out of 28 calves, 9 were categorized as having mild pulmonary consolidation severity. (< 10% of pulmonary consolidation, n=9) These 9 calves had a pulmonary consolidation range of 0 to 8% with a median of 4%, and a mean of 3.1%. Out of 28 calves, 19 were categorized as having moderate pulmonary consolidation severity (> 10% pulmonary consolidation, n=19) These 19 calves had a pulmonary consolidation range of 12 to 54% with a median of 28% and a mean of 27.7%. Histopathology revealed no abnormalities associated with the cardiac papillary muscle.

Culture

M. bovis was successfully cultured from 26 of the 28 lung samples. The pericardial fluid sample yielded no growth, but this fluid was obtained from a calf that had a positive lung culture for *M. bovis*. Microbial cultures of the 28 lung samples not including M. bovis results yielded no growth (n=10), *Pasteurella multocida (n=18),* and beta hemolytic *Streptococcus sp*. (n=4) One lung sample that did not yield a positive M. bovis culture result did culture positive for Pasteurella multocida and beta hemolytic *Streptococcus sp*. The second lung sample that did not yield a positive M. bovis culture did not yield a positive culture for any other bacteria.

Discussion

Several variables in the biophysical profile changed through the development of respiratory disease in calves. The majority of these variables represented statistically significant changes though no clinically relevant changes. The most interesting finding in this study was an interaction of fibrinogen concentration with sample day and pulmonary consolidation category.

This variable was higher in the calves that had greater than 10% pulmonary consolidation on day 14 of the trial. Cardiac troponin I did demonstrate a statistically significant increase in concentration over the course of disease. No significant gross or histologic changes related with cardiac injury were observed with the heart. This indicates that the myocardium was injured during acute respiratory disease before gross or histologic changes could be noted. The induction model appeared to satisfactorily induce pneumonia in this study based on changes in clinical illness scores and lung consolidation scores of the treatment group.

The results of serum biochemistry analysis revealed several variables where the effect of sampling day was modified by the level of lung consolidation. Albumin to globulin ratio was not clinically significant in that it was not outside of normal reference ranges. (Smith 2009) Though it was statistically lower at the initiation of the study in the high pulmonary consolidation category calves. Calves that became more severely diseased may have had a lower ability to mount as robust of a response to disease as the less severely affected calves for reasons that were not identified in the study's design. This may have been a result less globulin from consumption of recent unrecognized disease, or may be a genetic difference of the calf's immune systems or other variables not recorded such as body weight and body condition score.

On days 7 and 14, calves in the high pulmonary consolidation category had lower ALP compared to those in low pulmonary consolidation category. Increased ALP is often found in young growing calves. (Thompson JC and Pauli JV 1981) The lower ALP in the higher pulmonary consolidation group may be associated with a decreased anabolic state in the calves in the high pulmonary consolidation category, suggestive of a less robust growing calf though not clinically significant. The high pulmonary consolidation category had statistically lower serum calcium compared to the low pulmonary consolidation category group on day 14. Calcium is highly bound to albumin and serum concentrations will decrease in correlation with albumin concentration. The high pulmonary consolidation category also had a statistically lower albumin concentration, thus this is the likely cause for the calcium concentration difference. Several other variables demonstrated a significant association with pulmonary consolidation category or sample day. Although statistical significance was found for multiple parameters, the clinical significance of these differences may be limited because these values were within normal reference ranges.

Fibrinogen is an acute phase protein, synthesized in the liver as part of the immune system response to infection. (Baumann et al. 1994) Acute phase proteins including fibrinogen have been reported to increase in concentration in calf blood during periods of stress to include abrupt weaning, transportation and comingling. (Berry et al. 2004, Holland et al. 2011, Hickey et al. 2003, Arthington et al. 2003 and Carter et al. 2002) Fibrinogen was significantly higher in the high pulmonary consolidation category calves on day 14. The fibrinogen concentrations represented in this study are both statistically and clinically important as the concentrations for only the high pulmonary consolidation category on day 14 was greater than established reference ranges. (Smith et al. 2009) The normal reference range is 0.1 to 0.6 g/dL though this is not specific for *M. bovis*.

