Assessment of different stunning methods used for water buffaloes by means of MRI and CT

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Abstract

In view of genuine mozzarella production, keeping of water buffaloes has become increasingly popular in Switzerland. Even though *Bubalus bubalis* belongs to the same family of *bovidae* as domestic cattle, anatomical characteristics of the head differ considerably which makes the stunning of water buffaloes substantially more difficult. Available devices fail to produce adequate loss of consciousness reliably and, thus, do not fulfill animal welfare requirements. Therefore, butchers slaughtering water buffaloes mainly fall back on handguns. Though efficient, this technique is demanding and entails occupational safety hazards. The present study was undertaken to investigate anatomical specifics of water buffaloes as compared to cattle and to accurately assess brain lesions by diagnostic imaging after various stunning procedures in an effort to improve concussion. The use of captive bolt guns in an occipital position may be effective but is not practicable under common circumstances. Current efforts, therefore, aim at developing a captive bolt gun providing adequate stunning when used in a frontal position.

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

1. Introduction

Over the last decade, keeping water buffaloes has become more and more popular in Switzerland. According to the Swiss Animal Tracing Database, the livestock of water buffaloes amounted to approximately 1750 animals in 2013 (Federal Food Safety and Veterinary Office, personal communication). Interest in keeping this species rose as a consequence of an ongoing price decline for cow's milk [Zemp, 2012] being paralleled by an increasing demand for genuine mozzarella. This made the production of buffalo milk an economically interesting niche product, and since 1996, when the first animals were imported from Romania, the number of buffaloes has increased continuously. Notwithstanding, stock management requires animals to be eliminated and in addition, there is also a demand for buffalo meat. This results in the slaughtering of about 330 water buffaloes per year in Switzerland.

Adequate stunning must produce deep unconsciousness which is a legal requirement for slaughtering [Anonymous, 2005]. As consciousness arises from thalamocortical projections, stunning methods aim at disrupting information transfer to and within the cerebral cortex. In cattle, this is usually achieved by producing mechanical damage to the forebrain by means of percussive devices. Penetrating stunning devices may be used in either frontal, crown or occipital position. In order to uphold the function of the circulatory and respiratory centers and to allow sample collection for the diagnostics of bovine spongiform encephalitis (BSE), the brain stem must be spared. Therefore, the use of penetrating stunning devices in the occipital position is not allowed in Switzerland for adult cattle over 6 months of age [Anonymous, 2010]. Percussive stunning may be achieved by means of either a non-penetrating percussive apparatus or a perforating tool. Perforating devices may be either a captive bolt or a bullet both of which must necessarily strike through the skin and skull to reach the cranial cavity. As a consequence, effectiveness of a perforating stunning procedure is highly dependent on the given topographical circumstances and the device being used. Even though *Bubalus bubalus* belongs to the same family of *bovidae* as domestic cattle, anatomical characteristics of the head differ considerably between the two species. Thus, skull bones are substantially thicker and the frontal and the paranasal sinuses are noticeably wider in buffaloes as compared to the bovine [Saigal and Khatra, 1977]. Moreover, these anatomical features vary markedly with sex and age of the animals. To ensure adequate stunning in compliance with animal welfare requirements, these anatomical characteristics need to be taken into account carefully. This is well illustrated by the fact that conventional captive bolt devices usually fail to produce acceptable stunning in water buffaloes when used as in cattle [Camisasca and Calzolari, 1995]. To date, only few studies dealing with the anatomy of the skull and the paranasal sinuses of water buffaloes have been published [Kamel and Moustafa, 1966; Moustafa and Kamel, 1971; Singh et al., 1972; Lakshminarasimhan, 1974; Saigal and Khatra, 1977; Meyer and Fiedler, 2005; Forster, personal communication]. 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, therefore, was to revisit the anatomical specifics of the head of water buffaloes as compared to cattle. Heads from buffaloes and from domestic cattle were investigated after stunning, taking into account both sex and age. Anatomical specifics as well as brain lesions were assessed by gross anatomical dissection and diagnostic imaging by means of 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 landmarks being relevant for stunning. In addition, assessment of the brain lesions after exertion of various stunning methods was used to judge their potential in producing deep unconsciousness.

2. Material and Methods

Material

The heads of 41 water buffaloes were collected from 8 different abattoirs that had been selected as based on data from the Federal Food Safety and Veterinary Office FSVO. Heads were assigned to four different groups according to sex and age (Table 1). Two heads were used to collect the brains for gross anatomical necropsy and two heads were split in the median plane. The other 37 heads were used for diagnostic imaging. Four MRI-scans had to be discarded for technical reasons, this yielding 37 CT datasets and 33 MRI datasets, respectively (Table 1). Furthermore, diagnostic imaging was used to assess 12 heads from domestic cattle as controls, this yielding 12 CT datasets and 8 MRI datasets, respectively).

Stunning of water buffaloes was effected with one of the following means: conventional penetrating captive bolt devices (protruding length: 121 and 125 mm, respectively) in frontal or occipital position, pneumatic captive bolt gun (protruding length: 135 mm) in occipital position, bullet casing gun ("humane killer") [Anonymous, 2008] or revolver or pistol in frontal position. Specifications of stunning devices used are provided in Table 2 and stunning practices used for every single animal are provided in Table 3. 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 had been polled prior to further examination.

