USE OF CARDIAC TROPONIN I FOR EARLY DETECTION OF
MYOCARDIAL DAMAGE IN DAIRY COWS

THESIS

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ABSTRACT

Analysis of cardiac troponin I is considered the “gold standard” for the non-invasive diagnosis of myocardial injury in people and small animals. It has replaced traditionally used cardiac biomarkers such as creatine-kinase and its isoenzymes due to its high sensitivity and specificity for the detection of myocardial injury. In cattle the diagnosis of myocardial disease has been made on the basis of physical exam, radiography in cases of traumatic reticulo-pericarditis, electrocardiography and echocardiography. The purposes of this study were to evaluate a commercially available immunoassay for the detection of bovine cTnI and to show that cTnI will increase in cattle with myocardial injury. We hypothesized that a commercially available immunoassay is highly sensitive and specific for the detection of bovine cTnI and that increased serum concentrations of cTnI are associated with myocardial necrosis in cattle.

The study was divided into two parts. The first part of the study validated a commercially available immunoassay for the detection of bovine cTnI. Serum of 30 healthy dairy cows was analyzed for cTnI and a reverence range was established.

The second part of the study evaluated the relationship of cTnI concentration and electro- and echocardiographic and histopathologic findings after an administration of an overdose of monensin in 10 cows.
We demonstrated that the ADVIA Centaur immunoassay can reliably be used for the detection of cTnI in cattle and that an increased cTnI of $\geq 1.04$ ng/ml was highly specific for the presence of myocardial necrosis. Furthermore, an association between cTnI and left ventricular dysfunction was documented.

Further studies are needed to evaluate the diagnostic benefit of cTnI analysis in cattle with naturally occurring heart disease.
Für meinen Vater – Josef Varga.
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# TABLE OF CONTENTS

Abstract ........................................................................................................................... ii  
Dedication ....................................................................................................................... iv  
Acknowledgments......................................................................................................... v  
Vita..................................................................................................................................... vii  
List of Tables ................................................................................................................... ix  
List of Figures ................................................................................................................ x  

Chapters:  
1. INTRODUCTION AND LITERATURE REVIEW ....................................................... 1  
2. VALIDATION OF A COMMERCIALY AVAILABLE IMMUNOASSAY FOR 
THE MEASUREMENT OF BOVINE CARDIAC TROPNONIN .......................... 10  
   2.1: Materials and methods ..................................................................................... 10  
   2.2: Results ............................................................................................................... 15  
   2.3: Discussion ......................................................................................................... 17  
   2.4: Endnotes ........................................................................................................... 23  
3. CORRELATION OF SERUM CARDIAC TROPNONIN I AND MYOCARDIAL 
PATHOLOGY IN CATTLE WITH MONENSIN TOXICOSIS .................... 29  
   3.1: Materials and methods ..................................................................................... 29  
   3.2: Results ............................................................................................................... 36  
   3.3: Discussion ......................................................................................................... 41  
   3.4: Endnotes ........................................................................................................... 48  

BIBLIOGRAPHY ........................................................................................................... 57
LIST OF TABLES

Table 2.1: Intra- and inter-assay precision at different cTnI concentrations ...................... 24
Table 2.2: Serial dilution of serum cTnI for assessment of the lower limit of detection of the immunoassay .................................................................................................................. 25
Table 2.3: Effect of storage on recovery of cTnI ..................................................................... 26
Table 3.1: Electrocardiographic and echocardiographic findings in 2 healthy dairy cows after one single oral dose of monensin ........................................................................... 49
Table 3.2: Electrocardiographic and echocardiographic findings in 8 healthy dairy cows after one single oral dose of monensin ........................................................................... 50
Table 3.3: Maximum concentration of cardiac troponin I (cTnI) and creatine-kinase (CK) and results of gross and histopathologic examination of 10 dairy cows administered one oral dose of monensin ........................................................................... 51
Table 3.4: Concentration of cardiac troponin I (cTnI), CK (creatine-kinase) and AST (aspartate aminotransferase) (median and interquartile range) before (baseline) and after administration of a single oral dose of monensin (50 mg/kg) to 8 healthy dairy cows .................................................................................................................. 53
Table 3.5: Concentration of BUN (blood urea nitrogen), Crea (creatinine), K⁺, and TCa (total calcium), (median and interquartile range) before (baseline) and after administration of a single oral dose of monensin (50 mg/kg) to 8 healthy dairy cows .................................................................................................................. 54
LIST OF FIGURES

Figure 2.1: Plots of observed versus expected concentrations of cardiac troponin I (cTnI) after serial dilution.................................................................27

Figure 2.2. Point plot of serum cardiac troponin I (cTnI) concentrations in healthy dairy cows (n=30).................................................................28

Figure 3.1. Serum cardiac troponin I (cTnI) concentration in 8 cows after a single oral dose of monensin (50 mg/kg).....................................................55

Figure 3.2. Histopathological changes of the myocardium observed after monensin administration in cattle. Longitudinal sections; HE-stain; 400 x...............................56
CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Cardiac troponins are part of the cardiac contractile apparatus, the troponin–tropomyosin complex. The complex is comprised of three structurally and functionally different troponins (troponin I, T and C) and mediates the interaction between actin and myosin.¹ Troponins occur in cardiac and skeletal muscle, but not in smooth muscles.² Cardiac troponin I (cTnI) is a small (~ 20 kDa) myofibrillar protein associated with the thin filaments of sarcomeres and is the only troponin uniquely expressed in the myocardium.³ Phosphorylated cTnI inhibits the activity of actin-myosin ATPase preventing myofibrillar contraction during specific phases of the cardiac cycle inducing muscle relaxation.³

Muscle stimulation increases cytoplasmatic Ca²⁺ levels and binding of Ca²⁺ to TnC. Leading to conformational changes of the troponin/tropomyosin/actin complex and consequently causes movement of the troponin away from the myosin-binding site of the actin filament. These actions allow the interaction of actin with myosin and results in contraction.⁴ As Ca²⁺ is pumped back into the sarcoplasmatic reticulum, the complex reverts to its previous conformation, inhibiting ATPase action and allowing muscle relaxation. Cardiac troponin T (cTnT) has structural functions in the binding cardiac troponin C (cTnC) and the troponin complex to tropomyosin.³
In people, four isoforms of cTnT exist within the myocardium. However, only one of the isoforms is characteristic of the adult heart, the remaining isoforms are only expressed in fetal heart tissue. Furthermore, multiple isoforms of cTnT exist in skeletal muscle and several studies in people have demonstrated increased circulating concentrations of cTnT isoforms in skeletal muscle diseases, such as Duchenne muscular dystrophy, polymyositis and in patients with end stage renal failure. In people increased mortality has been associated especially with elevated cTnT concentrations. This was explained by cTnT reflecting subclinical myocardial lesions rather than acute myocardial ischemia.

Due to patent restrictions, only one cTnT immunoassay for the use in people is available. Willis et al showed that the assay had a good correlation and linear dose-dependent response to bovine cTnT. However they also demonstrated that the immunoassay is not sufficiently sensitive to accurately quantify bovine cTnT. Therefore the practical use of this assay in the bovine species for detection of myocardial injury remains to be determined.

Measurement of circulating cardiac troponin concentrations is considered the “gold standard” for the non-invasive diagnosis of acute myocardial injury in people. Previously used biomarkers of myocardial damage, such as creatine-kinase (CK) and the isoenzyme of CK (CK-MB), have limited value in detecting myocardial injury due to their lack of tissue specificity and sensitivity. They are also found in the myocardium, skeletal muscle tissue and gastrointestinal tract and are less specific to detect myocardial injury in the presence of skeletal muscle damage, whereas the measurement of cTnI remains unaffected. Even though the specificity of CK-MB can be enhanced by
calculating the CK-MB/CK ratio, the use of this ratio markedly reduces the sensitivity for
detection of myocardial injury in patients with concurrent cardiac and skeletal muscle
injury.\textsuperscript{10} Cardiac troponins have been shown to be a useful cardiac index even in patients
with skeletal muscle myopathies.\textsuperscript{11} Therefore, increased cTnI concentrations do not occur
in people with exercise-induced skeletal muscle damage or with skeletal muscle trauma.\textsuperscript{10}
The use of cTnI as a biomarker for the detection of myocardial injury has resulted in a
substantial increase in the frequency of diagnosing acute myocardial infarction in
people.\textsuperscript{12}

Various studies demonstrated that circulating cTnI is only detectable at very low
concentrations or below the detection limit of commercially available assays in healthy
people and animals.\textsuperscript{13,14,15,16} In people, an increased concentration of cTnI is defined as
the concentration that exceeds the 99\textsuperscript{th} percentile of a reference population or the
concentration at which the assay achieves a 10\% coefficient of variation if that exceeds
the percentile.\textsuperscript{17} In animals, no generally accepted definition for the determination of
cTnI reference ranges has yet been established. In clinically healthy horses cTnI
concentrations of \(\leq 0.015 \text{ ng/ml}\)\textsuperscript{18} were reported. In dogs without any evidence of cardiac
disease, cTnI concentration were measured between 0.00 and 1.37 ng/ml\textsuperscript{19,20} and in
healthy cows serum cTnI concentration of \(\leq 0.04 \text{ ng/ml}\) have been reported.\textsuperscript{21}

The majority of cTnI is present within the sarcomere of the myocyte, and only
between 3 \% and 8 \% of cTnI exists unbound in the cytoplasm.\textsuperscript{1} Whether this cytosolic
component is released into the extracellular space during ischemia rather than after an
insult leading to necrosis is under debate.\textsuperscript{22} In people with acute myocardial injury two
release patterns occur: on is a transient release and one a persistent release of cTnI. It is
postulated that the transient release of troponins occurs from leakage from the cytosolic pool, which results in an early rise of circulating cTnI. Increased membrane permeability may be generated by reversible oxygen deficits as seen in inflammation or toxic damage which leads to degradation and leakage of free cTnI.\textsuperscript{23} The transient release is associated with reversible ischemia as has been demonstrated in a porcine model of ischemic heart disease.\textsuperscript{24} Irreversible loss of the integrity of cardiac myocytes due to ischemic necrosis with subsequent reperfusion may lead to a prolonged, more persistent release of cTnI.\textsuperscript{25} This liberation is associated with release from the cytosolic pool combined with a slower release of myofibril-bound troponin complexes, resulting in a more sustained elevation of circulating cardiac troponins.\textsuperscript{23,26} In people, the predominant form of released cTnI is a mixture of proteolytic fragments complexed with cTnC.\textsuperscript{4} The predominant form of cTnI released into the circulation in animals is unknown. In people, a serum half life of 120 minutes has been reported for troponin and its complex forms in circulation.\textsuperscript{27} Furthermore, studies have revealed that circulating cTnI concentrations do not rise immediately after an acute myocardial insult. The release of cTnI may take up to 4 to 6 hours to become diagnostically measurable with peak concentration occurring 12 to 24 hours after myocardial injury. While the damaged myocardium is undergoing reperfusion and repair, cTnI is continuously released into the bloodstream. The cardiac troponin concentration can remain elevated for up to 14 days before decreasing to pre-injury levels in people with acute coronary syndrome and, therefore, can be used as an indicator of myocardial injury for a much longer period of time than CK or CK-MB.\textsuperscript{28,29,30}

