Assessment of time to death, brain tissue damage and clinical signs of consciousness in mature Jersey x Holstein cattle receiving a secondary penetrating captive bolt gunshot at the poll or frontal sinus locations

***Jesse A. Robbins**,[1] PhD; **Helstrom Sanchez**,[2] **Conrad Spangler**,[2] DVM; **Sergio Vazquez**,[2] **Nate Spatz**,[2] **Brett Boyum**,[2] DVM

[1] Independent Researcher, Carlisle, IA 50047
[2] Riverview LLP, Morris, MN 56267

***Corresponding author:** Dr. Jesse Robbins, **jesserobbins.ubc@gmail.com**

Abstract

Providing cattle with a good death is an ethical imperative for the dairy industry. When using a penetrating captive bolt gun (PCBG), a secondary (adjunctive) method is one of several options currently recommended. Some farms prefer to apply the secondary shot in the poll location whereas others prefer to apply a second shot in the frontal-sinus location. Unfortunately, little is known about the efficacy of these alternative secondary shot locations. The purpose of this study was to compare clinical signs of consciousness and time to death for 2 different secondary PCBG shot locations in mature Jersey x Holstein cross dairy cattle. Data was collected contemporaneously at 6 dairy operations located in the Midwest U.S. After receiving the first PCBG shot in the frontal-sinus location, cattle requiring euthanasia (n = 46) were randomly assigned to receive a secondary PCBG shot in either the poll (Frontal-Poll) or again in the frontal-sinus location (Frontal-Frontal) and continuously monitored for clinical signs of consciousness (corneal and palpebral reflex, rhythmic breathing, threat response, etc.) and heartbeat. Signs of consciousness were rarely observed immediately following the first frontal-sinus shot. The median time period when the last heartbeat was heard (7-8 min) did not differ between groups ($P = 0.253$) nor did gross brain trauma scores ($P = 0.247$). We conclude the location of the secondary PCBG shot (frontal vs poll) does not seem to impact time to death or gross brain trauma scores. These findings confirm results of previous research involving younger cattle and suggest additional research is needed to better understand the effect of different PCBG locations.

Key words: anatomical landmarks, animal welfare, captive bolt gun, death, euthanasia, humane

Introduction

Ensuring animals have a good death is an essential component of the dairy industry's ethical obligations. Providing animals with a good death requires methods that reliably render animals immediately and persistently unconscious until death with minimal stress to the animal and caretaker.[1,2] Failure to achieve this is not only distressful to animals, but human caretakers as well.[3,4] It is therefore very important for the dairy industry to continually re-evaluate the efficacy of different euthanasia methods.

Many farms utilize penetrating captive bolt guns (PCBG) for euthanasia.[5,6,7] PCBGs may be preferred to the more commonly used gunshot because they are considered safer for farm personnel. PCBGs also avoid regulatory and secondary toxicity concerns associated with chemical euthanasia methods (e.g. barbiturate overdose). Although pneumatic PCBGs are commonly used in many animal slaughter facilities, powder-charged PCBGs are more common in farm environments and both PCBGs function similarly; rapid expansion of gas propels a steel bolt at high velocity through the muzzle and into the animal's head causing concussion and brain trauma that impairs regions controlling respiratory and cardiac function.[8]

Citing one study showing that 0.16%-1.2% of feeder cattle shot in the frontal-sinus location with pneumatic PCBG showed signs of returning to consciousness,[9] the American Veterinary Medical Association (AVMA) has classified PCBGs "acceptable with conditions" and recommends all primary PCBG shots should be followed by a secondary (aka adjunctive) method.[8] Recommended secondary methods include injection of potassium chloride (KCl), pithing, exsanguination and the application of an additional PCBG shot.[8]

Practical experience of the authors indicates that some caretakers may elect to apply this secondary PCBG shot either in the frontal-sinus location (a second shot) or in the poll location. However, using the poll location, even as a secondary location, has been interpreted as violating the American Association of Bovine Practitioner's (AABP) Guidelines for the Humane Euthanasia of Cattle which state, "*poll position stunning with a penetrating captive bolt is not recommended*".[10] Such an interpretation is conceivable since this policy does not explicitly differentiate between the use of poll shots as primary vs secondary methods. This lack of clarity has important practical implications because the National Milk Producer Federation Farmers Assuring Responsible Management (NMPF-FARM) animal welfare program standards state that euthanasia techniques "*must follow the approved methods of the AABP*".[11] Non-compliance with these standards can result in serious penalties for dairy producers and, therefore, a study comparing a primary shot either in the frontal-sinus or in the poll is not currently an option on commercial farms.

