Highlights

- Examines the scientific literature on water buffalo welfare during the entire slaughter process.
- Buffalo handlers rely often more on coercive practices and instruments that cause pain and distress.
- Water buffalo are susceptible to thermal stress during the transport.
- The cranial anatomy of water buffaloes makes stunning difficult.
- Significant potential for pain and stress during slaughter without stunning.

The welfare of water buffaloes during the slaughter process: a review

L. de la Cruz[a], T. J. Gibson[b], I. Guerrero-Legarreta[c], F. Napolitano[d], P. Mora-Medina[e], D. Mota-Rojas[f*]

[a]University of the Valley of Mexico (UVM-Coyoacán), Lic. Medicina Veterinaria y Zootecnia, Ciudad de México, 04910.

[b]Department of Pathobiology and Population Science, Royal Veterinary College, University of London, Hatfield, UK.

[c]Department of Biotechnology: Food science, Universidad Autónoma Metropolitana-Iztapalapa, (UAM-I). México, DF, 09340, México.

[d]Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, 85100 Potenza, Italy.

[e]Department of Livestock Sciences, National Autonomous University of Mexico (UNAM), FESC-Cuautitlan Izcalli, Mexico.

[f]Stress Physiology and Farm Animal Welfare. Department of Animal Production and Agriculture. Universidad Autónoma Metropolitana- Campus Xochimilco (UAM). México city, DF 04960, México.

***Corresponding author:** Daniel Mota-Rojas, Ph.D. Tel./fax: +52 55 5483 7535. E-mail address: dmota100@yahoo.com.mx, dmota@correo.xoc.uam.mx. Universidad Autónoma Metropolitana campus Xochimilco. Stress Physiology and Farm Animal Welfare. Department of Animal Production and Agriculture. Calzada del Hueso 1100, Col. Villa Quietud, México, DF 04960, México.

Highlights

- Examines the scientific literature on water buffalo welfare during the entire slaughter process.
- Buffalo handlers rely often more on coercive practices and instruments that cause pain and distress.
- Water buffalo are susceptible to thermal stress during the transport.
- The cranial anatomy of water buffaloes makes stunning difficult.
- Significant potential for pain and stress during slaughter without stunning.

Abstract

This paper reviews the scientific literature on water buffalo welfare in all stages of the live animal supply chain from the farm gate to slaughter (loading/unloading, markets, transportation, handling, lairage, stunning and slaughter) with the objective of identifying risk factors and potential mitigation strategies. Although in some countries legislation exists to protect the welfare of farm animals during transport and killing, the handling practices used to load and unload buffaloes and move them in livestock markets and abattoirs are often harsh. This is frequently due to inadequate equipment designed principally for cattle, and the fact that water buffaloes are considered more temperamental than cattle. Additionally, more reactive animals have increased stress responses to handling, which can lead to more negative human interventions with increased numbers of skin lesions and bruises to the carcasses. During transport, buffaloes may suffer periods of thermal stress due to overstocking, inadequate ventilation and because in many tropical climates trips are made during the hottest time of the day. The anatomical and physiological characteristics of water buffalo make them particularly susceptible to thermal stress in the absence of water for wallowing**.** Although water buffaloes belong to the same *Bovidae* family as domestic cattle, certain anatomical features of the head make effective stunning very problematic. Buffaloes have extensive sinuses and frontal bones, meaning that the penetrating captive bolt devices recommended for cattle may prove ineffective in reliably inducing unconsciousness. There is a need for further development of procedures, stunning positions and appropriate devices to improve the efficiency of buffalo stunning. Finally, in many parts of the world where buffalo are routinely slaughtered in basic conditions without prior stunning. Slaughter without stunning can result in pain and stress associated with delays in the time to loss of consciousness, pain from the cutting of the neck and potential distress associated with aspiration of blood into the respiratory tract. Specific legislation, guidelines and handler/stockman/operator training programmes should be developed to improve the welfare of buffaloes during all *ante mortem* stages of loading, unloading, handling, stunning and slaughter.

Keywords: water buffalo, slaughter, stunning, stress, animal welfare, handling

Introduction

Water buffaloes (*Bubalus bubalis*) are multipurpose animals that are raised principally for draught and dairy purposes (Borghese, 2005). Compared to cattle, they are relatively disease-resistant and highly-adaptable to different environments where cattle cannot provide similar productivity (De la Cruz-Cruz et al., 2014). Water buffalo are also increasingly considered to have high potential for meat production (Andrighetto et al., 2008; Di Luccia et al., 2003; Kandeepan et al., 2009). Buffalo meat is redder than beef (Spanghero et al., 2004) owing to the high myoglobin content observed in this species (Kandeepan et al., 2013). Also, buffalo meat is lean and contains low amounts of saturated fat (40% less cholesterol and 55% less calories than beef), and this has created interest among health-conscious consumers (Ban-Tokuda et al., 2007; Irurueta et al., 2008; Purchas et al., 1993, Yilmaz et al., 2012) due to beneficial effects on cardiovascular risk profiles (Giordano et al., 2010). Nevertheless, in countries where buffaloes are mainly used in the dairy industry (e.g. India and Italy), the market for their meat is limited and welfare concerns are often raised regarding unwanted male calves who may suffer sub-optimal rearing conditions (Shivahre et al., 2014; Napolitano et al., 2017).

Throughout the animal production chain there is high risk of compromised animal welfare, this can be from deviations from regulations and guidelines and improper husbandry/handling practices. Various studies have identified these issues and indicated how harm to animal welfare can be minimised. Much of this knowledge has now been implemented in legislative codes, voluntary guidelines, industry practices and standard operating procedures. However, despite significant efforts in this field, significant risks to animal welfare still exist, and this is especially true for the water buffalo. In this species, despite the rapid growth in the industry, there is limited research on welfare relating to handling, transport and slaughtering (Bornett-Gauci et al., 2006). The areas where significant advances in buffalo welfare have been made are focused primarily on the effects of space allowance and human-animal interaction on the behavioural, physiological and immune responses in female milk-producers and calves in confinement and in extensive conditions (De Rosa et al., 2009a, 2009b; Napolitano et al., 2013, 2004) . Generally, post-farm gate factors have garnered greater attention because of the negative effects they may have on animal welfare (Hemsworth et al., 2011), carcasses and meat quality (Romero et al., 2013). While this review does not focus specifically on meat quality, it is well-known that the *post mortem* glycolytic potential of meat can be influenced by stress during the production cycle prior to slaughter, which can cause depletion of muscle glycogen stores and decreased meat quality, and result in economic losses due to carcass bruising (Becerril-Herrera et al., 2010; Teke et al., 2014).

Water buffalo are often considered as large docile animals (Desta, 2012) that are generally more curious than cattle (Napolitano et al., 2013). However, observations by Hoogesteijn and Hoogesteijn (2008) suggest that water buffaloes, display defensive behaviour against predators. To livestock, humans can often be associated as potential predators, evoking fear, especially if they are not habituated to the presence of people (Rushen et al., 1999). In fact, buffaloes may even revert to the feral condition if not managed according to routine practices (Hoogesteijn and Hoogesteijn, 2008). Therefore, animals with excitable temperament (defined as a set of behavioural responses of animals to external stimuli when are exposed to human handling, such as nervousness, fear, aggressions, etc.) have heightened acute-phase response when a stress stimulus is applied during handling (Francisco et al., 2012). This may lead handlers to rely more heavily on coercive practices and the use of force, especially if they are unskilled (Ahsan et al., 2014; Napolitano et al., 2013). In addition to issues associated with handling and restraint, water buffalo are more difficult to reliably stun and slaughter. This is principally due to characteristics of the skull and other factors (Schwenk et al., 2016).

The objective of this review is to examine the existing scientific literature on water buffalo welfare in all stages of the live animal supply chain from farm gate to slaughter (loading/unloading, markets, transportation, handling, lairage, stunning and slaughter) in order to identify risk factors and suggest mitigation strategies. Particular attention was given to key gaps in current knowledge of the *ante mortem* welfare of water buffalo.

Loading, unloading and pre-slaughter handling

The term stress refers to the behavioural, physiological and emotional status of an animal confronted with a situation that it perceives as threatening with respect to the correct functioning of its body or its mental state (Terlouw et al., 2008). The perception of stress from either an internal or external stimulus by an animal results in an adjustment in its physiology (Brown and Vosloo, 2017). The pre-slaughter handling of water buffaloes involves several stages that can compromise their welfare and causes stress (Table 1). This generally begins with the increased human contact that occurs at the point of origin as animals are loaded onto vehicles for transport to markets or abattoirs (Gregory et al., 2008). Though inevitable, loading and unloading are considered the most stressful stages of transportation (Alam et al., 2010a; Jacobson and Cook, 1998), with the former apparently being more stressful than the latter (Fisher et al., 2009), depending on previous rearing conditions, the level and type of human-animal interaction, and breed. Most buffaloes are raised in traditional, small outdoor systems located in low-lying swampy areas under hot-humid conditions, though others are kept in modern intensive systems (Napolitano et al., 2013). In Asian countries, buffaloes are usually owned and managed in small numbers by individual smallholder farmers and contribute in many ways to household incomes (Yilmaz et al., 2012). Therefore, the level of human-animal interaction can vary greatly, from very close association in traditional small farms or in intensive dairy systems with close contact with humans –concentrated mainly in Italy (Campania and Lazio regions, in particular)

(Napolitano et al., 2013)– to limited human-animal interaction in extensively-managed animals reared for meat (De la Cruz-Cruz et al., 2016).

Excitable temperament and duration of handling have a significant impact on the acute-phase response of stress associated with loading/unloading and transportation. Especially when animals become agitated during handling, as they are motivated by fear and exhibit resistance to entering a squeeze chute, vocalize, show visible eye white, and swish the tail, compared to animals with calmer temperaments (Grandin, 2014).

In addition, Grandin (2014) reported that extensively-raised animals generally have an excitable temperament and had higher stress levels when inside the working chute. This further complicates routine handling practices (Probst et al., 2013) and can lead to more negative animal-human interactions, such as the excessive use of instruments to increase the speed of movement (hitting with sticks or electric prods). Chandra and Das (2001b) reported that male buffaloes are generally more aggressive and difficult to handle under farm conditions, and that in India the most nervous animals are blindfolded before being loaded into the truck.

Many of the reactions of livestock to humans are attributed to fear (Silveira et al., 2012), which has been shown to be related to factors such as species, breed, sex, age and prior experience (Rushen et al., 2001). During handling, animal welfare can be compromised by a series of factors that include: the training and attitude of operators (Villarroel et al., 2001), the nature of the handling procedures (gentle *vs*. rough), the temperament of the animals, and the quality of the facilities (Schwartzkopf-Genswein et al., 2012). One common practice at large-scale abattoirs is to use instruments such as sticks and electric prods to increase the speed of movement, even though they can cause pain, tissue damage and stress (Adenkola and Ayo, 2010). In other countries, such as Bangladesh, buffaloes are fit with rope halters that sometimes pass through the nasal septum, or with a simple rope around the neck (Ahsan et al., 2014). A second method is tail-twisting, which occurs when an animal refuses to move (Alam et al., 2010c). In other cases, sticks or probes are applied to sensitive body regions (Chandra and Das, 2001b). In the European Union, current legislation on slaughter and pre-slaughter handling (European Union, 2009) prohibits the use of prods and other instruments with pointed ends, while electric probes may be used only with adult buffaloes. These devices can only be used when animals refuse to move, and only when there is open room ahead. Application of electric probes (electrical shock) should last no longer than one second in duration and should only touch the muscles of the hindquarters. Electric shocks from the probes should not be repeated if the animal fails to respond; a short shock from an electric prod is preferable to hard tail-twisting (Grandin, 2012). It is generally understood that strong pressure can cause pain, especially if the tail is broken (Alam et al., 2010c). Alam et al. (2010a) stated that the reasons for the harsh handling at the time of slaughter may be that buffaloes are sluggish walkers by nature and do not normally run or walk as fast as cattle, so handlers become impatient with them. On the other hand, the low commercial value of buffalo meat may lead handlers to be less careful. In addition, the fact that buffaloes are considered temperamental animals may also play a role (Ahsan et al., 2014).