Many variables in the CBC did show a significant association with sample day or pulmonary consolidation category. There may be physiologic differences present, though clinical importance in the remaining variables could not be identified based on established reference ranges. (Smith et al. 2009)

Cardiac troponin I has been used in bovine medicine to evaluate direct cardiac and indirect cardiac injury. (Varga et al. 2009a, Varga et al. 2009b, Peek et al. 2008, and Mellanby et al. 2009) Increased cTnI has been associated with human pulmonary hypertension. (Heresi et al. 2012) Cardiac troponin I was chosen to evaluate indirect cardiac injury of cattle via *M. bovis* induced pneumonia.

There was no evidence of disease on gross examination or histological examination of the papillary muscles of any of the hearts. There was a statistically higher plasma concentration of bovine cTnI on day 14 than day 0 and day 7 in all calves. This association of cTnI may be related with pulmonary hypertension, endotoxemia, or bacteremia. These changes may not have been specific for the direct insult from the pulmonary hypertension experienced from respiratory disease. The cTnI statistical values that were associated with day do not exceed previous reported reference ranges. (Varga et al. 2009a, Varga et al. 2009b, and Peek et al. 2008) It is interesting that no effect was found with pulmonary consolidation category, as it is established that chronic suppurative pneumonia has been shown to have higher cTnI concentrations as compared to controls. (Mellanby et al. 2009) Acute pneumonia may not have a dramatic effect on cardiac injury of cattle. Chronic pneumonia may result in fibrosis of lung parenchyma resulting in pulmonary hypertension and increased stress and injury to the right heart.

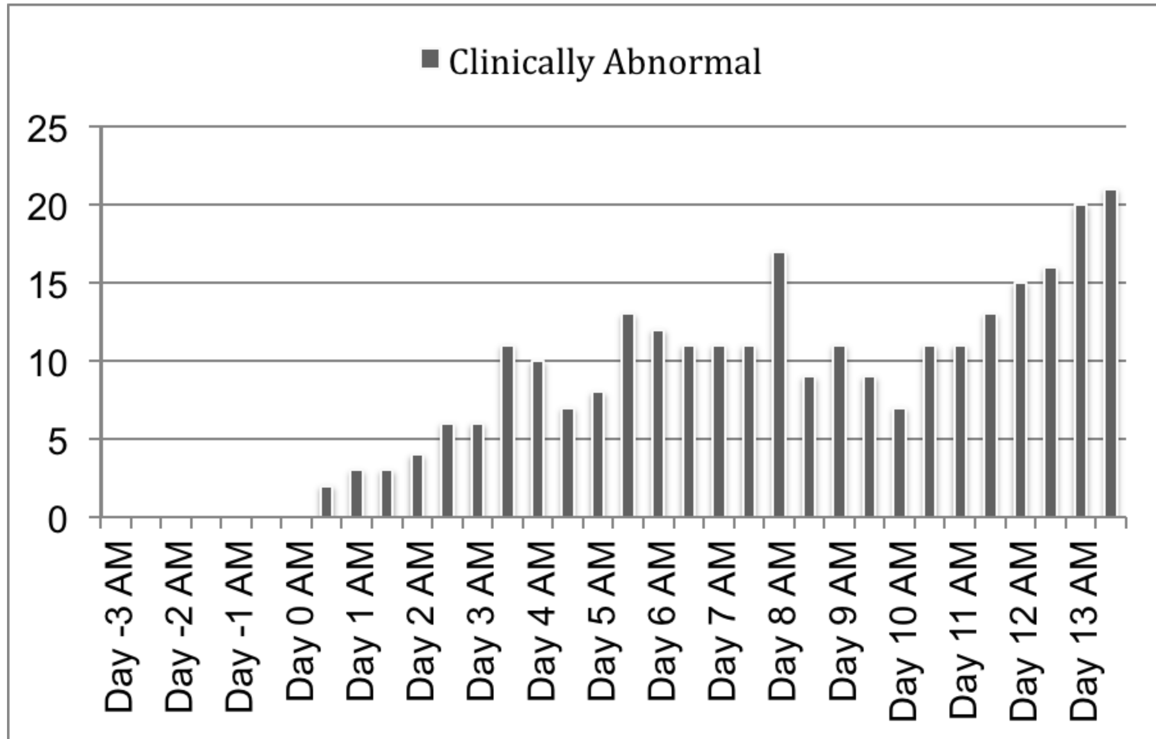
High resolution digital infrared thermography was utilized as a component of the biophysical profile in this study. Changes in both orbit and nose temperatures were recoded. High resolution digital infrared thermography has been used in bovine medicine and research. (Stewart et al. 2010a, Stewart et al. 2010b, Schaefer et al. 2007, Stewart et al. 2007) Many conditions have been evaluated with this modality. High resolution digital infrared thermography has been shown to possess predictive value for BRDC. (Schaefer et al. 2007)

