Assessment of Cattle and Pig Welfare at Stunning in Commercial Abattoirs

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Abstract

This thesis aimed to develop and implement a standardised assessment for stun quality for use in commercial pig and cattle abattoirs to protect animal welfare. A stun quality protocol identified and rated symptoms signifying recovery risk and level of concern for inferior animal welfare. The thesis also aimed to identify problem areas and methods for improving animal welfare standards at slaughter.

Eighteen assessments were conducted on 9520 pigs stunned with CO_2 gas and 2725 cattle stunned with penetrating bolt concussion stunners.

For pigs, insufficient CO_2 exposure times and concentrations contributed to a small percentage of pigs being inadequately stunned in three abattoirs using dip-lift systems; rectifying these elements resulted in 100% adequate stunning during follow-up studies.

In the studies in cattle abattoirs, bulls were found to be significantly more likely to be inadequately stunned than other cattle types (heifers, cows and steers). Risk factors identified as contributing to a higher frequency of inadequate stunning included inappropriately designed loading and stun-box facilities, use of cartridge fired stunners that were too low in power for bulls, poor maintenance of stunners, inappropriate ammunition storage and the lack of neck restraints to prevent inaccurate shooting. Stun quality was optimised by the use of a Jarvis® pneumatic stunner in combination with neck restraints.

Based on the display of different symptoms, the brains of twelve cattle were macroscopically analysed in two of the abattoirs studied, with the level of brain haemorrhage and tissue damage corresponding to the adequacy of the stun.

The use of protocols such as those developed in this study can help standardise stun quality assessments and allow for benchmarking of stun quality at commercial slaughter. This can help to ensure that animal welfare standards are met. The conclusions of this thesis will be of interest and relevance to the commercial abattoirs and relevant authorities globally.

Keywords Stun, Welfare, Cattle, Pig, Abattoirs, Assessment, Stun quality

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Dedication

To the hairy, woolly and furry creatures with four legs that provide us with food. This is in tribute to the fine food and clothes you produce, the great lifestyles you create, the beautiful landscapes you generate when holistically and well managed, and the companionship you give to so many of us.

Unless someone like you cares a whole awful lot, nothing is going to get better. It′s not.

Dr Seuss

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Atkinson, S., Velarde, A., Llonch. & Algers, B. (2012). Assessing pig welfare at stunning in Swedish commercial abattoirs using CO_2 group-stun methods. *Animal Welfare* 21, 487–495

II Atkinson, S., Velarde, A. & Algers, B. (2013). Assessment of stun quality at commercial slaughter in cattle shot with captive bolt. *Animal Welfare* 22, 473–481

III Atkinson, S., Velarde, A. & Algers, B. Stun quality assessment and identification of risk factors which reduce welfare and stun quality in cattle shot with penetrating bolt stunners in Swedish abattoirs (manuscript submitted to *Animal Welfare*).

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The contribution of Sophie Atkinson to the papers included in this thesis was as follows:

I Involved in planning the methodology, practical data collection, analysis, and writing of manuscript. Regular input was given from co-authors and supervisors.

II Involved in planning the methodology, practical data collection, analysis, and writing of manuscript. Regular input was given from co-authors and supervisors.

III Involved in planning the methodology, practical data collection, analysis, and writing of manuscript. Regular input was given from main co-author, and editing assistance with main supervisor and co-supervisors.

Abbreviations

Ar	Argon
CB	Cartridge-fired penetrating captive bolt stunner
CO_2	Carbon dioxide
N_2	Nitrogen
O_2	Oxygen
PB	Pneumatically fired penetrating bolt stunner
SJV	Swedish Board of Agriculture
SQP	Stun Quality Protocol
SQR	Stun Quality Rate

1 Introduction

The lives of farmed cattle and pigs generally end at an abattoir where they are slaughtered and processed into meat products. To protect animal welfare and minimise suffering during this process, they must be humanely stunned (rendered unconscious) before sticking and slaughter procedures commence (EC 2009). Sticking is the process of severing the major blood vessels in the thoracic (chest) cavity to drain an animal of blood, cause death and for meat hygiene purposes. The time between stunning and sticking is referred to as the stun-to-stick interval, which should be as short as possible to minimise the risk of animals recovering before sticking and bleeding is complete (EC 2009).

After stunning, the animals are hoisted by one hind leg and suspended with the head hanging downwards for transfer to a sticking area. For hygiene purposes in cattle slaughtered by chest sticking, one knife is used to cut the skin away from brisket to chin to expose the major blood vessels before sticking occurs with a different knife. For pigs, the first step after completion of chest-sticking is immersion in scalding hot water. Sometimes sticking does not result in profuse and rapid blood loss, delaying onset of brain death (Anil & McKinistry, 1993; Gregory, 1999; Parotat *et al.*, 2015). Therefore, in order to safeguard animal welfare, the stun must be of a sufficient depth and duration to ensure there is no risk of recovery during such events. This can only be verified through adequate monitoring of stun depth (stun quality), and animals showing signs of recovery must be properly identified and re-stunned.

The most accurate method for assessing unconsciousness involves measuring neurological brain activity using electroencephalogram (EEG). Under practical conditions in the abattoir, however, stun quality is assessed by observing physically displayed behavioural signs (i.e. clinical symptoms) to help establish stun effectiveness. Although symptoms such as failure to collapse, rhythmic breathing, spontaneous blinking, corneal reflexes, vocalisations and eyeball movements should be absent after stunning (EFSA

2004), a degree of controversy still exists as to which signs are the most reliable when deciding stun quality levels under commercial abattoir conditions (Verhoeven *et al.*, 2014).

In Sweden, all major pig abattoirs use Butina® Group-wise high concentration carbon dioxide (CO_2) stun systems, with which approximately 2.5 million pigs are slaughtered annually (Official Statistics of Sweden, 2015). Surveys carried out in similar systems in Spain and Germany respectively revealed that pigs were inadequately stunned in the range of 42 to 60% (Velarde *et al.*, 2000; Dalmau *et al.*, 2009) and 6 to 66% (von Holleben *et al.*, 2002; Nowak *et al.*, 2007; Hartmann *et al.*, 2010). Furthermore, in abattoirs in Germany, Parotat *et al.* (2015) detected signs of life including pain reactions, corneal reflexes and righting reflexes) in 0.25% of 12,300 pigs just prior to entering the scalding tank in.

In total, 428,000 cattle are slaughtered annually in Sweden and the most common stun mechanism is induced concussion by a cartridge or pneumatically fired bolt into the forehead (EFSA 2013a). Death may result as a consequence of the physical damage to the brain but it is not a guaranteed outcome (Appelt & Sperry, 2007). Surveys conducted in commercial abattoirs in Europe report that 32% of cattle were inadequately stunned in Portugal (Gouveia *et al.*, 2009); 9% in UK (Gregory *et al.*, 2007); and 9.2% in Germany, where up to 35% cattle were also identified as inaccurately shot when no devices were used to hold the head (von Wenzlawowicz *et al.*, 2012). In the UK, Gregory *et al.* (2007) reported a higher prevalence of inadequate stunning in bulls compared with female cattle (16% versus 6%). Each of the above mentioned studies used a different method for assessing stun quality, making comparisons problematic. Although regular monitoring of stunning in abattoirs is mandatory in the EU with the objective of safeguarding and standardising animal welfare protection at stunning and killing (EC 2009), deficiencies exist as to how assessments should be conducted. Thresholds for what can be considered an acceptable frequency of inadequate stunning also remain ambiguous. The lack of standardised assessment methods also prohibits proper benchmarking of existing standards at slaughter. This prevents the definition of suitable minimum thresholds, potentially limiting improvement in current animal welfare standards. Considering that 248 million pigs and 25 million cattle are slaughtered annually within the EU (Eurostat 2015), having no set minimum thresholds for stun quality is a significant concern from an animal welfare perspective.

2 Background

This thesis includes a compilation in part from study results conducted in Swedish abattoirs between 2003 and 2010 in response to a demand from the Swedish Board of Agriculture (SJV) for external assessments of stun-to-stick interval times in relation to animal welfare and stun quality. Further studies were later initiated in co-operation with cattle abattoirs in Sweden and Norway in 2011 to compare different systems for cattle restraint and stunning (however, this data is not included in the thesis). As a result of the work conducted in the thesis, abattoirs in Sweden and Finland have made requests to the author as recently as 2016 for auditing of animal welfare at slaughter.

2.1 Historical Aspects Relevant to Stunning and Slaughter

The campaign by animal protectionists for humane animal handling before slaughter was said to have begun in Europe during the 1850s to 1860s (Metcalf, 1989). However, stunning animals before slaughter was not a legal requirement until 1902 in Finland, 1929 in Norway, 1937 in Sweden, 1947 in the UK, 1953 in Denmark (Metcalf, 1989), 1958 in the USA (USC 1958) and 1974 in the EU (EEC 1974). In Sweden, the first formal call for more-humane slaughter of animals was prompted by Per Elis Zimdahl, a member of Riksdagen´s Second Chamber, in a bill in 1885. This was a result of his concern about the primitive methods of slaughter practised by Swedish farmers at that time. Pigs, for example, were slaughtered by the so-called Danish–American method, involving hoisting the pig by the hind leg on a hook and gouging a hole into the heart to purge the body of blood (Metcalf, 1989).

In the nineteenth century, the French word abattoir was introduced to describe a somewhat state-regulated centralised building where animals were slaughtered for human consumption (Vialles, 1994; Otter, 2008). As human populations increased and technology developed, so did the production of animals for consumption. In the 1800s, canning techniques, cold storage and refrigeration systems improved meat preservation, and soon this formed part of the industrialisation of food (Blay-Palme, 2008).

In 1865, the Union Stock Yard in Chicago became the forefront of large-scale mechanisation of abattoirs. It was the largest of its kind in the world. Henry Ford developed the conveyor belt and assembly line system in the abattoir to increase production efficiency (Stull and Broadway, 2004). In 1906, Upton Sinclair wrote a book about his experiences working in the factory entitled "The Jungle". His vivid documentary-style description of the poor standards of animal and human welfare evoked public outcry at the time. This instigated an official investigation of slaughter plant practices by President Franklin Roosevelt, leading to the development of regulations in US public abattoirs for the first time. Sinclair describes the slaughter process of pigs as follows;

> In these chutes the stream of animals was continuous; it was quite uncanny to watch them, pressing on to their fate, all unsuspicious – a very river of death. So as the wheel turned, a hog was jerked off his feet. At the same instant the ear was assailed by a most terrifying shriek. The shriek was followed by another, louder and yet more agonizing, for once started upon that journey, the hog never came back; And meantime another was swung up, and then another, and another, until there was a double line of them, each dangling by a foot and kicking in frenzy and squealing. The uproar was appalling, perilous to the eardrums; one feared there was too much sound for the room to hold--that the walls must give way or the ceiling crack. There were high squeals and low squeals, grunts, and wails of agony; there would come a momentary lull, and then a fresh outburst, louder than ever, surging up to a deafening climax. It was too much for some of the visitors--the men would look at each other, laughing nervously, and the women would stand with hands clenched, and the blood rushing to their faces, and the tears starting in their eyes. Meantime, heedless of all these things, the men upon the floor were going about their work. Neither squeals of hogs nor tears of visitors made any difference to them; one by one they hooked up the hogs, and one by one with a swift stroke they slit their throats. There was a long line of hogs, with squeals and lifeblood ebbing away together; until at last each started again, and vanished with a splash into a huge vat of boiling water. It was all so very business-like that one watched it fascinated. It was pork making by machinery, pork making by applied mathematics (Sinclair, 1906, Chapter 3, 36–37)

Despite increased global production and transportation of meat in the beginning of the 20th century (Fitzgerald, 2010), techniques for killing cattle and pigs remained primitive. This included hitting animals on the head with a pole-axe or mallet (Figure 1), slitting the throat for exsanguination, or nape-stab, otherwise known as puntilla (MacLachlan, 2001). Puntilla, a procedure where a knife is stabbed into the space between the occiput and first cervical vertebrae, was a common alternative to pole-axing. It is still used in South America on cattle (Limon *et al.*, 2010). It was used on reindeer in the 1920s after pressure from animal welfare activists and state veterinarians encouraged it as an alternative to heart piercing or bleeding directly from the throat without pre-stunning (Reinert, 2012). However, by 2008 it was banned even for Sami reindeer herders, who now have to stun with bullet or captive bolt.

Founded in Britain in 1840, the Society for the Prevention of Cruelty to Animals worked to develop humane stun methods in food animals (Hughes, 2011). This society was probably among the first groups in Europe that attempted to improve stun quality in animals at slaughter. This began with a modification of the pole-axe by adding an extra sharp metal piece to enhance its ability to cut through and perforate the skull, causing a deeper and longer lasting stun in animals prior to slaughter. Development of stun methods that were more effective continued after the London Council made an official investigation of perforation marks on the hides of 100 cattle, where multiple blows were found to have occurred on 45 animals, with up to 10 blows registered on one animal. Similar assessments later revealed that bulls received an average of 2.5 blows, while cows averaged 1.3 (MacLachlan, 2005).

The first science-based slaughter method for cattle was said to have been patented in 1838 by James Carson in the UK. The method involved immobilisation with ropes, inserting a hollow tube connected to an airbag into the thoracic cavity, and then pumping air into the chest, causing the lungs to collapse and killing the animal by asphyxiation within one to four minutes (MacLachlan, 2005). As the method was not compatible with bleeding out (important for meat preservation), it was not widely adopted. Meanwhile, the concern for humane slaughter grew, and various inventions for stunning animals continued to be developed.

In 1872, in Paris, France, the so-called “Bruneau Mask” was developed. The mask comprised a steel plate fitted with a tube, into which a bolt was inserted. This mask would be strapped onto a blindfolded cow and the bolt struck with a mallet so it penetrated into the skull and brain. It was followed up with pithing – a process of inserting a wire or rod into the hole made from the stun to maximise brain and upper spinal cord damage. It was banned in 2001 throughout Europe for all livestock destined for human consumption due to the risk of contamination of the carcass with brain tissue, potentially spreading diseases such as Bovine Spongiform Encephalopathy (BSE) (HSA 2016). In 1874, Robert Baxter developed a stun mask variation, which was easier and faster to fit on cattle of different sizes. Instead of a solid bolt, it consisted of a sharp-edged hollow steel cylinder which could be spring-loaded to rebound back into place after it was struck. In 1895, the first mechanical stunner was invented, known as "Greener´ s Humane Cattle Killer" (MacLachlan, 2005). The bell-shaped end was placed flush on the forehead with one hand and the top struck with a mallet (using the other hand) to fire a pin that discharged a .30 calibre bullet through a rifle barrel into the animal's forehead.

In 1904, the first captive-bolt pistol device was developed after a competition in London was held to find the best stunning device for cattle. It was won by a German and became known as the "Behr Flash Cattle Killer" (Hughes, 2011). This stunner used gunpowder to propel the penetrating bolt several centimetres into the skull and brain, and overcame the hazards free bullets posed to human operators. By 1907, the concept of the captive bolt stunner had been improved with ideas developed by Christopher Cash. Cash took his ideas to J.G. Accles an Australian-born firearms manufacturer living in Birmingham England, and together they tested, modified and finally produced a new model stunner suitable for most livestock species began in 1913, known as the "Cash Captive Bolt Penetrating Stunner". Cash stunners still exist today and new models are continually being developed by the Accles and Shelvoke® Company.

Following this, in London, an eight-month study conducted in 1923 by the County at a public abattoir showed that stunning by pole-axe was not acceptable on animal welfare grounds, compared with captive-bolt stunning. This may have been one of the earliest official studies conducted on stun quality. Out of 300 cattle and 100 pigs killed, 655 blows with a pole-axe were required to induce unconsciousness. In contrast, 1255 cattle and pigs were stunned using a bolt pistol, with only 1259 shots required; the four additional shots being necessary because of faulty cartridges (Hughes, 2011). However, pigs and sheep were thought not to suffer as much as cattle, and were not required to be stunned before slaughter if captive bolt guns were unavailable (Hughes, 2011). Hence, the pole-axe was not completely phased out in the UK until 1947.

Experiments began in the UK with electrical stunning of animals in 1863 and carbon dioxide (CO_2) in 1866 (MacLachlan, 2005). Experiments using electricity began to be developed in Sweden in 1938 (Metcalf, 1989) but were initially developed in France and Germany in the late 1920s for stunning cattle, sheep, pigs, calves and horses. By the early 1930s, high-throughput electrical stunning systems were available in the US. By the 1950s, these were also widely established in Europe. Electrical stunning is still used commonly around the world for pigs, sheep, and poultry, although gas stunning with high concentrations of CO_2 in broilers and pigs is now common for the benefits it provides to meat quality (Channon *et al*., 2002; HSA 2015).

