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JOURNAL: Meat Science

PUBLISHER: Elsevier

PUBLICATION DATE: 18 July 2018 (online)

DOI: https://doi.org/10.1016/j.meatsci.2018.07.016

Evaluation of brain damage resulting from penetrating and non–penetrating stunning of Nelore Cattle using pneumatically powered captive bolt guns

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ABSTRACT

Brain damage resulting from penetrating and non–penetrating stunning of cattle using pneumatically powered captive bolt guns was evaluated in the heads from forty–two adult Nelore cattle. The head of animals were collected during commercial operation of abattoir with different level of airline pressure that powered the pneumatic captive bolt guns, as follows: Penetrating captive bolt gun operating with 160 *psi* (P1; n=10), 175 *psi* (P2; n=10), 190 *psi* (P3; n=12), and non-penetrating captive bolt gun operating with 220 *psi* (NP; n=10). Skin and bone thickness, bolt penetration angle, bolt penetration depth and haemorrhage over the cerebral hemispheres were assessed. The brains were examined for presence of haemorrhage and/or laceration made by the bolt in the frontal lobes, parietal lobes, temporal lobes, occipital lobes, cerebellum, hypothalamus, midbrain, pons, medulla, third ventricle and lateral ventricles. The results showed that only P1 had shots that failed to perforate the skull (n=2; 20%). The bolt penetration depth and haemorrhage over the cerebral hemispheres was significantly ($P \leq 0.05$) greater when shooting with penetrating method. Presence of subarachnoid haemorrhage (which was present as at least a single blood clot) over the frontal, parietal and occipital lobes was higher for NP. For P1, P2 and P3 lacerations were observed only in the frontal and parietal lobes, and were restricted to the cortical region. Subarachnoid haemorrhage surrounding the brainstem structures (including medulla) was only found for P3. Only P3 caused laceration in the midbrain and pons. Neither haemorrhage nor laceration were observed in the brainstem structures of heads shot with NP. Thus, shooting adult Nelore cattle with a pneumatically powered penetrating

captive bolt gun operating with 190 *psi* is potentially more effective when trying to achieve unconsciousness by damaging the brainstem.

1. Introduction

Captive bolt guns are the most common devices used for stunning cattle prior to slaughter in abattoirs. Both non- and penetrating captive bolt guns are used. Non-penetrating method is mainly used for halal slaughter, while the penetrating method is used prior to non-religious slaughter, and there are differences regarding the way that these methods render the animal insensible.

Shooting with a non-penetrating gun causes acceleration and deceleration forces, which impart rotational and shear forces to the head and brain (Ommaya, Goldsmith & Thibault, 2002). Most brain damage caused by this method occurs from the effects of head movements, particularly angular or rotational acceleration, rather than the impact alone (Finnie, 1997). The concussion induces neuronal depolarization in the cerebral hemispheres and, depending on the magnitude of the impact, the brainstem (Gregory, 1998a; Posner, Saper, Schiff, & Plum, 2008). The shock wave created by the impact of a large bolt against the cranium can also push the brain tissue through the opening of the tentorium, compressing the brainstem, which may cause slowing or cessation of breathing and cardiac function (Carey, Sarna, Farrell, & Happel, 1989). If the lesion disrupts the functioning of the brainstem, cortical function also fails (Brown, Basheer, McKenna, Strecker, & McCarley, 2012) and the animal becomes unconscious.

The penetrating method is designed to cause a combination of concussion of the skull and destruction of brain tissue. The impact causes a shockwave through the brain provoking pressure gradients leading to tears and lesions in the brain tissue and disturbances in blood flow (Posner et al., 2008). Following penetration, skin and bone fragments can act as secondary missiles, which can cause further damage to the brain (Gibson et al., 2012), crushing tissues and blood vessels (Viel, Schroder, Puschel, & Braun, 2009). When the bolt retracts, it leaves a temporary void in the cavity created by its passage, and promotes further tearing of axons and blood vessels (Karger, 1995). This latter effect may be strengthened by increased intracranial pressure due to haemorrhage (Gibson et al., 2012). Since there is haemorrhage, there will be reduced blood supply and, in this situation, the brain could be starved of oxygen and unconsciousness would be sustained (Gregory, 1998b).

