Magnetic Resonance Imaging and Computer Tomography of Brain Lesions in Water Buffaloes and Cattle stunned with Handguns or Captive Bolts

Barbara K. Schwenk[1], Steffen G. Ross[2], Isabel Lechner[3], Dominic Gascho[2], Michael H. Stoffel[1]

[1] Division of Veterinary Anatomy, University of Berne, Berne, Switzerland
mailto:michael.stoffel@vetsuisse.unibe.ch
[2] Department of Forensic Medicine and Radiology, Institute of Forensic Medicine, University of Zurich, Switzerland
mailto:Steffen.Ross@irm.uzh.ch
[3] Veterinary Public Health Institute, University of Berne, Berne, Switzerland
mailto:isabel.lechner@vetsuisse.unibe.ch

Corresponding Author:
Michael H. Stoffel, Prof. Dr. med. vet.
Division of Veterinary Anatomy, University of Berne
Länggassstrasse 120, POB 8466
CH - 3001 Bern, Switzerland
Tel: +41 (0)31 631 22 05
Fax: +41 (0)31 631 26 15
Email: michael.stoffel@vetsuisse.unibe.ch

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Abstract

Owing to the demand for genuine mozzarella, some 330 water buffaloes are being slaughtered every year in Switzerland albeit a stunning procedure meeting animal welfare and occupational safety requirements still remains to be established. In an effort to improve concussion, we determined the distance from accepted contact points to the thalamus in water buffaloes and cattle and we assessed brain lesions by diagnostic imaging after stunning with captive bolts or handguns. In water buffaloes, the average distance from frontal skin to thalamus was 143 mm, the maximum value being 172 mm. Consequently, captive bolt stunners with a protruding length of 90 or 120 mm are insufficient. Handguns lead to immediate collapse of the animal and caused more severe brain damage than bolts. Thus, free projectiles are suited to stun buffaloes reliably. However, occupational safety hazards remain to be resolved. The results presented herewith shall provide the basis for the development of a device allowing proper stunning of water buffaloes.

Keywords: *Bubalus bubalis*, concussion, skull, anatomy, diagnostic imaging

1. Introduction

Over the last decade, water buffalo husbandry has become increasingly popular in Switzerland. According to the Swiss Animal Tracing Database, water buffalo livestock amounted to approximately 1,750 animals in 2013 (Federal Food Safety and Veterinary Office, Dr. Alexandra Briner, personal communication). The interest in rearing this species increased as a consequence of an ongoing price decline for cow's milk (Zemp, 2012) paralleled by an increasing demand for genuine mozzarella. This has made the production of buffalo milk an economically interesting niche product; since 1996, when the first animals were imported from Romania, the number of buffaloes has increased continuously. In response to stock management requirement to eliminate culled animals and market demand for water buffalo meat, approximately 330 water buffaloes per year in Switzerland.

To meet the Swiss legal requirements to ensure animal welfare at the time of slaughter, adequate stunning must produce deep unconsciousness (Tierschutzgesetz, 2014). As consciousness arises from thalamocortical projections, stunning methods aim to disrupt the information transfer to and within the cerebral cortex. In cattle, this is usually achieved by producing mechanical damage to the forebrain and or brain stem using percussive devices (Fries, Schrohe, Lotz, & Arndt, 2012). Percussive stunning may be achieved by means of either a non-penetrating percussive apparatus or a perforating tool. The perforating devices may be either a captive bolt or a bullet, both of which must strike through the skin and skull to enter the cranial cavity. As a consequence, the effectiveness of a perforating stunning procedure is highly dependent on the given anatomical conditions and the device being used. Penetrating stunning devices may be used in the frontal, crown, or occipital position. Furthermore, Swiss legislation applicable to the slaughtering of cattle bans the use of penetrating stunning devices in the occipital position in adult cattle over 6 months of age (Verordnung des BLV über den Tierschutz beim Schlachten, 2014). Although *Bubalus bubalus* belongs to the same family of Bovidae as domestic cattle, the anatomical characteristics of the head differ considerably between the two species.The skull bones were shown to be substantially thicker and the frontal and paranasal sinuses are noticeably wider in buffaloes compared to bovines (Saigal & Khatra, 1977). Moreover, these anatomical features vary markedly with the sex and age of the animals (Dyce, Sack, & Wensing, C. J. G, 2010). Furthermore, skin thickness may be expected to play a significant role as well. To ensure adequate stunning in compliance with animal welfare requirements (Atkinson, Velarde, & Algers, 2013; Gouveia, Ferreira, Roque da Costa, Vaz-Pires, & Martins da Costa, 2009), these anatomical characteristics need to be carefully taken into account. This is well illustrated by the fact that conventional captive bolt devices usually fail to produce deep unconsciousness in water buffaloes when used as in cattle (Camisasca & Calzolari, 1995). To date, several studies dealing with the anatomy of the skull and the paranasal sinuses of water buffaloes have been published (Kamel & Moustafa, 1966; Lakshminarasimhan, 1974; Meyer & Fiedler, 2005; Moustafa & Kamel, 1971; Saigal & Khatra, 1977; Singh, Soni, & Manchanda, 1972). However, neither age-related nor sex differences were considered, and findings have not been exploited to assess or improve stunning techniques.