Unfortunately, little is known about the relative efficacy of these different secondary shot locations on time to death and clinical signs of consciousness. Previous research comparing different secondary PCBG shot locations failed to detect differences in the persistence of clinical signs of consciousness or time of death;[12] however, this research involved mostly young dairy animals (mean age = 242 d ± 177 (range 14 to 625) and it is not clear whether similar results would be obtained in mature animals with different cranial characteristics. To address this uncertainty, we set out to examine the relative efficacy of 2 secondary shot locations (frontal vs poll) on time to death and clinical signs of consciousness in a sample of mature Jersey x Holstein cross cattle.

Materials and methods

Data collection occurred during the week of January 17, 2022 at 6 dairy operations located in the Midwest U.S. All sites were owned and operated by a single entity, and therefore housing, management and cattle genetics were nearly identical. Cattle enrolled in this study were mature, female, Jersey x Holstein cross cattle identified for euthanasia according to standard farm operating procedures. Animals < 21 months old, those exhibiting neurological symptoms, of a breed other than Jersey x Holstein cross, and/or animals in severe distress that would have been prolonged by their inclusion in this study, were not eligible for enrollment. Institutional Animal Care and Use Committee (IACUC) approval was not acquired for this study as it was conducted at private farms in accordance with all applicable local, state and federal laws and regulations.

Personnel with extensive experience (3-15 years) and documented annual training in the same euthanasia training program developed by veterinarians, were recruited from each site to assist in data collection. Prior to data collection, all personnel met to discuss and review the study design and data collection process. During this time, it was decided that any cows receiving improperly placed primary shots were to be immediately re-shot and excluded from the study. Personnel were equipped with a stopwatch, forceps, stethoscope and scoring sheet where they recorded the presence or absence of clinical signs every minute until death was confirmed. All PCBGs were thoroughly cleaned by a professional gun cleaner immediately prior to commencement of data collection.

All enrolled cattle received a primary PCBG shot in the frontal-sinus location and were then randomly assigned to receive a secondary PCBG shot in either the frontal-sinus location (Frontal-Frontal) or the poll location (Frontal-Poll) using a random number generator in Excel. Prior to euthanasia, all animals were sedated with a single intramuscular injection of xylazine hydrochloride[a] 0.91 mg/lb [2 mg/kg]). After 5 minutes, recumbent animals were haltered with their head tied off to a back leg or nearby structure (Figure 1) before being shot with a Jarvis HD Long bolt[b] (PAS 4144132; bolt length = 5.88 inches [14.9 cm]; shaft diameter = 0.45 inches) with orange charges (Jarvis .25 cal./3.5 grain). The PCBG muzzle was placed flush, perpendicular to the skull and aimed rostrally toward the intermandibular area. The frontal sinus landmark was located midline on the face, halfway between the top of the poll and an imaginary line connecting the outer canthus at an angle aiming toward the animal's spine. The poll shot landmark was located just behind midline of the external occipital protuberance and angled towards the animal's muzzle (Figure 2).

Assessment of clinical signs of consciousness and time to death began immediately following the first shot (min 0-1), and then continued until death was confirmed. Clinical signs of consciousness were selected on the basis of previous research and are detailed in Table 1.[12] Unconsciousness was defined as the

Figure 1: Jersey x Holstein cow restrained prior to sedation and euthanasia. Photo credit: Jesse Robbins

Figure 2: Anatomical landmarks and trajectories for frontal-sinus (A) and poll shots (B) with penetrating captive bolt gun. Shaded region reflects brain cavity. Photo credit: Ann Sanderson