The penetrating bolt should be oriented towards the brainstem, since the vital functions, such as breathing and cardiovascular activity are regulated by this structure (Laureys, 2005b). Thus, if brain damage is insufficient, or the bolt does not reach the relevant structures, either because of insufficient penetration depth, placement or orientation, the animal may remain conscious or show a shallow depth of concussion.

Pneumatically powered captive bolt guns are the most frequently used equipment for stunning cattle in large Brazilian beef abattoirs (EFSA, 2013). These guns use compressed air as the source of energy when the gun is fired, and this minimizes energy wasted as heat as occurs with cartridge powered guns. Since the air pressure level in the gun's air chamber before shooting affects the velocity of the bolt and the amount of energy transferred to the skull (Oliveira, Gregory, Dalla Costa, Gibson, & Paranhos da Costa, 2017), it could influence the severity of brain lesions and, consequently, the efficiency of stunning. Accordingly, the bolt penetration depth into the deeper structures in the brain could also be affected.

The aims of this study were to compare brain damage resulting from penetrating and non–penetrating stunning in cattle using pneumatically powered captive bolt guns, and to evaluate the effects of different air pressures when using a penetrating bolt gun.

2. Materials and methods

This research was carried out in accordance with the Brazilian legislation, being approved by the Committee for the Ethical Use of Animals from the Faculty of Agricultural and Veterinary Sciences of São Paulo State University (Protocol n. 022754/14), Jaboticabal, SP, Brazil.

2.1. Abattoirs, animals and treatments description

Forty–two adult Nelore cattle (over 400 kg liveweight) were slaughtered in a Brazilian commercial abattoir. Firstly, the animals were individually restrained in a stunning pen equipped with a head yoke and then stunned by an experienced slaughterman. Thirty–two cattle were shot with a pneumatically powered penetrating captive bolt gun (PCB; USSS–1, JARVIS® Jarvis Products Corporation; Middletown, CT, USA) and ten were stunned with a pneumatically powered non–penetrating captive bolt gun (NPCB; USSS–2A, JARVIS® Jarvis Products Corporation; Middletown, CT, USA). These guns and the levels of airline pressure (that powered them) are commonly used in beef slaughterhouses in Brazil. The bolt diameter and length of the PCB and NPCB were 15.9 and 34.9 mm, and 280 and 220 mm, respectively. The bolt weight was 0.30 and 0.83 kg for PCB and NPCB, respectively.

The PCB was tested with three airline pressures, 160 *psi* (P1, n = 10), 175 *psi* (P2, n = 10) and 190 *psi* (P3, n = 12). For the NPCB one level of airline pressure was tested (220 *psi*, n=10). According to the user's manual provided by the manufacturer, the operating airline pressure of the guns used at the abattoir is within a range of 160–190 *psi* advised for PCB and 190–245 *psi* advised for NPCB. The abattoir is monitored by the Brazilian Federal Veterinary Service.

2.2. Heads managements and pathology evaluations

Shots were aimed at the ideal shooting position, which was defined as the cross-over point between imaginary lines drawn between the base of each horn and the corner of the eye on the opposite side of the head (Gregory, 1998b). After bleeding, the skinned heads of the shot cattle were identified with matching paint marks in order to facilitate its tracking inside the abattoir. Heads were stored in labeled plastic bags and then moved to the freezing tunnel, where they remained for five hours at -45 C^{o}.

Frozen heads were band sawed at the Laboratory of Anatomy (*São Paulo State University*) with the cut passing longitudinally through the bolt hole for PCB or through the depressed shot position for NPCB. The lower jaw was removed beforehand in order to reduce wear of the saw. The trajectory and penetration depth of the PCB were measured from the outer surface of the head using a plastic probe inserted through the bolt entrance cavity. The bolt entrance site in the heads was examined for skin tissue and bone thicknesses with a digital Vernier caliper and the angle of penetration was measured with a probe fitted to a steel protractor inserted into the bolt wound cavity in the brain before open the heads for brain evaluations. Angles greater than 90^{o} indicates that the shot was directed caudally.

