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ANATOMICAL INJURY CAUSED BY PENETRATING VERSUS NON-PENETRATING CAPTIVE-BOLT STUNNING IN CATTLE: A REVIEW

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ABSTRACT

In Chile, stunning large cattle is mandatory. Most slaughter plants use a captive-bolt, with or without penetration of the skull, for the humane killing of cattle. The aim of this method is to administer a severe blow to the animal's head, inducing immediate loss of consciousness and causing temporary or permanent damage to the brain function of the animal. Specific indicators must be verified, such as the absence of rhythmic breathing, vocalisation and corneal reflex, among others. However, cattle stunning may be ineffective due to lack of trained personnel, equipment failure or inadequate infrastructure. This review aimed to compare bone and nerve injuries caused by penetrating and non-penetrating captive-bolt stunning in cattle. It was concluded that both methods are effective in inducing instantaneous unconsciousness of the animal. However, penetrating captive-bolt is considered safer when stunning cattle of different breeds and ages.

Keywords: mechanical stunning, cattle slaughter, frontal commotion, captive bolt.

INTRODUCTION

The industrial slaughtering of cattle is strictly regulated by national and/or international regulations. In Chile, Decree No. 94/2008 of the Ministry of Agriculture provides guidelines on the establishment and operation of slaughterhouses, cooling chambers and rendering plants, while it also sets the minimum equipment required for such establishments. Article 1 of the Decree defines slaughterhouses as "those facilities where large livestock (cattle and horses) and small livestock (pigs, sheep, goats) destined for human consumption are slaughtered. These establishments must be qualified in such a way as to ensure animal welfare, slaughtering, and hygienic preservation of meat" (BCN, 2009).

In slaughter plants, there must be an animal handler, also known as a livestock handler or animal welfare officer, present at all operations on the premises from the arrival of the animals to the processing, regardless of whether there are different handlers responsible for each procedure; these operators must be familiar with the behaviour and needs of the animals, ensuring animal welfare at each stage. In Chile, Article 5 of Decree No. 28/2012 states that "the person in charge of animal handling at the time of processing must demonstrate that he/she has undertaken a course in animal handling and welfare aspects or that he/she is a professional or technician with training in the agricultural

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area, and thus capable of carrying out these tasks efficiently, avoiding unnecessary pain and suffering to the animal" (BCN, 2013).

The hours before slaughter are stressful for animals, and stunning is the penultimate antemortem management that directly influences animal welfare. The procedure aims to: i) achieve a profound loss of consciousness of the animal, thus avoiding any suffering during exsanguination; ii) facilitate handling; iii) and provide greater safety to the operator during exsanguination (Figueroa et al., 2011).

In Chile, the stunning procedure became compulsory in slaughter plants for large and small livestock in 1992 by Meat Law No. 19,162 (Ley 19.162, 1992). Article 7 of Decree No. 94/2008 states that the stunning of large livestock must be carried out in a stunning box made of solid and resistant materials, preferably metal or concrete, with a smooth surface, and equipped with a system that ensures the restraint of the animal for stunning, allowing for its rapid and non-violent exit once desensitised" (BCN, 2009). Accordingly, the procedure must be carried out using methods that mitigate the suffering of the animals as internationally recognised and authorised by the Agriculture and Livestock Service (Figueroa et al., 2011). In this sense, Article 14 of Decree 29/2012 (BCN, 2013) refers to the procedures recommended by the Terrestrial Animal Health Code of the World Organisation for Animal Health (WOAH) for animal slaughtering (Table 1).

There are three basic technologies to achieve stunning: mechanical, electrical, and gas methods. Only the first two methods are common in developing countries (Food and Agriculture Organization of the United Nations [FAO], 2001). In Chile, the most widely used method in cattle is mechanical stunning (CIEN Patents, 2011). The method has lower installation and maintenance costs as well as lower risks for the operator, compared to other methods such as electronarcosis or the use of gas, because it can be powered by compressed air or by cartridges (Figueroa et al., 2011).

The aim of mechanical stunning systems is to induce immediate loss of consciousness by administering a severe blow to the animal's head (FAO, 2004). Mechanical systems have evolved from the blow with a mallet to the current captive-bolt guns, with either pneumatic or hydraulic action; the latter can be classified into two categories: penetrating captive-bolt and non-penetrating captive-bolt (hereafter PCB and NPCB, respectively) (CIEN Patents, 2011; Anderson et al., 2022). Both methods can be used for stunning cattle, sheep, goats, pigs and horses (FAO, 2004).

An air pistol or empty cartridge gun is used to fire a PCB; there is no free projectile. The operator fires the bolt in the middle of the forehead at the frontal bone, at a point of intersection of two imaginary lines drawn from the centre of the base of the horns to the upper vertex of the ocular orbit on the opposite side of the skull; a right angle should be formed with the skull, allowing penetration into the cerebral cortex of the animal (Figueroa et al., 2011; WOAH, 2019). The main advantage of PCB stunning is that it causes instantaneous loss of consciousness, penetrates the skull, and generates irreversible damage to the brain mass, significantly reducing the likelihood of return to consciousness, which is important for animal welfare (CIEN Patents, 2011).

For NPCB stunning, the gun should be

Table 1. Methods of bovine stunning in Chile according to current regulations in slaughter plants (modified from WOAH, 2019).