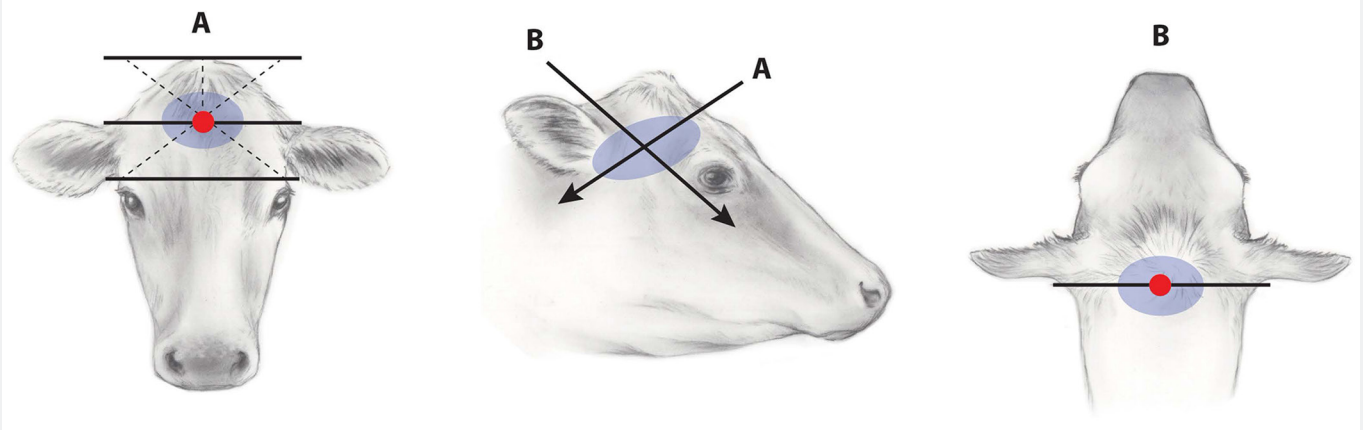

Table 1: Clinical signs used to assess consciousness. Clinical signs are presented in the order they were assessed for each animal. All signs were assessed as binary outcomes (1 = yes/present; 0 = no/absent) each minute until confirmation of death.

Clinical sign	Definition
Threat response	blinks/responds when hand is waved near eyes
Corneal reflex	blinks when gently touching cornea with finger
Palpebral reflex	blinks when dorsal eyelid in gently stroked with finger
Spontaneous, natural blinking	eyes move/blink like sensate animal
Pain response	responds to pinch with forceps to nostril
Rhythmic breathing	thorax/rib movement indicates rhythmic breathing
Vocalization	bawls, bellows, or makes other intentional sounds

absence of all clinical signs assessed.[13] Death was defined as the absence of all clinical signs assessed and a lack of an auscultable heartbeat.[12] Clinical signs were assessed every minute after the first shot in the following order: threat response, corneal and palpebral reflex, natural blinking, pain response, rhythmic breathing, vocalization and heartbeat. Time elapsed between shots was not measured, but was limited to the time necessary for one person to assess clinical signs while the other prepared to deliver the second shot (~20 seconds).

After death was confirmed, shot placements were mapped on the data sheet and heads from 10 randomly selected animals (5 from each group) were removed, frozen and transported to the Iowa State University Veterinary Diagnostic Laboratory for descriptive, post-mortem analysis of brain trauma. Following the methods outlined in a previous study by the authors,[12] heads were allowed to thaw in a cooler for 3 d before being skinned and split mid-sagittally with a bandsaw. Trauma was evaluated with brain in situ and ex situ using a 0-3 scale (0 = tissue is not disrupted; 1 = mild, limited to less than 25% of the affected region; 2 = moderate, 25-75% disruption; 3 = severe, 75% or more destruction of region). Statistical evaluation of all clinical data was conducted in R statistical software.[c] Trauma scores were compared using Welch's t-test. Comparison of time to last heartbeat auscultation by group was conducted using Kaplan-Meier survival analysis with packages "survival", "survminer" and "dplyr". Differences in signs of consciousness, were tested using Fisher's Exact Test to address cells with small values and 0s.