After defrosting, the cerebral hemispheres were removed from the cranial vaults for examination of gross lesions, according to the brain structures (frontal lobes, parietal lobes, temporal lobes, occipital lobes, cerebellum, hypothalamus, midbrain, pons, medulla, third ventricle and lateral ventricles) and type of damage (haemorrhage, identified by the presence of blood outside the vessels due to physical damage to the vascular wall; laceration, described as a brain injury from the bolt that causes loss of its anatomical architecture and destruction of brain mass). Haemorrhage over the brain was assessed subjectively as a percentage of total surface area of each brain hemisphere.

2.3. Radiography exams

For the radiography evaluations, a total of 15 heads, being five for each treatment (160, 175 and 190 *psi*) were collected according to each penetrating captive bolt treatment following the same procedures of frizzing/defrosting of heads. All heads were longitudinally band sawed 2 cm close to the bolt hole to avoid image overlay in radiographic results. About 2 mL of contrast (barium sulphate) was administered through a syringe connected to a silicone cannula positioned inside of the hole to identify the bolt trajectory and penetration depth by contrast.

2.4. Statistical analysis

For the nominal qualitative variables, the Fisher's Exact Test was used to compare the captive bolt guns and the airline pressures tested. Whenever the test detected a significant effect ($P \leq 0.05$) of treatment, the analysis was performed by comparing the treatments two by two. In the case of the quantitative variables (interval of ratio scales), the Kruskal–Walis test was used to compare the treatments.

3. Results

Comparing the penetrating treatments, only P1 had shots that failed to perforate the skull (n=2; 20%). In those cases, only the outer table of the cranial bone was completely perforated, with depressed fracture of the inner table (Figure 1a). The bolt entrance wounds in the skulls were a uniform round hole with approximately 16 mm diameter with radiating fracture lines observed on outer and inner table of the skull. There was no significant difference for skin and bone thickness or bolt penetration angle among the heads shot with P1, P2 or P3 (Table 1). However, the skin and bone thickness was significantly ($P = 0.05$) thinner for heads shot with NP. The reasons for these findings are not clear. Possible explanations lie on this group had smaller animals than the others, or it can be attributed to the compression and fracturing of the cranium after the application of NPCB. However, because the experiment was done under commercial conditions, this variable could not be randomized in the experimental field. The bolt penetration depth was significantly ($P \leq 0.05$) deeper when shooting with the highest airline pressure tested (P3, 190 *psi*; Table 1).

During this study, radiography was used in a limited number of heads to a better understand the effects of the levels of airline pressures on bolt penetration depth and to possibly support the previous findings. Accordingly, Figure 2 shows that heads shot with 160 *psi* had the shallowest penetration (Figure 2a), followed by head shot with 175 *psi* (Figure 2b). On the other hand, the bolt penetration depth of heads shot with 190 *psi* were notably greater (Figure 2c and 2d). On these specific cases, the bolt penetration angle was rostral rather than caudal, which is not desired when trying to cause direct damage to the brainstem.

Comparing the methods, the number of heads that presented subarachnoid haemorrhage (at least a single blood clot) over the frontal, parietal and occipital lobes was higher for NP than any of the penetrating airline pressures tested (Table 1). However, haemorrhage over the total cerebral hemispheres was higher for P3, with a significant difference between the stunning methods and airline pressure tested for the right hemisphere (Table 1). For either P1, P2 and P3, laceration was observed only on the frontal and parietal lobes, but not extending deeply into the basal ganglia and thalamus (Table 2). For P2, one head presented laceration of the lateral ventricle and three presented intraventricular haemorrhage. Laceration of hypothalamus was only found for P3 (Table 2 and Figure 1e).

Haemorrhage over the brainstem structures (medulla, pons, midbrain) was more frequent for penetrating than non-penetrating method. Additionally, abundant subarachnoid haemorrhage that completely surrounded all brainstem structures (medulla, pons, midbrain) was only found when shooting with P3. Among all airline pressures tested, only P3 caused laceration of the midbrain and pons (Table 2). Neither haemorrhage nor laceration was observed for brainstem structures in heads shot with the non-penetrating method (Table 2).