Method	Ages	Fastening	Animal welfare concerns due to inappropriate application
Bullet	All	No	Non-fatal injury
Penetrating captive-bolt, followed by pithing or bleeding	All except newborns	Yes	Ineffective stunning
Non-penetrating captive-bolt, followed by bleeding	Adults only	Yes	Ineffective stunning, recovery of consciousness prior to slaughter
Electricity, two-stage application	Calves only	Yes	Pain associated with cardiac arrest following ineffective stunning
Electricity, single application	All	Yes	Ineffective stunning

positioned approximately 20 mm above the position used for the PCB method, and perpendicular to the surface of the skull. After the impact, bleeding must be carried out as soon as possible to ensure the death of the animal (WOAH, 2019).

To ensure an effective stun, the signs of stunned (unconscious) animals must be recognised. A properly stunned animal falls with a rigid body, head extended, and hind limbs flexed. Involuntary movements may occur due to reflexes. The eyes show absence of the palpebral and corneal reflex, and there is a cessation of rhythmic breathing and absence of vocalisation (Grandin, 2011). While each sign alone is inconclusive, an animal showing at least one sign is considered to be conscious and it should be re-stunned immediately (Figueroa et al., 2011).

In Chile, stunning is mandatory, and most cattle slaughter plants use the captive-bolt gun, with or without skull penetration (Muñoz et al., 2012). In terms of animal welfare, it is of interest to analyse the information available on the methods derived from the application of the captive-bolt. Therefore, the aim of this review is to describe and compare the injuries caused by penetrating and non-penetrating captive-bolt stunning in cattle.

MATERIALS AND METHOD

A systematic qualitative and quantitative review of the literature was undertaken. Searches within the library service of the University of Concepción (Chile), and within governmental and non-governmental platforms of worldwide relevance were conducted in order to describe and compare the injuries caused by penetrating and non-penetrating captive-bolt in cattle.

Keywords and meta-search engines

Different concepts in both Spanish and English were entered into the search tools to find the most up-to-date information. The words used in the different meta-search engines were: mechanical stunning, captive-bolt stunning, bovine slaughter, frontal commotion, captive-bolt in cattle. Sites such as ScienceDirect, MeatScience, Pubmed, Scielo, Wiley Online Library, Google Scholar were used.

Inclusion criteria

Scientific articles, books and other reviews dealing with mechanical stunning by penetrating and non-penetrating captive-bolt in cattle were used as research material; available publications used English as the primary language from the year 2000 onwards, except for those cases where an explanation of a concept or mechanism was made. The reviewed literature included:

- Studies analysing the method of cattle stunning by penetrating and non-penetrating captive-bolt.
- Studies analysing the damage caused by penetrating and non-penetrating captive-bolt stunning of cattle.
- Governmental websites to explain cattle stunning at formal slaughter facilities in accordance with Chilean legislation and the recommendations of the World Organisation for Animal Health (WOAH).
- Additionally, Boolean operators AND, OR and NOT were included.

RESULTS

Physiology of animal consciousness and unconsciousness

The European Food Safety Authority (EFSA, 2013) defined consciousness as “a state requiring brainstem function and projections in relevant cortical regions”. Consciousness will generally be equated with wakefulness and the abilities to perceive, interact, and communicate with the environment, known as sentience (Zeman, 2001). “An animal’s sentience is essentially its ability to feel pain. In general, an animal can be presumed to be insentient when it does not show any reflex or reaction to stimuli” (EFSA, 2013). The state of consciousness is not binary, but rather continuous, of different forms and levels (Zeman, 2005). “An animal is described as “conscious” if a degree of consciousness is detectable” (EFSA, 2013). On the other hand, unconsciousness is defined as a state of loss of consciousness in which there is temporary or permanent damage to brain function; the individual is unable to perceive external stimuli (referred to as insensibility) or control voluntary mobility, and therefore unable to respond to normal stimuli, including pain (EFSA, 2004).

To mechanisms underlying the loss of consciousness depend on the stunning technique used, and thus it is necessary to understand the role of different brain structures in consciousness (Terlouw et al., 2016a). The cerebral cortex or peripheral part of the hemispheres is central to elaborate neurological functions, including self-awareness (the ability to perceive oneself as distinct from the outside world) and awareness of external stimuli (perception of the environment using the senses) (Zeman, 2005). In the context of killing, it is relevant to discuss in more detail the conscious perception of the environment. Different parts of the cortex deal with certain types of information and have different functions.

The primary cortexes are involved in decoding the initial signal and executing movements. The associative cortexes enable the conceptualisation of information and its integration into a wider context. Therefore, conscious perception of the environment requires the well-functioning of primary and associative cortexes in order to know, understand and make sense of what is perceived (Crick and Koch, 1995; Laureys, 2005a).

Another brain structure involved is the reticular formation, which plays an essential role in the level of arousal. It is in the brainstem and consists of a multitude of neural networks. The reticular formation and certain structures of the bridge project to the cortex and activate it, allowing it to function properly. These projections are called the ascending reticular activation system (Terlouw et al., 2016a) that reaches the cortex through two main pathways: i) one passes through the thalamus located above the brainstem, which in turn projects massively onto the cortex: ii) the other travels ventrally through the hypothalamus, before projecting onto the cortex (Brown et al., 2012). Consequently, if a lesion disrupts the functioning of the reticular formation or the ascending reticular activating system, the cortex will not function or will not be sufficient, and the subject will be unconscious (Terlouw et al., 2016a).