The goal of the present study was to revisit the anatomical specifics of the head of water buffaloes compared to cattle. The heads from buffaloes and domestic cattle were investigated after stunning, taking into account both sex and age. The anatomical specifics and brain lesions were assessed by gross anatomical dissection and diagnostic imaging using magnetic resonance imaging (MRI) and computer tomography (CT). The use of these imaging techniques enabled us to gain accurate information on the topography of the skull and brain with respect to the landmarks that are relevant to stunning. In addition, an assessment of the brain lesions after exertion of various stunning methods was used to judge their potential in producing deep unconsciousness.

2. Materials and Methods

Head collection and stunning techniques

The heads of the water buffaloes were collected from 8 different abattoirs that were selected based on data from the Federal Food Safety and Veterinary Office FSVO. One single person was in charge of stunning for any given slaughterhouse. Three butchers used different handguns, one butcher used a bullet casing gun and four butchers relied on three different types of captive bolt stunners (Table 1). The heads were collected from animals that had been stunned in the context of regular slaughterings. Immediate collapse was considered as evidence of effective concussion. This was the case for all animals included in this study. Heads were assigned to four different groups according to sex and age.

Two buffalo brains were removed immediately after slaughter and fixed in 10% formalin to macroscopically assess the severity of the lesions. Another two heads were sectioned in a mid-sagittal plane after diagnostic imaging to monitor the dimension of the sinuses, the extent of the cavum cranii and their topographical relationships.

Thirty-five heads were used for diagnostic imaging. Four MRI scans were discarded for technical reasons, thus yielding 35 CT datasets and 31 MRI datasets (Table 1).

The stunning of water buffaloes was performed with one of the following methods: conventional penetrating captive bolt devices (Cash Magnum 9000S, EFA Schmid & Wezel GmbH & Co. KG Maulbronn, Germany or Schermer KL, Karl Schermer GmbH & Co KG, Ettlingen, Germany; protruding length: 121 and 125 mm, respectively) in either the frontal or occipital position, with a pneumatic captive bolt gun (EFA VB 215, EFA Schmid & Wezel GmbH & Co. KG Maulbronn, Germany; protruding length: 135 mm) in the occipital position, with a bullet-casing gun in the frontal position ("humane killer", no manufacturer's data available) (Anonymous, 2008), with a revolver or pistol in the frontal position (44 S&W Magnum, Smith and Wesson, Springfield, USA; Ruger GP 100 Double Action Revolver, Sturm, Ruger & Co., Inc., Mayodan, USA; Swiss Army Pistol SIG P220, SIG Sauer GmbH & Co. KG, Eckernförde, Germany). Specifications of the stunning devices and ammunition as well as information regarding their application are provided in Table 1. The heads of the water buffaloes were documented photographically with respect to the bullet holes and skin lesions prior to further examination. All but two heads were polled prior to further examination.

The cattle heads were collected from three slaughterhouses (Table 1) in which the stunning was performed with captive bolt devices in frontal position (Kuchen, NATURaktiv AG, Winterthur, Switzerlan or Cash Magnum 9000S, EFA Schmid & Wezel GmbH & Co. KG Maulbronn, Germany; protruding length: 90 and 121 mm, respectively; Table 1) or with an air-operated captive bolt gun (EFA VB 215, EFA Schmid & Wezel GmbH & Co. KG Maulbronn, Germany; protruding length: 135 mm) according to standard procedures (Verordnung des BLV über den Tierschutz beim Schlachten, 2014; Fries et al., 2012). Diagnostic imaging was also used to assess the 12 heads from domestic cattle as controls, yielding 12 CT datasets and 8 MRI datasets as 4 MRI datasets had to be discarded for technical reasons. MRI data sets were obtained from two females over 30 months (category f2), four males between 15 and 30 months (category m1) and two males older than 30 months (category m2). Cattle heads were not photographed because stunning was performed according to established standard procedures.