Previous HRDIRT studies evaluating BRDC has focused on orbit temperatures and found temperature changes associated with respiratory disease. (Schaefer et al. 2007) In our study no significant changes in orbit temperatures were identified during the course of respiratory disease. Our study did find a difference of nose temperatures during the course of disease. The lower temperature reading of the nose in the high pulmonary consolidation category may be a sign of hypotension, hypovolemia or most likely a catabolic state in the high pulmonary consolidation category.

Conclusion

Bovine Respiratory disease complex continues to be a significant disease of cattle, and requires continued literature to be produced to further evaluate the individual animal's response to this disease. Changes in clinical illness scores and lungs support that the pneumonia induction protocol was successful. Fibrinogen is an acute phase protein that may increase with systemic disease. The statistical interaction of fibrinogen may have potential clinical application with the pulmonary consolidation category and sample day through *M. bovis* induced pneumonia. Clinicians may be able to evaluate fibrinogen concentrations from calves with *M. bovis* pneumonia to gain a greater understanding of the severity of the individual's pneumonia. In this study, no gross or histologic cardiac abnormalities were seen indicating no major myocardium remodeling or gross injury. Little literature is available on the calf's biophysical response to BRDC. In this study many biophysical parameters have shown significant interactions and associations with sample day and pulmonary consolidation category. The components of the biophysical profile performed in this study were not successful in determining susceptibility to severe respiratory disease in calves, or differentiating mild from severe respiratory disease.

Figure 4.1 Abnormal clinical Illness score (CIS) of Holstein calves challenged with *M. bovis* pneumonia (n=24).



Control calves (n=4) were not included in this chart and all received normal CIS's for the duration of the study. Study calves were inoculated on day 0 and were euthanized on day 14. An increasing total number of calves received abnormal scores with the duration of the study.

Figure 4.2 Fibrinogen concentration differences in low and high lung score categories (n=28.