Several theories of commotion have been developed over the last century, and these have been summarised and critically reviewed by Shaw (2002) and EFSA (2004): vascular hypothesis, reticular hypothesis, centripetal hypothesis, pontine cholinergic system hypothesis, and convulsive hypothesis. By no means they represent an exhaustive list, nor should they be considered mutually exclusive, but all five offer potentially valuable information about the pathogenesis of commotion. In fact, most of the above-mentioned hypotheses can provide a reasonable explanation for at least some of the elements of commotion. However, the author suggests that the neurophysiological data on commotion are compatible with the seizure theory (EFSA, 2004). The seizure theory suggests that the energy imparted to the brain by the sudden mechanical loading of the head generates turbulent rotational movements and movements of the cerebral hemispheres, increasing the chances of a collision or impact between the cortex and the skull that deforms the tissues. Loss of consciousness would not be caused by disruption or interference with the function of the brainstem reticular activating system. Rather, it is due to functional differentiation of the cortex because of mechanically induced diffuse depolarisation and synchronised discharge of neurons (Shaw, 2002; EFSA, 2004).

The use of PCB guns has additional effects, since the consequences of this technique are linked to both the commotion of the skull by the captive-bolt and the structural damage to the brain caused by its penetration (EFSA, 2013; Terlouw et al., 2015), which causes local fragmentation of the skull, crushing part of the brain tissue and blood vessels during its trajectory (Karger, 1995; Viel et al., 2009; Terlouw et al., 2016a). On the other hand, the retraction of the bolt temporarily leaves a void created by its passage that absorbs the surrounding brain tissue, causing further tearing of axons and resulting in damage to blood vessels (Karger, 1995). The latter effect may be forced by increased cranial pressure due to subarachnoid and intraventricular haemorrhages, especially adjacent to the entry wound and at the base of the brain due to commotion (Finnie, 1993; Gibson et al., 2012; EFSA, 2013; Terlouw et al., 2015; Terlouw et al., 2016a).

Importance of signs of correct animal consciousness and unconsciousness

After stunning, to avoid unnecessary stress or pain, unconsciousness must be ensured during shackling, hoisting and until exsanguination of livestock (Grandin, 2005; Terlouw et al., 2016b). In Chile, Article 22 of Decree No. 28/2012 states that "correct stunning must be verified using a reflex response or other indicators, according to the species and animal category, as well as stunning method used, which must be recorded and made available to the authority" (BCN, 2013). Reflexes originating in the brainstem or spinal cord are assessed, e.g. eye reflexes, and behavioural indicators such as loss of posture, vocalisation, and rhythmic breathing (Verhoeven et al., 2015a).

Several indicators are used to assess the state of consciousness or unconsciousness of the animal (EFSA, 2004; EFSA, 2013). These clinical signs are indirectly associated with brain functions involved in consciousness (Terlouw et al., 2016b). Some of these signs almost certainly indicate either a conscious or an unconscious state. To ensure unconsciousness, indicators of incorrect stunning must be absent, and indicators of unconsciousness or correct stunning must be present. To reduce animal welfare risks due to poor stunning, it is important to detect animals that are not properly stunned or regaining consciousness after stunning (EFSA, 2013). Therefore, it is very important to check the absence or presence of these signs from the application of the initial stun until death occurs (Limon et al., 2010; Terlouw et al., 2015; Oliveira et al., 2018a). For this, three key stages are recognised for the assessment of indicators: i) after stunning (immediately after stunning to hoisting), Stage 1; ii) during skin cutting, Stage 2; and iii) during exsanguination, Stage 3 (EFSA, 2013).

EFSA (2013) states that "the feasibility of an indicator is considered in relation to the physical aspects of its assessment. These include the position of the animal in relation to the assessor, the assessor's access to the animal, and the line speed. The feasibility of assessing an indicator is most likely to be influenced by the key stage of the slaughter process, i.e. after stunning, at the time of skinning or during bleeding, animals may be in different positions and proximity in relation to the assessor, which may affect the ease of using a certain indicator (EFSA, 2013).

Indicators of stunning

Posture: regarded as an easy-to assess indicator according to EFSA (2013). It can be recognised by the immediate and permanent loss of posture, and this collapse can be explained by damage to the reticular formation involved in the control of standing posture (Purves et al., 2001; Schepens and Drew, 2004; Terlouw et al, 2016b). Therefore, in stage 1, unconsciousness manifests as an immediate collapse of the animal to the ground and, if captive-bolt stunning is ineffective, the animal will not collapse, or it will attempt to regain posture within the stunning box (EFSA, 2013). On the other hand, a poorly stunned animal may lose its posture because of the impact of the bolt and remain collapsed in the stunning box (Lücking et al., 2024), without making any attempt to regain its posture, which will be detected once the animal exits the stunning box. Cattle showing signs of consciousness require a second stunning immediately. In stage 2, an unconscious bovine will be hanging limp, and therefore it is not expected to show any change in posture; however, if a bovine regains consciousness while being hoisted during stage 2 or 3, it will attempt to regain its posture by arching its neck or body; the animal will have to be re-sensitised (EFSA, 2013; Terlouw et al., 2016b).

Vocalisations: when not related to social communication, vocalisation most often expresses pain, thus pain can be perceived by conscious animals. Intentional vocalisations are indicative of consciousness, i.e. ineffective stunning, and the animal must be re-stunned immediately (Grandin, 2005). Viability according to EFSA (2013) indicates that it is an easy parameter to assess during the different stages of dressing (71, 65, and 53% for stages 1, 2 and 3, respectively).

Spontaneous blinking: the opening and closing of the eyelids (fast or slow) without stimulation involves circuits in the brainstem and cerebral cortex, and thus spontaneous blinking is expected only in conscious animals (Grandin, 2005; EFSA, 2013). However, not all conscious animals will show spontaneous blinking and, consequently, the absence of blinking does not necessarily indicate that the animal is unconscious (EFSA, 2013). For example, Terlouw et al. (2015) conducted a study in which twenty bulls were desensitised with a captive-bolt gun and found that all animals had no corneal reflex, suggesting a state of unconsciousness, but three animals showed spontaneous blinking. Therefore, further studies are required to understand the exact relationship between spontaneous blinking and the level of consciousness (Terlouw et al., 2016b). Eye reflexes (eyelid reflex, corneal reflex, and spontaneous blinking) are considered as easy to assess at stage 1 (63%), but difficult to evaluate at stages 2 (29%) and 3 (20%) due to a lack of access to the animals during these parts of the slaughter process (EFSA, 2013).

