Evaluation of cardiac troponin I as a predictor of clinical outcomes in cattle treated for bovine respiratory disease (BRD) in commercial feedyards

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Abstract

Bovine respiratory disease (BRD) is a major cause of morbidity and mortality in feedlot cattle and prognostic tools would be valuable for managing individual BRD cases. Cardiac troponin I (cTnI) is a protein released into the circulatory system after myocardial damage that may serve as a prognostic biomarker. The study objective was to determine potential associations between cTnI serum concentration of cattle at initial BRD treatment with the risk of retreatment or mortality within 60 days.

In this observational study, serum was collected from cattle in 2 central high plains feedyards at initial BRD treatment. Individual animal treatment outcomes were determined (treatment success, retreated, died, or culled due to BRD) at 60 days post-enrollment. Troponin concentrations were determined using a commercially available Bovine Cardiac Troponin ELISA assay and categorized as LOW (< 0.156 ng/mL) or HIGH (≥ 0.156 ng/mL).

Of 318 tested serum samples, troponin concentrations were LOW for 310 and HIGH for 8 samples. Cattle with HIGH cTnI concentrations had a significantly ($P < 0.05$) greater probability of not finishing the 60-day post enrollment period (0.56 +/- 0.22 SE) compared to cattle with LOW cTnI (0.11 +/- 0.03 SE).

Cattle with high cardiac troponin concentrations at initial BRD treatment were more likely to have negative clinical outcomes. However, a low number of cases were identified as having high cTnI.

High troponin concentrations may be valuable to improve management decisions; however, further research is necessary to optimize cut-off values predictive of clinical outcome and evaluate prognostic value in select subpopulations of feedlot cattle.

Key words: feedlot, cattle, cardiac troponin, bovine respiratory disease, prognostic

Introduction

Bovine respiratory disease (BRD) remains the major cause of morbidity and mortality in feedlot cattle, with many of these animals receiving antimicrobial therapy.[1] Cattle with BRD may show clinical symptoms of depression, lethargy, increased respiratory rate, increased nasal discharge and inappetence.[2] However, these non-specific clinical signs are often seen in animals with other disease syndromes, making accurate diagnosis and prognostication of suspected BRD cases challenging.

Cardiac troponins are proteins that are responsible for the regulation of contractile cardiac muscle tissue.[3] Cardiac troponin I (cTnI) is expressed in the myocardium and is released into circulation when the cardiac myocytes sustain damage.[4] A study in humans showed that increased cTnI concentrations can be induced by nonprimary cardiac disease, such as acute or chronic systemic disorders.[5] A review paper, published in 2024, found that human cTn1 concentrations can be elevated due to pulmonary, renal, neurologic, musculoskeletal, oncologic and gastrointestinal diseases, as well as acute illness and trauma.[6] Another study from the human medical literature demonstrated that detectable cTnI concentrations (1.0 gram per milliliter or higher) were associated with increased adverse events and mortality rate proportional to the amount of cTnI released.[7] Many studies have detected high cTnI concentrations in cattle with varying diseases, such as pericarditis, foot and mouth disease, LDA, dystocia and downer cow syndrome.[8-18] In dairy cattle, elevated cTnI concentrations with non-cardiac diseases (metritis, mastitis, pneumonia, peritonitis, LDA, dystocia and cesarean section, and downer cow syndrome) were prognostic for negative outcomes (death or culling).[17]

Cardiac troponins may serve as a potential biomarker to determine the extent of cardiac damage in BRD cases.[19] A previous study demonstrated that cattle have a higher risk of congestive heart failure when they have been treated for BRD multiple times compared to cattle never treated.[20] The hypothesis of the current study is that feedyard cattle presenting with clinical signs of BRD, but with significant cardiac damage (either primary from congestive heart failure or secondary to severe BRD), will be at increased risk for culling or mortality. The objective of this study was to evaluate the association between cTnI serum concentration and risk of retreatment or BRD-specific mortality and culling in individual cattle in a 60-day post enrollment period following first BRD treatment. If cTnI concentration at the time of first BRD treatment is associated with clinical outcome, this prognostic method could aid producers with early culling decisions, which would mitigate economic losses and reduce antimicrobial use in animals.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (IACUC-4846-AS&I). This study was performed at commercial feedyards, and all appropriate animal care and housing were provided by the feedyards and personnel.