Results

A total of 46 animals were euthanized during the data collection period. Two animals (1 animal from each group) were excluded from analysis due to misplaced primary frontal-sinus shots leaving a final sample of n = 44; Frontal-Poll n = 21; Frontal-Frontal n = 23. The mean age of enrolled cattle was 63.8 mo. ± 17.9 mo. (Range 21-97 mo.) and did not differ between groups (Frontal-Frontal: M = 65.2 SD = 18.2, Range 21:88; Frontal-Poll: M = 62.1, SD = 17.6, range 31-97; $P = 0.575$). Reasons for euthanasia by health record code category were as follows: Musculoskeletal (32%, n = 14); Respiratory (32%, n = 14); Mastitis (5%, n = 2); Digestive (23%, n = 10) and Reproduction (9%, n = 4).

Only one animal (Frontal-Poll group) showed any clinical signs of consciousness at any time after receiving the primary frontal-sinus PCBG shot (i.e. vocalizations during the 2-3 and 3-4 minute time period) thereby obviating the need for statistical comparisons. All animals (n = 44; Frontal-Poll n = 21; Frontal-Frontal n = 23) were confirmed dead within 12 minutes after receiving the primary PCBG shot. The median time period in which the last auscultable heartbeat occurred (7-8 min., range 3-4 and 11-12 min.) did not differ between groups ($P = 0.791$, Figure 3). Kaplan-Meier survival analysis confirmed there was no difference in time to death between animals euthanized using different secondary shot locations ($P = 0.253$, Figure 4).

Descriptive pathology results are listed in Table 2. Due to a data collection error, the Frontal-Frontal group sample included 4 animals (not 5 as planned). The cerebrum was damaged in 100% (9/9) of animals examined and the spinal cord was not damaged in any of the animals assessed (0%). None of the animals in the Frontal-Poll group showed damage to the midbrain or pons whereas 75% (3/4) animals showed damage to these regions in the Frontal-Frontal group. None of the animals in the Frontal-Poll showed damage to the medulla (0%) whereas 1 animal in the Frontal-Frontal showed damage to this area (25%). Welch's t test revealed no difference in gross brain tissue damage ($t = -1.324$, $P = 0.247$) between the 2 groups.

Discussion

This study assessed whether different secondary PCBG shot locations resulted in differences in clinical signs of consciousness and time to death in a sample of mature Jersey x Holstein cross dairy cattle. Our findings replicate earlier work in a sample of younger cattle which also failed to detect any differences in time to death between animals shot a second time in the frontal sinus vs the poll locations.[12] The median time period in which the last auscultable heartbeat occurred for all animals (7-8 minutes) was also consistent with previous research showing this is the approximate amount of time it takes for the heart to stop beating after being shot with a PCBG gun.[12,14]

Descriptive pathology on a small, randomly selected subsample of heads suggested no differences in gross brain tissue damage between the 2 groups. Specifically, destruction of the brainstem was not observed in any of the cadavers assessed which replicates previous research suggesting macroscopic edema and swelling in brain regions other than the brainstem may be sufficient to reliably induce unconsciousness and death.[12,16,17] We urge caution when interpreting our cadaver data since our subsample was very small. Future work should attempt to analyze all animals euthanized to provide a clearer picture of the complex relationship between damage to different brain regions, consciousness and time to death.

Figure 3: Number of cows (n = 41) who lacked an auscultable heartbeat by treatment and time period. Secondary shot occurred between the 0 to 1 min and 1 to 2 min time periods.

