When and what determines the death of an animal? A study investigating the heart activity during slaughter of farm animals.

När infaller dödstillfället vid slakt av lantbruksdjur? En studie av hjärtats aktivitet efter bedövning och avblodning.

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Studentarbete 525, Skara 2014

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ABSTRACT

Slaughter of animals should consist of two steps in order to be considered humane. The first step of the slaughter process is stunning, with the purpose of inducing unconsciousness and insensibility in the animal. The second step is exsanguination, which causes bleeding and eventually death. What death really means in these situations has been discussed intensively, but still no agreed definition has been accepted. Nonetheless, it is conceded that animals are declared dead when consciousness is irreversibly lost, and absence of respiration and blood circulation are observed. The Swedish Animal Welfare Act states that during slaughter, no further measures may be taken on the animal until its death has occurred. One question that arises is in respond to this is: *Is an animal really dead, even though the heart beats, when the remainder of the slaughter process commences after stunning and exsanguination?* The aim of this thesis was to investigate heart activity after stunning and exsanguination of cattle and fattening pigs at conventional commercial slaughter plants in Sweden in order to determine the exact time of cardiac arrest. In total, 108 cattle and 157 pigs from three different slaughter plants were observed using two different stethoscopes. A significant difference was found between the stethoscopes for both cattle and pigs ($p \leq 0.0001$). Furthermore, significant differences were found between the two slaughter plants where data was collected on pigs ($p \leq 0.0001$), but not between the slaughter plants where data was measured on cattle ($p=0.24$). The time from start of exsanguination to cardiac arrest was on average 5 minutes and 45 seconds for cattle and 2 minutes and 58 seconds for pigs. In conclusion, this study suggests that the definition of death in animal welfare contexts should be based both upon loss of brain and cardiac function. In future studies, this can be used to evaluate the possible implications this would have for the slaughter process and also the animal welfare.

Keywords: slaughter, stunning, exsanguination, bleeding, death, cardiac arrest

SAMMANFATTNING

För att slaktprocessen av lantbruksdjur ska klassificeras som human ska den ske i två steg: bedövning som gör djuret medvetslös och avblodning som orsakar blodförlust och leder till död. Vad som egentligen menas med död i slaktsammanhang är något som har diskuterats intensivt i världen men fortfarande finns det ingen vedertagen definition. Således finns det en acceptans om att djuren dödförklaras när medvetandet inte går att återfå och frånvaro av andning och blodcirkulation observeras. I den svenska djurskyddslagen finns det angivet att inga andra åtgärder får vidtas i slaktprocessen innan djuret är dött. En fråga som väcks då är: *Är djuret verkligen dött när slaktprocessen fortsätter efter bedövning och avblodning och hjärtat fortfarande slår?* Syftet med den här uppsatsen var att undersöka hjärtats aktivitet efter bedövning och avblodning på nötkreatur och slaktsvin i konventionella, kommersiella slakterier i Sverige för att fastställa den exakta tidpunkten för hjärtstillestånd. Totalt har 108 nötkreatur och 157 svin observerats på tre olika slakterier med två olika stetoskop. Resultaten visade signifikanta effekter mellan de två metoderna för både nöt och svin ($p \leq 0{,}0001$). Vidare visade det signifikanta skillnader mellan de två slakterierna där data samlats på svin ($p \leq 0{,}0001$), men däremot fanns det ingen signifikant effekt mellan slakterierna där data samlats för nötkreatur ($p=0{,}24$). Resultaten visar att tiden från starten på avblodningen till hjärtstillestånd var i genomsnitt 5 minuter och 45 sekunder för nötkreatur och 2 minuter och 58 sekunder för svin. Avslutningsvis föreslås att en definition av begreppet död i djurskyddssammanhang ska baseras på frånvaron av både hjärnans och hjärtats aktivitet. I framtida studier kan dessa resultat användas för att utvärdera om slaktprocessen påverkas av om hjärtat slår samt om det finns några eventuella djurvälfärdsproblem i samband med det.

Nyckelord: slakt, bedövning, avblodning, död, definition, hjärtstillestånd

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GLOSSARY

The glossary is specific for this study and used in order to clarify terms and words that are frequently used, and is of importance for understanding the subject.

Animal welfare:

How well an animal is coping with the conditions in which it lives. Describes the state of an animal which refers to health, comfort, feed and the level of which it can express innate, normal behaviour. Furthermore, it refers to the animal's state of suffering from pain, fear and distress.

Auscultation:

A non-invasive method for monitoring the heart sounds with a stethoscope.

Cardiac arrest:

The heart fails to contract successfully due to non-functional circulation of blood in the body or due to other reasons, i.e. cardiac dysfunction or biological reasons.

Chest stick:

Severing carotid arteries and other blood vessels in the animal's chest to cause bleeding. In cattle, two cuts are made; the first to remove the hide on the brisket and the second to open the blood vessels. In pigs, only one stick is made to open the blood vessels.

Consciousness (sensibility):

The ability of an awake animal to perceive stimuli from the external environment.

Electroencephalography (EEG):

Electrical activity of the brain usually recorded from the surface of the skull using non-invasive techniques.

Electrocardiography (ECG/EKG):

Electrical activity of the heart recorded by electrodes adhered to the skin or shown by a device externally attached to the body.

Electrocorticography (ECoG):

Electrical activity of the brain usually recorded on the surface of the brain or a membrane covering the brain.

Euthanasia:

The act of inducing death of an animal with a method that causes rapid and irreversible loss of consciousness with a minimum of pain and distress.

Exsanguination (bleeding):

The process when central arteries are cut to bleed the animal. Due to the loss of oxygen and nutrients to the brain it will eventually cause the animals' death.

Humane killing and bleeding:

When the handling and killing procedure results in insensibility to pain within a short period of time, without causing unnecessary pain and suffering of the animal.

Killing:

The deliberate process of slaughtering (i.e. stunning and exsanguination) which cause the death of an animal.

Neck cut:

Severing the carotid arteries and other blood vessels in the animal's neck to cause bleeding.

Slaughter:

The deliberate process of killing the animal with purpose to use the meat for human consumption.

Standard operating procedure (SOP):

The individual slaughter plants' standards to achieve uniformity of the performance of a specific function, i.e. regulates stun-to-stick intervals, according to EU regulation 1099/2009.

Stunning:

The method that makes an animal unconscious and incapable of sensory capacity without causing unnecessary pain, fear and suffering of the animal. The stunning method must result in instantaneous loss of consciousness.