This study was conducted in 2 U.S. central High Plains commercial feedyards in June and July 2023. Study enrollments were conducted at the individual animal level with no restrictions based on sex or days on feed. Any animal that met the location-specific criteria for BRD treatment was eligible for study inclusion at the time of first BRD treatment only. At the time of enrollment, approximately 5 mL of blood was collected from the coccygeal vein into a 10 mL preservative free blood collection tube[a]. All samples were kept on ice during the daily collection period. After daily collection was complete, all blood samples were centrifuged (2000 × g for 10 minutes) at the study sites, and serum was refrigerated during transport. Serum samples were frozen at -4 °F until cTnI concentration determination. Animal identification and demographics (weight, sex, days on feed) were recorded at the time of sample collection.

After a period of 60 days from enrollment of the last animal in this clinical study, animal treatment records and outcomes were pulled from a database containing all events for the specific feedyards. A 60-day period was chosen relative to previous literature showing that most repulls and deaths happen within 60 days from first treatment.[21] For an animal to be considered a first treatment success (FTS), there could be no additional BRD-associated treatments, BRD-associated death, or BRD-associated culling within the 60 days follow-up period. For an animal to meet the definition of did not finish (DNF), the animal either died of BRD-related causes or was culled due to BRD within the 60-day period. Diagnosis, treatment and management decisions were made by personnel at each commercial feedyard, independent of study investigators. Both feedyards had similar protocols for identifying sick cattle (anorexia, depression, increased rectal temperature), however, treatment protocols were not obtained for the individual feedyards. All personnel influencing cattle management decisions were blinded to cTnI results until the entire data collection phase was completed (60 days post enrollment of last animal).

All feedyard data were moved to a statistical software program[b] for cleaning and analysis. Animal lot and identification numbers were matched to all animals enrolled in the study. After matching cases with 60 days post-enrollment records, cases that were shipped to harvest, died from a non-BRD cause, or were culled due to a non-BRD cause, were removed from study. The remaining cases met criteria for the FTS and DNF models.

A commercially available bovine cardiac troponin-I ELISA[c] kit was used according to the manufacturer's instructions to measure cTnI concentrations in the stored serum samples. Standards of 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL cTnI concentrations were included and run on each ELISA plate. All samples were randomized to plate and run in duplicate. Aliquots (100 ul) of the provided standards and serum samples were dispensed into a microtiter plate, followed by the addition of 100 ul of horseradish peroxidase (HRP)-conjugate into each well. The plates were incubated on a plate shaker[d] at 150 rpm and 25 °C for one hour. Following incubation, the wells were emptied and washed 5 times with a 1x wash solution using a plate washer[e]. One hundred (100) ul of tetramethylbenzidine (TMB) was dispensed into each well and incubated on the plate shaker for an additional 20 minutes. Stop solution (100 ul) was added and the absorbance was measured at 450 nm using a plate reader[f] within 5 minutes. The cTnI concentrations were determined using an online curve fitting software[g]. The inter- and intra-assay coefficients of variation were 21 and 10%, respectively. Final results from the ELISA test were categorized as either LOW (< 0.156 ng/mL) or HIGH (≥ 0.156 ng/mL) cTn1 concentrations, determined by the lower limit of quantification of the assay.

Randomly selected cases were removed from the study due to limited assay capacity. Of the remaining, each case was included in both FTS and DNF models. To determine prognostic ability, DNF cases were evaluated for sensitivity (the percent of cases that did not finish the 60-day period that had HIGH cTnI concentrations), specificity (the percent of cases that finished the 60-day period that had LOW cTnl concentrations), positive predictive value (the percent of cases that had HIGH cTnl concentrations that also did not finish the 60-day period), and negative predictive value (the percent of cases that had LOW cTnl concentrations that finished the 60-day period).