During the 1970s CO_2 stunning became more popular due to better, faster processing. However, the systems still created stress in the pigs due to the need to separate individuals from the group for loading into an individual chamber for gassing. Forceful techniques such as electric prodding were often necessary, increasing stress levels and meat quality problems. Research in Denmark found that pig stress was reduced and meat quality improved when mechanically operated gates separated and facilitated small groups of pigs into the stun chamber (Barton-Gade and Christensen, 2002). This type of stun system was then developed and introduced into mainstream slaughter by the early 1990s (Butina 2016). Figure 2 shows a section of a pamphlet describing one of the systems adopted in abattoirs worldwide in the 1970s. It states that pigs are gently put to sleep by inhalation of harmless carbon dioxide. While outside of the scope of discussion in this thesis, recent research indicates that pigs can be exposed to varying levels of pain and distress during inhalation, and the acceptability of this stun method on the grounds of animal welfare is being called into question (EFSA 2014; Llonch *et al*., 2012; Atkinson *et al*., 2015).

In 1970, air-powered (pneumatic) tools, such as the automatic nail gun used for carpentry, were developed. This technology was applied to captive bolt stun equipment, with the rapid repeatability of the firing system attractive to abattoirs wanting more efficient stunning and processing. However, early studies showed that air pressure was not always consistent and neither was the stun quality (Karczewski, 2011). In 1993, a US based company (Jarvis®), developed the pneumatically operated penetrating bolt stunner capable of air pressure adjustment for stunning different cattle sizes. This was the first really revolutionised stunner in nearly 80 years (Karczewski, 2011). There has been a steady development and implementation of automated, hydraulic and pneumatically operated animal handling, restraint and stunning devices in abattoirs since.

In the 1990s, Temple Grandin, a US researcher, brought measurement and accountability for animal welfare into US abattoirs. McDonald’s Co-operation in 1999 were one of the first meat purchasers to adopt auditing measures which required 95% of animals to be correctly stunned on the first attempt in a day audit (Grandin, 2010). This standard soon set a benchmark for other abattoirs in the US, many of which were challenged to improve stun quality scores or lose access to market opportunities. According to Karczewski (2011), it became no longer acceptable to effectively stun most of the time; it had to be done every time.

Figure 1. A photo of a steer about to be stunned with a mallet. Photo reproduced with kind permission from Animals Australia.

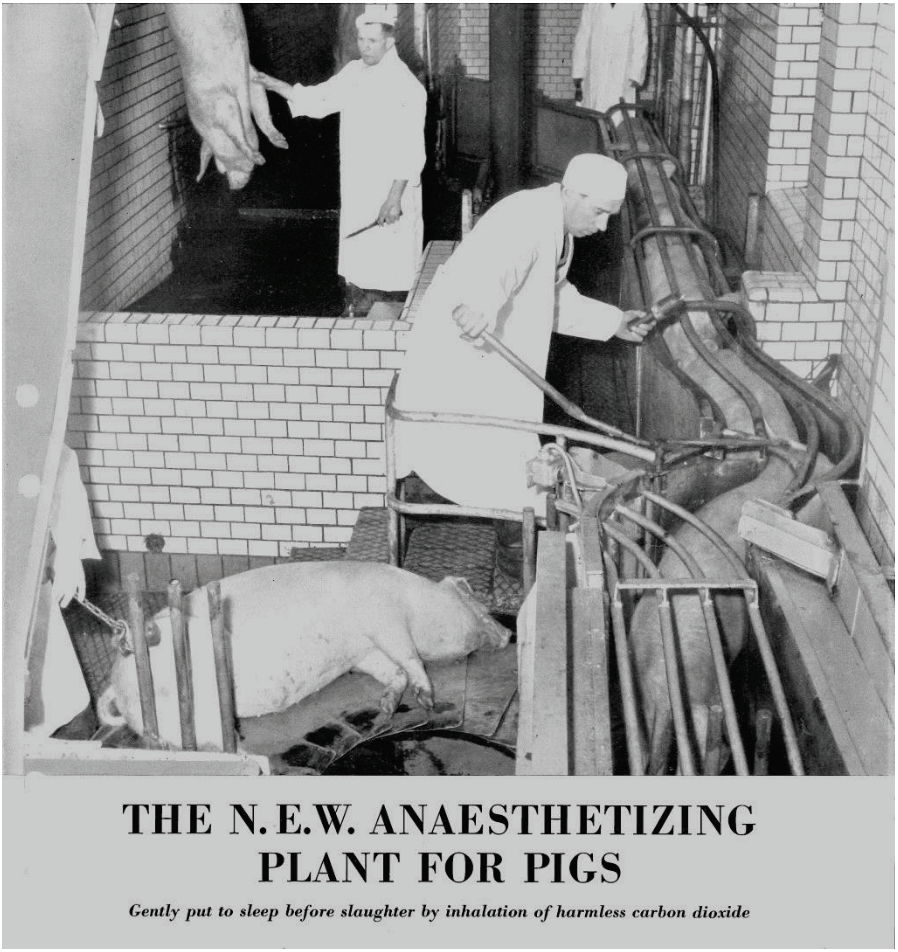


Figure 2. Pamphlet promoting CO_2 stunning of pigs, Werner, 1970 [Pamphlet] 2016-04-14.

2.2 Regulations Relevant to the Protection of Animal Welfare at Slaughter

The first international legal instruments to lay down ethical principles for the transport, farming and slaughtering of animals started in the 1960s through The Council of Europe (Food Chain Evaluation Consortium, 2007). Within the EU, directives relevant to member states regarding stunning animals before slaughter came into force in 1974. These were replaced in 1993 with Council Directive 93/119/EEC, which contained a much broader scope, covering different species and slaughter circumstances. It stipulated that the killing of domestic animals for human consumption should be performed to avoid unnecessary suffering of animals during slaughtering practices and that only proper approved methods could be used to stun and kill animals.

The slaughter industry changed in response to increasing demand for meat, with greater technological advancement leading to more efficient processing. Likewise, scientific knowledge relevant to animal welfare assessment improved, especially in the area relating to animal handling and stunning systems. In this context, the European Commission (EC) realised the need to update regulations relevant to animal welfare at the time of slaughter and killing. They requested the European Food and Safety Authority (EFSA) to develop recommendations on different stunning and killing methods, with relevant reports published in 2004, 2006 and 2013 (EFSA 2004; EFSA 2006; EFSA 2013a; EFSA 2013b). Furthermore, a study on the stunning and killing practices in abattoirs throughout Europe was carried out for the EC in 2007, with the general objective to identify methods for improving the protection of animal welfare at the time of slaughter, while ensuring a level playing field for all business operators. The subsequent report (Food Chain Evaluation Consortium, 2007) acknowledged specific problems such as the lack of consistent standardised methods for assessing stun quality, and the lack of clear responsibilities for operators in protecting animal welfare. It also acknowledged the importance of using scientifically developed animal-based indicators for assessing animal welfare at slaughter.

In 2010, Article 13 of the EU's Treaty of Lisbon recognised that all animals were sentient beings. This became significant for the progress of animal welfare, as full regard needed to be paid to animal welfare requirements in policy areas (EC 2010). In parallel, the World Organisation for Animal Health (OIE) adopted guidelines in 2013 for the slaughter of animals for human consumption (OIE 2013). An updated EU legislation protecting animal welfare at slaughter was developed in 2009, with official implementation beginning in 2013. It lay down provisions that animals must be spared any avoidable pain, distress or suffering during killing and related operations. This legislation is still current and some important provisions, particularly within Articles 5–7, 16 & 17, include:

- Increased food business operator responsibility for ensuring that mandatory standard operating procedures (SOPs) are in place. Operators are required to evaluate stun efficiency through assessments using animal-based indicators.
- The requirement that each slaughterhouse must appoint an Animal Welfare Officer (AWO) accountable for implementing animal welfare measures. The AWO needs to be specifically trained and qualified to design monitoring procedures at stunning, including implementation of corrective procedures when there is noncompliance.
- The requirement for manufacturers to provide instructions on the use and servicing of stunning equipment.
- The requirement for staff who handle animals to possess a certificate of competence regarding the welfare aspects of their tasks. This certification should be subject to independent examination by competent authorities.

In Sweden, legislation relevant to slaughter is covered under the Animal Welfare Act (SFS 1988:532, Saknr L1), Animal Welfare Ordinance (SFS 1988:53, Saknr L2) and the Swedish Board of Agriculture Regulations and recommendations on the Welfare of Animals at Slaughter and Killing (SJVFS 2012:77, Saknr L22), which include the animal welfare ordinances that require stunning before slaughter, without exemptions for religious slaughter.

Stunning methods permitted for pigs and cattle include free bullet or penetrating captive bolt. Pigs can also be stunned by head-only electrical means, where tongs with electrodes are positioned on either side of the head and an electrical current 50–200 Hz or 1.3 Ampere is passed through the brain. Alternatively, pigs can be gassed by immersion into a high CO_2 gradient of at least 90% CO_2 concentration (SJVFS 2012). Most Swedish standard operating procedures recommend sticking for blood exsanguination within 60 seconds after stunning to reduce recovery risk. Either carotid arteries (neck cut) or the blood vessel from which they arise (chest sticking) must be severed This is important, as scientific research shows this procedure is necessary to achieve a rapid bleed out, thereby minimising the risk of recovery and ensuring death occurs as quickly as possible. If sticking is improperly conducted, slow bleeding can result in animals regaining consciousness and suffering pain and distress if not identified and re-stunned. In Sweden, all cattle and pigs are bled with chest-sticking procedures. Neck-cutting is permissible in cattle, but it is rarely conducted in commercial abattoirs (Food Chain Evaluation Consortium, 2007).

The red meat industry in the EU is one of the world's largest, and livestock products make up approximately one quarter of the total value of its agricultural production (Food Chain Evaluation Consortium, 2007). The industry is characterised by a complex network of farmers, cooperatives, animal transport companies, abattoirs, processing and rendering plants, and retailers involved in distribution and marketing. This creates challenging circumstances in which to apply consistent animal handling and welfare standards, especially as abattoirs vary considerably in design and may not always have been built to take account of the welfare needs of the animals.

2.3 Pre-slaughter Handling, Stunning and Meat Quality

Stress induced in animals prior to stunning can affect meat quality (e.g. Velarde *et al*., 2000; Grandin, 2001). Therefore, abattoirs will generally choose handling and stun systems that reduce stress and optimise meat quality. Immediate pre-slaughter handling stress can particularly cause detrimental meat quality problems in pigs, such as pale, soft and exudative meat, known as PSE (Stoier *et al*., 2000). It occurs when pigs are exposed to short term stress to the extent that stress hormones accelerate breakdown of glycogen into glucose in the muscles. After stunning, the high glucose level converts to lactic acid, resulting in more rapid acidification of the meat, causing lower than optimal pH. PSE meat is unattractive to consumers, has a low water-holding capacity causing it to dry out during cooking, and is prone to faster bacterial growth, reducing preservation qualities. Blood splash, mainly associated with electrical stunning in pigs, is another meat quality defect affected by choice of handling and stun system. When muscles are overly exerted, which occurs through epileptic seizures during electrical stunning, small blood capillaries rupture as a result of high blood pressure, and leaking blood causes blood spots to occur over muscle surfaces. Blood splash is also unattractive to consumers and needs to be trimmed from the carcass.

A meat quality defect called dark, firm, dry (DFD) can occur when pigs or cattle are exposed to stress or physical activity over many hours, such as during long transport times, episodes of rough handling over a long duration, or when animals fight within a group for many hours. This is caused by an excessively high depletion of glycogen in the muscles at slaughter, resulting in the meat having a higher than optimal pH level (>5.8). The meat becomes discoloured and unattractive to consumers, and, like meat affected by PSE, is prone to bacterial growth and spoilage.

Bruising can also be a meat quality defect associated with pre-slaughter incidents such as falling, colliding with other animals or the restraint and handling facilities, or being hit by handlers. Bruising appears as a distinct discolouration on the carcass's surface. It is caused by an extravascular collection of blood following trauma to the body by a blow or collision with an object (Romero *et al*., 2013). As bruises create discolouration and are prone to microorganism growth, they need to be trimmed from the carcass. Inappropriate handling of an animal at any point from loading at the farm to stunning can lead to bruising (Nanni Costa *et al*., 2006). The time of bruising damage can be estimated by examination of its colour. For example, bright red bruises are likely to have been inflicted at the abattoir within a few hours prior to slaughter, and dark blue bruises are likely to be at least 24 hours old, indicating they were inflicted at the farm (Strappini *et al*., 2009). Strappini and co-workers (2013) reported that a high level of red bruising was found in abattoirs observed as using handling equipment incorrectly or handling the animals roughly just prior to stunning. The above-mentioned meat quality issues provide incentives for researchers and businesses to develop pre-slaughter handling and stun systems that minimise stress. However, an abattoir's choice of stun system will also be influenced by human safety issues, efficiency in which animals can be driven up to the stunning point and stunned, and relevant building costs.

2.4 CO_2 Group Gas Stunning in Pigs

Studies have confirmed that for pigs, CO_2 stunning results in substantially less blood splash and PSE than with electrical stunning (Velarde *et al*., 2000, Channon *et al*., 2003, Terlouw *et al*., 2008). Furthermore, a major animal welfare advantage of CO_2 stunning is that pigs can be handled and stunned in groups rather than individually. This reduces stresses associated with isolation (Christensen & Barton-Gade, 1997). CO_2 systems can also be operated with hydraulic moving gates that gradually separate pigs into small groups, driving the automatically into the stun system eliminating stresses associated with human handling. The stun-box descends like an elevator into a shaft or pit where the CO_2 gradient increases. There are several different models which vary in size; however, the basic technical design remains similar. Dip-lift models have only one box in the system, and, depending on the box size, between two and six pigs can be stunned at a time. The box descends into a pit four metres deep, pausing at the bottom where CO_2 concentrations are at least 90%, before ascending and tipping the pigs onto a table where shackling takes place.

The paternoster designs (Figure 3) have between three and seven boxes in the system, which can take four to eight pigs depending on box size. The boxes descend into the pit that is six to eight metres deep (depending on the size and number of cages within the system). The boxes are stopped to pause at levels where CO_2 concentrations are at least 80%, and then at the bottom where concentrations are at least 90%. The stunned pigs are delivered onto a moving conveyer belt and shackled and transferred one after the other a few metres to an area where sticking takes place. When pigs are exposed to fresh air, they may recover. Therefore the CO_2 concentration level and time pigs are exposed to CO_2 within the machine, needs to be at calculated levels to ensure adequate stunning remains until bleeding is complete in all pigs of a group. As pigs are stuck in succession when stunned in a group, the last pig will have the longest stun-to-stick time. With increasing stun-group size, the time taken to stick the last pigs in the group increases, which is a potential risk factor to maintaining unconsciousness throughout the slaughter process.

High CO_2 (>80%) exposure causes pigs to lose consciousness by reducing the blood pH so that acidification of brain cells and cerebrospinal fluid occurs, depressing brain activity (Rodríguez *et al*., 2008; Gerritzen, *et al*., 2006). When CO_2 is inhaled, even at concentrations as low as 12%, CO_2 replaces oxygen (O_2) from the inhaled air, and this depresses the central nervous system. At high CO_2 levels, hypercapnia and hypoxia occur and behavioural signs indicate breathlessness and excitation (EFSA 2007). As a result of individual biological variation, some pigs may regain consciousness while others do not, even if stunned in the same group (Holst, 2001). Sticking does not always result in rapid and profuse blood loss. If slow bleeding occurs, delaying death, it is imperative for animal welfare that unconsciousness is closely monitored and pigs re-stunned if signs of recovery appear. This is especially crucial in abattoirs where pigs are hoisted upside down and conveyed to a scalding tank for de-hairing within minutes after sticking. Research also indicates that cardiac arrest does not occur immediately after sticking, but the stun-to-stick interval does affect when cardiac arrest occurs, which takes up to four minutes as reported by Jerlström (2014), or up to ten minutes as reported by Vimini *et al*. (1983).

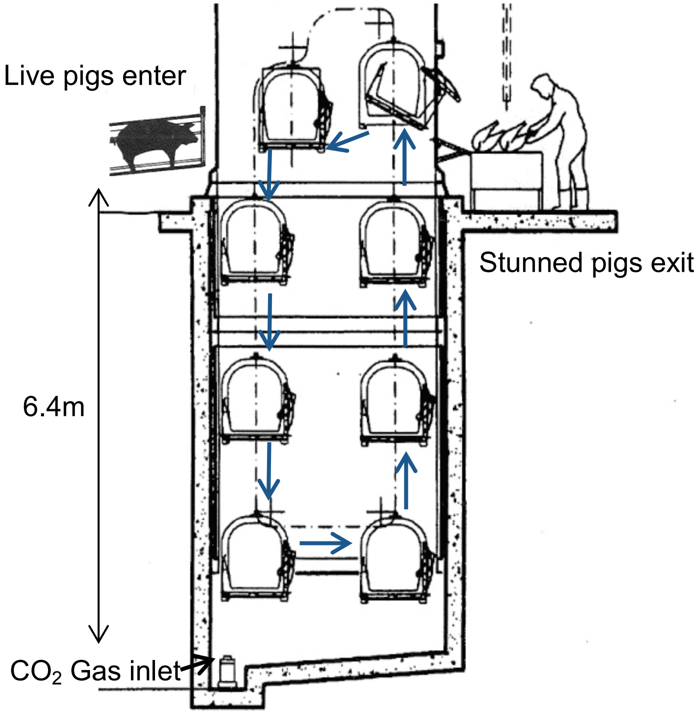


Figure 3. Diagram of a Butina Paternoster CO_2 stun system for pigs (each box can take up to 8 pigs).