Stun-to-stick interval:

The time from achievement of effective stunning to start of exsanguination. Maximum time for this is regulated in the slaughter plants SOPs.

Unconsciousness (insensibility):

The absence of consciousness and responsiveness in animals due to brain damage from the stunning method, e.g. gun, captive bolt, carbon dioxide or electricity. The individual is unable to perceive external stimuli and control its voluntary mobility and respond to normal stimuli, including pain.

1 INTRODUCTION

Death is a subject that most people avoid talking about, possibly due to the uncertainty of when it will occur. For domestic animals on the other hand, humans predominantly have the role in determining when they take their last breath. In the 20th century, during industrialisation, the slaughter industry grew and became an important business for the killing of animals. Since then, different methods for killing animals have been used and brought up for discussion. These differ between countries and depend, among other things, upon tradition and religion. An important aspect of slaughter is animal welfare (Grandin 2010), which is a practical and emotional issue that needs to meet the producers', consumers' and politicians' demands. However, the purpose of all slaughter plants is to produce food for human consumption. No matter where they are located in the world, the animal arrives at the slaughter plant alive and leaves it dead.

To determine the time of death of an animal, attempts have been made to establish specific principles. Conversely, this has ethical, regulatory and industrial concerns (Newhook & Blackmore 1982a) which make it hard to define criteria that can be accepted worldwide. Those engaged in the definition of death in humans have said it is too controversial to use for comparisons with animals. A compassionate way to describe death is if the stunning method produces permanent insensibility which ensures that the animal cannot regain consciousness (EFSA 2004). However, stunning can induce unconsciousness instantly, gradually or followed by exsanguination. Slaughter without stunning has been a topic of special concern for a long time (FAWC 1984; Gregory et al. 2010).

The standard methods for investigating the time of unconsciousness are electroencephalography (EEG) or electrocortiography (ECoG) (Bager et al. 1992). Newhook & Blackmore (1982b) also did complementary electrocardiography (ECG) and found that the heart was beating long after the EEG signals had ceased. Yet, no studies have examined the exact time from stunning and bleeding to time of cardiac arrest. In order to determine a definition for death, this is an important aspect to be considered in addition to the onset of unconsciousness, especially when deliberately killing animals without prior stunning.

This leads to the key question: when is an animal dead? Is it when the brain stops functioning, or is it when the heart activity has ceased, or does it perhaps depend on both these factors? What exactly is it that determines the death of an animal? The aim of this thesis is to investigate the heart activity after stunning and exsanguination of cattle and fattening pigs in conventional commercial slaughter plants in order to determine the exact time of cardiac arrest. The possible effects this will have on the slaughter activities will be discussed, as well as factors that influence heart activity. Also, a recommended definition of death will be proposed.

2 LITERATURE REVIEW

2.1 Background

The Council Regulation EC 1099/2009 regulates the protection of animals at the time of killing and slaughter. This applies in all countries that are members of the European Union and therefore sets the standard for Sweden as well. However, since Swedish legislation has had stricter regulations for animal welfare long before the introduction of this directive, the country has been allowed to continue with this. The current Swedish Animal Welfare Act (SFS 1988:534) and the Animal Welfare Ordinance (SFS 1988:539) are complemented with "*Statens jordbruksverks föreskrifter och allmänna råd om slakt och annan avlivning av djur*" (The Board of Agriculture's regulations and standard procedures for the slaughter and euthanasia of animals) (SJVFS 2012:27 L22).

The World Organisation for Animal Health (OIE) has outlined welfare standards for slaughter and killing of animals. These are, however, bare minimum standards that both the developed and developing countries have agreed upon. Additionally, each country has independent welfare laws and standards (Grandin 2010; OIE 2009). Another organisation, the UK based Farm Animal Welfare Council, states that the stunning method should always result in minimal distress to the animals (FAWC 1984). They have defined the basic principles that must be implemented in order to maintain a good standard of animal welfare during slaughter activities. One of these principles states that there should be a "guarantee of non-recovery from that process until death ensues" (FAWC 2003).

In the Swedish Animal Welfare Act, one paragraph is of particular interest for this project:

Section 14
§1 Domestic animals shall be stunned before being bled out prior to slaughter. No other measures may be taken in connection with slaughter until the animal is dead.

This brings about two important questions. First, when during the slaughter process is the animal considered to be dead? And second, is there a definition of death in animals? These two important aspects and how they relate to the loss of brain and cardiac function have been discussed extensively. Yet, no established or accepted definition for death of an animal has been identified. According to the Swedish Board of Agriculture (2009) and EFSA (2004) the animal is considered as dead when:

- All the reflexes have stopped;
- The animal is completely relaxed;
- The heart has stopped beating and the animal has no pulse;
- The breathing has ceased.

In summary, this means that no further measures, e.g. decapitation or de-hiding, can be performed on the animal until the heart has stopped beating and the animal is dead. It is not known at how many slaughter plants that it is properly ensured that the heart has stopped beating before any other measures are performed.

2.2 Slaughter techniques for different species

The slaughter techniques differ between species due to economical, anatomical, practical and other reasons (Atkinson et al. 2013). In the following section, the methods most frequently used in the commercial slaughter of cattle and fattening pigs in Sweden are described. It is important to keep in mind that regarding all animal species, slaughter without stunning is not allowed in Sweden (SFS 1988:534). Also, the stunning method should induce unconsciousness and insensibility in the animal, which must remain until the animal dies from blood loss (EC 1099/2009). The DialRel project aims to inform and encourage to constructive dialogue about religious slaughter (slaughter without prior stunning) and address the animal welfare considerations in connection to this (von Holleben et al. 2010).

The slaughter process of animals consists of several steps (Figure 1), but when using the term slaughter in the continuing text it will refer to the killing procedure (i.e. stunning and exsanguination) only.

Figure 1. The steps involved in the slaughter process (FAO 2001).

Humane and ethically correct slaughter usually consists of two steps. The first part is the stunning procedure. This must result in immediate insensibility (Newhook & Blackmore 1982c) and unconsciousness. The second part is exsanguination, where the actual death of the animal results from severance of blood arteries and veins, which lead to blood loss (EFSA 2004). The stun-to-stick interval is the time from the moment of achieved stunning to start of exsanguination. When both of these procedures have been performed, the slaughter man is obliged to verify that the animal is dead.