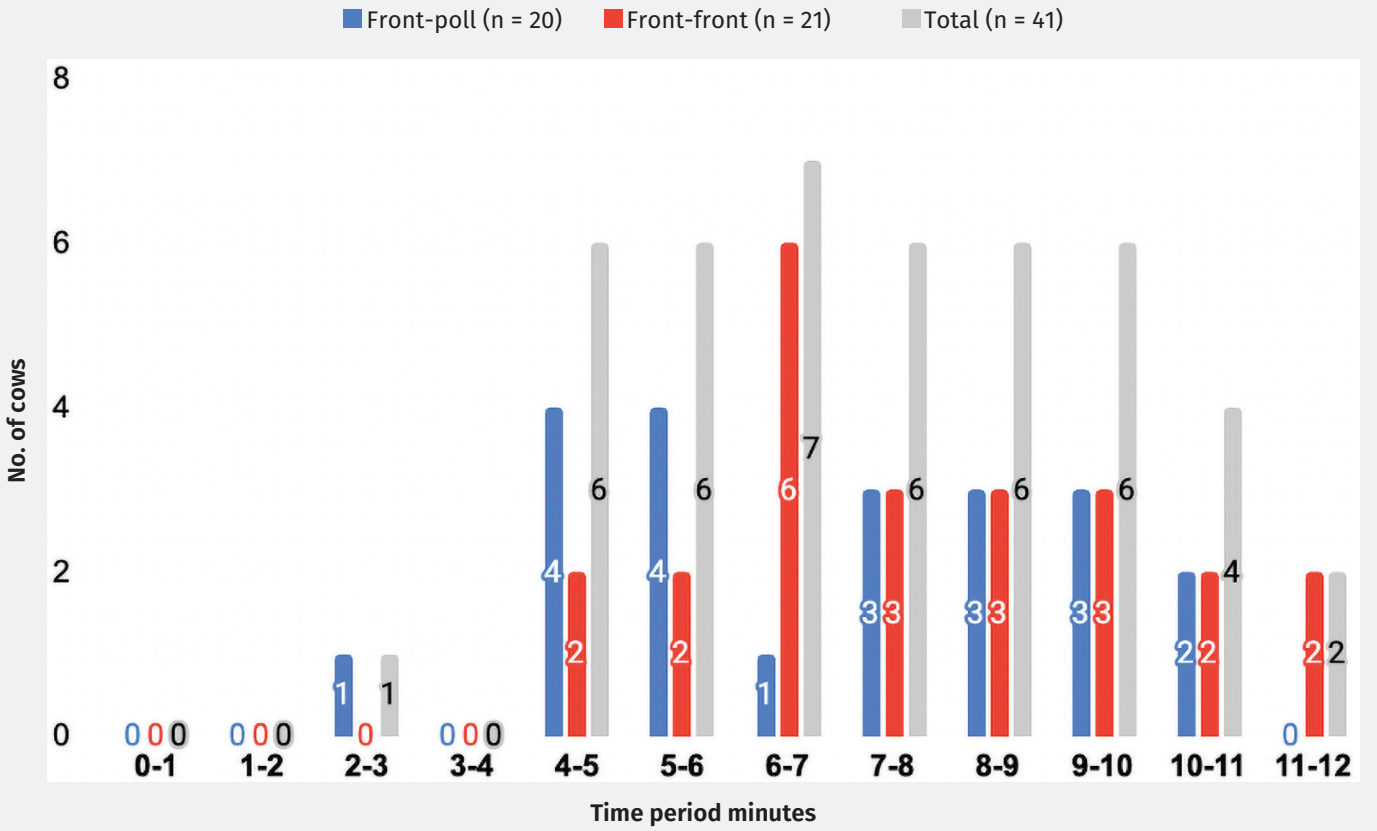

Figure 4: Kaplan-Meier survival plot comparing probability of survival at each time period for Frontal-Poll (blue line) and Frontal-Frontal (red line) groups (n = 44; *P* = 0.253).