2.5 Penetrating Bolt Concussion Stunning in Cattle

There are two types of penetrating captive bolt stunners commonly used for stunning cattle, differentiated by the firing mechanism. The cartridge bolt stunner (CB) is a handheld device loaded with gunpowder-filled blank cartridges (Figure 4). When fired, a firing pin ignites the gunpowder within the cartridge and explosive gases push out a sharp-rimmed metal bolt from the gun muzzle, with the bolt recoiling automatically back into the barrel within a second. Generally there are three cartridge strengths available, with higher strengths (described in milligrams or grains of gunpowder) recommended for larger animal types. These strengths are generally colour coded according to three ranges of live weight: light, medium and heavy. The pneumatic bolt stunner (PB) works essentially in the same manner except that it uses compressed air to propel a retractable bolt.

Pneumatic stunners are large and heavy to operate, and require the support of a wire suspension system (Figure 5). To facilitate correct shooting position, the animal's head is normally held in a neck restraint with hydraulically operated bars that close on either side to prevent animal movement. According to Shaw (2002), the extent of brain damage during concussion stunning is affected by:

- Skull shape and size.
- Density and mass of neural tissue.
- Extent and direction of the concussive blow.
- Mobility of the head and neck.
- Thickness of the scalp and skull.

It is possible to consistently stun animals under commercial abattoir conditions so that 100% are rendered insensible on the first shot (Grandin, 1998; Gregory & Shaw, 2000; Grandin, 2012). Sticking should also be carried out quickly as heart function can continue for up to six minutes after stunning (Jerlström, 2014). By law the animal should be brain-dead as a result of the stun or bleeding, before carcass dressing procedures commence (EC 2009).

Figure 4. Two examples of cattle that have been stunned with cartridge fired (CB) penetrating bolt concussion stunners (photo on left taken by Ulf Nylén and photo on right by Sophie Atkinson).

Figure 5. Two examples of cattle that have been stunned with pneumatically fired (PB) penetrating bolt concussion stunners (photo on left reproduced with permission from Richard Dunn -Jarvis®, and photo on right by Sophie Atkinson).

2.6 Alternative Stun Methods

Research indicates that stunning pigs in high concentrations of CO_2, causes an overall experience that is painful and stressful, as the gas interacts with respiratory and ocular membranes forming carbolic acid, causing severe irritation of the eyes, nasal mucosa and lungs (Raj & Gregory 1996). Studies where pigs were filmed during commercial CO_2 stunning indicated that pigs showed distress and escape behaviours of varying durations and intensities (Llonch *et al*., 2012; Atkinson *et al*., 2015). Despite EU regulations stipulating that abattoirs must take measures to avoid pain and minimise distress during the killing and slaughter process (Chapter II, Article 3 of the EC 2009), the EU, meat industry and government authorities accept CO_2 stunning. Research suggests that a less aversive anaesthetic effect can be achieved during induction with gas mixes containing nitrogen (N_2) or argon (Ar) (Raj, 1996; Raj & Gregory, 1999). N_2 and Ar are odourless, tasteless, inert, and inflammable gasses. However, they are not as heavy as CO_2, and N2 is lighter than air, so these gases are more difficult to contain inside a pit. Behaviour tests also show that discomfort in pigs is reduced in an anoxic atmosphere (where oxygen levels remain below 2%), with 80% N2 and 20% CO_2 (Llonch *et al*., 2012). Compared with conventional CO_2 stun systems, such gas mixes require longer exposure times to maintain brain inactivity until sticking and bleeding is complete. This would slow processing speeds in high throughput abattoirs and is a possible reason for the lack of initiative for commercial development, despite potential benefits for improving animal welfare (AVMA 2013).

Other methods tested for stunning pigs include the use of a high pressure water jet, such as those used by the building industry to cut and drill solid materials. The jet can cause immediate unconsciousness through brain destruction, as the jet enters the cranial cavity when applied frontally on the head. However, as with penetrating captive bolt stun methods, the jet can cause excessive convulsions leading to meat quality issues such as blood splash (Lambooij & Schatzmann, 1994; EFSA 2007). In some Jewish and Muslim faiths, pre-slaughter stunning is not an acceptable practice before sticking because the faith cannot accept the possibility of death occurring before exsanguination. As a result, there has been an interest to develop stun methods which guarantee that the animal is alive during sticking but is unconscious. Such methods include electromagnetic induction of the brain or microwave energy (Small *et al*., 2013). High-powered microwave energy can cause a rapid rise in temperature of the brain leading to unconsciousness within three seconds. After a ten-second application time, unconsciousness can remain for at least 78 seconds, allowing time for sticking before consciousness is regained. Despite promising results, further research and testing is required before solid conclusions on animal welfare aspects relevant to microwave stunning can be made (Rault *et al*., 2014).

2.7 Pain, insensibility and unconsciousness in the context of stunning and slaughter

> There is nothing in human experience, more central than our capacity to feel, and no aspect of this so crucial as our capacity to suffer, perhaps more particularly to suffer from extremes of physical pain. Although by definition the unconscious patient cannot tell you that they perceive pain, available data suggest that they may (McQuillen, 1991, p. 373-374)

Although animals are unable to communicate their experience of pain, the suffering caused by their pain is no less real (Gibson, 2009). The function of pre-slaughter stunning is to switch off all brain functions and communication pathways so there is no capacity for the animal to be aware, feel pain, fear, anxiety, confusion or distress, until brain death occurs due to completed exsanguination. The successful shutdown of these emotions was first defined by Blackmore & Newhook (1981) as the animal becoming "insensible". This term is now used globally to describe the desired effect of a stun in legislative texts and journals.

Understanding the workings of the brain is particularly important when considering penetrative bolt stunning, and it is helpful to consider three main areas and their basic functions: the forebrain, midbrain and brainstem (Figure 6). The brainstem is considered the most important for maintaining life as it regulates all other brain functions (Shaw, 2002; Verhoeven *et al*., 2015), while sections such as the cerebral hemisphere can be removed without disturbing conscious awareness (Gregory & Shaw, 2000). Terlouw *et al.* (2016a) reviewed literature relevant to defining consciousness, unconsciousness and brain function in the context of stunning and slaughter in livestock. While the anatomical basis of the conscious state is not well understood, to a limited extent it depends on feedback loops of neural activity between the brainstem and cerebral cortex (Finnie *et al*., 2002).

The assessment of the state of unconsciousness and insensibility after stunning can theoretically be obtained by observing behavioural signs – such as the presence or absence of voluntary motor movements and reflex reactions –

and by recording brain activity using an electroencephalogram (EEG) (Knudsen, 2005). The EEG is a non-invasive monitor that measures electrical activity in the brain. However, it involves complex methods for interpretation of raw data, which can be improved by the use of mathematical and statistical techniques such as the bispectral index (Kaul & Bharti, 2002). By exposing a patient to stimuli such as sound, light or pain, and observing time-referenced changes in EEG activity (referred to as testing for evoked potentials), a more in-depth assessment of the different brain areas in function can be concluded (Scneider & Sebel, 1997; Agarwal & Griffiths, 2004). For example, if there are EEG activity changes when the patient is exposed to bright light, this indicates an intact brainstem. In some cases in humans, EEG measurements have shown deep unconsciousness, but patients later reported that during the surgery they were aware (i.e. they could hear and see but not talk, react or feel the surgery) and that it was a traumatic experience (Whelan & Flecknell, 1992). In paediatric practice, an incidence of 0.8% was reported in a study of 1250 children aged 5–12 years (Bruhn *et al.,* 2006). Therefore even in human medicine it is well known that measuring depth of unconsciousness represents one of the most controversial and subjective aspects of medicine (Kaul & Bharti 2002).

Important earlier studies using EEG to assess the effectiveness of certain stun methods in livestock were conducted by the following authors: Lambooij & Spanjaard (1981), Newhook & Blackmore (1982), Blackmore & Newhook (1982), Gregory and Wotton (1986), Daly *et al.* (1986), Daly *et al*. (1988), Tidswell *et al.* (1987) and Daly & Whittington (1989). Their work contributed to development of observable criteria in abattoirs during commercial slaughter to determine effective stunning. For example, Lambooij *et al.* (1981) found that when corneal reflexes were present (elicited when the animal closed the eyelid in response to the cornea being lightly touched), EEG patterns indicated an incomplete loss of consciousness and hence an ineffective captive bolt stun.

European scientists came together to discuss and review literature regarding scientific research relevant to brain function and EEG work in livestock after stunning, and have produced comprehensive publications which detail and discuss practical signs that can be checked at slaughter (EFSA 2004; EFSA 2006; EFSA 2013a and EFSA 2013b; von Holleben *et al.,* 2010). Further reviews have recently been conducted by Verhoeven *et al*. (2015) and Terlouw *et al.* (2016a and b). In cattle, for example, the following criteria are generally accepted as indicating deep (adequate) stunning with penetrating captive bolt stunners:

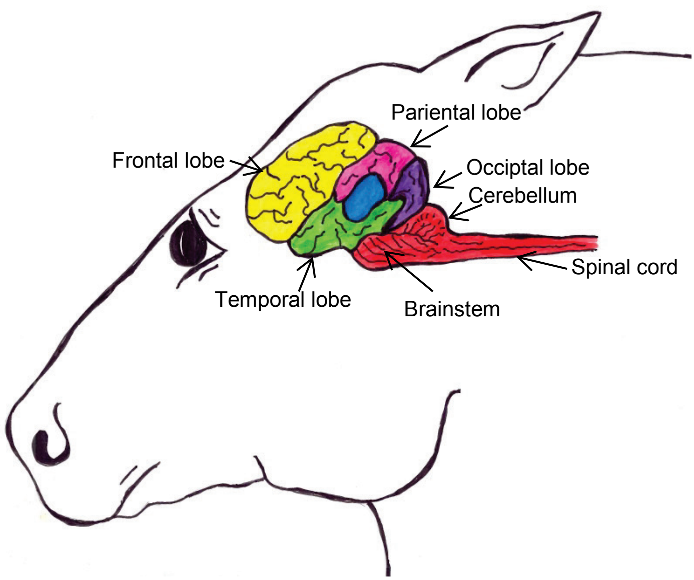
- Immediate collapse.
- Brief tetanic spasms that might be followed by uncoordinated hind limb movements.
- Immediate and sustained cessation of rhythmic respiration.
- Absence of coordinated attempts to rise.
- Absence of vocalisation.
- Glazed "glassy" appearance of the eyes.
- Absence of eyeball movement.
- Absence of eye reflexes (no response to touching of the eyelid or cornea area of the eye, i.e. no corneal or palpebral reflex).
- Absence of spontaneous blinking.

In pigs, the same criteria apply to indicate deep stunning after CO_2 gassing; however, there should be an absence of spasms and uncoordinated hind limb movements.

Recent studies using EEG analysis at stunning by Lambooij *et al.* (2012) and Verhoeven *et al*. (2015), showed that captive bolt stunning caused immediate unconsciousness (within one second) as displayed by EEG measurements. Rault *et al.* (2014) investigated EEG responses in adult cattle stunned with microwave energy and Gibson *et al.* (2009a, b) in calves shot with non-penetrating captive bolt stunners (used for religious slaughter purposes, but not permitted in Sweden). Rodriguez *et al*. (2008) and Llonch *et al*. (2013) have conducted studies using EEG to assess level of consciousness in pigs. In human medicine, several authors have emphasised that most methods of EEG recording are extremely sensitive and easily affected by environmental factors that can compromise the validity of the recordings (Thomsen & Prior, 1996; Knudsen, 2005; Alkire *et al.,* 2008). In an abattoir environment, assessing EEG activity in livestock after stunning is likely to be even more challenging. When assessing stun quality under practical slaughter conditions, a further complication, is that some signs such as gasping and eye reflexes can be present in either conscious or subconscious states, and alternatively even when recovery or death is imminent.

In 1959 in France, a new concept emerged in medical science, known as “brain death”. The concept of brain death took a decade to achieve medical recognition, which occurred following the publication of a report by the Ad Hoc Committee of the Harvard Medical School, known as the “Harvard criteria”. However, two more decades were to pass before the diagnosis was legally recognised in many countries (Knudsen, 2005). The European Regulation requires that carcass dressing or scalding can only be carried out only after verification of the absence of signs of life (EC 2009). Under practical conditions in the abattoir environment, this would take place after bleeding and after it has been verified that the animal is not breathing and shows no brainstem reflexes (Terlouw *et al.*, 2016b). As Zulkifli *et al.* (2014) confirmed through EEG studies, effective stunning may only result in a period of insensibility, not death. Therefore, to ensure animal welfare, successful and complete bleeding should occur before carcass dressing procedures begin.

There is still substantial debate about which indicators most adequately assess the unconscious state under practical conditions (EFSA, 2013b; Verhoeven *et al.*, 2015; Terlouw *et al.*, 2016b). Most of the studies on stunning and its effect on EEG activity have also used small sample sizes (Verhoeven *et al.*, 2015), which also reduces statistical robustness and the scope for individual variability. There are also few published studies correlating EEG recordings with physical signs or reflexes that can be determined under slaughter conditions. Nevertheless, clinical signs are still considered the most important method for assessing level of consciousness in both humans and animals (Thomsen & Prior, 1996; Agarwal & Griffiths, 2004; Hadzidiakos *et al.,* 2006; EFSA 2013a, EFSA 2013b).



Frontal lobe - reasoning, planning, movement, emotions
Temporal lobe – perception, memory
Pariental lobe - movement, orientation, perception of stimuli
Occiptal lobe - visual processing
Cerebellum- coordination of movement, posture, and balance
Brain stem- basic vital life functions such as breathing, heartbeat, and blood pressure
Spinal cord - structure between the body and the brain and part of central nervous system along with the brain itself

Figure 6. Diagrammatic representation and description of the bovine brain (illustration by Sophie Atkinson).

3 Aims

The overall aim of this thesis was to develop a protocol to assess stun quality standards in Swedish pig and cattle abattoirs in relation to animal welfare. It was hypothesised that adequate stunning would consistently occur with little variation between abattoirs.

The more specific aims included:

- To design a standardised protocol to sufficiently describe physical symptoms displayed after stunning, and rate these symptoms to help determine when adequate or inadequate stunning occurs and when re-stunning is necessary.

- To identify risk factors in the practical setting of the abattoir environment that may contribute to reducing stun quality and animal welfare.

4 Materials & Methods

This section gives an overview of the materials and methods used in the studies that are the basis of this thesis. In each paper (I–III) the full details of the methods are included.

4.1 Data collection and study subjects

In paper I, only pigs were assessed, while in the second and third papers only cattle were assessed. In total, 9,520 pigs (with a slaughter live weight average of 85 [± 20] kg) of halothane negative "PigHam" strains (Hampshire sire lines with Landrace × Yorkshire sows) were assessed during routine stunning. Ten visits in eight abattoirs were conducted. Each abattoir was numbered from one to eight from fastest to slowest processing rate. Abattoirs 1–5 and 8 were assessed once and abattoirs 6 and 7 twice (after adjustments in CO_2 stunning parameters). Two full days were spent in abattoirs 6 to 8 (processing 200–250 pigs per day) and one full day in abattoirs 1 to 5 (processing 1,500–3,000 pigs per day).

In paper II, an abattoir processing an average of 200 cattle daily was assessed during routine stunning over five consecutive days. A total of 998 cattle consisting of 885 bulls and 413 other cattle (cows, heifers, and steers) were observed from stunning until sticking.

In paper III, six major abattoirs (where over 70% of the cattle in Sweden are slaughtered) were assessed over a minimum of a full day. At least 200 cattle were observed per assessment, and two abattoirs were visited twice. A total of 2062 cattle consisting of 859 bulls and 1203 other cattle were observed between stunning and sticking. The study aimed to assess the stun quality in at least 50 randomly delivered bulls (uncastrated males ≥ 12 months of age) and 100 other cattle per abattoir. Each abattoir was identified numerically from 1 to 6 and the type of bolt-firing system denoted as CB (cartridge fired) or PB (pneumatically fired). If firearms using bullets were used, they were identified as B (bullet fired). Abattoirs 1 and 2 were visited twice within the same year, which was further categorised as visit (a) and (b). The purpose of the follow-up visits was to make a second assessment and collect heads for macroscopic skull and brain examination of cattle that showed adequate or inadequate stun symptoms after the first shot with CB or PB stunners.

4.2 Stun quality assessment in pigs (Paper I)

Each stun system was described and identified as to its type and the following details were recorded:

- System – dip-lift or paternoster.
- Loading method (i.e. mechanical push gates or manual gates with a person facilitating pigs into the stunner.
- Number of stun-boxes in the stun machine.
- Number of pigs loaded in each stun- box.
- The time pigs were inside the stunner and exposed to CO_2 gas.
- The pre-set gas concentrations in the stun machine.