2.2.1 The slaughter process of cattle

The legally accepted stunning methods for cattle in Sweden are: captive bolt, pneumatic-powered stunners, and free bullet rifle (bullet or shotgun) (SJVFS 2012:27). The mechanism of the captive bolt is that a driven gun-powder cartridge penetrates the skull and brain. The force of the bolt lead to brain concussion and combined with the penetration of the brain, it leads to bleeding and swelling of the brain stem which will make the animal unconscious and insensible (Grandin 2010). The structural damage of the brain leads to subarachnoid and intraventricular haemorrhages, and it is the major haemorrhages which ensure a permanent unconsciousness (EFSA 2013a). Therefore, it is important to remember that it is the bleeding of the brains basal parts that lead to irreversible unconsciousness (Atkinson et al. 2013). This is similar to the damage of a free bullet rifle except that a bullet not is fired when using captive bolt (Figure 2). The pneumatic-powered injection stunners are driven by air pressure and have the same mechanism as a captive bolt (de Oliviera Roca 2002; Gallo et al. 2003). However, air injection stunners are not allowed in Sweden. All of these stunning methods will cause a severe and irreversible damage to the brain and make the animal immediately unconscious (Smulders & Algers 2009).

If the stunning is succeeding, the brain concussion leads to an immediate collapse of the animal and it stops breathing. This is followed by a tonic seizure, where the animal shows an arched back, flexed legs and fixed eyes. In connection with this, the animal loose its' muscle tone. In this stage and after the animal has been shackled, the ears are hanging, the jaw is relaxed and the tongue is hanging flaccid. In order to determine absence of consciousness, palpebral, corneal and pupillary tests can be performed. These are testing if the animal show reflexes when touching the eyelashes, inner/outer corner of the eye, the cornea or if the pupil is responding when directing bright light on it. Since these tests would require brain control to respond to external stimuli's, it is a valid way to test absence of conscious (EFSA 2013a).

Figure 2. Left: stunning with gun for free bullet,
Right: stunning with captive bolt gun.

After ensuring that the animal has been stunned properly and is unconscious, the exsanguination can be started. There are two different kinds of exsanguination methods: through chest (thoracic) stick or neck cut (Figure 3). Chest stick is the most commonly used method in Sweden. In order to ease the procedure the hide where the stick is going to be is removed before the cutting of jugular veins and carotidal arteries is done (Jordbruksverket 2009a). The maximum interval from stunning to exsanguination is not regulated, but it is stated that it should start as soon as possible after stunning. The slaughter plants have restrictions for this in their Standard Operating Procedures (SOP). However, the maximum interval stated in the SOPs are often 60 seconds, just as it was before the removal of the restrictions from the national legislations (SJVFS 2012:27). The continuing slaughter process can then be performed in two different positions, either where the animal is hoisted in one of its hind-legs (vertical slaughter), or is laid vertically on its side or back (horizontal slaughter).

If the exsanguination is accurate on unconscious animals, they would not show recovery from this stage at any point during the bleeding process. On the other hand if the stunning is ineffective, the start of exsanguination is extended or the exsanguination is insufficient with the bleeding taking longer time, the animal can regain consciousness (EFSA 2013a). Therefore, it is crucial to ensure unconsciousness in the animal after stunning and before the exsanguination is initiated.

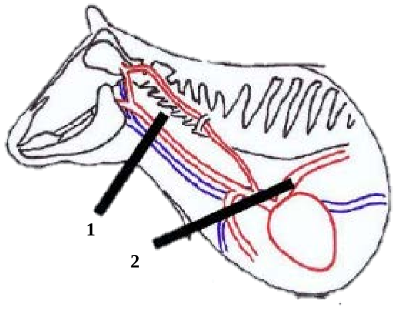

Figure 3. The positions of exsanguination for cattle with neck cut (1) and chest stick (2) (EFSA 2004).

2.2.2 The slaughter process of pigs

Fattening pigs are pigs fed to a weight of approximately 120 kg for slaughter (Christiansen 2005). Permissible slaughter methods for pigs are stunning with captive bolt, free bullet rifle (bullet or shotgun), electricity or carbon dioxide (CO_2) (Figure 4). The most common method at Swedish slaughter plants is stunning with CO_2 in a paternoster system (Jordbruksverket 2009b). This type of stunning exposes groups of pigs to concentrations of usually more than 80 % CO_2 and induce metabolic acidosis (low blood pH). Due to the inhibition of brain activity the animal will gradually lose consciousness and sensibility. However, different parts of the brain differ in sensitivity to CO_2 and the sign observed on the animal after exposure to the gas varies. It is important that the pigs are exposed for a long time to high concentration of CO_2 in order to remain unconscious and insensible during the whole exsanguination process. The first sign of unconsciousness is when the pigs lose their posture. This can be seen in connection to convulsions. Worth noticing is that the convulsions can be seen both before loss of posture and after. After some minutes, the convulsions will stop and lead to loss of muscle tone. The breathing will cease gradually, and eventually result in complete cessation of all respiratory activity. Observed signs of unconsciousness are among others fixed eyes, dilated pupils, no corneal reflex (EFSA 2013b). After ensuring that the animal is sufficient stunned, the exsanguination is performed with a chest stick severing the jugular veins and carotid arteries. During the exsanguination, the animal should not show signs of recovery from unconsciousness (Jordbruksverket 2009b).

Stunning with CO_2 is a potentially reversible technique depending on concentration and duration of exposure which means that the animal can regain consciousness and sensibility (Newhook & Blackmore 1982a) if exsanguination is not carried out immediately afterwards. In cases where the animal regains consciousness, vocalisation, breathing, spontaneous blinking and kicking of the hind legs can be observed.

There are two common methods used for stunning animals with electricity: head-only (electrodes are positioned on the head of the animal) and head-to-back (electrodes are applied to the head and to the mid-thoracic region) stunning. Both these methods produce an epileptic activity in the brain which leads to loss of sensibility, but do not lower the blood pH (Holst 2001). Currently in Sweden, only head-only electrical stunning is used.

Figure 4. Directly after being stunned with CO_2, the pigs are hoisted up by their hind leg and exsanguination starts (background).

2.3 Blood volume and heart rate in animals

The definition of heart rate (HR) is the number of heartbeat per minute (Lefcourt et al. 1999). The HR varies between species and individuals and depends on several different factors, e.g. age, body mass, degree of sexual excitement and stress (Broom & Johnson 1993) (Table 1). The weight of the heart and stroke volume is negatively correlated with body mass. Therefore, the cardiac output is higher in small animals than in large animals, which also depends on the metabolic rate per unit body mass, which is higher in small animals than large animals (Sjaastad et al. 2010). Another important factor that influences HR is stage of lactation, and it also fluctuates if the animal is pregnant. This was investigated in 1951, and brought up the question of which proportion of the variability in HR caused by body size is due to differences in rates of metabolism (Thomas & Moore 1951). It has also been shown that the pigs HR are changing due to external influences, i.e. transportation, pre-slaughter activities and time in lairage (Blokhuis et al. 1998).