Stunning of cattle was effected with captive bolt devices (protruding length: 90 and 121 mm, respectively) or an air-operated captive bolt gun (protruding length: 135 mm). Cattle heads were not photographed because stunning was performed according to well-known standard procedures.

Methods

In 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. As for cattle, the point of entry for captive bolt stunning was selected according to the standard procedure at the intersection of the two lines connecting the nasal ocular angle to the lower edge of the contralateral horn. As for the free projectiles, points of entry varied according to the slaughterers' preferences but were within the range of a connecting line between the medial ocular angles and one at midlevel of the horn bases.

Two buffalo brains were removed immediately after slaughtering and fixed in 10% formalin in order to assess the severity of the lesions macroscopically. Two heads were sectioned in a mid-sagittal plane after scanning so as to monitor the dimension of the sinuses, the extent of the cavum cranii and its topographical relationships.

Unless scanning was carried out within 2 days from slaughtering, 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. As stated above, the 37 heads yielded 37 complete CT and 33 MRI scans, respectively.
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 sagital T2-weighted (T2W) 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 done at a multimodality workplace

(LEONARDO, SynGo, Siemens Medical Solutions, Forchheim, Germany). Thereafter, data were analyzed with the Osirix® software (Pixmeo, Bernex, Switzerland). Data were subjected to statistical analysis by StataCorp. 2011 (Stata Statistical Software: Release 12. College Station, TX: StataCorp LP, Texas, USA). Intergroup comparisons of hide thickness, sinus width as well as the distance from the point of entry to the thalamus were performed by use of the Student's t-test. A resulting p-value < 0.05 was considered significant.

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 performed. 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. 1). 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 data sets 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 either 0 (no detectable lesion), 1 (detectable lesion, affected anatomical structures still identifiable) or 2 (severe damage or destruction, loss of identifiable anatomical detail) (Fig. 2). 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 of 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 main bullet fragment. Heads from domestic cattle were analyzed correspondingly as far as measurements were applicable to captive bolt stunning.

3. Results

Measurements of hide thicknesses in water buffaloes and domestic cattle as a function of sex and age are summarized in Fig. 3. The distance from the superficial border of the epidermis to the outer surface of the skull usually was larger in water buffaloes as compared to cattle. However, the difference was significant in female animals older than 30 months only and young male cattle even had a slightly thicker skin on average than water buffaloes. With respect to the width of the frontal sinus, the distance between the layers of compact bone was considerably larger ($p < 0.05$) in water buffaloes than in cattle of corresponding age and sex (Fig. 4).

Though no obvious landmarks were used when stunning was performed with handguns, the pathways of the projectiles as seen in 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 given in Table 3. As opposed to free projectiles, captive bolts did not leave a detectable mark within the brain as the bore canal collapsed completely. Extent and localization of brain lesions in water buffaloes and cattle are given in Table 3 and 5, respectively. In water buffaloes, destruction of the diencephalon was achieved in 3/5 when applying the captive bolt gun, 3/4 when using the Swiss army pistol and 6/18 when the Ruger GP 100 Double Action Revolver was used. Similarly, maximum damage to the frontal lobe ensued in 3/5 animals when using the captive bolt gun and in 15/18 when the Ruger GP 100 revolver was used (Table 3). Through and through shots resulted in 6/18 with the Ruger GP 100 revolver, i.e. three times with the 44 Rem. Mag. and with the 357 Mag. munition, respectively, 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. Mean values including the hide were 143 mm versus 105 mm in frontal position for water buffaloes and cattle, respectively (Table 4), and 106 mm in occipital position for water buffaloes. Corresponding maximum values were 172 mm versus 121 mm in frontal position for water buffaloes and cattle, respectively (Table 4). Values were significantly larger in male water buffaloes as compared to cattle. In young males, the distance was 136 mm vs 102 mm, in animals older that 30 months, the distance was 161 mm and 112 mm, respectively (Fig. 5). Disregarding the hide, mean and maximum values were 129 and 150 mm for water buffaloes and 86 and 88 mm for cattle, 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 (Table 4).

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. 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 commercially available captive bolt guns may be effective in young animals but may not be expected to reliably and consistently produce adequate loss of consciousness in adult water buffaloes and, thus, can not 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 imperatively needed.

Laws in effect require deep concussion prior to exsanguination within 60 seconds from stunning [Daly et al., 1987; Anonymous, 2010]. Consciousness in turn is bound to the activity of the cerebral cortex [Daly et al., 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 et al., 2009; Min, 2010]. This makes the thalamus an ideally suited and effective target region for inducing concussion besides the cortex itself. Concurrently, the mesencephalon and rhombencephalon must be spared in cattle in order to allow tissue collection for diagnostic purposes with regard to bovine spongiform encephalopathy. As this disease has never been reported in water buffaloes, this requirement does currently not apply to this species [Anonymous, 2010; Zhao et al., 2012].