Pigs were continually observed for physical symptoms that could indicate consciousness or a risk that recovery was imminent. When pigs were in a state of whole body relaxation, and there was no evidence of rhythmic breathing, righting reflex, vocalisations, convulsions, blinking, pain or eye responses to stimulation, pigs were considered in a state of deep anaesthesia and adequately stunned. Pigs that showed symptoms outside of the deep stun criteria were more closely examined and the eyes tested by carefully touching the corneal area with a pen tip angled at approximately 45°. If the pig blinked in response it was noted as a corneal reflex. Pain response was tested by pricking the inner snout of the pig with the sharp point of a metal pencil casing and withdrawal response was noted as pain reflex. Every pig in the group was routinely tested for reflexes.

To assist with the practical assessments, a stun quality protocol (SQP) was designed to identify and categorise symptoms signifying an estimated risk level (RL) for recovery of consciousness and concern for inferior welfare from highest (4) to lowest (1) Any pigs displaying one of the symptoms rated RL 3 or 4 were considered inadequately stunned. Pigs showing a single display of RL2 symptoms were closely examined and monitored, and inadequate stunning was only registered if other RL2 symptoms were observed (Table 1). The percentage and frequency of symptoms shown individually or in combination were evaluated for both individual abattoirs and as a total of pooled data. Group sizes in the stun-boxes were recorded by counting the number in each group as they came out of the stun-box. The stun-to-stick interval was timed for every pig in the group using a stopwatch. The time when the “end” of the stun occurred for all pigs in a group began when the stun-box stopped just before the gate opened to release pigs out of the stunner. Sticking was considered to be the point at which the knife was pushed into the chest, signalling the end of the stun-to-stick interval. Stun-to-stick intervals were recorded sequentially for each pig in the group. Any incidents or stops or causes for delays in sticking were recorded.

Table 1. Stun quality protocol (SQP) describing symptoms of inadequate stunning rated for risk for recovery level and inferior animal welfare from 4 (highest) to 1 (lowest).

Risk	Interpretation	Symptom	Definition
4	Inadequate stunning and the highest risk to animal welfare due to symptoms signifying consciousness	• Righting Reflex (RR)	Raising of the head or arching of back in animal's attempt to right itself or recover normal body position
		• Pain Reflex (PR)	Any response to a painful stimulus such as a severe prick on the nose with a sharp instrument
		• Blinking (B)	Animal blinks its eye on its own without stimulation
		• Vocalisation (V)	When animal squeals or groans using vocal cords not associated with involuntary sounds during the dying process
3	Inadequate stunning at a lower risk level due to symptoms signifying a recovery risk rather than specific signs of consciousness	• Nystagmus (N)	Rapid movements (twitching) of the eyeball from side-to-side
		• Corneal Reflex (CR)	Animal blinks in response to careful touching of the cornea
		• Rhythmic Breathing (RB)	Rhythmic air inhalation seen in the form of regular expansion/contraction of chest or flank area or feeling rhythmic air exhalations on the back of the hand
2	If shown independently indicates a low risk and not considered as inadequate stunning but if seen in combination with other symptoms in this rating, re-stun	• Convulsion (C)	Involuntary, violent seizure-like muscle contractions (excluding slight muscle twitches)
		• Eyeball Rotation (ER)	The eyeball is rotated in a fixed position so the sclera is predominantly seen and little or no iris remaining 40 s after stunning
		• Regular Kicking (RK)	Multiple movements of the limbs
		• Regular Gasping (RG)	Opening of the mouth with the sound or appearance of short gasps of air while flexing the head forwards occurring more than 3 times within 10 s intervals
1	If shown independently indicates a low risk of return to consciousness and not considered as inadequate stunning but pigs should be monitored	• Irregular Gasping (IR)	Occasional opening of the mouth while flexing the head forwards with the sound or appearance of short gasps of air intake at sporadic intervals
		• Irregular Kicking (IK)	Occasional movements of the limbs

4.3 Stun quality assessments in cattle (papers II and III)

To analyse the relationship between breed, sex and stun quality, the category of the animal was recorded, such as mature bulls over 12 months of age or over two years (i.e. cull breeding bulls), heifers, cows, or steers. Cows, heifers and steers were further categorised as "other" cattle and bulls as a separate class. Each animal was also categorised into breed types such as pure dairy breeds (Swedish Red and Holstein) and pure beef breeds (Charolais, Limousin, Simmental and Hereford) and crosses (mixes of any of the above or breeds not clearly identifiable).

The stun-to-stick interval was recorded using a stopwatch, timed from when the shot was heard to when the knife was inserted into the chest. Stops or causes for delays during this phase were also recorded. Each animal was evaluated from stunning until sticking and stun quality rated from 0 to 3 to specify a depth of stun and risk for inferior animal welfare, noted as the Stun Quality Rate (SQR). Animals with SQR0 showed signs of a deep stun with no risk of recovery. Animals with SQR1 indicated a stun depth that required close monitoring and testing for reflexes, which if negative, meant adequate stunning. SQR2 indicated a depth of stun that presented a moderate risk that the animal could recover. SQR3 indicated that the animal was in the process of recovery, or it was imminent, and represented the highest risk for inferior animal welfare. To protect animal welfare and reduce recovery risk, cattle showing either SQR2 or 3 symptoms were considered in need of re-stunning and classified as inadequately stunned (Table 2). The number of times each animal was shot was also registered.

To assist with identification of potential risk factors that contribute to reducing stun quality, a description of abattoir facilities and routine management relevant to stunning was made. This included noting details such as:

- Describing the design of the stun-box.
- Describing the method of loading i.e. manual, or automatic push gate.
- What tools were used by handlers to facilitate animal movement i.e. electrical prodders.
- Describing the restraint type used i.e. neck gate, or full head hold with chin lift.
- Describing floor surfaces in loading lane and stun-box.
- Describing where the shooter stood at animal at stunning.
- Describing the stun gun i.e. name, brand, power loading used for different cattle classes, manufacturer specifications, cleaning schedule, and information of the number and type of backup stunners available.

Table 2. Stun quality protocol describing symptoms from 0 (no recovery risk) to 3 (highest recovery risk).

Stun quality /inferior animal welfare level	Symptoms
SQS 3 HIGH RISK	
Inadequate stunning at the highest risk of recovery and compromised animal welfare. Re-stunning necessary to prevent suffering	• Failure to drop • Groaning/vocalisation • Respirations • Corneal/palpebral reflex • Spontaneous blinking • Pain reflex • Attempt to regain posture or raising of the head
SQS 2 MODERATE RISK	
Inadequate stunning, but with a moderate recovery risk and compromised animal welfare. Re-stunning necessary to eliminate recovery risk	• Nystagmus • Full rotation of eye (mostly sclera seen) • Gasping
SQS 1 UNKNOWN RISK	
If shown animal is closely monitored and tested for reflexes	• Tongue up in mouth at sticking • Excessive struggling at sticking • Ears up at sticking Partial eye rotation eyeball (iris is partially seen)
SQS 0 NO RISK	
Animal is deeply stunned and there is no concern of recovery or reduced animal welfare	• Immediate collapse • Tonic and clonic phase of spasms • Involuntary limb movements • Fixed eyeballs • Dilated pupils • Glassy appearance over iris and pupil

4.3.1 Shot accuracy paper II

In paper II the skull of each animal assessed at stunning was inspected after decapitation and skinning, and the shot location recorded using the diagram depicted in Figure 7. If the shot hole was registered in the "A" area, it was considered an "accurate" shot, i.e. located within a 2-cm radius of the intersection of two diagonals drawn between the base of the horns or upper edge of the ears contralateral to the opposite eyes. Shot holes located more than 2 cm outside of this area were considered as "inaccurate" and the relevant location registered. Using a body identification number for each animal, the breed, cattle class, stun quality, number of times shot, shot location on the skull, and numerical identification of the shooter were correlated.

4.3.2 Macroscopic brain damage assessments paper III

After assessing 107 cattle shot with CB in one of the abattoirs, 12 heads were selected for macroscopic brain damage analysis based on the display of SQR 0, 1, 2 or 3 symptoms. After assessing 314 cattle shot with PB in another abattoir, two bull heads were selected with SQR0 symptoms. The heads were removed from the slaughter line after skinning and stored in a chill room until examination, which was conducted within six hours after stunning. Accurate and inaccurate shots were identified using the diagram shown in Figure 7. Using a pen inserted into the bolt hole, the angle of bolt entrance into the skull at the forehead was visually assessed. The skull thickness was measured as the distance from the outer forehead to the brain surface midway along the length of the skull cavity. The brain was examined for peripheral bleeding while still inside the skull cavity. Both halves of the brain were then removed from the skull cavity. The surface area of brain covered in haemorrhage and level of tissue damage was rudimentarily examined, and the level of brain destruction described as being low, moderate or high. An examination was undertaken of the brains of three bulls assessed with SQR0 (one shot with CB and one with PB), two bulls and one steer with SQR1, three bulls with SQR2 and three bulls with SQR3.

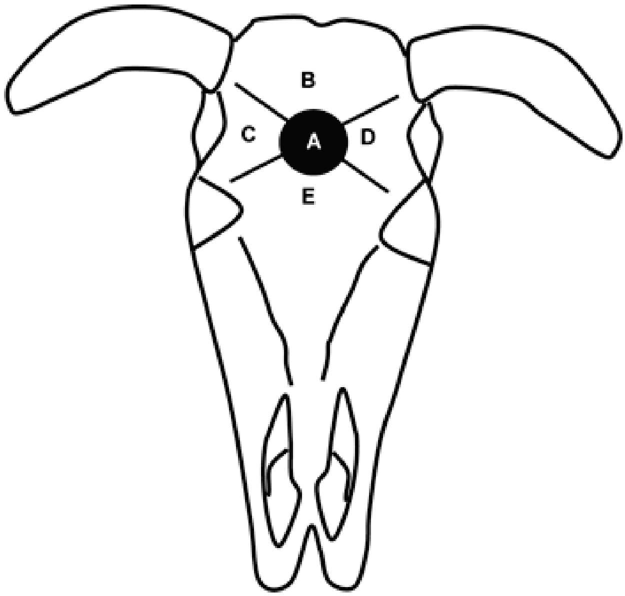


Figure 7. Shot holes found in the "A" area depicted accurate shots. Shots outside of this area (> 2 cm from the crossover point) were considered "inaccurate" and recorded as B (high), C and E (wide) and D low shots.

4.4 Statistical analyses

4.4.1 Paper I

Descriptive data analysis was used with Microsoft Excel version 2007. For the statistical analysis, the Statistical Analysis System software (SAS 9.2, SAS Institute Inc., Cary, NC, USA 1999–2001) was used. Differences in the prevalence of the different stun quality levels and different symptoms were analysed with a general linear model analysis of variance (PROC GENMOD) following a binomial distribution. The time of exposure, box type, group size and stun-to-stick interval were taken as fixed effects, whereas the CO_2 concentration was taken as a covariate. The correlation (PROC CORR) using the Fisher's exact test between all the different symptoms of recovery were analysed. Also, the correlation between the time of exposure and the number of pigs in each group was assessed. In all comparisons, results were taken to be statistically significant when $P < 0.05$.

4.4.2 Papers I and II

Effects on stun quality, shot accuracy and display of certain symptoms in different cattle classes and breeds were analysed using a marginal model and the generalised estimating equations approach using the GENMOD procedure of the Statistical Analysis System (SAS 9.2, SAS Institute Inc., Cary, NC, USA, 1999 to 2001). Animal class (bull or cow) and breed (beef or dairy) were introduced as fixed effects. Ninety-five percent confidence intervals were computed using the Pearson-Copper exact methods (Copper & Pearson 1934) for different cattle classes (bull, cow, steer). Correlation between symptoms rated SQR 1, 2 or 3 occurring with one another were analysed using Kendall's tau.

5 Results

The following sections summarise the results of the studies conducted initially in eight main pig abattoirs where an estimated 98% of pigs in Sweden are slaughtered, and then in six cattle abattoirs where at least 70% of cattle in Sweden are slaughtered. In the cattle abattoirs, the first study was conducted in one of the largest cattle abattoirs in Sweden. Further detailed results and discussion can be found in the individual papers located at the end of the thesis.

5.1 Pig stun assessments - paper I

Five abattoirs used the Butina® paternoster (abattoirs 1–5) and the other three (6–8) used the Butina® dip-lift stun systems. The number of boxes in each stun machine, exposure times and stun quality varied in each abattoir (Table 3). The largest paternoster system had seven boxes. In all paternoster systems, CO_2 concentrations were between 81 to 83% when pigs arrive at the first stop and 91 and 94% at the bottom stop.

Five abattoirs (4 and 5 with paternoster and all the ones with dip-lift systems) had consistent group sizes ranging from three to seven pigs. In all paternoster systems, CO_2 concentrations were between 81 and 83% when pigs arrive at the first stop and 91 and 94% at the bottom stop. Abattoirs 1 to 3 had varying group sizes from a minimum of three to a maximum of ten pigs. In the paternoster systems, the average stun-to-stick time for the last pig in the group varied from a minimum of 70 (± 4) seconds (s) to a maximum of 117 (± 12) s, while in the dip-lift systems the time varied from a minimum of 60 (± 8) s to a maximum of 86 (± 13) s. Of 7,476 pigs in the paternoster systems, 80% had stick times longer than 60 s, 62% longer than 70 s and 42% longer than 80 s. In the dip-lift systems, 75% of the pigs were stuck within 60 s, but 71% of the last pigs in each group were stuck after more than 60 s and 50% more than 70 s.

Pigs were consistently adequately stunned in the five abattoirs using paternoster systems, with only one of 7,476 pigs showing corneal reflex at sticking. In all three dip-lift systems, pigs were found inadequately stunned, with 1.5% of pigs in abattoir 6a, 3.3% in 7a, and 2% in abattoir 8 during the first study. A re-investigation was completed in abattoirs 6 and 7 after service of the stun systems and an increase in CO_2 exposure times (from 172 to 180 s in abattoir 6b and 208 to 224 s in abattoir 7b). Of the inadequately stunned pigs, 95% showed more than one symptom, and the corneal reflex was the most frequent symptom observed (present in 28 of 38 pigs). In 26 cases when corneal reflex was present there was also at least one other inadequate stun symptom displayed (Figure 8). Spontaneous blinking was seen in 10 of the 38 inadequately stunned pigs (0.5 and 1.7% in abattoirs 7 and 8 respectively). The symptoms of pain reflexes, righting reflex (RL4), nystagmus and eyeball rotation (RL3) were not observed. There was a significant positive correlation between the appearance of blinking and corneal reflex ($r = -0.31$, $P = 0.048$). All pigs in the study that were inadequately stunned were promptly re-stunned with back-up devices such as an electrical stunner (six abattoirs) and captive-bolt gun (two abattoirs). The longest stick times in abattoirs 1 to 8 were 160, 145, 119, 245, 83, 145, 104 and 116 s, respectively, and all these pigs were adequately stunned.

Table 3: Description of the eight abattoirs and ten study visits including: box type, loading mechanism, number of boxes, average group size/box, CO_2 concentration and exposure times, average stick time for last pig (LP), and number and percentage of inadequately stunned pigs.

Abattoir	Box type	Loading method	No. Boxes	Av. No. pigs/box	CO_2 (%) base	CO_2 Exp time (s±sd)	No. Inadequate stunned n/N (%)
1	Paternoster	Auto	7	7	93	282(±44)*	0/3444 (0)
2	Paternoster	Auto	6	4	93	238(±42)*	1/2325 (0.04)
3	Paternoster	Auto	4	3	93	250 (±34)*	0/500 (0)
4	Paternoster	Auto	3	3	91	240 (±10)*	0/700 (0)
5	Paternoster	Auto	3	3	93	240	0/507 (0)
6a	Dip-lift	Auto	1	7	91	172	10/602 (1.6)
6b**	Dip-lift	Auto	1	7	93	180	0/252 (0)
7a	Dip-lift	Manual	1	5	93	208	19/582(3.3)
7b**	Dip-lift	Manual	1	5	94	224	0/200 (0)
8	Dip-lift	Manual	1	4	92	224	8/408 (2.0)

** Taken as an average of box rotation time due to variations in time taken to load pigs effecting* CO_2 *exposure times (exposure times in abattoirs 5 to 8 never varied). **6b and 7b are reassessments. N is the total number of pigs studied in each abattoir and n is the portion of pigs of that number*

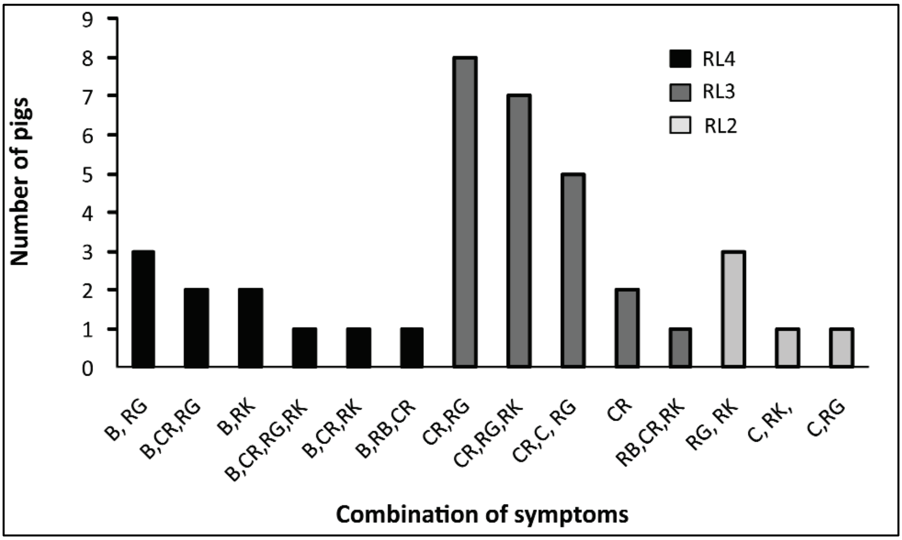


Figure 8. The combinations of symptoms seen in inadequately stunned pigs (n = 38), grouped in the category of the symptom with the highest risk level (RL). B: blinking, RB: rhythmic breathing, CR: corneal reflex, C: convulsions, RG: regular gasping, RK: regular kicking.