Rhythmic breathing: the respiratory muscles are innervated by control centres located in the medulla oblongata, situated in the lower part of the brainstem. These centres consist of different groups of neurons that control inspiration and expiration alternately. These groups of neurons form a neurological network underlying rhythmic breathing (Smith et al., 2013). They are stimulated by the reticular formation that receives information from the periphery and higher brain centres (Terlouw et al., 2016b). If ineffective stunning occurs, this can be recognised by the sustained presence of breathing; in the case of animals regaining consciousness, they will begin to breathe normally, which can be recognised by regular movements of the flanks and/or mouth and nostrils (Comin et al., 2023). If recovery of breathing is not apparent from these movements, it can be determined by holding a small mirror in front of the nostrils or mouth, since breathing will cause condensation to appear on the mirror because of expiration of the moist air, if this occurs, the animal must be re-stunned immediately (EFSA, 2013).

Gasping corresponds to intermittent inspiratory movements, which are not organised in the same way as normal breathing. It is often accompanied by guttural sounds that should not be confused with vocalisations (Grandin, 2005; Terlouw et al., 2016b). The feasibility of assessing breathing is regarded as easy in stage 1, with a 62%, reaching 50% and 46% in stages 2 and 3, respectively. This is because it is probably not possible to assess respiration in animals chained and elevated on the rail (EFSA, 2013).

Corneal reflex: a blink response that is elicited by lightly touching the cornea with a finger or brush (EFSA, 2013; Verhoeven et al., 2016). The reflex involves the transmission of sensory information to the brainstem triggering a motor response; if present, the eyeball retracts slightly and the eyelid closes. The sensory information passes through the trigeminal nerve to reach the trigeminal nucleus together with the reticula r formation (Cruccu and Deuschl, 2000; Terlouw et al., 2016b). In the context of killing, the absence of the corneal reflex after stunning with PCB or NPCB demonstrates that the process was effective (Shaw, 1989), since the corneal reflex is considered the most reliable ocular reflex for assessing the state of unconsciousness. Any disruption of the underlying neural circuitry will cause modification or absence of the reflex. The neural circuit of the corneal reflex crosses the reticular formation. If the corneal reflex is absent, there is a high probability that the disruption is associated with a broader dysfunction, comprising part of the reticular formation, and thus with a state of unconsciousness (Laureys, 2005b; Terlouw et al., 2016b). Shaw (1989) observed that the corneal reflex was absent in 97 of 100 animals, including adult cattle, calves, and small ruminants, stunned with a penetrating captive-bolt gun; all animals had been effectively stunned. However, the absence of the corneal reflex must be associated with other indicators of unconsciousness because only one verified indicator is not conclusive (Terlouw et al., 2016b). Ineffectively stunned animals and those that regain consciousness are expected to show a corneal reflex and need to be re-stunned (Gregory and Shaw, 2000). The feasibility of assessing the corneal reflex is easy at stage 1 (52%), but difficult at stages 2 and 3 difficult due to the lack of access to the animals (EFSA, 2013).

***Palpebral reflex*:** it is tested with a light touch on the external canthus of the eye or on the eyelashes (EFSA, 2013). Responses include eyelid closure and neural circuit, being similar to those of the corneal reflex. Accordingly, if an animal shows a positive palpebral reflex, it should be stunned again because it is not properly desensitized (Terlouw et al., 2016b). The feasibility of assessing the palpebral reflex is easy at stage 1 (29%), but difficult at stages 2 and 3 (54% each), because of the lack of access to the animals (EFSA, 2013).

Pupillary reflex: contraction of the pupil (miosis) when exposed to light. The pupillary reflex requires a functional condition of the retina; the neurological system involves the optic nerve (sensory information) and the oculomotor nerve (motor response). The integration centre is in the midbrain, near the reticular formation (Terlouw et al., 2016b), thus if an animal presents a positive pupillary reflex, it should be desensitized again (EFSA, 2013). The feasibility of assessing the pupillary reflex is relatively easy at stage 1 (36%), but difficult at stage 2 (67%) and stage 3 (56%), because exsanguination interferes with the blood supply to the retina (Verhoeven et al., 2015a).

Ultimately, the corneal reflex is considered the most reliable ocular reflex for assessing unconsciousness under practical conditions in slaughterhouses (Lambooij et al., 2012), but it must be associated with other indicators of unconsciousness.

Muscle tone: effectively stunned animals will show a tonic/clonic seizure followed by a general loss of muscle tone, cattle collapse with limbs bent, and the forelimbs are stretched after a few seconds (Atkinson et al., 2013; Terlouw et al., 2016b). When animals are hoisted, signs such as fully relaxed limbs, drooping ears and tail, relaxed jaw and tongue protrusion are more visible (Grandin, 2021). This shows that the feasibility of assessing muscle tone is easy at stage 2 (33%) and stage 3 (45%) (EFSA, 2013).

Eye movement: an effective captive-bolt gun stun will result in cattle with their eyes fixed because of the commotion, and this can be recognized by the eyes being wide open and glassy, with the iris clearly visible in the middle, and remaining so until death occurs (Nielsen et al., 2020). Cattle that are not effectively desensitized with the captive-bolt frontal method or that are regaining consciousness will show searching eye movements, nystagmus, or rotation of the eyeball (EFSA, 2013). Eye search movements aimed to track moving stimuli in the visual field are considered a sign of consciousness because they involve not only different structures in the brainstem but also of the cortex (Tehovnik et al., 2000; Terlouw et al., 2016b). While the presence of eye-seeking movements is considered that the animal is conscious, the absence of eye movements does not necessarily indicate unconsciousness (Terlouw et al., 2016b).