When loading, it is generally recommended that handlers avoid herding buffaloes during the hottest hours of the day and when solar radiation is most intense (Zava, 2011b), as these animals do not possess an efficient thermoregulation system when water or shade is lacking (Koga et al., 1999). The loading and unloading of livestock as mentioned above can be a significantly stressful event, though the level of stress and number of animal-human interaction depends on many factors. Chandra and Das (2001b) evaluated the amount of time and labour required to load and unload 100 buffaloes transported in a cattle truck for 30 min on a 20-km trip. They reported that 3 persons could load 10 buffaloes into a truck in 11 minutes, but that loading times were significantly longer for heavier animals (87 *vs*. 43 s per head). These authors also reported that hitting water buffaloes with sticks significantly reduced loading times compared with animals that were dragged with a rope tied around the neck (41 *vs*. 83 s per head), and that the manpower requirements were 2.8 and 1.9 per head, for loading and unloading, respectively. Instruments are often used to encourage livestock to move in the required direction or to increase loading/unloading speeds; however, the overuse of such aids can cause pain and distress and can result in carcass damage.

In addition to the stress associated with forced interactions with the environment and humans, race and ramp design can have a significant impact on water buffalo welfare and carcass quality. Ramps should not be too steep with a slope lower than 36.4% and 50% to the horizontal for calves and adult buffaloes, respectively. In addition, if the slope is steeper than 17.6%, ramps should be provided with foot battens, so the animals can climb and descend without risk or difficulty, as well as lateral protection to prevent them from escaping (European Union, 2009). Passageways should be clean, not slippery, and designed to minimise the risk of injuries. When race and chutes are too narrow, water buffalo may balk, which impedes loading and can result in an increased use of instruments intended to guide the animals and increase the speed of movement. This issue is especially important when herding large buffaloes (>600 kg) through narrow chutes designed for cattle, as this has been shown to increase falls and collisions. The presence of large horns can impact on human safety and handler behaviour, with workers unwilling to get too close to these animals.

This can result in handlers relying on movement aids (electric prods or pointed sticks), which have been shown to lead to an increased incidence (70%) of falls in water buffalo with blows being most prominent to the *tuber coxae* (De la Cruz-Cruz et al., 2014). Similar findings were reported in another study in which 61% of water buffaloes exhibited these behaviours during loading, while during unloading, 28% urinated and 72% urinated and defecated (Chandra and Das, 2001b). The increased frequency of these behaviours could indicate fear (Tarrant, 1990) that, in turn, could cause more slips and falls (Gregory, 2008), resulting in further suffering and damage to meat and other valuable animal by-products (Joshi et al., 2003; Mota-Rojas et al., 2006). In contrast, reducing stress during handling can improve productivity and product quality (Minka et al., 2009). One potential option for controlling these factors at intensive water buffalo operations could be introducing appropriate legislation and specific auditing protocols for buffalo welfare, like those already in place for other livestock species (Grandin, 2013, 2012, 2010).

Vehicle transportation

Transportation to/from markets and to abattoirs is for most animals the single, longest and most stressful journey they make in their lifetime. Grandin (2000) postulated that the condition in which transport takes place is more important than total journey time or the distance covered. During transportation, animals can experience hunger and thirst, fear, metabolic exhaustion, stress, and injury from bad driving (variations in speed, acceleration, braking), vibrations in the truck, novel or excessively loud noises, mixing with unfamiliar animals, high loading densities, long trip times, temperature extremes, novel environments and physical trauma. Most of these are generally unavoidable when transporting animals to abattoirs (Becerril-Herrera et al., 2010; Gonzalez et al., 2012; Knights and Smith, 2007; Mota-Rojas et al., 2006; Roldan-Santiago et al., 2013a, 2013b; Vazquez-Galindo et al., 2013). As animals usually experience a combination of stressors accumulated stress may have detrimental effects on their welfare and may cause depletion of muscle glycogen, leading to high final pH and, therefore, dark, firm, dry (DFD) meat (Brown and Vosloo, 2017; Romero et al., 2017).

In India, surplus water buffaloes are transported in trucks and railroad cars, sometimes for several weeks (Bornett-Gauci et al., 2006). The average number of buffaloes on these trucks is typically 16-18 (Alam et al., 2010a). In Bangladesh, buffaloes are walked in groups of approximately five animals to local abattoirs (Ahsan et al., 2014). A significant proportion of these animals are transported out of India to Bangladesh and other local non-Hindu countries and regions where they are slaughtered for human consumption.

Though water buffaloes are adapted to tropical climates, when exposed to high temperatures with no access to shade or water for wallowing they can suffer heat-induced stress. Indeed, thermal conditions are among the main transport-induced stressors as, according to a study by Winckler et al. (2003), mean temperatures inside compartments in summer were 3-6°C higher than outside ambient temperatures. Transporting cattle during the hottest part of the day can cause significant heat stress; Romero et al. (2012) suggest that a common mistake is loading animals in the morning, because this means that they will inevitably be on the road during the hottest part of the day due to the long distances often covered (Petherick, 2005). The swamp water buffalo has dark skin, and only one-sixth as many sweat glands as *Bos indicus*. Water buffalo skin is sparsely-covered with just 100-200 hairs/cm^2 compared to 550-1100 in *Bos taurus*, and 1400-2600 in *Bos indicus* (de la Cruz-Cruz et al., 2016). An additional issue is that, unlike cattle, buffalo hide is 2.5 times thicker, and the black pigmentation associated with a large amount of melanin captures greater quantities of ultraviolet rays (Zava, 2011a). These physical features make the buffalo highly-susceptible to thermal stress during transport, especially when exposed directly to the sun's rays, since its evaporative cutaneous cooling mechanism is less effective than those of other species (Das and Khan, 2010; Marai and Haeeb, 2010). Prolonged exposure to high temperatures can trigger a series of dramatic changes in this species' biological functions that include disturbances in water, protein and energy metabolism, as well as in mineral balances, hormonal secretions, enzymatic reactions and blood metabolites. The effect of heat stress is aggravated when is accompanied by high ambient humidity (De la Cruz-Cruz et al., 2014; Marai and Haeeb, 2010). High humidity accompanied by small diurnal changes in air temperature and evaporative heat loss are not highly effective in dissipating body heat (Napolitano et al., 2013). To reduce the potential for thermal stress, the European Union (2005) requires that all transport systems be designed to protect buffaloes from temperature extremes and adverse weather conditions. In particular, for trips longer than 50 km, trucks should have ventilation systems capable of maintaining the temperature within a range of 5-30°C (European Union, 2005).

A study by Alam et al. (2010b) evaluated long-distance transport on blood-based welfare parameters of cattle and water buffalo exported from India to Bangladesh during the hot season (April). They reported that long-distance export trade was associated with dehydration, lipolysis and muscle injury or activation. The results suggest that the animals were dehydrated, in metabolic distress and pronounced fatigue caused by extreme lipolysis and severe muscular damage. For this reason, it is recommended that water buffalo be give adequate food and water whenever they are off-loaded from vehicles over the course of a journey. In Gonzalez et al. (2012) examined in cattle the incidence of death, non-ambulatory injuries, and lameness in animals transported for ≥400 km. They found that factors such as age predisposed animals to stress, as calves and cull cattle had higher mortality rates. These authors also stated that when ambient temperatures fall below -15°C, the probability of dying increased sharply, and that loss of body weight is related to loading density, trip duration and driving style, rather than to non-ambulatory injuries or lameness. Similarly, in water buffaloes, Zava (2011b) reported that animals transported for 700 and 1,300 km lost 7% and 9% of body weight, respectively.

The stress associated with transport can significantly impact animal behaviour, both towards humans and conspecifics. During stressful transport, cattle may exhibit aggressive (head-butting, pushing, threats, mock fights), dominant and sexual (mounting, chin-resting) behaviours (Tarrant, 1990). These actions can impact on meat quality, causing carcass damage, laceration to the hides, bruising, and dark-coloured cuts, which can result in the downgrading of the carcass and/or the need for excessive trimming. Chandra and Das (2001b) found 244 contusions in 100 water buffaloes. These were mostly small deep bruises (59%), followed by medium-sized (19.3%), small (9.8%), medium-deep (6.1%) and heavy bruising (5.7%). Most of these lesions were on the hind limbs (43.4%), followed by the abdomen and udder region (21.3%), shoulders, neck and back (16%), and the perianal area (11.1%). Similarly, a study in Bangladesh found that 89% of cattle and buffalo had injuries, mainly on the upper body, including the point of the pin bone, the ventral tail, the tail head, the points of the lumbar vertebrae the, buttocks, and points on the shoulder and thorax. Additional injuries were observed in the sacral, thoracic and shoulder areas (Ahsan et al., 2014). Many of these injuries were likely caused by direct physical trauma from other animals and objects in the vehicles. The authors suggested that these injuries occurred due to high stocking densities in the transport vehicles (Ahsan et al., 2014), since water buffaloes have large horns and were closely confined in the vehicles (Alam et al., 2010a). Cattle transported at high stocking densities have limited room to move and adopt preferred orientations, such as aligning themselves with the direction of travel, which may increase their security of balance. Animals that slip and fall may be unable to get up and could be trampled (Strappini et al., 2009). Preventing bruises is important because they are indicative of rough handling and pain, and reflect poor welfare conditions during the pre-slaughter period (Strappini et al., 2013, 2012). In order to prevent stress and injuries caused by high stock densities, different space allowances have been set according to the weight of the animals and means of transport (European Union, 2005); for instance, a 550-kg buffalo transported on road vehicles should have a space allowance of 1.3-1.6 m^2. In addition, tied and untied animals, animals of significantly different size and age, of different species, or manifestly hostile to each other, should be transported separately using partitions to adapt compartments to specific requirements (European Union, 2005). In Colombia, legislation on the transport of cattle and buffalo states that the space allowance for animals weighing 300, 500, 600, and 700 kg must be 0.84, 1.27, 1.46 and 1.75 m^2, respectively (Resolution 002341/2007).

Water buffalo welfare at livestock markets

Livestock markets operate mainly through direct sales between buyers and sellers or auctions (Fornari et al., 2016). Sales of animals can result in longer transport times, multiple loading and unloading events, long fasting times, numerous handling procedures and, in all likelihood, mixing with unfamiliar animals (Roldan-Santiago et al., 2016; Romero et al., 2013). All of these conditions are associated with the risk of physical damage and bruising (Strappini et al., 2009). Generally, the welfare of animals sold in livestock markets is considered worse than that of animals transported directly (short journeys) to abattoirs. (Gregory, 2008). Welfare concerns include fatigue, fear and distress, fasting, dehydration, and physical injuries in finished cattle and, in store cattle, an additional concern is the acquiring of disease (Gregory et al., 2009a). In addition, it was reported that lambs were four times more likely to die in lairage or during transport if they had been purchased in a market, compared with direct supply to the abattoir from farms (Gregory et al., 2009b).

Thus, the principle threats to animal welfare are the methods employed during handling, loading and unloading, and transport (Adenkola and Ayo, 2010). In a study of cattle markets in the UK, the key welfare issues were found to be slips and falls during unloading, impacts during grading, and refusal to load after sale. Slips and falls occurred when there was manure plus rain or urine on the concrete floor (Gregory, 2008). Design faults can include right-angled bends in races, dead ends, flooring with inadequate slope or grip, and steps. Projecting fittings and square-edged corners were potentially injurious; conversely, rounded posts and curved races facilitated the flow of cattle with minimal impacts (Weeks et al., 2002).

The presence of skin lesions can indicate low animal welfare status and result in poor meat quality and lower hide prices (Adenkola and Ayo, 2010). Alam et al. (2010c) evaluated the frequency of nose and tail injuries in water buffalo at livestock markets in Bangladesh, finding that 54% of the animals had septum rings. Often animals were pierced with a hot iron rod so that a rope could be run through the septum to make them more tractable and easily-led by hand. However, almost half of those animals (47%) suffered lacerations and ulcerations where the rope rubbed against their noses. Pus in the nostrils was observed in 56% of the animals, and 57 and 58%, respectively, had severe or extensive nose injuries. To prevent nose lesions, animals should not be tied by nose rings during transportation (European Union, 2005).