5.2 Cattle stun assessments paper II

Inadequate stunning occurred in 12.5% of cattle. Ninety-eight of 585 (16.7%) bulls (young and mature) were inadequately stunned compared with 27 of 413 (6.5%) other cattle (cows, heifers, steers and calves) ($P < 0.0001$). A total of 124 cattle (12.5%) were re-shot, with bulls reshot the most frequently (16.7% compared with 6.2% other cattle – Figure 9). Bulls (both young and mature) also showed high risk symptoms (SQR3) more frequently ($P = 0.0011$) than other cattle (6.9 compared with 2.1%). Inaccurate shooting occurred in 8.0% of all cattle (8.5% of bulls and 0.6% other cattle – Figure 9). Of the 92% of cattle that were identified as being accurately shot, 35.5% showed signs of inadequate stunning, almost all of which were bulls. Of 26 mature bulls, 11 were inadequately stunned (42%), and nine were shot accurately. In total, 14 bulls were shot more than three times and one (a Holstein) was shot five times. No cows, steers or calves were shot more than twice. Beef bulls more frequently displayed a high risk level stun (SQR3) compared with dairy bulls (11.9% and 5.6% respectively). During the five-day study period, the highest percentage of cattle found inadequately stunned was 22% of bulls, compared with 8% of other classes on day three (Figure 10).

Inadequately stunned cattle showed more than one symptom from the stun quality protocol in 53% of cases. Blinking was the most frequently observed high risk symptom in bulls (3.5%), while the most frequent in other cattle classes was failure to collapse (1.2%). Full eyeball rotation (SQR2) was the most frequently observed symptom in total, present in 11.9% of bulls and 1.9% of other cattle. Three of the SQR1 symptoms (gasping, ears up and tongue up in the mouth at sticking) showed significant correlative values with the presence of inadequate stun symptoms. The majority of animals (89%) were stuck between 84 and 125 s after stunning. Technical design constraints in the sticking area were the main causes for delays in sticking, such as large cattle being stuck in the delivery gate after stunning, cattle rolling off the stun crate requiring a separate pulley to get them back on to the shackle line, and derailing of carcases when rounding a bend in the shackle line.

The odds of receiving inadequate stun quality were significantly increased for bulls, compared with cows (2.3), or beef bulls compared with dairy bulls (2.1). Of the cattle inadequately stunned, 6% first displayed symptoms in the stun-box, 57% on the delivery table, 13% on the shackle line, and 24% during sticking procedures.

The accuracy of shooting between the five different shooters ranged from 81 to 95% of cattle shot in the optimal area. One shooter (shooter 3) had only worked a few months at the abattoir and he shot inaccurately the most frequently, compared with other shooters (Table 4).

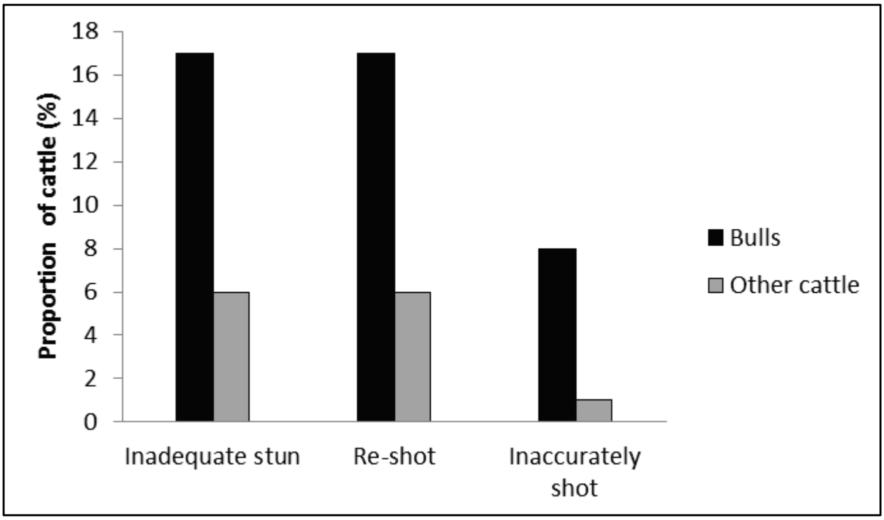


Figure 9. Frequency of inadequate stun, re-shots, and inaccurate shots in bulls and other (cows, heifers, steers combined).

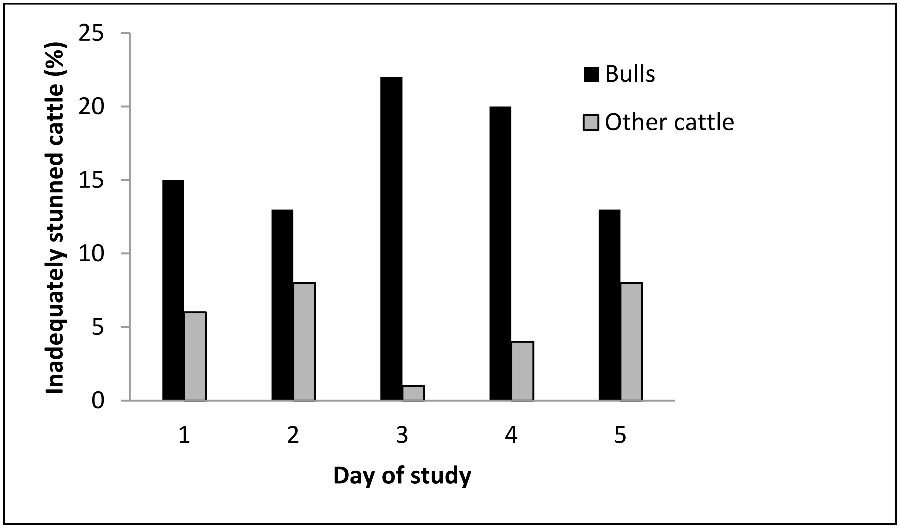


Figure 10. Daily percentages of inadequately stunned bulls, compared with other cattle (cows, heifers, steers).

Table 4. Number and percentage of cattle accurately shot by each shooter during the study.

Shooter ID	Total cattle shot	Accurate shots (%)	Time period employee worked at abattoir
1	200	90	5 years
2	240	94	5 years
3	39	81	3 months
4	223	90	3 years
5	296	95	15 years

5.3 Cattle stun assessments paper III

Table 5 shows for each abattoir which stunner type was used (i.e. CB or PB), the brand, ammunition and performance characteristics, and what type of animals were stunned with it. Table 6 displays the frequency in each cattle category (bulls or other cattle), that were adequately or inadequately stunned and at what recovery level. The proportion of bulls inadequately stunned with cartridge fired captive bolt stunners (CB) in each (abattoir and audit) in descending order respectively was 15.9% (3), 15.0% (1b), 13.8% (4), 9.5 (1a), 6.0% (5), and 3.6% (6). In bulls stunned with the pneumatic fired bolt stunner (PB), 7.0 % and 2.0% were found to be stunned inadequately during two audits. In only one of the eight visits were 100% of the bulls stunned adequately on the first shot (abattoir 2aPB) compared with four of eight visits of "other cattle" adequately stunned on the first shot (Figure 11). When the pneumatic stunner malfunctioned, the backup CB stunner was used, and 21 of 59 (35.6%) bulls were inadequately stunned with 15.3% receiving SQR3 stun rates. All 43 cows were adequately stunned (SQR0 and 1) with the same backup weapon (Table 6).

The proportion of bulls identified as inadequately stunned was significantly higher ($P < 0.0001$) compared with other cattle in all abattoirs. The odds of an inadequate stun were eight times higher for a bull than for other cattle. In total, 230 bulls were identified as pure beef breeds and 459 as pure dairy, and the remainder were crosses. Of the identified beef bulls, 18% were inadequately stunned compared with 9.3% of the dairy breeds ($P<0.0058$), while beef cattle had double the odds ratio for an inadequate stun to occur than dairy cattle. Only in abattoir 2PB were there no bulls showing SQR3 symptoms, all others (abattoirs 1, 3, 4, 5, 6) had proportions of high risk stunning ranging from 1 to 15.3%, whereas other cattle showed SQR3 symptoms in abattoirs 1 and 3 only (Table 6).

Bulls were reshot more frequently than other cattle classes in all abattoirs using CB. Abattoir 2CB had the highest frequency of bulls reshot (42%), followed by abattoirs 3 (12%), 1 (10%), 4 (9.0%), 5 (6.0%) and 6 (4.0%). Other cattle that were identified as inadequately stunned were all reshot, but in abattoirs 1, 3 and 4, more bulls were identified as inadequately stunned than were reshot. Seventy-nine cattle were shot twice, seven shot three times, three shot four times and one shot five times (i.e. 90 cattle were shot more than once).

The most frequent SQR3 symptom observed was spontaneous blinking, which was observed in 1.9%, 13.7%, 2.9%, 1.3%, and 2.0% cattle in abattoirs 1, 2CB, 3, 4 and 5 respectively. In total, 68 of 106 (64.0%) cattle with inadequate stunning (i.e. showing SQR3 or SQR2 symptoms) showed more than one symptom of inadequate stunning. Spontaneous blinking occurred in a total of 40 cattle, of which 36 also displayed other inadequate stun symptoms. In 18 of 19 cattle showing corneal reflex, other inadequate stun symptoms were also observed. All 12 cattle that groaned and all four that gasped also showed other inadequate stun symptoms. Eyeball rotation (considered a moderate recovery risk symptom) was the most frequent observed in all abattoirs, and occurred with other symptoms of inadequate stunning in 59.0% of cases (Table 7).

There was a large variation in stun-to-stick interval, with the fastest average time of 61 s (in abattoir 2) and a maximum average time of 105 s (abattoir 1). The most frequent high risk (SQR3) symptom identified was spontaneous blinking which occurred in a total of 40 cattle, of which 36 animals also displayed other inadequate stun symptoms.

The macroscopic brain assessments showed that the three bulls with SQR0 had a high level of brain tissue disintegration at the bolt entrance and frontal brain region. A well-defined thick haemorrhagic track traversed over the majority of the brain surface with severe tissue damage at the bolt entrance, and heavy bleedings at the brainstem. The blood was heavily clotted dark purple with more than 50% of the brain affected. When looking at the skull cavity before brain removal, the PB-shot bulls had a thicker, more evenly distributed layer of haemorrhaging around the brain surface and heavier bleeding at the brainstem compared with the CB adequately stunned bulls (Figure 12). However, all three brains with SQR0 were considered severely damaged.

Three animals displayed SQR1 symptoms (two Charolais bulls and one Friesian steer). One Charolais bull had a partial eye rotation so that part of the iris was still visible but the tongue was hanging out and ears were flopping down. It had severe brain damage despite a skull thickness of 3 cm and it was shot twice with both shots landing inside the recommended area at 90 degree angle into the forehead. The other showed excessive kicking, but the tongue was not out at sticking. However, it was also shot twice, but both shots landed high and wide and the bolt entered the forehead at a downward 45 degree angle. The bull had a skull thickness of 1.8 cm. Despite being inaccurately shot, it sustained severe brain damage with haemorrhage over 50% of the brain surface.

The steer had a skull thickness of 1.0 cm and showed severe struggling and tail flagging during sticking. It was shot once accurately, with the bolt entering on a 90-degree angle. However, there was less brain damage than that seen in the other SQR1-rated animals, with little tissue damage at the bolt entrance and moderate haemorrhaging at the brainstem.

Three dairy bulls showed SQR2 symptoms and all displayed full fixed eyeball rotations. Two had moderate levels of tissue damage and haemorrhaging of which one shot was placed accurately and the other wide with the bolt entering at a downward 45 degree angle to the forehead. The third showed such struggling behaviour at sticking that the blood-collecting knife fell out of its throat. Its ears were pointing upwards (had muscle tension) and there were groaning sounds during sticking. Brain damage could only be seen in the frontal region with little bleeding at the brainstem area, and the overall haemorrhage level was considered low.

Three bulls showed SQR3 symptoms and were pure beef breeds aged over two years (two Charolais and one Angus). One of the Charolais bulls displayed corneal reflex and spontaneous blinking immediately after shooting, and was shot three times: twice before shackling and once at sticking. The skull thickness was 3.2 cm, the thickest of the 12 skulls examined. None of the shots were accurately placed, as two were wide of the optimal area and the bolt failed to penetrate the brain, leaving no tissue or haemorrhage damage and no visible damage to the brainstem region (Figure 13). The other Charolais did not show corneal reflex but had full eyeball rotation and gasping (possible short respirations) at sticking; however, the tongue was out and the ears were down. The shot was accurately placed and the bolt entered at 90 degrees, but the brain damage was considered moderate, as tissue damage occurred only at the bolt entrance, with haemorrhaging on both sides of the brain covering only approximately 25% of the surface area, and not at the brainstem area.

The third bull, an Angus, had a skull thickness of 3 cm, and was shot five times (Figure 14), with the bolt penetrating at an angle of 90 degrees, but low in the brain cavity. It showed corneal reflexes, spontaneous blinking and eye rotation directly after shooting. It was reshot before shackling, but at sticking displayed extreme struggling behaviour, and groaning sounds were heard. Despite being inaccurately shot five times, the tissue damage and haemorrhaging were located mainly in the frontal brain area (Figure 15). The brain damage level was considered as low. Figure 16 shows the difference in brain damage between an adequately stunned bull (SQR0) and an inadequately stunned one (SQR3).

Table 5. Stun type (CB, PB or B) including ammunition, bolt, and energy specifications used in each abattoir on different animal types.

Stun type, manufacturer and description of stunner	Ammunition/ power load	Bolt exit length (mm)	Shooting specifications			Abattoirs using this stunner	Animals stunned
			Bolt diameter (mm)	Max velocity m/s	Max kinetic energy joules		
CB - Accles and Shelvoke "Cash", 0.22 calibre cartridge	Green cartridges 225 mg gun powder (3.0 grains)	65	11.91	50.5	296	1,2aCB, 3, 4	Females & steers
	Black cartridges 285 mg gun (4.5 grains)	65	11.91	56.62	369	1, 2aCB, 3, 4	Bulls
CB - Accles and Shelvoke "Cash Magnum", 0.25 calibre cartridge	Green cartridges 340 mg gun powder (4.5 grains)	65	11.91	63.95	474	6	Bulls
CB - Termet "Matador Super Securit 3000", 0.25 calibre cartridge	Red cartridges 250 mg gun powder (3.5 grains)	80	11.69	52	406	6	Females and steers
CB Shermer "Shermer Model ME" 0.22 calibre cartridge	Black cartridges ([NA] gun powder or grains	70	11.92	60	406	5	All cattle
PB Jarvis "USSS-1" Pneumatic	Pneumatic air pressure 175 PSI	97	15.9	38.5	427	2aPB, 2bPB	All cattle
B - Smith and Western handgun	9 mm bullet			695	4470	3	29 bulls

Table 6. Number and proportion of cattle adequately or inadequately stunned and risk level by abattoir visit and stun method used.