In people, increased concentrations of cTnI occur with acute myocardial infarction as well as acute and chronic heart failure.\textsuperscript{31} Elevated troponin concentrations
were also reported in non-ischemic cardiac conditions and non-cardiac diseases\textsuperscript{32,33} such as blunt chest trauma,\textsuperscript{34} pulmonary emboli,\textsuperscript{35} chronic kidney failure,\textsuperscript{36} septicemia,\textsuperscript{37} and pericarditis.\textsuperscript{38} These conditions can lead to an imbalance between oxygen demand and supply, thus resulting in myocardial ischemia or necrosis and subsequent release of the cTnI into the circulation. Various studies in small animals reported increased circulating cTnI concentrations in dogs with acute myocardial damage,\textsuperscript{39} cardiac contusion,\textsuperscript{40} cardiomyopathy,\textsuperscript{41} babesiosis,\textsuperscript{42} gastric dilatation volvulus,\textsuperscript{43} and in cats with hypertrophic cardiomyopathy.\textsuperscript{44,45} Elevated cTnI concentrations have also been reported in horses with myocardial disease.\textsuperscript{46,47}

In cattle, increased cTnI concentrations were measured in cows with idiopathic pericarditis,\textsuperscript{21} traumatic reticulo-peritonitis,\textsuperscript{48,49} and in a calf with suspected foot and mouth disease\textsuperscript{50} as well as in calves with experimentally induced endotoxemia.\textsuperscript{51}

Cardiac troponin concentrations above the reference range have been shown to be an independent predictor of higher risk of mortality in people with acute myocardial injury.\textsuperscript{52,29} Therefore, single or preferentially serial measurements of cTnI are valuable for risk assessment, identification of patients with greater disease severity and for therapy guidance in different cardiovascular diseases.\textsuperscript{53} Furthermore, the magnitude of cTnI concentration correlates well with the risk of future cardiac events or death in people.\textsuperscript{54}

A good relationship between cTnI concentration and the extent and severity of myocardial injury as shown by histopathologic evaluation of cardiac tissue in people and laboratory animals has been observed.\textsuperscript{5,55} Some investigators concluded the cut-off value of cTnI for the detection of histologically evident myocardial cell injury in laboratory animals is at least 4.1 ng/ml,\textsuperscript{55} whereas other studies found that blood concentrations of
0.35 ng/ml of cTnI were necessary to detect histological myocardial damage.\textsuperscript{56} The discrepancy between the cut-off concentrations may be due to the fact that the obtained concentrations were not directly comparable in these studies due to the use of different commercially available immunoassays. Variations in the measured cTnI concentration can occur and are possibly due to different capture antibodies for free and complexed cTnI used as well as due to the use of different target amino acid sequences of each analyzer.\textsuperscript{57,58} The disagreement between analyzers can result in an up to 10-fold difference in the obtained cTnI values. Therefore, reference ranges should be developed for each analyzer and any direct comparison between analyzers should be avoided.

Currently available immunoassays are intended for the use in people and, therefore, it is important to validate each assay for proposed diagnostic use in veterinary medicine. O’Brien et al.\textsuperscript{2} demonstrated a high amino acid sequence homology (> 96 \%) of cTnI in people, cattle and other mammalian species. They showed that cTnI immunoassays developed for the diagnosis of myocardial damage in people were useful in the detection of cTnI concentrations in numerous animal species because of the high phylogenetic conservation of the target protein.\textsuperscript{2}

A “gold standard” assay for the analysis of cTnI is not yet available; therefore no single assay can be considered more correct than another even though results are closely correlated. To the author knowledge, no commercially available cTnI assay has yet undergone validation for the detection of bovine cTnI. Several commercially available human troponin assays have been validated in companion animals. The ADVIA Centaur\textsuperscript{®} Assay (Siemens) was validated successfully for use in horses,\textsuperscript{18} whereas the Access\textsuperscript{®}AccuTnITM immunoassay (Beckman Coulter, Inc) revealed a high sensitivity
and specificity for the determination of canine cTnI. Furthermore, three cTnI analyzers (Access® AccuTnITM, Beckman Coulter, Inc; Stratus, Dade-Behring; Biosite Triage Meter, Biosite Inc) were compared by using purified canine free cTnI and plasma obtained from canine patients. These analyzers were able to detect canine cTnI, however, significant differences were obtained between analyzers.

As of now, the diagnosis of myocardial injury in food animal medicine has traditionally relied on physical examination, cardiac auscultation and further investigations using radiography, electrocardiography (ECG), and echocardiography. Although electrocardiography is an invaluable diagnostic tool, by itself it is neither sensitive nor specific enough to make a definitive diagnosis of myocardial injury. Studies in human and canine patients have demonstrated a low sensitivity (30-53 %) of the ECG in the detection of acute myocardial infarction. Normal electrocardiographic findings do not reliably rule out an acute myocardial insult. Echocardiography is a reliable, repeatable, specific, and non-invasive method for assessment of structural and functional abnormalities of the heart.

Thorough knowledge of the normal anatomy of the bovine heart as well as obtaining quality imaging views of the cardiac chambers are required. An appreciable amount of physical strength is required to obtain diagnostically useful images. Furthermore, the animal must be restrained for a period of time during the echocardiographic study. Lack of generally available hospital-standard diagnostic equipment, such as cardiac ultrasound machines or ECG, to the bovine veterinarian in the field suggests that a simple blood test such as the measurement of cTnI could potentially have a significant impact on the ante-mortem assessment of the presence and extent of
myocardial injury. Myocardial damage in cattle can have infectious, toxic, nutritional and traumatic etiologies. Examples include but are not limited to vitamin E and selenium deficiency, traumatic-reticulo-pericarditis, and ionophore and glycoside intoxication.

The measurement of cTnI is an easy to perform biochemical test which will enable the veterinarian not specifically trained in veterinary cardiology to determine specific information on the morphologic status of the myocardium. This information will allow early and rapid detection of acute or subacute myocardial damage and possibly provide risk assessment of the bovine patient.

The objective of this study was to validate the sensitivity and specificity of a commercially available immunoassay for the detection of bovine cTnI and to establish a reference range for circulating cTnI concentrations in serum of healthy dairy cows. We hypothesized that the immunoassay has adequate test performance for the detection of bovine cTnI and that in healthy dairy cattle serum cTnI concentration are below the assay detection limit or occurs only in trace amounts.

Although increased cTnI concentrations in blood should indicate ongoing myocyte injury, comparative studies in cattle on the quantitative relationship between serum cTnI and magnitude of histopathologic abnormalities have not yet been published. Therefore, the second aim of this study was to validate cTnI as a biomarker of myocardial cell damage after administration of monensin in cattle and to compare serum concentrations of cTnI with the magnitude of myocardial cell damage determined by histomorphometric methods.
We hypothesized that a) administration of monensin, a compound with known cardiotoxic potential, is associated with elevated concentration of circulating cTnI; b) increased serum cTnI is related to myocardial dysfunction, and c) the quantity of serum cTnI correlates to the severity of histopathologic lesions of the myocardium.
CHAPTER 2

VALIDATION OF A COMMERCIALLY AVAILABLE IMMUNOASSAY FOR
THE MEASUREMENT OF BOVINE CARDIAC TROPOIN I

2.1 MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the Ohio State University, Columbus, OH. Because control bovine serum was not commercially available at the time of investigation, such serum was generated by the investigators. Two-hundred milliliters of blood from a healthy steer (2 years old, 420 kg BW) was collected and serum was separated and used as control serum for all measurements during the study. Absence of detectable cTnI (< 0.01 ng/ml) was assessed by analyzing a serum sample in duplicate.

Commercially available purified bovine cTnI was used to spike cTnI-free serum and generate control serum of known cTnI concentration. Serum samples were immediately frozen and stored at -20°C unless otherwise indicated. To assure expected concentrations of cTnI, the frozen samples were sent to a commercial laboratory for analysis within 48 hours. After arrival at the laboratory, the samples were thawed at room temperature and immediately analyzed. Calibration of the analyzer was performed using human cTnI standards. The assay used for all cTnI analyses is a 3-site sandwich
immunoassay using direct chemiluminometry with 1 polyclonal goat anti-troponin I antibody labeled with acridium ester and 2 biotinylated mouse monoclonal anti-troponin I antibodies for detection of cTnI. The capture antibodies recognize amino acid sequences 41-49 and 87-91 located in the stable central region of the cTnI molecule. Magnetic latex particles conjugated with streptavidin are used as the solid phase reagent. The antibodies bind to cTnI in the sample, and biotin as part of the immune complex then will bind to the streptavidin–labeled magnetic particles. A chemiluminescent reaction is initiated and a direct relationship exists between amount of cTnI in the sample and amount of relative light units detected by the assay. A sample volume of at least 100 μL is required.

Test performance of the assay has been evaluated previously in people. Confirmation of similar assay characteristics using bovine cTnI was performed by evaluation of the assay’s precision, sensitivity, interference with skeletal muscle, linearity, and recovery. Control samples of 4 different concentrations of cTnI (0.2, 1, 10, and 30 ng/ml) were used. Intra-assay precision (within-run) was evaluated by analyzing all samples 3 times in the same run within 1 day, and inter-assay precision (between-run) was evaluated by analyzing all samples twice each day for 3 consecutive days. For evaluation of the lower limit of detection of the immunoassay (i.e., sensitivity of the assay) samples with concentrations of approximately 0.5, 0.1, 0.01, and 0.001 ng/ml cTnI were generated. Each concentration was assayed in 3 replicates to determine the lowest measurable cTnI concentration. Assay linearity was evaluated using serum with 4 different concentrations of cTnI (0.43, 2.33, 14.25, and 29.20 ng/ml). Serial dilutions to obtain 80%, 60%, 40%, 20%, and 10% of the original concentration were performed in
each of the 5 samples of known cTnI concentration. Test recovery (in percent) was
determined for each dilution by comparison of expected versus measured cTnI.

