PEER REVIEWED

Assessment and comparison of electrocardiographic and clinical cardiac evidence of death following use of a penetrating captive bolt for euthanasia of cattle

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Abstract

The objective of this study was to describe temporal associations between cardiac auscultation and ECG to evaluate cardiac activity and determine cardiac death following euthanasia with a penetrating captive bolt device. Twenty-two feedlot calves between 6 and 19 months of age were enrolled. Electrocardiographic monitoring and recording was conducted using a modified base-apex lead configuration. Cardiac death was defined as ventricular standstill and occurred when ventricular electrical activity ceased. Clinical cardiac death was defined as the absence of an auscultable and rhythmic heartbeat, and was assessed immediately following the final captive bolt administration and again at 1-minute intervals for a minimum of 10 minutes post-captive bolt administration. Discernable cardiac activity was demonstrated for an average of 513.35 seconds (8 minutes 34 seconds) post-captive bolt administration. Clinical cardiac death and ventricular standstill occurred within the same 60-second measurement in 33% (7/21) of calves. Thus, approximately 70% of calves were assumed to be deceased based on clinical cardiac parameters prior to ventricular standstill.

Key words: bovine, ECG, captive bolt, euthanasia

Résumé

L'objectif de cette étude était de décrire l'association temporelle entre l'auscultation cardiaque et l'ECG pour évaluer l'activité cardiaque et déterminer la mort cardiaque. L'étude comportait 22 veaux de parc d'engraissement entre 6 et 19 mois d'âge. La surveillance électrocardiographique et l'enregistrement ont été faits avec une configuration modifiée avec direction apex-base. La mort cardiaque impliquait l'arrêt ventriculaire et était déclarée lorsque l'activité électrique ventriculaire cessait. La mort cardiaque clinique était déclarée en l'absence de battement rythmique du cœur suite à l'auscultation faite immédiatement après le dernier coup du pistolet à tige captive. L'auscultation est faite à une minute d'intervalle et ce pendant 10 minutes suivant l'utilisation du pistolet à tige captive. Une activité cardiaque discernable a été démontrée pendant en moyenne 513.35 secondes (8 minutes 34 secondes) suite au dernier coup du pistolet à tige captive. La mort cardiaque clinique et l'arrêt ventriculaire ont pris place dans le même intervalle de 60 secondes dans 33% des cas (7 veaux sur 21). Par conséquent, le décès basé sur les paramètres cardiaques cliniques était assumé chez approximativement 70% des veaux avant l'arrêt ventriculaire. Il est nécessaire de confirmer l'arrêt cardiaque avant de déclarer la mort. Les vétérinaires et les autres intervenants impliqués dans l'euthanasie des bovins devraient donc s'assurer que le pistolet à tige captive causent assez de dommage pour rendre l'animal complètement inconscient et de surveiller l'animal jusqu'à ce que la mort soit confirmée.

Introduction

Euthanasia means a "good death" or one that occurs with minimal pain and distress.[13] Death may be further defined as an irreversible loss of cerebral brain and brainstem functions or, more generally, as a "permanent cessation of all vital bodily functions".[14] The use of a penetrating or non-penetrating (in calves) captive bolt followed by the application of an adjunctive method to assure death is an acceptable method for bovine euthanasia.[13] When used correctly, a penetrating captive bolt causes significant and permanent brainstem and cerebral trauma. This trauma may be sufficient to cause death but a secondary step, such as exsanguination, pithing, potassium chloride or magnesium sulfate overdose, or a second shot with the captive bolt device, should be planned to ensure death occurs.[9,10,13]

Parameters used to confirm clinical death among bovines include lack of pulse or heartbeat, absence of rhythmic respiration, and absence of reflexes such as corneal or palpebral reflexes, as well as rigor mortis and gray mucous membranes.[1,5,13] Methods outlined by the American Veterinary Medical Association's Panel on Euthanasia to confirm death in mammals include auscultation, electrocardiography (ECG), doppler ultrasound, and pulse oximetry.[13]

Several published reports describe bovine euthanasia.[8,10,13,17] Blackmore described the use of ECG to measure cardiac electrical activity following stunning at slaughter.[3] However, the current literature does not contain information describing cardiac electrical activity utilizing ECG in cattle euthanized with a penetrating captive bolt device. In addition, ECG patterns have not been correlated with clinical signs of death in cattle.

The purpose of this study was to describe temporal associations between cardiac auscultation and ECG to evaluate cardiac activity and determine cardiac death. In addition, ECG changes during euthanasia of cattle with a penetrating captive bolt device were characterized.