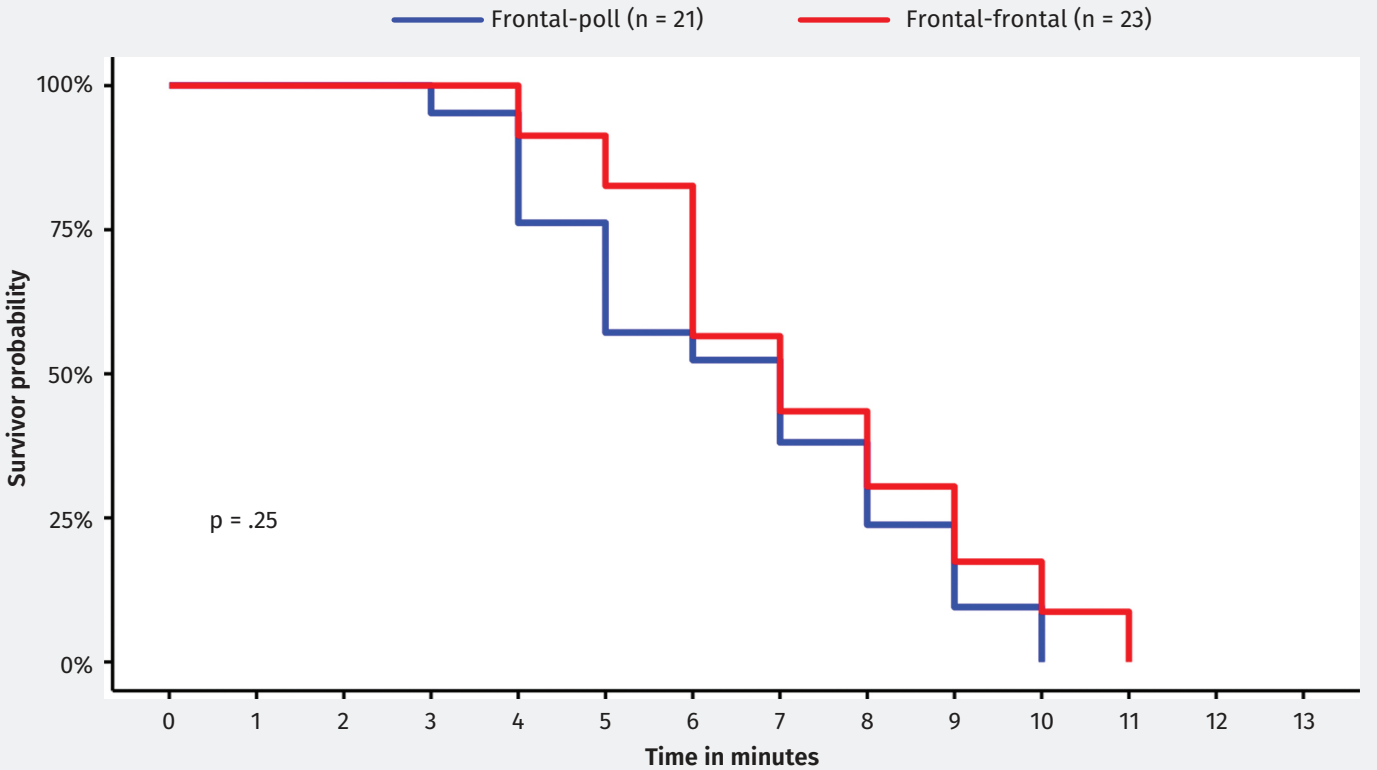

Table 2: Trauma scores by anatomical location for individual animals (n = 9). Anatomical portion of the brain were scored according to a 0-3 scale (0 = tissue is not disrupted; 1 = mild, limited to less than 25% of the affected region; 2 = moderate, 25-75% disruption; 3 = severe, 75% or more destruction of region).

	Frontal-Poll					Frontal-Frontal			
Animal	A	B	C	D	E	F	G	H	I
Cerebrum	1	1	1	2	1	1	1	1	1
Cerebellum	0	0	0	1	0	0	1	0	0
Thalamus	0	1	0	0	2	2	0	0	2
Hypothalamus	1	1	0	0	2	1	0	0	2
Midbrain	0	0	0	0	0	1	1	0	1
Pons	0	0	0	0	0	1	1	0	1
Medulla oblongata	0	0	0	0	0	0	1	0	0
Spinal cord	0	0	0	0	0	0	0	0	0