Interference with skeletal muscle troponin I was determined by spiking a serum
sample with a known cTnI concentration with a skeletal muscle homogenate. To obtain
the latter, a 50 g skeletal muscle block (Mm biceps femoris) was harvested from a cow
humanely euthanized after a femoral fracture, and frozen at -70°C within 2 hours of
collection until further processing. Muscle tissue from the non-affected limb was used. A
1.5 g sample of the frozen muscle block was removed and ultra-frozen in liquid nitrogen
(-196°C) for 2 minutes. Thereafter the tissue was manually macerated and mixed with 6
ml of phosphate buffered saline. A tissue tearor was used to break the skeletal muscle
tissue down further over a period of 2 minutes at 30,000 rpm. Finally, a Dounce
homogenizer was used to homogenize the remainder of the muscle tissue. The resulting
homogenate was centrifuged at 2,800 rpm at 4°C for 10 minutes, and the supernatant
was collected for further analysis. Three different volumes of skeletal muscle
homogenate (10, 100, and 200 μl) were added to 1 ml serum with a cTnI concentration of
0.89 ng/ml. Interference of the assay with skeletal muscle was evaluated by comparing
baseline concentrations with the cTnI concentration of the mixed sample.

Stability of the analyte in serum was determined by analysis of serum samples
with low (0.5 ng/ml), medium (5 ng/ml), and high (20 ng/ml) cTnI concentrations stored
at different temperatures over different time periods.

The samples were stored for 48 hours at -80°C, -20°C, 4°C, and room temperature
(23°C) as well as for an additional 7 and 14 days at -80°C and -20°C. Recovered cTnI
was compared to cTnI concentration prior to storage, which were immediately analyzed
after preparation. The effect of repeated freeze-thaw cycles on cTnI recovery (in percent) using samples with low (0.44 and 0.67 ng/ml) and medium (5.28 and 4.22 ng/ml) concentrations of cTnI stored at -80°C and -20°C, respectively was evaluated. The serum samples were frozen for at least 24 hours before they were thawed over 30 minutes at room temperature. After completing the defrosting process, the samples underwent 2 additional freeze-thaw cycles on the same day. After each cycle, cTnI was analyzed and compared to baseline concentrations.

A total of 30 healthy dairy cows (26 Holstein, 4 Jersey) were used for generation of reference values of cTnI. Inclusion criteria were a normal history and physical examination including cardiac and pulmonary auscultation. Twenty Holstein cows were pregnant (between 40 and 215 days) and their average daily milk yield was 24.5 ± 6 kg. The remaining cows were in the dry off period. The mean estimated body weight was 544 ± 71 kg based on body condition score and height of the animal assessed by 2 independent investigators and averaged. Twenty-five cows were < 5 years old and 5 cows were > 5 years old. A 10 ml blood sample was drawn from either the jugular or the coccygeal vein into a serum vacutainer. Blood was left at room temperature for a maximum of 45 minutes before tubes were centrifuged at 2800 rpm for 20 minutes at 23°C. Serum then was removed, separated into 2 aliquots, and frozen at -20°C within 4 hours of collection. Samples were analyzed for cTnI within 2 days of collection.
**Statistical Analysis**

Statistical analyses were performed using commercially available software. Precision was calculated as coefficient of variation (CV % = SD/average x 100 %). Linearity of serial dilutions at different cTnI concentrations was determined by linear regression analysis. Linearity of the assay was assumed when the correlation coefficient was > 0.95. Recovery (in per cent) was calculated (obtained cTnI concentration/expected cTnI concentration x 100 %). Kruskal-Wallis analysis of variance (ANOVA) was performed to determine interference with skeletal muscle homogenate as well as to determine differences induced by temperature, storage time, and cTnI concentration.

A post hoc Mann-Whitney test was used to evaluate significant differences between all pairs of duration of storage on cTnI concentrations recovered. One-way analysis of variance followed by a post hoc Tukey test was used to determine significant differences in recovery of cTnI caused by multiple freeze-thaw cycles.

In the majority of healthy cows, the cTnI concentrations found were less than the detection limit of the assay. Therefore LIFEREG procedure was used to handle such censored data and to estimate the 99th percentile as upper limit of the reference range. Cardiac troponin I concentrations reported as < 0.01 ng/ml were handled as interval-censored data for statistical purposes.

Effect of age, body weight, lactation, and pregnancy status of the cows was evaluated by multiple regression analysis accounting for censored data. Results are expressed as mean ± standard deviation (SD) unless otherwise stated. Statistical significance was defined as p < 0.05.
2.2 RESULTS

Intra-assay precision over the range of 0.2 to 30 ng/ml cTnI was between 3.09 % and 4.84 % with a mean CV of 4.78 ± 2.26 %. The inter-assay precision over the same range of cTnI concentrations was between 5.41 and 21.01 % with a mean of 13.36 ± 6.59 % (Table 2.1).

Assessment of the lower limit of detection of the immunoassay revealed undetectable cTnI concentrations for all samples with an estimated concentration of < 0.01 ng/ml. The lowest concentration measured in this experiment was 0.13 ng/ml (Table 2.2).

Regression analysis showed good linearity for all sets of serial cTnI dilutions (Figure 2.1). The slope of the regression line ranged from 1.006 to 1.019 and the correlation coefficient from 0.98 to 0.994 for obtained versus expected cTnI concentrations. The average percent recovery for each dilution series was 100.81 %, 85.26 %, 87.72 %, and 114.42 % with a mean percent recovery of 96.90 ± 16.59 % for all dilutions.

Recovered cTnI in serum samples containing 0.89 ng/ml of cTnI spiked with 10, 100, and 200 µl skeletal muscle homogenate was 0.98, 0.94 and 0.98 ng/ml respectively ($p = 0.188$). There was no effect of the quantity of homogenate added on the cTnI concentration recovered.

All troponin I concentrations decreased from baseline concentration when stored at 23°C and 4°C for 48 hours ($p < 0.05$). A significant decrease in recovery was observed when samples were stored at -80°C ($p < 0.001$) for the same time period. Cardiac TnI concentrations were not affected when stored at -20°C ($p > 0.05$). Storage temperature
had a significant impact on recovery of cTnI \( (p < 0.001; \textbf{Table 2.3}) \). Storage of samples at -20°C for 7 days had no significant effect on the recovery of cTnI. Storage for 14 days at the same temperature resulted in a significant decrease in the recovered cTnI concentration \( (p = 0.001) \). When samples were stored at -80°C for 7 and 14 days, no significant difference in recovery was observed \( (p > 0.05; \textbf{Table 2.3}) \).

Repeated freeze-thaw cycles had no significant influence on the cTnI recovery \( (p > 0.05) \). After 1, 2, and 3 freeze thaw-cycles, the recovery at low cTnI concentration (0.5 ng/ml) at -20°C was 97.76 %, 96.27 %, and 96.21 %, respectively, and at -80°C was 105.6 %, 110 %, and 116.9 %, respectively. At medium concentration (5 ng/ml), the recovery of cTnI at -20°C was 96.88 %, 94.22 %, and 96.97 % and at -80°C was 102.8 %, 105.7 %, and 113.6 %, respectively.

In healthy cows, all serum cTnI concentrations were \( \leq 0.03 \) ng/ml. Twenty-three cows had cTnI concentrations \( \leq 0.01 \) ng/ml (\textbf{Figure 2.2}). Assuming the measured cTnI concentration followed an exponential distribution, the mean cTnI concentration in healthy cows was 0.02 ng/ml and the 99th percentile was 0.07 ng/ml. Variables such as age, body weight, lactation, and pregnancy status had no effect on baseline cTnI concentration \( (p > 0.05) \).
2.3 DISCUSSION

To the author’s knowledge, this is the first report on the validation of a commercially available cTnI assay for the detection of bovine cTnI. Our results indicate that the ADVIA Centaur®TnI-Ultra™ immunoassay can be used for analysis of bovine cTnI. The immunoassay had similar analytical performance to that observed in previous studies of small animals and horses and performs with adequate precision, linearity, and recovery across different concentrations of purified bovine cTnI.

The intra-assay (within-run) precision was < 10%, which is similar to that reported in studies in people using the same assay. The inter-assay precision (between-runs) at lower cTnI concentration (0.2 ng/ml) also was < 10%. However, at higher cTnI concentrations (1.0, 10.0, and 30 ng/ml) precision decreased.

For clinical acceptance of assay performance, the International Federation of Clinical Chemistry and Laboratory Medicine recommends a coefficient of variation < 10%. This recommendation was made to obtain correct risk classification in people with myocardial infarction. High assay precision is of particular importance if low cut-off values (< 0.01 ng/ml) are used for the detection of myocardial injury thus providing an earlier identification of patients at risk. Although we observed an imprecision above such recommendations at higher cTnI concentrations, its clinical significance for cattle may not be as important.

The manufacturer reports a low (< 0.007%) cross reactivity for the ADVIA Centaur immunoassay when skeletal muscle troponin I is added to a solution with known concentration of cTnI in people. Capture antibodies used in commercially available cTnI assays have negligible cross reactivity with skeletal muscle troponin.
We did not observe a clinically relevant increase in cTnI concentration when various amounts of skeletal muscle homogenate were added. The increase in cTnI concentration was not proportionate to the volume of skeletal muscle homogenate added. The mild increase in cTnI concentration observed may be due to interference with other substrates such as myoglobin, muscle proteins, or lipids. Based on our results, we conclude that concurrent skeletal muscle disease will not limit the diagnostic use of the ADVIA Centaur immunoassay in cattle with myocardial injury.