Materials and Methods

The reported study is a hypothesis generating study conducted using cattle enrolled in a clinical trial. The original study was designed to evaluate the use of a proprietary penetrating captive bolt device with a built-in low pressure air channel pithing mechanism as a single-step method for humane euthanasia of cattle in a mass depopulation scenario. Since there was no control group and all study animals had the same intervention applied (captive bolt), researchers were not blinded. Here, we describe aspects of the study relevant to the question of whether confirmation of clinical death criteria occurred simultaneously with the cardiac death defined by ECG. The rationale for this secondary use of the cattle was to maximize the obtainable information from research animals. Prior to initiating the study, protocol approval was obtained from the Iowa State University (ISU) Institutional Animal Care and Use Committee (IACUC 2-12-7296-B).

Study Location and Animal Sourcing

The study was conducted over a 3-day period in January 2014. Angus-cross calves between 6 and 19 months of age were obtained during the feedlot phase of production from private Iowa-based operations and transported to the Iowa State University Lloyd Veterinary Medical Center the morning they were scheduled to be euthanized. Prior to euthanasia, enrolled calves were housed in the food animal ward of the veterinary teaching hospital.

Enrollment of Animals

Twenty-two feedlot calves (10 heifers, 12 steers) ranging from 339 to 1100 lb (154 to 499 kg) were enrolled. All calves were designated as "chronics" or "treatment failures" at the feedlot or farm of origin, and were unlikely to reach typical market weight with their pen mates. Reasons for culling were chronic lameness, respiratory disease, or a combination of both. An observational examination was conducted on each calf to assess its fitness to walk approximately 100 feet (31 meters) from the holding area to the euthanasia site.

Clinical and Electrocardiographic Data Collection

Prior to euthanasia, calves were individually restrained in a self-catching head gate. Electrocardiographic monitoring and recording was conducted using a Televet 100 version 5.0.0 veterinary telemetric ECG system[a], and a modified base-apex lead configuration on a 4-lead ECG monitor was used to generate the electrocardial activity readouts. Limb leads were connected using subcutaneously placed stainless steel wire loops and connected to alligator clips fixed on the monitor cable. Isopropyl alcohol was used to improve conductivity. Leads were placed in the following locations: yellow electrode in the left distal jugular furrow; black electrode in the right distal jugular furrow; green electrode on the left lateral thoracic wall at the level of the olecranon; and the red electrode was placed approximately 4 inches (10 cm) ventral from thoracic vertebra 10.

Electrocardiographic monitoring and recording was conducted by a single researcher and was initiated a minimum of 1 minute prior to captive bolt administration. Electrical activity was recorded to establish baseline cardiac activity and was continuously recorded throughout the euthanasia process for up to approximately 15 minutes post-captive bolt administration, but no less than 10 minutes post-captive bolt administration. Once placed on the live animal, the real-time ECG was recorded using a portable laptop computer.

Calves were euthanized using a prototype portable pneumatic captive bolt device[b] with low pressure air channel pithing and a retractable bolt. The intended anatomical site was on the midline of the frontal skull approximately half-way between 2 lines—1 drawn from 1 lateral canthus to the opposite lateral canthus and the other drawn laterally across the poll.[9] If the captive bolt device was inadvertently misdirected, the animal was immediately shot again. Clinical cardiac death was defined as the absence of an auscultable and rhythmic heartbeat. To evaluate clinical indicators of cardiac viability, the presence or absence of an auscultable heartbeat and the presence or absence of a rhythmic heartbeat were assessed by the same researcher capturing the ECG data. Clinical cardiac parameters were assessed in "real time" immediately following the final captive bolt administration, and then at 1 minute intervals for a minimum of 10 minutes following captive bolt administration or until clinical cardiac death was determined. Auscultable heartbeat was assessed for audibility and consistency using a stethoscope[c] placed in the left axillary region to detect the presence of cardiac activity. If the heartbeat was detectable and rhythmic (occurring at regular intervals as expected from the sinus node), rhythmic heartbeat was noted as "present". Using the ECG data, cardiac death was defined as ventricular standstill and occurred when ventricular electrical activity, and thus contractions and cardiac output, ceased. Individual animal standardized ECGs were saved as .ecg files and electronically stored for analysis following the euthanasia procedure. Prior to evaluation of ECG data, ECG data were evaluated for each calf to screen for pre-existing problems such as arrhythmias, cardiomyopathies, cardiomegaly or chamber enlargements, or other potential cardiac anomalies that could have affected the resulting cardiac response to the use of a captive bolt.