Several additional limitations of this study also merit mention. Although steps such as the use of standardized data collection sheets with clear descriptions of clinical signs and training on their assessment were implemented, the use of multiple, unblinded observers may have introduced some measurement error and bias. For example, one animal was identified as vocalizing during the 2-3 and 3-4-min time periods; however, after conferring with the observer, these "vocalizations" appeared consistent with reflexive, guttural sounds associated with gasping and not voluntary vocalizations.[13] This is consistent with the fact that this animal did not show any other clinical signs of consciousness. Regardless, future research should consider the feasibility of video recording each euthanasia procedure and evaluation of clinical signs of consciousness to allow for subsequent reliability analyses. Moreover, additional clarification is needed as extant research typically fails to differentiate between different types of sounds exhibited by animals during euthanasia. The inclusion of more objective measures such as electrocardiogram and electroencephalogram would also be very useful in future research.[18,19] Furthermore, although this study involved animal numbers equivalent to, or greater than, previous research used to support existing professional and industry euthanasia policies, and is only the second study involving dairy cattle, it may not have been possible to detect small group differences given the small sample size. This means the lack of group differences is likely attributable to the efficacy of the primary frontal-sinus shot rather than the second shot location and this should be investigated in future studies. Inclusion of a primary-frontal sinus shot-only and primary, poll shot-only would provide valuable insights and should be a priority for future academic research, given that this type of study would not be possible on commercial farms under the current interpretation of industry guidelines without risking sanctions. Given the vital importance of science-based euthanasia standards, we strongly encourage additional larger-scale studies aimed at understanding the relative efficacy of different primary euthanasia methods for dairy cattle that can be applied practically on farms.

Conclusion

Dairy farms have an ethical obligation to ensure their animals live good lives that end with a good death. To achieve this, the dairy industry must continually reevaluate their euthanasia methods to ensure they are based on the best available science. Much of the current scientific literature is comprised of research conducted in slaughter facilities that differ from dairy farms in multiple ways, including type of PCBG used (pneumatic vs powder-charged), cattle breeds (beef vs dairy), sex (mixed vs mostly female), response to humans in close proximity (flighty vs calm), and age variability (low vs high variability) of animals commonly euthanized. More research addressing practical methods of euthanasia specific to dairy farms is needed. This study provides evidence that time to death and brain tissue damage does not differ between dairy cattle receiving a secondary poll vs frontal PCBG shot.

Endnotes

[a] AnaSed® LA, xylazine hydrochloride 100 mg/ml, VetOne® MWI, Boise, ID

[b] Jarvis® HD Long bolt, Richlands, Queensland, Australia and Calgary, AB, Canada

[c] R Core Team. R: a language and environment for statistical computing. Vienna R Foundation for Statistical Computing; 2019. **https://www.R-project.org**

References

1. Shearer JK. 2018. Euthanasia of cattle: practical considerations and application. *Animals, 8* (4), 57. **https://doi.org/10.3390/ani8040057.**

2. Walker JB, Roman-Muniz IN, Edwards-Callaway LN. 2019. Timely euthanasia in the United States dairy industry–challenges and a path forward. *Animals.* 10 (1), 71. **https://doi.org/10.3390/ani10010071**

3. Román-Muñiz IN, Cramer MC, Edwards-Callaway LN, Stallones L, Kim E, Thompson S, Simpson H, Mijares S. 2021. Dairy Caretaker Perspectives on Performing Euthanasia as an Essential Component of Their Job. *Animals.* 11 (2), 289. **https://doi.org/10.3390/ani11020289**

4. Edwards-Callaway L, Simpson H, Román-Muñiz N, Cramer C, Mijares S, Rivera-Gonzalez LJ, Aberle J. 2022. Preliminary Exploration of Weekly Peer Group Discussions as a Strategy for Coping with Feelings Associated with Euthanasia in Dairy Caretakers. *Int J Environ Res Public Health*. 19 (4), 2177. https://doi.org/10.3390/ijerph19042177

5. Fulwider WKT, Grandin T, Rollin BE, Engle TE, Dalsted NL, Lamm WD. 2008. Survey of dairy management practices on one hundred thirteen North Central and Northeastern United States dairies. *J Dairy Sci*. 91 (4), 1686-1692. **https://doi.org/10.3168/jds.2007-0631**

6. Hoe, FGH, Ruegg PL. 2006. Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being. *J Dairy Sci*. 89 (6), 2297-2308. **https://doi.org/10.3168/jds.S0022-0302(06)72301-3**

7. Roche SM, Genore R, Renaud DL, Shock DA, Bauman C , Croyle S, Barkema HW, Dubuc J, Keefe GP, Kelton DF. 2020. Describing mortality and euthanasia practices on Canadian dairy farms. *J Dairy Sci*. 103 (4), 3599-3605. **https://doi.org/10.3168/jds.2019-17595**

8. Leary SL. American Veterinary Medical Association. AVMA Guidelines for the Euthanasia of Animals, 2020. Available at: **https://www.avma.org/ sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf. Accessed June 15, 2022**.