Eyeball rotation is recognized by the appearance of most of the sclera, with little or no visibility of the iris. After captive-bolt stunning, cattle that are not breathing and show no positive corneal reflex exhibit eyeball rotation (Gregory, Lee, and Widdicombe, 2007; Terlouw et al., 2015; Terlouw et al., 2016b). Even when eyeball rotation is present at the same time as several signs of unconsciousness, its presence indicates that there is a risk of a shallower depth of unconsciousness or a return of consciousness (Gregory et al., 2007; Atkinson et al., 2013). In this sense, the degree of eyeball rotation can be crucial (Terlouw et al., 2016b). A study conducted by Atkinson et al. (2013) showed that the presence of complete rotation required a second stunning, while partial rotation required greater control of the animal.

Nystagmus is a rapid vertical or horizontal oscillation of the eyeball due to repeated contractions of extraocular muscles. It is considered as an indicator with low discriminatory power (Terlouw et al., 2016b). A study conducted by Gregory et al. (20079 in cattle stunning by captive-bolt showed that nystagmus was rare, occurring in 3% of animals; however, when it was present, there was a one-in-three chance that the quality of stunning was insufficient. This estimate is consistent with other studies (Bourguet et al., 2011; Terlouw et al., 2015; Terlouw et al., 2016b) that have related nystagmus to superficial unconsciousness (Vecerek et al., 2020a). The feasibility of assessing eye movements is easy at stage 1 (62%), being more complicated to assess in stages 2 (47%) and 3 (26%) because of the position where the operator is located with respect to the animal (EFSA, 2013).

Response to painful stimuli: skin prick with a hypodermic needle or ear pinch can be used to test response to painful stimuli. Currently, pain tests need further research to improve their discriminatory power. Certain responses to painful stimuli require cortical input and are indicative of consciousness. Other responses to painful stimuli may be simple nociceptive reflex arc responses, based on a circuit that involves the spinal cord, but not the brain (Grandin et al., 2023). Terlouw et al. (2015) reported that some cattle showed a ventral neck movement in response to the cutting of the skin and blood vessels when the animal was exsanguinated. This reaction was weak in bulls after longer stunning intervals. It is very likely that this movement is a nociceptive reflex response based on a neural circuit that runs through the spinal cord, but not the brain. Therefore, the reaction to skin cutting cannot be used as an indicator of consciousness (Terlouw et al., 2015). Similarly, Verhoeven et al. (2015b) found that pharmacologically anaesthetised sheep with an electroencephalogram indicative of unconsciousness responded to an ear pinch, which shows that certain pain responses do not necessarily indicate consciousness.

Experts surveyed by EFSA (2013) indicated that the response to a painful stimulus was difficult to assess in all stages of the slaughter process.

Threat test response: the hand is moved rapidly towards the animal's eye and checked for the presence of a blinking response or a withdrawal reaction (Limon et al., 2010; Verhoeven et al., 2015a; Verhoeven et al., 2016). To perform the test properly, it must be verified that the animal has adequate vision, i.e. no eyeball rotation, nystagmus, or blood in the eyes. This reflex requires functional efferent cranial (facial) nerve and motor cortex integration (Verhoeven et al., 2015a). The relationship between the threat test and consciousness was shown in sheep and calves; the reflex was lost several seconds before reaching unconsciousness (Verhoeven et al., 2015a; Terlouw et al., 2016b). It is a sensitive test but needs to be associated with more indicators.

Responses to non-painful sensory stimuli: these include the withdrawal response to a puff of air into the nostrils, ear movements in response to a clap of the hand, or a movement of the nostrils or tongue in response to smells or tastes, respectively. This test has much lower discriminatory power than the threat test (Terlouw et al., 2016b). It is difficult to assess at any stage. During dressing, it is essential to use a multi-criteria approach to verify indicators of awareness and unconsciousness (Verhoeven et al., 2015a) to reduce animal welfare risks due to poor stunning. As unconsciousness must be confirmed from initial stun until death occurs (Verhoeven et al., 2015a), EFSA (2013) recognises indicators to assess each stage of the slaughter process: i) stage 1 (immediately after stunning): posture, rhythmic breathing, corneal and palpebral reflex, and vocalisations; stage 2 (during skin cutting): muscle tone, rhythmic breathing, and body movements; and stage 3 (during exsanguination): muscle tone, rhythmic breathing, and spontaneous blinking. If unconsciousness is not confirmed or there is doubt, the operator should proceed quickly to a second stunning and check the stunning equipment (Fries et al., 2012; Terlouw et al., 2016b).

