

Heart disease in beef cattle: visual heart score and sarcocystis

by

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Abstract

Congestive heart failure (CHF) in feedyard cattle has been diagnosed at lower elevations, indicating elevation is not a necessary factor for CHF. Hypoxia from a variety of causes leads to vasoconstriction and pulmonary hypertension which can culminate in CHF. Sustained differences in the cardiac pressure gradient may result in morphologic changes of the heart such as ventricular and atrial enlargement. Understanding previous literature and the association between gross and histologic cardiac changes are important to advancing industry knowledge on this disease process.

A narrative literature review was completed with the objective of providing an overview of the prevalence of sarcocystis infections found in beef cattle globally and regionally. Two databases (PubMed and AGRICOLA) were reviewed. A total of 59 articles from 1957 to 2026 were identified describing prevalence or epidemiology of sarcocystis in beef cattle. The intent of this review was to identify literature gaps surrounding the topic of sarcocystis infection in cattle, particularly within North America. The review identified 11 articles from Africa, 26 from Asia, 13 from Europe, 1 from North America, 4 from Oceania, and 4 from South America. Respectively the prevalence of sarcocystis in beef from each continent was 59%, 75%, 76%, 62%, 80%, and 96%.

The objective of the first study was to evaluate gross heart score in cattle at slaughter with cardiac fibrosis, necrosis, or cardiomyofibrillar abnormalities. A total of 121 cases were stratified by heart score (HS) using a scale of 1 to 5. A HS of 1 represented a normal heart and a HS of 5 represented of severe remodeling with misshapen ventricles. Cattle were evaluated for gross lesions of the heart, lung, and liver including failure of lungs to deflate, pulmonary edema, pneumonia, and liver abscesses or congestion. Samples collected for histology included cardiac

(n=4), pulmonary (n=4), and hepatic (n=1). Histologic evaluation scored cardiac and hepatic fibrosis and necrosis, embedded myocardial protozoal cysts (EMPC) were quantified, and pulmonary lesions were categorized. A total of 40 cases had a normal HS (1 or 2), and 63 cases had an abnormal HS (3, 4, or 5). A total of 64 cases had normal lung deflation scores (normal deflation), and 39 cases had abnormal lung deflation scores (mild, moderate, or severe failure of the lungs to deflate). Of the 103 total cases, 67 cases had EMPC in at least one cardiac section. Cattle with abnormal lung deflation scores were more likely to also have an abnormal HS (0.76 ± 0.07 , $p \leq 0.01$) compared with cattle with normal lung deflation scores (0.52 ± 0.06). Cattle with EMPC present in at least one cardiac section were more likely to also have abnormal HS (0.73 ± 0.05 , $p \leq 0.1$) compared with cattle without EMPC (0.39 ± 0.08).

The objective of the second study was to determine if an association existed between EMPC and HS or CHF in cattle feedyard mortalities. A total of 121 cattle feedyard mortalities that underwent necropsy in 2022 and 2023 were included in this study. A veterinarian scored the hearts post-hoc on a scale of 0 to 5. A HS 0 was a heart with infectious disease, a score of 1 represented normal, and HS 5 represented a severely enlarged and misshapen heart. Mortalities were necropsied in the field and the cardiopulmonary, gastrointestinal, musculoskeletal, urinary, and reproductive systems were evaluated. Samples from the papillary muscle of the heart were collected for histologic evaluation. Cardiac samples were digitized and the presence of EMPC were counted along with a measurement of each sample area. The EMPC over the sample area was calculated for an embedded cardiac protozoal cyst ratio (EMPCR). After case removals, 106 cattle were left in the study population with a total of 73 heifers and 33 steers. Heart scores were categorized into normal HS (NHS) (HS 1 or 2), abnormal HS (AHS) (HS 3, 4, or 5), and infected (HS 0). There was a total of 60 NHS, 34 AHS, and 12 infected cases. A total of 15 cases were

defined as having CHF which was defined as an AHS with two or more effusions of the pleural, pericardial, or peritoneal space with a congested liver. After statistical analysis, no association was found between AHS and EMPCR or cattle with CHF and EMPCR.

Congestive heart failure in feedyard cattle has an increasing impact on the industry. While no associations were established between AHS and histologic changes of the heart, lungs, or liver, a significant association was found between the presence of EMPC and AHS in cattle at slaughter. This same association was not found in feedyard cattle mortalities. While these protozoan parasites have been considered incidental in the past, their association with AHS in feedyard cattle, potential implication on heart health, and pathogenesis should continue to be investigated.

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Introduction

Before the early 2000s, non-infectious cardiac failure in beef cattle had a low reported incidence. However, in more recent years bovine congestive heart failure (CHF) has become more frequently diagnosed at lower elevations, indicating that elevation is not always a significant factor. Clinical signs of CHF are variable but include dyspnea, edema of the brisket and ventral abdomen, jugular venous distention, moist cough, depression or inappetence, exercise intolerance, and abducted elbows. Bovine congestive heart failure may be initiated by hypoxia (e.g. high elevation, bronchopneumonia, obesity, heat stress, or parasitic infestation) which causes vasoconstriction and resultant pulmonary hypertension. Increased pulmonary vascular pressure results in increased right ventricular afterload causing stress on the cardiac walls, remodeling and degeneration of the extracellular matrix (ECM) and myocardial interstitial connective tissue and collagen, which in turn leads to right ventricular hypertrophy. Sustained periods of myocardial injury and remodeling eventually lead to reduced compensatory capability of the cardiac wall, and clinical congestive cardiac failure. Assessing visual heart score in cattle with and without CHF and any potential associations with bovine cardiac health may provide additional insight to heart disease in the cattle industry moving forward.

Chapter 1 summarizes current literature on the global and regional prevalence of sarcocystis infections in beef cattle and defines the current knowledge gaps. Sarcocystis is a cardiac parasite commonly seen in cattle but can lead to condemnation at slaughter if macroscopic sarcocysts or related inflammation is seen. Limited research studying sarcocystis infection of cattle exists in North America with only one prevalence study available for review. However, the prevalence of sarcocysts is well studied in Europe and Asia. The objective of this study was to perform a narrative literature search to review the prevalence of sarcocysts within

each continent. This review highlighted both knowledge gaps and the areas of sarcocystis research that are well understood.

Chapter 2 assessed 103 cattle from a harvest facility with the primary focus of finding potential associations between gross heart score and histopathology of the heart, lungs, and liver. The objective of this research study was to determine if gross heart score was associated with gross lung deflation score, cardiac histopathology (cardiac fibrosis, necrosis, or cardiomyofibrillar abnormalities), or hepatic histopathology (hepatic fibrosis, necrosis, or hepatocellular abnormalities). Potential associations between gross and histopathology can be important to further research on non-infectious cardiac disease in beef cattle and future management of the disease.

The focus of Chapter 3 was to further analyze potential associations between gross heart score and cardiac histologic findings. Cattle mortalities (106) were analyzed in this study for the presence of embedded myocardial protozoal cysts (EMPC) presumed to be sarcocysts based on the commonality of sarcocystis infection in cattle. The objective of this research study was to determine if there was an association between gross heart score and EMPC. Research regarding sarcocysts and the potential implication on cardiac health is important to understand due to the lack of research around sarcocystis in general.

Chapter 1 - Review of Global and Regional Prevalence of Sarcocystis Infections in Beef Cattle

Abstract

Sarcocysts are a protozoan parasite commonly found in the cardiac muscle of cattle. Sarcocystis has been described as incidental, but infection can cause condemnation of entire or parts of carcasses. Little research in North American exists describing the frequency of infection and associations with disease. The aims of this narrative literature review are to provide an overview of the prevalence of sarcocystis infections found in beef cattle globally and regionally. This literature reviewed two databases including PubMed and AGRICOLA. A total of 53 articles from 1957-2026 were identified on PubMed describing prevalence of sarcocystis in beef cattle. An additional 6 articles were identified on AGRICOLA describing sarcocystis prevalence in beef cattle. These articles also described the tissues collected for sampling and the diagnostic or series of diagnostic tests used for confirmation of sarcocystis presence. The purpose of this review was to describe the prevalence of sarcocystis across different regions. South America had the highest reported prevalence of sarcocystis at 96% and Africa had the lowest reported prevalence at 59%.

Keywords: Sarcocyst, Beef Cattle, Global, Regional, Prevalence

Introduction

While the prevalence of sarcocystis infections in cattle are relatively well studied in several regions, North America is not one of them. Sarcocysts are a protozoan parasite with numerous species affecting both animals and humans. Cattle infected with sarcocysts are commonly an incidental finding at harvest, but occasionally sarcocysts have been described to cause disease leading to anorexia, anemia, weight loss, abortion and even death in cattle.¹ Clinical

sarcocystosis has been reported in several countries worldwide including the USA and Canada.^{1,2} Species infecting cattle include *S. cruzi*, *S. hirsute*, *S. bovifelis*, *S. bovini*, *S. rommeli*, and two zoonotic species: *S. hominis* and *S. heydorni*.^{3,4} Zoonotic species of sarcocysts between cattle and humans may cause intestinal sarcocystosis from eating raw or undercooked beef.⁵ Clinical illness in humans is rare, as sarcocystis is generally asymptomatic or goes unreported. Human infection is generally limited to case reports, and intestinal sarcocystosis has been documented most frequently in Europe.⁵ Due to the inability to differentiate zoonotic and non-zoonotic sarcocysts species in cattle at harvest, condemnation of parts of the carcass are warranted when macrocysts which are visible to the eye or sarcocyst related inflammation (Bovine Eosinophilic Myositis) is identified. Understanding sarcocyst prevalence can lead to improved observation strategies and identify areas or regions where prevalence is higher.

The objective of this narrative review is to assess global and regional prevalence of sarcocystis infections in beef cattle. Previous research suggests sarcocystis infection in cattle is commonly 100% of animals, however prevalence of the parasite in Europe is reported to be between 56.5% and 96%.³ This literature review identified articles from multiple databases, organized them based on inclusion/exclusion criteria, and summarized the prevalence and epidemiology of sarcocyst infections around the globe.

