THESIS

EFFECTS OF PENETRATING CAPTIVE BOLT GUN MODEL AND NUMBER OF STUNS ON STUNNING-RELATED VARIABLES OF CATTLE IN A COMMERCIAL SLAUGHTER FACILITY

Submitted by

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ABSTRACT

EFFECTS OF PENETRATING CAPTIVE BOLT GUN MODEL AND NUMBER OF STUNS ON STUNNING-RELATED VARIABLES OF CATTLE IN A COMMERCIAL SLAUGHTER FACILITY

The objective of this study was to assess two different penetrating captive bolt gun models (Jarvis USSS-1 and USSS-21) and two stunning methods (1KNOCK and 2KNOCK, applying one and two knocks, respectively) on stunning-related variables in cattle. Heads were collected at a commercial slaughter facility and knocking efficiency, knock hole diameter, brain damage, knock hole placement and hemorrhage were assessed. Knocking efficiency was not impacted by gun model or number of knocks ($P = 0.39$ and 0.12, respectively). Knock number influenced knock hole diameter, brain damage and hemorrhage in the cavity ($P \leq .01$). Presence of brainstem damage was greater ($P < .01$) when using the USSS-21 gun. Results suggested similar performance between the USSS-1 and USSS-21gun models. Potential benefits of using the newest model could be related to damaging vital brain areas like the brainstem.

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CHAPTER 1 – REVIEW OF LITERATURE

INTRODUCTION

Animal welfare is one of the most debated topics in our society. Consumers are increasingly making purchasing decisions based on how animals are processed and cared for, this trend is driving large food retailers and restaurants to require animal welfare audits on a regular basis from farm to the plate. The meat industry is making efforts to minimize stress and discomfort for the animals and enhance the interaction between human and animal that occurs from farm to slaughter.

Slaughtering cattle is the top critical point for animal welfare worries. This process may expose the animals to multiple stressors, such as noise, fear of novelty, new pen conditions, unfamiliar animals, severe temperatures, human handling, food/water deprivation, etc. Words of wisdom quoted from Dr Temple Grandin: “Nature’s cruel, but we don’t have to be”. Following this responsibility, packing plants are gradually obtaining an effective systematic approach to good animal care practices in their facilities.

Captive bolt is a device that has been used for stunning cattle in abattoirs since the last century. In 1903, the German Veterinarian Dr. Hugo Heiss invented the penetrative captive bolt stunning device (Kamenik et al., 2019b). As a director of the abattoir in Straubing, Germany, he successfully made every effort to make sure those animals were stunned quickly and processed without having pain. For more than 100 years his invention has been improved and remains one of the most versatile pieces of stunning equipment. It is now one of the most used stunning devices for slaughtering cattle (N. G. Gregory et al., 2007; Kline et al., 2019; Martin et al., 2018; Verhoeven et al., 2015).

Penetrating captive bolt guns (CBG) are designed to fire a retractable steel bolt into the skull of animals causing a massive damage to the brain. Due to the speed of the knock, the animal is rendered instantly insensible to pain and unconscious before perceiving the impact. The extensive destruction of the brain may be enough to cause death, however a rapid exsanguination is required to ensure zero chances of returning conscientiousness and death is fully achieved (Grandin, 2009). After the shot, the captive bolt rod returns to its retracted position and is ready for the next animal. There are basically two energy sources that drive the penetrating captive bolt mechanism: handheld cartridge operated presented in Figure 1.1 or large pneumatically powered guns demonstrated in Figure 1.2.

CAPTIVE BOLT STUNNING DEVICES

Large commercial packing plants are more likely to be equipped with pneumatically powered captive bolt guns. This type of equipment is more suitable to stun several animals in a fast pace, some plants will have more than 10 animals stunned per minute. The volume of compressed air used for each cycle can be as high as 41 liters (Jarvis Products Corporation, 2000). The air supply for the guns is a critical point for the effectiveness of the stunning. A dedicated large capacity air compressor is required to provide enough compressed air for the gun. The velocity of the bolt and the kinetic energy can be affected by a low air line pressure and therefore a poor stunning performed (Oliveira et al., 2017). For example, the model Jarvis Pneumatic Stunner USSS-1 requires 190 pounds per square inches (psi) for its ideal operation condition, while the model Jarvis Pneumatic Stunner USSS-21 requires a range between 150 to 190 psi and support a maximum 250 psi. It has been demonstrated by Oliveira et al. (2017) that a Jarvis USSS-1 captive bolt gun operating below 190 psi air line pressure is inappropriate to stun Zebu adult beef cattle, which tend to have a thicker bone mass on the forehead than other cattle breeds. The minimum power recommended for the air compressor when equipped with the stunner Jarvis USSS-1 is 5 horsepower (HP) with a capacity to produce more than 400 liters per minute at 190 psi (Jarvis Products Corporation, 2000).

Small abattoirs are usually equipped with a handheld captive bolt gun, this equipment is held by the operator's hand and is powered with a blank cartridge. When the trigger is pressed, it fires the captive bolt against the animal's head. Large operations may use a handheld captive bolt gun as a back-up to re-stun animals after a non-successful shot. This equipment is less powerful compared to pneumatically powered guns and the shot location is more critical to achieve an effective stunning. After several cycles under an excessive heat and friction, the handheld stunner may become worn causing failure to maintain the recommended bolt velocity. Due to high moisture found in most of the abattoirs, the cartridge used as a propellant may become wet, and using a damp propellent in the cartridge may cause failure to achieve the success of the stun (N. G. Gregory & Grandin, 1998). Preventive maintenance will extend the usable life of the equipment and prevent premature failure of parts (Troy J. Gibson, Mason, et al., 2015). The problem of using a worn gun with low performance gun due to a previous damaged can be aggravated when stunning large bulls with heavy skulls. Large bulls require a higher bolt velocity compared to steers, cows, and heifers. The ideal bolt velocity for bulls compared to steers is 72 and 55 meters per second (m/s) respectively (Grandin, 2002). It is important to always have a great condition spare gun for emergencies where the operator can easily substitute the equipment in case of failure or damage. A damp cartridge is more likely to produce less noise and can be easily identified by the operator. Previous studies found that up to 4% of cartridges might be suspected of being damp and not effective as the noise produced was quieter compared to sound cartridge. It is important to inspect the tip of the bolt periodically (Gregory et al., 2007), Atkinson (2013) reported that a damage on the outer rim edge of the penetrating bolt was responsible for 19% of the inadequate stuns compared to 3% when the gun was repaired. This highlights the importance of observing any change on the stunning effectiveness during the process.

STUNNING EFFECTIVENESS AND CONSCIOUSNESS

The main intention of an effective stunning is to collapse the animal instantaneously after the first shot by inducing complete loss of consciousness (Terlouw et al., 2016b). More specifically, the penetrating bolt is designed to damage specific structures involved in maintenance of consciousness. Those regions are cerebral cortex and thalamus, which are regulated by the brainstem (Verhoeven et al., 2015). The core structures are located within the brainstem known as a reticular formation or ascending reticular activating system (Finnie, 2001).

The reticular formation is located in the central core of the brainstem, extending from the medulla oblongata to the upper part of the midbrain, this structure is extensively connected to the spinal cord, cerebellum, thalamus, and cerebral cortex giving it a net-like appearance. Its functions can be classified into 4 categories: motor control, sensory control, visceral control, and control of consciousness. Motor control is related to activities such as standing, walking, and running. Sensory control refers to the 6 senses such as sight, sound, taste, touch, smell, including pain. Visceral control is attributed to heart rate, blood pressure and breathing. Control of consciousness is associated to sleeping, vigilance, awareness, and various conscious states. Under these circumstances, if one of those regions is damaged, the cortex does not function appropriately, causing an instantly loss of consciousness (Finnie, 2001; French, 1957; Terlouw et al., 2016a). However, it is important to mention, to induce instantaneous insensibility, physical damage caused by the bolt to the brainstem is not the only action needed to produce unconscious. A calibrated pneumatic powered non-penetrating captive bolt gun is also able to deliver a shock wave to the brain preventing it from functioning normally, without penetrating the brain (Gibson et al., 2009a, 2009b; Kline et al., 2019; Terlouw et al., 2016a; Wagner et al., 2019). Non-penetrating captive bolt devices are not covered in this review.

Once the penetrating captive bolt impacts the skull it produces a deadly shockwave through the brain. The concussion will disrupt the brain function by causing a depolarization of the neurons by a potassium efflux from its cells. (Gregory, 1998). A high load of excitatory neurotransmitters and calcium are dumped into the neuron cells disabling mitochondrial function, ceasing the energy production (Posner et al., 2008). It is very important to use a specific manufacturer pneumatic stunner tester to verify the velocity of the bolt daily, the kinetic energy applied to the skull is proportional to the mass and bolt velocity. An effective percussive stunning is performed when the maximum amount of energy is applied to the ideal location of the head (*Humane Handling of Livestock*, 2013). The ideal placement for an effective stun is demonstrated in Figure 1.3 and Figure 1.4, it should be at the intersection of two imaginary lines drawn from the outside corner of the eye to the center of the base of the opposite horn (Leary et al., 2020), but it may vary depending on cattle breed and the format of the head (Grandin, 2009).

Following the concussion, the bolt causes a fragmentation of the skull's bone and massive mechanical destruction of brain tissue and blood vessels caused by the penetration of the bolt into the cranium (Kamenik et al., 2019a; Pu et al., 2009). The retraction of the bolt causes a negative pressure tunnel that collapse its surrounding causing additional damage to the brain and blood vessels (Karger, 1995). The consequences of the bolt's destruction are hemorrhage inside the brain and the brain cavity, increasing the cranial pressure, and depriving blood supply to the brain. The lack of oxygen also contributes with the collapse of the nervous system (Gregory, 1998).