Table 1. *Heart activity during rest for different species and the volume of blood in an animal per kilo body weight (BW) (Sjaastad et al. 2010)*

Species	Heart beat per minute (BPM)	mL blood/kg BW
Cattle	60-70	62-77
Pigs	60-80	50-60

How much blood an animal contains depends on, among other factors, body size, physical fitness and age. Usually, more physically active animals have a larger blood volume than those less physically active. Since blood volume increases proportionally with lean body mass, fatter animals tend to have lower blood volume than lean ones of the same body mass. Older animals are proven to have more fat than younger ones, which means that they have lower blood volume relative to body weight than younger animals (Sjaastad et al. 2010). One study that investigated the effect that cardiac arrest had on blood loss in pigs indicated that the average blood yield from pigs at slaughter is usually 4.1% of their live weight, which corresponds to approximately 4.7 litres of blood (Warriss & Wotton 1981).

2.4 Methods for measuring heart rate

Measuring heart rate has had a central role for indicating health and welfare parameters in many vertebrate species over the years (Broom & Johnson 1993). It was first introduced in the beginning of the 1950s where HR and increased feed intake in dairy cattle was studied (Thomas & Moore 1951). The heart activity is controlled by the autonomic nervous system (ANS), i.e. the activity of both the sympathetic and parasympathetic branches. One consequence of this is that HR varies even in the absence of physical or physiological stress, which is termed heart rate variability (HRV) (Hagen et al. 2005). Today, a considerable amount of research is focused on the emotional state of the animal in relation to its social interactions, human handling and similar stress-inducing situations (Kovács et al. 2014).

In order to measure HR, monitors can be implanted surgically or used externally. There are advantages and disadvantages with both of these methods. The biggest disadvantage with surgical interventions is those in terms of animal welfare, but also difficulties with the surgery and the cost. It is possible that it may change physiological functions as well. On the other hand, the advantage is that it allows for recording during the animal's recovery period after surgical trauma or similar situations. The external monitors can be based either on detection of arterial pulses or electrocardiography (ECG) signals. The obstacle faced when using implanted monitors is more than likely the thickness of the animals' hide and body fat, and ECG problems are often related to low signals and instability of electrodes (Lefcourt et al. 1999). Here, the focus will be to explain two of the external methods for monitoring HR.

2.4.1 Stethoscope

A relatively cheap and simple method for measuring HR is auscultation using a stethoscope. By placing the stethoscope on the left chest wall in the region of the heart, HR sounds can be observed (Thomas & Moore 1951). This method is the most important part of examining the cardiovascular system in cattle (and other species) in order to detect normal heart sounds and cardiac rhythm (Rezakhani & Zarifi 2007).

Auscultation with stethoscope has been used as the method for validating the stunning technique and to ensure cardiac arrest after slaughter of pigs (Vogel et al. 2010). Similarly, auscultation of the chest by stethoscope was proven to be an effective method for detecting cardiac dysfunction when calves were stunned by "head-to-back" electricity, i.e. two electrodes were placed on the back of the head and a third electrode on the mid thoracic region inducing electricity (Newhook & Blackmore 1982c). The study indicated that when the cardiac dysfunction was stable, the animals were unable to regain consciousness. However, one study revealed issues with electronic stethoscopes in slaughter environment due to background noises and declared this to be an unsuitable method for recording heartbeats (Blackmore & Petersen 1981).

2.4.2 Electrocardiography (ECG/EKG)

The electrocardiography shows the heart activity and provides information about the heart's functions. It records the electrical activity of the heart by electrodes adhered to the skin or shown by a device externally attached to the body, and records electrical impulses from the polarisation and depolarisation of the cardiac tissue. The outcome is an electrocardiogram, which illustrates the heart rhythm and distinguishes any abnormalities, e.g. regularity of heartbeats or damage to the heart (Sjaastad et al. 2010).

2.5 Definition of death in humans

In the Swedish Act (SFS 1987:269) on criteria for determining human death it is declared that a person is legally dead when the brain's functions are lost. Furthermore it states that death has occurred if breathing and blood circulation have ceased and the armistice lasted so long that it can be safely determined that the brains function is lost. When it has been determined that death has occurred, medical efforts can be continued if necessary. It is apparent that this is very similar to the statements made in the animal welfare act; as humans must also be determined dead before any further measures may be taken.

According to Troug (1997), the definition of death in humans is: "The permanent cessation of functioning of the organism as a whole". Furthermore, the criterion for death is: "The permanent cessation of functioning of the entire brain". When determining the death of an individual, he states that tests must be performed to ensure that this criterion is fulfilled. One of the tests is based on the traditional method of the absence of normal circulation and respiration and the other on testing the neurological standard with numerous tests that ensures the loss of brain function.

2.6 Definition of death in animals

As mentioned in the introduction, if the slaughter is to be humane, no further measures shall be performed on the animal until it is dead (FAWC 1984; Grandin 2010). The act of inducing humane death in animals is called euthanasia, i.e. a "good death" and is said to occur without causing pain and distress (Andrews et al. 1993).

To define what death means has been an issue that has engaged society and scientists for centuries. There is a controversial aspect since death of humans cannot be compared with death of animals due to cultural reasons, and therefore the same definition cannot be used. However, research has shown that signs used to confirm brain death in humans can be used in animals as well (Guerit 1999). There are also similarities between humans and animals in the way the body functions. For example, death of an organism, no matter if it is human or animal, occurs in stages when the tissues' functions are decreasing and in the end stop working (Newhook & Blackmore 1982a).

2.6.1 Unconsciousness and insensibility

Unconsciousness is defined as the loss of brain function through a deficiency of nutrients and oxygen in the brain, which will cause brain damage and eventually death (EFSA 2004). Studies have focused on examining when time of insensibility occurs in cattle, sheep and pigs (Daly et al. 1988; Bager et al. 1992; Warriss & Wotton 1981). Most of them have focused on stunning with electricity and measured the degree of unconsciousness with EEG, and sometimes ECG, but also stunning with CO_2 in pigs (Table 2).

Table 2. *Insensibility period (in seconds) for cattle and pig using different stunning methods (Grandin 1997)*

Species	**Conventional electric stunning**	**Conventional mechanical stunning**	**Stunning by exposure to CO_2**
Cattle (6 months to adults)	21-41 (Lambooy & Spanjaard 1982)	< 1 (EFSA 2004)	-
Pigs	35 +/- 12 (Swatland et al. 1984)	< 1 (EFSA 2004)	> 60 (Rodriguez et al. 2008)

Note: all of these studies are measured with electroencephalographic and electrocorticograms.