Heart rate was determined by measuring the R-R interval (the time between the peak of 1 R wave to the peak of the next R wave). This was measured using the calipers in the software, which then calculated the heart rate (rate = 60/(R-R interval)). Measurements included mean R-R interval heart rates prior to and immediately following captive bolt administration as soon as there was a readable ECG (within 60 seconds post-captive bolt administration), then at time of clinical cardiac death and at the time the ECG was devoid of ventricular activity. Following captive bolt administration, the ECG for each calf was evaluated for heart rate, signs of anoxia or cardiac compensatory events, and presence of ventricular standstill. Cardiac anoxia was recorded when aberrations or alterations in T-wave configuration, S-T segment elevation or depression, and heart rate were noted. Cardiac compensatory mechanisms are the heart's attempt to compensate for lack of oxygen, or in this case, the failure of respiration following captive bolt administration. Cardiac compensatory indicators included increases in R and T wave amplitudes, measured on the ECG, and heart rate.

In addition, calves were evaluated immediately post-shot for other indicators of clinical death or unconsciousness, including the presence or absence of corneal reflex, palpebral reflex, righting reflex, vocalization, respiration, and involuntary movement. The results of this work are described more fully in a future manuscript.

Data Collection and Analysis

All clinical cardiac data were collected using a spreadsheet[d]. Recorded ECG data was saved with the following information documented on each individual animal's .ecg file: individual animal identification, gender, date of euthanasia, and farm of origin. Electrocardiography data were analyzed by a registered veterinary technician with cardiology expertise. Measurements were made directly on the computer using the Televet 100 version 5.0.0 software provided with the unit. Electrocardiographic analysis and interpretation was conducted until ventricular standstill was noted or available ECG recording had been completely examined. Descriptive statistics such as ranges, means, and proportions were generated using the Excel toolbar. The unit of analysis was the individual animal. Outcome variables subjected to descriptive analysis included auscultable heartbeat, rhythmic heartbeat, ECG heart rate, ventricular standstill, and clinical cardiac death.

Results and Discussion

One of the 22 calves was eliminated from the study because the ECG was inadvertently removed prior to determination of clinical death. No pre-existing cardiac abnormalities were detected with the ECG analysis, therefore 21 calves were enrolled in the study.

Cumulative incidence of cessation of auscultable heartbeat, rhythmic heartbeat, and onset of ventricular standstill over time are depicted in Figure 1. Once an auscultable heartbeat ceased in a calf, it was not detected again at a later time point. When electrical cardiac activity was examined through analysis and interpretation of the recorded ECG files, discernable activity was demonstrated for an average of 513.35 seconds (8 minutes 34 seconds) post-captive bolt administration, and ranged from 287 seconds (4 minutes 47 seconds) to 703 seconds (11 min 43 seconds). Mean heart rate for all calves prior to use of the captive bolt was 122 beats per minute (BPM), and ranged from 78 to 197 BPM. Immediately following captive bolt administration (within 60 seconds), mean heart rate was 126 BPM and ranged from 77 to 127 BPM. As expected, heart rate slowed when approaching time of clinical cardiac and cardiac electrical death. At time of clinical cardiac death, mean ECG heart rate was 56 BPM and ranged from 0 to 146 BPM. Immediately prior to permanent cessation of ventricular activity, mean BPM decreased to 25 and ranged from 0 to 69 BPM. The ECG from 19 of the calves indicated there was oxygen deprivation immediately following the use of the captive bolt, including S-T segment depression or elevation, T wave inversion, or T wave amplitude changes. Since rhythmic respiration ceased immediately following captive bolt administration, signs of oxygen deprivation are not unexpected. The ECGs from 6 of the 19 study calves (32%) showed more than 1 of these changes. Nine (43%) calves had ECGs indicating the heart was responding in a compensatory manner. Fifteen (71%) calves showed ventricular standstill within 10 minutes, and 20 calves (95%) showed cardiac standstill within 15 minutes. One calf did not reach ventricular standstill prior to the end of the ECG recording and had discernable cardiac activity that extended beyond 15 minutes 34 seconds after captive bolt administration (Figure 1).

Corneal reflexes, righting reflexes, vocalization, and respiration were absent immediately post-captive bolt administration. Time post-captive bolt administration for onset of clinical cardiac death (cessation of both rhythmic and auscultable heartbeat) was compared to time of cardiac electrical death (ventricular standstill). Clinical cardiac death parameters had been met for all enrolled calves prior to disconnecting ECG leads. Twenty of the 21 calves experienced clinical cardiac death at 10 minutes following captive bolt administration. One calf still had an auscultable heartbeat until 11 minutes, 27 seconds after captive bolt administration.