Operator experience has been shown strictly related to achieving a consistent stunning outcome. Shooting an animal at the optimal location is a complex task, the stun operator must deal with a moving head within three planes (Hewitt, 2016). There is no mark where the shot should be placed, the operator uses the eyes and ears of the animal as a reference and cross the two imaginary lines to apply the shot at the intersection. Fatigue is an additional factor for accuracy, the operator may be fatigued after hours of handling the pneumatic gun. There is a mechanism to help the operators that hold the pneumatically powered gun models by a spring-loaded cable that partially offset its weight (14.7 kg) facilitating its maneuverability reducing operator fatigue. Appropriate training is extremely important to prepare the operator for stunning. Research has shown that high accuracy skill can be achieved in years after several repetitions (Atkinson et al., 2013; Figure 1.5).

According to Algers and Atkinson (2007), the type of equipment had a high influence on the effectiveness of stunning. The author examined 594 cattle in 2 different abattoirs in Estonia, the plant A used only cartridge handheld captive bolt gun manufactured by Accles and Shelvoke LTD, type Cash Magnum 9000 and plant B used the same handheld device combined with pneumatic powered captive bolt gun manufactured by Jarvis model USSS-1. Results combined from both plants showed that 18% of bulls were poorly stunned when using the handheld device. On the other hand, when using the pneumatic stunner, the efficiency was greater with only 1.3% of the bulls being reported with poor signs of stunning. To assess stunning effectiveness the author observed mainly corneal reflex, spontaneous blinking and full or partial eyeball rotation up to sticking. There was a great difference when comparing the diameter of the bolt used for both guns, for the pneumatic gun, the diameter and was 33% greater compared to the handheld device. These results suggests that the characteristics of the gun's bolt such as higher diameter, mass, and velocity contributed for a more efficient shock wave delivered to the brain causing a higher concussion and better stun quality.

THE IMPACT OF SHOT PLACEMENT TO THE STUN QUALITY

The shot location is one of the most important factors for a successful stun and it may vary depending on the species and breed of the animal (Baier and Wilson, 2020). In case the shooting position in the frontal plane of the head lies more than 2 cm from the ideal shot location there is a greater risk of not achieving a successful stun (Gregory et al., 2007). Figure 1.6 demonstrates a low shot placement and its trajectory missing most part of the brain. It is important to mention that a successful stun does not fully depend on the location of the shot. Eventually, a shot that is not placed in an ideal position may induce immediate unconsciousness as a result of the concussion to the brain produced by the kinetic energy delivered by the bolt (Fries et al., 2012). However, results found by (Vecerek et al., 2020) demonstrated that shot placement within 3 cm from the ideal location contributed with 2.4% of failure to induce unconsciousness, in the other hand if the shot was placed more than 7 cm from the ideal location more than 72% of the stuns where not effective rendering the animal unconscious.

High line-speed abattoirs can slaughter more than 350 animals per hour (Martin et al., 2018), they are more economically efficient but there are implications associated to this process. Higher line-speed may result in lower stunning accuracy as the operator has to shoot each animal in shorter time (von Wenzlawowicz et al., 2012), the author also stated that is often difficult to achieve the ideal location using a heavy and powerful pneumatic machine, and most of the cases small variations do not influence the sunning effectiveness. Considering 350 animals slaughter per hour, the operator will have only 10 seconds to shoot each animal. As the line-speed goes up, handlers are required to push a higher number of animals to the single-file chute, the use of electric prod increase impacting the animals as they become more agitated making it more difficult to position the stunner (Grandin, 2013).

A study conducted at northern Germany in two plants, a total of 8,879 cattle heads were investigated for 3 factors: a) number of shots to the heads, and precision of shots: b) angle of the gun, and c) placement of the shot on the skull. Results were divided by precision and assigned in three categories: 1) Less than 2.5 cm from the ideal position and maximum vertical deviation at an angle of 0–10°, 2) Between 3 and 4.5 cm and a deviation in the shot angle of 10–20° from the vertical direction, and 3) more than 5 cm and an angle of shot with a deviation from the vertical direction more than 20° (Fries et al., 2012).

Results were similar in both plants examined by the researchers, at the plant 1 a total of 64.7% of skulls were assigned to category 1 and 31.3% were assigned to category 2 and finally 4% of the skulls were shot more than 5 cm from the ideal position, which indicates poor stunning precision (Grandin, 2002). More than one hole was observed in 284 (2.2%) heads and three heads were observed with 3 holes. Impressively, one head was found with no lesion. These results were similar to those found by (Golveia et al., 2009), where stunning efficacy assessed by clinical indicators was observed in 68.2% of the stunned cattle. (Fries et al., 2012) states that one of the reasons for lower accuracy might be an excessive agitated animal and the sudden up and down movement of the head at the restrainer.

CAUSES OF CAPTIVE BOLT STUNNING FAILURES

Grandin (1998) has implied that the lack of daily preventive maintenance is one of the most common causes of captive bolt failures to effectively render the animal instantly insensible to pain in the first shot. All stunning devices including pneumatically powered and handheld captive bolt guns must be completely disassembled, inspected for wear and tear, and cleaned every day or every 500 shots ensuring correct and continued function and best performance (Grandin, 2007). The main indicator of poor performance is the decreased velocity of the bolt, and as a consequence, lower kinetic energy is delivered to the animal's head (Gibson et al., 2015)

Handheld cartridge-fired devices are more likely to be subjected to wear and tear due to the residual gunpowder, excessive heat generated by the combustion of the cartridge propellant and friction between the buffers with the barrel and bolt. The excessive moisture found on the slaughter plants may be a contributing factor for failure on handheld captive bolt devices. It has been hypothesized that the residual gunpowder absorbs moisture forming acids that may cause pitting to the inside of the barrels (Gibson et al., 2015). Cartridges that are being used on the stunner must be stored and kept out of the high moisture environment. Damp cartridges have been associated with lower noise produced by the captive bolt gun < 111 decibels being responsible for poor stunning (Gregory et al., 2007). Excessive cleaning also may be a factor that compromises the optimal performance of a handheld captive bolt stunner, due to excessive wear caused by extra abrasive cleaning brushes causing excessive "play" in the barrels allowing the gases to escape the chamber and therefore not projecting the bolt with desirable velocity (Gibson et al., 2015).

The major cause of failure for pneumatically powered captive bolt guns is the inappropriate air line pressure. The volume of compressed air used by this powerful machine is extremely high, 41 liters of air is used for each shot fired for the model Jarvis USSS-1 one of the most popular pneumatic stunners used in North America (Jarvis Products Corporation, 2000). The recommended bolt velocity is 55 meters per second (m/s) for steers, heifers, and cows (Gibson et al., 2015) and 70 m/s for bulls (Grandin, 2013). It is known that the air pressure level in the gun's air chamber before shooting affects the velocity of the captive bolt and consequently the kinetic energy delivered to the animal's head (Oliveira et al., 2017). These authors reported a significant proportion of animals showing symptoms of poor stunning such as signs of rhythmic respiration after using the lower air line pressure recommended by the manufacturer (160 psi). These authors reported further that the assessment was prematurely finished using this pressure, and immediately increased to the maximum pressure recommended (190 psi) by the manufacturer.

DETERMINING UNCONCIOUSNESS AND INSENSIBILITY IN CATTLE

Assessing insensibility and assuring unconsciousness is essential for the best humane slaughter approach. To better understand the state of awareness (i.e., consciousness), it is helpful to point out the three major divisions of the brain: forebrain (or prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon), with each division perform specific functions (Pasquini, 1982). The forebrain is undoubtedly the largest division and consists of two subdivisions: telencephalon and diencephalon. The cerebral cortex is the first part that process sensory information. It also controls motor functions and perform cognitive functions such as reasoning and problem-solving. It has been demonstrated that the cerebral cortex still responds during anesthetized and unanesthetized states, therefore the absence of activity or response in the cortex does not affect consciousness. On the other hand, the speed of a response is influenced by anesthesia, which means it is a useful pattern to qualify consciousness experimentally (Gregory and Shaw, 2000). It is known that the cortex by itself is not capable of percept and interpret consciousness, the signs coming from the sensory system must be transmitted to other parts of the brain before perception (Sieb, 1990). It is important to understand that the cortex is essential for perception, cognition, recognition and thinking, but the cortex alone is not essential for other aspects of consciousness such as the ability to breath or the ability to remain stand (Bradley, 1959).

On the authority of the institution Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA), “all animals must be rendered insensible to pain by a single blow or gunshot or an electrical, chemical, or other means that is rapid and effective”. This wording is described on the statute “Humane Slaughter Acts” published in 1958 and updated in 1978 (United States Department of Agriculture Food Safety and Inspection Service, 1979). According to European Union's Food Safety Authority (EFSA), "Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent damage to brain function and the individual is unable to perceive external stimuli (which is referred to as insensibility) and control its voluntary mobility and, therefore, respond to normal stimuli including pain". As claimed by the veterinarian Brazilian authorities from the Department of Agriculture, Livestock and Supplies (MAPA) enforced by the Federal Inspection Service (SIF), "all animals slaughtered under the SIF inspection must be insensible to pain before exsanguination or any invasive procedure and shall be maintained unconscious until the death of the animal". The Cambridge Dictionary defines consciousness as "the state of understanding and realizing something". The Oxford Living Dictionary defines consciousness as "The state of being aware of and responsive to one's surroundings".

Consciousness has been widely studied with main focus in the human medicine. Nowadays the same concepts are applied for production animals. However, there is a major problem in these discussions about consciousness definition, making it difficult to combine different theories against each other to have a solid definition (Doerig et al., 2020). There are some nuances among researchers on the exact signs that are in the transition between awake (i.e conscious) and insensible (i.e., unconscious) (Grandin, 2020). All scientific theories must rely on empirical data regardless of their metaphysical assumptions which goes beyond physics. (Alkire et al., 2008) studied the effects of anesthesia inducing unconsciousness by aiming, directly or indirectly, a posterior lateral corticothalamic complex centered around the inferior parietal lobe, and possibly a medial cortical core. The author suggests that consciousness requires an integrated system with a large range of states, and the use of anesthetics would avoid this integration among specialized brain regions by blocking the interactions or by reducing the information traffic among cells.