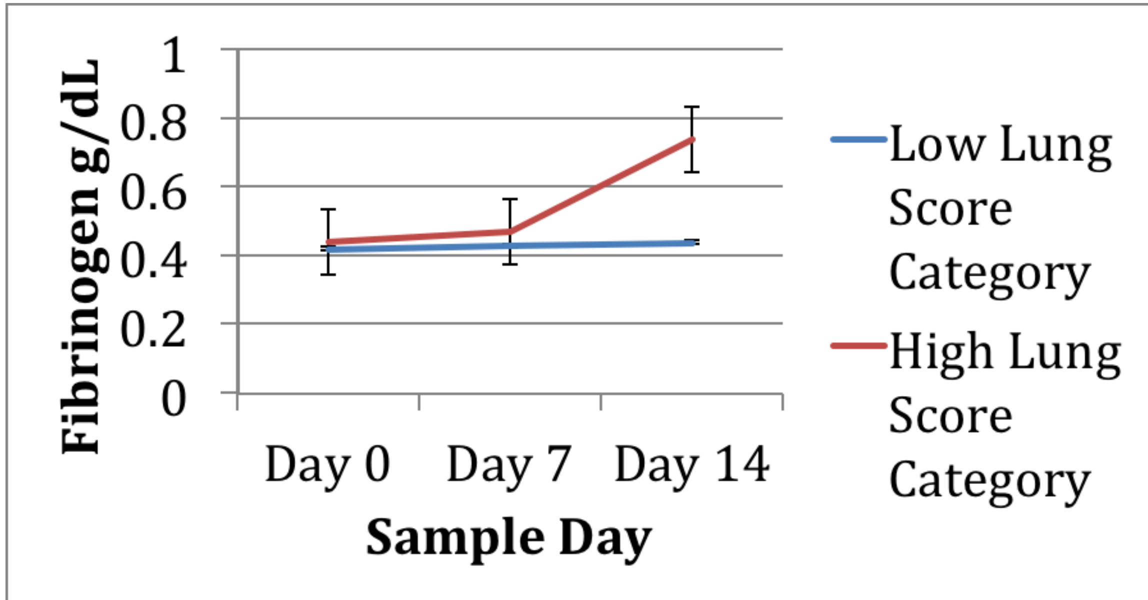


Table 4.1 Significant Serum Biochemistry Variables from Holstein Calves Challenged with *M. bovis* Pneumonia (n=28)

Variable	Test	Levels	LSM	P-Value
Alb/glob ratio	LungScoreXSampleDay	Day 0 Low	1.11^{A}	0.03
		Day 0 High	1.28^{B}	
		Day 7 Low	1.08^{A}	
		Day 7 High	1.13^{A}	
		Day 14 Low	1.00^{A}	
		Day 14 High	0.90^{A}	
ALP U/L	LungScoreXSampleDay	Day 0 Low	207.89^{A}	0.05
		Day 0 High	196.11^{A}	
		Day 7 Low	195.56^{A}	
		Day 7 High	149.05^{B}	
		Day 14 Low	183.56^{A}	
		Day 14 High	105.16^{B}	
CA mg/dL	LungScoreXSampleDay	Day 0 Low	10.64^{A}	0.02
		Day 0 High	10.72^{A}	
		Day 7 Low	10.74^{A}	
		Day 7 High	10.55^{A}	
		Day 14 Low	10.57^{A}	
		Day 14 High	9.83^{B}	
NA mmol/L	LungScoreXSampleDay	Day 0 Low	139.22^{A}	0.03
		Day 0 High	138.79^{A}	
		Day 7 Low	139.33^{A}	
		Day 7 High	136.79^{B}	
		Day 14 Low	136.67^{A}	

		Day 14 High	136.47^{A}	
ALBUMIN g/dL	Pulmonary consolidation	<10%	3.44^{A}	<0.01
		>10%	3.26^{B}	
	Sample day	DAY 0	3.52^{A}	<.0001
		DAY 7	3.35^{B}	
		DAY 14	3.18^{C}	
ANION GAP mmol/L	Pulmonary consolidation	<10%	19.70^{A}	0.01
		>10%	18.40^{B}	
	Sample day	DAY 0	19.80^{A}	<0.01
		DAY 7	17.70^{B}	
		DAY 14	19.66^{A}	
BUN mg/dL	Sample day	DAY 0	12.60^{A}	0.04
		DAY 7	12.25^{AB}	
		DAY 14	14.00^{B}	
CL mmol/L	Pulmonary consolidation	<10%	97.11^{A}	0.05
		>10%	96.28^{B}	
	Sample day	DAY 0	97.26^{A}	<0.01
		DAY 7	97.18^{A}	
		DAY 14	95.65^{B}	
CREAT mg/dL	Sample day	DAY 0	0.70^{A}	0.02
		DAY 7	0.64^{B}	
		DAY 14	0.64^{B}	
GLOBULIN g/dL	Pulmonary consolidation	<10%	3.31^{A}	0.02
		>10%	3.04^{B}	