The binomial categories of cTnI concentration (HIGH; ≥ 0.156 ng/mL, or LOW; < 0.156 ng/mL) were used for statistical analysis. DOF at first BRD treatment was categorized into less than or equal to 60 days and greater than 60 days. Weight of the animal at first BRD treatment was categorized into ≤ 500 lb, 501 to 700 lb, 701 to 900 lb, 901 to 1100 lb, and > 1100 lb. Using a statistical software program[b], individual generalized linear mixed effect models ("glmer" function of the "lme4" package) were created to determine potential associations of fixed effects with the probability of DNF and FTS. The fixed effects included individual animal sex, DOF category, weight category, and cTnI category (HIGH/LOW) at the time of first BRD treatment. Animal lot number (given to a group of cattle with similar demographics for the entirety of the feeding period) was nested into feedyard as a random effect due to the lack of independence among samples. A statistically significant cutoff value was set at $P \leq 0.05$.

Results

Blood samples were collected from 343 steers and heifers at initial BRD treatment. Cattle were excluded (n = 16) from further analysis if during the 60-day post-treatment period the animal either died from a non-BRD cause, was removed for a non-BRD related cause, or was shipped to harvest. Due to limited ELISA plate capacity, 9 additional randomly selected animals were removed from further laboratory analysis. Three-hundred-and-eighteen cases were included in the final laboratory analysis. Of the 318 cases, there were 230 animals (72%) classified as FTS, and 88 that required additional BRD-related therapy and/or died or were removed early for BRD causes. There were 39 cases (12%) that matched the case definition of DNF (died or were removed early for BRD causes), and 279 that finished the 60-day study period.

Of the 318 samples, 8 samples had HIGH cTnI concentrations with the remaining 310 samples classified as LOW cTnI concentrations. Of the 8 cases with HIGH cTnI, 2 were retreated for BRD, 2 died from BRD without additional treatment, and 4 additional animals were retreated and died of BRD within the 60 d post-treatment evaluation window. Table 1 depicts the number of cases that fit into each category of Days on Feed (DOF) and weight.

DOF category, weight category, and cTnI category at initial BRD treatment were not statistically associated with FTS ($P > 0.05$). Significant associations were identified between DNF and DOF category ($P < 0.05$), weight category ($P < 0.01$),

Table 1: Cattle demographics and 60-day outcomes from 318 feedyard cattle in U.S. central High Plains commercial operations at the time of initial BRD treatment by cTnI concentration categorized as HIGH (≥ 0.156 ng/mL) and LOW (< 0.156 ng/mL).

		cTnI concentration category	
Cattle demographics	**All cattle (n=318)**	**HIGH (n=8)**	**LOW (n=310)**
DOF			
0-60	237 (75%)	7 (88%)	230 (74%)
> 60	81 (25%)	1 (12%)	80 (26%)
Weight, lb			
≤ 500	53 (17%)	3 (38%)	50 (16%)
501 to 700	64 (20%)	4 (50%)	60 (19%)
701 to 900	83 (26%)	1 (13%)	82 (26%)
901 to 1100	83 (26%)	0 (0%)	83 (27%)
> 1100	35 (11%)	0 (0%)	35 (11%)
Sex			
Steer	155 (49%)	4 (50%)	151 (49%)
Heifer	163 (51%)	4 (50%)	159 (51%)
60-day outcomes			
†First treatment success	230 (72%)	0 (0%)	230 (74%)
†Did not finish	39 (12%)	6 (75%)	33 (11%)

† Percentages in the 60-day outcomes do not add to 100% due to animals not classified as a FTS or DNF (e.g. animals that were retreated but did finish the 60-day post enrollment period).

and cTnI category ($P < 0.01$). Cattle greater than 60 DOF at the time of first BRD treatment had a greater probability of DNF (0.49 ± 0.16 SEM) than cattle less than 60 DOF (0.14 ± 0.07 SEM). Cattle within the 2 lowest bodyweight categories (≤ 500 lb and 501 to 700 lb) had a greater probability of DNF (0.72 ± 1.14 SEM and 0.66 ± 0.14 SEM, respectively) than cattle within all heavier weight categories (701 to 900 lb = 0.14 ± 0.09 SEM, 901 to 1100 lb = 0.16 ± 0.09 SEM, >1100 lb= 0.06 ± 0.06 SEM). Cattle with HIGH cTnI had a greater probability (0.56 ± 0.22 SEM) of DNF than cattle with LOW cTnI (0.11 ± 0.03 SEM).