Materials and Methods

Literature Search Strategy

Relevant literature was identified using two databases: PubMed and AGRICOLA (AGRICultural Online Access). The same search term string was used for both databases and was as follows: ((((((cattle) OR ("beef cattle")) OR ("feedlot cattle")) OR ("bos taurus")) OR (bovidae)) OR (bovine)) AND (((sarcocystis) OR (sarcocystosis)) OR (sarcocyst))) AND ((prevalence) OR

(epidemiology)). The search term was formulated by MJJ with the assistance of a literature expert from the K-State College of Veterinary Medicine library. No additional filters from PubMed and AGRICOLA were applied to allow access to as many journal articles as possible. Articles were copied and pasted from each database into Microsoft Excel (Microsoft 365, version 2602) for article management and review.

Article Management

Once articles from both PubMed and AGRICOLA were placed into Microsoft Excel for review, the first step was to screen titles for inclusion/exclusion criteria. Any articles not readily available were requested through the K-State Libraries Interlibrary Loan Service. The next step was to screen articles based on abstracts. Following abstract review, articles underwent full text review.

Inclusion/Exclusion Process

Inclusion criteria for this literature review included epidemiological studies in beef cattle that mentioned prevalence, background, or diagnostic testing of sarcocystis. Requirements of each study included: the number of cattle in the study, the source of cattle, prevalence of sarcocystis, diagnostic testing used, and region of the study to be included in this review. Exclusion criteria were as follows: other published literature reviews, books, case studies, articles without English translation, studies focused on non-cattle species, studies with working dairy cattle (not at slaughter), studies with retail beef or beef products, and articles not meeting the inclusion criteria.

Results

Search and Selection

The initial search resulted in 264 publications from the two databases (174-PubMed; 90-AGRICOLA). Non-English publications were removed which totaled 15 articles. Between the two databases, 60 articles were duplicated and therefore removed. An additional 130 articles were removed from the review due to inclusion/exclusion criteria. This left 59 articles in the final review.

Continental Prevalence

Over 30 countries across six continents and about 16,000 cattle were represented in this literature review. Articles from Asia represent 44% (26/59) of the publications, Europe 22% (13/59), Africa 18% (11/59), South America 7% (4/59), Oceania 7% (4/59), and North America 2% (1/59). Most published articles studied cattle at slaughter (57/59), 1 paper studied a cow-calf herd, and 1 specifically studied cow-calf herds with a history of abortion (Table 1). Papers reporting prevalence of sarcocystis in South American cattle had the highest mean prevalence of 96% and papers reporting sarcocystis prevalence in African had the lowest mean prevalence with 59% infected (Figure 1).

Discussion

The current literature review found that out of the 6 continents evaluated, all had a sarcocystis prevalence of over 50%. Almost every prevalence study in this literature review described sarcocystis in cattle at harvest. However, two studies looked at cattle on cow-calf operations, with one specifically studying herds with history of abortion. The cow-calf herd with a history of abortion had a 0% prevalence of sarcocystis while the herd without history of abortion had 100% prevalence. Only two herds does not allow specific conclusions on the relationship of sarcocysts

and outcomes in cow-calf herds and the importance of sarcocystis requires further research in cow-calf herds.

Samples collected for each prevalence study varied with heart muscle being the most common sample taken followed by esophagus and diaphragm. Skeletal muscle, ocular muscle, and blood samples were also collected for a few studies. Diagnostic techniques also were variable with 18s rRNA PCR being the most used and solo histological evaluation being the least common. Other techniques involved visual only inspection, peptic digestion, Multiplex PCR, or cox1 sequencing. Overall, the prevalence of sarcocyst infection is relatively high, however, most of the studies in this review were from Asia and Europe. There were very few studies available from North America, South America, and Oceania. This could introduce increased heterogeneity between the groups and introduce potential bias, which is a limitation of this study.

With few studies available and reports of sarcocystis being prevalent even in well developed countries, the U.S should not be as quick to dismiss this parasite as incidental without further research.⁶ Most studies were completed in cattle that made it to harvest. Even though prevalence of sarcocystis was documented, few studies documented associated inflammation with sarcocysts or the number of carcasses condemned. Therefore, more extensive research is required to know if these cases were incidental or pathogenic in nature. While rare, a few more pathogenic species of sarcocysts such as *S. cruzi* can cause acute disease such as fever, abortion, neurologic signs, ataxia, and death.⁷ Clinical sarcocystosis has been documented in the USA, Canada, England, Norway, Ireland, Australia, and Uruguay.^{2,8} Sarcocystis can also manifest as chronic disease with decreased weight and hair loss being the most common clinical signs.¹ Neither acute or chronic

sarcocystosis are well documented which may be due to similar clinical presentation to other, more common, protozoa such as *Toxoplasma*, *Neospora*, and *Cystoisospora*.⁹ Chronic sarcocystosis may also be mistaken for external parasite infestation due to inconspicuous clinical signs. Further review of regional prevalence is required to better understand the potential impact of sarcocystis on cattle health particularly in North America, South America, and Oceania.

Conclusion

This literature review of the prevalence of sarcocystis in beef cattle found Africa had the lowest prevalence of 59% and South America had the highest prevalence of 96%. This review included 59 articles with only one from North America, which requires more research to determine a true prevalence. A majority of the articles were from Asia and Europe. The regional prevalence determined from this review was similar to past research for both regions.

Figures

Figure 1.1: Continental Prevalence of Sarcocystis

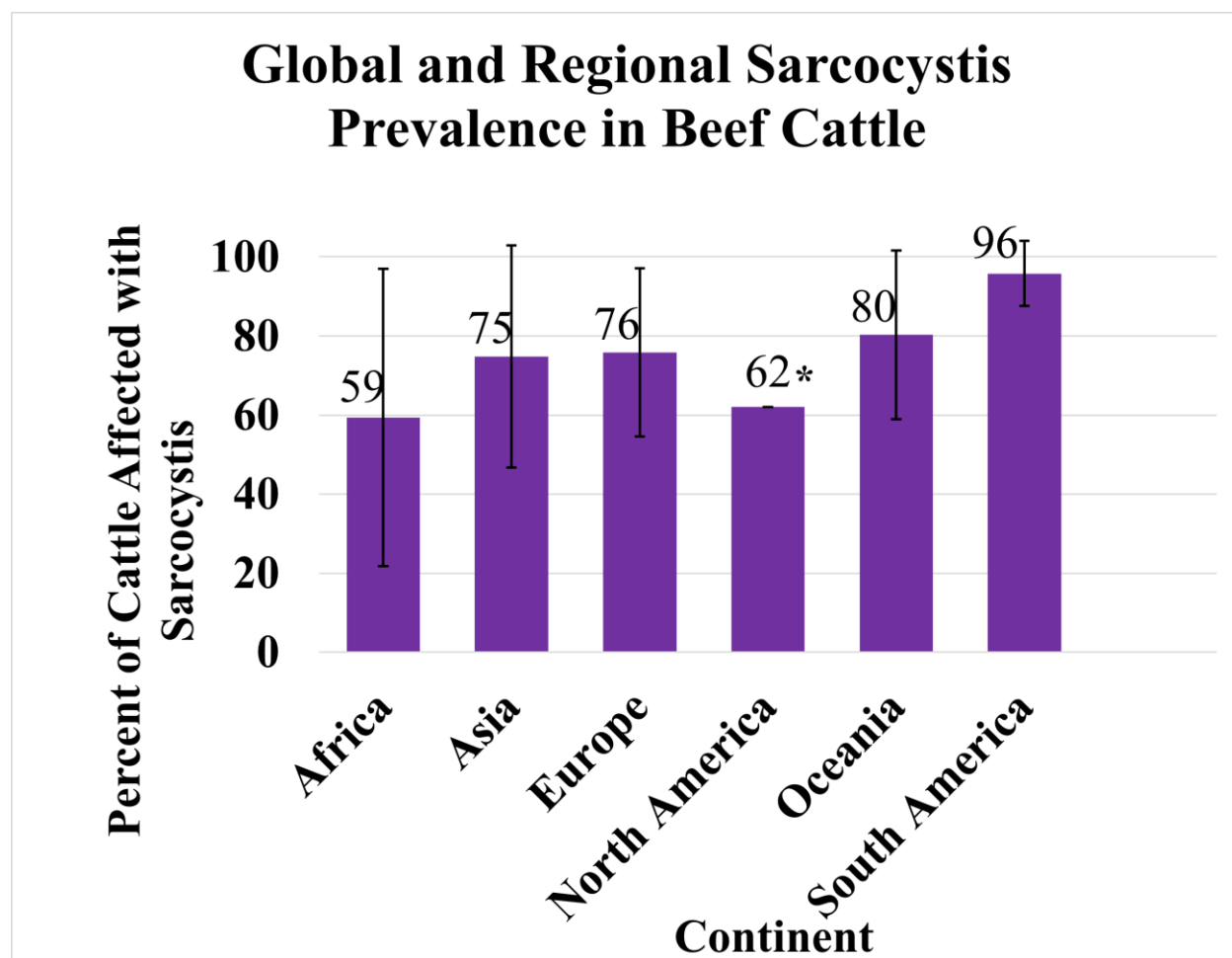


Figure 1.1: This graph shows the average prevalence of sarcocyst infections in cattle across each continent. *Standard deviations are shown except for North America, as it only had one article.

Tables

Table 1.1: Number of Articles per Continent

Continent	No. of articles	Reference
Africa	11	10–20
Asia	26	4,6,7,21–43
Europe	13	3,44–55
North America	1	56
Oceania	4	57–60
South America	4	61–64

Table 1.1: This table shows the number of articles across each continent along with the published references.

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Chapter 2 - Association of visual heart score with gross lung pathology and histology of hepatic and cardiopulmonary tissues in cattle at harvest

Abstract

Non-infectious cardiac failure in feedyard cattle has become more frequently diagnosed. Limited research has assessed gross and histologic lesions in grossly abnormal hearts of harvested cattle. A total of 121 cases were stratified by heart score (HS) using a scale of 1 to 5, with 1 representative of a normal heart and 5 representative of severely misshapen ventricles. Cattle were evaluated for gross lesions of the heart, lung, and liver. Samples collected per animal for histology included: cardiac (n=4), pulmonary (n=4), and hepatic (n=1). Histologic evaluation scored cardiac and hepatic fibrosis and necrosis, embedded myocardial protozoal cysts (EMPC) were quantified, and pulmonary lesions were categorized based on histologic patterns. Of 103 cases, 40 had normal HS (NHS) (1 or 2), and 63 had abnormal HS (AHS) (3, 4, or 5). There were 64 cases with normal lung deflation scores, and 39 cases with abnormal lung deflation scores. At least one cardiac section contained EMPC in 67 cases. Cattle with abnormal lung deflation scores were more likely to have an AHS (0.76 ± 0.07 , $p \leq 0.01$) compared with cattle with normal deflation scores (0.52 ± 0.06). Cattle with EMPC present in at least one cardiac section were more likely to also have AHS (0.73 ± 0.05 , $p \leq 0.1$) compared with cattle without EMPC (0.39 ± 0.08). No histological findings for heart, lung or liver were associated with abnormal heart score; however, lung deflation and EMPC were associated with abnormal heart score.