	Sample day	DAY 0	2.97^{A}	<0.01
		DAY 7	3.10^{A}	
		DAY 14	3.46^{B}	
GLUCOSE mg/dL	Sample day	DAY 0	88.89^{A}	<.0001
		DAY 7	76.64^{C}	
		DAY 14	82.10^{B}	
HCO3 mmol/L	Pulmonary consolidation	<10%	27.59^{A}	0.04
		>10%	28.56^{B}	
	Sample day	DAY 0	28.22^{A}	0.01
		DAY 7	28.90^{A}	
		DAY 14	27.11^{B}	
K mmol/L	Sample day	DAY 0	5.03^{A}	<0.01
		DAY 7	4.77^{B}	
		DAY 14	4.73^{B}	
PHOS mg/dL	Pulmonary consolidation	<10%	7.61^{A}	<0.01
		>10%	6.92^{B}	
	Sample day	DAY 0	7.71^{A}	<0.01
		DAY 7	7.21^{B}	
		DAY 14	6.89^{B}	
PROTEIN g/dL	Pulmonary consolidation	<10%	6.75^{A}	<0.01
		>10%	6.30^{B}	

Serum biochemistries. Significant interactions of pulmonary consolidation category and study day are included, along with significant differences of pulmonary consolidation category and study day analyzed independently. Students T tests were utilized to determine significant differences between the least squared means. P values included if 0.05 or less.

Table 4.2 Significant Complete Blood Count Variables from Holstein Calves Challenged with *M. bovis* Pneumonia (n=28)

Variable	Test	Levels	LSM	P-Value
FIBRIN g/dL	LungScoreXSampleDay	Day 0 Low	0.42^{A}	<0.01
		Day 0 High	0.44^{A}	
		Day 7 Low	0.43^{A}	
		Day 7 High	0.47^{A}	
		Day 14 Low	0.44^{A}	
		Day 14 High	0.74^{B}	
BAND CONC K/uL	Sample day	DAY 0	0.01^{A}	0.02
		DAY 7	0.00^{A}	
		DAY 14	0.09^{B}	
BASO CONC K/uL	Sample day	DAY 0	0.15^{A}	0.01
		DAY 7	0.12^{AB}	
		DAY 14	0.07^{B}	
HCT %	Sample day	DAY 0	34.32^{A}	<0.01
		DAY 7	31.68^{B}	
		DAY 14	31.47^{B}	
HGB g/dL	Sample day	DAY 0	12.26^{A}	0.01
		DAY 7	11.50^{B}	
		DAY 14	11.32^{B}	
LYMPH CONC K/uL	Pulmonary consolidation	<10%	5.57^{A}	0.02
		>10%	4.80^{B}	
MCH pg	Pulmonary	<10%	11.85^{A}	0.02

	consolidation			
		>10%	12.28^{B}	
MCHC g/dL	Pulmonary consolidation	<10%	36.48^{A}	0.00
		>10%	35.81^{B}	
	Sample day	DAY 0	35.94^{A}	0.04
		DAY 7	36.44^{B}	
		DAY 14	36.05^{AB}	
MCV fL	Pulmonary consolidation	<10%	32.41^{A}	<0.01
		>10%	34.40^{B}	
RBC M/ul	Pulmonary consolidation	<10%	10.23^{A}	0.01
		>10%	9.38^{B}	
	Sample day	DAY 0	10.37^{A}	0.02
		DAY 7	9.45^{B}	

Complete blood cell counts. Significant interactions of pulmonary consolidation category and study day are included, along with significant differences of pulmonary consolidation category and study day anaylized independently. Students T tests were utilized to determine significant differences between the least squared means. P values included if 0.05 or less.

Table 4.3 Other Significant Variables from Holstein Calves Challenged with *M. bovis* Pneumonia (n=28)

Variable	Test	Levels	LSM	P-Value
Cardiac Troponin I ng/ml	Sample day	DAY 0	0.00^{A}	0.04
		DAY 7	0.00^{A}	
		DAY 14	0.02^{B}	
HRIRT Max Ventral Nose Celsius	Pulmonary consolidation	<10%	25.82^{A}	0.01
		>10%	23.07^{B}	
HRIRT Max Dorsal Nose Celsius	Pulmonary consolidation	<10%	22.85^{A}	<.01
		>10%	18.69^{B}	

Bovine cardiac troponin I (cTnI), and high-resolution digital infrared thermography. Significant interactions of pulmonary consolidation category and study day are included, along with significant differences of pulmonary consolidation category and study day anaylized independently. Students T tests were utilized to determine significant differences between the least squared means. P values included if 0.05 or less.