	Abattoir visit and stun type								
Factor	1aCB N=250	1bCB* N= 255	2aPB N=120	2aCB N=102	2bPB* N=314	3CB N = 364	4CB N=209	5CB N=158	6 CB N=256
Number of bulls	107	100	40	59	74	94	80	83	193
Adequate stun (%)	97 (90.6)	85 (85.0)	37 (92.5)	38 (64.4)	73 (98.6)	79 (84)	69 (86.3)	78 (93.9)	186 (96.3)
SQS0 (%)	95 (88.7)	68 (68.0)	35 (87.5)	37 62.7)	72 (97.2)	78 (82.9)	67 (97.1)	64 (77.1)	181 (93.7)
SQS1 (%)	2 (1.8)	17 (17.0)	2 (5.0)	1 (1.69)	1 (1.3)	1 (1.06)	2 (2.5)	14 (16.8)	5 (2.5)
Inadequate stun (%)	10 (9.4)	15 (15.0)	3(7.5)	21 (35.6)	1 (1.3)	15 (15.9)	11(13.8)	5 (6.0)	7 (3.6)
SQS2 (%)	7 (6.5)	6 (6.0)	3 (7.5)	5 (8.5)	1 (1.3)	7 (7.4)	2 (2.5)	2 (2.4)	5 (2.6)
SQS3 (%)	3 (2.8)	9 (9.0)	0	16 15.3)	0	8 (8.5)	9 (11.3)	3 (3.6)	2 (1.0)
Reshot (%)	9 (8.4)	12 (12.0)	1 (2.5)	25 (42.0)	0	11 (11.7)	7 (8.8)	5 (6.0)	7 (3.6)
Number of other cattle	148	150	80	43	240	270	129	75	64
Adequate stun (%)	142 (95.9)	144 (96.0)	80 (100.0)	43(100.0)	239 (99.5)	268 (99.2)	126 (97.6)	75 (100.0)	64
SQS0 (%)	140 (94.5)	138 (92.0)	1 (1.0)	43 (100.0)	238 (99.1)	266 (98.5)	126 (97.6)	65 (86.6)	64
SQS1 (%)	2 (1.3)	6 (4.0)	0	0	1 (0.4)	2 (0.4)	0	10 (13.3)	0
Inadequate stun (%)	6 (4.0)	6 (4.0)	0	0	1 (0.4)	2 (0.4)	3 (2.3)	0	0
SQS2 (%)	1 (0.7)	3 (2.0)	0	0	0	1 (0.4)	0	0	0
SQS3 (%)	5 (3.3)	3 (2.0)	0	0	1(0.4)	1 (0.4)	3 (2.3)	0	0
Reshot (%)	5 (3.3)	1 (0.6)	0	0	1 (0.4)	2 (0.7)	2 (0.7)	0	0
Total inadequate stun (%)	16 (6.3)	21 (8.4)	3 (2.5)	21 (20.5)	2 (2.7)	17 (4.7)	14 (6.7)	5 (3.2)	7 (2.7)

Table 7: Symptoms observed and percentage of times they occurred with inadequate stun signs.

SQS	Symptom	No of times symptom occurred Number/(%)	% of times symptom occurred with other signs of inadequate stunning	Kendall´s tau correlations occurring with inadequate stun symptoms (SQR 2 or 3)
3	Failed to drop	8 (0.04)	71	0.22
3	Righting reflex	2 (0.10)	100	0.09
3	Spontaneous blinking	40 (2.00)	88	0.60
3	Corneal reflex	19 (0.90)	95	0.44
3	Respirations	18 (0.90)	94	0.32
3	Groaning	4 (0.20)	100	0.17
3	Gasping	12 (0.60)	100	0.23
2	Full eye rotation	78 (3.80)	59	0.80
2	Nystagmus	13 (0.60)	85	0.35
1	Partial eye rotation	37 (1.80)	49	0.26
1	Excessive struggling at stick	35 (1.70)	54	0.23
1	Ears up at sticking	8 (0.29)	75	0.24
1	Tongue in mouth at stick	6 (0.19)	83	0.12

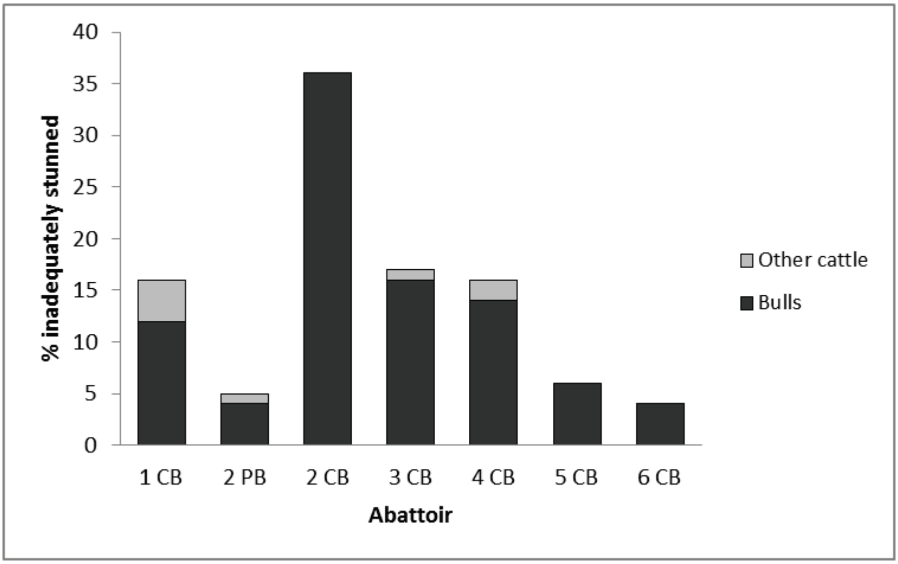


Figure 11. Percentage of bulls and other cattle inadequately stunned in each abattoir (abattoir 1 includes pooled data from 2 visits, and abattoir 2 is separated into when the PB or CB was used).

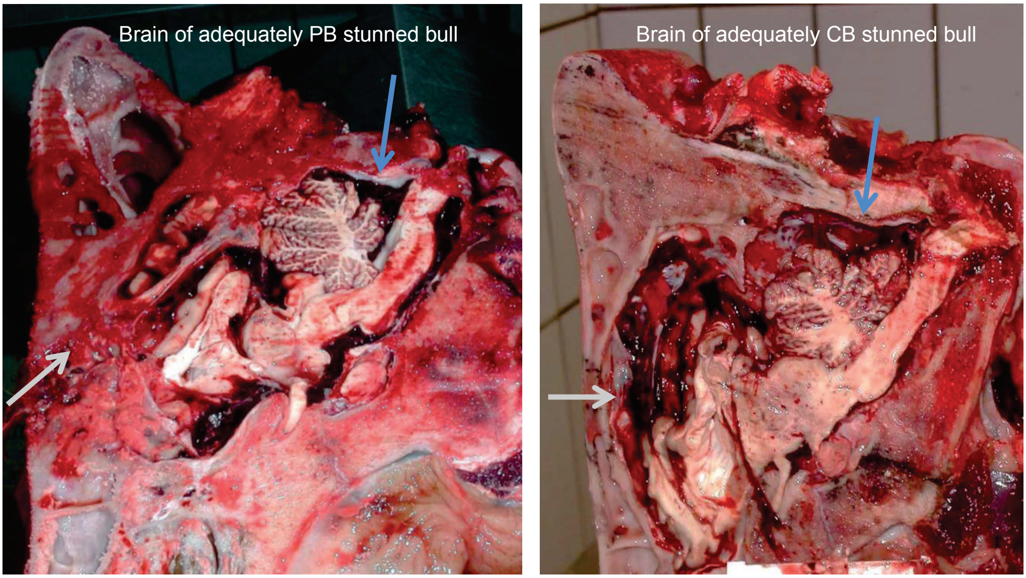


Figure 12. Blue arrows on left picture (PB-shot well-stunned bull) show heavier haemorrhaging than arrows on right picture (CB-shot well-stunned bull). Arrows also indicate bolt entrance location and angle of penetration (photos Sophie Atkinson).

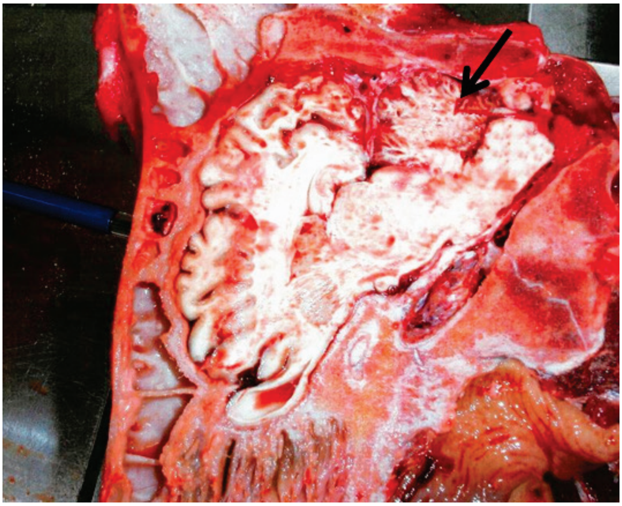


Figure 13. The brain of a Charolais bull CB-shot three times (3.2 cm skull thickness) with SQR3 symptoms and a low level of tissue damage and low haemorrhaging at base of brain (photos Sophie Atkinson).

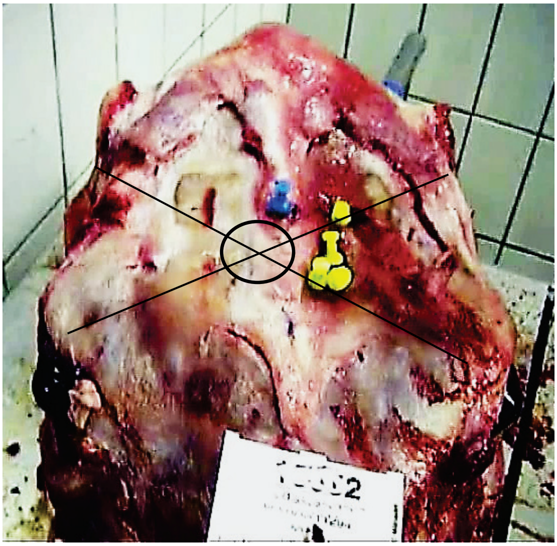


Figure 14. The forehead of a bull after skin removal showing the five shots placed outside of the optimal target area. This bull showed SQR3 symptoms (corneal reflex, spontaneous blinking and groaning), SQR2 symptoms (full eyeball rotation and gasping), and SQR1 symptoms (excessive struggling at sticking) (photo Sophie Atkinson).

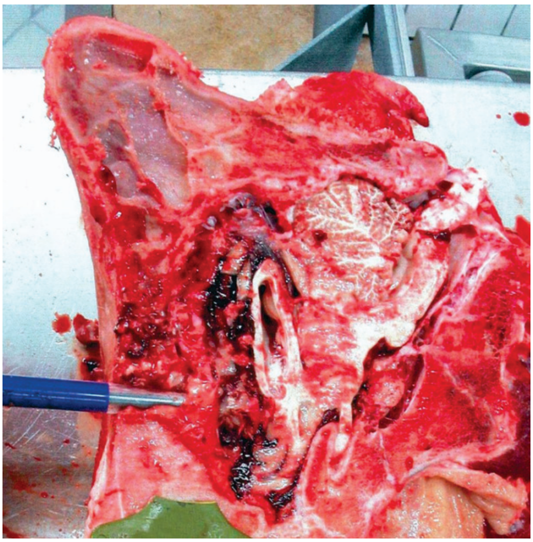


Figure 15. The same bull pictured above with brain damages located mainly in the frontal area and lacking at the cerebellum and brainstem (photo Sophie Atkinson).

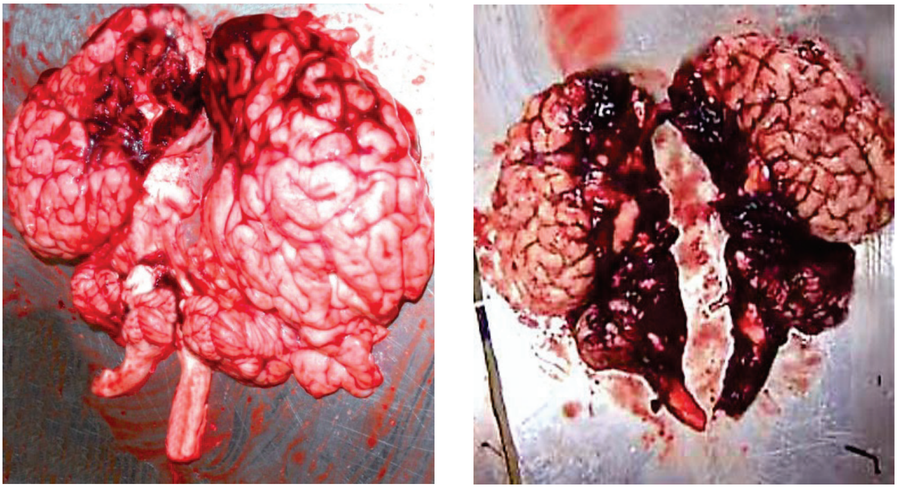


Figure 16. Photo on left shows a brain from an inadequately stunned bull shot three times with CB, which displayed corneal reflex and spontaneous blinking and had low level haemorrhaging and damage (especially at the brainstem), compared with the brain on the right from an adequately stunned bull shot once with PB (photos Sophie Atkinson).

6 Discussion

6.1 Paper I: Assessing pig welfare at stunning in Swedish commercial abattoirs using CO_2 group- stun methods

In this study, the recording of at least 200 stick times in the smaller abattoirs and 500 in the larger ones was considered an adequate sample size to gauge the stun-to-stick time variations. Pigs were consistently adequately stunned in the paternoster systems despite most stun-to-stick stick times exceeding 60 s. The shortest CO_2 exposure time recorded in the paternoster systems was 238 s, indicating pigs were exposed to CO_2 concentrations higher than 80% for at least 192 s. Due to insufficient exposure times, studies in Germany and Spain on similar Butina® paternoster systems reported much higher percentages of inadequately stunned pigs where between 6.2 and 17% pigs displaying corneal reflex, and 28% with pain and righting reflex (Hartmann *et al.*, 2010; Velarde *et al.*, 2000b).

In the dip-lift group-stun systems, all three abattoirs had pigs detected as inadequately stunned. This was an unexpected finding, and especially in abattoir 8, which had the longest CO_2 exposure times (224 s) at 91% CO_2 at the pit base. After the audit, the stun-box manufacturer (Butina®) was contacted and they informed that draughts, cold gas or excess water in the stun-pit base can reduce individual CO_2 consumption, preventing adequate stunning in some pigs. After this was reported to the abattoirs, they subsequently had specialised Butina technicians come and service the machines. Gas-transfer pipes were better insulated to reduce cooling from outdoor air temperatures, and the gas-transfer valves were upgraded, which ensured the CO_2 entered the stun-box at no less than 20°C. After the servicing, a second assessment was requested by the abattoirs, during which 100% of pigs were determined as adequately stunned.

6.2 Paper II: Assessment of stun quality at commercial slaughter in cattle shot with captive bolt

Until recently, Swedish national regulations required that sticking should occur within 60 s after stunning to minimise recovery risk (SJV 2008). Since new EU regulations came into force in 2013 specifying only that sticking should be started as quickly as possible (EC 2009), the 60 s requirement is now just a recommended standard operating procedure. In this study, no cattle were stuck within 60 s after stunning, and the majority of stun-to-stick times were between 85 and 125 s. The proportion of cattle identified as inadequately stunned was considered high, particularly in bulls, which were three times more likely to be inadequately stunned compared with other cattle classes. Similar results have been reported in UK by Gregory *et al*. (2007).

The number of bulls in total that required re-shooting was 16.7%. Of the bulls showing inadequate stun symptoms, 79% were, in fact, shot accurately. To maximise brainstem damage, Gilliam *et al.* (2012) suggests a higher optimum shot location than in this study. Yet, of the cattle inaccurately shot and inadequately stunned, 70% were shot higher than in the optimal area recommended by EFSA (2004). Although re-shooting was always performed quickly, the fact that 14 bulls required more than two shots and one required five shots, indicating a significant concern for animal welfare. As all cattle shot multiple times were mature cull bulls, it was apparent that the stunner lacked power. It failed to cause the necessary level of arterial bleeding at the brainstem to ensure adequate stunning.

In Portugal Gouveia *et al.* (2009) reported that 50% of bulls older than 30 months showed signs of recovery after stunning with a 0.22 calibre CB stunner, contributing the results to the use of a too low powered stunner for the thicker frontal skulls of the older cattle.

The bulls in the thesis study were mostly between 18 and 24 months old and stunned in delivered consignments. After batches of these bulls were stunned one after the other, inadequate stunning tended to occur. Then when cows were stunned, there were no inadequate stuns. It seemed the weapon was somewhat rested or cooled down when shooting smaller cattle, perhaps due to the use of a lower ammunition charge. This led to the conclusion that the 0.22 calibre CB stunner did not have the performance required to cope with the repetitive firing into the thicker bull skulls. This was supported by the fact that a device for measuring the gun's power registered operation at full capacity during servicing, yet 11% of bulls the following day were inadequately stunned, even though shot accurately.

On one of the morning shifts, an inspection was made of the stunner after a high frequency of inadequate stunning was occurring. The outer rim of the stunner's concave bolt was found to be damaged and it was recommended to the staff that the stunner be serviced before the next shift commenced. Incidentally this resulted in fewer inadequate stuns occurring. This day also had the highest frequency of inadequate stunning in bulls (22.0%) compared with the other four study days (Figure 10).

Staff generally appeared to be under constant pressure from management to keep up with slaughter speeds and sometimes had difficulty in finding the time to assess stun quality. Standard operating procedures should also be in place to allow staff the opportunity to properly check stunning, and they must have the capacity to recommend a stop in slaughter if there are animal welfare concerns due to frequent cases of inadequate stunning.