Errors in captive-bolt stunning of cattle

Captive-bolt stunning can have very good results if used correctly. The weapon is easy to maintain and allows instant induction of unconsciousness. However, despite specific instructions on the use of this piece of equipment, field observations show varying percentages of effective stunning (71% - 88%) (Grandin, 2000; Gallo et al., 2003; Gregory et al., 2007; Bourguet et al., 2011; Terlouw et al., 2016a). In fact, it has been described that the major difficulty is controlling the location and orientation of the shot (Bourguet et al., 2011; Vecerek et al., 2020a) as deviation in either direction from the ideal point decreases the likelihood of effective stunning (Fries et al., 2012; Vecerek et al., 2020a; Vecerek et al., 2020b). Vecerek et al. (2020a) have stated that the incidence of failed stuns increases in both bulls and cows as the stun shot is further away from the ideal point of stunning. In addition, Gregory et al. (2007) conducted a study with 1608 cattle and found that, when the position of the captive-bolt gun shot in the frontal plane of the head was more than 2 cm from the ideal position, there was an increased risk of a shallow commotion. Similarly, a previous work carried out by Terlouw et al. (2015) showed that the position and thickness of the skull influence the characteristics of the shock wave, and probably the type of mechanical damage produced by stunning. Furthermore, Shearer (2018) concluded that the most important factor in the successful use of captive-bolt stunning is accuracy of the gun on the corresponding anatomical site, i.e. two imaginary lines are drawn from the centre of the base of the horns (or cornual button) to the upper vertex of the ocular orbit on the opposite side; in order to use the PCB stunning method, the shot must be placed approximately 20 mm caudal to the aforementioned point (WOAH, 2019), (Fig. 1). Shearer (2018) found that the location of the brain in relation to the forehead varies between breeds, which is not considered in current recommendations for gun location.

Another cause that will influence the effectiveness of stunning is related to the quality of handling during the driving of the animals to the stunning box (Losada-Espinosa et al., 2018). Probst et al. (2014) and Romero et al. (2017) mentioned that the ease with which animals are driven to the stunning box may be associated with the number of shots required to cause correct stunning. Bourguet et al. (2011) indicated that animals that received only one shot at the time of stunning received fewer electric prods on the way to the stunning box compared to those that received two shots to achieve unconsciousness. Similarly, Grandin (1998; 2006) found that the use of electric prods causes pain and suggested that 75% of animals should enter the stunning box without the use of electric instruments. Therefore, careful, and quiet handling and leading of animals into the stunning box will provide animals with peace of mind and greater ease of correct stunning (Grandin, 2021).

Inadequate functioning of the gun can also affect correct stunning. According to the Chilean legislation, Article 8 of Decree No. 28/2012 states that "all equipment used for the stunning of animals must be maintained and checked regularly, so that its function is not compromised" (BCN, 2013). For example, a

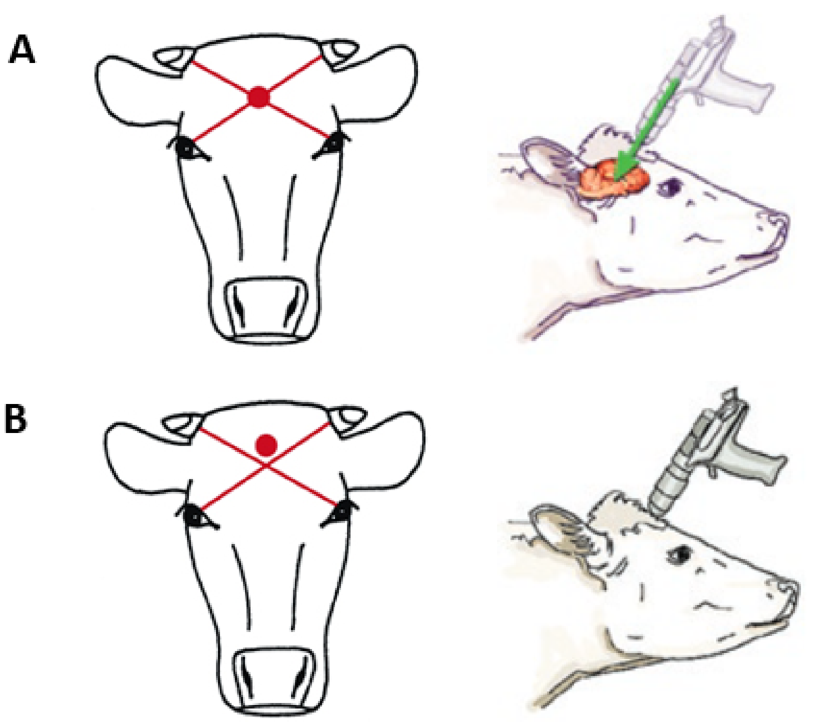

Fig. 1. Anatomical location for captive-bolt stunning of cattle. A. Stunning point using a penetrating captive-bolt gun (PCB). B. Stunning box using a non-penetrating captive-bolt gun (NPCB) (modified from HSA, 2014).

dirty stunner will lose captive bolt velocity and a high velocity of the captive-bolt is required for effective stunning (Darly et al., 1987; Grandin, 2021). Importantly, air pressure is critical for good pneumatic gun actuation (Oliveira et al., 2017). Furthermore, Oliveira et al. (2018b) demonstrated that the depth of penetration of the captive-bolt is significantly deeper when fired at the highest pressure, causing greater damage at the time of stunning.

The stunning gun must be adapted in terms of position on the head and power (energy, velocity) according to the size and gender of the animal. Many studies have reported a gender difference in inadequate stunning, with male animals generally having a higher prevalence of incomplete commotion due to their skull thickness and head size (Grandin, 2002; Gregory et al., 2007; Gibson et al., 2012; Atkinson et al., 2013; Gibson et al., 2015). Gregory et al. (2007) showed that bulls were less likely to develop a deep commotion than steers or young cows. Likewise, Vecerek et al. (2020a) showed that bulls showed more signs of consciousness after stunning compared to cows and/or heifers, while Finnie (1997) stated that much more energy is required to penetrate the thicker skull of mature bulls.