Another study by Alam et al. (2010a) assessed the number of body lesions in beef cattle and water buffaloes sold in livestock markets. They found that 99% of the buffaloes had potentially painful skin injuries. They also had more abrasions (95%), lacerations (57%), swelling (15%), and hyperkeratosis (32%) than the cattle in the same market. The most severely-affected body areas were the head, neck, forelimbs, thorax, abdomen, hips, buttocks and back ($P<0.05$), particularly in animals from India. Those researchers attributed their findings to the use of inadequate vehicles, in which animals rubbed against or impacted panel bolts that protruded into the box, and to abuse by handlers during loading and unloading. Another study showed that some animals sold at livestock markets exhibit clinical signs of illness, including eye congestion, tearing, nasal discharge, or salivation (Chandra and Das, 2001a).

For most cattle that are directly consigned from farm to abattoir, the period of food and water deprivation is likely to be <24 h. However, this can extend to >48 h in livestock markets. One of the most obvious impacts of food and water deprivation is weight loss (Ferguson and Warner, 2008). Another potential stressor is noise. The principal sources of noise included clanging gates, vocalizing from nearby sheep and more distant cattle, amplifiers used to broadcast the bidding, vehicles driving nearby, and people shouting while driving animals along corridors and into vehicles (Gregory et al., 2009b).

Arrival at the abattoir

Upon arrival at the abattoir, animals face not only unloading, inspections and penning in the lairage area (Schwartzkopf-Genswein et al., 2012) but also an unfamiliar environment with novel situations that present a whole series of stimuli that can cause fear and stress. Together, these constitute the causes of the so-called "abattoir effect" (Hemsworth et al., 2011; Roldan-Santiago et al., 2011). Furthermore, older or poorly-designed abattoirs may have inadequate illumination, slippery floors and steep ramps in the herding corridors. Also, the design of animal holding and raceways in some abattoirs are compromised due to the existing architecture or where the priority has been to optimize available spaces or facilitate human activities above the requirements associated with animal behaviour (Hultgren et al., 2014; Miranda-de la Lama et al., 2012). In other, less developed countries, additional complications may impact welfare. Abattoirs are generally small-scale operations, ranging from simple buildings to open-air concrete slabs beside rivers, where animals are either slaughtered immediately on arrival, or cast or tethered awaiting slaughter. Ahsan et al. (2014) reported that in abattoirs in Bangladesh animals were tethered in positions that prevented them from lying down or having access to bedding in the lairage area. Also, they showed signs of faecal soiling, had insufficient rest areas, and were often housed on concrete slabs with sharp, broken edges that could cause injury, or were left outside, where they were exposed to rain and showed initial signs of hypothermia.

Handling in the stunning box

An often overlooked factor in animal welfare at slaughter is handling and restraint systems (Grandin, 2013). At slaughter, all livestock can be exposed to handling that impacts their welfare (Hultgren et al., 2014), sometimes through the use of sticks and electric prods that cause pain and distress (Villarroel et al., 2001). In addition, handlers often use practices such as slapping the rear or front (*i.e.* slapping the animal behind or in front of the hip bone with the hand), shouting (speaking or yelling harshly or loudly), and beating rear (striking the animal behind the hip bone with an instrument) while herding animals through chutes into stunning and slaughter boxes (Hultgren et al., 2014). When entering narrow raceways and stunning and slaughter boxes, animals can collide with protruding objects and the guillotine-type door. Bourguet et al. (2011) found that 21% of cattle were struck by the guillotine door, perhaps because one animal may follow the one in front of it into the stunning box, causing the operator to suddenly close the door, which hits the second animal to impede it from entering the box.

Collisions and attempts to escape can cause pain and injury, including bruising, and animals tend to panic when they slip. Also, they may balk and refuse to move, which can lead to the overuse of instruments intended to increase speed of movement. This is an issue especially with large water buffalo and other animals with long horns. When attempting to move livestock more quickly, workers have been seen to twist the animals' tails (Hultgren et al., 2014), this can result in kinked tails due to dislocation, or even breakage. Alam et al. (2010b) found that in Bangladeshi livestock markets, 39% of cattle and water buffaloes had either kinked or missing end tails.

The behaviour of animals upon entering the stunning or slaughter box can have a significant impact on the effectiveness of stunning and slaughter performance and can ultimately affect animal welfare. Agitated animals in the stunning box can complicate the accurate placement of the captive bolt gun (CBG), which can unnecessarily increase the time spent inside (Grandin, 2013) and lead to inadequate stunning. Restraining aids can enhance stunning performance, as buffaloes stunned with a head-restraint spend less time in the box than those stunned without that apparatus (De la Cruz-Cruz et al., 2014). Head-restraints can also significantly improve the accuracy of CBG stunning and so improve welfare (Atkinson et al., 2013; Grandin, 2002; von Wenzlawowicz et al., 2012). A study in Colombian abattoirs found that when buffaloes were stunned without head or body restraint systems, an average of 2.8 shots were required (range 1-8) to stun the animal. But where head and body restraints were used this figure decreased to 2.45 (range 2-3) (De la Cruz-Cruz et al., 2014). However, Ewbank et al. (1992) reported that the use of head-restraints during CBG stunning for cattle can slow down the operation, when animals refuse to enter their heads in the restraint. This can result in more distress than shooting without restraint. For these reasons the design and operation of equipment can significantly affect stress. Grandin (2012) points out that diverse factors must be considered to facilitate entry into the stunning box, including: a) illumination, restraints and race entrances; b) blocking the view of distractors (animals should not be able to see people or activities through openings); c) eliminating sources of hissing air; d) reducing equipment noise; and e) equipping restraint devices with pressure controls. An additional recommendation is that animals be stunned as soon after restraint as possible (Muñoz et al., 2012).

Adequate restraint is equally essential when slaughtering animals without stunning. In modern abattoirs, animals are restrained individually in either rotating (180° or 90°) or upright positions. These generally provide a high level of restraint by means of a back pusher, neck yoke, belly plate/belly rail or chin lift (Gibson et al., 2015b). However, stunning water buffaloes in these restraining conditions is more complicated than slaughtering cattle, due to differences in body size and shape, their long horns, and their shorter neck. Also, there can be issues with stress and pain associated with hyperextension of the neck by the chin lift, excessive pressure exerted by some restraining systems, and organ compression in inverted designs (Gibson et al., 2015b). However, most slaughtering of buffaloes without stunning is performed in small-scale abattoirs in poorer areas where it is impractical and too expensive to have highly-mechanised, purpose-built restraining systems (Ahan et al., 2014; Gregory et al., 2012). Restraint often involves the manual casting of conscious animals on the ground or a concrete slab into lateral recumbency using ropes to secure their legs. This can result in significant pain and discomfort, especially where animals fall onto concrete slabs, and/or are cast and left for extended periods prior to slaughter (Ahan et al., 2014). At slaughter, the neck is manually twisted to present the ventral aspect where the cut is be made (Gregory, 2008). However, water buffalo's long horns and shorter neck can make it almost impossible to expose the neck optimally, resulting in the cut being made as a stab in the side of the neck while the animal is in lateral recumbency. This can have a serious impact on the cut itself and the level of stimulation at the wound site, which can significantly impact welfare (see the section on Slaughter of water buffalo without stunning for further discussion).

In some countries, water buffaloes are still immobilized prior to slaughter by an act called puntilla (FAO, 2001). Puntilla involves inserting a sharp knife into the back of the neck at the foramen magnum in an attempt to sever the spinal cord. The aim is to induce immediate collapse and immobilisation, allowing the animal to be bled without risk of injury to operators (Gibson et al., 2015d). Traditionally, this method was considered humane (Gregory, 1990); however, research with cattle has found that the stab is often ineffective, as 91% of animals show signs of brain and spinal cord function, suggesting that they were still conscious and able to experience pain and distress (Dembo, 1894; Limon et al., 2010, 2009). The puntilla method of stabbing cattle to immobilize them before slaughter, or cutting tendons, should be discontinued, as they are not permitted by the OIE (Grandin, 2010). Unfortunately, they are still used in some small- and medium-sized slaughterhouses in developing countries like Viet Nam, Mexico, Chile, Peru and Bolivia (Limon et al., 2012).

Stunning

The act of stunning is designed to render animals insensible to pain and leave them unconscious prior to slaughter (Önenç and Kaya, 2004; Roldan-Santiago et al., 2011; Mota-Rojas et al., 2012; Bolaños-López et al., 2014; Terlouw et al., 2016b). It is essential to ensure that an animal is insensitive to pain and permanently unconscious before performing hoisting, skinning or other invasive dressing procedures (Grandin, 2013). Consciousness can be defined in various ways, but is generally associated with the state of wakefulness and the ability to perceive and respond to normal stimuli and interact with the environment and other organisms (Verhoeven et al., 2015a, 2015b). Most stunning methods induce unconsciousness by causing dysfunction in the cerebral hemispheres, thalamic structures, reticular formation and/or ascending reticular activating system via physical, electrical, hypoxic or chemical means (Terlouw et al., 2016a). Stunning and slaughter for human consumption is generally a two-step process with the former being followed by exsanguination (ventral neck incision and/or thoracic stab) (Hilsenbeck et al., 2007). Some stunning methods can be considered as single-step killing methods (*i.e.*, free bullet, head-to-body electrical stunning and some gas mixtures); however, captive bolt, which is practiced legally on water buffaloes in most countries, requires exsanguination to prevent potential recovery of consciousness from inadequate stunning (England, 2015; European Union, 2009; New Zealand, 2016).

In bovids, blood is supplied to the brain via the basi-occipital plexus and through branches of the carotid and basilar arteries (Gregory, 2008). In addition, the vertebral arteries supply oxygenated blood to the brain (Baldwin and Bell 1963a, 1963b). Although the correct use if the captive bolt causes an immediate cessation of breathing, if the animals are not bled, cardiac activity may continue for 8-10 min (Terlouw et al., 2016b). After application of the stun procedure, the animals must be bled as soon as possible (Terlouw et al., 2016a). In cattle and water buffaloes, exsanguination is usually performed by severing the carotid arteries and jugular veins on the ventral neck (ventral neck incision) or cutting the brachiocephalic trunk (chest stick or thoracic stab) (Gregory et al., 2009c; Robins et al., 2014). The devastating effect of the haemorrhage during bleeding is mostly related to the lack of oxygenation of the organs, this can cause irreversible cessations of brain, structures responsible for vital functions like breathing, thermal and cardiovascular regulation and this can cause final cardiac arrest. (Terlouw et al., 2016a; 2016b). However, the vertebral arteries remain intact during slaughter, though this only becomes an issue if the animal is not effectively stunned or is slaughtered without stunning. Because in some cases the vertebral arteries can continue to supply oxygenated blood to the brain after severance of the common carotid arteries (see the section on Slaughter of water buffalo without stunning for further discussion) (Terlouw et al., 2016a).

Captive bolt stunning (CBG) is the method most commonly used with ruminants (Gibson et al., 2015c; Gilliam et al., 2012; Kim et al., 2013; Lambooij et al., 2012; Trentini et al., 2011;). The effectiveness of CBG stunning for rendering animals unconscious can be affected by the following factors: (a) selection of the appropriate CBG/cartridge combination for the species and/or animal class; (b) correct placement of the CBG shot; (c) expertise of the stunner operator; (d) storage conditions of the stunner and cartridges, in particular, damp conditions should be avoided; (e) behaviour of the animals, since the ease with which they are driven into the stunning box is associated with the number of stuns that may be needed; and (f) regular maintenance of the stunner, including removing excessive carbon build-up, regular rotation of the rubber buffers (recuperator sleeves), and replacing damaged firing pins and bolts (Bourguet et al., 2011; Gibson et al., 2015c; Grandin, 2002, 1994, 1980; Gregory and Shaw, 2000). Guns for cattle and water buffaloes are powered either by compressed air or black powder cartridges. Conventional CBGs designed for cattle generally have insufficient bolt penetration depth and power to stun water buffaloes. Several manufacturers have designed specific CBGs for water buffaloes and very large cattle, for example models such as the Magnum XL with 6-gr cartridges (Acceles and Shelvoke Ltd) and the Schermer KL (Karl Schermer GmbH & Co) are currently on the market.