The assay demonstrated excellent linearity and recovery for all sets of serial dilutions for the detection of bovine cTnI. Cardiac TnI can occur in circulation after myocardial injury as free, binary (complex of cTnI with cTnC), as well as ternary forms (complex of cTnI with cTnC and cTnT). The troponin complex released after injury undergoes rapid posttranslational modification. The type of myocardial insult determines which forms of circulating cTnI dominate. Cardiac troponin I is susceptible to proteolysis by serum proteases as well as phosphorylation and oxidation after it is released into the bloodstream. This leads to conformational changes and a variety of peptides with different stabilities. The rate of degradation of cardiac troponin depends on different factors such as size of the cTnI fragments released and their complex formation with other troponin subunits.

In people, free cTnI has a lower stability than the binary or ternary forms. The N-terminal as well as C-terminal regions of troponin I are rapidly cleaved during proteolysis. Therefore, using an immunoassay that employs antibodies against the stable core region of the protein will increase analytical performance in comparison with assays using antibodies recognizing either the C- or N-terminus of cTnI. The stable core region
lies between amino acid residues 30 and 110. The ADVIA Centaur® immunoassay uses antibodies that recognize immunologic epitopes located within this stable region.

In people, the majority (> 97%) of cTnI released is complexed with TnC after myocardial infarction. Free cTnI has a very short half-life after release (approximately 5 min) and occurs only in small quantities in circulation. Other minor fractions of released troponins are the TnI-TnT binary complex and the TnI-TnC-TnT ternary complex. Shi et al. reported that some assays k,l,m preferentially recognized cTnI in complex form over free cTnI in people. Considerable differences in cTnI recovery among various commercially available immunoassay methods may occur mainly due to the presence of various forms of cTnI in the sample and the use of capture antibodies with different affinities for free and complexed forms of cTnI. In cattle it is unknown which of the several forms of cTnI is released with regard to a particular myocardial insult. In our study, we only validated the assay using free bovine cTnI. Therefore, our results are limited because conclusions about recovery of complex isoforms of cTnI by the immunoassay cannot be drawn.

We detected a significant decrease in cTnI recovery when cTnI samples were stored at room temperature and 4°C for 2 days. These findings are similar to previous studies in laboratory animals and people. Storage at -20°C for 7 days had no significant effect on cTnI recovery. In contrast, a significant, although possibly clinically irrelevant, decrease in cTnI recovery was noticed after storage for 2 days at -80°C. The same batch did not experience a significant decrease in cTnI when stored at the same temperature for 7 and 14 days. Inter-assay variance or possible laboratory error are likely reasons for the latter finding. Further investigation using a larger sample size is needed to
fully understand the effect of storage on cTnI recovery. Our results suggest that cTnI measurements are best performed on the day of sampling or with samples stored at -20ºC for a brief period of time.

All cTnI concentrations determined in clinically healthy dairy cows were ≤ 0.03 ng/ml, resembling reported concentrations by others in cattle.21,49,51 The majority of cows had a serum cTnI concentration at or below the lower limit of detection of the assay used. From this we conclude that cTnI in healthy dairy cows should not be detectable or occur only in trace amounts. Because of the lack of standardization of commercially available cTnI assays, results of this study are only valid for cTnI measurements using the ADVIA Centuar® TnI-Ultra™.78 Different manufacturers utilize a variety of capture and proprietary antibodies with differing abilities to detect free or complexed forms of cTnI, resulting in significant and clinically relevant differences among cTnI analyzer.79 Because of this, results of studies using different cTnI assays cannot directly be compared without bias.

Lactation and pregnancy status, age, and weight of the cows did not influence serum cTnI in this study. Additional investigations involving a larger numbers of cows and groups of different breeds, sex, and body weights are required to determine whether these confounding factors affect the concentration of circulating cTnI.

The clinical use of the immunoassay in cattle with naturally-occurring myocardial disease was not determined. Mellanby et al49 reported a significant difference in circulating cTnI concentrations in 4 of 5 cows with reticulo-pericarditis when compared to a healthy control group (n=34) using the Immulite® troponin I immunometric chemiluminescent assay system.49 However, this study did not correlate histopathologic...
evidence of myocardial injury to serum cTnI concentrations. Studies in people\textsuperscript{80} and rats\textsuperscript{81} indicated good correlation between the severity of morphologically-detectable myocardial cell damage and blood cTnI concentrations. Additional investigations are needed to confirm similar findings in cattle.

Circulating cTnI is substrate (myocardium) specific, but lacks specificity for the particular type of cardiac and non-cardiac disease. Previous studies in people\textsuperscript{12,32} reported on increased cTnI concentrations in patients with renal failure, sepsis, blunt chest trauma, hyperthyroidism, and diabetic ketoacidosis. Also, strenuous exercise may be associated with increased cTnI\textsuperscript{12,84}. The effect of extracardiac disease on circulating concentrations of cTnI is not well documented in cattle although endotoxemia was found to be related to plasma cTnI concentration in calves\textsuperscript{51}. Additional studies are needed to investigate the relationship between circulating cTnI and systemic disease.

Certain limitations of this study need emphasis. One limitation was that only free bovine cTnI was used for the assessment of analytical performance of the ADVIA Centaur\textsuperscript{®} immunoassay. Information on assay performance using complex forms of cTnI was not obtained, and therefore, no information with regard to analytical differences between free and complexed bovine cTnI could be obtained. Another limitation relates to the assessment of the lower limit of detection. The magnitude of the dilution steps used was possibly too large to detect minor differences in cTnI concentrations. The lower limit of detection obtained may be an underestimation of the true limit. Sample size was small leading to low statistical power to detect significant differences. Moreover, based on statistical recommendations, reference values should be established from at least 120 independent observations\textsuperscript{82}. We only used 30 cows in our study and did not test the upper
limit of detection of the assay. Moreover, we only used custom made standards for the measurement of cTnI because standards for cattle were not commercially available.

In summary, the ADVIA Centaur®TnI-Ultra immunoassay has sufficient analytical performance for the measurement of bovine cTnI. The assay reliably detects free cTnI but additional studies are needed to evaluate the ability of the immunoassay to detect complex cTnI isoforms. Storage of serum affects recovery of the analyte. Storage at -20°C is recommended if serum cannot be analyzed within a few hours of sampling. Results of this study are similar to those of previous investigations in other species but cannot necessarily be extrapolated to results of studies using different cTnI assays for the detection of bovine cTnI. Additional research on the evaluation of circulating cTnI in cows with naturally-occurring myocardial injury secondary to viral or bacterial infections, traumatic reticulo-peritonitis, glycoside and ionophore toxicity, and nutritional deficiencies affecting myocardial integrity and function as well as the effects of non-cardiac diseases on circulating cTnI concentrations are needed.
2.4. **ENDNOTES**

a. ADVIA Centaur®TnI-Ultra™, Siemens Medical Solutions Diagnostics, NY, USA.

b. BiosPacific, Emeryville, CA, USA.

c. ADVIA Centaur Calibrator UL, Siemens Medical Solutions Diagnostics, NY, USA.

d. Phosphate Buffer Solution, Fisher Scientific, Pittsburgh, PA, USA.

e. Tissue-tearor, Fisher Scientific, Pittsburgh, PA, USA.

f. Dounce homogenizer, Fisher Scientific, Pittsburgh, PA, USA.

g. Sorvall®Legend™ T/RT, Fisher Scientific, Pittsburgh, PA, USA.

h. BD Franklin Lakes, NJ, USA.

i. Minitab 15.1, Minitab Inc., State College, PA, USA.

j. SAS 9.1, SAS Institute Inc., Cary, NC, USA.

k. Stratus® II, Dade International, Miami, FL, USA.

l. Opus®, Behring Diagnostics Systems, Westwood, MA, USA.

m. Access®, Beckman Coulter, Inc., Brea, CA, USA.

n. Immulite®, Siemens Medical Solutions Diagnostic, Los Angeles, CA, USA.
<table>
<thead>
<tr>
<th>cTnI concentration (ng/ml)</th>
<th>Intra-assay CV (%)</th>
<th>Inter-assay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>3.09</td>
<td>5.42</td>
</tr>
<tr>
<td>1.0</td>
<td>3.25</td>
<td>11.44</td>
</tr>
<tr>
<td>10.0</td>
<td>7.95</td>
<td>21.01</td>
</tr>
<tr>
<td>30.0</td>
<td>4.84</td>
<td>15.56</td>
</tr>
</tbody>
</table>

Table 2.1. Intra- and inter-assay precision at different cTnI concentrations.

cTnI, serum cardiac troponin I concentration; CV(%), coefficient of variation in percent.
<table>
<thead>
<tr>
<th>cTnI (ng/ml)</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.45</td>
<td>0.47</td>
<td>0.46</td>
</tr>
<tr>
<td>0.1</td>
<td>0.13</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>0.01</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>0.001</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Table 2.2. Serial dilution of serum cTnI for assessment of the lower limit of detection of the immunoassay (analyzed in triplicates).

n.d., not detectable.
<table>
<thead>
<tr>
<th>Storage</th>
<th>Average Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cTnI (ng/ml)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Time (d)</td>
</tr>
<tr>
<td>22-23</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>-20</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>-0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

Table 2.3. Effect of storage on recovery of cTnI.
Figure 2.1. Plots of observed versus expected concentrations of cardiac troponin I (cTnI) after serial dilution.

A, baseline cTnI concentration 0.43 ng/ml; B, baseline cTnI concentration 2.33 ng/ml; C, baseline cTnI concentration 14.25 ng/ml; D, baseline cTnI concentration 29.20 ng/ml; Samples were run in duplicates. p < 0.0001 at all concentrations.
Figure 2.2. Point plot of serum cardiac troponin I (cTnI) concentrations in healthy dairy cows (n=30). The line in the center represents the mean value.
CHAPTER 3

CORRELATION OF SERUM CARDIAC TROPONIN I AND MYOCARDIAL INJURY IN CATTLE WITH MONENSIN TOXICOSIS

3.1 MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee at The Ohio State University, Columbus, Ohio.

The study population consisted of 10 non-pregnant and non-lactating Jersey ($n = 6$) and Holstein ($n = 4$) cows with a mean bodyweight of 494 (SD ± 85 kg). Seven cows were less than 5 years old and 3 cows were over 5 years of age based on dental examination. A pilot study was performed with two randomly selected cows (Group A) in order to determine the dose of monensin and sampling intervals most appropriate for the purposes of the study. All animals were housed in individual tie stalls bedded on straw with free access to water, timothy hay, and mineral blocks. After initial physical examination, 10 ml of blood was obtained from the right jugular vein for determination of baseline data including a complete blood count (CBC) and serum biochemical analyses (CK; aspartate aminotransferase [AST]; blood urea nitrogen [BUN]; and creatinine [Crea] and concentrations of potassium [K$^+$]; total calcium [TCa], and cTnI) prior to monensin$^a$ administration.
Electrocardiography and transthoracic echocardiography were also performed in all cows. For these procedures, the cows were standing, non-sedated, and restrained in a movable cattle transporter. The ECG recordings included limb leads I, II, and III with the electrodes attached to the skin as described by Rezakhani et al. ECG’s were recorded over approximately 2 minutes with a paper speed of 25 and 50 mm/sec and a calibration of 10 mm equal to 1 mV. Electrocardiograms were evaluated for heart rate and rhythm, abnormalities of conduction, and alterations in amplitude and duration of P, QRS, R, ST, and QT intervals.