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cTnI ELISA results at time of first treatment for BRD to determine DNF status were calculated (Table 2).

Discussion

The aim of the current study was to evaluate the association between cTnI and clinical outcome in BRD cases in feedyard cattle. Results of this study show that cattle with HIGH cTnI (≥ 0.156 ng/mL) at the time of first BRD treatment were significantly more likely to not finish the 60-day post-enrollment period than animals with LOW cTnI (< 0.156 ng/mL) at first BRD treatment. Although the current study considered both mortality and culling, these results are similar to previous research in stocker cattle with naturally occurring BRD, that reported a significant association between cTnI concentration at the time of first BRD treatment and subsequent mortality.[22] In that study, calves that died of BRD had cTnI concentrations of 0.1 +/- 0.14 ng/mL (similar cutoff to the present study), while cTnI concentrations in calves that did not die were 0.03 +/- 0.05 ng/mL. A previous report suggests a "normal" reference interval of cTnI in cattle serum of 0.00-0.05 ng/mL, determined by a point of care analyzer.[23]

Though the study results showed an association between cTnI concentrations and DNF, there was no significant association between cTnI concentrations and FTS. While previous research has shown the association between cTnI concentrations and negative outcomes like death or culling, we are unaware of research regarding the prognostic ability of cTn1 to determine the probability of retreatment. Of the 88 cases that did not qualify as FTS in the 60-day post enrollment period, 33 (38%) of the cases were retreated for BRD, without subsequent death or culling. The subjectiveness of treating cattle for BRD not only impacts the initial case selection, but also the likelihood of being an FTS. If feedyard personnel are selecting BRD cases with mild clinical signs for treatment, these cases are likely to have a higher spontaneous cure rate; whereas animals with more severe BRD are more likely to be true BRD cases, but also may have a lower FTS. Because this research was conducted in 2 different feedlots, with multiple animal health personnel in each operation deciding to treat an animal or not, there could be observer variation. This inter-observer variation could have impacted the ability to find statistical significance relative to FTS because of the subjectiveness of treating cattle for BRD.

Results of the present study show that the majority of cTnI concentrations in cattle at the time of first BRD treatment are not detectable with the assay used in this study (LLOQ; lower limit of quantification, 0.156 ng/mL). Of 318 samples assessed

Table 2: Results of 318 feedyard cattle in U.S. central High Plains commercial operations at the time of initial BRD treatment by cTnI concentration categorized as HIGH (≥ 0.156 ng/mL) or LOW (< 0.156 ng/mL) that did or did not finish (DNF) the 60-day post-enrollment period. Calculated values for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

		DNF		
		Yes	No	**Total**
HIGH cTnI concentrations	Yes	6	2	**8**
	No	33	277	**310**
	Total	**39**	**279**	**318**

Sensitivity	0.15
Specificity	0.99
PPV	0.75
NPV	0.89

in this study, only 8 animals had cTnI concentrations above the lower limit of quantification for this assay (0.156 ng/mL) at the time of first BRD treatment. One explanation for this finding is that cTnI is only released after damage to the cardiac muscle; therefore, only BRD cases with cardiac damage or primary heart disease misdiagnosed as BRD would have measurable cTnI concentrations. Another explanation for the low number of animals with measurable cTnI could relate to timing of blood collection to disease progression. In the present study, a single blood sample was collected at the time of initial BRD diagnosis. Individual progression of BRD can be variable; therefore, the window between initial disease insult and sample collection is likely to be different for each animal sampled in the current study. In humans, cTnI concentrations increase within 2-3 hours after insult, peak within 12-48 hours, and then begin returning to normal baseline concentrations.[24] It is possible that the time between cardiopulmonary insult and when an animal starts showing clinical signs is delayed and cTnI concentrations have already peaked and returned to the normal range. Another possibility is that the peak of cTnI concentration happened after initial BRD treatment, and cattle were treated before cardiopulmonary insult, therefore missing the peak concentration. Animals that were retreated in the 60-day post enrollment period could potentially have had higher cTn1 concentrations at their retreatment date, due to a more severe disease process. Another population of cattle that could benefit from the prognostic ability of cTnI would be cattle getting retreated for a first or second time due to BRD. Another limitation of this study is the relatively high LLOQ compared to previously reported normal cTnI concentrations in cattle. It is likely that our assay was unable to detect milder cardiac insults.