Penetrative and non-penetrative captive bolt devices administer a concussive blow that involves transferring the kinetic energy of the moving bolt to the cranium, resulting in focal and diffuse damage throughout the brain. Focal damage is understood as direct physical damage at the injury site caused by bolt penetration, while diffuse injury is the result of a combination of shock waves, coup, countercoup and shear forces, cerebral haemorrhaging and neuron depolarisation. This large-scale injury and damage to specific brain structures prevents normal functioning and produces unconsciousness (Terlouw et al., 2016a; Schwenk et al., 2016).

During CBG stunning, animals experience a tonic spasm of approximately 10 s before relaxing, often followed by more violent clonic convulsions (Lambooij et al., 2012). Care must be taken during the convulsive stages since the rapid movement of the limbs and body can endanger the safety of an abattoir's personnel (Robins et al., 2014). In cattle, the correct position for CBG stunning is the mid-forehead at the intersection of two imaginary lines between the base of the horn and the opposite eye (Grandin, 2010; Gregory et al., 2007). Although water buffaloes belong to the same family of Bovidae as domestic cattle, the anatomical characteristics of the head differ considerably between the two species (Schwenk et al., 2016). It is well-documented that the efficacy of the stunning device depends greatly on the anatomical features of individual animals. In cattle the precise location of the brain relative to the forehead varies among breeds (Gilliam et al., 2012), genders and skull shapes (Terlouw et al., 2015).

In this regard, studies of buffaloes have determined that the frontal stunning method may not be as efficient as in cattle because the frontal bones are significantly thicker (Gregory, 2008). In addition, they have extensive frontal sinuses within the frontal and parietal bones rostrodorsal to the brain, with the left and right frontal sinuses separated by a highly-developed, complete median inter-frontal septum. In cattle, in contrast, the two frontal sinuses are communicated caudally where the inter-frontal septum is deficient (Alsafy et al., 2013). For this reason, applying the conventional penetrating CBG recommended for cattle (bolt length = 9 or 12 cm) to the forehead of water buffaloes may not be effective in producing sufficient trauma and energy transfer to the brain to induce unconsciousness (Schwenk et al., 2016). A recent study by Schwenk et al. (2016) using computerised tomography (CT) determined that the mean distance from the forehead skin surface to the inner bone table in buffaloes and cattle is 7.4 cm (5.6-10 cm) and 3.6 cm (2.9-4.4 cm), respectively, and from the skin to the thalamus, 14.48 cm (11.71-17.20 cm) and 10.20 cm (10.1–12.10 cm), respectively. In addition, there can be significant differences in skull anatomy due to sex and age, as males aged over 30 months had greater skull size and skin thickness in the frontal position (8.6 cm) than males under 30 months (7.4 cm). This highlights the importance of selecting the correct CBG/cartridge combination for each animal type. Swiss legislation recommends that for CBG stunning bolt penetration must be at least 12 cm in order to achieve a sufficient level of unconsciousness in adult buffaloes, while the diameter should be at least 9 mm with a velocity of 60-70 m/s (Ufficio Federale della Sicurezza Alimentare e di Veterinaria, 2010).

The efficacy of stunning methods is tested by assessing specific brain stem reflexes (Erasmus et al., 2010). Key parameters commonly assessed as signs of a good stun, include immediate collapse, lack of rhythmic breathing (verified at the muzzle and/or flank), tonic and clonic convulsions, no conscious vocalizations, absence of palpebral and corneal reflexes, absence of nystagmus, no eyeball rotation/facing forward, no spontaneous blinking, lack of muscle tone (jaw muscle tension or tongue protrusion when on the bleeding rail), no responsiveness to pain (responses to scraping a fingernail along the animal's inner nostril or to tongue pinching), and no righting reflex (defined as lateral head-turning associated with apparent struggling movements) (Ahsan et al., 2014; Gregory et al., 2007; Neves et al., 2016; Terlouw et al., 2016a). The presence or absence of these indicators must be checked after stunning and during bleeding for both conventional and religious slaughter (Terlouw et al., 2016b). Nystagmus or intermittently positive corneal or palpebral reflexes can indicate a shallow depth of concussion, but this does not necessarily indicate compromised welfare since the animal may still be unconscious (Gibson et al., 2015a, 2015d). Spontaneous, simple, unintentional movements after clonic convulsions originate from anoxia in the spinal cord, peripheral nerves and/or central rhythm generators. and are considered unrelated to consciousness (Grandin, 2010; Terlouw et al., 2015).

Placement of the captive bolt shot has a significant effect on stunning performance. Gregory et al. (2009c) evaluated the efficacy of different captive bolt stunning sites (frontal, crown and poll) in water buffalo with different gun/cartridge combinations. They reported that bulls over 30 thirty months old (skull thickness at the frontal position = 8 cm) shot in the frontal position did not collapse after the first shot. Shooting in the crown (16-19 cm from the foramen magnum) produced a shallow depth of concussion with continued respiration and eyeball rotation post-collapse in some animals. In contrast, animals shot in the poll (occipital) position (1-4 cm from the foramen magnum) (Figure 1), especially those aged over 30 months, showed signs of rhythmic breathing that began slowly at 38 s after shooting, while eyeball rotation was seen in 19% of animals. In poll-shot water buffaloes 79% had damage to the cerebellum, and 71% to the medulla and/or pons. However, 3 of the animals that were shot in the poll position were considered to have been shot improperly because the bolt went into the spinal cord (n=2) or did not penetrate the brain (n=1) (Gregory et al., 2009c). Similar work by Schwenk et al. (2016) reported that shooting in the poll position (depression below the intercornual protuberance but above the attachment points of the *Ligmenta nuchae*), dramatically reduces the bone-to-thalamus distance to a median of 87 mm (range: 81-98 mm), compared to 127 mm (range 93-106 mm) for the frontal position. Furthermore, a pilot study in Colombian abattoirs found that a higher percentage of buffalo shot in the frontal position showed signs of sensibility compared to those stunned in the poll position (De la Cruz-Cruz et al., 2014). Taken together, these results highlight the potential risks and benefits of the poll shot for water buffalo. The risks are that (1) when shooting in this position the brain may be missed entirely, hitting only the spinal cord; and (2) in both positions, the bolt length of commercially-available guns may be insufficient for large adult buffaloes. Shooting only the spinal cord could potentially result in animals being paralysed but not losing consciousness; therefore, the immediate and permanent loss of standing posture must be interpreted with caution (Terlouw et al., 2016b). Moreover, insufficient bolt length might result in inconsistent stunning and potential welfare deterioration. However, overall results from these studies suggest that the poll position (depression below the intercornual protuberance but above the attachment points of the *Ligmenta nuchae*) is the more reliable shooting site for water buffaloes. Shots in this position should be directed towards the base of the tongue in buffaloes under three months of age, but towards the nose in older animals. The shot also needs to be directed rostrally to ensure that the brain, and not only the spinal cord is penetrated (Gregory et al., 2009c). However, some authors consider the occipital position of the stunner unsuitable for practical reasons, as some buffaloes show interest in the device and may tend to move their heads (Meichtry, 2015). Another anatomical study using computed tomography, suggested that the most suitable position on the frontal sinus is on a line that joins the mid-temporal regions, about midway between the median plane and the lateral margin of the head (Alsafy et al., 2013). However, there is no stunning efficiency data in the literature to support this suggestion.

Due to issues associated with the non-stunned slaughter of bovids (see the section, Slaughter of water buffalo without stunning, below), some Muslim communities accept the use of non-penetrating stunning in the Halal slaughter of cattle and buffalo, as long as the device does not penetrate or fracture the cranium (Nakyinsige et al., 2013). However, non-penetrating CBGs are recognized as being largely ineffective in reliably inducing unconsciousness in animals with large heads, such as bulls and water buffaloes (EFSA, 2004). For this reason, the European Union does not permit their use with bovid species (European Union, 2009). Reversible electrical methods are also allowed for the Halal slaughter of cattle, provided they use non-lethal currents greater than 100 Hz in frequency (Dalmau et al., 2012). However, there is currently no published information on the electrical stunning of water buffaloes.

Free bullet dispatch

Unlike CBGs, the free bullet technique is classified as a killing method that does not require any secondary procedure(s), as long as the bullet is fired directly into the cranium to destroy the brain. However, to ensure good meat quality and animal welfare it is important that animals be bled after shooting. Free bullet shooting is often used to dispatch water buffaloes due to the ineffectiveness of some CBGs in rendering them unconscious. Generally, rifles and pistols are used (Gibson et al., 2015a; Machado et al., 2013; Meichtry, 2015; Thomson et al., 2013;). Pistols range from specifically-designed single- or twin-shot slaughter types (Cash .32 Humane Killer) to military grade weapons (Meichtry, 2015; Schwenk et al., 2016).

The AVMA recommends that the combination of the firearm and ammunition selected must achieve a muzzle energy of at least 300 ft-lb (407 J) for animals weighing up to 400 lb (180 kg), but for animals over 400 lb, 1,000 ft-lb (1,356 J) is required (American Veterinary Medical Association, 2013). Animals are generally shot at close range, but in the case of extensively-reared, stressed or dangerous livestock, it may be more appropriate to shoot from a distance. However, according to the Euthanasia Guidelines of the AVMA

(2013), it may not be possible or appropriate to target the head of the animal when attempting to kill it from a long distance because missed shots may result in jaw fractures or other non-fatal injuries. It is advisable to use a silencer to minimise noise and, hence, disturbances to other animals. The gun and ammunition should be appropriate for the distance and species involved. It is also important to ensure that the marksmen are adequately trained and licenced, and that the ammunition is designed and constructed so that it does not exit the animal's head. The use of hollow-point or non-jacketed rounds can reduce the risk of ricochets or bullets exiting the animal because they are designed to deform and fragment upon impact (Gibson et al., 2015a). This kind of ammunition has the added advantage of increasing the surface area and severity of the wound (Farjo and Miclau, 1997; Hollerman et al., 1990; Thomson et al., 2013). Safety, however, is the most important consideration when animals are to be killed using the free bullet method, due to the potential for ricochets off bone or solid objects. Therefore, animals should not be shot in enclosed spaces or areas with substantial solid objects (e.g. metal gates). Sandpits, earthen floors and walls, or rubber tire enclosures can be used to minimise the risk of ricochets and prevent further travel of the projectile if it exits the animal's head (Gibson et al., 2015a).

To ensure effective killing, it is recommended that animals be shot in the head in a way that maximises damage to the structures of the brainstem (i.e., midbrain, pons and medulla). Schwenk et al. (2016) reported immediate collapse, unconsciousness and extensive brain damage in water buffaloes shot with a variety of pistols. Those animals were shot in different positions and at distinct angles, and the damage to the diencephalon and frontal lobe was more uniform and extensive than in beasts shot with penetrating CBGs. As in the case of CBGs, the free bullet method requires that the position and angulation of the shot be adequate for each specific type of animal. Also, the projectile must have enough kinetic energy to ensure that it will penetrate and cause sufficient damage to the brain to produce death instantaneously. Recent work by Meichtry et al. (2015) has resulted in the design and development of a new, experimental, twin-barrelled, 9-mm, free bullet casing rifle for the frontal shooting of water buffaloes. Having two barrels means that one can be used as an immediate backup if the first shot fails. This gun was designed to maximise effectiveness and reliability, minimise suffering, and reduce risks to operators. These authors achieved complete unconsciousness for almost all buffalo categories including males aged up to 5 years, though one bull aged 9.8 years did not collapse after the first shot in the frontal position and had to be shot again, this time in the occipital site. After the second shot, the animal lost almost all reflexes but still had eyeball rotation. Analysis of the CT found that both shots (frontal and occipital) had failed to penetrate the cranial vault, so the brain damage was limited to mild haemorrhagic lesions (Meichtry et al. 2015), suggesting that despite being shot twice the animal was only incompletely concussed.