An adequate stunning procedure must immediately induce an irreversible loss of consciousness without causing pain, distress, anxiety or apprehension. It must be reliable, compatible with subsequent examination of tissue samples, safe to use and it should preclude any abuse as far as possible [Gregory et al., 2007]. These criteria are largely met for the well-established captive bolt stunning of livestock although 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 matching sex and age groups of domestic cattle *vs* water buffaloes were assessed. As the number of young female water buffaloes being slaughtered is neglectable and because this category poses the least challenge with respect to stunning, this group was not examined any further. 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 and Moustafa, 1966; Moustafa and Kamel, 1971; Meyer and Fiedler, 2005; Forster, personal communication]. The frontal sinus is significantly wider and its depth in older males may easily exceed the length of conventional captive bolts. Although by trend hide thickness tended to be larger in water buffaloes than in cattle, the differences were largely insignificant, male cattle under 30 months of age even having a slightly thicker skin than their exotic counterparts. The fur itself was not taken into account as it was not evident in diagnostic images. Distances measured thus constitute slight underestimates. Overall, the distance from a frontal contact point to the thalamus as the target region is substantially and significantly larger in water buffaloes than in cattle. The maximum value determined for domestic bulls was slightly above 12 cm, the average being approximately 10 cm. In contrast, the average value for water buffaloes amounted to more than 14 cm, the maximum value exceeding 17 cm. In contrast, adopting an occipital position would dramatically reduce the distance from the contact point to the thalamus to no more than 8 – 10 cm. 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, thus, completely spared the rhombencephalon as well. Bovine spongiform encephalopathy has never been reported in water buffaloes [Zhao et al., 2012], this making the collection of brain stem samples obsolete. Notwithstanding, the rhombencephalon with its autonomous circulatory control center ought to be spared in order to support adequate bleeding.

Taken together, these results show that even specially designed captive bolt guns with a protruding length of 12 cm may not be considered adequate to stun water buffaloes irrespective of age and sex in frontal position let alone conventional guns with a bolt length of only 9 cm. Provided that the energy delivered is adequate, however, the diencephalon might be reached with these devices when used in an occipital position. Thus, the use of penetrating bolts in occipital position might be considered to provide a solution. Unfortunately, feasability of an occipital approach remains questionable from a purely practical point of view. 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 to increase the length of the bolt to 18 cm and to adapt the delivered energy in order to reach the thalamus. Lengthening of the bolt in turn is likely to dramatically impede its retraction. Indeed, all the skull holes being observed in this study were sharp-edged as punched-out and no cracks were ever seen 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 optimising bolt shape in order to facilitate retraction.

The use of handguns by experienced butchers 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 3) 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. Even though 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 herewith show that deep concussion is difficult to achieve with commercially available captive bolt guns when used in a frontal position. Although the thalamus might be reached more easily from an occipital contact point, this approach is not compatible with common slaughtering settings. Thus, the challenge of developing a reliable device allowing the stunning of water buffaloes with a frontal approach remains to be met.

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6. References

Anonymous (2005) Tierschutzgesetz. http://www.admin.ch/opc/de/classified-compilation/20022103/index.html. Accessed 08 January 2014.

Anonymous (2008) BANG! You're dead!: A Primer on Humane Animal Killers. http://www.nrvoutdoors.com/HUMANE%20KILLERS/BANG.htm. Accessed 13 January 2014.

Anonymous (2010) Verordnung des BLV über den Tierschutz beim Schlachten. http://www.admin.ch/opc/de/classified-compilation/20080808/index.html. Accessed 08 January 2014.

Anonymous (2011) Slaughter and Killing of Minority Farmed Species. www.hsa.org.uk.

Camisasca, S., C. Calzolari (1995) Stordimento dei bufali mediante apparecchi esplodenti a proiettile captivo. Obiettivi veterinari(*3)*: 19–22.

Daly, C.C., N.G. Gregory, S.B. Wotton (1987) Captive bolt stunning of cattle: effects on brain function and role of bolt velocity. Br. Vet. J. *143*(*6)*: 574–580.

Daly, C.C., E. Kallweit, F. Ellendorf (1988) Cortical function in cattle during slaughter: conventional captive bolt stunning followed by exsanguination compared with shechita slaughter. Vet. Rec *122*(*14)*: 325–329.

Grandin, T. (1998) Objective scoring of animal handling and stunning practices at slaughter plants. J. Am. Vet. Med. Assoc. *212*(*1)*: 36–39.

Gregory, N.G. (2005) Recent concerns about stunning and slaughter. Meat Sci *70*(*3)*: 481–491.

Gregory, N.G., C.J. Lee, J.P. Widdicombe (2007) Depth of concussion in cattle shot by penetrating captive bolt. Meat Sci *77*(*4)*: 499–503.

Gregory, N.G., J.Y. Spence, C.W. Mason, A. Tinarwo, L. Heasman (2009) Effectiveness of poll stunning water buffalo with captive bolt guns. Meat Sci *81*(*1)*: 178–182.

Kamel, S.H., M.S. Moustafa (1966) A detailed description of the skull of the Egyptian buffalo, Bos (bubalus) bubalis L. with a comparative study of that of the cow. Zentralbl Veterinarmed A *13*(*8)*: 746–752.

Lakshminarasimhan, A. (1974) Morphological variations of the sinus durae matris of the Indian buffalo (Bubalus bubalis). Zentralbl Veterinarmed C *3*(*1)*: 57–62.

Meyer, F., H.-H. Fiedler (2005) Die tierschutzgerechte Betäubung von Wasserbüffeln bei der Schlachtung. Rundschau für Fleischhygiene und Lebensmittelüberwachung(*11)*: 248–249.