This study shows that animals with HIGH cTnI concentrations were more likely to have negative clinical outcomes (DNF). Based on results shown in Table 2, the specificity of the cTnI ELISA was near perfect, demonstrating that most of the cattle that finished the 60-day period had LOW cTnl concentrations. The low sensitivity of the assay resulted from a number of animals that did not finish the 60-day period having LOW troponin concentration. This finding is not completely unexpected as these animals could have died of BRD without substantial cardiac involvement or had cTnI concentrations below the LLOQ of the assay. The PPV indicates that 75% of cattle with HIGH cTnl did not finish the 60-day period. Though the NPV value shows 89% of cattle with LOW cTnI concentrations did finish the 60-day post enrollment period (there was a significantly higher number of cattle with LOW concentrations) the PPV is more useful in this scenario due to the negative outcome of death or culling associated with HIGH cTnI concentrations. These results show that cTnI could be a beneficial prognostic tool for producers to modify their treatment and management regimen or culling decisions. Since HIGH cTnI was associated with DNF, an individual animal with cTnI concentrations above 0.156 ng/mL may warrant a culling decision, allowing the producer to receive a salvage value, rather than incurring the negative costs of treating, retreating, or mortality. Sensitivity, specificity, and predictive values were not calculated for FTS as cTnI concentration was not statistically associated with FTS.

One of the limitations of the present study relates to assay sensitivity. The lower limit of quantification for the ELISA was 0.156 ng/mL. Because there were cases that DNF and had LOW cTnI concentrations, using an assay with a lower LLOQ may improve the prognostic value for animals with cTnI concentrations below the 0.156 ng/mL cutoff used here, but still above the previously suggested reference range. Another limitation to the study is sample timing. Due to the short half-life of cTnI, it is possible that the peak cTnI concentration may have lapsed before clinical signs of disease were detected. Further research is warranted in optimizing sample timing and further identification of animal subpopulations that would benefit the most from the prognostic ability of cTnI.

Elevated cTnI concentrations at initial BRD diagnosis may be an effective prognosticator of negative clinical outcomes, like death and culling. Further research is warranted to optimize the use of cTnI for aiding BRD treatment decisions, which includes determining the population that could benefit the most from the prognostic ability of cTnI. Research evaluating the optimal case selection and alternative testing methodologies would improve the clinical utility of cTnI as a chute-side prognosticator. Additionally, using cTnI in combination with other predictive tools could potentially improve the prognostic utility for individual cases of BRD.

End Notes

[a] BD Vacutainer, BD, Franklin Lakes, NJ

[b] R Studio, Version 2022.02.3, Boston, MA

[c] Bovine Cardiac Troponin-I ELISA, Ctnl-11-HS, Life Diagnostics, Inc., West Chester, PA

[d] MB10-2A Thermo-Shaker, Allsheng, Xihu District, Hangzhou

[e] 405 TS, Agilent BioTek, Santa Clara, CA+

[f] SpectraMax i3x, Molecular Devices, San Jose, CA

[g] MyAssays, Cambridge, United Kingdom

Acknowledgements
Special acknowledgement to Luis Feitoza and Dannell Kopp for their help with blood collection and the feedyard personnel for allowing us to collect at their locations.

Conflicts of Interest
The authors have nothing to disclose.

Funding
This research was funded by the Dr. John and Mildred Gish Large Animal Research Fund.

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