Keywords: bovine congestive heart failure; heart score; lung deflation; protozoal cyst

Introduction

Prior to the early 2000s, non-infectious cardiac failure in cattle had a low reported incidence, was more commonly seen at elevated altitudes, and was mostly considered part of the syndrome known as high mountain or high-altitude disease ¹. However, in more recent years bovine congestive heart failure (BoCHF) has become more frequently diagnosed at lower elevations, indicating that elevation is not always a significant factor ^{1,2}. Clinical signs of BoCHF are variable but include dyspnea, edema of the brisket and ventral abdomen, jugular venous distention, moist cough, depression or inappetence, exercise intolerance, and abducted elbows ³⁻⁸. Bovine congestive heart failure may be initiated by hypoxia (e.g. high elevation, bronchopneumonia, obesity, heat stress, or lungworm infection) which causes vasoconstriction and resultant pulmonary hypertension ². Increased pulmonary vascular pressure results in increased right ventricular afterload causing stress on the cardiac walls, remodeling and degeneration of the extracellular matrix (ECM) and myocardial interstitial connective tissue and collagen, which in turn leads to right ventricular hypertrophy ^{2,9}. Sustained periods of myocardial injury and remodeling eventually lead to reduced compensatory capability of the cardiac wall, and clinical congestive cardiac failure in the individual; morphologic lesions accompany only the chronic phases of injury ⁹.

Gross morphologic cardiac lesions, especially ventricular and atrial enlargement have been described using a scale from 1 to 5. A gross heart score (HS) of 1 is a normal heart with no

misshapen ventricles; a HS of 5 is a severely remodeled heart with markedly misshapen and distorted ventricles ¹⁰. A recent study documented cardiac enlargement and misshapen ventricles (CEMV) in feedlot cattle mortalities, but cattle with evidence of congestive heart failure were only a subset of these cases. In the forementioned study, cases of BoCHF were defined as having CEMV with congested liver and two or more effusions such as pericardial, pleural, or peritoneal ¹⁰. Another study of feedyard mortalities, using the same definition for BoCHF, found that 35.7% of mortalities had cardiovascular lesions (BoCHF, heat stress, right or left ventricular concentric hypertrophy, anemia, endocarditis, myocarditis, nutmeg liver, or pericarditis) with 15.3% of those being BoCHF ¹¹. Few studies have looked at changes in cardiac morphology of cattle at harvest, but a 4.14% incidence of HS of 4 or 5 has been described ³. Further studies have shown an association between CEMV and failure of lungs to deflate after death in harvested cattle; a hypothesis is that necrosis and fibrosis of the lung is a secondary consequence of chronic congestive heart failure which prevents normal deflation ¹². An alternate hypothesis is that failure of the lungs to deflate could be associated with pulmonary hypertension followed by cardiomegaly. The goal of this study was to determine the relationship between HS and histologic architecture of the heart, lung and liver in feedlot cattle at harvest.

Three main objectives were defined in this study. The first objective was to determine if gross lung deflation or histologically confirmed pneumonia were associated with HS. The second objective was to determine if HS at harvest was associated with cardiac fibrosis, necrosis, or cardiomyofibrillar abnormalities. The third objective was to determine if abnormal HS at harvest was associated with hepatic fibrosis, necrosis or underlying hepatocellular abnormalities.

Materials and Methods

A cross-sectional observational study was performed that evaluated the association of HS with gross lung pathology and histopathology of the cardiopulmonary and hepatic systems. This study was exempt from approval by the Institutional Animal Care and Use Committee, because all data and sample collection occurred after death. USDA regulations for post-mortem cattle at slaughter, including the Food Safety and Inspection Service (FSIS) and Office of Laboratory Animal Welfare (OLAW) guidelines were followed.

Gross Evaluation

Three evaluators (R. Raymond, J. Buchanan, S. Crisp) collected samples from a targeted cross-section of cattle at harvest having passed USDA antemortem inspection (n=121) which were then stratified by HS ranging from 1 = normal, 2 = mild change, 3 = moderate change, 4 = severe change, and 5 = severe change and flaccid, summarized in Figure 1¹⁰. Individual animals were evaluated for gross lesions of the heart, lung, and liver including failure of lungs to deflate, pulmonary edema, interstitial or bronchopneumonia, and liver abscesses. All cases received a gross pulmonary deflation score of either normal/deflated, mild failure to deflate, moderate failure to deflate, or severe failure to deflate. Normal lungs were defined as completely collapsed and pliable with no turgidity, mild to moderate failure to deflate had increasing aeration and turgidity, and severe failure to deflate were fully aerated lungs with complete turgidity and stiffness of the tissue (Figure 2). Each case was assigned a gross lung lesion description (bronchopneumonia, interstitial pneumonia, and pleuritis) and percent of lung affected was estimated with categories ranging from normal <5% lesions, 5-15% lesions, 15-30% lesions, 30-50% lesions, and 50%+ lesions. Nine tissue samples from each animal were collected for histopathologic evaluation from the same anatomic location for each animal: four cardiac

[septum, papillary muscle, right ventricular free wall (RVFW), and left ventricular free wall (LVFW)], four pulmonary [right and left cranioventral lobes, and right and left caudodorsal lobes], and one representative liver section.

Histopathology

All samples collected for histopathology were approximately 1 cm² and fixed for at least 24 hours in 10% neutral buffered formalin. Once samples arrived at the laboratory, they underwent processing for serial dehydration and paraffin embedding. Each sample was then sectioned at 4 μm and stained using the Leica ST5010 Autostainer XL (Leica Biosystems, Deer Park, IL, USA) with hematoxylin and eosin. Prepared samples for histopathology were evaluated by a board-certified veterinary pathologist (B. Plattner) and veterinarian (T. Gunderson) cross-trained and supervised by the same pathologist. Evaluators were blinded to all gross lesions including gross heart scores, lung lesion scores, and liver evaluation. All histology evaluations were finalized by the pathologist.

Histologic sections for heart and liver each received an autolysis score defined as 0 (no autolysis), 1 (mild autolysis, increased scattered bacterial overgrowth), 2 (moderate autolysis, loss of nuclear and cellular details, moderate bacterial overgrowth), or 3 (marked autolysis, cell and nuclear morphology difficult or impossible to discern, severe bacterial overgrowth). Cardiac and hepatic samples were assigned a fibrosis score of 0 (normal), 1 (mild subendocardial or interstitial increased loose to compact fibrous tissue), 2 (moderate compact interstitial fibrosis with loss and replacement of cardiomyocytes and/or hepatocytes), or 3 (marked patchy regional interstitial and replacement or bridging fibrosis/fibroplasia); and a necrosis score of 0 (normal, no necrosis), 1 (mild individual scattered individual cell coagulative necrosis), 2 (moderate

patchy coagulative necrosis), or 3 (marked patchy and bridging coagulative necrosis).

Additionally, cardiac samples were scored based on the number of embedded protozoal cysts, which was further defined as rare (0-1 cyst per tissue section, approximately 400mm²), few (2-10 cysts per tissue section), moderate (>10 cysts per tissue section), or severe (>50 cysts per tissue section). The pulmonary tissue sections were categorized based on the most prominent pneumonia pattern including bronchopneumonia (BP), interstitial pneumonia (IP), or other (OTH) as previously described in Feitoza et.al (Table 1) ¹³.

Data Categorization

Gross heart scores were grouped into normal (HS 1,2) (NHS) or abnormal (HS 3,4,5) (AHS). Lung deflation score was categorized into normal (deflated) or abnormal (mild, moderate, and severe failure to deflate). Gross lung lesion scoring (percent lung affected with lesions) was categorized as normal (<5% pathology) and abnormal (5-15%, 15-30%, 30-50%, and 50%+ pathology). Cardiac and hepatic fibrosis and necrosis were categorized as normal (0,1) or abnormal (2,3). Protozoal cyst scoring of the heart was dichotomized into rare (none, rare) or present (few, moderate, severe).

Using the pneumonia pattern scoring system for each of the four individual lung samples, a final case diagnosis was assigned including BP, exudate or proliferative IP, bronchopneumonia with an interstitial pattern (BIP), lymphoplasmacytic interstitial pneumonia (LIP), or other (OTH). Chronicity was also assessed in each lung section, and if any of the four lung samples had detectable fibroplasia, the case was considered chronic. In order to be considered BP, one or more of the samples had BP but with no other pneumonia features except for LIP or OTH. For a diagnosis of IP, one or more of the samples had exudative or proliferative IP but with no other

pneumonia patterns except for LIP or OTH. If one or more lung sections had BP and another one or more lung sections of the same case had exudative or proliferative IP, the diagnosis was BIP. If one or more lung sections had chronic LIP and no other pattern than OTH, they were diagnosed as LIP. Cases diagnosed as OTH could only have characteristics of OTH present. OTH was defined as edema, congestion, atelectasis, emphysema, or no lesions.

Analysis

A series of logistic regression models were constructed in Rstudio (version 4.5.0, Boston, MA, USA) to evaluate the probability of an AHS. Each independent variable of interest was first modeled in a univariate logistic regression using a logit link function. Type three likelihood ratio tests were performed using the `car::Anova()` function to provide p-values for each predictor¹⁴. Estimated marginal means with pairwise contrasts were calculated with the `emmeans` package to present results on the probability scale. Contingency tables were also generated to visualize raw counts of outcomes across predictor categories.