Standard 2-dimensional (2-D) and M-mode echocardiography was performed from the right side of the thorax using a commercially available echocardiographic system equipped with a transducer with a nominal frequency of 3 MHz. The left ventricular diameter at the end-diastole (LVDd) and peak-systole (LVDs) and the left ventricular area at end-diastole (LV area in Saxd) and peak-systole (LV area in Saxs) were measured from M-mode recordings and 2-D images of the left ventricle (LV), respectively. Images were obtained from a right parasternal short axis view at the level of the chordae tendineae. The maximum left atrial diameter in systole (LADs) and the LV volumes at end-diastole (LVvol Laxd) and end-systole (LVvol Laxs) were measured and calculated, respectively, from right parasternal long axis images using the modified Simpson’s method. Left-ventricular systolic function was assessed subjectively and by calculating the following variables:

Left ventricular fractional shortening (FS): \[ FS\% = \frac{LVDd - LVDs}{LVDd} \times 100\% \]

Left ventricular ejection fraction (EF): \[ EF\% = \frac{LVvol Laxd - LVvol Laxs}{LVarea Saxd} \times 100\% \]
Left ventricular shortening area (SA): \[ SA\% = \frac{LVareaSaxd - LVareaSaxs}{LVareasaxd} \times 100\% \]

All values presented and considered for statistical analysis reflect the average of three consecutive measurements. All measurements were done en-bloc at the end of the study period by one board-certified cardiologist (KES), who was blinded to animal identification, treatment, and time.

After assurance of general and cardiac health of the animals, an indwelling catheter was placed into the right jugular vein. During the study period, the catheter was flushed every six hours with 10 ml heparinized saline and replaced if necessary. All cows were given a single oral dose of monensin suspended in 300 ml of water via orogastric tube. A 500 ml bolus of plain water was used to flush residual monensin remaining in the tube. The two animals enrolled in the pilot study received 30 and 40 mg/kg monensin. The dosages were chosen based on studies by Van Vleet et al. and Litwak et al. suggesting effectiveness of monensin to produce clinically relevant myocardial injury. Due to the equivocal evidence of myocardial damage found in the pilot cows using such doses, the dosage for the eight remaining cows (Group B) was increased to a single oral dose of 50 mg/kg monensin. In all cows, body temperature, heart rate, and respiratory rate were monitored every other hour after monensin administration. Flunixin-meglumine (1.1 mg/kg, IV) was given for pyrexia and alleviation of gastrointestinal pain as needed.

Blood was collected from the indwelling jugular catheter at 4, 6, 8, 12, 16, 20, 24, 36, 48, 72, and 80 hours (Group A) and at 12, 24, 36, 48, 72, 96, 120, and 144 hours (Group B) after monensin administration for serum biochemical analyses. The aliquots were divided equally and put into potassium-EDTA containing tubes and tubes without
anti-coagulant. Serum tubes were left at room-temperature for 45 minutes, centrifuged at 2,800 rpm for 20 minutes at 23°C, and the serum removed, divided and stored at 4°C for subsequent biochemical analyses and at – 20°C for later analysis of cTnI. Within 12 hours of collection, the biochemical profile was analyzed on an automated clinical analyzer. Frozen serum samples for cTnI analysis were sent out in batches to a commercial laboratory within 48 hours of sampling and analyzed immediately upon arrival. Concentration of cTnI was determined with the ADVIA Centaur®TnI-Ultra™ immunoassay. This three-site sandwich immunoassay uses direct chemiluminometric technology for the detection of free and complexed cTnI. It includes one polyclonal goat and two monoclonal mouse anti-troponin-I-antibodies. The capture antibodies recognize amino acid sequences 87-91 and 41-49 located in the stable region of the cTnI molecule. A previous validation study of the assay performed in our laboratory revealed sufficient analytical performance for the detection of bovine cTnI with a lower limit of detection of 0.01 ng/ml and a reference range (95% CI) for serum cTnI obtained from 30 healthy cows of 0 to 0.07 ng/ml.

Electrocardiography and echocardiography were repeated at 48 hours (Groups A and B), 72 hours (Group A), and 120 hours (Group B) after monensin administration. The study was terminated at 80 hours (Group A) and 144 hours (Group B) after monensin administration or if any animal fulfilled early removal criteria such as tachycardia (heart rate > 100 bpm), tachypnea (respiratory rate > 60 bpm), heart failure, severe discomfort, or inability to rise. Cows were euthanized with an overdose of pentobarbital (40 mg/kg, IV). Within one hour, tissue samples from nine locations throughout heart (both auricles and atria, left and right ventricular free wall, interventricular septum, left ventricular...
apex, and posterior papillary muscle) as well as four locations of striated non-cardiac muscle (*Mm. biceps humeri*, *Mm. quadriceps femoris*, costal parts of diaphragm, and tongue (only Group B)) were harvested. Any gross lesions were noted and photographed. Histopathological examinations were performed on all tissue samples by one board-certified pathologist (PCS) blinded to the animal identification, treatment status, and serum cTnI. Heart and skeletal muscle samples from healthy cattle obtained from a regional slaughterhouse served as controls (n = 5). Tissue blocks were preserved in 10% buffered neutral formalin, dehydrated, blocked in paraffin, cut into 3µm sections, and stained with hematoxylin-eosin (HE). Semi-quantitative histopathological evaluation under light microscopy was then performed (magnification 400x). Each tissue sample was rated in 10 different microscopic fields and was assessed for five criteria: vacuolar changes, fiber swelling, rhabdomyolysis, edema, and cellularity. Each criterion was subjectively scored as not evident (score 0), mild (score 1), or moderate to severe (score 2). Scores from ten different microscopic fields were averaged generating a field score for the specific segment of the heart. All field scores were then added, generating a total heart score or a non-cardiac muscle score. In order to generate consistent and repeatable data, all samples were assessed *en-bloc*. Prior to final data analysis, the observer underwent repeated assessments of ten randomly selected tissue samples until observer variability, expressed as the coefficient of variation of field scores, was less than 10%.
Statistical analysis

Statistical analysis and graphical depiction were performed with commercially available software. Serum concentrations of cTnI reported by the laboratory as less than 0.01 ng/ml were handled as equal to 0.01 ng/ml for statistical purposes. Blood taken from cows that fulfilled early removal criteria was handled as if taken at the next regular sampling period. In two such cows (cow #6 and # 8), ECG and echocardiography were repeated immediately prior to euthanasia. Data were handled as if acquired at 120 hours post monensin administration.

All continuous variables are reported as median and interquartile range (25th and 75th percentiles) unless stated otherwise. The Kolmogorov-Smirnov test was used to assess normal distribution of residuals and was repeated after log transformation if residuals failed normality testing. Data for the pilot animals (Group A) are reported for each individual cow. A repeated measure ANOVA was used to determine temporal differences within individual variables. A Wilcoxon signed rank test with Bonferroni adjustments for multiple comparisons was used when significant differences were detected. For the assessment of association between cTnI or log cTnI with continuous variables, a mixed effects regression model with a cow random effect was used. To detect differences in electro and echocardiographic continuous variables over time, a nonparametric repeated measures two-way analysis of variance (Friedman’s test) was used. Where necessary, imputation was performed using a repeated measure ANOVA model where missing observations were modeled using a quadric function over time. To test for associations between maximal log cTnI and maximal log CK and total heart or skeletal muscle score, Pearson’s correlation was used.
To detect differences in mean scores between different parts of the heart, repeated measures ANOVA with a post hoc Tukey’s test were used. Differences of median heart and non-cardiac muscle scores between monensin treated cows and control cows were analyzed with the Mann-Whitney rank sum test. A $p$ value of $\leq 0.05$ was considered significant, unless otherwise stated.
3.2 RESULTS

All cows were healthy prior to monensin administration based on history, physical examination, CBC, serum biochemistry, ECG, and echocardiography and with cTnI concentrations between ≤ 0.01 and 0.03 ng/ml. All animals developed clinical signs of monensin toxicity including anorexia, lethargy, and diarrhea within 24 hours of monensin administration.⁸⁶

Pilot Group (Group A)

Cardiac TnI concentration did not change and biochemical variables stayed within reference range throughout the entire study period in cow # 1 (dose of monensin 30 mg/kg, PO). Cow # 2 (dose of monensin 40 mg/kg, PO) had increased cTnI concentration at 12 hours (0.12 ng/ml), with a peak concentration at 24 hours (1.04 ng/ml) after receiving monensin. Creatine-kinase was mildly increased (356 IU/L) at 72 hours after monensin administration and reached a concentration of 1,699 IU/L at study termination (80 hours after monensin). Other biochemical variables remained within normal limits.

Electrocardiographic and echocardiographic studies in both pilot cows revealed mild ST segment elevation on ECG and decreased SF, SA, and EF at 48 and 72 hours after monensin. Shortening fraction, SA and EF had decreased at both time points by 70%, 42%, and 55% (cow # 1) and 66%, 81% and 54% (cow # 2), respective of the baseline values (Table 3.1.).

At necropsy no gross lesions were observed in the myocardium or skeletal muscle sections in either cow. Cow # 1 had only mild histopathological changes such as vacuolar alterations with mild swelling of individual myocardial fibers. Myocardial necrosis was
not observed. Cow # 2 had evidence of vacuolar degeneration of single myofibrils and a variable degree of interstitial edema, swelling, and multiple foci of necrosis (Table 3.3). Tongue and skeletal muscle sections were unremarkable.