The technical skills and experience of the operator also play a very important role (Terlouw et al., 2016a; Vecerek et al., 2020a). In this sense, the WOAH (2019) states that "all personnel involved in the humane killing of animals must have the necessary skills and competence. The required competence may be acquired through formal training and/or practical experience". In Chile, Decree No. 28/2012 states that training is compulsory to operate captive-bolt equipment. The operator should be trained to be patient and to avoid chasing the animal's head, taking the time for achieving an effective direct shot to the head of the bovine. An operator also recognises when he has not achieved a good stun. Additionally, Article 17 of Decree No. 28/2012 states that "The personnel in charge of stunning must take the necessary measures when an animal has not been correctly stunned in order to avoid its unnecessary suffering" (BCN, 2013).

While the stunning procedure should immediately induce a loss of consciousness without causing pain, it should also be reliable, safe to use, and avoid abuse as much as possible (Gregory et al., 2007; Schwenk et al., 2016). A second application of the stunner is acceptable as a safety measure if the operator has had the opportunity to confirm unconsciousness after the initial stun (Grandin, 2021). While these criteria are largely met when using the PCB and NPCB methods of cattle stunning, there is a risk of stunning failure which is a critical problem (Schwenk et al., 2016). Therefore, Grandin (1999) indicated that to evaluate the efficacy of captive-bolt stunning in large plants, at least 100 animals should be evaluated, taking into account the following classification guidelines: "Excellent", from 99 to 100% of animals are instantly stunned with one shot; "Acceptable", 95 to 98% of animals are instantaneously stunned with one shot; "Unacceptable", 90 to 94% of animals are instantaneously stunned with one shot; and "Severe problem", less than 90% of animals are instantaneously stunned with one shot.

Emphasising that if the effectiveness of the first shot falls below 95%, immediate action must be taken to improve the percentage (Grandin, 1999).

Bone injuries caused by captive-bolt stunning in cattle

The position to place the captive-bolt gun for the correct stunning of cattle corresponds is 20 mm caudal to the aforementioned point (two imaginary lines are drawn from the centre of the base of the horns (or cornual button) to the upper vertex of the ocular orbit on the opposite side) (Fig. 1). The bones of this region are formed by both frontal bones, or more precisely by the frontal scale (Kamenik et al., 2019). The sagittal suture joins both frontal bones in the median plane. In the region studied, the frontal scale consists of two sheets of compact bone: the external facies and the internal facies, between which is a large paranasal frontal cavity called the frontal sinus (Kamenik et al., 2019).

In the case of NPCB stunning, a well-circumscribed fracture of the frontal bone at the impact site is revealed upon macroscopic examination of the skulls, corresponding to the size of the mushroom-headed bolt. These fractures are classified as closed, which is common when using this method (Concha, 2010; Herrera, 2012). In addition, fracture lines are found around the cranial lesion (Fig. 2) (Finnie, 1995).

The entry wound in the skull produced by PCB stunning is a discrete round hole of approximately 1 cm in diameter and occasionally very fine fracture lines are observed radiating from the bony defect in the skull (Finnie, 1993). Similarly, it is common to find bone fragments driven by the captive-bolt inside the skull (Grist et al., 2019).

Injuries caused by captive-bolt stunning of cattle

The vertebrate central nervous system is lined by three layers of membranes called meninges, which provide a protective covering

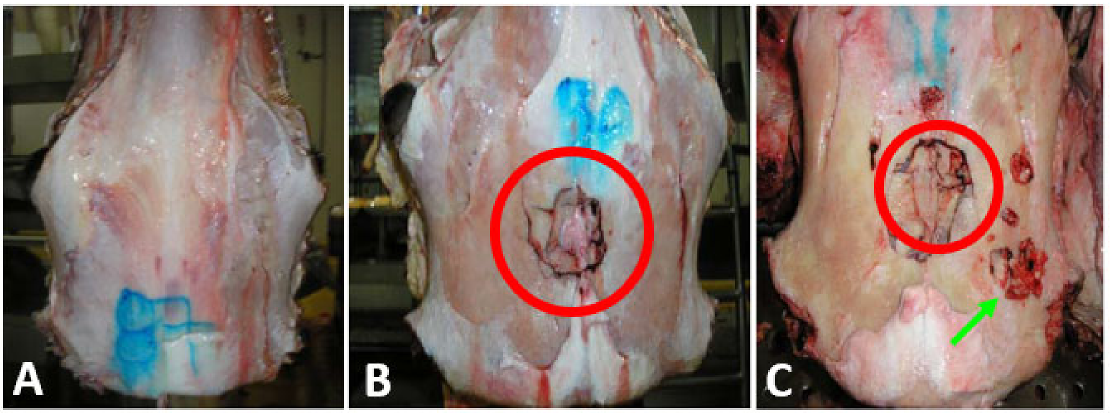

Fig. 2. Bovine skulls showing different degrees of fracture after using non-penetrating captive-bolt. A. Bovine skull with a grade 0 fracture, i.e. no fracture is visible, and the area of the shot is reddened and slightly sunken. B. Bovine skull with a slight collapse of the frontal bone around the shot and the presence of fissures dividing the bone into small pieces or fragments, but without separation. C. Bovine skull with loss of bone tissue, defined by separation between pieces of bone tissue and loss of some of them around the shot (modified from Concha, 2010).

for the underlying soft neural tissue of the brain and spinal cord (Dasgupta and Jeong, 2019). The outermost layer is the dura mater, a tough fibrous membrane (Johns, 2014), then follows the arachnoid, which is a thin membrane, and the arachnoid trabeculae (spongy connective tissue) beneath. The innermost meninx is the pia mater, a thin layer of vascular connective tissue that is intimately associated with the surface of the brain and spinal cord (Johns, 2014; Probst et al., 2014; Dasgupta and Jeong, 2019). The arrangement of the meningeal layers creates the subarachnoid space between the arachnoid and pia mater. This space contains cerebrospinal fluid and blood vessels. If a rupture occurs, it will cause blood to pool in the subarachnoid space, which is called a subarachnoid haemorrhage (Johns, 2014; Moini and Piran, 2020).