Head/skull examination

Based on topographical relationships as determined on sagittally sectioned heads of water buffaloes, the frontal point of entry for captive bolt guns was chosen at the intersection of two lines connecting the lower edge to the upper edge of the contralateral horn. The occipital point of entry was located at the level of the lower edge of the horns, i.e., above the insertion of the nuchal ligament. For cattle, the point of entry for captive bolt stunning was selected according to the standard procedure, i.e. at the intersection of the two lines connecting the nasal ocular angle to the lower edge of the contralateral horn. Handguns used by three butchers were fired at a distance of approximately 5 cm from the head. All the corresponding points of entry of the free projectiles were between a line connecting the medial ocular angles and a line at midlevel of the horn bases.

Unless scanning was carried out within 2 days of slaughter, the heads from both water buffaloes and cattle were stored at -20°C in a deep freezer and thawed for 72 hours before scanning. For the scanning procedure, all the heads were wrapped in heavy-duty plastic bags. The heads were examined with a 3-Tesla MR scanner (Achieva 3.0 TX, Philips Medical System, Best, The Netherlands) using a 16-channel SENSE-XL-torso coil. The sequences included an axial, coronal and sagittal T2-weighted (T2 W) sequence with fat saturation. Slice thickness was 3.0 mm for all the sequences. Computer tomograms were obtained with a dual-source CT scanner (SOMATOM FlashDefinition, Siemens, Forchheim, Germany) with 2x128 slices. Data reconstruction was performed with 0.6 mm slice thickness in a soft (B30) and a hard (B70) reconstruction algorithm. Multiplanar and 3-dimensional reconstructions were performed at a multimodality workstation (LEONARDO, SynGo, Siemens Medical Solutions, Forchheim, Germany).Data were analyzed with the Osirix® software (Pixmeo, Bernex, Switzerland).

The following landmarks were identified prior to proceeding with data assessment: optic canal including its lateral edge, nasal, frontal, occipital, basisphenoid and presphenoid bones, hypophyseal fossa, cribriform plate and crista galli of the ethmoid bone, hard palate and nasal septum.

Several anatomical measurements were taken. Hide thickness and sinus width were assessed in CT datasets whereas MRI datasets were used to determine brain damage and the fate of free projectiles. Hide thickness (HT) without coat was measured at a right angle to the frontal bone at the level of the dorsal end of the crista galli (HT1) and at the level of the rostral end of the hypophyseal fossa (HT2). Similarly, the distance between the layers of compact bone delimiting the frontal sinus was measured twice at the same locations as for the skin (Sinus width SW1 and SW2) (Fig. 1a). Furthermore, the distance from any chosen contact point (frontal or occipital stunning) to the thalamus as the target region was determined either from the skin surface or from the bone when specimens had been skinned previously.

MRI and CT datasets were used to determine the localization and extent of brain injuries produced by the weapons and devices used and to assess the path and effect of bolts and bullets. Lesions were graded as 0 (no detectable lesion), 1 (detectable lesion, affected anatomical structures still identifiable) or 2 (severe damage or destruction, loss of identifiable anatomical detail) (Fig. 1b). Damage was assessed separately for the following brain regions: right and left frontal lobes, right and left olfactory, parietal, occipital and temporal lobes, diencephalon, mesencephalon, cerebellum and rhombencephalon.
The pathways of bolts and the free projectiles fired with handguns or with the bullet casing gun were tracked from entry to endpoint or exit point and assessed in MRI and CT datasets whenever possible. Points of entry as well as points of exit were identified where applicable. The length of the trajectories from entry point to endpoint and first deflection point, respectively, were determined. Shots were considered through and through when the bullet exited the cranial cavity. Projectiles were considered retained when the bullet did not exit the cranial cavity. Finally, imaging data were scrutinized for bone fragments, bullet disintegration and fate of the main bullet fragment. Heads from domestic cattle were analyzed correspondingly as far as measurements were applicable to captive bolt stunning.

Statistical analysis

Data were subjected to statistical analysis using StataCorp. 2011 (Stata Statistical Software: Release 12. College Station, TX: StataCorp LP, Texas, USA). The data were first checked for outliers, missing variables and errors in data entry, and comprehensive descriptive statistical analysis was then performed. Considering the limited number of observations (n = 31), and since the main objective of the study was to detect anatomic differences between subgroups of the study population, a Student's t-test was used after verifying that the outcome variable was normally distributed using the Shapiro–Wilk Normality Test (p-value < 0.05 was considered indicative of non-normality). For non-normally distributed data or statistical comparisons with an observation number < 10, the more conservative and non-parametric Wilcoxon Rank Sum Test was used. Intergroup comparisons of the hide thickness, sinus width, as well as the distance from the point of entry to the thalamus were performed, and a p-value of < 0.05 was considered statistically significant.

3. Results

All the cattle examined collapsed immediately at the first stunning attempt.