Study Group (Group B)

In the eight cows treated with a single dose of 50 mg/kg of monensin, differences in median cTnI, CK, AST, BUN, creatinine, calcium, and potassium were detected over time as compared to baseline concentrations ($p \leq 0.05$). After adjustment of $p$ for multiple comparisons significant differences between baseline variables and any specific time point after monensin administration were not found ($p > 0.006$, Table 3.4 and Table 3.5). Although statistical differences were not detected, an increase of cTnI above the upper limit of the reference range (0.07 ng/ml) was found in all cows of Group B after monensin administration (Figure 3.1, Table 3.4), with a median peak concentration of 13.0 ng/ml (range 0.97 – 39.0 ng/ml). An increase in serum CK concentration above the reference range was observed in seven cows during the study period (Table 3.4), with a median peak concentration of 565 IU/L (range 367 – 3,834 IU/L). Electrocardiographic abnormalities were identified in all cows (Table 3.2). The most common findings at either 48 hours and/or 120 hours after monensin administration were an increase in heart rate (n=6) and delayed intraventricular conduction and repolarization, evident by lengthening of the duration of QRS complex (n=8) and prolongation of the QT interval (n=5). Furthermore, low voltage R waves (n=7) and ST segment elevation (n=5) were observed, indicative of myocardial injury. Reduction of R wave amplitude measured at 48 hours after monensin was statistically significant from baseline ($p = 0.021$). No
association between R wave amplitude and cTnI concentration was found, however, a significant association was detected between serum cTnI concentration and heart rate ($r^2 = 0.60, p = 0.013$). Cardiac TnI did not correlate to any other electrocardiographic variable.

In **Table 3.2**, the median and interquartile ranges of the echocardiographic variables are summarized. All cows had signs of reduced chamber size and impaired LV systolic function at either 48 and/or 120 hours after monensin. The most common findings were decreased LADs (n=8) and decreased LVDd (n=8) and an increase of LVDs (n=5). Furthermore, a decrease of SA, SF, and EF was evident in seven, six and five cows, respectively. The mean decrease (SD) from the baseline, averaged for both time points, was 74 % for SA (19), 78 % for SF (22), and 65 % for EF (20; **Table 3.2**). However, only the LVDd measured at 48 hours revealed a statistical significant difference from baseline ($p = 0.014$; **Table 3.2**). The cTnI concentration was associated with LVDd ($r^2 = 0.79; p = 0.011$) and SF ($r^2 = 0.51; p = 0.02$). No other correlation between cTnI and any other echocardiographic variable was found.

Cow # 10, # 8, and # 6 fulfilled early removal criteria at 70, 100, and 120 hours after monensin due to sinus tachycardia, tachypnea, severe discomfort, and pyrexia. Cow # 4 died acutely at 94 hours after monensin.

Gross pathologic lesions such as pale striations, petechiae, and ecchymosis were observed in four cows of Group B. The most prominent histopathologic lesions were hyaline and vacuolar degeneration, moderate to severe swelling of myocardial fibers with variable interstitial predominantly neutrophilic cellularity, and multifocal to diffuse myocardial necrosis (**Figure 3.3**). Evaluation of the heart scores of all ten cows (group A
and b) revealed a significantly greater mean field score for the left atrium than the LVFW, the papillary muscles, or the interventricular septum ($p < 0.05$). Other differences between mean scores of various areas were not observed. All cows (n=6) with a total heart score above 30 had histological evidence of myocardial necrosis, whereas cows with a total heart score below 30 had no evidence of necrosis. Gross lesions were not observed in any of the non-cardiac striated muscle samples, however occasional histopathologic evidence of localized necrosis was observed in skeletal muscle tissue of four cows (Table 3.3). Gross or microscopic evidence of rhabdomyolysis was not found in any of the control hearts or their non-cardiac muscle tissue. Median total heart score in control animals was 0.4 (inter quartile range, 0 and 6.1). Non-cardiac muscle samples of the control cows were histologically unremarkable. Median total non-cardiac muscle score was 6.7 (interquartile range, 0 and 7.8). When comparing the median total heart scores between study cows and control cows a significant difference was detected ($p = 0.010$). No difference was found between non-cardiac muscle scores of both groups ($p = 0.162$). Furthermore, a significant relationship between maximum (log) cTnI and total heart score was observed ($r = 0.71; p = 0.022$), whereas maximum (log) CK was not correlated to heart score ($r = 0.48; p = 0.164$).
Considering all cows studied, a peak concentration of cTnI above 1.04 ng/ml (n=7) predicted a total heart score of ≥ 30 and evidence of myocardial necrosis (Table 3.3) whereas cows with a peak cTnI concentration below 1.04 ng/ml (n=3) had a total heart score < 30 and no evidence of myocardial necrosis in any of the histopathological fields assessed.
3.3 DISCUSSION

Studies in laboratory animals\textsuperscript{56,87} and dogs\textsuperscript{88} have reported on the diagnostic use of cTnI and concluded that cTnI is both sensitive and specific for the diagnosis of acute myocardial injury. The results of this study confirm such findings. Also, a relationship between LV systolic function and circulating cTnI was found. The magnitude of elevation of cTnI was associated with the severity of histopathological lesions and more specifically, with the severity of myocardial necrosis.

In people, increased concentrations of cTnI can be detected within three to four hours after acute ischemic myocardial injury with peak concentrations occurring after 48 to 72 hours. Detectable cTnI concentrations persist for up to two weeks before declining to pre-injury levels.\textsuperscript{89,90} Rats and dogs with experimental acute myocardial infarction revealed a similar release pattern of cTnI.\textsuperscript{91}

In our model of myocyte injury, the onset of myocardial necrosis could not be exactly defined although it may be variable. The temporal variability of cTnI release and magnitude of cTnI elevation, despite similar doses of monensin, is considerable and is likely multi-factorial in nature. Oral administration may have been associated with individual differences in monensin absorption. Age and bodyweight, as seen in other species, may also have influenced the effects of monensin.\textsuperscript{88} In addition, previous ingestion of monensin in individual cows cannot be excluded with certainty, as monensin is frequently used as a feed additive in dairy cattle. Prior exposure to monensin may lead to rumen adaption associated with a lower toxicity of any additional dose of the drug due to induction of rumen microfloral alterations.\textsuperscript{92}
Mild elevation of serum cTnI may occur without histological evidence of myocardial cell injury secondary to increased myocyte membrane permeability with release of cytosolically dissolved cTnI. In this study, two cows had maximum concentrations of cTnI of 0.27 ng/ml and 0.29 ng/ml without histological evidence of myocardial necrosis. Interestingly, in both animals ECG abnormalities and LV systolic dysfunction were found. The discrepancy between increased cTnI concentration and absence of myocardial necrosis might reflect that mild cTnI release is not necessarily associated with irreversible necrotic damage of the myocardium.

In human cardiomyocytes, approximately 6% to 8% of total cardiac troponin is cytosolically dissolved and thus unbound in cytoplasm. Early after myocardial cell injury affecting cell membrane permeability, parts of this free pool are leech into the blood stream, but the majority of cTnI is retained intracellularly because of structural linkage to the contractile apparatus. Thus, release of cTnI may occurred monophasically, with only minor elevation after reversible myocyte injury, or bi- or polyphasically with more severe injury of the myocardium affecting the structurally bound portion of cTnI. The latter is characterized by an early peak of serum cTnI and a subsequent second, more significant increase. To the author knowledge, the amount of free and unbound cTnI in cardiomyocytes of cattle is unknown.

Moreover, we identified a higher sensitivity of cTnI to detect myocardial injury as compared to CK as described by other investigators. Serum cTnI increased earlier after monensin intoxication and an association between cTnI concentration, ventricular dysfunction, and myocardial necrosis was detected, whereas CK did not correlated to any of these measures. This finding is in accordance with previous studies which have shown
that CK lacks specificity for myocardial cell injury and that the measurement of CK is not sensitive enough to detect micro-pathology of the heart in cattle. In contrast, CK-MB, an isoenzyme of creatine-kinase, which is specific to the cardiac muscle, has a higher specificity for myocardial cell injury and reportedly correlates well with myocardial infarct size in people. In the current study, CK-MB was not measured, therefore no definitive statement with regard to the diagnostic performance of CK-MB in cattle using our model can be made.

All cows had decreased water consumption and persistent diarrhea as typical signs of monensin toxicity. Also, azotemia developed in most cows, possibly due to dehydration. Mildly increased cTnI concentrations have been demonstrated in people with end-stage renal disease. Although the underlying pathophysiology of this abnormality is still not clearly understood, it may reflect ongoing, often subclinical, myocardial damage. There is a possibility that up-regulated apoptosis might explain modest elevations of serum troponin in patients with renal insufficiency, but this concept has been understudied. It is unlikely that elevated serum cTnI is the result of decreased clearance by the failing kidneys. Cardiac troponin I is cleared by the reticulo-endothelial system (RES), and renal failure should only have minor effects on RES. Pericarditis secondary to uremia is a well documented condition in people affecting the subepicardium leading to the release of cTnI. Moreover, in earlier studies on the association between renal disease and serum cTnI less specific first and second generation cTnI assays were used which are known to be affected by uremic compounds reducing analytical performance and leading to false positive results. Higher cut off values of cTnI have been recommended in such patients. In our study, a third
generation cTnI assay was used and no significant correlation between maximal cTnI and severity of azotemia was detected. Most likely the cows in our study developed pre-renal azotemia due to dehydration from monensin induced diarrhea. However kidney function was only assessed using BUN and creatinine; no further diagnostic procedures were performed. A possible association of renal dysfunction and cTnI concentration cannot be excluded with certainty.

In agreement with another study, all cows had some degree of electro- and echocardiographic abnormalities after administration of monensin. The majority of cows had ST segment elevations which may be interpreted as a sign of myocardial injury (e.g. ischemia, infarction, and reperfusion) to the myocardium. No linear correlation between serum cTnI and ST segment alteration was found in this study, whereas in dogs an association between the severity of the ECG abnormalities and circulating cTnI concentration was previously reported. It is likely that a correlation between variables could not be demonstrated due to the nonspecific nature of ST changes. This is similar to what has been reported previously in dogs. Furthermore, the small number of animals could have reduced the likelihood of detecting statistically significant differences and clinically meaningful associations.