Min, B.-K. (2010) A thalamic reticular networking model of consciousness. Theor Biol Med Model *7*(*1)*: 10.

Moustafa, M.S., S.H. Kamel (1971) Sinus paranasalis of the Egyptian buffalo, Bos(Bubalus)bubalis L. Zentralbl Veterinarmed A *18*(*6)*: 530–535.

Saigal, R.P., G.S. Khatra (1977) Paranasal sinuses of the adult buffalo (bubalus bubalis). Anat Anz *141*(*1)*: 6–18.

Singh, K., B.K. Soni, S.K. Manchanda (1972) Brain of the buffalo (Bubalus bubalis) in stereotaxic coordinates. Indian J. Physiol. Pharmacol *16*(*2)*: 127–138.

Zemp, M. (2012) Tiroler Bauernbund: IP Lait. Bern.

Zhao, H., L.-L. Liu, S.-H. Du, S.-Q. Wang, Y.-P. Zhang, G. Forloni (2012) Comparative Analysis of the Shadoo Gene between Cattle and Buffalo Reveals Significant Differences. PLoS ONE *7*(*10)*: e46601.

7. Table headings

Table 1: ***Number of analyzed heads according to age and sex groups***

Table 2: ***Stunning devices and munition used***

Table 3: ***Analysis of CT- and 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 (f1, f2, m1, m2): see Table 1.
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 ricochetted from the petrosal bone and left the cranial cavity through the Foramen magnum, ## The projectile ricochetted 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 ricochetted.

Table 4: ***Distances from frontal and occipital points of entry to the thalamus***
WB = water buffaloes, Ca = cattle

Table 5: ***Analysis of CT- and MRI-data and assessment of brain lesions resulting from frontal use of three different penetrating bolt guns in cattle taking into account age and sex***
Age and sex groups (f1, f2, m1, m2): see Table 1.
Damage to brain regions: 0 = undamaged, 1 = damaged, 2 = destroyed.

Table 1

		Abbreviation	Water buffaloes	Cattle
MALE	15 to 30 months	m1	13	4
	> 30 months	m2	9	4
FEMALE	15 to 30 months	f1	2	0
	> 30 months	f2	13	4
Total			37	12

Table 2

Free projectiles	**Calibre of Munition**	**Calibre [mm]**	**Bullet weight [g]**	**Number of scanned heads**
44 S&W Magnum	44 Rem. Mag.	10.9	15.6	3
357 S&W Magnum	357 Mag.	10.9	15.6	2
Ruger GP 100 Double Action Revolver 357 Magnum	357 Mag.	9.0	10.2	13
	38 Spl.	9.0	8.0	3
Swiss Army Pistol SIG P220	9 mm	9.0	8.0	5
Bullet casing gun (humane killer)	7.5 mm	7.5	8.1	5
Captive bolt guns	**Diameter / length of bolt [mm]**	**Cartridges [g]**	**Calibre of cartridges [inches]**	**Number of scanned heads**
Captive bolt gun Cash Magnum 9000S	11.4 / 121	black / 0.8	0.22	1
Captive bolt gun Schermer KL	12 / 125	red / 1.4	0.27	4
Air operated captive bolt gun EFA VB 215	12 / 135	-	-	1

Table 3

HANDGUN OR DEVICE		AGE AND SEX GROUPS	POINTS OF ENTRY	DAMAGE TO FRONTAL LOBE	DAMAGE TO DIENCEPHALON	DAMAGE TO CEREBELLUM	DAMAGE TO RHOMBENCEPHALON	LOCALIZATION OF MAIN BULLET FRAGMENT	EXIT POINT	BEHAVIOUR OF BULLET
44 S&W MAGNUM	44 Rem. Mag.	f2	F	2	0	0	0		Occiput	*
		f2	F	2	0	0	0		Lacrimal bone	**
		f2	F	2	0	2	0		Presphenoid bone	***
44 S&W MAGNUM AND RUGER GP 100 DOUBLE ACTION, RESPECTIVELY	357 Mag.	f1	F	2	0	0	0		Presphenoid bone	
		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	38 Spl.	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	9 mm	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	7.5 mm	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	Cash Magnum 9000S	f1	O	0	2	0	0	does not apply		
		f2	O	0	2	0	0			
	Schermer KL	m1	F	2	2	1	2			
		m1	F	2	1	2	0			
		m2	F	2	0	0	0			
	EFA VB 215	m1	O	0	0	2	0			

Table 4

	Frontal						Occipital		
	with hide (23 WB, 6 Cattle)			without hide (7 WB, 2 Ca)			without hide (37 WB)		
	min (mm)	max (mm)	mean (mm)	min (mm)	max (mm)	mean (mm)	min (mm)	max (mm)	mean (mm)
Water buffalo (WB)	117	172	143	114	150	129	81	98	89
Cattle (Ca)	101	121	105	83	88	86	-	-	-

Table 5

BOLTGUNS	AGE AND SEX GROUPS	DAMAGE TO			
		FRONTAL LOBE	DIENCEPHALON	CEREBELLUM	RHOMBENCEPHALON
Penetrating bolt gun	m2	2	0	0	0
(bolt length 90 mm)	m2	2	1	0	0
Cash Magnum 9000S	f2	0	1	0	0
(bolt length 121 mm)	f2	2	0	0	0
Air operated penetrating bolt gun EFA VB 215	m1	2	2	0	1
(bolt length 135 mm)	m1	2	2	0	0
	m1	0	0	0	0
	m1	0	1	0	0

8. Figure legends

Figure 1: ***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*.