The measurements of hide thicknesses in water buffaloes and domestic cattle as a function of sex and age are summarized in Fig. 2a, b. The distance from the superficial border of the epidermis to the outer surface of the skull was typically larger in water buffaloes than in cattle. However, the difference in hide thicknesses 1 and 2 were significant in female animals only ($n = 10$, $p = 0.04$ and 0.03, respectively) whereas the difference in hide thicknesses 1 and 2 were not significant ($n = 12$ and 15, $p \geq 0.05$) in either age group of males (Fig. 2a, b). With respect to the width of the frontal sinus, the distance between the layers of compact bone was considerably larger in water buffaloes than in cattle of corresponding age and sex ($n = 17$ for f2 and m2, $n = 17$ for m1, all p-values < 0.05) (Fig. 2c, d).

Although no obvious landmarks were used when stunning was performed with handguns, the pathways of the projectiles as seen in the diagnostic imaging were observed to be very constant for a given butcher. The number of retained missiles and through and through shots as a function of devices used and animals stunned are shown in Table 1. As opposed to free projectiles, captive bolts did not leave a detectable mark within the brain as the bore canal collapsed completely. The extent and localization of brain lesions in water buffaloes and cattle are given in Table 1. In water buffaloes, the destruction of the diencephalon was achieved in 2/5 when applying a captive bolt gun, in 3/4 when using the Swiss army pistol and in 6/14 when the Ruger GP 100 Double Action Revolver was used. Similarly, maximum damage to the frontal lobe ensued in 3/5 animals when using a captive bolt gun and in 11/14 when the Ruger GP 100 revolver was used (Table 1). The through and through shots resulted in 3/3 when using the 44 S&W Magnum with 44 Rem. Mag ammunition, in 2/11 with the Ruger GP 100 revolver and the 357 Mag. ammunition, in 0/3 with the Ruger GP 100 revolver and 38 spl. ammunition, in 4/4 with the Swiss army pistol, and in 1/5 with the bullet casing gun. Bullet fragmentation occurred with the Ruger GP 100 Double Action Revolver and with the bullet-casing gun.

The distance from the chosen point of entry (skin surface) to the thalamus was determined irrespective of the stunning device used. The mean values including the hide were 143 mm versus 105 mm in the frontal position for water buffaloes and cattle, respectively (Table 5), and 106 mm for the one buffalo stunned in occipital position. The corresponding maximum values were 172 mm versus 121 mm in the frontal position for water buffaloes and cattle of both sexes, respectively. The average distance from the frontal point of entry to the thalamus was significantly larger in all male water buffaloes compared to cattle (Wilcoxon Rank Sum Test, $p < 0.05$). In young males (m1, $n = 13$), the distance was 136 mm *vs* 102 mm; in animals older than 30 months (m2, $n = 8$), the distance was 160 mm and 112 mm, respectively. As poll stunning is not allowed in adult cattle, the distance from the occipital point of entry to the thalamus was determined in water buffaloes only. Not considering the skin, the corresponding values were 89 mm for the mean and 98 mm for the maximum distance.

4. Discussion

The present study provides a comprehensive and accurate analysis of brain damage resulting from various stunning procedures currently being used for slaughtering water buffaloes in Switzerland. The brain lesions produced were assessed by diagnostic imaging and were compared to the effects of conventional captive bolt stunning in domestic cattle. Our results show that bolt length in commercially available devices may be sufficient to stun young animals (Table 1) but may not be expected to reliably and consistently produce adequate loss of consciousness in adult water buffaloes; thus, they cannot be recommended as a standard practice. When used properly, free projectiles are suitable to achieve correct stunning. However, the use of handguns is demanding and entails safety hazards for the personnel involved. Thus, neither technique currently being used meets all the requirements for a reliable, humane and occupationally safe stunning of water buffaloes, and further development to resolve the issue is urgently needed.

Swiss laws in effect (Tierschutzgesetz, 2014; Verordnung des BLV über den Tierschutz beim Schlachten, 2014) require deep concussion prior to exsanguination within 60 seconds from stunning. Consciousness in turn is bound to the activity of the cerebral cortex (Daly, Kallweit, & Ellendorf, 1988). On their way to the cortex, however, nearly all sensory afferents need to pass the thalamus as a central gateway. Therefore, this inner area of the diencephalon is the clue to conscious perception related to all the senses but olfaction (Gregory, Spence, Mason, Tinarwo, & Heasman, 2009; Min, 2010). This makes the thalamus an ideally suited and effective target region for inducing concussion besides the cortex itself.