9. Grandin T. Return-to-sensibility problems after penetrating captive bolt stunning of cattle in commercial beef slaughter plants. 2002. *J Am Vet Med Assoc*. 221:1258–1261. **https://doi.org/10.2460/javma.2002.221.1258**

10. American Association of Bovine Practitioners. Guidelines for the Humane Euthanasia of Cattle, 2019. Available at: **https://aabp.org/Resources/ AABP_Guidelines/EUTHANASIA-2019.pdf. Accessed Nov 11, 2022.**

11. FARM. 2020. Farmers Assuring Responsible Management Program Animal Care Reference Manual; Version 4.0. Accessed November. 15, 2022. **https: //nationaldairyfarm.com/wp-content/uploads/2021/12/FARM_Animal-Care-4-Manual_Layout_FINAL_112921.pdf**

12. Robbins JA, Williams R, Derscheid RJ, Boyum B, Spangler C. 2021. Comparison of frontal-sinus and poll shot locations as secondary methods for euthanizing dairy cattle with a penetrating captive bolt gun. *Bov Pract*. 55 (2), 115-119. **https://doi.org/10.21423/bovine-vol55no2p115-119**

13. Terlouw C, Bourguet C, Deiss V. 2016. Consciousness, unconsciousness and death in the context of slaughter. Part 2. Evaluation methods. *Meat Sci.* 118:147–156. **https://doi.org 10.1016/j.meatsci.2016.03.010**

14. Derscheid RJ, Dewell RD, Dewell GA, Kleinhenz KE, Shearer LC, Gilliam JN, Reynolds JP, Sun Y, Shearer JK. 2016. Validation of a portable pneumatic captive bolt device as a one-step method of euthanasia for use in depopulation of feedlot cattle. *J Am Vet Med Assoc*. 248: 96-104. **https://doi.org/10.2460/javma.248.1.96**

15. Dewell RD, Moran LE, Kleinhenz KE, Derscheild RJ, Dewell GA, Shearer LC, Shearer JK. 2015. Assessment and comparison of electrocardiographic and clinical cardiac evidence of death following use of a penetrating captive bolt for euthanasia of cattle. *Bov Pract*. 49:32-36. **https://doi.org/10.21423/bovine-vol49no1p32-36**

16. Lambooy E., Spanjaard W. 1981. Effect of the shooting position on the stunning of calves by captive bolt. *Vet Rec*. 109:359-361. **https://doi.org/10.1136/vr.109.16.359**

17. Kline HC, Wagner DR, Edwards-Callaway LN, Alexander LR, Grandin T. 2019. Effect of captive bolt gun length on brain trauma and post-stunning hind limb activity in finished cattle *Bos taurus*. *Meat Sci*. 155:69-73. **https://doi.org/10.1016/j.meatsci.2019.05.004**

18. Wagner BK, Cramer MC, Fowler HN, Varnell HL, Dietsch AM, Proudfoot KL, Shearer J, Correa M, Pairis-Garcia MD. 2020a. Determination of dairy cattle euthanasia criteria and analysis of barriers to humane euthanasia in the United States: Dairy producer surveys and focus groups. *Animals,* 10 (5), 770. **https://doi.org/10.3390/ani10050770**

19. Wagner BK, Cramer MC, Fowler HN, Varnell HL, Dietsch AM, Proudfoot KL, Shearer J, Correa M, Pairis-Garcia MD. 2020b. Determination of dairy cattle euthanasia criteria and analysis of barriers to humane euthanasia in the United States: The veterinarian perspective. *Animals,* 10 (6), 1051. **https://doi.org/10.3390/ani10061051**