4. Discussion

To the best of our knowledge, this is the first published study to evaluate brain damage of cattle stunned with pneumatically powered non- and penetrating captive bolt guns operating with different levels of airline pressures.

The study found that bolt penetration depth, damage to the brainstem structures and haemorrhage in ventricles and over the entire cerebral hemispheres, was greatest for heads shot with the penetrating gun at 190 *psi*. Work by Oliveira, Gregory, Dalla Costa, Gibson & Paranhos da Costa (2017), reported for this same gun model at 190 *psi*, that it had significantly higher performance indices (such as higher bolt velocity, kinetic energy and energy density) compared to when operating with 160 and 175 *psi*. This explains the extensive damage with 190 *psi* compared to lower pressures, since there is more energy transfer from the moving bolt to the cranium and, consequently, to the deeper structures in the brain. Additionally, heads shot with 160 and 175 *psi* only presented laceration of the frontal and parietal lobes, and it was restricted to the cortical region. Indeed, the results of this study are in agreement with Oliveira, Gregory, Dalla Costa, Gibson, Dalla Costa &

Paranhos da Costa (2018) who suggested an increased risk of incomplete stunning at slaughter based on physical signals of brain function after stunning, which, among other reasons, would be due to the greater velocity and kinetic energy of PCB gun (54.6 ± 1.33 $m.s^{-1}$ and 447.91± 22.02 joules, respectively) compared to NPCB gun (18.06 ± 0.19 $m.s^{-1}$ and 135.17 ± 2.85 joules).

Although the basis of the conscious state is not well understood, it is believed to depend on feedback loops of neural activity between the brainstem reticular activating system and the cerebral cortex (Blumbergs, 1997). In this study, in animals shot with the PCB with 160 and 175 *psi* there was no gross macroscopic damage to the hypothalamus, midbrain, pons and medulla. Accordingly, Gibson et al. (2015) observed that among the alpacas shot with penetrating captive bolt gun that presented signs of incomplete concussion none had any macroscopic damage to the pons and severe damage to the thalamus, midbrain and medulla. However, in this study, there was damage to hypothalamus, midbrain, pons and haemorrhage in the medulla (17%, 33%, 8% and 33%, respectively; see Table 2) in heads shot with 190 *psi*. This suggests that the penetrating gun operating with higher airline pressures is more efficient in provoking damage to regions of the brain responsible for maintaining the conscious state.

When shooting at the ideal position for adult Nelore cattle, the approximate angle of bolt penetration needed when trying to reach the brainstem is around 110º - 120º (Oliveira et al., 2018). Since there was no significant difference in bolt penetration angle between the airline pressures tested for the penetrating method, and the highest airline pressure (190 *psi*) was the only treatment to cause damage to the brainstem, it was concluded that only this airline pressure is effective in producing sufficient macroscopic damage to induce unconsciousness.

Subarachnoid haemorrhage in the occipital lobe was more frequently observed in heads shot with the NPCB. Presumably, the greater acceleration/deceleration forces imparted by a larger diameter bolt caused rotational and shear forces to the head and brain (Ommaya, Goldsmith & Thibault, 2002), producing a wider distribution of blood clots on the opposite side to that which was directly impacted. Moreover, the thinner skin and bone of cattle shot with the NPCB gun makes the cranium less resistant to the effects of the bolt impact, since, as stated by Currey (2003), the thicker the bone the stiffer it will be. Similarly, Finnie (1997) stated that much greater energy is required to penetrate the thicker skull bones of mature bulls.

According to the Jewish (Shechita), Shariah (Islamic) and Muslim (Halal) law, the animal needs to be considered alive before the procedure and requires that the animal does not experience pain or suffering. These requirements dictate how and whether the pre-stunning of animals is acceptable before slaughter for these faiths (Downing, 2015). After all, animals destined for meat consumption by followers of those faiths must not have any injuries other than the one made by the neck cut, which has to be performed by a prescribed method. However, the findings of this study showed that relatively large subarachnoid haemorrhage were developed when NPCB was used, which, according to the requirements listed above, would pre-empt those carcasses from consumption by members of those faiths.