It was observed that during the servicing of stunners in several abattoirs, dirty rags were used to clean the inner cylinder where rubber buff (energy absorbing) rings align the cylinder through which the bolt slides during protraction and retraction. It is crucial to have this cylinder cleaned of grime and dirt, as even the tiniest amount may be a factor in reducing CB stun performance.

The least-experienced shooter had worked only a couple of months in the abattoir and seemed fearful of many cattle, often hesitating just before shooting. This appeared to disturb the cattle, causing them to become unsettled and evade his approach, which probably contributed to the much higher frequency of inaccurate shooting he had, compared with the more experienced shooters. Inexperienced shooters should start with smaller, easier cattle, until confidence is gained and proven, before being allowed to stun larger bulls or nervous animals.

6.3 Paper III: Assessment of stun quality and identification of risk factors affecting animal welfare in cattle shot with penetrating bolt stunners in commercial abattoirs

The stun quality standards identified in these studies showed that achieving adequate stunning on the first shot in cattle was a challenge. The frequency of reshooting when CB stunners were used per study visit was 3.6%, 6.0%, 8.4%, 8.8%, 11.7%, 12.0% and 42.0%. These frequencies would be unacceptable to many quality assurance programs (Grandin, 2010).

The Accles and Shelvoke Cash brand 0.22 calibre was the most commonly used stunner of the six abattoirs assessed in this study. Although the strongest cartridges available were used for bulls, those stunned with cartridge-fired (CB) bolt stunners had eight times the risk of being inadequately stunned than other cattle. Abattoir 6 had the lowest frequency of inadequate stunning in bulls shot with CB. However, it used an Accles and Shelvoke 0.25 calibre Cash Euro stunner which fires at a maximum bolt velocity of 63.95 m/s with 474 j kinetic energy. The other abattoirs used a 0.22 calibre CB, which fires at maximum bolt velocity of 56.2 m/s with 369 j kinetic energy. Stun quality was likely optimised in abattoir 6 by the use a more powerful stunner for bulls. They also used a separate stunner for shooting other cattle, reducing repetitive firing of the same stunner over an extended period of time (which can cause excessive heat build-up). Excessive heat build-up can contribute to a reduction in CB performance (Gibson *et al.,* 2015). On account of the large size of 29 cattle, abattoir 4 shot with a pistol firing a 9 mm bullet and all animals were well stunned. Generally firearms are avoided in the abattoir environment due to worker´s safety concerns regarding the risk of ricochet if bullets pass through the animal. However, the stun quality is usually effective due to the substantial brain damage caused by the bullet passing through the major brain structures (Schiffer *et al*., 2014).

Although the results suggest that the use of PB in combination with neck restraints in abattoir 2 optimised stun quality, when the backup CB stunner was used, the management failed to ensure animal welfare was safeguarded, as no other backup weapons were available that day. The frequency of inadequate stunning and re-shooting in bulls on that occasion was unacceptably high (36.5%). Due to use of neck restraints in the stun-box, shot accuracy was not the cause for the high frequency of inadequate stunning; therefore, it was likely to be either a malfunctioning weapon or faulty cartridges. The cartridges were stored in a workshop where a large door was left open to the outdoor temperature and humidly during the study, potentially exposing them to moisture. This was a probable cause for the high frequency of inadequate stun occurrence in bulls. CB cartridges must be stored in constant room temperatures as moisture exposure can reduce explosive forces.

Between 20.0% and 42.0% of bulls in each abattoir study were estimated to be at least 18 months or older. When bulls reach puberty, skin, hair and bone density in the forehead region is thicker than in cows or steers. The penetrating stunner must have sufficient power to consistently penetrate through this material for effective stunning. Daly & Whittington (1989) argue that the transfer of kinetic energy from the bolt to the cranial vault, as opposed to direct physical damage caused by the bolt, is what makes a stun effective. Other authors suggest velocity as being the most crucial to successful stunning (Gregory & Shaw, 2000). However, the PB stunner had the lowest velocity of all stunners but the highest kinetic energy (a velocity of 38.5 m/s at 427 j compared with 56.6 m/s at 369 j for the 0.22 CB). The PB stunner also had the longest and widest bolt dimensions. This indicates that bolt mass plays an important role in delivering a powerful stun. One of the main weaknesses with CB stunners noted by Gregory (2008) is that they do not cope with high line speeds, due to heat build-up reducing the gun's performance. The PB stunner, however, does not have this problem and can fire at optimal power levels consistently over long periods. Therefore these stunners would be more reliable in abattoirs where more than 20% of the total day's slaughter consists of bulls over 12 months of age. Otherwise 0.25 calibre CB stunners should be used exclusively for bulls.

Bulls that showed severe damage to the frontal areas of the brain but no bleeding or damage to the brainstem area, did show at least two high-risk symptoms for recovery (SQR3) which included corneal reflex, spontaneous blinking, or respiration. Heavy haemorrhaging or tissue damage at the brainstem is considered the most reliable indication of massive brain trauma (Gibson *et al.,* 2012). In two bulls with SQR3 symptoms, the bolt failed to penetrate the brain altogether and there was little to no haemorrhaging at the brainstem area.

Three bulls showing multiple signs of inadequate stunning were more than two years old, of pure beef breeds (two Charolaise and one Angus) and had among the thickest of the skulls investigated (3.2 and 3 cm respectively, compared with the thinnest, measured at 0.5 cm). Despite the fact that two were shot multiple times (the Angus five times), there was still a lack of brain haemorrhaging. Re-shooting may have no effect because of a reduction in impact energy due to absorption by fractures in the skull (Adams & Sheridan, 2008). It could also be due to the extremely muscular necks of some older bulls (Figure 17), which may reduce acceleration forces primarily responsible for destroying the brainstem region (Daly & Whittington 1989). This is plausible as the bovine head is positioned behind the neck and not under it as in humans, so it could absorb most of the axonal forces rather than the cranium. Early studies conducted on chimpanzees showed this type of effect when brain damage after a concussive hit was reduced by placing a support board behind the head compared with no board supporting the head (Letcher *et al*., 1973).

The anatomy of the head, and the position, shape and size of the brain inside the skull, varied among the 12 brains observed (Figure 18). The length of the brain varied by up to 1 cm in the adult bulls assessed, and the thinnest part of the brain did not always appear to be located behind the crossover section of the contralateral diagonals. When investigating brain damage in horses shot with a free bullet, Miller & Mills (2000) also reported considerable variation in geometry of the head. The skull thickness also differed in bulls by up to 3 cm. Due to anatomical differences in skull shape, the brain cavity could be positioned closer or further away to the poll area. The angle of the bolt where it entered the forehead also varied, pointing upwards, centrally or downwards. Most of the brains showed that the bolt entered at a downward angle in CB-shot bulls, probably as result of the animal ducking its head at the last moment before shooting. This would have reduced the transmission of energy and damage to the brain stem area, which appeared to be a critical factor for achieving adequate stunning. In several of the brains, there was substantial damage at the front where the bolt entered the forehead, but no damage or bleeding over the brain surface or at the brain stem. The differences in anatomy and position of the brain relative to the physical appearance of the forehead could explain why some bulls, even though shot accurately, may have still showed a shallow stun depth.

The trajectory of the bolt was also probably affected by the head position of the animal at the time of shooting. Low-angled shots were identified in the stun-boxes where no head restraints were used, as the animals were more likely to lower the head at shooting to evade the shooter. This is a behaviour often observed in nervous cattle about to be stunned. Sometimes in the stun system where a pneumatic air stunner was used, the handler would blow high pressured air into the face of the animal as a method of getting it to lift its head. The air made a very loud sound, and was painful to the human ear if ear protection was not used. Hence this must have been very stressful for the cattle involved and such a practice should be discouraged due to welfare concerns for the animal.

Figure 17. Mature bulls can have extremely muscular necks, which may absorb energy from penetrating concussion stunning, reducing the effect of the stun (photo Sophie Atkinson).

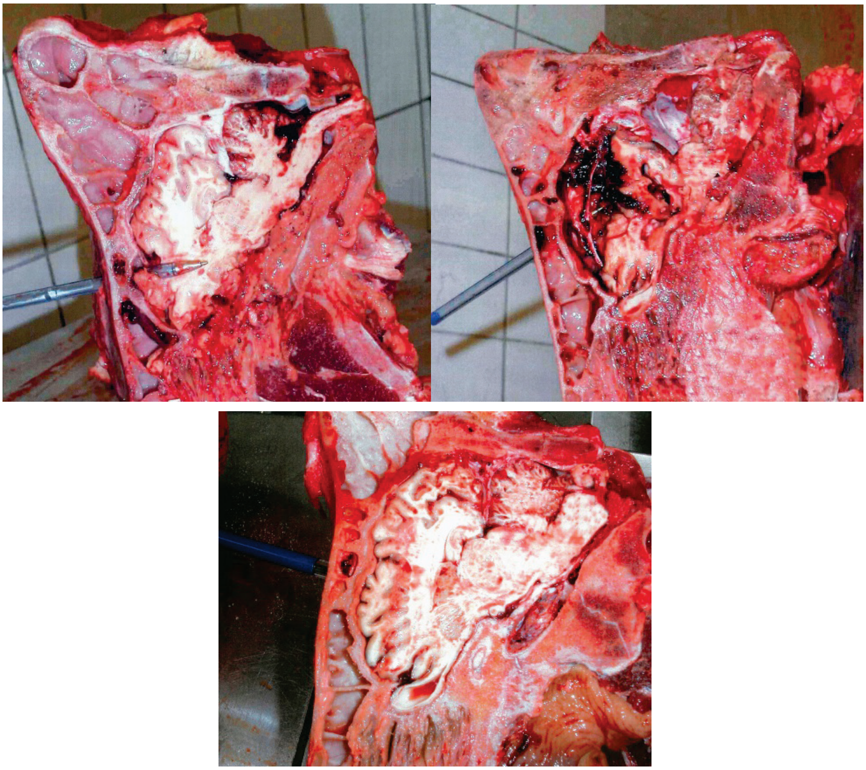


Figure 18. Differences in the angle of bolt entrance and anatomy of the skull, brain cavity and brain shape in bulls shot with CB stunners (photos Sophie Atkinson).

6.4 Methodological considerations

6.4.1 The stun quality protocols

The stun quality protocols (SQP) were designed to standardise the stun quality assessments. The rating of symptoms aimed to provide a clearer explanation of stun quality and reduce potential confusion or doubt as to which symptoms exactly indicated adequate and inadequate stunning. Research relevant to stun quality generally describes the more obvious symptoms that indicate consciousness. However, there appears to be a deficiency in describing which symptoms indicate when an animal is in a questionable state of consciousness. During the studies it became apparent that animals show symptoms outside categories most commonly described in literature. Therefore, these symptoms were allocated a score to identify when the stun quality level and risk for recovery was unknown. The SQP attempted to quantify clinical symptoms that animals may show after stunning to give an overall empirical result of stun quality for each animal observed.

The assessments were also designed to ensure a reasonable number of animals were assessed to represent welfare standards during routine slaughter. Providing data showing the percentage of animals within separate stun quality levels was useful for comparative purposes between abattoirs. Incidentally, four abattoirs using 0.22 calibre CB stunners had SQR3 (recovery) between 9 and 15.3%, which was a concern from an animal welfare perspective. These frequencies indicated that the better stun equipment and management procedures needed to be put in place to reduce inadequate stun frequency.

6.4.2 Correlation of symptoms within the stun quality protocol

The results indicated that, in pig stunning, the symptom of regular gasping (SQR2) appeared to indicate different levels of stun quality in different abattoirs. This symptom appeared in up to 2.5% of pigs without any other symptoms in abattoir 2; however, in abattoirs 6, 7 and 8 most pigs with regular gasping symptoms also displayed inadequate stun symptoms. Gregory *et al.* (1987) reported 75% of pigs with gasping, of which 16% also had corneal reflex after insufficient CO_2 exposures. Grandin (2010) states that gasping is a symptom of a dying animal but in the thesis studies, regular gasping and regular kicking in pigs in abattoirs where inadequate stunning occurred, were good indicators to initiate a closer examination to assess the stun quality. In fact, 18 pigs with corneal reflex first showed symptoms of regular gasping while on the shackle line.

Although it cannot be established what risk level of recovery there is if pigs display only one of the symptoms identified at SQR2 in Table 1, it was decided that if pigs displayed a combination of these symptoms (convulsions, regular gasping or regular kicking) they should be considered inadequately stunned and re-stunned as a precaution to avoid potential recovery.

Eyeball rotation (SQR2) is a difficult symptom to interpret as to what the level of recovery, if at all, it indicates. Early studies conducted by Gregory (1999) stated that it should never occur after penetrative concussive stunning. However, in later studies (Gregory *et al*., 2007) it was suggested that although eyeball rotation was a symptom seen more frequently in bulls, it might not indicate recovery, but a more shallow depth of stun (although a definition for shallow stunning was not given). Gibson *et al*. (2012) found that the presence of eyeball rotation in sheep was often the first sign of incomplete concussion preceding brainstem and cranial or spinal responses. However, they also determined that it was not always associated with other signs of recovery. This concurred with the cattle studies undertaken in papers II and III. In study II, 70% cattle showed no other inadequate stun symptoms other than a full eyeball rotation, but in paper III, 60% showed other inadequate stun symptoms.

It was also noted that cattle could display different degrees of eyeball rotation; subsequently, the degree of eyeball rotation was differentiated in the SQP. When the eyeball was fully rotated with only the sclera visible (Figure 19) it was defined as a “full eyeball rotation”. When only some sclera was visible along with part of the iris, this was defined as a “partial eyeball rotation”. The results from paper II confirmed that this was a valid discrimination, as partial eyeball rotation had low correlative values when occurring in cattle that showed inadequate stun symptoms. In comparison, full eyeball rotation had high correlative values with other symptoms of inadequate stunning. Similarly, ear tension had a positive correlation with inadequate stunning in paper II, but in paper III it did not. However, as some cattle with partial eyeball rotation or ear tension also sometimes showed symptoms of inadequate stunning, it was valid to rate these symptoms at a lower risk level and suggest closer monitoring and reflex testing as a precautionary measure when these symptoms are observed.

Gouveia *et al*. (2009) suggest that more than one symptom should be considered when determining inadequate stunning. However, to protect animal welfare, symptoms such as corneal reflex, blinking, respirations, righting reflex or failure to drop should always indicate inadequate stunning and the animal reshot. Lambooij *et al*. (2012) concluded that the corneal reflex appeared to be a conservative clinical parameter for indicating the state of consciousness as the EEG data indicated unconsciousness was present when calves showed corneal reflexes. However, the study conclusions were based on a small sample of data and do not represent potential variations that would occur under practical slaughter conditions. Consequently, Lambooij´s conclusion should be interpreted with caution. The thesis studies showed that in most cases where the corneal reflex was observed, other signs of inadequate stunning were displayed, substantiating it as a probable sign of recovery. In agreement with EFSA (2004) and (EFSA 2013a), it should not occur after penetrating bolt stunning.

In the cattle study conducted in paper II, gasping was originally rated as SQR1. However, as the results showed high correlative values with the display of inadequate stun symptoms, the stun quality protocol in paper III rated this symptom at a higher risk level for recovery (i.e. SQR2). This was a valid judgement as in paper III all cattle showing gasping also showed other inadequate stun symptoms.

Standard procedures in all cattle abattoirs studied was to cut the skin from the jowls to the sternum prior to chest sticking. Some authors suggest that it is difficult to ascertain if excessive reactions at sticking are due to unconscious nociceptive arc-reflexes (Bourguet *et al.*, 2011). In the thesis studies, excessive reactions at sticking were only recorded when the reactions were well over normal levels seen during concussive stunning. However, when an animal showed excessive reactions, if no other symptoms from the SQP were presented and there were no reflexes, it was registered as SQR1, which required close monitoring of the animal.

Figure 19. A bull showing what was considered as a full eyeball rotation (Photo Sophie Atkinson).

6.4.3 Animal handling and design of abattoir facilities

During the assessments, certain environmental risk factors appeared to have an indirect influence on the outcome of the stun quality. Many architectural designs in the lairage were noted as not being designed with animal behaviour in mind. Livestock have a poor depth perception in close confinements and problems to focus if there are many structural changes. Designs that consider how animals see and move under natural circumstances are integral to facilitating smooth animal flow and minimising conflict during human–animal interactions. In most of the cattle abattoirs, the lanes leading into the stun-box were high-walled of solid construction, which blocked any possibility for the handler to use the animal's natural flight zone to encourage forward movement. There was also little incentive for cattle to enter the noisy dark and often slippery stun-boxes. Figure 20 shows the difference between a badly designed stun-box where an obvious "dead" end occurs and a better designed stun-box with more even lighting, better flooring, a more open front so the animal can see a way through, and built in a sound-insulated room out of the carcass processing area.

Figure 20. An outdated stun-box typical of older Swedish cattle abattoirs (left) compared with a more modern stun-box design conducive to improving cattle welfare (photos Sophie Atkinson).