Independent variables included categorized covariates evaluating lung pathology (gross deflation, percent affected, and pneumonia diagnosis), cardiac lesions (fibrosis, necrosis, and embedded myocardial cysts, at the animal-level and sample-specific), hepatic lesions (fibrosis and necrosis), and pneumonia pattern scoring from each lung lobe (RCv, RCd, LCv, LCd). A separate binary logistic regression model, fitted using `glm()` with a binomial error distribution and logit link function, was then used to assess associations between AHS and pneumonia patterns with an interstitial component (AIP, CIP, CLIP) or lesion chronicity (acute/chronic). The IP component and lesion chronicity were fixed effects, and an interaction term between the two was included in the model.

Predictors were evaluated in univariate models and then placed into a final multivariate model if the p-value was ≤ 0.3 . As with the univariate models, the multivariate model was summarized using coefficient estimates, standard errors, Wald tests, and Type III likelihood ratio tests. Pairwise comparisons of predicted probabilities across factor levels were obtained using `emmeans`. Covariates included in the multivariate model included gross lung deflation, protozoal cysts, at the animal-level and sample-specific, and pneumonia pattern scoring from each lung lobe. Gross lung deflation and protozoal cysts were evaluated for each animal, but aliased coefficients and multicollinearity precluded analysis of protozoal cysts and pneumonia pattern scoring using multiple samples from the same animal. Predictors with a p-value of ≤ 0.05 were utilized in the final model including gross lung deflation and protozoal cyst categories for each animal. Co-linearity between gross lung deflation and the presence of protozoal cysts was assessed using a variance inflation factor (VIF).

Results

Out of 121 cases, 15 had missing or incomplete tissue sample sets, 1 had an incomplete animal ID, 1 had a missing lung deflation score and lung percent affected score, and 1 was diagnosed histologically as infectious pericarditis; these were excluded, leaving a total of 103 cases for further evaluation. Forty cases had NHS and 63 had an AHS (Table 2). Sixty-four cases had normal lung deflation scores, while 39 were abnormal. Of the 39 cattle with abnormal lung deflation scores, 33 had $\leq 5\%$ gross lung lesions. Out of the total 103 cases, 8 had significant cardiac fibrosis in at least one heart section, and only 4 cases had significant necrosis in at least one of the heart sections (Table 3). Sixty-seven cases had embedded protozoal cysts within at

least one of the four cardiac sections. Of the 67 cases with embedded protozoal cysts, 57 had protozoal cysts in all 4 cardiac sections (Figure 3 and 4). Histopathology of the liver revealed 2 cases with abnormal scores for both fibrosis and necrosis, 1 case with an abnormal fibrosis score, and 1 case with an abnormal necrosis score. After final pneumonia diagnosis, there were 5 acute BP (ABP), 5 chronic BP (CBP), 12 acute IP (AIP), 2 chronic IP (CIP), 3 chronic BIP (CBIP), 75 chronic LIP (CLIP), and 1 OTH (Figure 1). Of the 39 cases with failure of lungs to deflate, histologic diagnosis included 7 AIP, 2 ABP, 3 CBIP, 4 CBP, 2 CIP, and 21 CLIP.

Independent variables with a p -value ≤ 0.3 that were included in the multivariate regression model were gross lung deflation, the presence of embedded protozoal cysts at the animal-level, and lung histopathology at the sample level. The lung histopathology had aliased coefficients determining perfect multicollinearity between two or more of the lung samples. Therefore, lung histopathology was removed from the multivariate model.

The final multivariate model included gross lung deflation and protozoal cysts at the animal-level with an outcome of AHS. Cattle with lungs that failed to deflate had a higher probability of an AHS (0.76 ± 0.07 , $p \leq 0.01$) than cattle with normally deflated lungs (0.52 ± 0.06). Cattle with cardiac embedded protozoal cysts at the animal-level were more likely to be associated with an AHS (0.73 ± 0.05 , $p \leq 0.1$) than cattle without protozoal cysts present (0.39 ± 0.08). The VIF for lung deflation and embedded protozoal cysts at the animal-level was 1.06 suggesting both factors contributed independently to the outcome.

Discussion

Cattle affected with pneumonia or which have experienced pneumonia previously often have increased and irreversible pulmonary fibrosis which is a normal physiologic or healing response to severe or persistent inflammation ¹⁵. Interstitial pulmonary fibrosis causes damage to the alveolar walls, decreased air exchange capacity, decreased lung distensibility, and pulmonary hypertension, leading to increased cardiac afterload and right sided cardiac hypertrophy ¹⁵. In this sample set, lungs with abnormal lung deflation scores had minimal gross lesions and a wide variety of subcategories of pneumonia histologically. This finding warrants further investigation but suggests that gross and histologic evaluation of pulmonary tissue do not provide adequate explanation for the proposed pathophysiologic pathway of cardiac remodeling due to microscopic or gross pulmonary changes.

Where disagreement between gross and histologic diagnosis of pneumonia patterns in cattle have been evaluated, histopathology is considered a more accurate methodology ¹⁶. Differences between the percent of lungs exhibiting pulmonary lesions and the number of cases diagnosed with CLIP at histologic evaluation are notable; CLIP lesions may not be readily observed grossly, however the increased inflammation in the pulmonary interstitium may affect the ability of lungs to collapse. Recent work has shown a prevalence of 12.6% CLIP in feedyard mortalities and concluded that based on the distinct lesions (especially from exudative or proliferative IP patterns), CLIP represents a different pathogenic process than IP ¹³. While 73% of cattle in this study were diagnosed with CLIP histologically, it is not a representative population of all feedyard cattle because the sample was first stratified by HS, and thus should not be directly compared to prevalence in feedyard cattle populations (Table S1).

A positive association between the presence of embedded protozoal cysts within the heart and AHS was noted in this study. These protozoan parasites are commonly observed in bovine hearts and are typically presumed to represent *Sarcocyst* spp. Often observed in the absence of significant inflammation, these cysts are typically reported as an incidental finding. The association between AHS and sarcocyst embedment in cardiac tissue warrants additional consideration of the effect of the parasite on cardiac function. Canids and felids are the most common definitive hosts for *Sarcocyst* spp, but some species transmitted by canids may be more pathogenic ¹⁷. Identified sarcocyst species of cattle include *S. cruzi*, *S. hominis*, *S. bovifelis*, *S. bovini*, *S. hirsuta*, and *S. heydornii*. Of these, *S. hominis* and *S. heydornii* have been shown to be zoonotic ^{18,19}. *S. cruzi* is the most common species found in cattle with a prevalence of 56.5% of carcasses at harvest followed by *S. hominis* at 21% ¹⁸. Species identification was not performed during this study.

Clinical sarcocystosis leads to anorexia, weight loss, abortion, hyperexcitability, and death ^{17,18}. Subclinical infection has been reported in up to 60% of harvested cattle; in this study 65% of carcasses had at least some protozoal cysts present ¹⁸. Sarcocystosis is only diagnosed by histopathology and is typically unrecognized clinically. A small subset of these cases can have more widespread disease, known as bovine eosinophilic myositis (BEM). These gross lesions appear as gray, white, or green foci that lead to condemnation at slaughter. Disseminated inflammatory lesions have been found in the heart, tongue, diaphragm, esophagus, and skeletal muscle ^{17,19}. Cases condemned for BEM were not documented in this study.

Cattle infected with *S. cruzi* are commonly noted to have perivascular mononuclear cell infiltration in various organs including the kidneys, liver, and lung ¹⁷. Increased perivascular mononuclear cell infiltration is hypothesized to lead to decreased blood-gas exchange, and possibly pulmonary hypertension or right sided cardiac hypertrophy ¹⁵. We did occasionally observe protozoa embedded within structures of the conduction system (Purkinje fibers, bundle branches) as well as protozoal-associated inflammation or necrosis. It is plausible that parasite-associated inflammation may contribute to cardiac injury and remodeling which may be a cause of AHS or even BoCHF in severe cases. However, a limitation of this study is that protozoal-associated inflammation was not quantified, so further research in this area may be warranted.

Cardiac fibrosis has been reported in bovine hearts and is usually considered either an early and more mild reactive fibroplasia, or a more severe and extensive chronic form known as replacement fibrosis ⁹. Reactive fibrosis can be seen as interstitial fibrosis, defined as a dissection of collagen between myocytes, and perivascular fibrosis noted specifically around interstitial and coronary blood vasculature. Replacement fibrosis is visualized in areas of cardiomyocyte loss after necrotic events and is considered chronic granulation or scar tissue, where fibroblasts replace degenerate and lost cardiomyocytes and become the majority cell type in the cardiac wall ^{4,9}. Rigidity in a structurally and functionally normal heart can be attributed to a majority of type 1 collagen. Loss of type 1 collagen due to injury leads to decreased tensile strength and subsequent ventricular dilation. Unlike cardiomyocytes, fibroblasts are capable of re-entering the cell-cycle which contributes to hyperplasia or fibrosis ²⁰. In humans, cardiac remodeling with fibrosis has been documented to lead to diastolic dysfunction, pulmonary hypertension, arrhythmias, and sudden death ⁹.

Table 3 describes the majority of cardiac fibrosis and necrosis scores were observed to be zero or have very mild changes. Cardiac fibrosis has been previously studied in cattle with clinical signs of BoCHF. Krafur et al described feedyard mortalities of cattle with clinical signs of BoCHF noted to have significant perivascular, interstitial, and replacement fibrosis present; 2 case control cattle out of 20 also had notable interstitial fibrosis and mild perivascular and replacement fibrosis ⁴. In a separate study, Krafur et al noted cattle symptomatic for BoCHF had severely atrophied hepatic cords, centrilobular necrosis, and centrilobular fibrosis ²¹. Additional work is warranted to understand the relationship between cardiac fibrosis and necrosis, cardiac function, and cardiac ventricular wall remodeling.

Conclusions

In this study we observed a significant positive association between abnormal gross lung deflation and AHS; however, we were unable to document specific histologic lesions in the lung, heart, or liver consistently associated with AHS, or specific subcategories of pulmonary lesions including BP, BIP, IP, or CLIP. An association with cardiac and hepatic fibrosis or necrosis and AHS was not demonstrated. Presence of embedded myocardial protozoal cysts was positively associated with AHS.

Figures

Figure 2.1: Visual Heart Scores Pictured 1-5.