Currently, there are two main methods to assess brain activity: visual behavioral indicators (most used in a commercial scale) and electroencephalogram (EEG). Researchers argue that using EEG is the most direct indication of the brain activity after stunning and a more reliable indicator of consciousness, although this method is restricted to experimental set-ups. The EEG is a representation of the functional activity of the brain. When monitoring brain activity using EEG, electrodes are attached to different parts of the head's surface collecting electrical activity from neurons. The level of consciousness can be related to the amplitude of the electrical waves detected by the EEG (Voss and Sleigh, 2007). Short waves (i.e., high frequency, low-amplitude) are found on active and awake animal, long waves (i.e., low frequency, high-amplitude) are detected when the animal is under reduced consciousness, sleeping or anesthetized. Using EEG, Gibson (2019) examined the brain activity of mature bulls after stunning, the author was able to identify that of 31 bulls, 2 shot with non-penetrative captive bolt gun had long periods (i.e., 20 seconds) of normal EEG activity, followed by high amplitude low frequency waves after stunning, indicating incomplete insensibility. The animals that were successfully stunned followed a pattern of transactional or high amplitude low frequency activity before becoming isoelectric. Similar results were found in other studies(Gibson et al., 2009a; Gibson et al., 2019; McLean et al., 2017; Voss and Sleigh, 2007).

The other common method to assess brain activity is by visual behavioral and reflexes. This technique can be easily applied in any commercial slaughter plants, small and large. The animal's reflexes are controlled by the central nervous system and are indicators of consciousness. The brainstem and spinal cord are responsible to produce involuntary reflex movements such as cornea reflex, blinking, palpebral, pupillary light and threat reflex (Dugdale, 2010). All these reflexes require functional nerves, spinal cord, and brainstem. Some of these reflexes may be challenging to access when the animal exhibit convulsions or vigorously body movements (i.e., kicking) (Grandin, 2017). It is common to observe animals vigorously kicking after stunning (Terlouw et al., 2015), it should never be used as an indicator for proper stunning (Bartz et al., 2015). It has been demonstrated in a large commercial abattoir that Holsteins are more prone to kick after stunning compared to other breeds (Martin et al., 2018). There are high injury risks associated with the assessment of the reflex movements due to the proximity between the person evaluating and the animal that was just stunned. In order to confirm the animal is unconscious or brain-dead, the following indicators are required: absence of rhythmic breathing, absence of corneal reflex, absence of palpebral reflex (tested by a light touch of the eyelid) and eyelash reflex (lightly brushing the eyelashes) (Terlouw, 2020).

Another valuable indicator of loss of consciousness that is one of the most noticeable is the loss of posture, it is strictly related to the extensive damage to the cerebral cortex (Gregory and Wotton, 1985). The following behavioral examples are indicators of unsuccessful stunning: remains in standing posture, head and/or body righting reflex (when hung in the rail), voluntary vocalization (should not be mistaken by involuntary passage of air along the vocal cords), spontaneous blinking (no touching), eye pursuit to a moving object, response to threat or menace test (no touching) (Leary et al., 2020). If the animal is showing any of these signs, it should be immediately restunned. Before any invasive dressing procedures, all indicators of unsuccessful stunning and all signs of return to sensibility must be absent to pass an audit according to The North American Meat Institute (NAMI, 2019).

BRAIN DAMAGE AND HEMORRHAGE FOR EVALUATING STUNNING EFFECTIVENESS

Many studies have evaluated brain damage and hemorrhage as an indicator of successful stunning (Atkinson, 2007; Daly et al., 1987; Finnie, 1993, 2001; Finnie et al., 2002; Gibson, Whitehead, et al., 2015; Oliveira et al., 2018; Wagner et al., 2019).

The location of the shot will dictate which area of the brain will be damaged, some rare cases, the operator can miss the brain by shooting far from the ideal position, and therefore failing to induce complete insensibility (Gibson et al., 2015). In the study presented by Gibson (2015), the author investigated the pathophysiology of captive bolt injury when stunning alpacas. It was found that 10 alpacas that had signs of poor stunning, none of them had visible damage to the brainstem region, although two of those animals had severe damage to the frontal lobe. The present study highlights that damage to the brainstem region are associated with better stunning quality. Other observations done by Kline (2019) testing if the length of the captive bolt effects the brain damage in commercial slaughtered cattle, found that looking into 45 skulls none of them showed visual signs of damage to the brainstem. However, the study concluded that longer bolt is more likely to increase brain damage. This has also been explored by Schiffer (2017) by investigating stunning quality using gunshot on brain damage and hemorrhage. The author concluded that 25 out of 30 animals shot with a gun were associated with a deep stun, the other 5 cases that were poorly stunned that was found by dissection “insufficient” brain hemorrhage, in addiction, all these 5 cases the shot deviated more than 4 centimeters from the optimal placement. The author also concluded that results regarding brain damage had no influence on the stun quality.

In the present literature review, it has been anecdotally observed that recent studies have been increasingly using brain damage and hemorrhage to assess stunning quality. In order to investigate brain damage and hemorrhage, the interior of the skull of the animal has to exposed, either by dissection (Schiffer et al., 2017) or using a band saw to split the skull in two parts (Kline et al., 2019; Martin et al., 2018; Wagner et al., 2019). There is a considerable challenge associated with this process and studies have used similar methods but not the same and therefore results are not consistent. There have been 3 main ways to treat the skull prior splitting: ambient temperature, chilled and frozen. The outcome of the brain structures after splitting skulls with these 3 treatments differs considerably, based on the author experience. Briefly explaining: the brain is a very soft tissue, and it is subjected to be macerated when being split by the saw. When the brain is warm at body temperature (~37° Celsius) it is more likely to be damaged by the saw due to its extremely softness, if the skull is chilled to a refrigerator temperature (2° - 8° Celsius) the brain become harder and less subjected to maceration caused by the saw. On the other hand, if the skull is frozen the cut will be cleaner but harder to visually inspect the damage cause by the stunning. There is another variable that may influence the perception of disruption on the brain. When cutting the skull to split in two halves the researcher may decide to cut in the exact sagittal plane having two identical hemispheres, this cut may not coincide to the center of the captive bolt hole, and therefore will not expose the trajectory of the bolt for further investigation. In this case, the researcher may decide to aim the blade in the middle of the captive bolt hole in case it is displaced less than ~ 4 millimeters from the sagittal line of the head with the saw passing through the shot hole or in the middle of the skull. Brain hemorrhage was also observed in previous studies to be visually inspected and reported as a numerical score and not having a consistent method to evaluate the extension of blood covering the external part of the brain or the brain cavity.

It is important to mention that using brain damage and hemorrhage is extremely relevant to measure and assess stunning quality, however it has been observed inconsistency within studies. In addition, there needs to be a consensus between the researchers to better understand stunning effectiveness.

CONCLUSION

In conclusion, captive bolt stunning method has been widely used by most of abattoirs around the world. The objective is to induce loss of sensibility instantaneously avoiding any animal suffer assuring a humane slaughter. Proper equipment maintenance and operator training has been proven to increase the stunning quality. The location of the stunning shot will dictate which areas of the brain will be damaged and one of the most important area to be targeted is the brainstem. To evaluate the effectiveness of the stunning process the reflex signs such as cornea reflex, blinking, palpebral, pupillary light, and threat reflex must be inspected. Brain damage and hemorrhage assessment is a useful technique for deciding a stunning quality, but it has to be improved and discussed withing researchers to find a consensus and achieve better results consistency.