An adequate stunning procedure must immediately induce an irreversible loss of consciousness without causing pain, distress, anxiety or apprehension. It must be reliable, safe to use and should preclude abuse as far as possible (Gregory, Lee, & Widdicombe, 2007). These criteria are largely met for the well-established captive bolt stunning of livestock, although the inadequate depth of concussion has remained a problem even under standard slaughtering conditions (Grandin, 1998; Gregory, 2005; Gregory et al., 2007). Notwithstanding, this technique was used as a reference, and the matching of sex and age groups of domestic cattle *vs* water buffaloes were assessed. As the number of young female water buffaloes being slaughtered is negligible and this category poses the least challenge with respect to stunning, this group was not further examined. On the other hand, maximizing the number of male animals older than 30 months was deliberately pursued, as any method providing adequate stunning in this category may be expected to be effective in all the other groups as well.

The anatomy of the buffalo's head is substantially different from its counterpart in domestic cattle (Kamel & Moustafa, 1966; Meyer & Fiedler, 2005; Moustafa & Kamel, 1971). The frontal sinus is significantly wider, and its depth in older males may easily exceed the length of conventional captive bolts. Although by trend the hide thickness tended to be larger in water buffaloes than in cattle, the differences were largely insignificant; male cattle under 30 months of age had a slightly thicker skin than their exotic counterparts. The fur itself could not be taken into account, as it was not mapped in diagnostic imaging. The distances measured thus constitute slight underestimates. Overall, the distance from a frontal contact point to the thalamus as the target region was substantially and significantly larger in water buffaloes than in cattle. The maximum value determined for domestic bulls was slightly above 120 mm, with an average of approximately 100 mm. By contrast, the average value for water buffaloes was more than 140 mm, with the maximum value exceeding 170 mm. As cattle may not be stunned in the occipital position, the corresponding distance from the occiput to the thalamus was not determined in this species. Notwithstanding, adopting the occipital position in water buffaloes would dramatically reduce the distance from the contact point to the thalamus compared to the frontal approach, with the distance from the occipital point of contact being no more than 80 – 100 mm. This results from the fact that the frontal sinus does not extend up to the occipital contact point, as defined in the present study. In conventional poll stunning as recommended by the Humane Slaughter Association (Anonymous, 2011) and as adopted by Gregory et al. (Gregory et al., 2009), the bolt does penetrate through the cerebellum and may affect the brain stem as well. In the occipital approach investigated in the present study, however, the bolt passed dorsally to the cerebellum and completely spared the rhombencephalon as well. Bovine spongiform encephalopathy has never been reported in water buffaloes (Zhao et al., 2012), thus making the collection of brain stem samples obsolete. Notwithstanding, the rhombencephalon with its autonomous circulatory control center should be spared to support adequate bleeding.

MRI and CT provided a means to assess brain damage resulting from captive bolts or free projectiles. The present study relied on material collected from regular, workmanly slaughterings of animals which underwent bleeding after adequate loss of consciousness only. Our goal was not to validate concussion as based on clinical findings but to compare brain lesions with respect to the stunning procedure used. Captive bolts produced only unimpressive brain lesions as seen in diagnostic imaging. These observations are in accordance with other reports (Finnie, 1993). Free projectiles, however, left severe damage on their trajectory. This is consistent with the immediate collapse of animals when stunned with handguns. This observation and MRI and CT data indicate that free projectiles are at least as effective in producing concussion as captive bolts. However, future stunning devices for water buffaloes will need a thorough clinical assessment with respect to an immediate and complete loss of consciousness.

Taken together, these results show that conventional guns with a bolt length of no more than 90 mm are inadequate. Furthermore, even specially designed captive bolt guns with a protruding length of 120 mm may only be effectual in younger animals but may not be considered to be reliable enough to stun water buffaloes in the frontal position irrespective of the animal’s age and sex. Provided that the energy delivered is adequate, however, the diencephalon might be reached with these devices when used in the occipital position. Thus, the use of penetrating bolts in the occipital position might be considered to provide a solution. Unfortunately, the feasibility of an occipital approach remains questionable for practical reasons. Efforts to reach the neck in a standard environment failed consistently as the animals were alienated when personnel was acting outside of their field of vision and constantly attempted to turn their heads back. To reach the animal's neck would require the animals to be immobilized. The requirement of a very accurate positioning of the captive bolt gun can hardly be met in a common setting considering the behavior of the conditionally tame water buffaloes. Therefore, a frontal approach seems inevitable. This, however, will require the length of the bolt to be increased to 180 mm and adaptation to the delivered energy to reach the thalamus. Retraction of the bolt is a prerequisite for the butcher to retain the stunning device in his hands. Furthermore, retention of the bolt in the skull would exacerbate the difficulties of shooting the animal a second time. However, lengthening of the bolt is likely to dramatically impede its retraction, and, thus, is highly undesirable. Indeed, all the skull holes observed in this study were sharp-edged as punched-out, and no cracks were observed in the surrounding bone tissue. This results in a very tight guiding of the bolt by the two holes being produced in the inner and outer tables delimiting the frontal sinus. Even a slight tilt will thus be enough to block the withdrawal of the bolt. Current investigations aim at optimizing bolt shape to facilitate retraction.