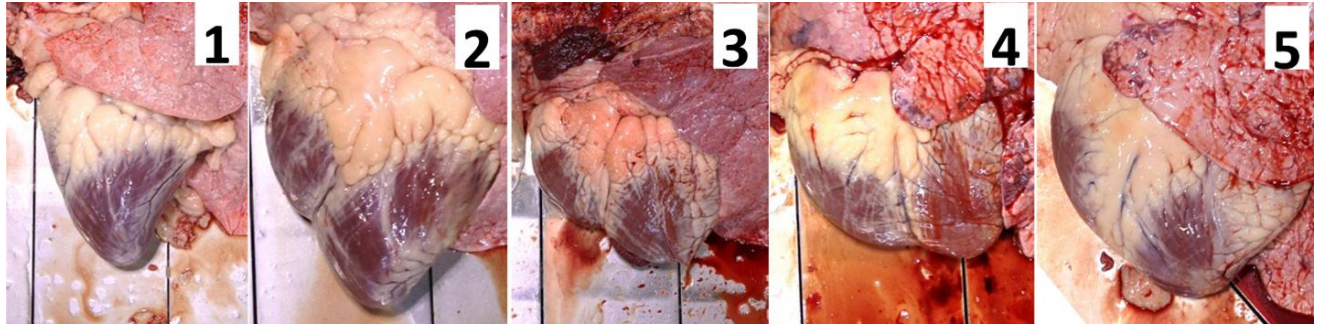


Figure 1: Pictured above starting on the left side is a heart score (HS) 1. The right ventricle can be seen on the left side of the image and is tight with no rounding and the heart comes to a V-shaped apex. Moving to the right, HS 2 and 3 have increasingly enlarged right ventricles seen by the rounding on the left side of the image. A HS 4 has a characteristic W-shape. The picture on the right side is a HS 5 with severe cardiac changes, loss of a tight right ventricle seen by rounding and loss of the apex resulting in a U-shape.

Figure 2.2: Lung deflation examples

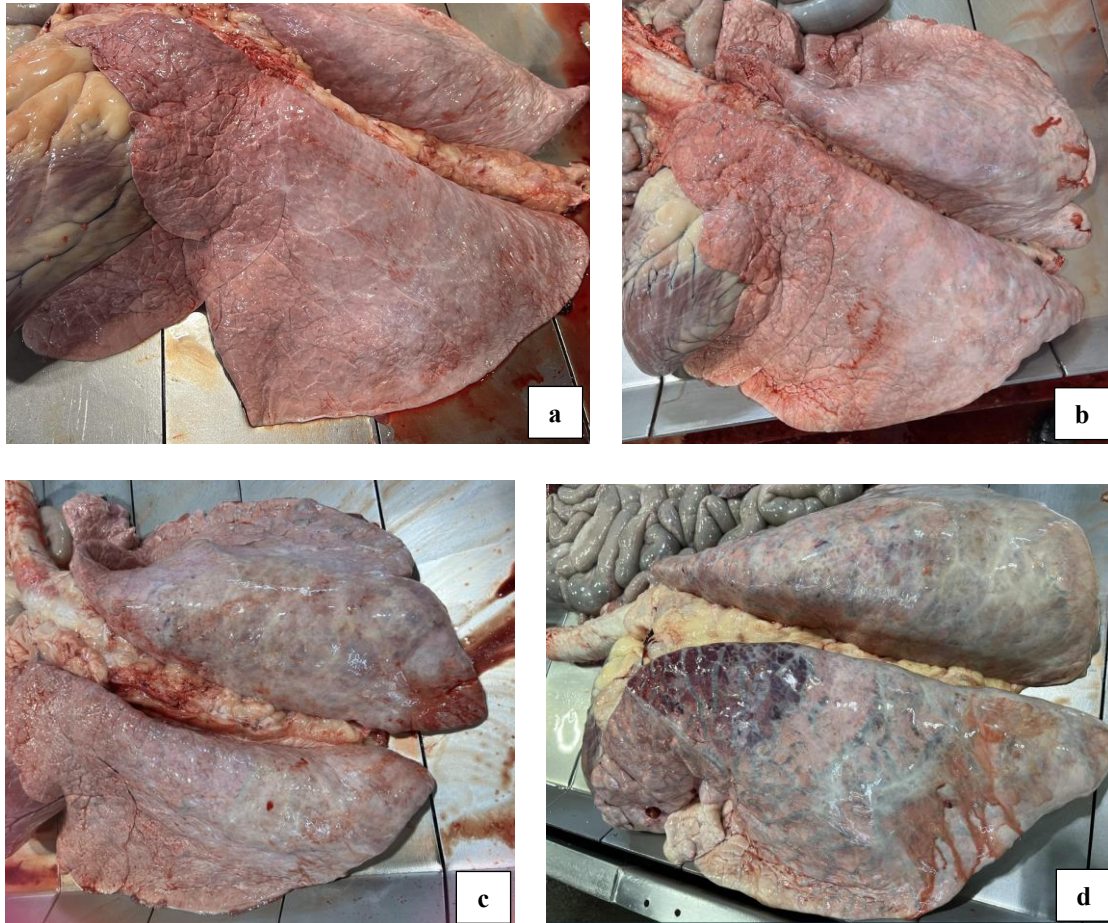


Figure 2: a: Normal deflated lungs; b: mild failure to deflate; c: moderate failure to deflate; d: severe failure to deflate. The margins of the normal lungs are defined, sharp, and they are completely collapsed with no turgidity or stiffness of the tissue. With increased severity, the margins of the lungs are lost and become rounded. Increased severity is determined by increased aeration and turgidity of the lung tissue.

Figure 2.3: Venn Diagram of cattle with embedded myocardial protozoal cysts in cardiac samples

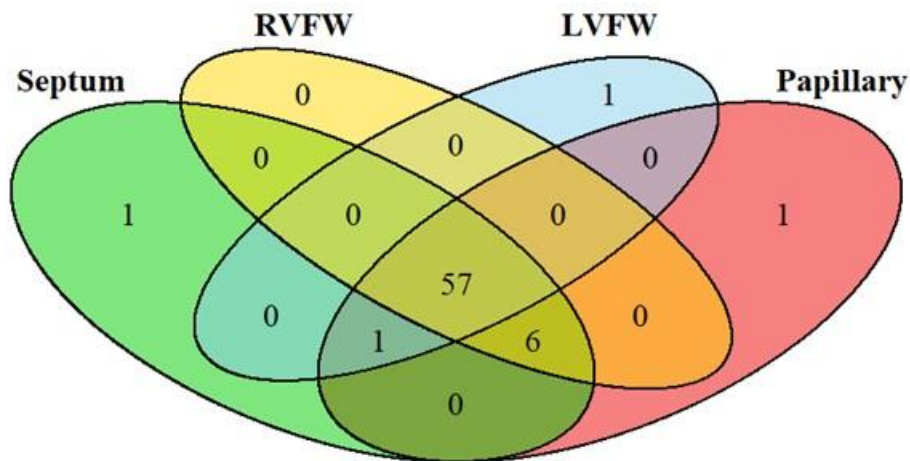


Figure 3: Four heart sections were collected and reviewed histologically including the septum, right ventricular free wall (RVFW), left ventricular free wall (LVFW), and papillary muscle. Each sample was given a score for embedded protozoal cysts defined as rare (0-1 cysts per tissue section), few (2-10 cysts per tissue section), moderate (>10 cysts per tissue section), or severe (>50 cysts per tissue section). These were then categorized into rare or present (few, moderate, or severe). Most animals with sarcocysts involved all four sections of the heart.

Figure 2.4: Cardiac Histopathology

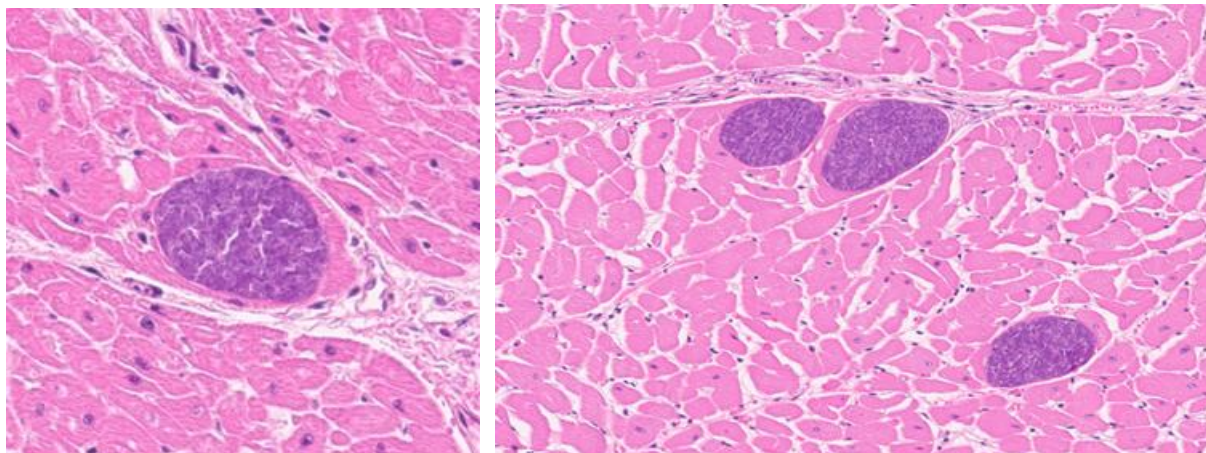


Figure 4: Embedded myocardial protozoal cysts on H&E stain.

Tables

Table 2.1[†]: Histologic pneumonia pattern scores assigned to each lung sample

Pattern	Major Lung Patterns	Type	Age	Pneumonia
Score				Type
1	Acute exudative interstitial pneumonia (hyaline membranes, septal necrosis)	IP	Acute	AIP
2	Subacute proliferative interstitial pneumonia	IP	Acute	AIP
3	Chronic fibrosing interstitial pneumonia (may include bronchiolitis obliterans)	IP	Chronic	CIP
4	Chronic lymphoplasmacytic interstitial pneumonia	LIP	Chronic	CLIP
5	Acute fibrinosuppurative bronchopneumonia	BP	Acute	ABP
6	Subacute proliferative and histiocytic bronchopneumonia	BP	Acute	ABP
7	Chronic bronchopneumonia with fibrosis (may include bronchiolitis obliterans)	BP	Chronic	CBP
8	Bronchiectasis and bronchiolectasis (caseonecrotic bronchopneumonia)	BP	Chronic	CBP
9	Fibrinous pleuritis and pleuropneumonia	BP	Acute	ABP
10	No lesions/Edema/Congestion/Atelectasis/Emphysema	OTH		OTH

Four lung sections (right and left cranioventral and right and left caudodorsal lobes) were submitted for histopathologic evaluation. Each section was given a pattern score based on the major lung lesions present.