During slaughter without prior stunning, it is likely that the animal feels pain and suffers sine the time needed to produce unconsciousness from bleeding often is longer than the insensibility period (Grandin 1997). Similarly, if stunning is applied but does not properly induce unconsciousness and the animal continues to the next stop, the exsanguination, it will be exposed to stress, pain and suffering until it loses consciousness due to blood loss (Algers et al. 2006). Other factors that influence when the time of unconsciousness occur are the skills of the slaughter man (inducing unconsciousness on the first stunning trial or not), correct bleeding method and the precision of the stick during exsanguination. Furthermore, slaughter plants have different production capacities dependent on construction of the plant and personnel size, among other factors (Atkinson et al. 2013) which also influence the slaughter process.

Newhook & Blackmore (1982a) suggests that when permanent insensibility has occurred, the animal can be considered dead according to the legal terms of slaughter. The most important task is to ensure that the animal is permanently unconscious when it's brought from the exsanguination to the next step i.e. de-hiding, scalding, evisceration. Therefore, if knowledge is obtained on how long the heart continues to beat after stunning, it can be guaranteed that the animal is completely dead and insensible for the rest of the slaughter process.

2.6.2 Determination of death

The slaughter process must lead to permanent insensibility in order to be defined as humane (FAWC 1984; Grandin 2010), and this was discussed by Newhook & Blackmore (1982a). They took the initiative to study onset of insensibility by performing electroencephalographic (EEG) studies of sheep after exsanguination. This led them to the following definition of death for animals at slaughter: "*Irreversible insensibility due to cerebral anoxia caused by complete severance of both common carotid arteries and the vessels from which they arise.*" Accordingly, ECG measurements were performed on these sheep and they indicated that the heart continued to beat rhythmically for often 10 minutes or more after the EEG signals had ceased. Furthermore, they detected an early increase in HR and a later increase in amplitude as the bleeding proceeded. This is similar to their subsequent study on calves where heart activity was detected for about 6 minutes after EEG recording had ceased (Newhook & Blackmore 1982b).

An existing explanation of death in animals is a physiological state in which respiration and blood circulation no longer functions and the respiratory and circulatory centres are irreversibly inactive. In other words, the animal cannot regain consciousness (Figure 5). For the slaughter process this means that death can be seen as the absence of brain stem reflexes through destruction of the brain, e.g. no corneal reflex, and absence of cardiac activity through bleeding, e.g. no pulse or heartbeats (EFSA 2004).

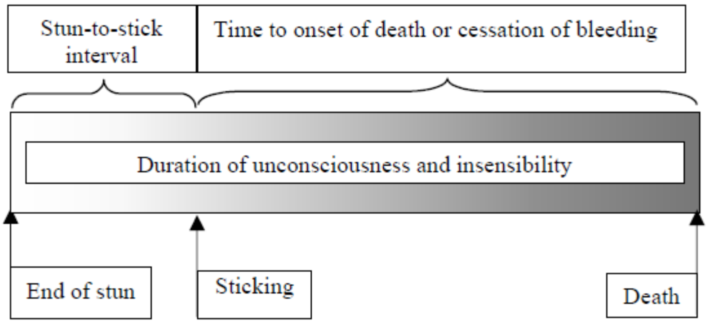

Figure 5. The killing procedure required in order to be classified as humane slaughter (EFSA 2004).

2.6.3 Criteria to determine insensibility and death

In order to determine when the death of an animal takes place, some criteria have been established. However, these are not accepted worldwide and differ between countries (Grandin 2010). For stunning with a penetrating captive bolt in cattle and pigs, an immediate collapse must follow for the stun to be classified as completely successful. Furthermore, the loss of corneal reflex must occur instantly after the stun (EFSA 2004). To continue, EFSA states that the corneal reflex is typically the last to disappear during loss of consciousness (or time of death). However, it is the first to reappear if the stunning is improper and the animal regains conscious. The criteria to determine insensibility and death differ slightly between species, but are mostly dependent on stunning technique.

The most important signs to assess insensibility after stunning and exsanguination, applicable on both cattle and pig (Grandin 1994) are as follows:

- The animal hung downward and make no attempt to regain an upright position;
- No rhythmic breathing;
- No eye blinking;
- No corneal reflex;
- No vocalisations;
- Gasping movements are permissible;
- Uncoordinated reflexive limb movements can be observed but is not of importance;
- The tongue is hanging straight down and is flaccid.

Furthermore, recent reports by EFSA (2013a; 2013b) identify three key stages and indicators of how to ensure successful stunning leading to unconsciousness when slaughtering cattle with captive bolt and pig with carbon dioxide. These are:

1. Key Stage 1: between end of stunning and shackling.
2. Key Stage 2: sticking (either by chest stick or neck cut).
3. Key Stage 3: during the exsanguination.

Also here, important signs are absence of breathing, muscle tone corneal or palpebral reflex, spontaneous blinking and vocalisations. Also, criteria are used to determine unconsciousness and eventually the death of the animal (EFSA 2013a).

3 MATERIAL AND METHODS

3.1 Pilot study

A pilot study was carried out during a period of three days in order to test the data collection procedure. For this, a stethoscope of model Supra-Bell and a timer were used. The purpose of this was to confirm and adjust the observation technique and the material used for collecting data, but also to determine when during the day the observations could be performed, from a practical point of view.

3.2 Study areas and animals

Initially, six slaughter plants were contacted and three of them agreed to participate. These are mentioned as slaughter plant 1, 2, 3 and 4 in the continuing text. Even though three slaughter plants were involved in the study, they will be mentioned as four in order to guarantee anonymity, as one of them slaughter both cattle and pigs. The study was performed on cattle and pigs of different breed, sex and age. In total, 108 cattle and 157 pigs were examined (Table 3). The reason for choosing cattle and pigs as study animals was purely practical. Sheep were initially intended to be included in this study but since the slaughter plants accepted only slaughtered sheep in small volumes during this time of the year, it was not possible to get sufficient amounts of data.