Chapter 5 - Thesis Conclusion

Literature of cardiac involvement with bovine respiratory disease complex remains sparse. General physiologic principles have long been established which have linked chronic respiratory disease with heart failure in cattle. Little is known concerning the progression of heart disease in early respiratory disease.

Evaluation of bovine cardiac troponin I can be utilized as a biomarker for cardiac injury. This biomarker has been used extensively in human and veterinary medicine. Advantages of the use of this biomarker include ease of sample collection, inexpensive, and objective measurements. With the addition of a point of care method of analysis, cattle health providers will be presented with rapid diagnostic information.

The first study showed that a point-of-care assay has the ability to accurately quantify and identify bovine cardiac troponin. This was accomplished through in vivo and in vitro testing of bovine cardiac troponin I with comparison of a previously validated immunoassay. The in vitro testing demonstrated the point-of-care assay's ability to accurately identify bovine cardiac troponin over a wide range of clinically applicable values. In vitro testing demonstrated the ability of the point-of-care assay to identify cardiac troponin I in a calf respiratory disease model. This point-of-care assay will allow further clinical and research evaluation of bovine cardiac troponin I.

The second study evaluated the progression of bovine respiratory disease through objective measurements. The most clinically applicable finding was an increase of fibrinogen associated with sample day and lung score category. Many variables showed statistical changes over time and by lung score category though all may not be clinically relevant. Cardiac troponin I did exhibit a temporal increase through the disease course.

Bovine respiratory disease complex and associated cardiac injury remains a challenging endeavor for the clinician. Further evaluation of cardiac injury as a result of respiratory disease may provide improved prognostic information to the animal health provider. The point-of-care assay will allow a rapid and objective evaluation of cardiac injury in various disease states of the bovid.

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Appendix A - Holstein calf body weight data with no statistical evaluation

Table A.1 Holstein calf body weight data from chapter 4 (n=28)

ID	Date	Parameter	Result
1393	-14	Weight kg	87.7
1415	-14	Weight kg	88.6
1429	-14	Weight kg	75.5
1430	-14	Weight kg	65.9
1431	-14	Weight kg	83.2
1432	-14	Weight kg	66.4
1433	-14	Weight kg	82.3
1434	-14	Weight kg	75.9
1435	-14	Weight kg	82.7
1436	-14	Weight kg	85.5
1437	-14	Weight kg	77.7
1438	-14	Weight kg	73.2
1440	-14	Weight kg	72.7
1441	-14	Weight kg	75.5
1442	-14	Weight kg	73.2
1443	-14	Weight kg	68.6
1444	-14	Weight kg	75.9
1445	-14	Weight kg	88.6
1446	-14	Weight kg	67.3
1447	-14	Weight kg	84.5
1448	-14	Weight kg	61.4
1454	-14	Weight kg	76.8
1455	-14	Weight kg	72.7

1456	-14	Weight kg	91.8
1457	-14	Weight kg	80.5
1460	-14	Weight kg	90.9
1462	-14	Weight kg	78.2
1463	-14	Weight kg	75.0
1393	7	Weight kg	96.8
1415	7	Weight kg	92.3
1429	7	Weight kg	88.2
1430	7	Weight kg	70.9
1431	7	Weight kg	93.6
1432	7	Weight kg	78.2
1433	7	Weight kg	88.2
1434	7	Weight kg	92.7
1435	7	Weight kg	96.4
1436	7	Weight kg	95.0
1437	7	Weight kg	94.1
1438	7	Weight kg	85.5
1440	7	Weight kg	91.8
1441	7	Weight kg	83.6
1442	7	Weight kg	83.2
1443	7	Weight kg	79.1
1444	7	Weight kg	90.5
1445	7	Weight kg	106.4
1446	7	Weight kg	x
1447	7	Weight kg	x
1448	7	Weight kg	76.4
1454	7	Weight kg	94.5
1455	7	Weight kg	89.5
1456	7	Weight kg	101.8