†Modified from Feitoza, L. F. B. B. *et al.* Determining chronicity and frequency of pulmonary histopathology lesions in feedyard cattle mortalities (in press).

Table 2.2: Descriptive statistics of gross pathological lesions by heart score

Heart Score:	HS1	HS2	HS3	HS4	HS5
Number of Cattle:	21	19	23	25	15
Gross Lung Deflation:					
Normal (deflated)	20	11	16	11	6
Abnormal (mild, moderate, or severe failure to deflate)	1	8	7	14	9
Percent Lung Affected with lesions:					
Normal (<5% lesions)	21	17	22	22	14
Abnormal (≥5% lesions)	0	2	1	3	1

Table 2.3: Number of cases for fibrosis score by sample location

Fibrosis Score	Septum	Papillary Muscle	Right Ventricular Free Wall	Left Ventricular Free Wall	Liver
0	49	44	57	64	78
1	52	55	44	39	18
2	2	4	2	0	3
3	0	0	0	0	1
NA	0	0	0	0	3

Table 2.3: This table shows the number of cases that had fibrosis in each of the three scores for each heart and liver section before they were dichotomized into normal(0,1) and abnormal(2,3).

Table 2.4: Number of cases for necrosis score by sample location

Necrosis Score	Septum	Papillary Muscle	Right Ventricular Free Wall	Left Ventricular Free Wall	Liver
0	55	49	49	56	73
1	47	53	53	46	23
2	1	1	1	1	4
3	0	0	0	0	0
NA	0	0	0	0	3

Table 2.4: This table shows the number of cases that had necrosis in each of the three scores for each heart and liver section before they were dichotomized into normal(0,1) and abnormal(2,3).

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Chapter 3 - Association of embedded myocardial protozoal cysts with visual heart score in feedyard cattle mortalities

Abstract

Sarcocystis is a common cattle cardiac protozoan parasite. However, little research in the United States has evaluated sarcocystis prevalence or potential relationships with cardiac health. The study objective was to evaluate potential associations between gross heart score (HS) and the presence of embedded myocardial protozoal cysts (EMPC) within a population of cattle feedyard mortalities. The study included 121 cattle feedyard mortalities that underwent full, systemic necropsy. A HS from 0 to 5 was assigned to each calf. A HS 0 was a heart with infectious disease, HS 1 represented a normal heart, and HS 5 represented severe cardiac remodeling. Heart scores were categorized into normal HS (NHS) (HS 1 or 2), abnormal HS (AHS) (HS 3, 4, or 5), and infected (HS 0). Cardiac papillary muscle samples were collected for histologic evaluation. The number of EMPC were counted and area of each sample measured. The EMPC over the sample area was calculated for an embedded cardiac protozoal cyst ratio (EMPCR). After case removals, 106 cattle were included in the analysis and heart scores were distributed as 60 NHS, 34 AHS, and 12 infected with a subset of 15 cases defined as congestive heart failure (CHF). An EMPC was identified in 96/106 (90.5%) cases and EMPCR was right skewed with a median of 0.08 and a mean ratio of 0.13. After statistical analysis, no associations were found among EMPCR and HS or CHF status. This study identified EMPC was common in feedyard mortalities, but no relationships with heart score or CHF status were identified.

Keywords: congestive heart failure, sarcocyst, protozoal cyst, feedyard mortality

Introduction

Sarcocysts are the most common protozoan parasite found in the cardiac muscle of cattle, but this parasite has historically been diagnosed as an incidental finding at slaughter. Sarcocysts have an affinity for striated muscles specifically found in the heart, but sarcocystis can disseminate to the diaphragm, esophagus, tongue, and skeletal muscle. A two-host life cycle is required; canines are the most common intermediate host for sarcocyst species infective to cattle, but felids and primates are also intermediate hosts for species of sarcocysts found in bovids.¹ Sarcocysts found in cattle include *S. cruzi*, *S. hirsuta*, *S. bovifelis*, *S. bovini*, *S. rommeli*, and two zoonotic species *S. hominis* and *S. heydorni*.^{1,2} Currently very little research has been conducted on the prevalence of sarcocysts within the United States let alone the implication they may have on cardiac health in cattle.

A recent study evaluated heart and lung histology of cattle at slaughter and found a significant association between an abnormal heart score and the presence of embedded myocardial protozoal cysts (EMPC).³ Since sarcocysts are the most common cardiac parasite seen in cattle, the EMPC were presumed to be sarcocystis. While the work of Jensen, et.al noted an association between AHS and EMPC, the study population did not allow evaluation of EMPC with a gross diagnosis of congestive heart failure (CHF), as the cardiac samples were collected at slaughter.³ Additional work may be helpful to more clearly define a potential relationship between EMPC and cardiac changes.

The objective of this study was to further evaluate potential associations between HS and the presence of EMPC within a population of feedyard cattle mortalities. This study identified two main objectives. The first objective was to determine the association between AHS and embedded myocardial protozoal cyst ratio (EMPCR), and the second objective was to determine the association between CHF and EMPER.

Materials and Methods

Data Collection

A cross-sectional observational study evaluated feedyard cattle mortalities from six High Plains feedyards with necropsies conducted between June 1, 2022 and July 29, 2022, and June 1, 2023 and July 28, 2023. Additional work from this study has been previously reported.⁴⁻⁷ The study was exempt from the Institutional Animal Care and Use Committee, as all data and sample collection occurred after naturally occurring deaths or humane euthanasia deemed necessary by feedyard personnel.

Field necropsies were performed within 18 hours of death by two teams of trained research technicians. Post-mortem evaluation included assessment of cardiopulmonary, gastrointestinal, musculoskeletal, urinary, and reproductive systems. Necropsies were conducted similar to previous literature with examination of the peritoneum, pleural space, and pericardial sac for effusion; hearts were excised above the atria and removed for further evaluation.⁴⁻⁷ After the heart was removed from the pericardium, the myocardium was examined for petechia/ecchymosis, and a cut was made into the ventricles to evaluate the endocardium, valves, and to take samples from the papillary muscle. The central nervous system was not evaluated due

to rendering protocols and to maintain safety of necropsy technicians. Each case was documented, photographed, and any abnormal gross lesions of the examined anatomic systems were noted. Final case diagnoses were confirmed by a veterinarian [BJW]. After necropsy, respective feedyards provided demographics of cattle including sex, arrival weight, and days on feed (DOF).

Hearts were scored post-hoc, using photographs, by a veterinarian (BJW) based on a modified Colorado State University system with heart score (HS) ranging from 0=infectious heart disease (e.g Pericarditis), 1=normal, 2=mild right ventricular dilation, 3=moderate right ventricular dilation, 4=severe right ventricular dilation, and 5= severe right ventricular dilation with a flaccid appearance (Figure 1). Cardiac tissue samples were collected from the papillary muscle for cases with moderate to severe cardiomegaly (HS 3, 4, or 5), and control samples were collected from cases with HS 1 or 2.

Samples were fixed in 40ml of 10% neutral buffered formalin. Once samples arrived at the Kansas State Veterinary Diagnostic Laboratory, they underwent initial processing, serial dehydration, were embedded in paraffin, cut into a 4 μ m slice, and stained using the Leica ST5010 Autostainer XL (Leica Biosystems, Deer Park, IL, USA) with hematoxylin and eosin. Samples were digitalized onto Aperio e-slide manager (Leica Biosystems, Deer Park, IL, USA). A board-certified veterinary pathologist (BLP) scored cardiac samples for autolysis. Autolysis was defined as 0 (no autolysis), 1 (mild autolysis, increased scattered bacteria), 2 (moderate autolysis, many bacteria, loss of nuclear structure), or 3 (marked autolysis, myriad bacteria, cell

morphology difficult or impossible to discern). Cardiac tissue samples with an autolysis score of 3 were excluded from the study.

A veterinarian blinded to HS (MJJ) evaluated all digital samples from 2022 and 2023 for EMPC count using Aperio eSlide Manager (12.5.1.5997) by Leica Biosystems (Figure 2). Slides were evaluated at 4x magnification counting all cysts on a sample from left to right and from the top of the sample to the bottom. Increased magnification was used when necessary for cyst identification. Samples were then uploaded into Aperio ImageScope (12.4.6.7001) by Leica Biosystems. The pen tool was used to outline the perimeter of each sample, and the annotation function measured the area of each cardiac sample in μm^2 . Area was then converted from μm^2 to mm^2 . A total of 24 cases had the cardiac sample sliced twice and placed on the same slide. The left-most slice was read and measured for this study, as the slices were from the same cardiac sample and had nearly the same shape and architecture. Exclusion criteria for this study consisted of cardiac samples with an autolysis score of 3 and any cattle with missing necropsy records.

Data Categorization

Data for DOF was categorized into $\leq 50\text{d}$, 51-100d, 101-150d, and $>150\text{d}$. Arrival weight was converted from pounds to kilograms then categorized into $<272\text{kg}$, 272-317kg, 318-362kg, and $>363\text{kg}$. Heart scores were categorized as either NHS (HS 1 or 2), AHS (HS 3, 4, or 5), or infected (HS 0). The EMPCR was calculated using the EMPC as the numerator and the sample area (mm^2) as the denominator. In addition to an EMPCR, a scoring system similar to Jensen et.al was used grouping EMPC into categories of rare (0-1 cysts per sample), few (2-10 cysts per

sample), moderate (11-49 cysts per sample), and severe (≥ 50 cysts per sample).³ These categories were then dichotomized into rare and present (few, moderate, and severe).

Analysis

A series of univariate linear mixed effects models were constructed in Rstudio (version 4.5.0, Boston, MA, USA) to evaluate potential associations of descriptive factors (sex, DOF, and arrival weight) with the outcome of EMPCR. The `car::Anova` function was used to produce p-values for each independent variable tested with Type III likelihood ratio tests.⁸ An `emmeans` package was used to calculate estimated marginal means with pairwise contrasts for results on a probability scale. Each model, including the univariate and final multivariate models, had a random effect of feedyard nested within year to account for a lack of independence due to the sampling strategy. Variables in univariate models with a p-value ≤ 0.3 were retained for evaluation in the final multivariate model.