The use of handguns by the experienced butchers participating in the present study reliably produced immediate collapse. Reproducibility was impressively confirmed in diagnostic imaging, where the pathways of the projectiles were observed to be very constant for a given butcher. This was all the more astounding as the weapons were not put on the head at a right angle but were fired at different oblique angles according to the slaughterer's experience and preference. Notwithstanding, the use of handguns in dealing with barely tamed animals is highly demanding and ultimately remains hazardous. Bullets exiting the cranial cavity were noted in a substantial number of cases (Table 1), and projectiles exiting the animal's body after proper stunning were reported by butchers in several instances. Unfortunately, the bullet's final trajectory could not be tracked any further through the body, as only the heads were available for investigation. Although such incidents were reported when the Swiss Army Pistol was used in combination with full jacket bullets only, this observation makes such an approach highly questionable from an occupational safety point of view.

In conclusion, the results presented in this study show that deep concussion is difficult to achieve in water buffaloes with commercially available captive bolt guns when used in the frontal position. Although the thalamus might be reached more easily from the occipital contact point, this approach is not compatible with common slaughtering settings. Free projectiles, however, produce adequate loss of consciousness in accordance with animal welfare requirements. Yet, occupational safety hazards associated with handguns remain to be resolved. Thus, the challenge of developing a reliable device allowing the stunning of water buffaloes and fulfilling both the welfare and safety requirements remains to be met.

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7. Table headings

Table 1 ***Analysis of MRI data and assessment of brain lesions resulting from various stunning devices used for water buffaloes taking into account age and sex***

Age and sex groups: m1 = male animals up to 30 months of age; m2 and f2 = male and female animals older than 30 months of age, respectively.

Points of entry: F = frontal, O = occipital.

Damage to brain regions: 0 = undamaged, 1 = damaged, 2 = destroyed.

Behavior of bullet: R = Ricochet, BFS = Bullet already fragmented within the sinus, FMJ = full metal jacket – no deformation, * Presphenoid and basisphenoid bones were partly broken away, ** Projectile stopped above the eye, *** No ricochet - Focal lifting of the calvarium resulted from the increase in intracranial pressure, # The projectile ricocheted from the petrosal bone and left the cranial cavity through the Foramen magnum, ## The projectile ricocheted from the temporal bone and then became stuck within the brain, ### Metal fragments were found within the sinus and the cranial cavity as well as in the presphenoid bone where the projectile ricocheted.

8. Figure Captions

Figure 1: ***Computer tomography and Magnetic resonance imaging of water buffalo heads.***

*a: CT image of unskinned head from a male water buffalo older than 30 months***.** Green lines show how measurements of hide thickness and sinus width were established. 1: HT1 and SW1 were measured at a right angle to the frontal bone at the level of the dorsal end of the *Crista galli*. 2: HT2 and SW2 were measured at a right angle to the frontal bone at the level of the rostral end of the *Hypophyseal fossa*. *b: Assessment of brain lesions based on an MRI image of a head from a male water buffalo under 30 months of age that was stunned with the Ruger GP 100 Double Action with 38 spl ammunition.* The frontal lobe was rated as undamaged (score 0), and the diencephalon, the rhombencephalon and the cerebellum were rated as destroyed (score 2). When assessing brain damage, the complete dataset was taken into account.

Figure 2: ***Statistical analyzes of length measurements***

*a, b: Hide thickness in water buffaloes and cattle according to age and sex groups***.** a: Hide thickness 1 (HT1), b: Hide thickness 2 (HT2). Specification of measurements: see Fig. 1.