Many stun-boxes had a metal triangular block built into the floor to assist correct delivery for hoisting, which cattle sometimes fell on, and struggled to keep upright because of it, during shooting. When no head restraints were used it was also observed that cattle often lowered their head making a flat shot on the forehead challenging for the shooter.

Cattle standing and waiting in line for stun-box loading could also be seen to react to the loud noises when a restrained animal in the stun-box struggled and hit the metal walls of the pen. Bangs associated with firing of the stunner and falling of animals after stunning, were visually identified as causing fear and distress in waiting animals. Furthermore, five of the six cattle abattoirs had built the stun-boxes inside the same room where carcass processing took place. High noise levels from machinery emanated into the stun-box area acting as a deterrent for the animals to enter the stun-box.

In abattoirs two abattoirs water and blood pooled at the stun-box entrance, visibly causing some cattle to baulk and refuse to move forward (Figure 21). High activity levels in the lairage and noise levels over 90 decibels have been shown to cause fear and stress responses in livestock (Waynert *et al*., 1999; Weeks *et al*., 2009; Peña *et al*., 2014; Vermeulen *et al*., 2015).

Figure 21. Blood soaked floor in stun box where bull stands, which caused many animals to refuse to load the stun-box (photo Sophie Atkinson).

Use of an electric cattle prodder (a tool giving an electric shock when pressed onto an animal's body to facilitate movement) is unpleasant and painful for the animal. Although the frequency of electric prodding was not registered as part of the thesis studies, it was noted that, in general, all abattoirs used electric prodders at various frequencies to load cattle into the stun-box. It appeared to trigger a panic and struggling response in some cattle when they entered the stun-box. This may have been a factor contributing to inaccurate shooting, leading to inadequate stunning. Grandin (2001) suggested that a minimised use of electric prodding improves successful stunning on the first shot. Bourguet *et al.* (2011) reported that cattle difficult to drive into the stun-box were more likely to receive a second shot. Presumably this is because some cattle receiving painful or forceful handling will attempt to escape, which, if the animal is restrained in the stun-box, creates high anxiety. As the animal struggles and moves around, accurate placement of the stunner becomes a challenge for the shooter. Hultgren *et al.* (2014) reported that 8.3% of cattle in a Swedish abattoir displayed significant struggling behaviour while in the stun-box, and 10% required re-stunning. In abattoir 6 (one of the nosiest abattoirs), struggling cattle appeared to be a main risk factor for an inadequate stun to occur, as the shooter appeared to have problems placing the stunner correctly. When the electric prodder must be used frequently, it indicates a clear sign that there are problems with the facility design.

Gregory *et al*. (2007) found that 6% of 306 bulls struggled in the stun-box during stunning, and they had more than twice the frequency (19%) of inadequate stunning compared with those that did not struggle. In the third study of the thesis, struggling behaviour was recorded in one of the abattoirs. Of 193 bulls, six bulls were inadequately stunned, five of which showed significant struggling behaviour at shooting. In all other abattoirs, the shooter had responsibility only for shooting and not for shackling or sticking, and the staff rotated tasks on an hourly basis. In abattoir 6 the shooter was responsible all tasks and the staff did not rotate duties for the whole day. This appeared to be a very high workload for one person. The shooter also had to climb a ladder and step over to a narrow ledge to access the animal for shooting. Cattle often took fright and reacted when he suddenly approached from below as he climbed the ladder directly in front of their head. The shooter also appeared exhausted and frustrated, and was noted to use the electric prodder frequently.

One of the pig abattoirs with the highest frequency of inadequate stunning compared with other abattoirs also had stressful handling procedures during stun-box loading. Handlers loaded pigs manually and electric prodders were used frequently to move the pigs through a doorway from a quiet lairage area into a pen located inside where high noise levels occurred due to carcass processing machine operations. An abrupt change in air quality where ventilation was reduced, and humidity, air temperature and noxious odours also increased. From this pen, the pigs were loaded manually with great difficulty into a dip-lift stunner. It was very stressful for both the pigs and the handlers.

Reports were distributed to participating abattoirs from each of the study visits conducted in the thesis. Two of the cattle abattoirs subsequently invested in PB stunning with head restraints after the studies. In one system, an expensive mechanical gate was installed to assist stun-box loading. However it moved above the head of an incoming animal to get behind it to push it forward. This startled many cattle, sending them backwards, resulting in the handlers having to use physical force to get the animal forward again. This defeated the purpose of reducing handling stress with the use of a mechanical loading gate.

Once the animal was loaded in the stun-box, hydraulically operated moving panels pressed above and below the head, essentially placing the animal in a full headlock (Figure 22). Although operator ease and shot accuracy may be improved in such systems, many cattle showed behavioural signs of stress including rolled eyes, flaring nostrils, panting, and loss of footing due to struggling, indicative of a low tolerance for such a restrictive and unfamiliar restraint system. Very limited research has been conducted on the effect such systems have on stress levels in cattle. EC (2009) has a clause stating that the stunning process should avoid causing pain and distress during the slaughter process. Such restraint systems should be scientifically researched to appraise how physiologically stressful they might be for cattle at stunning. This could also contribute to development of solutions for optimising the system design for animal welfare and efficiency. The few studies that have been conducted have indicated that full restraint where the whole head of the animal is restrained (rather than just the neck) caused high cortisol levels and behavioural signs indicative of high stress (Ewebank *et al.,* 1992; Mason *et al.*, 1995; and Atkinson *et al.,* 2009). In UK full head restraint systems are not recommended (HAS 2016). Although full head restraints improve shot accuracy, this benefit can be diminished by unreasonably increased levels in stress. How the restraints are operated is important to consider for improved animal welfare. In Atkinson´s study, it was found that neck restraints and chin lifts could be operated too quickly, which caused some animals to startle and panic at the time of shooting.

Figure 22. A relatively newer design of a highly restrictive head restraint in a stun-box used with a pneumatic stunner (photo supplied with kind permission from Jarvis).

In some of the abattoirs it was not possible to properly access the animals for stun quality assessment after sticking. In the case of pigs, the floor could be covered in a thick layer of blood to the extent that it was too dangerous for staff to walk on the surface to access the pigs before they entered the scalding bath. This was a concern, as symptoms of inadequate stunning sometimes appeared while pigs were on the shackle-line after sticking. Abattoir buildings need to be constructed to allow space enough for staff to safely access animals for inspection.

According to a report conducted by the Food Chain Evaluation Consortium (2007), who made an economic analysis of stunning and killing practices within EU member states, the total percentage of costs allocated to the stunning process was 5% compared with 89% for carcass-dressing procedures. It can be questioned if it is ethically acceptable, to spend substantially more money on facilities once the animal is dead, rather than proper facilities to ensure stress is minimised when the animal is still alive.

7 Conclusions

- The use of a stun quality protocol helped formulate a standardised method for deciding when animals were adequately or inadequately stunned.

- The scoring of certain clinical symptoms helped ascertain different risk levels for recovery and identify associated concerns for animal welfare. This was useful for comparing stun quality standards between different abattoirs, animal types and stun methods.

- All pigs stunned in paternoster systems were consistently adequately stunned due to CO_2 exposure times and concentrations set appropriately for the stun group size and relevant stun-to-stick intervals

- Abattoirs using dip-lift systems had stun quality problems related to insufficient CO_2 exposure times and concentrations resulting from poor stun machine maintenance. Rectifying these elements resulted in 100% adequate stunning during follow-up studies.

- Unexpectedly high variations in animal welfare and stun quality occurred in all cattle abattoirs.

- Beef bulls and older cull bulls were at highest risk of inferior stun quality.

- Macroscopic brain assessments showed that adequately stunned cattle had a well-defined thick haemorrhagic track traversing over the brain with severe tissue damage at the bolt entrance and heavy bleeding at the brainstem.

- Bulls showing recovery signs had thick skulls (≥3 cm), low-level haemorrhaging and brain tissue destruction, with little to no bleeding at the brain stem, even in bulls shot more once.

- Risk factors contributing to inadequate stun frequency in cattle included lack of head restraints, use of stunners with insufficient power for shooting large consignments of bulls, insufficient stunner maintenance and incorrect ammunition storage.

The following recommendations can optimise stun quality:

- Loading areas and stun-boxes should be built in well-ventilated, evenly lit, sound-insulated zones, separate from noisy carcass dressing areas and areas where animals can see high levels of activity or disturbances.

- In abattoirs regularly processing bulls, a Jarvis pneumatic stunner or a minimum 0.25 calibre cartridge-fired penetrating bolt stunner with neck restraint should be used.

- Abattoirs that cannot succeed in adequately stunning animals in a consistent manner should be required to service the stunners or upgrade the system to rectify the situation.

While animals should be constantly monitored by abattoir staff, this study highlighted the importance of external stun quality assessments that can help to ensure certain standards of animal welfare are met. These conclusions should be relevant to most commercial abattoirs globally.

8 Reflections from the stun quality assessments

The thesis studies identified a large variation in abattoir design and management practices. During the studies, there were many factors identified which can have an effect on stun quality other than the stun system itself. For example, stressed animals were more likely to struggle during the stun process and this appeared to increase the risk of ineffective stunning. Environmental factors such as noise levels, lighting conditions and position of lights, air quality, surface colours, odours, space dimensions (including roof heights), floor surface materials, position of floor drains, lane designs (including the height of the lane walls), strategic placing of solid panels in handling lanes, all appeared to have indirect effects on animal welfare. Abattoir environments could be greatly improved with more specific knowledge of how these factors can be managed to reduce stress and improve the ease in which animals can be moved through the lairage into the stun-box and during restraint at stunning. Unfortunately, it was out of the scope of this study to include a thorough assessment of stun quality in calves. Most calves sent to slaughter in Sweden are dairy calves around four months of age. As they have been reared without their mothers, they tend to be very difficult to move because of a lack of flight instinct. Many instances were observed where handlers had significant problems loading calves into the stun-box. The shooter was also observed to have problems reaching the calves to place an accurate shot. Of a total of 49 calves that were observed in an abattoir, 14% showed high risk symptoms of inadequate stunning. Research is required into the welfare of calves during loading and stunning.

When stun quality or animal welfare problems were identified during the study, it was a concern that slaughter plant procedures were not in place so for staff to identify and react to stun quality problems. While the present EU legislation does require abattoirs to monitor the stunning of animals and animal welfare officers to be employed to ensure that stun assessments are implemented, these requirements are not a safeguard for animal welfare at slaughter. Media footage in 2015 revealed obvious animal welfare problems at stunning in several major abattoirs in Finland (Finnish TV station YLE, Programme MOT, aired on 26-10-2015, and also in France (French TV, El Mundo, aired 26-02-2016). This footage demonstrated that officials working in the slaughter industry on a daily basis can become complacent about animal welfare. The issues identified may also be related to a lack of knowledge of and a limited ability to identify animal welfare problems. Therefore animal welfare at slaughter would be better safeguarded if well managed yearly third-party audits during specific phases of pre-slaughter handling and stunning were introduced in addition to the regular internal and official controls. This would also provide a better platform for spreading knowledge to abattoirs about good animal handling and welfare practices and increase transparency in the food chain, which is important for quality assurance and consumer confidence.

9 Populärvetenskaplig sammanfattning

Vid all slakt i Sverige gäller att samtliga djur ska bedövas (göras medvetslösa) före avblodning (som sker genom att de större blodkärlen skärs upp så att blodet kan rinna ut). Av djurskyddsskäl ska sådana bedövningsmetoder användas som leder till en nivå av medvetslöshet (bedövningskvalitet) som säkerställer att djuret förblir helt okänsligt för smärta, och inte återfår medvetandet under tiden från bedövning och avblodning fram till dess döden inträder.

Svenska rekommendationer anger att avblodning bör ske inom 60 sekunder efter bedövning för att minimera risken för att medvetandet återkommer. Djurskydd vid slakt är en fråga som engagerar allmänheten och regelbunden kontroll av bedövningskvaliteten på slakterier är något som krävs enligt EU:s lagstiftning på området. Det finns dock endast begränsade riktlinjer för hur detta ska göras (med avseende på kontrollmetod, kontrollfrekvens, antal djur som ska kontrolleras, och vilka nivåer av bristande bedövning som kan accepteras). Målet med detta avhandlingsarbete var att utveckla och tillämpa protokoll för bedömning av bedövningskvalitet på kommersiella slakterier. Det syftade även till att identifiera riskfaktorer för dålig djurvälfärd och diskutera metoder för att säkerställa ett gott djurskydd vid slakt.

Ett protokoll för bedömning av bedövningskvalitet hos gris utvecklades för att identifiera och riskklassificera olika tecken på återkommande medvetande och dess eventuella konsekvenser för djurvälfärden. Protokollet tillämpades på åtta grisslakterier, av vilka fem använde Butina paternostersystem (flerkorgssystem) och två använde ett så kallat dip-lift-system (en korg som hissas vertikalt), där grisarna bedövades i grupp i höga koncentrationer av koldioxid. Trots att tiden från bedövning till avblodning (sticktiden) var över 60 sekunder i paternostersystemet kunde inga problem med bedövningskvaliteten ses. Vid de slakterier som använde dip-lift-system sågs problem med bedövningskvaliteten hos 1,7–3,3 % av grisarna. Detta kunde hänföras till alltför kort exponeringstid och för låg koldioxidkoncentration under bedövningen av grisarna.

Ett annat protokoll för bedömning av bedövningskvalitet utvecklades för att bedöma nötkreatur i samband med användning av penetrerande bultpistol. Detta protokoll tillämpades vid ett stort slakteri. Sticktid, skottplacering, omskjutningar och variation i bedövningskvalitet mellan olika kategorier av nötkreatur och mellan olika skyttar undersöktes. Dålig bedövningskvalitet registrerades hos 12,5 (16,7 % av tjurarna, jämfört med 6,5 % för övriga kategorier nötkreatur). Trots korrekt skottplacering uppvisade 13,6 % av tjurarna bristande bedövningskvalitet, jämfört med 3,8 % hos övriga nötkreatur. Andelen nötkreatur med felaktig skottplacering varierade från 19 % för den minst erfarna skytten till 5 % för den mest erfarna. Sticktiden var i genomsnitt 105 (± 17) s vilket ger upphov till frågor beträffande djurskyddet mot bakgrund av andelen djur som inte uppvisade fullt tillfredsställande bedövningskvalitet. Problem med bedövningskvaliteten kunde kopplas till alltför klen ammunition till tjurarna och till bristande vapenunderhåll.

I den tredje studien utvärderades bedövningskvalitet och djurskydd vid sex större nötkreatursslakterier med hjälp av det protokoll som togs fram i studie 2. Fem av slakterierna använde krutdriven penetrerande bultpistol och ett använde en lufttrycksdriven (pneumatisk) penetrerande bultpistol, ett system där djuren fixeras vid halsen i samband med bedövning. Hos ett antal av de djur som bedövades med dessa metoder undersöktes även de makroskopiska hjärnskadorna i relation till bedövningskvaliteten. Vid de slakterier som använde krutdriven bultpistol sågs bristande bedövning hos 4-36 % av tjurarna och 0-4 % av de övriga kategorierna nötkreatur (kor, kvigor, stutar). Vid det slakteri som använde pneumatisk bultpistol uppvisade 4 % av tjurarna och 0 % av övriga nötkreatur tecken på bristande bedövning. Förekomsten av bristande bedövning hos tjurar var förvånansvärt hög vid de slakterier som använde krutdriven bultpistol. Av de hjärnor som undersöktes sågs betydligt mer omfattande makroskopiska skador (total destruktion av hjärnan) hos de nötkreatur som skjutits med pneumatisk bultpistol jämfört med de som skjutits med krutdriven bultpistol. Hjärnorna hos de nötkreatur som uppvisade tecken på bristande bedövning med störst risk för negativa konsekvenser för djurvälfärden uppvisade liten omfattning av blödningar generellt och inga blödningar i hjärnstamsområdet. Denna studie illustrerar tydligt vikten av att använda rätt avpassad utrustning till olika kategorier av nötkreatur.

I de studier som ingår i denna avhandling har totalt 9520 grisar och 2725 nötkreatur studerats. Avhandlingen identifierar och diskuterar praktiska metoder för att minska förekomsten av bristande bedövningskvalitet hos grisar och nötkreatur, liksom olika angreppssätt som kan minska förekomsten av stress i samband med hantering av nötkreatur vid bedövning. Det faktum att bristande bedövning förekom vid både gris- och nötslakterier, och hos en relativt hög andel av nötkreaturen, visar på behovet av tredjepartskontroller av bedövningskvaliteten. För att säkerställa ett gott djurskydd vid slakt krävs även att kontrollpersonal och företagsledning får bättre utbildning. Ett tydligare angivet tröskelvärde för vad som kan anses vara acceptabelt respektive oacceptabelt vad gäller förekomst av bristande bedövning, i kombination med lämpliga sanktionsåtgärder för att säkerställa efterlevnad, skulle också leda till en höjning av nuvarande standard på djurskyddet vid slakt.

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