Figure 1.1. Pneumatically powered Captive Bolt Gun Jarvis USSS-1

Figure 1.2. Handheld Captive Bolt Gun Cylinder Style Jarvis .25 Caliber

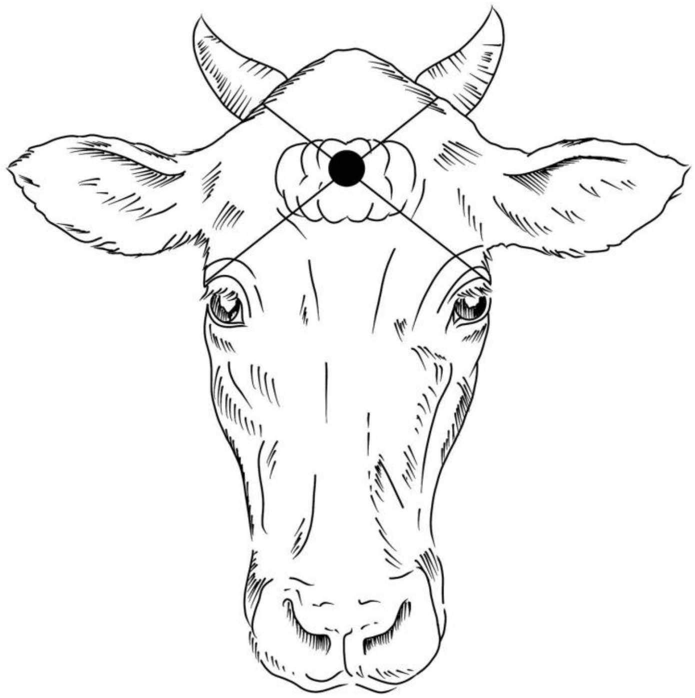

Figure 1.3. Ideal location for captive bolt stunning placement front view

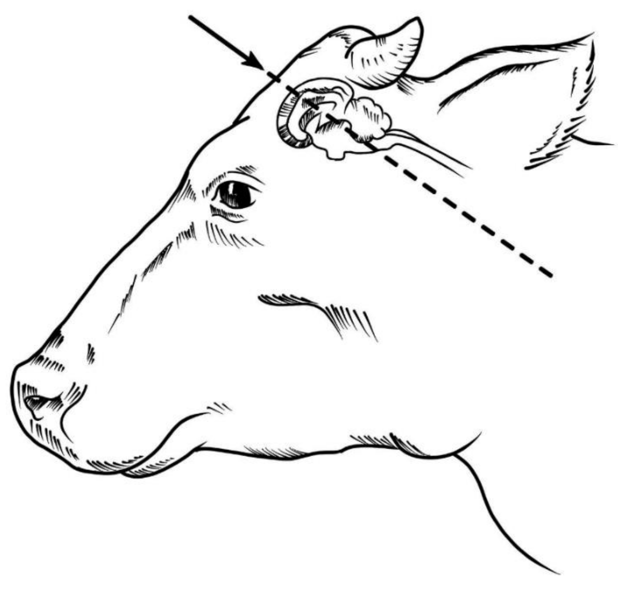

Figure 1.4. Ideal location for Captive Bolt stunning placement lateral view

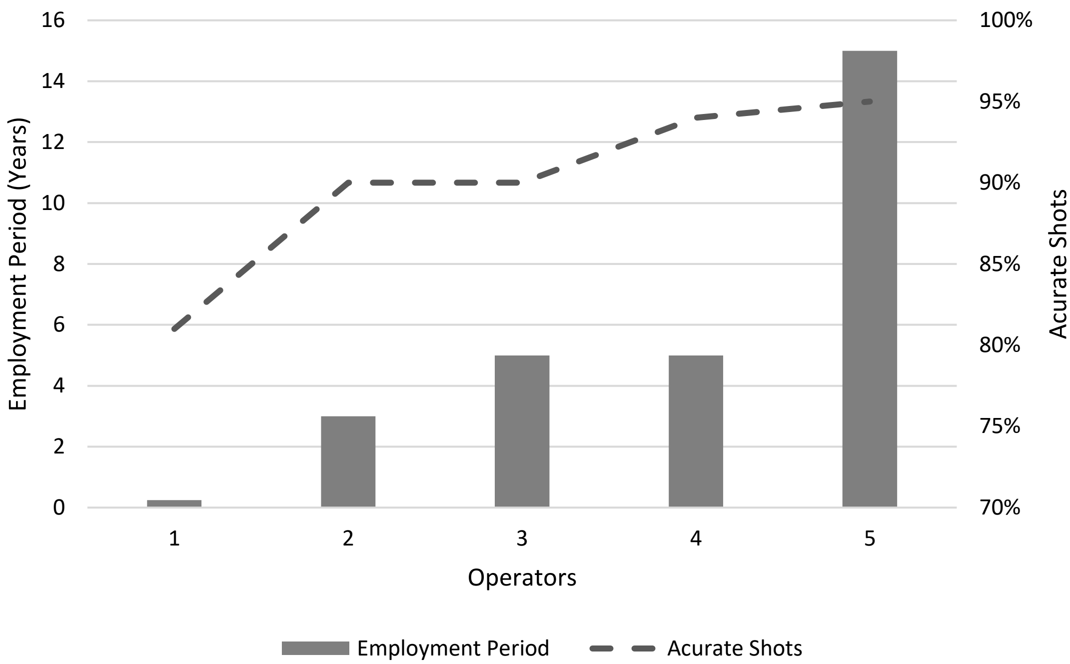

Figure 1.5: Shooting accuracy versus years of experience by each stunner operator [Adapted from Atkinson et al., 2013].

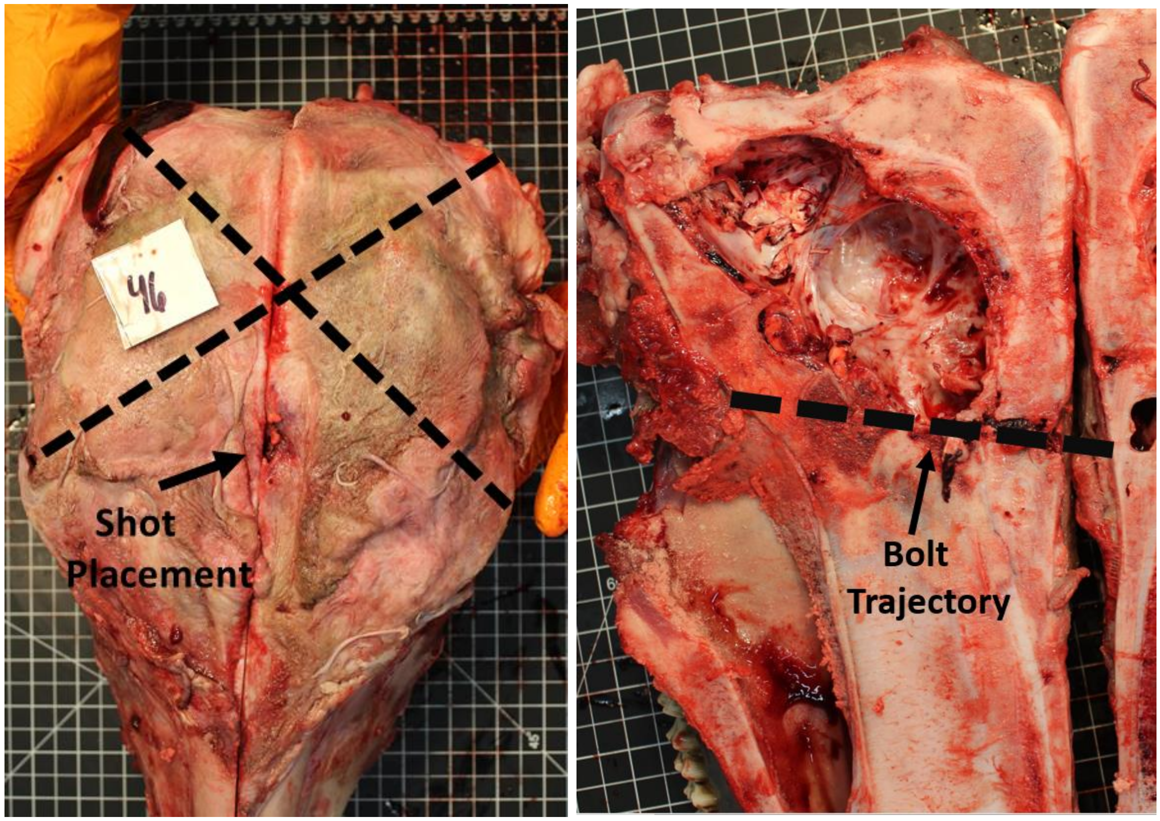

Figure 1.6. A low shot placement and its trajectory that misses most part of the brain. This shot is not considered ideal and is very likely to not produce a successful stun.

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CHAPTER 2 – Effects of penetrating captive bolt gun model and number of stuns on stunning-related variables of cattle in a commercial slaughter facility

INTRODUCTION

Humane slaughter and stunning effectiveness are top priorities for beef processing plants due to critical regulatory impacts and association with animal welfare risks. According to section 9 CFR 313.15 of the U.S. Code of Federal Regulations for the humane slaughter of livestock (United States Department of Agriculture, 1979), “animals shall be stunned in such a manner that they will be rendered unconscious with a minimum of excitement and discomfort”. Consequently, the stunning method utilized in slaughter facilities must be successful to produce immediate unconsciousness in the animals in order to be in compliance with regulatory standards.

The use of a penetrating captive bolt gun is the most common method used to stun cattle in the United States at commercial slaughter facilities. The penetrating captive bolt gun is designed to fire a metal bolt into the cranium of the animal causing massive brain damage and disruption and subsequent insensibility (Atkinson et al., 2013). This method of stunning causes disruption of neurological function and subsequent insensibility from both the direct mechanical damage of brain tissue from the captive bolt itself but also from the diffuse damage of brain tissue caused by the transferred kinetic energy of the bolt entering the cavity. Studies have shown that penetrating captive bolt stunning, due to its extensive damage to the brain, is effective at inducing an unconscious state throughout shackling, sticking, and bleeding (Gallo, Teuber, Cartes, Uribe, and Grandin, 2003; Grandin, 2009; Terlouw, Bourguet, and Deiss, 2016a). One of the biggest challenges of stunning is to control the location and orientation of the gun (Terlouw et al., 2016a). In cattle, the ideal placement for an effective stun should be at the intersection of two imaginary lines drawn from the outside corner of the eye to the center of the base of the opposite horn (AVMA, 2020). A stun administered above this intersection will more likely disrupt the brainstem than one below, leading to more efficient stunning and lower risk of regaining sensibility (Gilliam et al., 2012). Center track restrainers which are commonly used in slaughter plants within the United States do not restrict cattle head movements making the accuracy of the stunning sometimes challenging. Additionally, in high-speed processing plants, the stunning process occurs in a fast-paced environment in which approximately 6 or more animals need to be stunned in one minute (based on a standard chain speeds of 360 head per hour observed in large plants), under the expectation that stunner operators will perform their job correctly every time (Edwards-Callaway and Calvo-Lorenzo, 2020).

In 2000, the Jarvis USSS-1 (JARVIS® Jarvis Products Corporation; Middletown, CT, USA) pneumatic powered stunner was introduced into the market. Since then, most large commercial cattle slaughter facilities in the United States have used this device to stun cattle. Almost two decades later Jarvis introduced a new model, the USSS-21 (JARVIS® Jarvis Products Corporation; Middletown, CT, USA), a new version of the pneumatically powered stunner advertised to include a lighter stunning rod, a new shape designed for easier penetration, and an increased speed at the same air pressure when compared to the USSS-1 (Jarvis Products Corporation, 2000). However, despite the potential benefits associated with the new gun model design, no studies in a commercial setting have been performed to assess its stunning effectiveness.