5. Conclusions

Shooting adult Nelore cattle with a pneumatically powered penetrating captive bolt gun operating with lower airline pressures failed to produce sufficient damage to brainstem structures. Only PCB operating with 190 *psi* produced damage to the hypothalamus and brainstem, and this pressure has previously been associated with a greater certainty of inducing unconsciousness. At 190 *psi* there was greater depth of penetration into the brain and this evidently increased the likelihood of causing direct damage to the brainstem. Thus, these results could be used as guidelines, demonstrating that lower levels of airline pressures should not be used for stunning and dispatch of adult cattle.

Acknowledgements

This study was conducted as part PhD thesis of the lead author in the Animal Science Graduate Program at the Faculty of Agricultural and Veterinary Sciences, UNESP, Jaboticabal, SP, Brazil. The research was partially funded by CNPq (that granted the scholarships to Steffan Edward Octávio Oliveira and Filipe Antonio Dalla Costa) and by FAPESP (grant number 2016/24.910-4) for supporting Prof. Neville G. Gregory as a visiting professor at FCAV-UNESP, Jaboticabal campus. The authors also express the gratitude to Everton Adriano Andrade and Carlos Alberto de Oliveira Silva for their support during data collection, and to Karen Camille Góis, Maria Camila Ceballos, Monique Valéria de Lima Carvalhal for their contribution in data collection. This study would not be possible without the support of JBS, which opened its facilities for data collection.

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Table 1. Means ± SE of skin and bone thickness, bolt penetration angle[1], bolt penetration depth and haemorrhage over the left and right hemispheres of the brain according to method of stunning and airline pressure.

	P1 (N=10)	P2 (N=10)	P3 (N=12)	NPCB (N=10)	$P>\chi^2$
Skin and bone thickness (mm)	16.2±0.5 [ab]	17.19±0.9 [ab]	18.4±1.1 [a]	16.0±0.5 [b]	0.05
Bolt penetration angle (º)	80.0±13.6	100.00±2.4	96.3±3.6	–	0.66
Bolt penetration depth (cm)	4.8±0.8 [c]	7.84±0.2 [b]	10.6±0.5 [a]	–	<0.001
Haemorrhage over left hemisphere (%)	15. 0±2.8	23.00±3.5	35.8±6.4	19.0±3.8	0.06
Haemorrhage over right hemisphere (%)	24.5±3.8 [b]	27.00±2.9 [b]	58.8±6.5 [a]	25.0±2.6 [b]	0.001

Means followed by different letters in the same line differ significantly by Wilcoxon test (P≤ 0.05);
Penetrating method: P1=160 *psi*; P2=175 *psi*; P3=190 *psi*
Non-penetrating method: NP=220 *psi*
Haemorrhage: (%) of total surface area
[1]Data from the two animals in which the skull was not penetrated was not included in calculating mean penetration depth

Table 2. Frequencies of cattle with haemorrhage and laceration according to the region of damage from *post mortem* examination of the effects of stunning method and airline pressure.

Region of damage	Haemorrhage					Laceration				
	P1 (%) n=10	P2 (%) n=10	P3 (%) n=12	NPCB (%) n=10	*P-value*	P1 (%) n=10	P2 (%) n=10	P3 (%) n=12	NPCB (%) n=10	*P-value*
Cerebrums										
Frontal lobes	70	50	83.33	100	0.063	80.00^{a}	100.00^{a}	91.67^{a}	0.00^{b}	<.0001
Parietal lobes	20.00^{a}	20.00^{ab}	16.67^{a}	70.00^{b}	0.038	20	20	8.33	0	0.522
Temporal lobes	30	20	16.67	40	0.724	0	0	0	0	-
Occipital lobes	0.00^{a}	0.00^{a}	16.67^{a}	80.00^{b}	<0.001	0	0	0	0	-
Ventricles										
Lateral	30	40	66.67	50	0.423	0.00^{a}	10.00^{ac}	41.67^{bc}	0.00^{ad}	0.009
Third	20	30	58.33	20	0.191	0	0	33.33	0	0.010
Hypothalamus	10.00^{b}	0.00^{b}	66.67^{a}	10.00^{b}	<0.001	0	0	16.67	0	0.233
Cerebellum	10.00^{a}	0.00^{a}	16.67^{a}	80.00^{b}	<0.001	0	0	0	0	-
Brainstem structure										
Midbrain	10.00^{a}	10.00^{a}	50.00^{a}	0.00^{b}	0.010	0.00	0.00	33.33	0.00	0.010
Pons	0.00^{a}	10.00^{ac}	50.00^{bc}	0.00^{ad}	0.002	0.00	0.00	8.33	0.00	1.000
Medulla	0.00	0.00	33.33	0.00	0.010	0	0	0	0	-