Figure 2: ***Assessment of brain lesions as based on an MRI image of a head from a male water buffalo under 30 months of age which was stunned with the Ruger GP 100 Double Action with 38 spl munition.***
The frontal lobe was rated as undamaged (score 0), the diencephalon, the rhombencephalon and the cerebellum were rated as destroyed (score 2). When assessing brain damage, the complete data set was taken into account.

Figure 3: ***Hide thickness in water buffaloes and cattle according to age and sex groups***
Specification of measurements: see Fig. 1.
m = male animals, f = female animals; * = statistically significant difference (Student's t-test, $p < 0.05$)

Figure 4: ***Sinus width in water buffaloes and cattle according to age and sex groups***
Specification of measurements: see Fig. 1.
m = male animals, f = female animals; * = statistically significant difference ($p < 0.05$)

Figure 5: ***Distance from frontal point of entry to thalamus in male water buffaloes and cattle according to age***

Figure 1

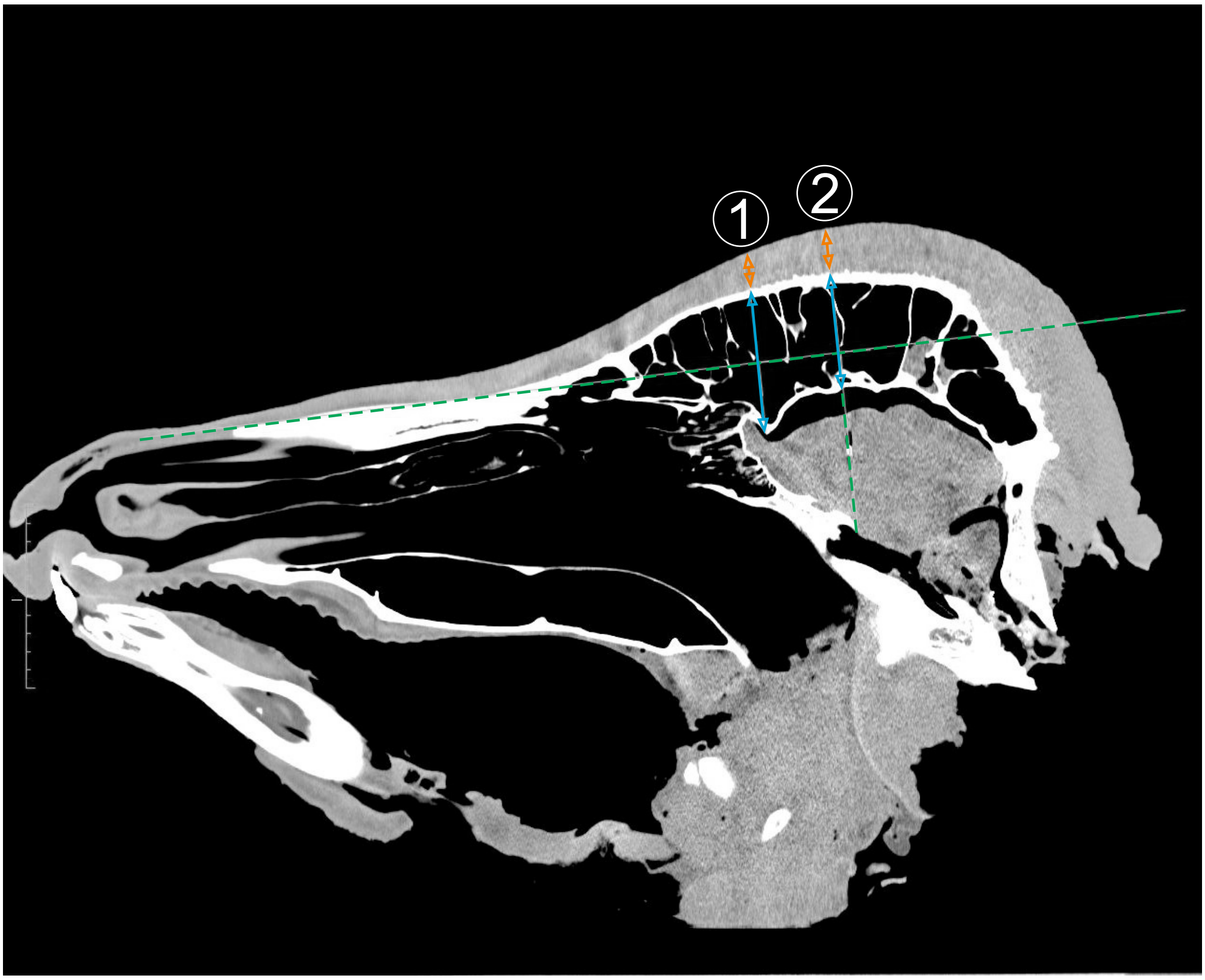


Figure 2