With the ever-increasing focus on stunning effectiveness in slaughter plants, there has been a trend within the industry towards implementing a routinely administered security knock (i.e., a second stun administered after an initial effective stun) to stun cattle as part of the standard operating procedure (Martin et al., 2018). The use of two consecutive knocks would likely cause increased brain damage adding extra assurance that animals will not regain consciousness after the initial stun. Whether the security knock is indeed necessary or not, has not been formally investigated. One concern about routinely applying a security knock is that it may decrease the emphasis on ensuring that the first knock is effective, which is how employees are currently trained and audits conducted. With a new gun model available to plants and the new method of applying two stuns being adopted within industry, this study was designed to test the effects of gun model (Jarvis USSS-1 and USSS-21 pneumatic stunners) and stunning technique (single knock or single knock and security knock) on stunning-related variables including knock hole diameter, brain hemorrhage, gross brain damage, stunning accuracy and knocking efficiency in a large commercial cattle slaughter facility.

METHODS

Ethical statement: Since all animal measurements occurred post-stunning an exemption petition was filed and granted with the Colorado State University (CSU) Animal Care and Use Committee.

Facilities: The study was conducted between August and September of 2019 in a large commercial slaughter facility located at the western region of the United States and the Colorado State University Global Food Innovation Center (GFIC) in Fort Collins, CO. The GFIC is a newly designed university meat processing center (3300 m^2) used for teaching and research in meat processing. The data related to the stunning process and the skull collection were carried out at the commercial slaughter facility and the detailed measurements of skulls were carried out at the GFIC. The commercial slaughter plant operated in two eight hour shifts and slaughtered an average of 5000 cattle per day under normal conditions (~360 cattle/h). Upon arrival at the packing plant, cattle were rested in holding pens until a USDA veterinarian performed ante-mortem inspection. After inspection, cattle were moved through the handling areas to the single file chute and entered to the center track conveyor restrainer in order to be slaughtered. Cattle for this study were heifers and steers of Bos taurus breed and no specific selection of live cattle to be included in the study was performed.

Study design and treatments: The experimental design was a 2 × 2 factorial arrangement in which the main effects tested were gun model (USSS-1 and USSS-21) and stunning technique (one single knock, 1KNOCK; a knock followed by a security knock, 2KNOCK). Per plant protocol, the stunning operator was trained to apply the first knock in the ideal stunning location and the second knock slightly higher and displaced to the side, i.e. not in the same location as the first stun. Data were collected at the slaughter plant on four days in a 7-week time period. Data was collected on each day during a 3-h time between approximately 9 AM and 12 PM, representing the time in between the first break of the day and lunch time. On each of the four data collection days, only one gun model was used. In the middle of the 3-h collection period, the knock number treatment (1KNOCK vs 2KNOCK) was changed. For each collection day, the treatment order was alternated. All animals slaughtered within the aforementioned time frame on the designated collection days were included in the study for observations of knocking efficiency but only 400 heads (i.e. 100 heads per treatment subgroup) were targeted to be collected during the designated time frame.

Stunning equipment: The stunning was performed by the same trained plant employee for all data collection days. The USSS-1 gun model has a striking end area of 1.98 cm^2, in a spherical shape. The USSS-21 has an oblong shaped striking area (two short-curved edges and two longer curved edges) with a striking end area of 2.10 cm^2. When fired, the bolt protrudes 9.53 cm (this includes the 0.64 cm protrusion from estimated bumper compression) for both models. The USSS-1 and the USSS-21 models consume 41 L and 23.5 L of air, respectively (Jarvis Products Corporation, 2000, 2019). During this study, both gun models operated within their optimal air pressure range (190 to 198 psi).

Slaughter facility data collection: For each day of data collection, a researcher was positioned next to the restrainer in order to record knocking efficiency by recording the number of knocks administered to each animal during the stunning process. Insensibility after the first stun was not visually assessed by the researcher, simply the number of knocks administered to each animal. If an extra knock besides the stuns required by the treatment was applied, it was considered not compliant to the protocol (i.e. 2 or more knocks for the 1KNOCK treatment and 3 knocks or more knocks for the 2KNOCK treatment). In order to investigate any signs of returning to consciousness a digital action camera GoPro Hero 5 (GoPro, San Mateo, CA, USA) was placed after stunning and before exsanguination. The camera view included the time from when the cattle were shackled through the time they entered the line (the stack) to be exsanguinated.

Four researchers were located on the processing floor to collect the heads. One-hundred heads were collected on each of 4 days. Heads were collected from the conveyor belt after jaw removal. Every third or fourth head was collected dependent upon processing speed. Heads were individually bagged and placed in large cardboard containers that held 50 heads each. Dry ice was added into the containers to cool down the heads. The heads were transported to the GFIC in a refrigerated truck (~ 2 °C), where they were stored at 2 °C for approximately 48 h until further processing.

Global Food Innovation Center data collection: In the GFIC, information was collected on knock hole characteristics and heads were split and photographed. Before heads were split, a transparent sheet with a Cartesian coordinate system was used to determine knock hole placement for each of the collected heads. The size of the sheet was 52 × 74 mm (landscape orientation) and contained 54 and 39 alphanumeric coordinates for the x (alpha) and y (numeric) axes, respectively. The distance between coordinates was 5.47 mm. Using the corner of the eyes as an anatomical reference to line up the sheet on the surface of the skull, the three coordinates corresponding to the corner of each eye and the dorsal apex of the skull, were recorded.

The diameter of each knock hole was assessed using Vernier digital calipers (IP54 Waterproof Stainless Steel Electronic Digital Caliper with LCD Screen, Home Depot Product Authority LLC., Atlanta, GA). The smaller jaws of the calipers were placed inside each knock hole in order to measure its largest internal diameter. For heads in the 2KNOCK treatment, they were differentiated by their proximity to the ideal knock location, i.e. the knock closest to the ideal position and the knock farthest from the ideal location, because the order of stun placement was not recorded. Heads were then split with a band saw (AEW 400 M, A.E.W Engineering CO Ltd) with 315 cm × 1.6 cm × 0.02 cm blades (Edgemaster Performer bandsaw blades, Bunzl, Processor Division, North Kansas City, MO). Heads were cut down the sagittal plane of the penetration hole when it was located approximately less than 3 cm away from the center of the head (n = 266), otherwise, the cut was made through the center (n = 144). The intention of the splitting location was to maintain consistency in the size of each split hemisphere for consistency in assessment.

After being split, photographs of the skull and brain structures were taken. A camera (Canon EOS Rebel T6i Digital SLR with EF-S 18-55 mm IS STM Lens - Wi-Fi Enabled, Canon Inc., Melville, NY) was held by a bracket fixed to a table facing down. A set of lights located approximately 0.5 m away from the table provided better illumination to improve image quality. Light intensity was equally maintained for all pictures taken during the study. In order to avoid direct camera manipulation and maintain the consistency of its position, a cellphone connected to the camera via wi-fi was used to take pictures remotely. Figure 2.1 shows the pictures taken of each head: 1) anterior view of the head; 2) sagittal cut of the skull; 3) cerebral cavity; and 4) sagittal cut of the brain. All pictures included both hemispheres of the skull and brain.

Post-collection data processing: Brain damage was visually determined from both hemispheres by a trained researcher that inspected the pictures on a computer screen that were previously taken. The trained researcher was blinded to the associated treatment subgroup of the heads assessed. A transparent sheet printed with bovine brain structures from a bovine anatomy book (Pasquini, 1982) was used as a reference to determine if there was brain damage (Yes or

No) in the following brain areas (Figure 2.2): cerebrum (CE), central area (CA), brainstem (BS) and cerebellum (C). Due to the skill needed to define the smaller brain structures (e.g. thalamus and hypothalamus), these structures were grouped into a larger region identified as the "central area (CA)". A portion of the midbrain (generally regarded as a part of the brainstem) was included in the CA region as it was determined that the division identified in Figure 2.2 was visually clear to the researcher assessing damage. The observed structure was considered damaged if at least in one of the hemispheres there was any sign of maceration, tissue mutilation or distortion that caused loss of its anatomical architecture and was noticeable to the researcher as described by Wagner et al. (2019).

Hemorrhage was identified by the presence of coagulated blood covering the internal brain cavity and the external area of the brain. The software Image-Pro 10 (Media Cybernetics, Inc., Rockville, MD, USA) was used to automatically measure and quantify the percentage of hemorrhaged area covering the surface of the brain and cavity structures (Figure 2.3). The border of each structure was manually outlined using the "select" tool. After setting the total area of each structure, the "count" tool detected the darker spots related to hemorrhage on the selected area using red as a color threshold. Through initial analysis it was determined that a range from 0 to 70 was the optimal level of pixel values that identified hemorrhage, rather than shadow and pooled fluid. The same settings were used to analyze all pictures. A macro was programmed to load the picture file, select the threshold parameter, count the area related to the hemorrhage, export the total area of each structure (i.e. left and right side of brain and brain cavity) and enter the counted area in a Microsoft Excel Office 365 (Microsoft Corporation, Remond, WA, USA) spreadsheet. The percentage of area covered by hemorrhage for each structure was then calculated in the spreadsheet and the weighted average (i.e. based on the size of each structure) for the total brain and brain cavity was obtained. The distances from observed knocks to their ideal positions were also determined using the information gathered with the Cartesian coordinate system. The distance from the actual knock to the ideal location, determined by the research team at the intersection of the lines between the base of where the horn would be and the eyes, was calculated to be used in the statistical analysis.

Knocking placement was assessed using a plant-specific accuracy scoring system similar to a "bullseye" diagram. The bullseye diagram was overlaid on the previously described Cartesian grid system (Figure 2.4) in order to identify the coordinates within each circle of the "bullseye" diagram. The bullseye was divided into 8 segments, four above the midline of the bullseye and four below. A distribution of the knock location by bullseye region was calculated. It is important to mention that the "bullseye" circles were only used as a reference during the post-data collection, researchers used a clear transparent sheet with coordinates without circles to identify the location of the knock hole on the Cartesian grid system. As it was previously mentioned, the location of the ideal knocking position for each head was dependent upon its specific size. Therefore, an adjustment of the innermost circle of the "bullseye" diagram to the unique ideal position of each head was performed.