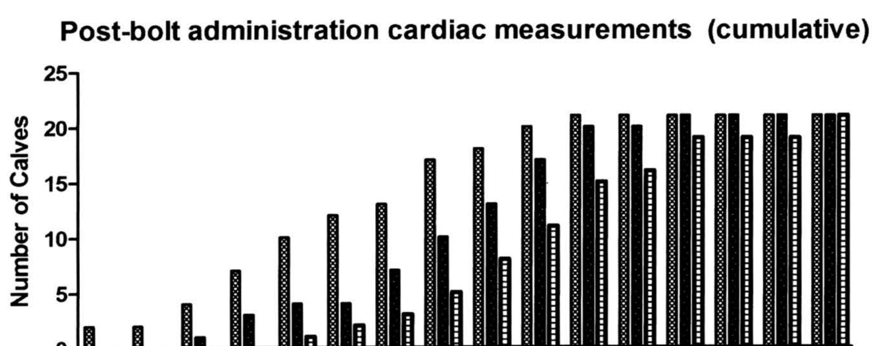
Clinical death and cardiac electrical death do not necessarily occur simultaneously in bovines following euthanasia with a captive bolt device. In the present study, cardiac death, defined as ventricular standstill, typically occurred after clinical cardiac death. Seventy percent of ECGs from study animals demonstrated continued cardiac electrical activity following time of clinical cardiac death. Clinical cardiac death and ventricular standstill occurred within the same 60-second measurement in 33% of calves; therefore, approximately 70% of the calves were assumed to be dead based on clinical cardiac parameters prior to the complete cessation of electrical cardiac activity. Although the heart still has discernable electrical activity, it is not auscultable, likely due to decreased cardiac contractility resulting in reduced valvular sounds. This result is similar to findings from previously reported equine and canine studies.[4,7] Both of these studies reported continued electrical cardiac activity following signs of clinical cardiac death.

The inconsistency in time of onset between clinical death and cardiac death may be explained by the possibility of pulse-less electrical activity (PEA). Pulse-less electrical activity has been described in humans and has also been suggested to occur in equines.[2,4,15,16] Pulse-less electrical activity occurs when precordial impulses (palpable cardiac impulse noted on thoracic wall) are absent and the patient or animal is unresponsive but organized cardiac electrical activity is still noted. Once PEA is identified, the prognosis for recovery is poor and is rarely reversed in humans.[2,16]

Though potential for consciousness in calves following captive bolt administration was not measured using electroencephalography, it is unlikely that animals were sensible. Lambooij et al reported that correlation dimension and changes in beta, delta, and theta waves indicate immediate unconsciousness after proper captive bolt administration.[12] Due to the significant brain trauma[6] resulting from the correct placement and use of a penetrating captive bolt in this study, it is assumed that return to full sensibility and consciousness would be improbable. In addition to evaluating the placement, and thus likely trajectory of the captive bolt, it is important to assess indications of clinical cardiac death in the context of other clinical signs, such as corneal reflexes.

Enrolled animals were chronically ill cattle obtained from local feedlots. In contrast to chemical euthanasia methods such as barbiturates or inhalation agents, captive bolt euthanasia does not rely on normal body functions to deliver the euthanasia agent to the brain. When a penetrating captive bolt is properly applied to euthanize cattle, lethal physical brain damage to the medulla oblongata disrupts both central control of heart rate as well as respiration. Cardiac anoxia subsequent to lack of respiration then causes the automaticity of the sinus node to fail and the heart to stop its electrical activity and stop beating.[18] Thus, health status prior to euthanasia likely did not affect the outcomes measured in this study.

Whenever a penetrating captive bolt is used for bovine euthanasia, it is recommended that a secondary euthanasia step be taken once unconsciousness is achieved to ensure death of the animal. Inaccurate placement of the captive bolt may not result in sufficient brain trauma to cause death without the use of a secondary euthanasia step. Following application of euthanasia procedures, cattle should be monitored until death is confirmed.



Minutes post-bolt administration	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No rhythmic heartbeat	2	2	4	7	10	12	13	17	18	20	21	21	21	21	21	21
No auscultable heartbeat	0	0	1	3	4	4	7	10	13	17	20	20	21	21	21	21
Ventricular standstill	0	0	0	0	1	2	3	5	8	11	15	16	19	19	19	21

Figure 1. Cumulative incidence auscultable heartbeat, rhythmic heart, and ventricular standstill following captive bolt administration. Numbers in the table below reflect total number of calves out of 21 animals in the study.