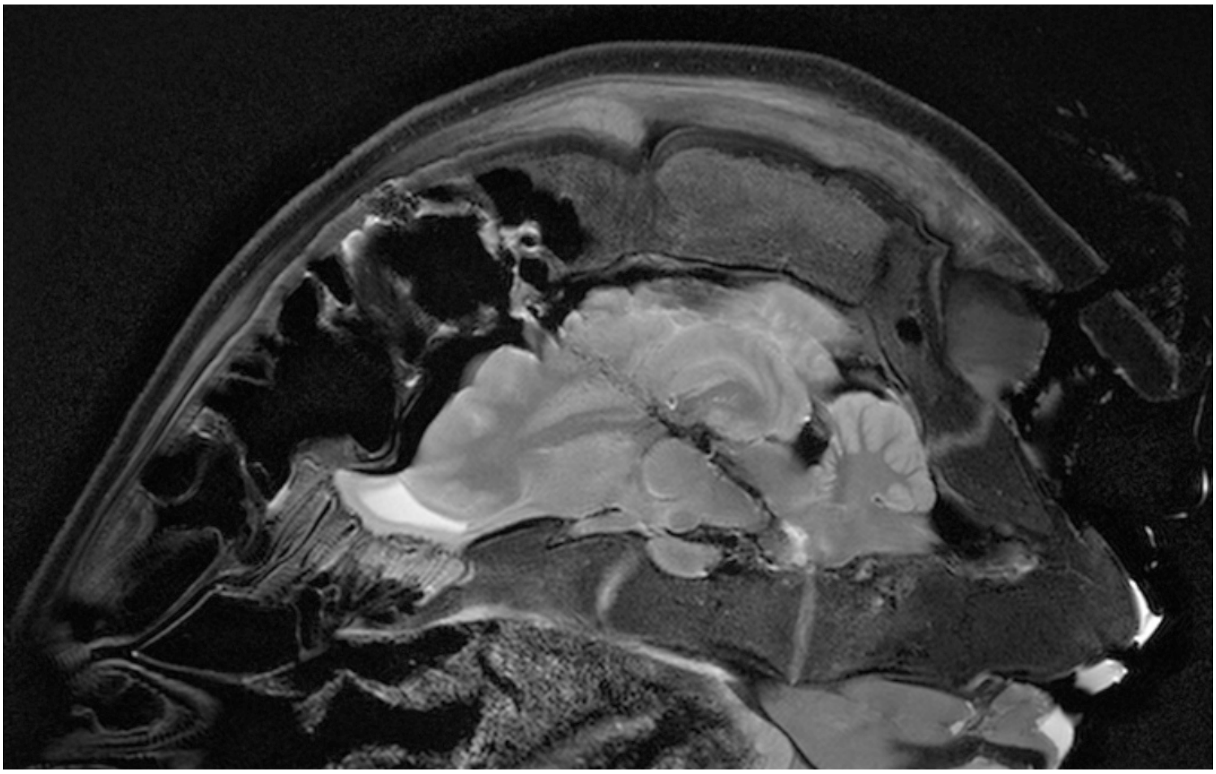


Figure 3

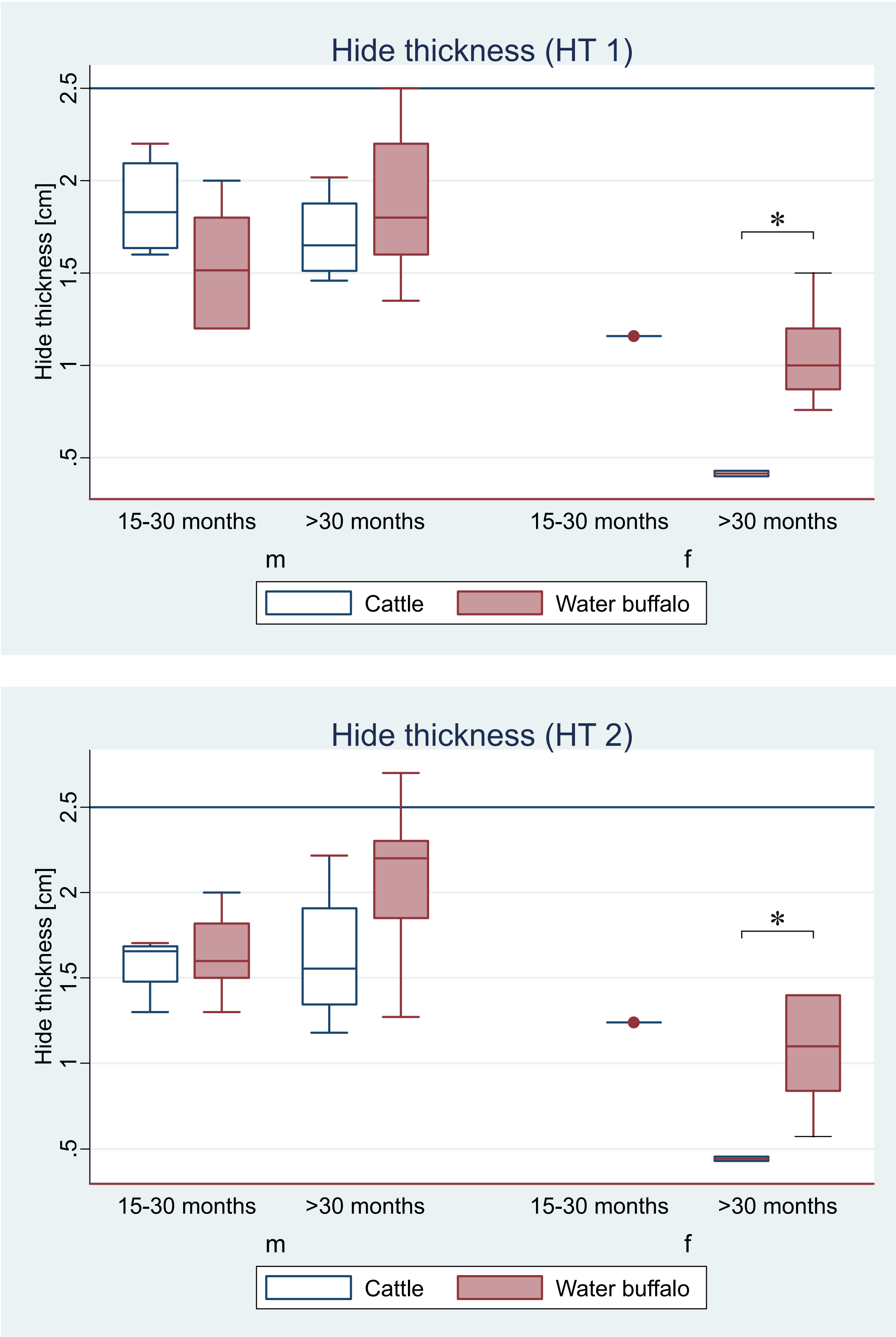
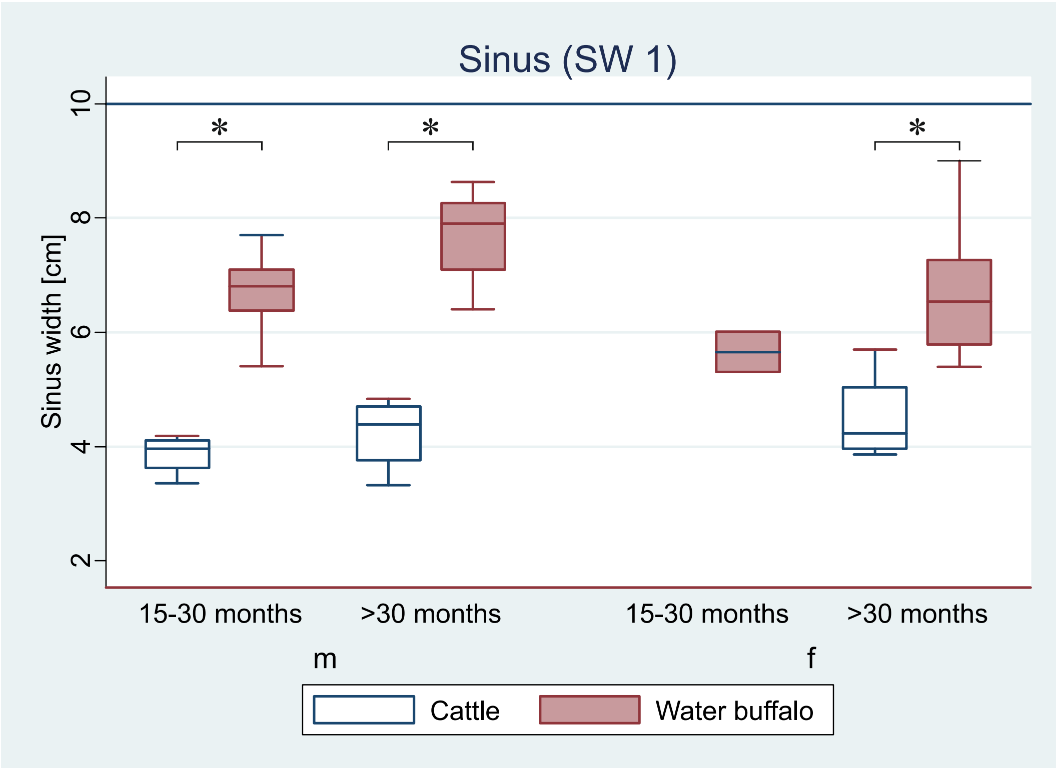

Hide thickness (HT 1)
Hide thickness [cm]
.5
1
1.5
2
2.5
*
15-30 months
>30 months
15-30 months
>30 months
m
f
Cattle
Water buffalo
Hide thickness (HT 2)
Hide thickness [cm]
.5
1
1.5
2
2.5
*
15-30 months
>30 months
15-30 months
>30 months
m
f
Cattle
Water buffalo

Figure 4


Sinus (SW 1)
Sinus width [cm]
2
4
6
8
10
*
*
*
15-30 months
>30 months
15-30 months
>30 months
m
f
Cattle
Water buffalo