Statistical analyses: All statistical analyses were performed using R (R Core Team, 2019) with the following packages: car, emmeans, ggplot2, dplyr, multcompView, MASS, MuMIn, ResourceSelection, stargazer, and nnet. Before analysis, 17 heads from the 2KNOCK treatment were removed from the study because only one knock was visible. It was assumed that the security knock happened to be placed on the same spot of the first knock but since that could not be determined post-stunning, the heads were removed from all analyses. During data quality control checks, an additional 14 heads were removed from the analyses. A total of 369 heads were used in the final analyses. Continuous variables including knock diameter, percentage of hemorrhage in brain cavity and percentage of hemorrhage in the brain were analyzed using a general linear model. The model considered the effects of gun model (USSS-1/USSS-21), number of knocks (1KNOCK/ 2KNOCK) and the interaction between these main effects. Additionally, the distance from the actual knock to the ideal position was also included in the model as a linear covariate in order to adjust for the variation associated with the knock location. The distance from the ideal position was not considered for the 2KNOCK treatment because per protocol the second knock was administered outside of the ideal location. Additionally, it was not possible to determine which knock occurred first post-stunning. Results were presented as least-square means considering a significance level of $\alpha = 0.05$.

A total of 265 heads were utilized to analyze brain damage. The remaining heads were not included in this analysis because they were not cut down the sagittal plane of the penetration hole and, therefore, brain trauma would not be fully exposed for its visual inspection. A total of 2363 observations were included in the knocking efficiency analysis. Logistic regression models were then utilized to analyze brain damage and knocking efficiency. Models included the effects of gun model (USSS-1/USSS-21), number of knocks (1KNOCK/2KNOCK) and the interaction. Additionally, the distance from the actual knock to the ideal position was also included in the model for brain damage as a linear covariate. Results were presented as frequencies and the significance level was considered as $P \leq 0.05$.

RESULTS

Knocking efficiency: None of the animals observed on this study expressed any signs of returning to sensibility after being stunned and before exsanguination. Knocking efficiency was not impacted by gun model or number of knocks ($P = 0.39$ and 0.12), respectively (Table 1).

Knock hole diameter: There was no effect of gun model on the diameter of the knock hole closest to the ideal knock position ($P = 0.33$; Table 2). However, the number of knocks had an effect on knock hole diameter ($P = 0.01$). The diameter of the knock hole farthest from the ideal position (assessed only in the 2KNOCK treatment) was not impacted by gun model ($P = 0.83$).

Brain damage characteristics: The presence of brain damage caused by gun model and number of knocks is shown in Table 3. It should be noted that all heads exhibited CE damage (Table 3). There was no effect of gun model on brain damage in the CA and C ($P = 0.65$ and 0.98), respectively. However, the USSS-21 gun caused a greater occurrence of damage in the BS region ($P < 0.01$). Most of the brain areas had a greater presence of damage in the 2KNOCK treatment ($P < 0.01$); the C area was not impacted by the number of knocks ($P = 0.14$).

Hemorrhage characteristics: The impact of gun model and number of knocks on presence of hemorrhage on the surface of the brain and within the brain cavity are shown in Table 3. There was no effect of gun model or knock number on percentage of hemorrhage on the surface of the brain ($P = 0.54$ and 0.08, respectively). There was no effect of gun model on percentage of hemorrhage in the cavity ($P = 0.37$) but there was an effect of number of knocks ($P < 0.01$).

DISCUSSION

In this study, knock hole diameters ranged from 14.9 to 16 mm, which is consistent with the specifications of bolt diameters provided by the manufacturer of both gun models (USSS-1 and USSS-21; Jarvis Products Corporation, 2000, 2019). Oliveira et al. (2018) reported knock hole diameters close to 16 mm when utilizing the USSS-1 gun-model. Smaller penetration hole diameters ranging from 8.5 to 12.2 mm have been also reported using the same gun-model; these results were obtained when the bolt diameter of the gun was 10 mm (Wagner et al., 2019). Due to the fact the heads were assessed post- stunning in this study and stunning was not observed for the sample heads, it was not known which knock was applied first. The knock holes were identified by their location relative to what an ideal knock location would have been, the one closest to the ideal position and the one farther from the ideal position in the 2KNOCK treatment. Although the number of knocks did impact the diameter of the knock hole closest to the ideal position (i.e. the 2KNOCK treatment diameters were greater than 1KNOCK treatment diameters) the differences were relatively low. Furthermore, Wagner et al. (2019) suggested that knock hole diameter could be influenced by factors such as penetration angle and gun orientation, i.e. if the bolt is not perpendicularly positioned to the skull, the hole diameter could increase. Although knock hole diameter was a characteristic measured in this study, it does not necessarily provide much information about the stunning effectiveness.

Greater hemorrhage within the brain cavity was found in the 2KNOCK treatment. This was expected since the mechanical distortion of the cerebral blood vessels would be considerably higher when a security knock is applied during the stunning process, assuming both knocks were administered in an equivalent manner, the damage caused would be at least two times greater in a brain that was stunned twice. Additionally, the protocol in this study for administering the second knock was to apply it in a different location (rather than applying the knock in the same location as the initial stun) which likely increased the number of brain regions damaged. Previous studies have speculated that increments in the bolt length and potentially the number of knocks applied could be factors associated with increased degrees of brain hemorrhage (Martin et al., 2018; Wagner et al., 2019). A benefit associated with increased post-stunning hemorrhage levels is the reduction in the blood supply of brain tissues, since the lack of nutrients and oxygen disrupts the inter and intra-cellular biochemical balance (Ommaya and Gennarellim, 1974; Terlouw et al., 2016b). Hemorrhage percentages within the brain and brain cavity in the current study ranged from 31.2 to 39.2% and fall within the range of brain hemorrhage percentages (15 to 58.8%) reported by Oliveira et al. (2018) for both head hemispheres. Algers and Atkinson (2007) studied brain hemorrhage in cattle after captive bolt stunning and found lower incidences of hemorrhage on the brain when there was no or very little damage in the brainstem area. Greater hemorrhage is expected when arterial structures from the back and basal parts of the brain (subdural or subarachnoid) are disrupted (Algers and Atkinson, 2007). Anatomical differences between species play a major role in determining the ideal stunning location. A study with water buffaloes explored the difference between stunning in the frontal, crown and poll positions determining that the poll position caused damage to the cerebellum (79%) and/or pons (71%) in a majority of experimental animals while knock placement at the crown position resulted in a shallow depth of concussion (Gregory et al, 2009). In another study with alpacas, the authors suggest the crown as a preferred shooting position as it maximizes the probability of damaging the brainstem area (Gibson et al., 2015). Similar findings were shown in a study with sheep stunned with a captive bolt (Gibson et al., 2015). Differences in hemorrhage among studies could be explained by the different methods utilized to quantify total hemorrhage (e.g.

visual assessment vs pixel image analysis) in addition to differences associated with variations in skull shape and size differences between species.

Knock hole placement is critical in effective stunning as not all locations will result in an effective stun. The ideal location for a knock in cattle is at the intersection of two imaginary lines drawn from the outside corner of the eye to the center of the base of the opposite horn. In this study, it was found that the majority of knocks (39 and 51% for 1KNOCK and 2KNOCK, respectively) were administered within the middle and second ring of the knock hole bullseye used in this study. It is important to note that for the 2KNOCK treatment in this study, the stunning operator was instructed to apply the second knock slightly outside the innermost, ideal location. Therefore, it follows that for the knock farthest from the ideal location in the 2KNOCK treatment that there is a greater percentage of knocks in the outer rings. In other studies, stunning accuracy has been evaluated using a similar bullseye system (Kline et al., 2019; Wagner et al., 2019). Although the distribution of knocks across the various bullseye regions was not reported in these studies, a number associated with each ring was. Kline et al. (2019) and Wagner et al. (2019) reported average stunning accuracies of 4 when utilizing a plant-specific accuracy system that considered a score of 5 as the most accurate stun, i.e. the center of the bullseye. The results from Kline et al. (2019) and Wagner et al. (2019) are in line with the current study.

Knock hole placement will have a direct impact on areas of the brain that are damaged due to stunning. In a study with alpacas stunned with a captive bolt, shots that missed the brain or superficially damaged the brainstem were strongly associated with ineffective stunning (Gibson et al., 2015). In the current study, significant brain damage was observed in all of the experimental heads, regardless of the treatment. All analyzed heads had damage to the cerebrum; it was expected that following the protocol, knocks applied close to the ideal position cause damage in the frontal part of the brain, in accordance with Gregory and Shaw (2000). Damage in the brainstem area was approximately 21.5% greater when using the USSS-21 gun-model as compared with the USSS- 1. A possible explanation for this result could be linked to the increased efficiency of skull penetration and the increased bolt velocity of the USSS-21 model, as reported by the manufacturer but not measured in this study. Both factors can increase the shock wave created by the impact of the bolt against the cranium and push the brain tissue through the caudal region of the brain compressing the brainstem (Carey et al., 1989). Furthermore, it has been indicated that following penetration, skin and bone fragments could act as secondary missiles that cause more damage to this brain region (Gibson et al., 2012). Although these data were not collected in this study perhaps this was a factor that impacted differences in brain damage between gun models. A substantial increase in brain trauma was found in the 2KNOCK treatment, all regions of the brain had a higher incidence of noticeable signs of tissue maceration and mutilation when a second knock was applied. Further work done by Wagner et al. (2019) investigating the use of a longer bolt utilized in a penetrating captive bolt gun showed that a longer bolt is associated with an increased level of certain types of brain damage. As would be anticipated, procedures that cause more brain disruption (e.g. two knocks or a longer bolt) are shown to cause an increase in subsequent brain damage.