It should be noted that brain damage differs between the PCB and NPCB methods. In closed injuries, a combination of accelerating and decelerating forces imparting a large rotational and shearing impulse, with relatively low kinetic energy to the head, occurs when using NPCB (Finnie, 1997; Oliveira et al., 2018a). On the other hand, PCB stunning is designed to produce a noxious shock wave inside and direct damage to brain tissue due to the smaller area of the head impacted by the captive bolt, resulting in the delivery of a high focal kinetic energy and a relatively low cranial impulse (Finnie, 1997; Oliveira et al., 2018a).

When using NPCB stunning, a focal subarachnoid haemorrhage, a subarachnoid haemorrhage at the base of the brain, around the midbrain, cerebellum, pons, and medulla oblongata, occurs at the site of the impact fracture. By the blow of the captive-bolt, a caudal displacement of the brain occurs, causing a tearing of the blood vessels of the midbrain, cerebellum, pons, and medulla oblongata. Similarly, capillary haemorrhages are found in the parenchyma, particularly in the grey matter of the cerebral hemispheres, and petechial haemorrhages in the thalamus and basal ganglia (Finnie, 1995; 1997).

In the case of using PCB stunning, when the bolt hits the skull, it causes a shock wave that depolarises part of the neurons in the brain, preventing them from functioning properly (as in NPCB) (Terlouw et al., 2015). When the captive bolt penetrates the skull, there is mechanical destruction of the brain (Kamenik et al., 2019). In addition, the retraction of the bolt temporarily leaves a void created by its passage, sucking in surrounding brain tissue and causing further tearing of axons and blood vessels (Terlouw et al., 2016a), which in turn results in marked subarachnoid, interventricular and brain base haemorrhage (Finnie, 1997; Terlouw et al., 2015). Petechial haemorrhages are also found at the level of the pons and medulla oblongata (Grist et al., 2019).

Comparison of penetrating and non-penetrating captive-bolt stunning in cattle

The aim of captive-bolt stunning is to induce immediate stunning by administering a severe and forceful blow to the skull of the animal, which must remain unconscious until death because of exsanguination (HSA, 2014). PCB and NPCB are both powered by compressed air or blank cartridges, but there are differences between them. For instance, PCB guns have a smaller bolt, one tip and are concave, while NPCB guns have a large bolt with a convex mushroom head (EFSA, 2004; American Veterinary Medical Association [AVMA], 2016). Both types are always shot into the frontal bone of the animal, perpendicularly (at right angles) to the skull bone, but the NPCB gunshot should be located 2 cm above the ideal site for PCB stunning, i.e. the intersection of two imaginary lines drawn from the centre of the base of the horns to the upper vertex of the ocular orbit on the opposite side of the skull (WOAH, 2019).

For PCB and NPCB stunning, the maximum time to perform exsanguination is 60 and 30 seconds, respectively, because of the higher likelihood to return to consciousness (World Society for the Protection of Animals [WSPA], 2009). Both methods cause similar and sufficient injury for both to be considered effective for inducing instantaneous unconsciousness of the animal (AVMA, 2016). However, NPCB is considered unreliable (EFSA, 2004) because the pistol requires more careful placement compared to PCB (Grandin, 2002; AVMA, 2016). From an animal welfare point of view, PCB is the best available method for the stunning of adult and young cattle (Table 2) (Grandin, 1999; EFSA, 2004; Gibson et al., 2019).

CONCLUSIONS

Both penetrating and non-penetrating captive-bolt stunning cause sufficient injury to be considered effective methods. However, penetrating captive-bolt is a safer method because loss of consciousness is rapid and sustained over time. Macroscopic injuries caused by penetrating captive-bolt stunning are fractures in the frontal bone and subarachnoid and petechial haemorrhages in the thalamus round and open entry wounds in the frontal bone; bone fragments in the trajectory; laceration of nerve tissue in the frontal part of the brain; and subarachnoid and interventricular haemorrhages. On the other hand, non-penetrating captive-bolt generates closed fracture in the frontal bone and subarachnoid haemorrhages, around the midbrain, cerebellum, pons, and medulla oblongata.

Implications

This work provides the theoretical and methodological basis required to carry out an experimental analysis for the evaluation of stunning using the frontal concussion method.

Author contribution

The authors participated in the bibliographic review and contributed to the conceptualisation and design of the study. Data collection was conducted by Reinaldo Letelier Contreras, Paulina Bruna Castillo and Camila Marín Contador; the methodology was developed by Reinaldo Letelier Contreras, Sergio Donoso Erch and Camila Marín Contador; data analysis and discussion of the results were conducted by Reinaldo Letelier Contreras, Sergio Donoso Erch, Fernando González Schnake, Horst Erich König and Camila Marín Contador. All authors revised the final version of the manuscript.

Table 2. Differences between penetrating and non-penetrating captive-bolt stunning in cattle (modified from EFSA, 2004).

Criteria	Penetrating	Non-penetrating
Unconsciousness	Rapid and sustained loss of consciousness, low risk of return of consciousness	Duration of unconsciousness is relatively short; there is a risk of return of consciousness.
Exsanguination	Up to 60 seconds after stunning	Within 30 seconds after stunning
Recommended animal category	All cattle	Only young male and female cattle
Shot accuracy	There may be a slight deviation from the recommended point.	With some deviation from the recommended point, insensibility less effective
Return consciousness	No or low probability	There is a likelihood

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