Slaughter of water buffalo without stunning

In several countries, it is mandatory that a stunning method be used to render animals unconscious during and after the slaughter process. However, some have dispensations and special permits that allow slaughtering without stunning for consumption by members of certain religious faiths (Terlouw et al., 2008). Although slaughter without stunning is permitted by the OIE, the European Union and numerous other nations, it is a highly-controversial issue from the perspective of animal welfare and meat labelling (Farouk, 2013). Slaughter of water buffaloes without stunning is done primarily to satisfy the demand for Halal meat in Muslim communities, particularly in Africa, the Indian subcontinent and Southeast Asia, where animals are killed by cutting the soft tissues of the ventral aspect of the neck (skin, muscle, trachea, oesophagus, carotid arteries, jugular veins, other blood vessels, sensory and motor nerves, and connective tissue) with a sharp knife (Gibson et al., 2015b; Nakyinsige et al., 2013). This must be performed by a member of the Muslim faith who invokes the name of Allah during the act (Grandin and Regenstein, 1994). Some Muslim communities require that the animal be facing Mecca, but this is not universally observed. During slaughter without stunning, the principle welfare concerns are: the stress and pain associated with restraint (Berg and Jakobsson, 2007; Daly et al., 1988; Grandin, 1998; Grandin and Regenstein, 1994; Gregory et al., 2012b; Velarde et al., 2014); the pain from the cut itself and the stimulation of nociceptors in the wound (Gibson et al., 2015a, 2015b; Zulkifli et al., 2014); the distress associated with delays to loss of consciousness (Daly et al., 1988; Gregory et al., 2010); and the distress caused by aspiring blood into the respiratory tract (Grandin and Regenstein, 1994; Gregory et al., 2010).

Unlike Shechita slaughter (the Jewish method), Halal has no special requirements for the knife used (Gibson, 2009). As a result, knives of inadequate length and sharpness may be utilized. This is especially important when slaughtering water buffaloes because their thick hide, long horns and short neck can make it difficult to perform a clean cut. This can extend the time required to perform the cut and result in incomplete severance of the carotid arteries, followed by stabbing, hacking and chopping motions with the knife, all of which increase the pain associated with this practice (Ahsan et al., 2014). Gregory et al. (2008) reported that this can be further complicated by the inability of operators to manually reposition the animal's head –because of its horns– to ensure adequate exposure of the ventral surface of the neck for cutting. Their study shows that when the head cannot be repositioned correctly, the knife had to be stabbed into the side of the neck repeatedly, causing many small cuts. One animal had to be cut 17 times before the soft tissues of its neck were severed (Gregory et al., 2008). Similarly, a study in Bangladesh reported that the knife point was often used to stab animals in the throat area in the initial phase of the cutting process. In some cases, after cutting the wound site was stimulated by washing or filling the trachea with water (Ahsan et al., 2014). The tissue damage associated with the neck cut and additional stimulation of the wound site has been shown to cause significant pain and distress during the slaughter of cattle without stunning (Gibson et al., 2009a, 2009b; Zulkifi et al., 2014).

In bovid species slaughtered without stunning, there may be a prolonged latency between loss of consciousness and death, though this will vary from one individual to another. In their work on veal calves, Lambooij et al. (2012) reported that brain activity continued for up to a mean of 80 s, while Gregory et al. (2010) using collapse as an early sign of loss of consciousness during non-stunned Halal slaughter in cattle observed that most animals collapsed within 20 s after the cut, but a significant proportion (8%) took more than 60 s. It has been suggested that the combination of continued cerebral perfusion by the intact vertebral arteries plus the formation of false aneurysms (carotid occlusions) on the cephalic and cardiac ends of the severed carotid arteries could prolong latency to loss of consciousness (Baldwin and Bell, 1963a, 1963b; Gregory et al., 2010). False aneurysms are thought to form when a severed carotid artery retracts into its surrounding sheath of connective tissue, causing the blood from the severed vessel to impregnate and swell the adventitia (Gregory et al., 2006). The swelling of the adventitia impedes –and may even block– the flow of blood from the artery which present a theoretical risk of recovery of consciousness or prolonged periods of consciousness (Gibson et al., 2015b). As in cattle, false aneurysms can also form in the severed carotids of water buffaloes. Gregory et al.

(2008) reported that the prevalence of false aneurysms 3 or more cm in diameter in Halal-slaughtered water buffaloes was greater than in cattle slaughtered in a similar way. However, the sample size of the water buffalo population in that study was relatively small (n=11). Work in Bangladesh, meanwhile, reported that the average time from neck cut to cardiac arrest was 7 to 8 min (Ahsan et al., 2014). However, this figure must be used with caution because cardiac arrest does not directly relate to time of loss of consciousness. In sheep, calves, adult cattle and chickens, irregular and sometimes isolated heart contractions (cardiac fibrillation) may continue until 5 to 10 min after the end of bleeding, whether the animal was stunned or not, or after the installation of isoelectric EEG (Vimini et al., 1983; Terlouw et al., 2016a). Ahsan et al., (2014) also reported that in some abattoirs the dressing-out process was performed in less than 30 s after the neck cut; thus there is a significant risk that some animals would still have been conscious during this period and would have suffered accordingly.

Recent work with non-stunned Halal cattle has shown that performing a high neck cut (HNC) (in relation to the first two trachea rings or first cervical vertebra) compared to the standard low neck cut (LNC), significantly reduced the time to final collapse (LNC 18.9 ± 1.1 s; HNC 13.5 ± 1.3 s) and the proportion of animals that took over 20 s to collapse (Gibson et al., 2015). Similarly, Gregory et al. (2012a) reported reduced false aneurysm development in Halal-slaughtered cattle cut in the HNC position. Taken together these results suggest that the HNC could significantly improve exsanguination and time to loss of consciousness. However, this work has not yet been replicated with water buffaloes.

Clearly, there is a need for additional research on the time to unconsciousness in non-stunned water buffaloes. However, based on the physiological similarities with other bovids and the limited number of studies carried out up to now we can assume that, like cattle, water buffaloes are at risk of prolonged times to unconsciousness during non-stunned slaughter.

Conclusions

Animals raised for meat production are unavoidably subjected to certain painful and stressful events and procedures during their lifetime. In the case of water buffaloes, the risk of deteriorated welfare may be higher than in other species due to various factors. However, it is important to note that there has been little research on the effects of *ante mortem* handling procedures, stunning and slaughter methods in this species. Specific legislation and handler training programmes in developed and developing countries need to be produced and implemented to try to reduce the welfare risks to buffaloes during all *ante mortem* stages, including handling, loading, unloading, transport, trading at the livestock market and slaughter.

The critical aspects to be improved are: (a) the use of harsh methods to handle and move buffaloes (e.g. electrical prods, sticks, ropes passed through the nose), especially those reared in extensive conditions; (b) transportation in terms of loading density, thermal stress and journey times; (c) the mixing of unfamiliar animals at livestock markets; (d) inadequate stunning methods and positioning when using the CBG or free bullet techniques such that they fail to induce complete unconsciousness; and (e) slaughter without stunning in developed and developing countries, where the focus should be on developing practical and humane restraining techniques, improved cutting procedures that reduce time to death, and research into stunning methods that do not induce cardiac arrest but are acceptable for Halal meat production. Many of these points relate to improving education and communication with stockmen, slaughterers and communities to highlight the importance of improving welfare not just for the animals' sake, but also in the interests of improving meat quality and productivity, and enhancing human safety.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

Daniel Mota-Rojas, Patricia Mora-Medina and Isabel Guerrero-Legarreta were funded as members of Mexican National System of Research (SNI).

References

Adenkola, A.Y., Ayo, J.O., 2010. Physiological and behavioural responses of livestock to road transportation stress: A review. Afr. J. Biotechnol. 9, 4845-4856.

Ahsan, M., Hasan, B., Algotsson, M., Sarenbo, S., 2014. Handling and Welfare of Bovine Livestock at Local Abattoirs in Bangladesh. J. Appl. Anim. Welf. Sci. 17, 340-353.

Alam, M.R., Gregory, N.G., Jabbar, M.A., Uddin, M.S., Kibria, A., Silva-Fletcher, A., 2010a. Skin injuries identified in cattle and water buffaloes at livestock markets in Bangladesh. Vet. Rec. 167, 415-419.

Alam, M.R., Gregory, N.G., Jabbar, M.A., Uddin, M.S., Widdicombe, J.P., Kibria, A., Khan, M.S.I., Mannan, A., 2010b. Frequency of dehydration and metabolic depletion in cattle and water buffalo transported from India to a livestock market in Bangladesh. Anim. Welf. 19, 301-305.

Alam, M.R., Gregory, N.G., Uddin, M.S., Jabbar, M.A., Chowdhury, S., Debnath, N.C., 2010c. Frequency of nose and tail injuries in cattle and water buffalo at livestock markets in Bangladesh. Anim. Welf. 19, 295-300.

Alsafy, M.A.M., El-Gendy, S.A.A., Sharaby, A.A., 2013. Anatomic Reference for Computed Tomography of Paranasal Sinuses and Their Communication in the Egyptian Buffalo (*Bubalus bubalis*). Anat. Histol. Embryol. 42, 220-231.

American Veterinary Medical Association (AVMA), 2013. Guidelines for the Euthanasia of Animals. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf (accessed 21.08.17).

Andrighetto, C., Jorge, A.M., Roca, R.D., Rodrigues, E., Bianchini, W., Francisco, C.D., 2008. Physical-chemical and sensory characteristics of meat from Murrah buffaloes slaughtered at different feedlot periods. Rev. Bras. Zootec. 37, 2179-2184.

Atkinson, S., Velarde, A., Algers, B., 2013. Assessment of stun quality at commercial slaughter in cattle shot with captive bolt. Anim. Welf. 22, 473-481.

Baldwin, B.A., Bell, F.R., 1963a. The anatomy of the cerebral circulation of the sheep and ox. The dynamic distribution of the blood supplied by the carotid and vertebral arteries to cranial regions. J. Anat. 97, 203-215.

Baldwin, B.A., Bell, F.R., 1963b. The effect of temporary reduction in cephalic blood flow on the EEG of sheep and calf. Electroencephalogr. Clin. Neurophysiol. 15, 465-473.

Ban-Tokuda, T., Orden, E.A., Barrio, A.N., Lapitan, R.M., Delavaud, C., Chilliard, Y., Fujihara, I., Cruz, L.C., Homma, H., Kanai, Y., 2007. Effects of species and sex on plasma hormone and metabolite concentrations in crossbred Brahman cattle and crossbred water buffalo. Livest. Sci. 107, 244-252.

Becerril-Herrera, M., Alonso-Spilsbury, M., Ortega, M.E.T., Guerrero-Legarreta, I., Ramírez-Necoechea, R., Roldan-Santiago, P., Pérez-Sato, M., Soní-Guillermo, E., Mota-Rojas, D., 2010. Changes in blood constituents of swine transported for 8 or 16 h to an Abattoir. Meat Sci. 86, 945-948.

Berg, C., Jakobsson, T., 2007. Post-cut stunning at religious slaughter. Svensk Veterinärtidning. 59, 21-28.

Borghese, A., 2005. Buffalo Production and Research. FAO, Rome, Italy.

Bornett-Gauci, H.L.I., Martin, J.E., Arney, D.R., 2006. The welfare of low-volume farm animals during transport and at slaughter: a review of current knowledge and recommendations for future research. Anim. Welf. 15, 299-308.

Bolaños-López, D., Mota-Rojas, D., Guerrero-Legarreta, I., Flores-Peinado, S., Mora-Medina, P.-, Roldan-Santiago, P., Borderas-Tordesillas, F., García-Herrera, R., Ramírez-Necoechea, R. Recovery of consciousness in hogs stunned with CO2: Physiological responses. Meat Sci. 98, 193–197.

Bourguet, C., Deiss, V., Tannugi, C.C., Terlouw, E.M.C., 2011. Behavioural and physiological reactions of cattle in a commercial abattoir: Relationships with organisational aspects of the abattoir and animal characteristics. Meat Sci. 88, 158-168.

Brown, E.J., Vosloo, A., 2017. The involvement of the hypothalamopituitary-adrenocortical axis in stress physiology and its significance in the assessment of animal welfare in cattle. Onderstepoort J. Vet. R. 84, a1398.

Chandra, B.S., Das, N., 2001a. Behaviour of Indian river buffaloes (*Bubalus bubalis*) during short-haul road transportation. Vet. Rec. 148, 314-315.

Chandra, B.S., Das, N., 2001b. The handling and short-haul road transportation of spent buffaloes in relation to bruising and animal welfare. Trop. Anim. Health Prod. 33, 155-163.

Chen, Y., Stookey, J., Arsenault, R., Scruten, E., Griebel, P., Napper, S. 2016., Investigation of the physiological, behavioral, and biochemical responses of cattle to restraint stress. J. Anim. Sci. 94, 3240-3254.