The current study found that the knocking efficiency was similar between treatments. It was found that with both gun models stunning efficiency was between 97 and 98%. It is important to note that stunning efficiency in this study was an assessment of administering the appropriate stunning protocol; animals were not observed for signs of return to insensibility after the initial stun. Stunning operators apply additional stuns to animals for a variety of reasons and since insensibility after the first stun was not assessed our conclusions are limited. Regardless of why additional stuns had to be administered in this study, the values reported for knocking efficiency are above the acceptable limit (96% knocking efficiency) set in the North American Meat Institute (NAMI) animal handling and auditing guidelines (NAMI, 2019). For many years the acceptable limit for stunning in the NAMI guidelines was 95% and due to improvement seen in slaughter plants for this animal handling outcome the threshold was changed. Additionally, although most regulatory enforcement actions that occur in the packing industry are related to stunning, the total number of enforcement actions in the last three years has decreased (Bowman-Blackwell, 2019; Galindo, 2019), which is in part reflective of the awareness and attention given to stunning effectiveness.

CONCLUSION

In conclusion, for the majority of the stunning-related variables no differences were detected between the gun models USSS-1 and USSS- 21, with the exception of the trauma caused in the brainstem region. When two knocks were applied during the stunning process there was increased hemorrhage and brain damage found in this study which was as to be expected. These results suggest that performance between the USSS-1 and USSS-21gun models is similar; however, potential benefits of using the newest model could be related to an enhanced capacity to cause direct mechanical and diffuse damage in the brain- stem region. The application of a security knock significantly increased the occurrence of damage in all the regions of the brain, although the 1KNOCK treatment caused enough damage to efficiently stun all cattle in this study.

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Table 1 - Effects of gun model (USSS-1 and USSS-21) and number of knocks (1KNOCK and 2KNOCK) on knocking efficiency[1].

Response variable	USSS-1[2] 1x n = 749	USSS-1[2] 2x n = 585	USSS-21[3] 1x n = 512	USSS-21[3] 2x n = 517	SEM	P-Value Gun model	P-Value #of knocks	P-Value Interaction
Knocking efficiency, %	98	98	97	98	0.7	0.40	0.12	0.29

[1]Knocking efficiency was assessed by observation of the number of knocks administered to each animal during the stunning process. If an extra knock besides the stuns required by the treatment was applied, it was considered not compliant to the protocol. (i.e. 2 or more knocks for 1KNOCK treatment and 3 or more knocks for 2KNOCK treatment).

[2] Jarvis USSS-1 stunning gun model (Jarvis Products Corp, Middletown, CT) was equipped with a bolt diameter of 15.9 mm and length of 280 mm.

[3] Jarvis USSS-21 stunning gun model (Jarvis Products Corp, Middletown, CT) had an oblong shaped bolt with a 19 x 13.2 mm diameter and the same length as the USSS-1 model.

Table 2 – Effects of gun model (USSS-1 and USSS-21) and number of knocks (1KNOCK and 2KNOCK) on knock hole diameter. (Least Square Mean and Standard Error of the Mean, SEM).

Response variables	USSS-1[1] 1KNOCK n = 97	USSS-1[1] 2KNOCK n = 84	USSS-21[2] 1KNOCK n = 95	USSS-21[2] 2KNOCK n = 94	SEM	P-Value Gun model	P-Value # of Knocks	P-Value Interaction
Diameter for knock closest to the ideal position, mm	15.3	15.8	14.9	16.0	0.3	0.33	0.01	0.30
Diameter for knock farthest to the ideal position, mm	-	14.8	-	14.9	0.3	0.83	-	-

[1]Jarvis USSS-1 stunning gun model (Jarvis Products Corp, Middletown, CT) was equipped with a bolt diameter of 15.9 mm and length of 280 mm.

[2] Jarvis USSS-21 stunning gun model (Jarvis Products Corp, Middletown, CT) had an oblong shaped bolt with a 19 x 13.2 mm diameter and the same length as the USSS-1 model.

Table 3 - Effects of gun model (USSS-1 and USSS-21) and number of knocks (1KNOCK and 2KNOCK) on occurrence of brain damage and hemorrhage (% of total surface) (Least Square Mean and Standard Error of the Mean, SEM).

	USSS-1[2]				USSS-21[3]					P-Value		
Brain Region[1]	n	1KNOCK	n	2KNOCK	n	1KNOCK	n	2KNOCK	SEM	Gun model	# of knocks	Interaction
Cerebrum, %	58	100	69	100	59	100	79	100	-	-	-	-
Central Area, %	58	26	69	69	59	29	79	63	0.06	0.65	< 0.01	0.29
Brainstem, %	58	13	69	42	59	34	79	64	0.06	< 0.01	<0.01	0.55
Cerebellum, %	58	0	69	45	59	30	79	46	<0.01	0.98	0.14	0.98
Hemorrhage Cavity[4], %	97	31.2	84	37.7	95	32.5	94	36.9	0.01	0.37	<0.01	0.33
Hemorrhage Brain[4], %	97	35.9	84	39.2	95	35.1	94	36.4	0.08	0.54	0.08	0.31

[1]Data are displayed as % of sample brains that were recorded to have damage present in the region listed.

[2] Jarvis USSS-1 stunning gun model (Jarvis Products Corp, Middletown, CT) was equipped with a bolt diameter of 15.9 mm and length of 280 mm.

[3]Jarvis USSS-21 stunning gun model (Jarvis Products Corp, Middletown, CT) had an oblong shaped bolt with a 19 x 13.2 mm diameter and the same length as the USSS-1 model.

Brain damage assessment was visually determined by the presence of any sign of maceration, tissue mutilation or distortion that caused loss of its anatomical architecture and was visually noticeable. Areas assessed included: cerebrum (CE), central area (CA), brainstem (BS) and cerebellum (C).

[4]Hemorrhage was assessed by the presence of coagulated blood covering the internal brain cavity and the external area of the brain. A software was used to quantify the percentage of hemorrhage area covering the surface of the structure throughout dark color threshold (pixels that had a value between 0 and 70).

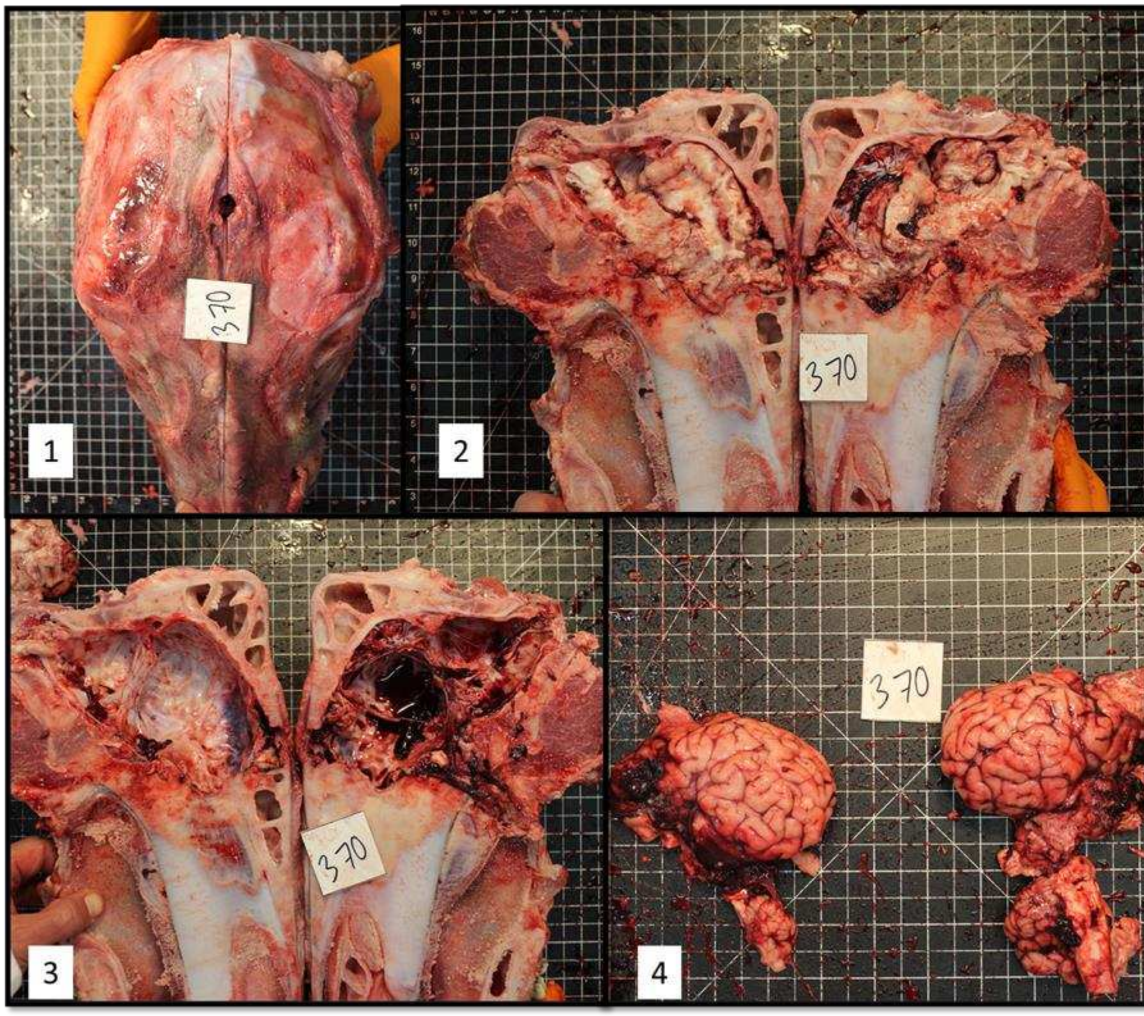

Figure 2.1. A set of four pictures were taken for each head at the Global Food Innovation Center in order to analyze brain damage and percentage of hemorrhage on brain and brain cavity. The pictures are: 1. anterior view of the head; 2. sagittal cut of the skull; 3. cerebral cavity and 4. sagittal cut of the brain.