Multivariate models were built similarly to the univariate models for both of the study objectives with `car::Anova` and Type III likelihood ratio tests.⁸ The model for the first objective evaluated potential relationships among EMPCR and covariates of HS category (AHS, NHS, infected), sex, DOF category (≤ 50 d, 51-100d, 101-150d, and > 150 d), and arrival weight category (< 272 kg, 272-317kg, 318-362kg, and > 363 kg). The second model evaluated EMPCR as the outcome and tested potential relationships with CHF status, sex, DOF category, and arrival weight category. Due to the skewed distribution of the EMPCR, data were log transformed using the `log` function for each model. After log transformation of the data, residuals were evaluated using `qqnorm` to ensure proper data fit. Third and fourth logistic regression models were run using `glmer` evaluating a dichotomized outcome of EMPC (rare or present) with covariates of either HS

category (NHS/AHS) or CHF status while including sex, DOF category, and arrival weight category in all models. The model building process was the same for all models with a backwards selection approach removing the variable with the highest p-value in an iterative fashion until final models contained only variables with a significant ($P < 0.05$) association with the outcome of interest.

Results

A total of 121 cases from 2022 and 2023 had available records from gross necropsy and histopathology. Out of these, 14 had an autolysis score of 3 and were removed and 1 case was unable to be tied back to necropsy records due to a labeling error. The final data set consisted of 106 cases. There were a total of 73 heifers and 33 steers.

A total of 12 cases had a HS 0, 42 cases had HS 1, 18 cases had HS 2, 19 cases had HS 3, 6 cases had HS 4, and 9 had HS 5. There were 60 NHS, 34 AHS, and 12 cattle in the infected category.

A total of 15 cases out of the 106 had CHF. Within this study, 96 out of 106 cattle had EMPC present or a 90.5% prevalence. A distribution was plotted for EMPCR with a median of 0.08 and a mean ratio of 0.13 protozoal cysts per millimeter of sample (Figure 3).

Two individual models were constructed with EMPCR as the outcome. The first model evaluated EMPCR and HS category as the independent variable of interest, but no significant ($P > 0.05$) associations between any covariates and EMPCR were identified. The second model evaluated potential associations between covariates including CHF status and EMPCR and no significant ($P > 0.05$) relationships were identified. The third and fourth models evaluated potential relationships of EMPC category (rare/present) with HS category (model 3) and CHF status

(model 4) while including the same covariate structure. Neither model found significant ($P < 0.05$) associations between EMPC presence and any of the evaluated covariates.

Discussion

The United States has one paper reporting prevalence of sarcocystis infection of beef cattle. The current study had a significantly higher EMPC prevalence of 90.5% compared to previous work (62%).⁹ The results illustrate EMPC is common in this dataset of feedyard cattle mortalities; however, no work has been published describing the scale of infection as defined by EMPCR. The distribution of EMPCR was highly skewed (Figure 3) illustrating that while many cattle were infected, low EMPC count density were identified in most cases. A small number of cases were identified with higher EMPCR which was used in the evaluation to determine potential associations with cardiac variables of interest (AHS and CHF status).

No associations were identified between EMPCR or the presence of EMPC with HS category or CHF status. This is contradictory to research by Jensen et.al which found an association between HS category and the presence of EMPC in cattle at slaughter.³ A key difference between the projects is the population sampled: the previous work used cattle from a slaughter facility in the Pacific northwest while the current research study used feedyard cattle mortalities in the High plains region.³ Both studies looked at cysts dichotomized into the same categories, and the current study took it one step further to look at a ratio of protozoal cysts per area of cardiac sample. This is a strength, as it increases statistical power looking at a protozoal cysts/area ratio instead of a dichotomized protozoal cyst presence classification.

While sarcocysts are the most common cardiac protozoa of ruminants, little research has been conducted on prevalence in North America. Sarcocystosis is generally considered to be a subclinical myopathy meaning cysts are present without inflammation or clinical signs present. However, cases of inflammation associated with these protozoa have been documented as Bovine Eosinophilic Myositis (BEM).^{11,12} Bovine eosinophilic myositis has been described at slaughter as gray to green lesions on the heart and can lead to condemnation of a carcass or parts of it.^{1,13} A paper by Reiten et. al looked at 100 cattle slaughtered in Greeley, CO, but the study population was selected for only cattle BEM, which is a subset of sarcocystis cases.¹⁰ Therefore, this study is not representative of sarcocystis prevalence.

While this study did not find any significant associations between EMPCR and HS category or CHF status, it did have a few limitations. The first limitation was that EMPC were presumed to be sarcocystis due to sarcocysts being the most common cardiac parasite of ruminants, but confirmation of sarcocystis requires molecular testing which was not pursued. Another limitation was that EMPCR has never been described before, and therefore the study was unable to compare this finding to previous literature. The third limitation was that very little literature on sarcocystis exists within the United States or North America. Sarcocysts have been presumed incidental, but there is no literature supporting this claim. The current study also had a small sample population, so while no associations were found, it may be that the power was too small to find any associations if they do exist.

Conclusions

This study did not observe any significant associations of heart score category or congestive heart failure with EMPCR. The next steps in this research may be to include a larger study population for increased power. Further research is warranted to determine if there are associations between parasitic infestation and cardiac health in beef cattle.

Figures

Figure 3.1: Examples of gross heart scores 0 to 5 from feedyard cattle mortalities.

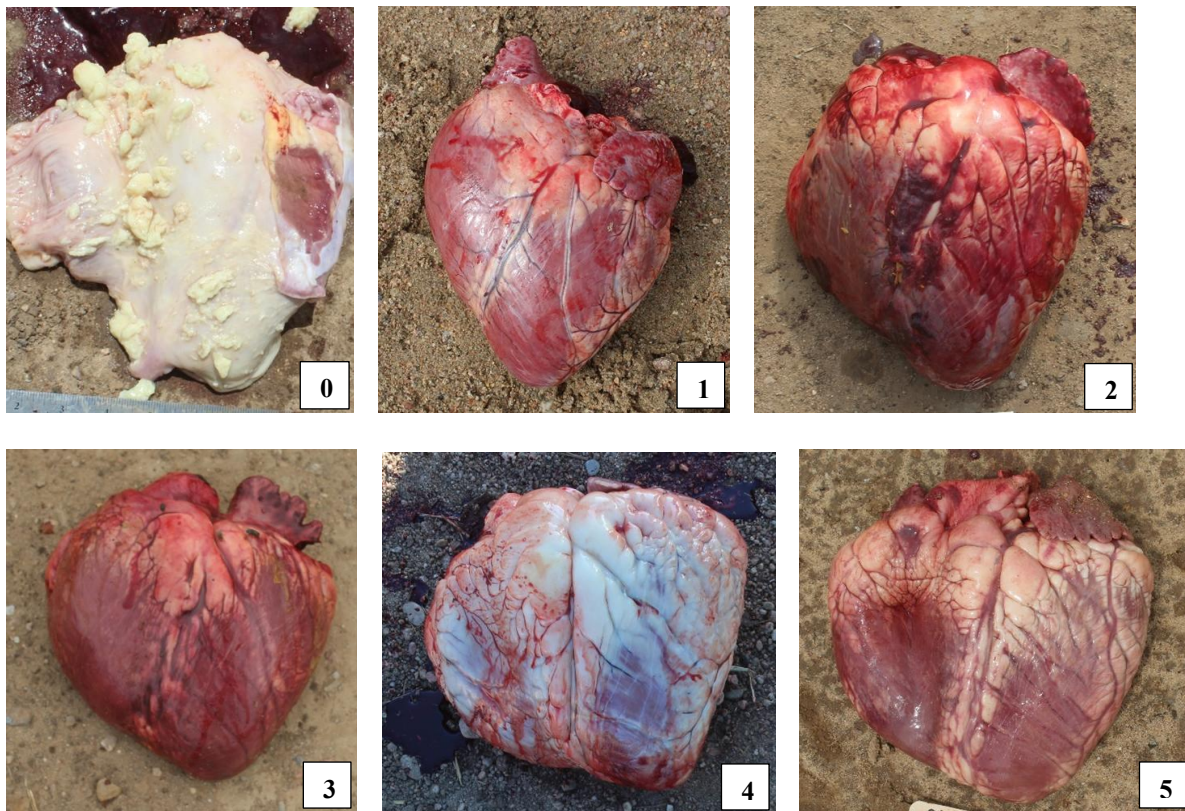


Figure 3.1: Pictured above starting on the top left side is a heart score (HS) 0. The pericardium is fused to the myocardium through adhesions. The top middle image is a HS 1. The right ventricle is on the left side of the image, and the heart comes to a V-shaped apex with no misshapen ventricles. Moving to the top right and bottom left, HS 2 and 3 have rounding and enlargement of the right ventricles and loss of the sharp apex with HS 4 having a W-

shape. On the far bottom right, is a HS 5 with severe cardiac changes and a flaccid right ventricle; this is characterized by a U-shaped apex.