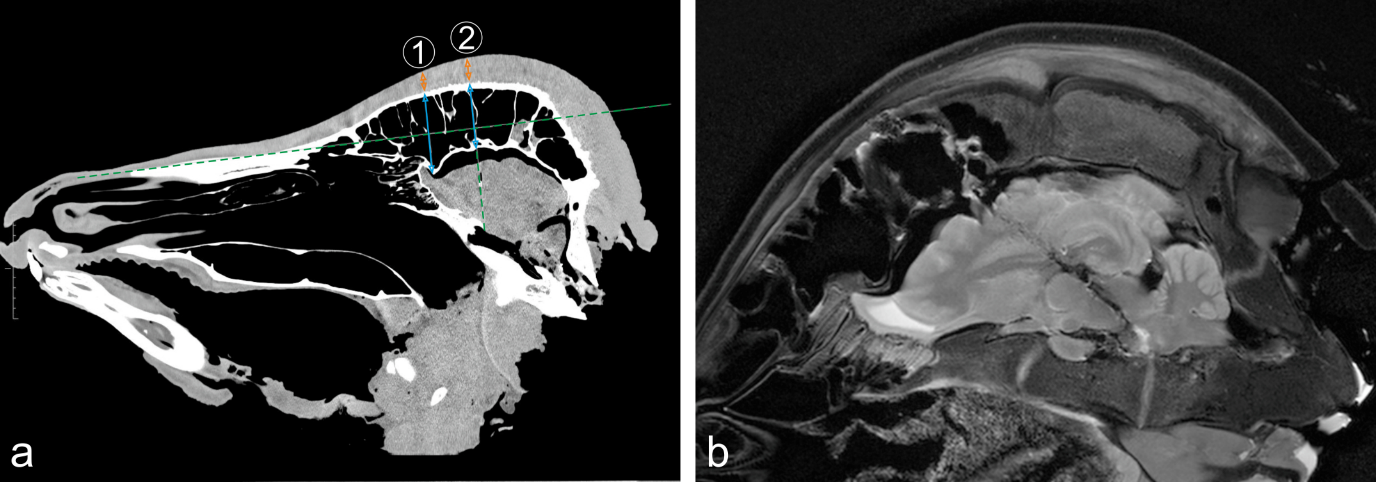
c, d: Sinus width in water buffaloes and cattle according to age and sex groups c: Sinus width 1 (SW1), d: Sinus width 2 (SW2). Specification of measurements: see Fig. 1.

m1 = male animals up to 30 months of age; m2 and f2 = male and female animals older than 30 months of age, respectively; * = statistically significant difference (Student’s t-test or Wilcoxon Rank Sum Test, $p < 0.05$)