Dalmau, A., Llonch, P., Velarde, A., 2012. Sacrificio religioso de animales para consumo, in: Mota-Rojas, D., Maris-Huertas, S., Guerrero-Legarreta, I., Trujillo, M. (Eds.), Bienestar Animal Productividad y Calidad de Carne. Elsevier, México, pp. 427-448.

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

Das, G.K., Khan, F.A., 2010. Summer Anoestrus in Buffalo - A Review. Reprod. Domest. Anim. 45, e483-e494.

de la Cruz-Cruz, L.A., Guerrero-Legarreta, I., Ramirez-Necoechea, R., Roldan-Santiago, P., Mora-Medina, P., Hernandez-Gonzalez, R., Mota-Rojas, D., 2014. The behaviour and productivity of water buffalo in different breeding systems: a review. Vet. Med. 59, 181-193.

de la Cruz-Cruz, L.A., Napolitano, F., Berdugo-Gutiérrez, J., Tarazona-Morales, A.M., Toledo, L., 2016. Chapter 6. Behavior and welfare of water buffalo. In: Mota-Rojas, D., Velarde, A., Huertas-Canen, S., Cajíao, M.N. (Eds.), Animal Welfare: a global vision in Ibero-America (In spanish). Elsevier, España, pp. 63-76.

De Rosa, G., Grasso, F., Braghieri, A., Bilancione, A., Di Francia, A., Napolitano, F., 2009a. Behavior and milk production of buffalo cows as affected by housing system. J. Dairy Sci. 92, 907-912.

De Rosa, G., Grasso, F., Pacelli, C., Napolitano, F., Winckler, C., 2009b. The welfare of dairy buffalo. Ital. J. Anim. Sci. 8, 103-116.

Desta, T.T., 2012. Introduction of domestic buffalo (Bubalus bubalis) into Ethiopia would be feasible. Renew. Agric. Food Sys. 27, 305-313.

Dembo, J.A., 1984. The Jewish method of slaughter compared with other methods from the humanitarian, hygienic, and economic points of view. London: Kegan, Paul, Trench, Trübner & Co Ltd.

Di Luccia, A., Satriani, A., Barone, C.M.A., Colatruglio, P., Gigli, S., Occidente, M., Trivellone, E., Zullo, A., Matassino, D., 2003. Effect of dietary energy content on the intramuscular fat depots and triglyceride composition of river buffalo meat. Meat Sci. 65, 1379-1389.

European Food Safety Authority (EFSA), 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA J. 45, 1-29.

England, 2015. The Welfare of Animals at the Time of Killing (England) Regulations. https://www.gov.uk/government/collections/welfare-of-animals-at-the-time-of-killing#history (accessed 06.03.03).

Erasmus, M.A., Turner, P.V., Widowski, T.M., 2010. Measures of insensibility used to determine effective stunning and killing of poultry. J. Appl. Poult. Res. 19, 288-298.

European Union, 2005. Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations. Official Journal of the European Union L 3, 1-44.

European Union, 2009. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. Official Journal of the European Union L 303, 1-30.

Ewbank, R., Parker, M.J., Mason, C.W., 1992. Reactions of cattle to head-restraint at stunning: a practical dilemma. Anim. Welf. 1, 55-63.

Francisco, C.L., Cooke, R.F., Marques, R.S., Mills, R.R., Bohnert, D.W., 2012. Effects of temperament and acclimation to handling on feedlot performance of Bos taurus feeder cattle originated from a rangeland-based cow-calf system. J. Anim. Sci. 90, 5067-5077.

Food and Agriculture Organization of the United Nations (2001). Slaughter of livestock. in: Chambers, P.G., Grandin, T. (eds.), Guidelines for Humane Handling, Transport and Slaughter of Livestock, Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific (RAP), Bangkok, Thailand.

Farjo, L.A., Miclau, T., 1997. Ballistics and mechanisms of tissue wounding. Injury J. 28, 12-17.

Farouk, M.M., 2013. Advances in the industrial production of halal and kosher red meat. Meat Sci. 95, 805-820.

Ferguson, D.M., Warner, R.D., 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Sci. 80, 12-19.

Fisher, A.D., Colditz, I.G., Lee, C., Ferguson, D.M., 2009. The influence of land transport on animal welfare in extensive farming systems. J. Vet. Behav. 4, 157-162.

Fornari, G.B., Menegassi, S.R.O., Pereira, G.R., Oliveira, T.E.d., Barcellos, J.O.J., 2016. Factors affecting the selling prices of calves in auctions in Santa Catarina State, Brazil. R. Bras. Zootec. 45, 632-638.

Gibson, T.J., 2009. Electroencephalographic responses of calves to the noxious sensory input of slaughter by vental neck incision and its modulation with non-penetrative captive bolt stunning. Institute of Veterinary, Animal and Biomedical Science. Massey University, Palmerson North, New Zeland, p. 280.

Gibson, T.J., Bedford, E.M., Chancellor, N.M., Limon, G., 2015a. Pathophysiology of free-bullet slaughter of horses and ponies. Meat Sci. 108, 120-124.

Gibson, T.J., Dadios, N., Gregory, N.G., 2015b. Effect of neck cut position on time to collapse in halal slaughtered cattle without stunning. Meat Sci. 110, 310-314.

Gibson, T.J., Mason, C.W., Spence, J.Y., Barker, H., Gregory, N.G., 2015c. Factors Affecting Penetrating Captive Bolt Gun Performance. J. Appl. Anim. Welf. Sci. 18, 222-238.

Gibson, T.J., Whitehead, C., Taylor, R., Sykes, O., Chancellor, N.M., Limon, G., 2015d. Pathophysiology of penetrating captive bolt stunning in Alpacas (*Vicugna pacos*). Meat Sci. 100, 227-231.

Giordano, G., Guarini, P., Ferrari, P., Biondi-Zoccai, G., Schiavone, B., Giordano, A., 2010. Beneficial impact on cardiovascular risk profile of water buffalo meat consumption. Eur. J. Clin. Nutr. 64, 1000-1006.

Gonzalez, L.A., Schwartzkopf-Genswein, K.S., Bryan, M., Silasi, R., Brown, F., 2012. Relationships between transport conditions and welfare outcomes during commercial long haul transport of cattle in North America. J. Anim. Sci. 90, 3640-3651.

Grandin, T., 1980. Mechanical, electrical and anesthetic stunning methods for livestock. Int. J. Study Anim. Probl. 1, 242-263.

Grandin, T., 1994. Euthanasia and slaughter of livestock. J. Am. Vet. Med. Assoc. 204, 1354-1360.

Grandin, T., 1998. The feasibility of using vocalization scoring as an indicator of poor welfare during cattle slaughter. Appl. Anim. Behav. Sci. 56, 121-128.

Grandin, T., 2000. Livestock handling and transport. CAB International, Wallingford, UK.

Grandin, T., 2002. Return-to-sensibility problems after penetrating captive bolt stunning of cattle in commercial beef slaughter plants. J. Am. Vet. Med. Assoc. 221, 1258-1261.

Grandin, T., 2010. Auditing animal welfare at slaughter plants. Meat Sci. 86, 56-65.

Grandin, T., 2012. Auditing animal welfare and making practical improvements in beef-, pork- and sheep-slaughter plants. Anim. Welf. 21, 29-34.

Grandin, T., 2013. Making Slaughterhouses More Humane for Cattle, Pigs, and Sheep. Annu. Rev. Anim. Biosci. 1, 491-512.

Grandin, T., 2014: Behavioural Principles of handling cattle and other grazing animals under extensive conditions, in Grandin, T. (Ed.), Livestock Handling and Transport. CAB International., London UK, pp. 39-64.

Grandin, T., Deesing, M.J., 2014. Genetics and behavior during handling, restraint, and herding, in Grandin, T., Deesing, M.J. (Eds.), Genetics and the behavior of domestic animals. Academic Press, San Diego, pp. 115-158.

Grandin, T., Regenstein, 1994. Religious slaughter and animal welfare: a discussion for meat scientists. Meat Focus International. CAB International, pp. 115-123.

Gregory, N.G., 1990. Slaughtering methods and equipment. Vet. Hist. 6, 73-84.

Gregory, N.G., 2008. Animal welfare at markets and during transport and slaughter. Meat Sci. 80, 2-11.

Gregory, N.G., Benson, T., Mason, C.W., 2009a. Cattle handling and welfare standards in livestock markets in the UK. J. Agr. Sci. 147, 345-354.

Gregory, N.G., Benson, T., Smith, N., Mason, C.W., 2009b. Sheep handling and welfare standards in livestock markets in the UK. J. Agr. Sci. 147, 333-344.

Gregory, N.G., Fielding, H.R., von Wenzlawowicz, M., von Holleben, K., 2010. Time to collapse following slaughter without stunning in cattle. Meat Sci. 85, 66-69.

Gregory, N.G., Lee, C.J., Widdicombe, J.P., 2007. Depth of concussion in cattle shot by penetrating captive bolt. Meat Sci. 77, 499-503.

Gregory, N.G., Schuster, P., Mirabito, L., Kolesar, R., McManus, T., 2012a. Arrested blood flow during false aneurysm formation in the carotid arteries of cattle slaughtered with and without stunning. Meat Sci. 90, 368-372.

Gregory, N.G., Shaw, F., 2000. Penetrating captive bolt stunning and exsanguination of cattle in abattoirs. J. Appl. Anim. Welf. Sci. 3, 215-230.

Gregory, N.G., Shaw, F.D., Whitford, J.C., Patterson-Kane, J.C., 2006. Prevalence of ballooning of the severed carotid arteries at slaughter in cattle, calves and sheep. Meat Sci. 74, 655-657.

Gregory, N.G., Spence, J.Y., Mason, C.W., Tinarwo, A., Heasman, L., 2009c. Effectiveness of poll stunning water buffalo with captive bolt guns. Meat Sci. 81, 178-182.

Gregory, N.G., von Wenzlawowicz, M., Alam, R.M., Anil, H.M., Yeşildere, T., Silva-Fletcher, A., 2008. False aneurysms in carotid arteries of cattle and water buffalo during shechita and halal slaughter. Meat Sci. 79, 285-288.

Gregory, N.G., von Wenzlawowicz, M., Holleben, K., Fielding, H.R., Gibson, T.J., Mirabito, L., Kolesar, R., 2012b. Complications during shechita and halal slaughter without stunning in cattle. Anim. Welf. 21, 81-86.

Gilliam, J.N., Shearer, J.K., Woods, J., Hill, J., Reynolds, J., Taylor, J.D., Bahr, R.J., Crochik, S., Snider, T.A., 2012. Captive-bolt euthanasia of cattle: determination of optimal-shot placement and evaluation of the Cash Special Euthanizer Kit (R) for euthanasia of cattle. Anim. Welf. 21, 99-102.

Hemsworth, P.H., Rice, M., Karlen, M.G., Calleja, L., Barnett, J.L., Nash, J., Coleman, G.J., 2011. Human–animal interactions at abattoirs: Relationships between handling and animal stress in sheep and cattle. Appl. Anim. Behav. Sci. 135, 24-33.

Hilsenbeck, V.E., Forster, S., Stolle, A., 2007. Examinations of bleeding-time in cattle after capitive bolt stunning. Fleisch. 87, 119-122.

Hollerman, J.J., Fackler, M.L., Coldwell, D.M., Benmenachem, Y., 1990. Gunshot wounds: 1. bullets, ballistics, and mechanisms of injury. Am. J. Roentgenol. 155, 685-690.

Hoogesteijn, R., Hoogesteijn, A., 2008. Conflicts between cattle ranching and large predators in Venezuela: could use of water buffalo facilitate felid conservation? Oryx. 42, 132-138.

Hultgren, J., Wiberg, S., Berg, C., Cvek, K., Lunner Kolstrup, C., 2014. Cattle behaviours and stockperson actions related to impaired animal welfare at Swedish slaughter plants. Appl. Anim. Behav. Sci.152, 23-37.

Irurueta, M., Cadoppi, A., Langman, L., Grigioni, G., Carduza, F., 2008. Effect of aging on the characteristics of meat from water buffalo grown in the Delta del Parana region of Argentina. Meat Sci. 79, 529-533.

Jacobson, L.H., Cook, C.J., 1998. Partitioning psychological and physical sources of transport-related stress in young cattle. Vet. J. 155, 205-208.