Echocardiography is currently the most valuable and accurate tool for non-invasive assessment of LV function in small animals and people. Due to the level of expertise required to perform the examination and interpret the findings as well as the cost of the sophisticated equipment used, it is not commonly employed in bovine practice. In the current study, a relevant correlation between cTnI and SF (shortening fraction) was found. The latter is the most commonly used echocardiographic variable of
LV systolic function. These results are similar to observations in dogs with cardiomyopathy and degenerative valve disease where an increased cTnI concentration was associated with the severity of myocardial dysfunction. Although, the central tendency of echocardiographic variables of LV systole revealed possibly meaningful associations with cTnI, the scatter of parameter values despite very similar study conditions was wide, making the interpretation of findings in individual animals rather challenging. Also, most cows experienced significant loss of intravascular volume (and thus preload) which may have confounded our interpretation of echocardiographic variables of LV systolic function.

A relationship between the amount of cTnI release and the severity of myocardial injury has been established in various studies by histopathological examination as the method of choice for demonstration of myocardial necrosis. In our study, the extent of myocardial damage was assessed semi-quantitatively as histomorphometry of the entire heart was not feasible. However, a large number of tissue sections and microscopic fields per section was evaluated (total number of microscopic fields per heart was 90), and a significant correlation between circulating cTnI and total heart score was evident. We concluded that the magnitude of serum cTnI is closely relates to the magnitude of myocyte injury and may therefore be clinically useful in the estimation of severity of myocardial damage in cattle. Marked myocardial cell necrosis was detected histopathologically in cows with a cTnI concentration of ≥ 1.04 ng/ml. In contrast to our results, studies in laboratory animals reported such cut off values being lower (0.35 ng/ml) or higher (4.1 ng/ml). These differences may be due to analytical issues associated with the cTnI assay used, differences in histopathological methods and criteria
applied, and observer variation. The use of various analyzers utilizing different capture antibodies with varying abilities to detect free or complex forms of cTnI may lead to different cTnI concentrations despite the use of identical substrates. Results of different cTnI assays may not be directly comparable due to lack of method standardization.

Certain weaknesses of this study need to be brought out. One limitation is the lack of ante-mortem assessment of cTnI in the control group for comparison and validation of the histopathologic injury scoring system used. Furthermore, myocardial lesions were only semi-quantitatively analyzed. An increased number of tissue blocks and the use of a computer-based, fully automated, quantitative histomorphometric analysis system may have influenced our current findings. Histopathology was performed by only one pathologist and studies on observer variability were not performed. In this study the investigator was blinded to the animal group and the results of the cTnI analysis. An extended training period with repeated examinations was performed to assure consistency prior to the final assessment. Only two-minute ECG recordings were obtained which may have resulted in missed rhythm abnormalities affecting the interpretation of the ECG findings. The number of cows was small leading to underpowered data and decreased abilities to identify significant differences especially when using methods such as Bonferroni correction for multiple comparisons.

In conclusion, the present study confirmed that cTnI is a specific and sensitive biomarker for the detection of myocardial cell damage in cattle. A serum concentration of cTnI > 1.04 ng/ml is an indicator of histopathologically detectable myocardial necrosis in cattle. In our model, elevated cTnI was associated also with signs of LV dysfunction. The measurement of serum cTnI may become a clinically useful tool for the non-invasive
diagnosis of myocardial cell injury in cows, including animals with congestive heart failure, cardiomyopathy, myocarditis, viral or bacterial infections, selenium deficiency, intoxications with gossypol and cardiac glycosides, and overdoses of ionophore feed additives. Early detection of cardiac damage may also help in the risk stratification and prognostication in such patients. Further clinical studies are needed to evaluate the diagnostic benefit of the assessment of circulating cTnI in cattle with naturally occurring heart disease.
3.4 ENDNOTES

a. Rumensin® 80, Elanco Animal Health, Greenfield, IN, USA.
b. Ohio Cattle Transporter, Bud Corporation, Columbus, OH, USA.
c. Hewlett Packard, Siemens Medical Systems, MA, USA.
d. Vivid 7 Vantage TM with Echo Pac software package BT04, GE Medical Systems, Milwaukee, WI, USA.
e. BD Angiocath™, Becton Dickinson Infusion Therapy System Inc., Sandy, UT, USA.
f. Banamine®, Intervet Schering-Plough Animal Health, Millsboro, DE, USA.
g. Monoject 7.5% EDTA Liquid, Tyco Healthcare Group LP, Mansfield, MA, USA.
h. BD vacutainer® Serum, BD Franklin Lakes, NJ, USA.
i. Hitachi 911, Roche Diagnostics Corp., Indianapolis, IN, USA.
j. ADVIA Centaur®TnI-Ultra™ Siemens Medical Solutions Diagnostics, NY, USA.
k. SomnaSol, Butler Animal Health Supply, Dublin, OH, USA.
m. GraphPad Prism 4, GraphPad Software Inc, La Jolla, CA, USA.
n. Minitab 15.1, Minitab Inc., State College, PA, USA.
o. SAS 9.1, Cary, NC, USA.
<table>
<thead>
<tr>
<th>Time</th>
<th>Baseline</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Electrocardiography**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (b/min)</td>
<td>57 / 86</td>
<td>91 / 94</td>
<td>81 / 74</td>
</tr>
<tr>
<td>QRS interval (ms)</td>
<td>100 / 80</td>
<td>100 / 100</td>
<td>90 / 110</td>
</tr>
<tr>
<td>R amplitude (mV)</td>
<td>-1.6 / -0.9</td>
<td>-2 / -1.25</td>
<td>-1.6 / 1.1</td>
</tr>
<tr>
<td>ST segment (mV)</td>
<td>0.2 / 0</td>
<td>0.4 / 0.2</td>
<td>0.3 / 0.1</td>
</tr>
<tr>
<td>QT interval (ms)</td>
<td>440 / 400</td>
<td>380 / 400</td>
<td>390 / 460</td>
</tr>
</tbody>
</table>

**Echocardiography**

<table>
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<th>Parameter</th>
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<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>LADs (cm)</td>
<td>11.6 / 12.8</td>
<td>10.2 / 11.1</td>
<td>10.5 / na</td>
</tr>
<tr>
<td>LVDd (cm)</td>
<td>9.1 / 10.2</td>
<td>8.2 / 8.9</td>
<td>8.3 / 8.8</td>
</tr>
<tr>
<td>LVDs (cm)</td>
<td>5.7 / 5.8</td>
<td>5.3 / 6.7</td>
<td>6.8 / 5.9</td>
</tr>
<tr>
<td>SA (%)</td>
<td>58 / 49</td>
<td>31 / 44</td>
<td>18 / 36</td>
</tr>
<tr>
<td>SF (%)</td>
<td>38 / 4</td>
<td>35 / 24</td>
<td>18 / 33</td>
</tr>
<tr>
<td>EF (%)</td>
<td>67 / 71</td>
<td>34 / 34</td>
<td>39 / 49</td>
</tr>
</tbody>
</table>

Table 3.1. Electrocardiographic and echocardiographic findings in 2 dairy cows (Group A) after one single oral dose of monensin (30 and 40 mg/kg). Data of each animal is presented.

LADs, left atrial diameter at end-systole. LVDd, left ventricular diameter at end-diastole.

LVDs, left ventricular diameter at end-systole. SA, shortening area. SF, shortening fraction.

EF, ejection fraction. na’ not assessed.
Group B

<table>
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<tr>
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<th>120 hr</th>
</tr>
</thead>
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<td>8</td>
<td>6</td>
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**Electrocardiography**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>48 hr</th>
<th>120 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (b/min)</td>
<td>71 (57-84)</td>
<td>81 (76-95)</td>
<td>84 (74-99)</td>
</tr>
<tr>
<td>QRS interval (ms)</td>
<td>80 (65-94)</td>
<td>90 (80-110)</td>
<td>118 (80-157)</td>
</tr>
<tr>
<td>R amplitude (mV)</td>
<td>-0.9 (-1.3-(-0.7))</td>
<td>-1.55* (-1.9-(-1.2))</td>
<td>-1.3 (-1.6-(-1.0))</td>
</tr>
<tr>
<td>ST segment (mV)</td>
<td>0 (0-0)</td>
<td>0.1 (0-0.2)</td>
<td>0.05 (-0.02-0.2)</td>
</tr>
<tr>
<td>QT interval (ms)</td>
<td>400 (370-420)</td>
<td>385 (348-428)</td>
<td>455 (430-490)</td>
</tr>
</tbody>
</table>

**Echocardiography**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>48 hr</th>
<th>120 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>LADs (cm)</td>
<td>12.1 (11.3-13.0)</td>
<td>11.3 (9.6-12.7)</td>
<td>10.5 (8.9-12.6)</td>
</tr>
<tr>
<td>LVDd (cm)</td>
<td>8.9 (8.0-9.7)</td>
<td>7.6* (6.8-8.1)</td>
<td>7.3 (6.3-8.8)</td>
</tr>
<tr>
<td>LVDs (cm)</td>
<td>4.8 (4.1-5.3)</td>
<td>4.7 (3.7-5.7)</td>
<td>4.9 (4.0-5.3)</td>
</tr>
<tr>
<td>SA (%)</td>
<td>64 (57-65)</td>
<td>56 (48-61)</td>
<td>55 (47-72)</td>
</tr>
<tr>
<td>SF (%)</td>
<td>47 (44-50)</td>
<td>40 (33-50)</td>
<td>36 (26-49)</td>
</tr>
<tr>
<td>EF (%)</td>
<td>61 (57-70)</td>
<td>57 (45-67)</td>
<td>40 (34-73)</td>
</tr>
</tbody>
</table>