1457	7	Weight kg	96.8
1460	7	Weight kg	x
1462	7	Weight kg	x
1463	7	Weight kg	91.8
1393	14	Weight kg	98.2
1415	14	Weight kg	81.4
1429	14	Weight kg	83.6
1430	14	Weight kg	75.5
1431	14	Weight kg	96.8
1432	14	Weight kg	74.1
1433	14	Weight kg	87.3
1434	14	Weight kg	90.9
1435	14	Weight kg	88.6
1436	14	Weight kg	100.9
1437	14	Weight kg	92.7
1438	14	Weight kg	85.9
1440	14	Weight kg	93.2
1441	14	Weight kg	86.8
1442	14	Weight kg	85.0
1443	14	Weight kg	78.2
1444	14	Weight kg	91.4
1445	14	Weight kg	104.5
1446	14	Weight kg	87.3
1447	14	Weight kg	110.5
1448	14	Weight kg	78.6
1454	14	Weight kg	104.1
1455	14	Weight kg	89.5
1456	14	Weight kg	98.2
1457	14	Weight kg	96.8

1460	14	Weight kg	117.3
1462	14	Weight kg	102.3
1463	14	Weight kg	95.9

Body weight data of 28 Holstein calves was not analyzed due to incompleteness of data collection. No data was obtained on day 0 and the control calves were not collected on day 7. This chart is supplied for completeness of findings. An x indicates no data for the collection time.

Appendix B - Holstein calf rectal temperature data with no statistical evaluation

Table B.1 Holstein calf rectal temperatures from chapter 4 (n=28)

ID	Date	Parameter	Result
1456	7	Temp Celsius	35.15
1437	7	Temp Celsius	37.88
1445	7	Temp Celsius	37.97
1448	7	Temp Celsius	37.99
1443	7	Temp Celsius	38.11
1415	7	Temp Celsius	38.50
1393	7	Temp Celsius	38.52
1434	7	Temp Celsius	38.54
1457	7	Temp Celsius	38.55
1455	7	Temp Celsius	38.61
1441	7	Temp Celsius	38.65
1431	7	Temp Celsius	38.69
1454	7	Temp Celsius	38.77
1446	7	Temp Celsius	38.83
1463	7	Temp Celsius	38.83
1430	7	Temp Celsius	38.87
1440	7	Temp Celsius	38.88

1436	7	Temp Celsius	38.95
1435	7	Temp Celsius	38.99
1433	7	Temp Celsius	39.03
1432	7	Temp Celsius	39.11
1438	7	Temp Celsius	39.17
1444	7	Temp Celsius	39.17
1460	7	Temp Celsius	39.28
1462	7	Temp Celsius	39.33
1429	7	Temp Celsius	39.40
1442	7	Temp Celsius	39.51
1447	7	Temp Celsius	39.53
1443	14	Temp Celsius	36.45
1457	14	Temp Celsius	37.86
1434	14	Temp Celsius	38.55
1460	14	Temp Celsius	38.57
1455	14	Temp Celsius	38.59
1446	14	Temp Celsius	38.77
1462	14	Temp Celsius	38.97
1463	14	Temp Celsius	39.11
1447	14	Temp Celsius	39.23
1454	14	Temp Celsius	39.25
1438	14	Temp Celsius	39.35
1393	14	Temp Celsius	39.39
1433	14	Temp Celsius	39.49
1441	14	Temp Celsius	39.51
1442	14	Temp Celsius	39.53
1435	14	Temp Celsius	39.55
1448	14	Temp Celsius	39.59
1436	14	Temp Celsius	39.91

1431	14	Temp Celsius	40.03
1437	14	Temp Celsius	40.05
1444	14	Temp Celsius	40.17
1456	14	Temp Celsius	40.49
1432	14	Temp Celsius	40.51
1440	14	Temp Celsius	40.53
1415	14	Temp Celsius	40.65
1429	14	Temp Celsius	40.83
1430	14	Temp Celsius	40.95
1445	14	Temp Celsius	41.03

Rectal temperature data was not analyzed due to incompleteness of data collection. No data was obtained on day 0. This chart is supplied for completeness of findings.