Joshi, D.D., Maharjan, M., Johansen, M.V., Willingham, A.L., Sharma, M., 2003. Improving meat inspection and control in resource-poor communities: the Nepal example. Acta Trop. 87, 119-127.

Kandeepan, G., Anjaneyulu, A.S.R., Kondaiah, N., Mendiratta, S.K., Lakshmanan, V., 2009. Effect of age and gender on the processing characteristics of buffalo meat. Meat Sci. 83, 10-14.

Kandeepan, G., Mendiratta, S.K., Shukla, V., Vishnuraj, M.R., 2013. Processing characteristics of buffalo meat - a review. J. Meat Sci. Technol. 1, 1-11.

Kim, G.D., Lee, H.S., Jung, E.Y., Lim, H.J., Seo, H.W., Lee, Y.H., Jang, S.H., Baek, S.B., Joo, S.T., Yang, H.S., 2013. The effects of CO_2 gas stunning on meat quality of cattle compared with captive bolt stunning. Livest. Sci. 157, 312-316.

Knights, M., Smith, G.W., 2007. Decreased ACTH secretion during prolonged transportation stress is associated with reduced pituitary responsiveness to tropic hormone stimulation in cattle. Domest. Anim. Endocrinol. 33, 442-450.

Koga, A., Kurata, K., Ohata, K., Nakajima, M., Hirose, H., Furukawa, R., Kanai, Y., Chikamune, T., 1999. Internal changes of blood compartment and heat distribution in swamp buffaloes under hot conditions: Comparative study of thermo-regulation in Buffaloes and Friesian cows. Asian Australas. J. Anim. Sci. 12, 886-890.

Lambooij, E., van der Werf, J.T.N., Reimert, H.G.M., Hindle, V.A., 2012. Restraining and neck cutting or stunning and neck cutting of veal calves. Meat Sci. 91, 22-28.

Limon, G., Guitian, J., Gregory, N.G., 2009. A note on the slaughter of llamas in Bolivia by the puntilla method. Meat Sci. 82, 405-406.

Limon, G., Guitian, J., Gregory, N.G., 2010. An evaluation of the humaneness of puntilla in cattle. Meat Sci. 84, 352-355.

Limon, G., Guitian, J., Gregory, N.G., 2012. A review of the humaneness of puntilla as a slaughter method. Anim. Welf. 21, 3-8.

Machado, M., Guimarães, S.R.M., Medeiros, V.A.P., Giannoccaro, S.M.A., César, M., P., , Mendez de Córdova, F., Tony, R.A., 2013. Bone and Brain Lesions in Horses Following Euthanasia with Fire Gun. Braz. J. Vet. Pathol. 6, 102-105.

Marai, I.F.M., Haeeb, A.A.M., 2010. Buffalo's biological functions as affected by heat stress - A review. Livest. Sci. 127, 89-109.

Meichtry, C. 2015. Assessment of a specifically developed bullet casing gun for the stunning of water buffaloes. Doctoral dissertation, University of Bern, Switzerland.

Minka, N.S., Ayo, J.O., Sackey, A.K.B., Adelaiye, A.B., 2009. Assessment and scoring of stresses imposed on goats during handling, loading, road transportation and unloading, and the effect of pretreatment with ascorbic acid. Livest. Sci. 125, 275-282.

Miranda-de la Lama, G.C., Leyva, I.G., Barreras-Serrano, A., Perez-Linares, C., Sanchez-Lopez, E., Maria, G.A., Figueroa-Saavedra, F., 2012. Assessment of cattle welfare at a commercial slaughter plant in the northwest of Mexico. Trop. Anim. Health Prod. 44, 497-504.

Mota-Rojas, D., Becerril, M., Lemus, C., Sánchez, P., González, M., Olmos, S.A., Ramírez, R., Alonso-Spilsbury, M., 2006. Effects of mid-summer transport duration on pre- and post-slaughter performance and pork quality in Mexico. Meat Sci. 73, 404-412.

Mota-Rojas, D., Becerril-Herrera, M., Roldan-Santiago, P., Alonso-Spilsbury, M., Flores-Peinado, S., Ramírez, R., Ramírez, J. A., Mora-Medina, P., Pérez, M., Molina, E., Soní, E., Trujillo, M. (2012). Effects of long distance transportation and CO2 stunning on critical blood values in pigs. Meat Sci. 90, 893–898.

Muñoz, D., Strappini, A., Gallo, C., 2012. Animal welfare indicators to detect problems in the cattle stunning box. Arch. Med. Vet. 44, 297-302.

Nakyinsige, K., Che Man, Y.B., Aghwan, Z.A., Zulkifli, I., Goh, Y.M., Abu Bakar, F., Al-Kahtani, H.A., Sazili, A.Q., 2013. Stunning and animal welfare from Islamic and scientific perspectives. Meat Sci. 95, 352-361.

Napolitano, F., De Rosa, G., Grasso, F., Pacelli, C., Bordi, A., 2004. Influence of space allowance on the welfare of weaned buffalo (*Bubalus bubalis*) calves. Livest. Prod. Sci. 86, 117-124.

Napolitano, F., Pacelli, C., Grasso, F., Braghieri, A., De Rosa, G., 2013. The behaviour and welfare of buffaloes (*Bubalus bubalis*) in modern dairy enterprises. Animal. 7, 1704-1713.

Napolitano F., Pacelli C., Braghieri A., Grasso F. and De Rosa G. (2017). Animal - Environment Interaction: Buffalo Behavior and Welfare. In: G. A. Presicce. The Buffalo (Bubalus bubalis) – Production and Research. Bentham E-Books, 69-104.

Neves, J.E.G., Paranhos da Costa, M.J.R., Roça, R.O., Faucitano, L., Gregory, N.G., 2016. A note comparing the welfare of Zebu cattle following three stunning-slaughter methods. Meat Sci. 117, 41-43.

New Zeland, 2016. Commercial Slaughter: Code of Welfare 15 December 2016. Ministry of primary industries. https://www.mpi.govt.nz/document-vault/1409 (accessed 06.03.17).

Önenç, A., Kaya, A., 2004. The effects of electrical stunning and percussive captive bolt stunning on meat quality of cattle processed by Turkish slaughter procedures. Meat Sci. 66, 809-815.

Petherick, J.C., 2005. Animal welfare issues associated with extensive livestock production: The northern Australian beef cattle industry. Appl. Anim. Behav. Sci. 92, 211-234.

Probst, J.K., Hillmann, E., Leiber, F., Kreuzer, M., Spengler Neff, A., 2013. Influence of gentle touching applied few weeks before slaughter on avoidance distance and slaughter stress in finishing cattle. Appl. Anim. Behav. Sci. 144, 14-21.

Purchas, R.W., Thomson, N.A., Waghorn, G.C., Death, A.F., 1993. A comparison of carcass and meat characteristics and organ weights of castrate and entire male buffalo and cattle. Proc. New Zeal. Soc. Anim. Prod. 53, 407-411.

Resolution 002341/2007, 2007. Condiciones sanitarias y de inocuidad en la producción primaria de ganado bovino y bufalino destinado al sacrificio para consumo humano. http://www.ica.gov.co/getattachment/0b5de556-cb4a-43a8-a27a-cd9a2064b1ab/2341.aspx (accessed 06.03.17).

Robins, A., Pleiter, H., Latter, M., Phillips, C.J.C., 2014. The efficacy of pulsed ultrahigh current for the stunning of cattle prior to slaughter. Meat Sci. 96, 1201-1209.

Roldan-Santiago, P., de la Cruz-Cruz, L.A., Tarazona-Morales, A.M., Buenhombre, J., Acerbi, R., de Varona, E., Mota-Rojas, D., 2016. Animal welfare in livestock markets. In: Mota-Rojas, D., Velarde, A., Huertas-Canen, S., Cajíao, M.N. (Eds.), Animal welfare: a global vision in Iberoamerica (in spanish). Elsevier, España, pp. 155-168.

Roldan-Santiago, P., Gonzalez-Lozano, M., Flores-Peinado, S.C., Camacho-Morfin, D., Concepcion-Mendez, M., Morfin-Loyden, L., Mora-Medina, P., Ramirez-Necoechea, R., Cardona, A.L., Mota-Rojas, D., 2011. Physiological Response and Welfare of Ducks During Slaughter. Asian J. Anim. Vet. Adv. 6, 1256-1263.

Roldan-Santiago, P., Martinez-Rodriguez, R., Yanez-Pizana, A., Trujillo-Ortega, M.E., Sanchez-Hernandez, M., Perez-Pedraza, E., Mota-Rojas, D., 2013a. Stressor factors in the transport of weaned piglets: a review. Vet. Med. 58, 241-251.

Roldan-Santiago, P., Mota-Rojas, D., Guerreo-Legarreta, I., Mora-Medina, P., Borderas-Tordesillas, F., Alarcon-Rojo, A.D., Flores-Peinado, S., Orozco-Gregorio, H., Martinez-Rodriguez, R., Trujillo-Ortega, M.E., 2013b. Animal welfare of barrows with different antemortem lairage times without food. Vet. Med. 58, 305-311.

Romero, M.H., Gutierrez, C., Sanchez, J.A., 2012. Evaluation of bruises as an animal welfare indicator during pre-slaughter of beef cattle. Rev. Col. Cien. Pec. 25, 267-275.

Romero, M.H., Uribe-Velasquez, L.F., Sanchez, J.A., Miranda-de la Lama, G.C., 2013. Risk factors influencing bruising and high muscle pH in Colombian cattle carcasses due to transport and pre-slaughter operations. Meat Sci. 95, 256-263.

Romero, M.H., Uribe-Velasquez, L.F., Sanchez, J.A., Rayas-Amor, A.A., Miranda-de la Lama, G.C., 2017. Conventional versus modern abattoirs in Colombia: Impacts on welfare indicators and risk factors for high muscle pH in commercial Zebu young bulls. Meat Sci. 123, 173-181.

Rushen, J., Taylor, A.A., de Passillé, A.M., 1999. Domestic animals' fear of humans and its effect on their welfare. Appl. Anim. Behav. Sci. 65, 285-303.

Rushen, J., de Pasillé, A.M., Munksgaard, L., Tanida, H., 2001. People as social actors in the world of farm animals, in: Keeling, L., Gonyou, H.W. (Eds.), Social behaviour in farm animals. CABI Publishing, UK, pp. 353-372.

Schwartzkopf-Genswein, K.S., Faucitano, L., Dadgar, S., Shand, P., Gonzalez, L.A., Crowe, T.G., 2012. Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. Meat Sci. 92, 227-243.

Schwenk, B.K., Lechner, I., Ross, S.G., Gascho, D., Kneubuehl, B.P., Glardon, M., Stoffel, M.H., 2016. Magnetic resonance imaging and computer tomography of brain lesions in water buffaloes and cattle stunned with handguns or captive bolts. Meat Sci. 113, 35-40.

Shearer, J. K. (2005). Euthanasia of cattle: Indications and practical considerations. Proceedings of the North American Veterinary Conference: Large animal. Vol. 19. (pp. 28–29). Gainesville, Florida, USA: Eastern States Veterinary Association (January 8, 2005-January 12, 2005, Orlando, Florida).

Shivahre, P.R., Gupta, A.K., Panmei, A., Bhakat, M., Kumar, V., Dash, S.K., Dash, S., Upadyay, A., 2014. Mortality pattern of Murrah buffalo males in an organised herd. Vet. World. 7, 356-359.

Silveira, I.D.B., Fischer, V., Farinatti, L.H.E., Restle, J., Alves, D.C., de Menezes, L.F.G., 2012. Relationship between temperament with performance and meat quality of feedlot steers with predominantly Charolais or Nellore breed. Rev. Bras. Zootecn. 41, 1468-1476.

Spanghero, M., Gracco, L., Valusso, R., Piasentier, E., 2004. In vivo performance, slaughtering traits and meat quality of bovine (*Italian Simmental*) and buffalo (*Italian Mediterranean*) bulls. Livest. Prod. Sci. 91, 129-141.

Strappini, A.C., Frankena, K., Metz, J.H.M., Gallo, C., Kemp, B., 2012. Characteristics of bruises in carcasses of cows sourced from farms or from livestock markets. Animal. 6, 502-509.