Table 3. *Distribution of slaughter plants and study animals*

Slaughter plant	**Cattle**	**Pigs**
1	69	-
2	39	-
3	-	103
4	-	54
Total	**108**	**157**

3.3 Methods

Time was measured from stunning until cardiac arrest. The parameters of interest were the end of stunning, start of exsanguination and time when last heart beat was detected (Appendix I). To do this, auscultation with stethoscope was performed and time was measured with a stopwatch. Two different stethoscopes was used, one Supra-Bell Ges. Gesch© stethoscope (method 1) and one electronic stethoscope Littmann© 3100 (method 2). The age, breed (milk/beef), sex (bull/steer/cow or gilt/barrow) start of stunning, start of exsanguination and time when heart beats ceased was recorded in a protocol (Appendix 1). One person performed all the data collection.

Since data was collected from commercial slaughter plants during normal working hours, the process had to be adjusted depending on breaks and periods with higher or lower speed during the day. Therefore, data collection was spread throughout different time periods, before and after breaks, and the first or the last animals of the day. Moreover, data were randomly recorded on both cattle and pigs without noting breed and sex until the observation started.

3.3.1 Method 1

The timer was started when the slaughter man stunned the cattle with either a captive bolt gun or a free bullet rifle. In a few cases, the stunning was unsatisfactory and a back-up method had to be used. However, the time was still measured from the first stunning attempt. After stunning, the animal was shackled in the hind leg and hoisted from the mechanised system in the ceiling. Prior to exsanguination, the slaughter man usually removed a piece of hide on the neck to ease the precision of the stick. However, this was not recorded as the start of exsanguination, and the start of exsanguination was instead defined as the first stick to initiate blood flow from the animal. As soon as possible after the start of exsanguination, the Supra-Bell stethoscope was placed on the animal's left chest, a bit below the foreleg and the heartbeats were recorded until the last sound could be detected. During the observations, no other activities such as de-hiding, removal of forelegs, etc. took place.

Pigs were stunned by exposure to CO_2 with a concentration of on average 90 % for approximately 2 minutes and 30 seconds (± 30 sec) in a paternoster system. After being hoisted by their hind leg they were exsanguinated by a chest stick in a similar way to cattle, although no hide was removed from the chest. If the stunning was not satisfactory, a captive bolt was used to re-stun the animal. However, this did not have any influence on the measurement. Time was measured from the first stick when the blood started to flow out of the animal. Immediately after this, the auscultation began by placing the Supra-Bell stethoscope on the left chest wall, under the left foreleg (Figure 6). Auscultation was made until no heart activity could be detected. Only fattening pigs, which consisted of gilts and barrows, were included in the study, i.e. no recordings were carried out on sows or boars. Since two to four pigs could be stunned at the same time in the paternoster system, auscultation was done randomly on one of the pigs in a group. This means that sometimes the first pig to be hoisted was measured and sometimes the second, third or last pig.

Figure 6. Illustrating how data collection was performed on cattle.

3.3.2 Method 2

The same process as in Method 1 was used for auscultating heart activity on cattle and pigs. The only difference was that instead of a Supra-Bell stethoscope, the electronic stethoscope Littmann ©3100 was used. This instrument could detect heart sounds on two different levels, high and low, and also detect the pulse of the animal.

3.3.3 Palpation

In order to validate these methods, palpation of the heart was carried out. When no heart activity could be detected with the stethoscope, a hand was placed on the heart by using the same aperture made for the exsanguination. This confirmed that the heart beats had ceased. For cattle, this was done with the entire hand touching the heart and for pigs by placing three fingers on the heart. However, palpations were only done randomly as a way of validating the method, and not on all animals.

3.4 Statistical analysis

When analysing data from the observational study, it was first compiled and edited using Excel. The statistical programme SAS 9.3 was used to compare and distinguish differences in time until cardiac arrest in cattle and pigs. Variables of interest for cattle were breed and sex, while for pigs only sex was considered. Age was not included in the analysis of cattle due to the wide variation (6 months to 19 years) and since the groups would be too small for analysis. Furthermore, breed was not analysed on cattle due to the same reason as for the variable age (the group size would have been too small). The pigs' ages were not expected to have a significant effect since fattening pigs are generally slaughtered at an average weight of 120 kg when they are 180 days old (Christiansen 2005) and variation is very small.

Since data were unbalanced, an analysis of variance (ANOVA) was performed using the GLM procedure. The variation between and within different groups of observations could then be identified (SAS, 1999). All the data were normally distributed.

Several analyses were performed for the observations collected on cattle and pigs. First, the two methods were compared using procGLM in order to see if significant differences could be detected. If it showed significant differences, one of the methods would be excluded. Second, observations from the two slaughter plants were compared. This was of interest especially for cattle since one of the slaughter plants had horizontal slaughter and the other had vertical slaughter, although both slaughter plants had applied sticking on vertical animals. In the end, it was necessary to have more than one slaughter plant included in the analysis in order to get valid data. To calculate the time from stunning until the first stick of the exsanguination, average values for cattle and pigs, respectively, were calculated.

3.5 Ethical considerations

Due to confidentiality and ethical reasons, the slaughter plants' location, slaughter capacity, etc. will remain anonymous.

4 RESULTS

4.1 Editing of data

A correction of data was done in order to carry out the analysis:

$$Time\ from\ stunning\ to\ cardiac\ arrest - (stun - to - stick\ interval) =$$
$$= amount\ of\ time\ from\ start\ of\ exsanguination\ to\ cardiac\ arrest$$

Quantitative data are shown in Tables in Appendix II and III. All data were unbalanced.

4.2 Time until cardiac arrest for cattle

4.2.1 Comparison of methods

For comparisons between the stethoscopes, 69 observations from slaughter plant 1 were included. The class variables were method, sex and breed and the model used were "heart stops" equal with class variables and interactions between these. The GLM procedure generated a Type III ANOVA which indicated significant differences between methods ($p \leq 0.0001$). Breed ($p=0.29$) and sex ($p=0.56$) did not have any significant effect on either of the two methods. Similarly, no significant interactions between method and sex ($p=0.65$) or method and breed ($p=0.41$) were revealed. Mean values of time for cardiac arrest using method 1 and 2 are illustrated in Table 4 and 5 (Appendix II). In the rest of the analysis, only data collected with method 2 will be included.

4.2.2 Comparison of slaughter plants

For comparison between slaughter plant 1 and 2, 73 observations using method 2 was included in the analysis. In the model, the effect of class variables (slaughter plant, sex, and breed) on cardiac arrest was analysed. The overall GLM procedure gave a Type III ANOVA which showed no significant effects in the model ($p=0.24$). Sex ($p=0.21$), breed ($p=0.84$) and slaughter plant ($p=0.44$) indicated no significant effects of when heartbeat ceased. Likewise, there were no significant interactions between sex and breed ($p=0.06$) that could have affected the heart activity. Mean values of time for cardiac arrest at slaughter plant 1 and 2 are illustrated in Table 6 and 7 (Appendix II).