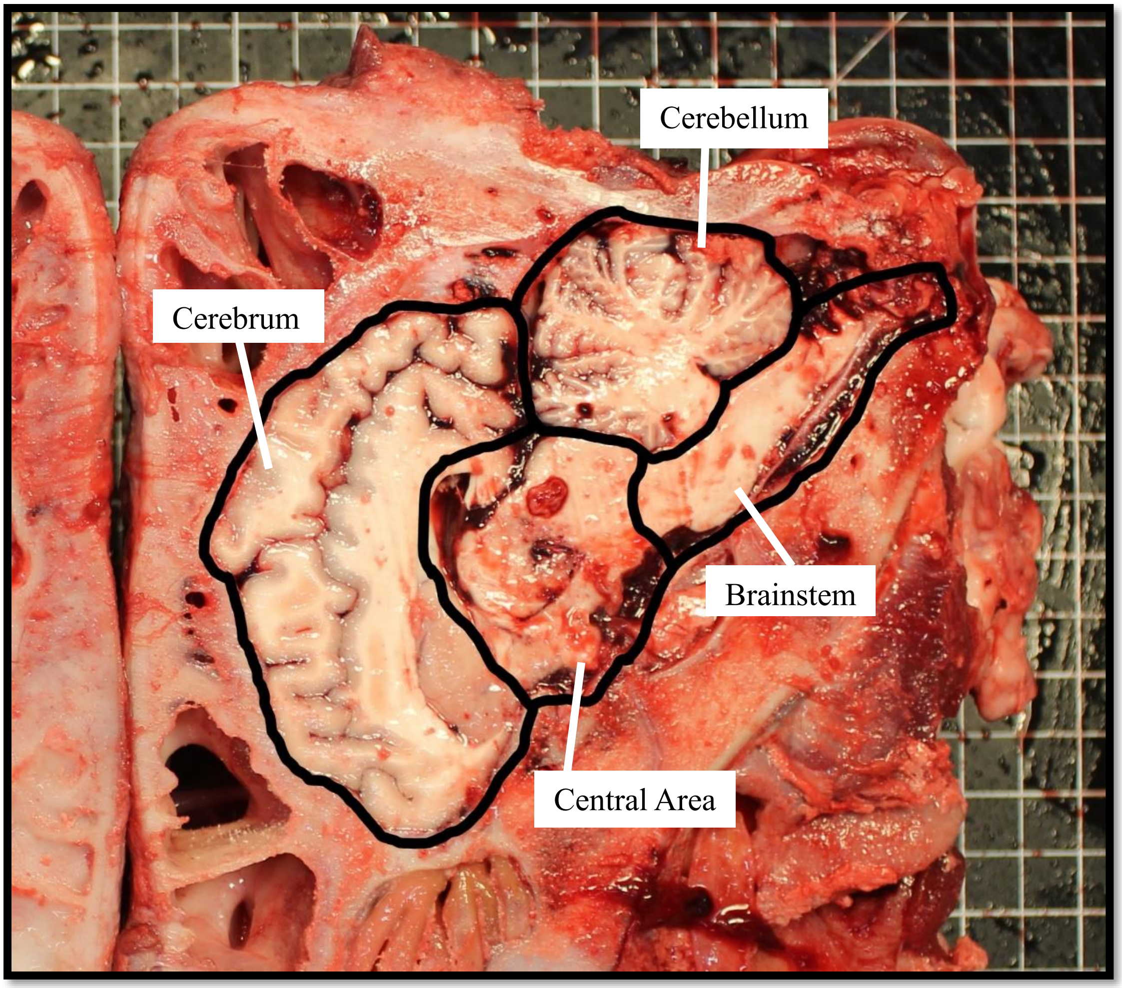

Figure 2.2. Representation of the anatomical divisions of the brain utilized for damage assessment. A transparent sheet printed with bovine brain structures from a bovine anatomy book (Pasquini, 1982) was used as a reference to determine if there was brain damage on each structure.

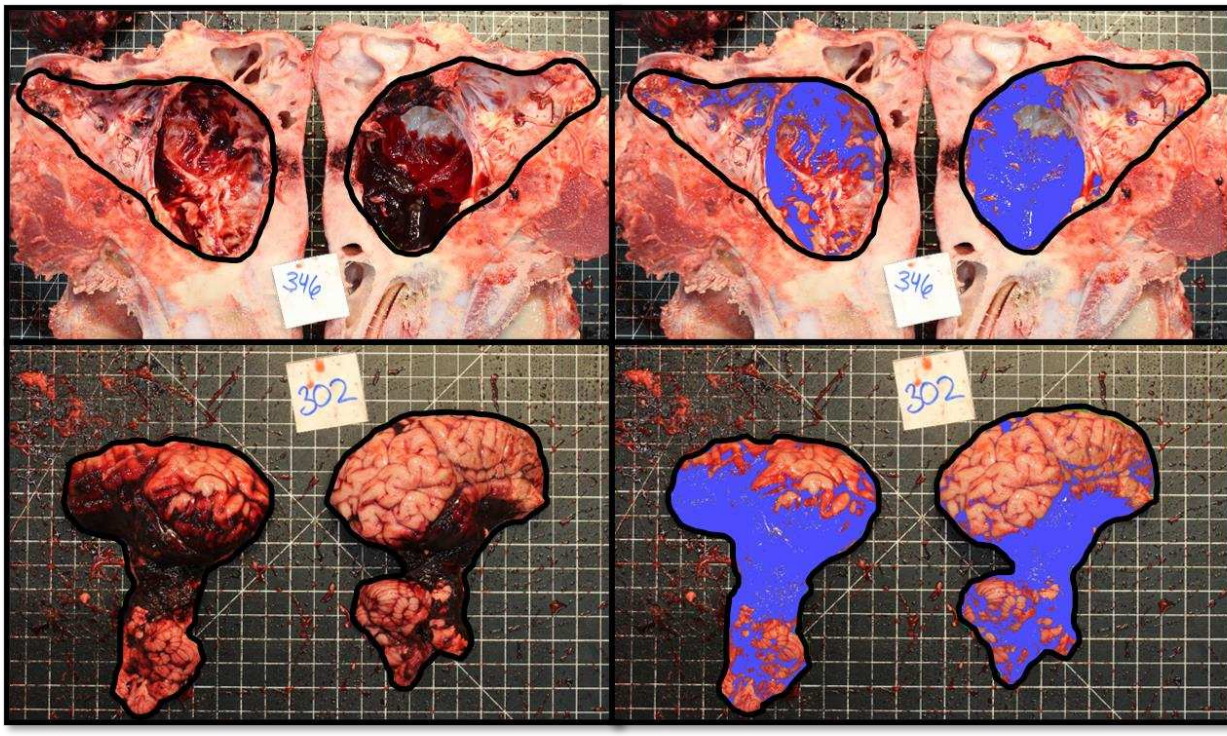

Figure 2.3. Hemorrhage was identified by the presence of coagulated blood covering the internal brain cavity and the external area of the brain throughout pixel image analysis using the software Image-Pro 10 (Media Cybernetics, Inc., Rockville, MD, USA) to process images. To detect the darker spots related to hemorrhage on the selected area, a red color threshold ranging from 0 to 70 pixel values was used. Green line represents the border that was manually outlined using the “select” tool.

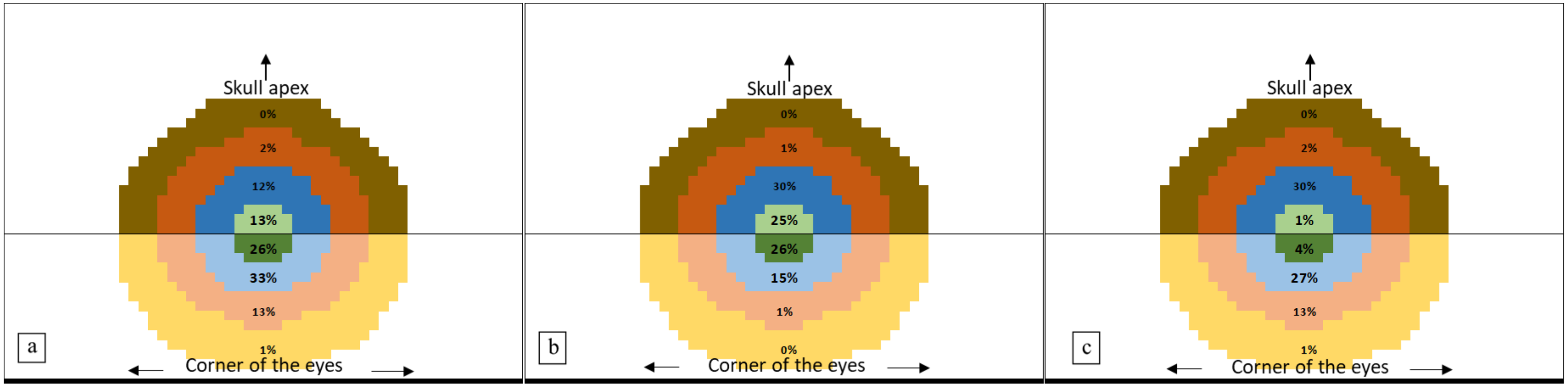

Figure 2.4. Distribution of knock hole placement using the coordinate Cartesian system with "bullseye" circles. A plant-specific accuracy scoring system similar to a "bullseye" diagram was overlaid on the Cartesian system in order to identify the coordinates of the knocks that falls within each circle of the "bullseye" diagram. The center represents the most accurate stun. An adjustment of the innermost circle of the "bullseye" diagram to the unique ideal position of each head was performed based on the ideal location for each head. a) Represents 1KNOCK treatment including the knock closest to the ideal position; b) Represents 2KNOCK treatment including the knock closest to the ideal position; c) Represents 2KNOCK treatment including the knock farthest to the ideal position.

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