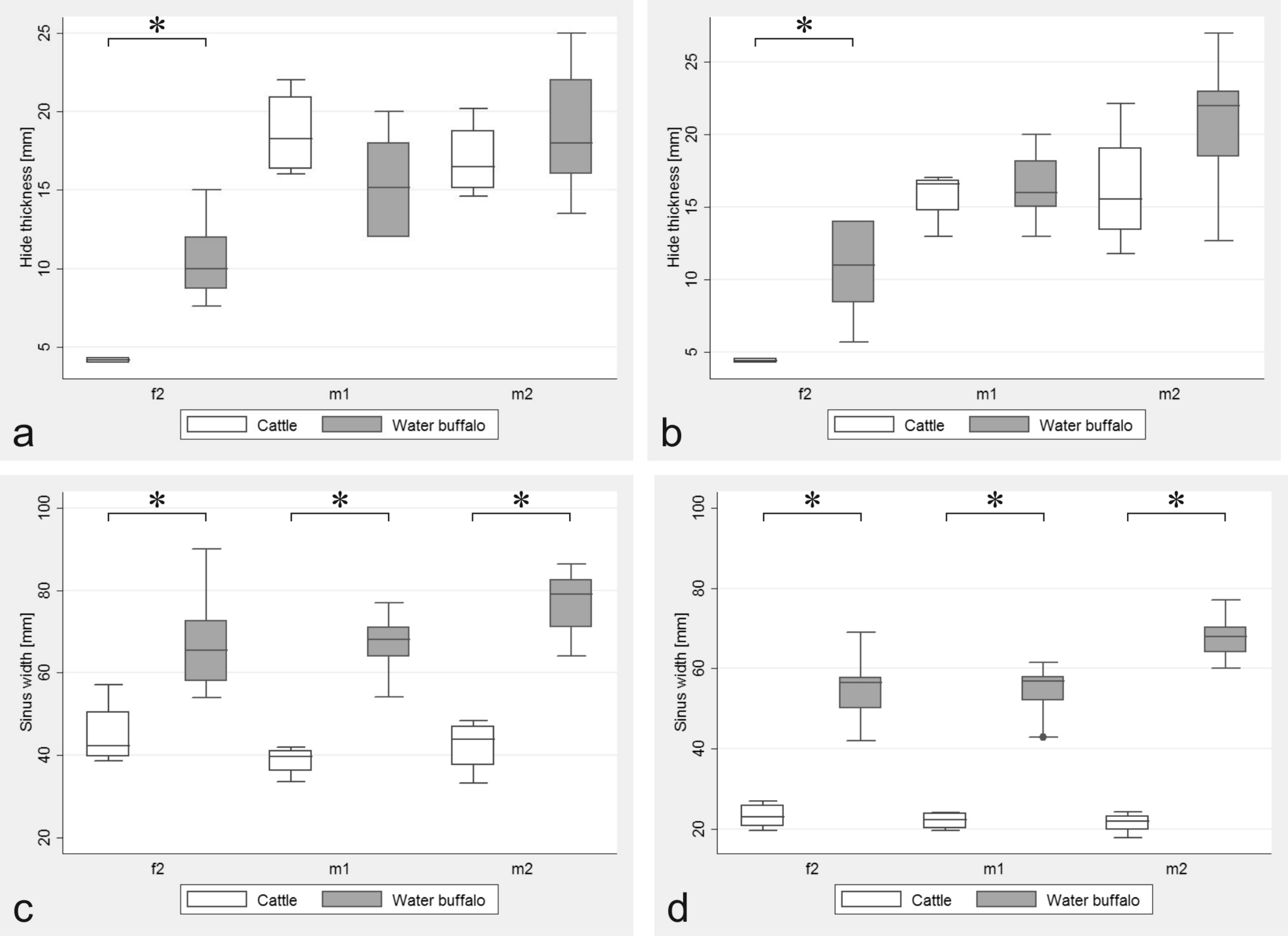
Table 1

Table 1

	HANDGUN OR DEVICE	ABATTOIR	AMMUNITION OR TYPE OF BOLT GUN	CALIBRE/DIAMETER [mm]	BULLET WEIGHT [g]	BOLT LENGTH [mm]	CALIBRE [inches] / WEIGHT [g] OF CARTRIDGES	AGE AND SEX GROUPS	POINTS OF ENTRY	DAMAGE TO				LOCALIZATION OF MAIN BULLET FRAGMENT	EXIT POINT	BEHAVIOUR OF BULLET
										FRONTAL LOBE	DIENCEPHALON	CEREBELLUM	RHOMBENCEPHALON			
WATER BUFFALOES	**44 S&W MAGNUM**	**1**	44 Rem. Mag.	10.9	15.6			f2	F	2	0	0	0		Occiput	*
								f2	F	2	0	0	0		Lacrimal bone	**
								f2	F	2	0	2	0		Presphenoid bone	***
	RUGER GP 100 DOUBLE ACTION	**2**	357 Mag.	9.0	10.2			f2	F	2	2	0	0	Falx cerebri (between the hemispheres)	None	##
								f2	F	2	1	1	0	Occiput	None	
								f2	F	2	1	1	1	Occiput	None	
								f2	F	2	0	0	2	Occiput	None	
								f2	F	2	0	0	0	Rhombencephalon	None	R
								m1	F	2	0	0	0		Presphenoid bone	
								m2	F	2	2	2	2	Petrosal bone	None	BFS
								m2	F	2	2	1	2		Foramen magnum	BFS #
								m2	F	2	0	0	0	Presphenoid bone	None	
								m2	F	2	0	0	0	Occiput	None	
								m2	F	0	2	0	2	Occiput	None	
	RUGER GP 100 DOUBLE ACTION	**2**	38 Spl.	9.0	8.0			m1	F	2	2	2	2	Rhombencephalon	None	
								m1	F	0	2	2	2	between Cerebellum and Cerebrum	None	
								m1	F	0	0	0	0	Petrosal bone	None	
	SWISS ARMY PISTOL SIG P220	**3**	9 mm	9.0	8.0			m1	F	1	2	0	1		Occiput	FMJ
								m1	F	0	2	0	0		Basisphenoid bone	FMJ
								m1	F	2	1	0	0		Presphenoid bone	FMJ
								m1	F	0	2	0	0		Basisphenoid bone	FMJ
	BULLET CASING GUN	**4**	7.5 mm	7.5	8.1			f2	F	2	1	0	0	Intersection Basi-/presphenoid bones	None	
								f2	F	2	1	0	0	Presphenoid bone	None	
								f2	F	2	0	0	0		Presphenoid bone	
								f2	F	2	2	0	0	Presphenoid bone	None	R
								m2	F	2	1	0	0	Parieto-occipital transition	None	R ###
	BOLTGUNS	**5**	Cash Magnum 9000S	11.4	-	121.0	0.22/0.80	f2	O	0	2	0	0			
		6	Schermer KL	12.0	-	125.0	0.27/1.40	m1	F	2	2	1	2			
								m1	F	2	1	2	0	does not apply		
		7						m2	F	2	0	0	0			
		8	EFA VB 215	12.0	-	135.0	pneumatic	m1	O	0	0	2	0			
CATTLE	**BOLTGUNS**	**9**	Kuchen	11.0	-	90.0	0.27/1	m2	F	2	0	0	0			
								m2	F	2	1	0	0			
		10	Cash Magnum 9000S	11.4		121.0	0.22/0.80	f2	F	0	1	0	0			
					-			f2	F	2	0	0	0	does not apply		
		11	EFA VB 215	12.0	-	135.0	pneumatic	m1	F	2	2	0	1			
								m1	F	2	2	0	0			
								m1	F	0	0	0	0			
								m1	F	0	1	0	0			



1
2
a
b



Hide thickness [mm]
Sinus width [mm]
f2
m1
m2
Cattle
Water buffalo
*
a
b
c
d