4.2.3 Time to cardiac arrest

Mean values of time until cardiac arrest after stunning and exsanguination in cattle was divided into categories by sex, and data recorded with method 2 was included in the analysis (Table 8). To clarify, the data are from animals that were slaughtered using all types of stunning weapons, at all the slaughter plants and all breeds. The interval of time to cardiac arrest of cattle ranged from 183 – 478 seconds.

Table 8. *Mean values of time (in seconds) from start of exsanguination to cardiac arrest in cattle*

	Cardiac arrest		
Sex	**Mean**	**Std.**	**N**
Cows	345	44	40
Bulls & steers	323	53	33

4.3 Time until cardiac arrest for pigs

4.3.1 Comparison of methods

When comparing methods 1 and 2 from slaughter plant 3, a total of 103 observations were included in the analysis. The class variables were sex and stethoscope, and the model was "heart stops" equal with class variables and interactions between these. The same analyses as above were performed and showed a significant difference between methods ($p \leq 0.0001$). However, the method did not have any significant interaction between sex ($p=0.09$), and sex did not have any significant effect of time for cardiac arrest ($p=0.14$). Mean values of time for cardiac arrest at slaughter plant 1 using method 1 and 2 are illustrated in Table 9 and 10 (Appendix III).

4.3.2 Comparison of slaughter plants

To analyse possible effects between slaughter plants on cardiac arrest, a comparison between data from slaughter plant 3 and 4 was made. In this analysis, 105 observations were included with data from method 2. The effect of class variables (slaughter plant and sex) on cardiac arrest was analysed. The ANOVA showed a significant effect in the model ($p \leq 0.0001$). Sex ($p=0.02$) and slaughter plant ($p \leq 0.0001$) indicated significant effects of when heart activity ceased. Mean values of time for cardiac arrest at slaughter plant 3 (Table 10) and 3 are illustrated in Table 10 and 11 (Appendix III).

4.3.3 Time to cardiac arrest

The average time until cardiac arrest occur after stunning and exsanguination in pigs are shown in Table 12. Only data recorded with method 2 are included and data are added together from slaughter plants 3 and 4. The interval of time to cardiac arrest of pigs ranged from 96 – 280 seconds.

Table 12. *Mean values of time (in seconds) from start of exsanguination to cardiac arrest in pigs*

	Cardiac arrest		
Sex	**Mean**	**Std.**	**N**
Gilts	178	25	50
Barrows	169	19	55

4.4 Stun-to-stick interval

The variables included in the analysis were the time between stunning and start of exsanguination, time when heart activity ceased, divided by slaughter plants. This means that the two methods for recording heartbeat, breed, sex, slaughter plant and stunning method were not included as variables because they were assumed not to have any effect on when exsanguination started.

4.4.1 Cattle

In total, 108 observations were included in the analysis. The overall GLM procedure generated a Type III ANOVA which indicated that start of exsanguination, i.e. the stun-to-stick interval, had a significant effect on time to cardiac arrest ($p=0.04$). When calculating mean values for start of exsanguination, it was categorised by slaughter plant to show differences (Table 12). The stun-to-stick interval ranged from 44 – 186 seconds.

Table 13. *Mean values (in seconds) of the stun-to-stick interval in cattle*

	Start of exsanguination		
Slaughter plant	**Mean**	**Std.**	**N**
1	74	16	69
2	54	11	39

4.4.2 Pigs

For the analysis of pigs, 157 observations were included in the GLM procedure. The ANOVA showed no significant effect between time of start of exsanguination and time for cardiac arrest (p=0.50). For the calculations of mean values, a division of slaughter plant was made in order to visualise differences (Table 13). The stun-to-stick interval ranged from 18-85 seconds.

Table 14. *Mean values (in seconds) of the stun-to-stick interval in pigs*

	Start of exsanguination		
Slaughter plant	**Mean**	**Std.**	**N**
3	49	10	103
4	33	8	54

5 DISCUSSION

In accordance to what is known, this is the first study which investigated time of cardiac arrest after stunning and exsanguination. Other studies have evaluated animal welfare during the slaughter process (Grandin 1994; 1997; 2010), the time of onset of insensibility (Newhook & Blackmore 1982a; 1982b; 1982c), the effect of cardiac arrest (Warriss & Wotton 1981; Wotton & Gregory 1986), but not the time to cardiac arrest to my knowledge. Also, there is a variety of reports about the important aspects to consider when slaughtering animals (EFSA 2004; FAWC 1984; OIE 2009). Consequently, even though much is known about the slaughter process and activities in relation to it, the time to cardiac arrest after stunning and exsanguination has not previously been described.

The study was initiated because observations of the slaughter process at one slaughter plant were made and it was noticed that the animal carcasses were proceeding to the next station relatively quickly after the start of exsanguination. Since the law in Sweden states that no further measures may be taken in connection with slaughter until the animal is dead (SFS 1988:534), and there is no agreed-upon definition of when an animal is dead, this study highlights this issue. To find out for how long the heart continues to beat after stunning and exsanguination, measurements needed to be done. The aim of this study was to investigate heart activity after stunning and exsanguination of cattle and pigs in conventional commercial slaughter plants. Additionally, a definition of death in animals will be given so that the results from this study could be used in the creation of further legislations and additional studies building upon the work already done.

MAIN FINDINGS OF THIS STUDY:

- → Significant differences between stethoscopes (method 1 and 2), for data collected both on cattle and pigs.
- → No significant differences between slaughter plants with data collected on cattle.
- → Significant differences between slaughter plants with data collected on pigs.
- → The average times from exsanguination to cardiac arrest in **cattle** were:
    - *Cows (of both beef and milk breed)*: 345 sec (5 min, 45 sec)
    - *Bulls and steers (both beef and milk breed)*: 323 sec (5 min, 23 sec)
    - The stun-to-stick interval had a significant effect on the time until cardiac arrest
- → The average times from exsanguination to cardiac arrest in **pigs** were:
    - *Gilts*:178 sec (2 min, 58 sec)
    - *Barrows*: 169 sec (2 min, 49 sec)
    - The stun-to-stick interval had no significant effect on the time until cardiac arrest
- → The stun-to-stick interval was:
    - *Cattle*: 54 sec and 74 sec for slaughter plant 1 and 2 respectively
    - *Pigs*: 49 sec and 33 sec for slaughter plant 3 and 4 respectively.