Strappini, A.C., Metz, J.H.M., Gallo, C., Frankena, K., Vargas, R., de Freslon, I., Kemp, B., 2013. Bruises in culled cows: when, where and how are they inflicted? Animal. 7, 485-491.

Strappini, A.C., Metz, J.H.M., Gallo, C.B., Kemp, B., 2009. Origin and assessment of bruises in beef cattle at slaughter. Animal. 3, 728-736.

Tarrant, P.V., 1990. Transportation of cattle by road. Appl. Anim. Behav. Sci. 28, 153-170.

Teke, B., Akdag, F., Ekiz, B., Ugurlu, M., 2014. Effects of different lairage times after long distance transportation on carcass and meat quality characteristics of Hungarian Simmental bulls. Meat Sci. 96, 224-229.

Terlouw, E.M.C., Arnould, C., Auperin, B., Berri, C., Le Bihan-Duval, E., Deiss, V., Lefevre, F., Lensink, B.J., Mounier, L., 2008. Pre-slaughter conditions, animal stress and welfare: current status and possible future research. Animal. 2, 1501-1517.

Terlouw, C., Bourguet, C., Deiss, V., Mallet, C., 2015. Origins of movements following stunning and during bleeding in cattle. Meat Sci. 110, 135-144.

Terlouw, C., Bourguet, C., Deiss, V., 2016a. Consciousness, unconsciousness and death in the context of slaughter. Part I. Neurobiological mechanisms underlying stunning and killing. Meat Sci. 118, 133-146.

Terlouw, C., Bourguet, C., Deiss, V., 2016b. Consciousness, unconsciousness and death in the context of slaughter. Part II. Evaluation methods. Meat Sci. 118, 147-156.

Thomson, D.U., Wileman, B.W., Rezac, D., Miesner, M.D., Johnson-Neitman, J.L., Biller, D.S., 2013. Computed tomographic evaluation to determine efficacy of euthanasia of yearling feedlot cattle by use of various firearm-ammunition combinations. Am. J. Vet. Res. 74, 1385-1391.

Trentini, R., Di Fede, E., Iannetti, L., Ruggieri, E., Di Nardo, A., Villa, P.D., 2011. Pre-slaughtering stunning effects on small ruminants welfare: a review. Large Anim. Rev. 17, 177-186.

Ufficio Federale della Sicurezza Alimentare e di Veterinaria, 2010. Ordinanza dell'USAV concernente la protezione degli animali nella macellazione (455.11.2).

https://www.admin.ch/opc/it/classified-compilation/20080808/index.html (accesed 06.03.17).

Vazquez-Galindo, G., de Aluja, A.S., Guerrero-Legarreta, I., Orozco-Gregorio, H., Borderas-Tordesillas, F., Mora-Medina, P., Roldan-Santiago, P., Flores-Peinado, S., Mota-Rojas, D., 2013. Adaptation of ostriches to transport-induced stress: Physiometabolic response. Anim. Sci. J. 84, 350-358.

Velarde, A., Rodriguez, P., Dalmau, A., Fuentes, C., Llonch, P., von Holleben, K.V., Anil, M.H., Lambooij, J.B., Pleiter, H., Yesildere, T., Cenci-Goga, B.T., 2014. Religious slaughter: Evaluation of current practices in selected countries. Meat Sci. 96, 278-287.

Verhoeven, M.T.W., Gerritzen, M.A., Hellebrekers, L.J., Kemp, B., 2015a. Indicators used in livestock to assess unconsciousness after stunning: a review. Animal. 9, 320-330.

Verhoeven, M.T.W., Gerritzen, M.A., Kluivers-Poodt, M., Hellebrekers, L.J., Kemp, B., 2015b. Validation of behavioural indicators used to assess unconsciousness in sheep. Res. Vet. Sci. 101, 144-153.

Villarroel, M., Maria, G.A., Sierra, I., Sanudo, C., Garcia-Belenguer, S., Gebresenbet, G., 2001. Critical points in the transport of cattle to slaughter in Spain that may compromise the animals' welfare. Vet. Rec. 149, 173-176.

Vimini, R.J., Field, R.A., Riley, M.L., Varnell, T.R., 1983. Effect of delayed bleeding after captive bolt stunning on heart activity and blood removal in beef cattle. J. Anim. Sci., 57, 628–631.

Voisinet, B.D., Grandin, T., Oconnor, S.F., Tatum, J.D., Deesing, M. J., 1997. *Bos indicus* cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. Meat Sci. 46, 367-377.

von Wenzlawowicz, M., von Holleben, K., Eser, E., 2012. Identifying reasons for stun failures in slaughterhouses for cattle and pigs: a field study. Anim. Welf. 21, 51-60.

Weeks, C.A., McNally, P.W., Warriss, P.D., 2002. Influence of the design of facilities at auction markets and animal handling procedures on bruising in cattle. Vet. Rec. 150, 743-748.

Winckler, C., Capdeville, J., Gebresenbet, G., Horning, B., Roiha, U., Tosi, M., Waiblinger, S., 2003. Selection of parameters for on-farm welfare-assessment protocols in cattle and buffalo. Anim. Welf. 12, 619-624.

Yilmaz, O., Ertugrul, M., Wilson, R.T., 2012. Domestic livestock resources of Turkey Water buffalo. Trop. Anim. Health Prod. 44, 707-714.

Zava, M., 2011a. Anatomía y Fisiología. El búfalo doméstico. Orientación Gráfica Editora, Buenos Aires, Argentina, 39-60.

Zava, M., 2011b. Manejo y Bienestar Animal El búfalo doméstico. Orientación Gráfica y Editora, Buenos Aires, Argentina, 459-496.

Zulkifli, I., Goh, Y.M., Norbaiyah, B., Sazili, A.Q., Lotfi, M., Soleimani, A.F., Small, A.H., 2014. Changes in blood parameters and electroencephalogram of cattle as affected by different stunning and slaughter methods in cattle. Anim. Prod. Sci. 54, 187-193.

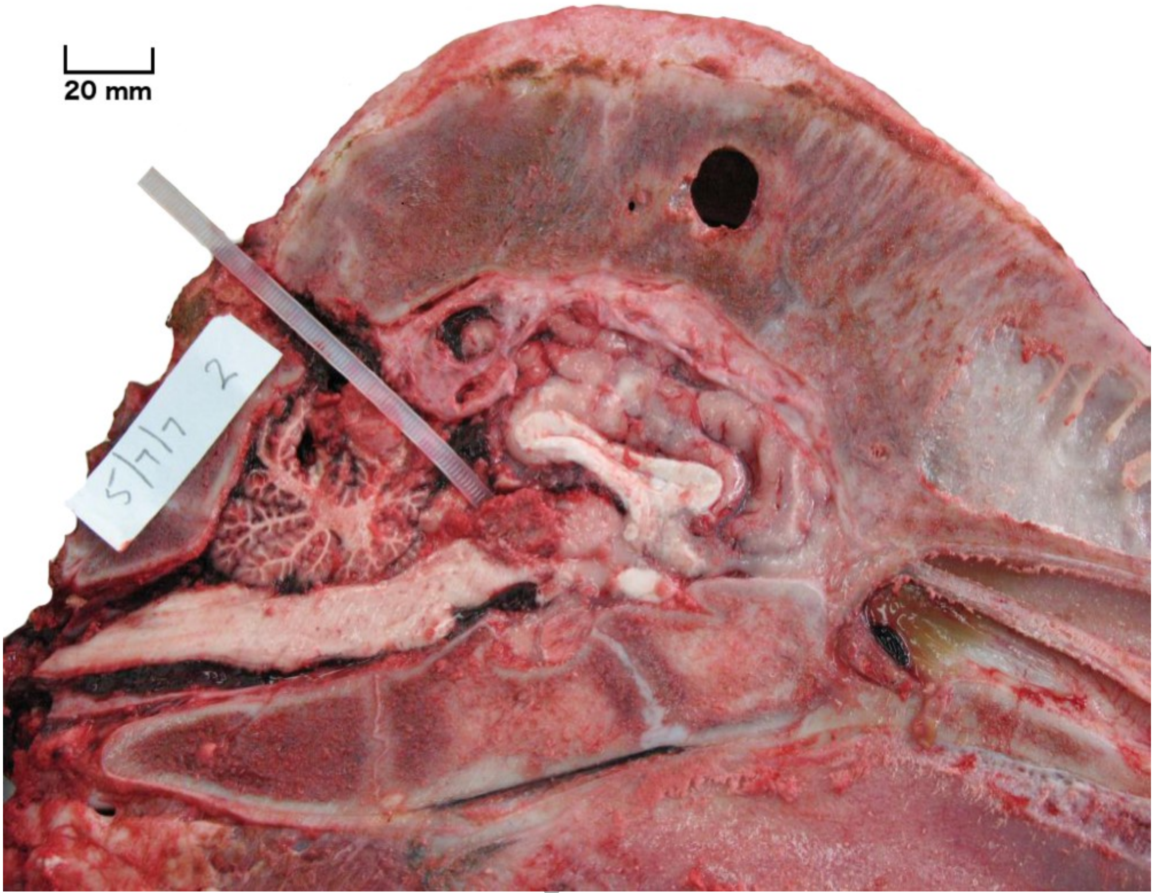

Figure 1. Water buffalo shot in the poll position with the bolt entering into the occipital lobe and terminating in midbrain. Total bolt and bone fragment travel of 10 mm. Photo courtesy of N. Gregory

Table 1. Effects of ante-mortem procedures and stunning on water buffalo welfare

Ante mortem stage	Negative effects	Reference
Handling during loading and unloading	↓ blood pH with increasing restraining time in the working chutes. ↑ blood lactate values at 5 and 15 minutes in the working chutes. ↑ pCO_2 values in unweaned calves compared to weaned calves, steers and bulls. ↑blood glucose values with longer times in the working chutes in 1 year buffaloes. ↑ nervousness and restlessness of buffaloes raised in extensive conditions.	de la Cruz-Cruz (2015)
	↑ loading times for heavier as compared with lighter animals (87 *vs.* 43 s). ↓ unloading time with sticks as compared with lassoes.	Chandra and Das (2001b)
	↑ number of falls, collisions and blows to the most prominent parts of their body (*Tuber coxae*) when moving >600 kg buffaloes through narrow chutes (80-85 cm).	de la Cruz-Cruz et al. (2014b)
Sale for slaughter in livestock markets	↑ percentage of (99%) with visible body lesions (abrasions, lacerations, inflammation, hyperkeratosis) as compared with cattle. The most affected areas were: head, neck, forequarters, hindquarters, thorax, abdomen.	Alam et al. (2010a)
	2.4 lesions per animal; most commonly, small, deep contusions (59%). The most affected areas were: hindquarters, abdomen, udder.	Chandra and Das (2001b)
	47% of the buffaloes with lacerations and ulcerations of the nostrils caused by ropes used to tie them during transport.	Alam et al. (2010c)
	Frequent signs of distress: ocular congestion, tearing, nasal discharge, salivation.	Chandra and Das (2001a).
Transport	In Bangladesh rope halters passing through the nasal septum cause severe injuries	Ahsan et al. (2014).
	Temperature extremes causing thermal stress.	Zava (2011a)
	↑ blood glucose levels in hot seasons.	Alam et al. (2010b)
	Loss of body weight for transports over 700 km (7% to 9%).	Zava (2011b)
	In Bangladesh 89% of animals had injuries on various parts of their bodies.	Ahsan et al. (2014).
Stunning methods	The frontal bones and sinuses of water buffaloes are up to 8 cm thick.	Gregory (2008).
	The left and right frontal sinuses are separated by a highly-developed, complete, median interfrontal septum.	Alsafy et al. (2013)
	In water buffaloes and cattle, respectively, the median distance from the frontal skin surface to the inner bone table is 7.4 cm *vs*. 3.6 cm,	Schwenk et al. (2016).

and from skin to the thalamus, 14.48 *vs*. 10.2 cm.	
Discharges in the forehead do not cause immediate loss of posture. When shot in the crown position, animals continue to breathe rhythmically with ocular rotation post-collapse. When shot in the poll position, buffaloes –especially those over 30 months old– present rhythmic respiration and ocular rotation; 79% of the animals stunned in the poll position suffered damage to the cerebellum, and 71% damage in the medulla and/or pons.	Gregory et al. (2009c)