Conclusions

Electrical activity of the heart after captive bolt administration often persists beyond typical measures of death, such as corneal reflexes, respiration, and heartbeat. Death requires confirmation of cardiac arrest and veterinarians and others involved with bovine euthanasia should ensure that use of the captive bolt results in sufficient damage to render the animal completely unconscious and then monitor the animal until death is confirmed. Proper administration of the primary and any necessary secondary euthanasia steps as well as consideration of available clinical parameters to accurately ascertain insensibility and death are critical to assure that a humane death occurs.

Endnotes

[a]Engel Engineering Services GmbH, Offenbach, Germany
[b]Jarvis USSS-3, Jarvis Products Corporation, Middleton, CT
[c]3M Littman Classic II SE stethoscope, 3M Center, St. Paul, MN
[d]Microsoft. Microsoft Excel. Redmond, Washington:Microsoft, 2010

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References

1. American Association of Bovine Practitioners. AABP Practical Euthanasia of Cattle. 2013 Available at: http://www.aabp.org/resources/AABP_Guidelines/Practical_Euthanasia_of_Cattle-September_2013.pdf. Accessed July 24, 2014.
2. Aufderheide TP. Etiology, electrophysiology and myocardial mechanics of pulseless electrical activity. In: *Cardiac arrest. The science and practice of resuscitation medicine.* 2nd ed. Cambridge, UK: Cambridge University Press, 2007; 426-446.
3. Blackmore DK. Electrocardiograms as an aid in assessing the stunning of cattle and pigs. *N Z Vet J* 1989;37:59-61.
4. Buhl R, Andersen LO, Karlshøj M, Kanters JK. Evaluation of clinical and electrocardiographic changes during the euthanasia of horses. *Vet J* 2013; 196:483-491.
5. Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D, Warwick C. Recommendations for euthanasia of experimental animals: Part 2. DGXT of the European Commission. *Lab Anim* 1997; 31:1:1-32.
6. Derscheid RJ, Dewell RD, Dewell GA, Klienhenz K, Shearer L, Shearer JK. Validation of a portable pneumatic captive-bolt device as a single-step method of euthanasia of feedlot cattle. Poster. Presented at Iowa State University College of Veterinary Medicine Annual Research Symposium, January 07, 2015.
7. Evans AT, Broadstone R, Stapleton J, Hooks TM, Johnston SM, McNeil JR. Comparison of pentobarbital alone and pentobarbital in combination with lidocaine for euthanasia of dogs. *J Am Vet Med Assoc* 1993; 203:664-666.
8. Gardner DL. Practical and humane methods for bovine euthanasia. *Vet Med* 1999; 94: 92-93.
9. Gilliam JN, Shearer JK, Bahr RJ, Crochik S; Woods J; Hill J; Reynolds J; Taylor JD. Evaluation of brainstem disruption following penetrating captive bolt shot in isolated cattle heads: comparison of traditional and alternative shot placement landmarks. *Proceedings.* 4th International Symposium on Beef Cattle Welfare 2014.
10. Gregory N, Shaw F. Penetrating captive bolt stunning and exsanguination of cattle in abattoirs. *J Appl Anim Welf Sci* 2000;3:215-230. Available at: http://dx.doi.org/10.1207/S15327604JAWS0303_3. Accessed July 24, 2014.
11. Gregory NG, Wotton SB. Time to loss of brain responsiveness following exsanguination in calves. *Res Vet Sci* 1984;37:141-143.
12. Lambooij E, van der Werf JTN, Reimert HGM, Hindle VA. Restraining and neck cutting or stunning and neck cutting of veal calves. *Meat Sci* 2012; 91:22-28.
13. Leary SL. American Veterinary Medical Association. AVMA guidelines for the euthanasia of animals: 2013 edition. Available at: https://www.avma.org/KB/Policies/Documents/euthanasia.pdf. Accessed June 12, 2014.
14. Merriam-Webster.com. Death. Available at: http://www.merriam-webster.com/medical/death. Accessed June 27, 2014.
15. Rosman HS. Precordial impulses. In: Walker HK, Hall WD, Hurst JW, eds. Clinical methods: the history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths, 1990. Available at: http://www.ncbi.nlm.nih.gov/books/NBK322/. Accessed July 24, 2014.
16. Shah SN, Shah AN. Pulseless electrical activity. Medscape. Chief editor: JN Rottman. Available at: http://emedicine.medscape.com/article/161080-overview#showall Accessed July 31, 2014.
17. Shearer JK. Euthanasia of cattle: indications and practical considerations. *Proceedings.* North American Veterinary Conference Large Animal 2005;19:28-29.
18. Ware WA. *Cardiovascular disease in small animal medicine.* 1st ed. London: Manson Publishing, 2007; 14-15.