Frequency followed by different letters in the same line differ significantly by Wilcoxon test ($P \leq 0.05$);
Penetrating method: P1=160 *psi*; P2=175 *psi*; P3=190 *psi*
Non-penetrating method: NP=220 *psi*
Haemorrhage: Subarachnoid Haemorrhage, recognized when at least one single blood clot was observed

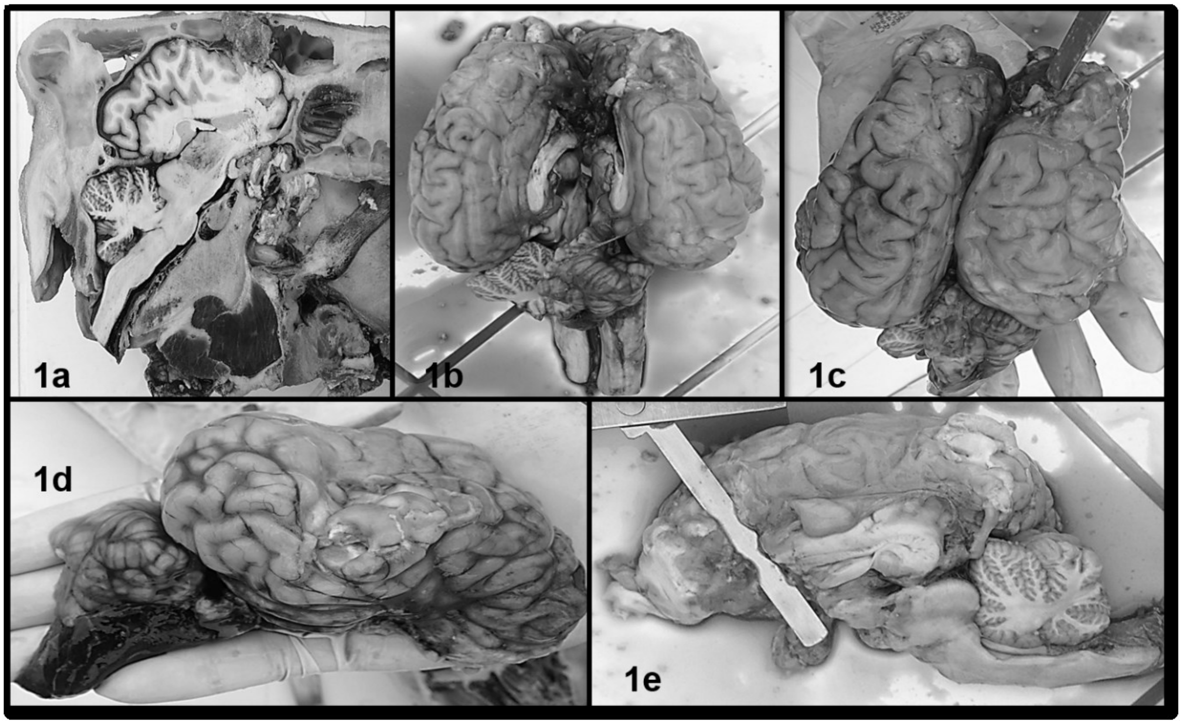

Figure 1. Adult male Nelore cattle: Shot failed to reach the brain when shooting with PCB gun operating with 160 *psi* (1a). Ventral and superior aspects of the brain showing frontal lobe laceration when shot with PCB gun operating with 160 *psi* (1b and 1c). Right cerebral hemisphere showing abundant subarachnoid haemorrhage around the brainstem (medulla, pons, midbrain) when shot with PCB gun operating with 190 *psi* (1d). Protractor probe inserted into the bolt wound cavity in the right cerebral hemisphere highlighting laceration reaching the hypothalamus of a head shot with PCB gun operating with 190 *psi* (1e).