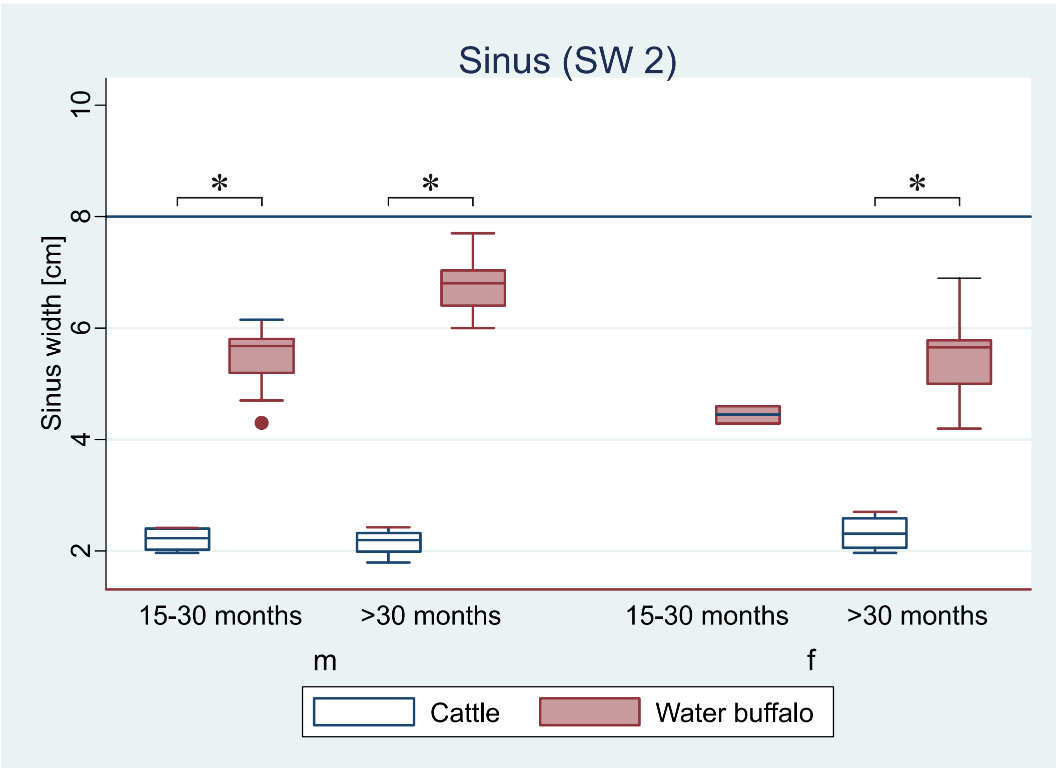
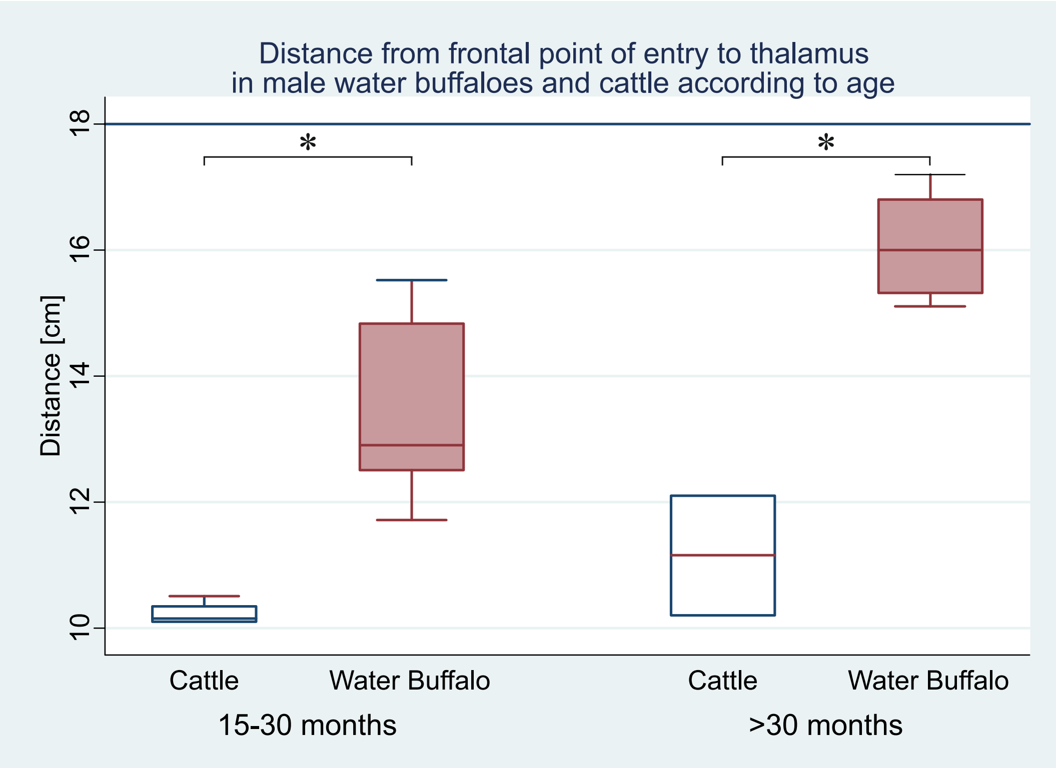

Sinus (SW 2)
Sinus width [cm]
2
4
6
8
10
*
*
*
15-30 months
>30 months
15-30 months
>30 months
m
f
Cattle
Water buffalo

Figure 5


Distance from frontal point of entry to thalamus
in male water buffaloes and cattle according to age
Distance [cm]
10
12
14
16
18
*
*
Cattle
Water Buffalo
Cattle
Water Buffalo
15-30 months
>30 months