Figure 3.2: Example of embedded myocardial protozoal cysts on H&E stain

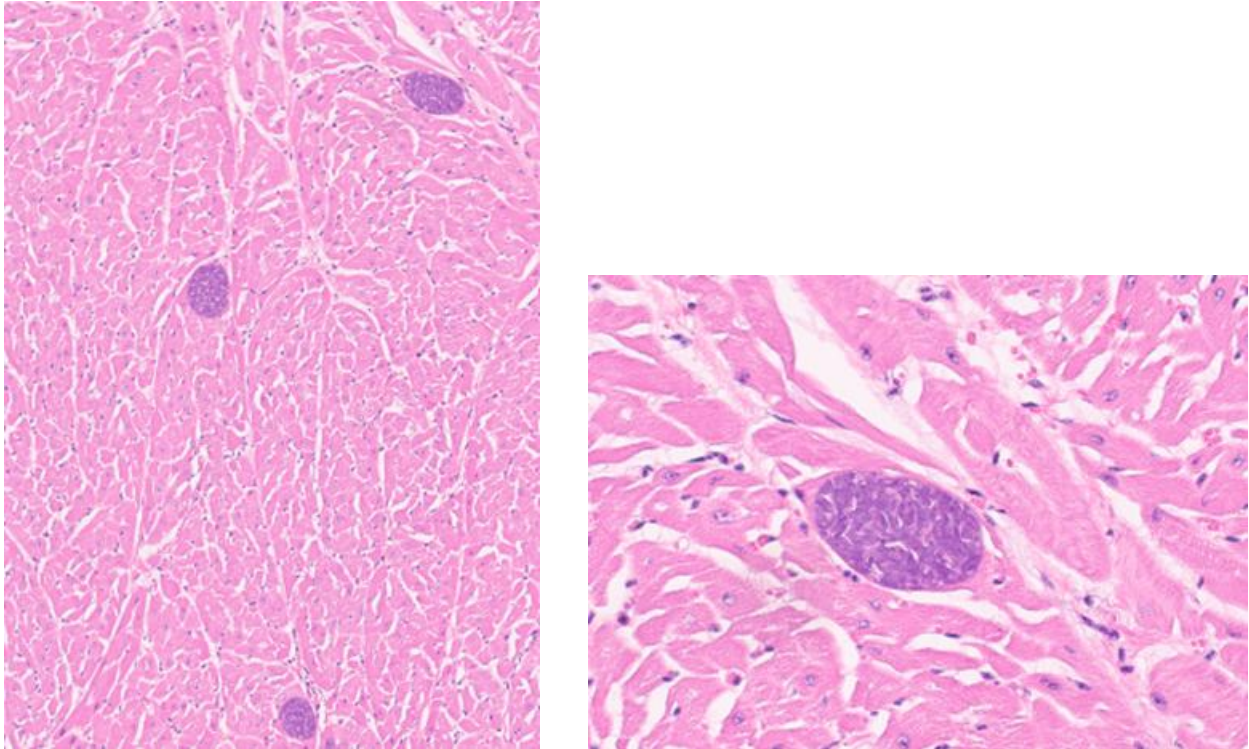


Figure 3.2: The photo on the left shows H&E stained cardiac tissue at 4x magnification which was used for counting protozoa. The photo on the right is an embedded protozoal cyst at 8x magnification from the same tissue.

Figure 3.3: Distribution of Embedded Myocardial Protozoal Cysts per animal

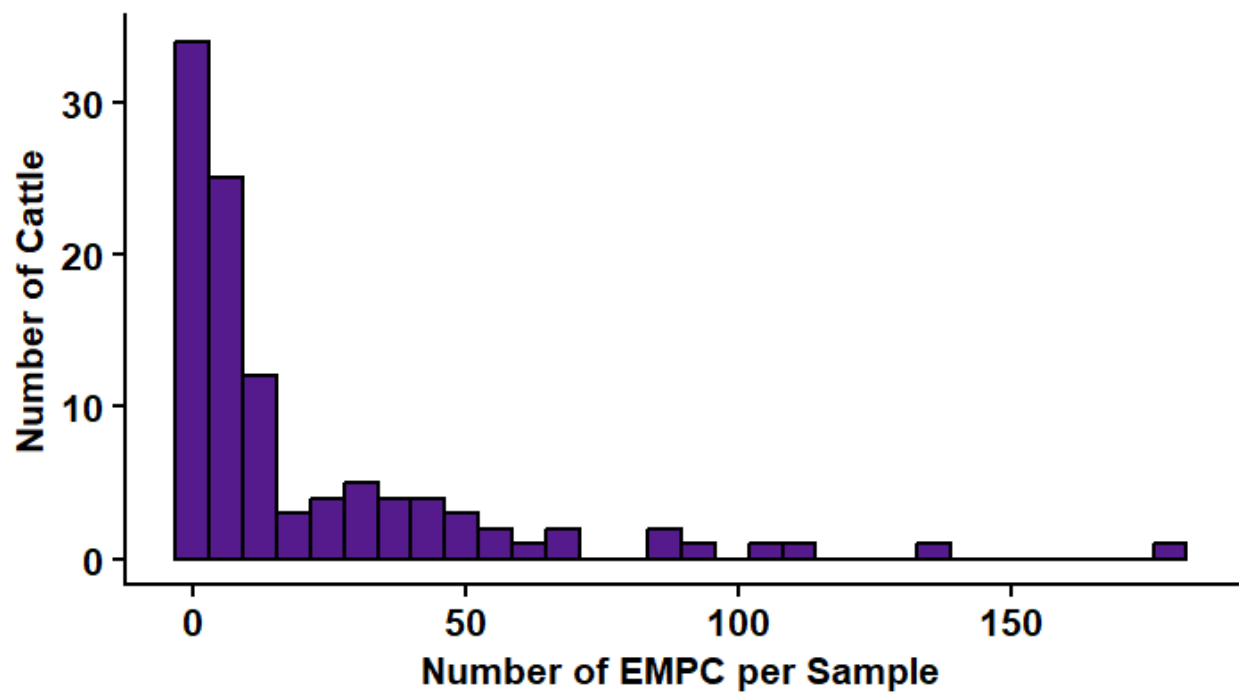


Figure 3.3. The distribution of the number of embedded myocardial protozoal cysts per individual animal.

Figure 3.4: Distribution of Embedded Myocardial Protozoal Cysts per cardiac sample area

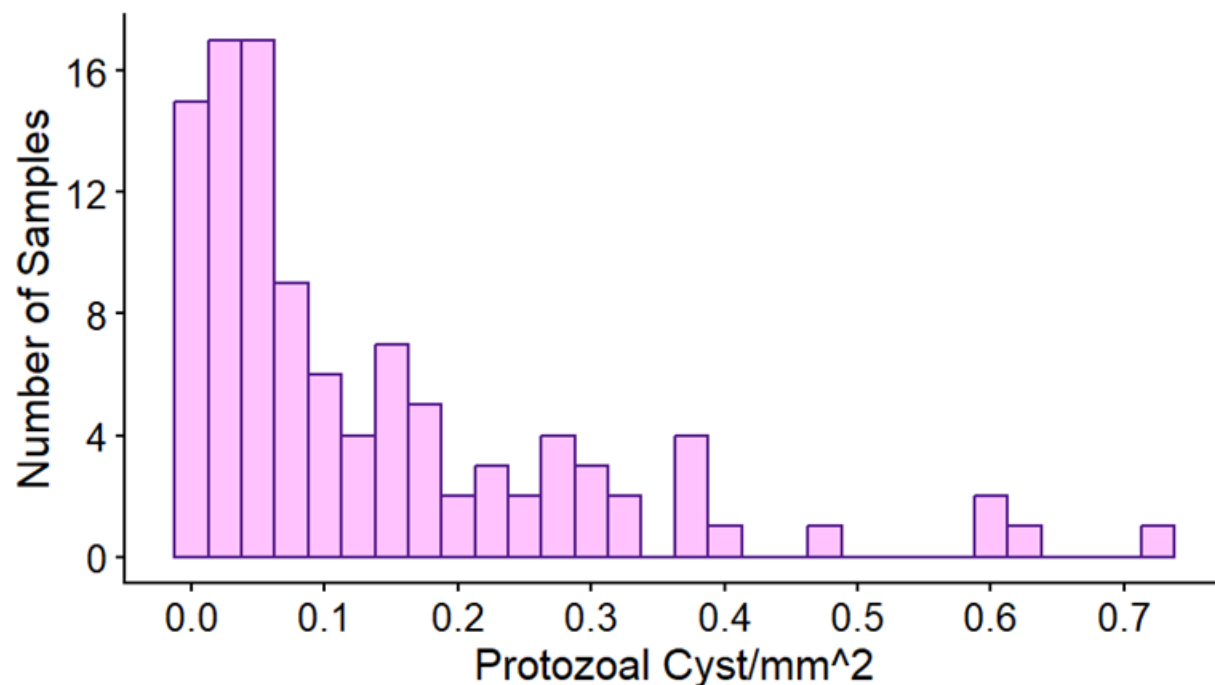


Figure 3.4. The number of samples over the distribution of embedded myocardial protozoal cysts per millimeter of cardiac tissue.

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Conclusions

Heart disease continues to be at the forefront of beef cattle research with little forward momentum. Ongoing research hypothesizes that irreversible pulmonary fibrosis or lung damage can lead to pulmonary hypertension and secondary cardiac remodeling in cattle. However, our research found no associations between cardiac and pulmonary histologic lesions. There was a significant association between the gross failure of lungs to deflate and enlarged hearts, but further investigation is warranted for the pathophysiologic pathway of cardiac remodeling due to microscopic or gross pulmonary changes.

Presence of embedded myocardial protozoal cysts (EMPC) was positively associated with abnormal heart score in a study with a population of cattle at harvest, but it was not associated in a population of feedyard cattle mortalities. Continued studies with larger sample populations may be required to see if the disparity between the populations was due to a lack of power.

Upon a positive association of EMPC and abnormal heart scores in harvested cattle, a literature review was conducted evaluating the prevalence of sarcocystis infection in cattle over different regions. Overall, the prevalence across six different continents was high. However, sarcocystis infection of cattle cannot be ruled out as incidental without further evaluation of the pathophysiology of the inflammatory pathway cardiac parasites may induce. While much more research is required in this field of study, these data highlight avenues of research that have not yet been explored or established in feedyard cattle.

Supplemental Chapter 2 Material

Heart Score:	HS1	HS2	HS3	HS4	HS5
Pneumonia Patterns:					
CLIP Diagnosis	19	16	20	13	7
ABP Diagnosis	2	0	2	0	1
CBP Diagnosis	0	0	0	3	2
AIP Diagnosis	0	1	1	7	3
CIP Diagnosis	0	2	0	0	0
CBIP Diagnosis	0	0	0	1	2
OTH Diagnosis	0	0	0	1	0
Cardiac Fibrosis:					
Normal (0, or 1)	21	18	22	22	14
Abnormal (2 or 3)	0	1	1	3	1
Cardiac Necrosis:					
Normal (0, or 1)	21	19	23	25	12
Abnormal (2 or 3)	0	0	0	0	3
Liver Fibrosis:					
Normal (0, or 1)	21	18	23	25	12
Abnormal (2 or 3)	0	1	0	0	3
Liver Necrosis:					
Normal (0, or 1)	21	17	22	25	14
Abnormal (2 or 3)	0	2	1	0	1
Protozoal Cysts:					
Rare (none or rare)	13	9	7	5	2
Present (few, moderate, or severe)	8	10	16	20	13