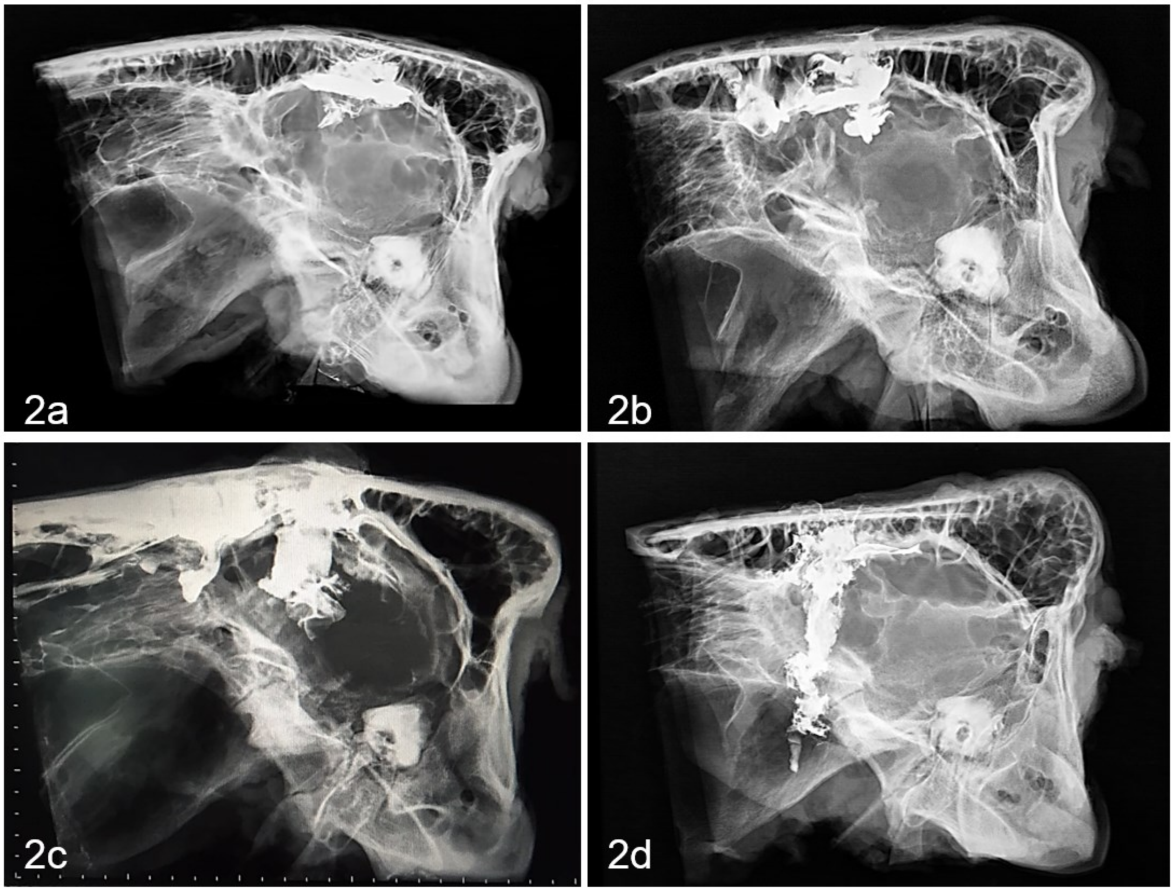

Figure 2. Radiographic images of adult male Nelore cattle shot with pneumatically powered penetrating captive bolt gun with infusion of 5 to 10 ml of barium sulphate to produce contrast through the bolt cavity: Cattle shot with PCB gun operating with 160 and 175 *psi* showing a very shallow bolt penetration depth (2a and 2b, respectively). Greater penetration depth caused by PCB operating with 190 *psi* (2c and 2d).