Table 3.2. Electrocardiographic and echocardiographic findings in 8 healthy dairy cows after one single oral dose of monensin (50 mg/kg, Group B). Interquartile range (25th and 75th percentile). LADs, left atrial diameter at end-systole. LVDd, left ventricular diameter at end-diastole. LVDs, left ventricular diameter at end-systole. SA, shortening area. SF, shortening fraction. EF, ejection fraction. *p ≤ 0.025 compared to baseline.
<table>
<thead>
<tr>
<th>Cow number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin dose (mg/kg)</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Time of death after monensin (h)</td>
<td>80</td>
<td>80</td>
<td>144</td>
<td>94</td>
<td>144</td>
<td>120</td>
<td>144</td>
<td>100</td>
<td>144</td>
<td>70</td>
</tr>
<tr>
<td>Maximum cTnI (ng/mL)</td>
<td>0.01</td>
<td>1.04</td>
<td>45.53</td>
<td>1.65</td>
<td>0.27</td>
<td>11.8</td>
<td>0.29</td>
<td>14.82</td>
<td>32.34</td>
<td>68.54</td>
</tr>
<tr>
<td>Maximum CK (IU/L)</td>
<td>366</td>
<td>1,699</td>
<td>40,080</td>
<td>3,632</td>
<td>314</td>
<td>565</td>
<td>404</td>
<td>367</td>
<td>3,834</td>
<td>32,162</td>
</tr>
<tr>
<td>Macroscopic lesions at necropsy*</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Total histopathologic heart score</strong></td>
<td>18.6</td>
<td>30.8</td>
<td>41.1</td>
<td>44.4</td>
<td>5.8</td>
<td>49.3</td>
<td>9.7</td>
<td>45.3</td>
<td>30.3</td>
<td>41.8</td>
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<tr>
<td>Left auricle</td>
<td>0.9</td>
<td>3.5</td>
<td>7.4</td>
<td>5.2</td>
<td>0</td>
<td>3.8</td>
<td>0.9</td>
<td>5.6</td>
<td>5.7</td>
<td>7.4</td>
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<tr>
<td>Left atrium</td>
<td>3.5</td>
<td>4.5</td>
<td>7.3</td>
<td>4.6</td>
<td>0.5</td>
<td>5.7</td>
<td>1.7</td>
<td>6.5</td>
<td>4.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Right auricle</td>
<td>1.9</td>
<td>3.4</td>
<td>6.5</td>
<td>5.7</td>
<td>0.1</td>
<td>6.3</td>
<td>1.0</td>
<td>5.9</td>
<td>3.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Right atrium</td>
<td>2.1</td>
<td>3.1</td>
<td>4.8</td>
<td>5.8</td>
<td>1.0</td>
<td>5.2</td>
<td>1.0</td>
<td>5.1</td>
<td>3.8</td>
<td>3.1</td>
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<tr>
<td>LV FW</td>
<td>2.6</td>
<td>3.1</td>
<td>2.3</td>
<td>5.6</td>
<td>0</td>
<td>5.8</td>
<td>0.5</td>
<td>3.5</td>
<td>2.1</td>
<td>3.3</td>
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<tr>
<td>IVS</td>
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<td>3.7</td>
<td>2.9</td>
<td>3.7</td>
<td>0.1</td>
<td>5.1</td>
<td>1.0</td>
<td>4.8</td>
<td>2.3</td>
<td>3.5</td>
</tr>
<tr>
<td>RV FW</td>
<td>1.0</td>
<td>2.9</td>
<td>4.8</td>
<td>4.4</td>
<td>1.7</td>
<td>5.8</td>
<td>1.8</td>
<td>5.1</td>
<td>2.8</td>
<td>6.0</td>
</tr>
<tr>
<td>LV apex</td>
<td>1.5</td>
<td>3.4</td>
<td>3.1</td>
<td>5.0</td>
<td>1.8</td>
<td>6.0</td>
<td>0.7</td>
<td>4.2</td>
<td>3.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Papillary muscle</td>
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<td>3.2</td>
<td>2.0</td>
<td>4.4</td>
<td>0.6</td>
<td>5.6</td>
<td>1.1</td>
<td>4.6</td>
<td>3.1</td>
<td>2.9</td>
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Continued

Table 3.3. Maximum concentration of cardiac troponin I (cTnI) and creatine-kinase (CK) and results of gross and histopathologic examination of 10 dairy cows administered one oral dose of monensin.
<table>
<thead>
<tr>
<th>Muscles</th>
<th>3.1</th>
<th>5.7</th>
<th>15.4</th>
<th>12.5</th>
<th>0</th>
<th>6.6</th>
<th>5.1</th>
<th>17.1</th>
<th>9</th>
<th>8.5</th>
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</thead>
<tbody>
<tr>
<td>M. biceps femoris</td>
<td>1.4</td>
<td>1.8</td>
<td>6.2</td>
<td>3.4</td>
<td>0</td>
<td>2.2</td>
<td>1.4</td>
<td>3.7</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>M. quadriceps</td>
<td>0.5</td>
<td>1.6</td>
<td>0.5</td>
<td>1.6</td>
<td>0</td>
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<td>1.1</td>
<td>5.3</td>
<td>2.2</td>
<td>0.8</td>
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<tr>
<td>Diaphragm</td>
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<td>2.3</td>
<td>1.7</td>
<td>4.2</td>
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<td>1.2</td>
<td>1.5</td>
<td>4.6</td>
<td>2.3</td>
<td>1.1</td>
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<tr>
<td>Tongue</td>
<td>n.d.</td>
<td>n.d.</td>
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<td>3.3</td>
<td>0</td>
<td>1.5</td>
<td>1.1</td>
<td>3.5</td>
<td>2.8</td>
<td>4.7</td>
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Table 3.3 Continued
<table>
<thead>
<tr>
<th>Time (h)</th>
<th>n</th>
<th>cTnI (ng/mL)</th>
<th>CK (IU/L)</th>
<th>AST (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference range</td>
<td>8</td>
<td>0-0.06</td>
<td>90-310</td>
<td>50-120</td>
</tr>
<tr>
<td>Baseline</td>
<td>8</td>
<td>0.01 (0.01–0.02)</td>
<td>205 (143-434)</td>
<td>74 (63-116)</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>0.04 (0.02-0.53)</td>
<td>231.5 (189-388)</td>
<td>80 (70-106)</td>
</tr>
<tr>
<td>24</td>
<td>8</td>
<td>0.40 (0.04-2.40)</td>
<td>193 (169-320)</td>
<td>87 (81-114)</td>
</tr>
<tr>
<td>36</td>
<td>8</td>
<td>0.78 (0.04-1.89)</td>
<td>187 (169-258)</td>
<td>100 (84-129)</td>
</tr>
<tr>
<td>48</td>
<td>8</td>
<td>0.53 (0.05-1.10)</td>
<td>203 (152-245)</td>
<td>113 (88-135)</td>
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<tr>
<td>72</td>
<td>8</td>
<td>0.47 (0.12-7.40)</td>
<td>290 (193-1,475)</td>
<td>144 (106-227)</td>
</tr>
<tr>
<td>96</td>
<td>7</td>
<td>0.60 (0.21- 3.0)</td>
<td>367 (259-3,632)</td>
<td>172 (116-255)</td>
</tr>
<tr>
<td>120</td>
<td>5</td>
<td>3.00 (0.16- 6.00)</td>
<td>565 (293-10,934)</td>
<td>179 (123-992)</td>
</tr>
<tr>
<td>144</td>
<td>4</td>
<td>16.00 (0.4- 39.00)</td>
<td>2114 (312-31,019)</td>
<td>252 (136-2438)</td>
</tr>
</tbody>
</table>

Table 3.4. Concentration of cardiac troponin I (cTnI), CK (creatine-kinase) and AST (aspartate aminotransferase) (median and interquartile range) before (baseline) and after administration of a single oral dose of monensin (50 mg/kg) to 8 healthy dairy cows.
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BUN (mg/dL)</th>
<th>Crea (mg/dL)</th>
<th>$K^+$ (mEq/L)</th>
<th>TCa (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference range</td>
<td>8</td>
<td>4-31</td>
<td>0.7-1.6</td>
<td>3.9-5.2</td>
<td>8.6-10.0</td>
</tr>
<tr>
<td>Baseline</td>
<td>8</td>
<td>8 (5-10)</td>
<td>1.4 (1.2-1.7)</td>
<td>3.9 (3.4-4.1)</td>
<td>9.9 (9.5-10.5)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13 (12-16)</td>
<td>1.3 (1.1-1.5)</td>
<td>3.9 (3.7-4.2)</td>
<td>9.6 (9.0-10.1)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>16 (13-19)</td>
<td>1.3 (1.1-1.6)</td>
<td>3.5 (3.4-3.7)</td>
<td>8.6 (8.3-9.2)</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>16 (14-18)</td>
<td>1.3 (1.1-1.5)</td>
<td>3.1 (2.9-3.8)</td>
<td>8.6 (8.3-8.9)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>20 (15–23)</td>
<td>1.4 (1.2-1.6)</td>
<td>3.0 (2.8-3.2)</td>
<td>8.2 (7.8-8.7)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>37 (26-50)</td>
<td>2.3 (1.7-3.6)</td>
<td>3.0 (2.7-3.2)</td>
<td>7.6 (6.9-8.2)</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>55 (25-77)</td>
<td>2.2 (1.4-5.1)</td>
<td>3.0 (2.7-3.2)</td>
<td>7.5 (7.3-8.6)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>48 (24-88)</td>
<td>1.9 (1.7-5.2)</td>
<td>3.1 (2.6-3.7)</td>
<td>8.4 (7.4-8.7)</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>32 (16-44)</td>
<td>1.6 (1.3-1.7)</td>
<td>2.8 (2.6-3.2)</td>
<td>8.5 (7.8-9.0)</td>
</tr>
</tbody>
</table>

Table 3.5. Concentration of BUN (blood urea nitrogen), Crea (creatinine), $K^+$, and TCa (total calcium), (median and interquartile range) before (baseline) and after administration of a single oral dose of monensin (50 mg/kg) to 8 healthy dairy cows.
Figure 3.1. Serum cardiac troponin I (cTnI) concentration in 8 cows after a single oral
dose of monensin (50 mg/kg). Median and interquartile range (25th and 75th percentile).
Figure 3.2. Histopathological changes of the myocardium observed after monensin administration in cattle. Longitudinal sections; HE-stain; 400 x.

A. Normal left auricular myocardium (control cow).

B. Mild swelling of myocardial fibers with numerous small cytoplasmatic vacuoles and multifocal loss of striation. Left auricular myocardium (cow # 4).

C. Moderate to severe swelling of myofibrils with diffuse loss of striation. Merging of vacuoles. Early dissolution of the cytoplasm of the myofibers. Left atrial myocardium (cow # 10).

D. Loss of striation of all myofibers and coagulative necrosis with fragmentation. Left auricular myocardium (cow # 10).
BIBLIOGRAPHY


34. Rajan GP, Zellweger R. Cardiac troponin I as a predictor of arrhythmia and ventricular dysfunction in trauma patients with myocardial contusion. J Trauma 2004;57:801-808.


79. Collinson PO, Gaze DC. Biomarkers of cardiovascular damage and dysfunction—an overview. Heart Lung Circ. 2007;16:S71-S82