5.1 Data collection and related actions

At slaughter plants, the environment is noisy due to the sound of machines operating and people speaking loudly. At full speed of work, the noise levels can be as high as 115 dB depending on where in the slaughter area the measurements were taken (Hultgren et al. 2014). Therefore, there were some difficulties with distinguishing the heart beats from the surrounding environment, especially when observing the time of cardiac arrest. This was taken into consideration before the study began and was planned for when performing the data collection. In order to minimise the surrounding sounds, the data collection was done when the slaughter work started in the mornings when few machines were active. Furthermore, data was collected on the last animals before breaks and the first animals after breaks. This limited the risk of falsely distinguishing the time of the last heartbeats and also the risk of measuring the wrong sound.

Cattle in particular are big animals, and various sounds come from their organs and body when being slaughtered. Therefore, the heartbeats can accidently been mistaken for such sounds. However, this risk is considered as relatively small since examinations on the live animals were done before the study started in order to get familiar with auscultation techniques and heart rhythm.

During the data collection, the slaughter man was asked by the manager at several occasions during the exsanguination process to start cutting in the animal at the same time as auscultations were done. When doing so, an increase in the heart's rate could be heard. If this has any implications on the speed and quality of the exsanguination process is not clear and was not further examined. To confirm that the heartbeats were increasing when cutting the animal, the same aperture as the one used for the chest stick was used in order to palpate the heart and feel the heartbeats. An increase in the frequency of contractions could then be felt. This is not evidence that the animal is conscious, but it is assumed to be a reaction of the skin receptors that are still active during the exsanguination process. The argument that physical contact with the heart would stimulate additional contractions is not hypothesised to be the reason for any mistakes in recording.

Another aspect that needs to be taken into consideration is that only one person performed the data collection. There are different factors that influenced this person's state of mind; one example of this is the attention level, which fluctuates over the duration of the day. However, this is not believed to have affected the data collection in a systematic way.

5.1.1 Possible limitations

One of the biggest limitations was that only three slaughter plants agreed to participate in this study. If it had been possible to compare more slaughter plants, it is possible that the significance level of the study could increase. Another weakness was the issue during data collection to determine the time when the last heartbeat was detected, due to the boisterous surrounding. There is a risk that the heart was beating for longer than could be detected with the stethoscopes.

5.2 Time to cardiac arrest

This study shows that the time from start of exsanguination to cardiac arrest was on average 5 minutes and 45 seconds for cattle, and 2 minutes and 58 seconds for pigs. The factor that was expected to have an effect on time for cardiac arrest was mainly stun-to-stick interval. Sex did not have any significant effects on time until cardiac arrest for either cattle or pigs, and neither did breed for cattle. However, for cattle the sex and breed interaction between slaughter plant 1 and 2 was just about significant. If more data was included in the study it is likely that a significant difference could be identified.

When analysing the data from method 1 (Suprabell stethoscope) and 2 (Littmann 3100 electronic stethoscope) significant differences were found. This related to data collected both in cattle and pigs. The assumption was then made that method 2 was more valid than method 1, and it is suggested that the electronic stethoscope allowed for a clearer observation of heartbeats and detection of high-pitched heartbeats. Factors that could impact the data recording performed both with method 1 and 2 are principally the same, e.g. noise level in the slaughter plants and animal factors (fat, hide thickness etc.).

For cattle, slaughter plant 1 had much higher noise level than slaughter plant 2, which mostly is believed to depend on the sizes of the slaughter plants (e.g. number of persons working, number of machines active at the same time). Other factors that are assumed to influence the data recordings were dirt, blood and water on the animal's body. After the cattle were stunned, they fell out from the stun box on the floor which was occasionally covered with blood in various amounts. Therefore, their body got wet and in some cases it was necessary to briefly wipe the body where the stethoscope was placed. This was only because the wetness of the skin appeared to have an influence on the stethoscope's function.

The pigs, on the other hand, always got wet when falling out from the paternoster system and onto the table. Consequently, it was almost always necessary to remove some amount of the water with a shirt sleeve before auscultation could be started. The noise level was observed to be lower at slaughter plant 4 compared to slaughter plant 3, which eased the data collection with the stethoscope.

The data analysis of recordings on cattle between the two slaughter plants indicated no significant differences. This was expected since there should not be any differences in cardiac arrest between the slaughter plants.

Since slaughter plant 1 had vertical slaughter and slaughter plant 2 slaughtered cattle in a horizontal position, it was assumed to be a difference in time for cardiac arrest. However, at both slaughter plants exsanguination was done in a vertical position, i.e. the animal was hoisted by its hind legs with a chain. It remained this way for the entire duration of data collection at slaughter plant 1. During the normal slaughter process at slaughter plant 2 it was observed that the animal was typically placed in a horizontal position after a maximum of two minutes from the start of exsanguination. This is believed to have an effect on the efficiency of bleeding and hence on the time to cardiac arrest but was not examined and therefore not evaluated further in this study.

The data analysis of recordings on pigs indicated significant differences between slaughter plant 3 and 4, which was not expected. The time to cardiac arrest was longer at slaughter plant 3 compared to the corresponding time at slaughter plant 4. The possible reason is that the stun-to-stick interval is approximately 20 seconds longer at slaughter plant 3 in comparison to that at 4. I can therefore make the assumption that there may be a correlation between stun-to-stick interval and time to cardiac arrest, which seems biologically plausible. However, this was not further investigated in this study.

The exsanguination process will declare gradually. This was observed during the data recordings, and it was also observed that the blood was flushing out from the animal in high speed for three to four minutes from start of exsanguination. After that, the bleeding was gradually declining. This means that even if the blood is dripping from the animal, it has lost a high volume of blood and it can most likely not regain conscious. However, there are many factors influencing this, especially the precision of the stick. This needs to be taken into consideration if the slaughter plants should implement a maximum time for how long the animals need to be bled out before cardiac arrest has occurred and they can continue to the next stop of the slaughter chain. If this would be accepted, further investigations and discussions of a suggested maximum time need to be done. This study suggests that the cattle should be bled out for at least 6 minutes and pigs for at least 3 minutes after start of exsanguination. Hence, this time is not a guarantee that cardiac arrest has occurred on all animals since the intervals of time to cardiac arrest vary widely, both for cattle and pigs. Though, it is an approximate indicator that can be used to ensure death.

5.3 Stun-to-stick interval

The stun-to-stick for cattle had a significant effect on time to cardiac arrest. Differences between slaughter plants were not analysed. However, slaughter plant 1 was about 20 seconds slower to start the exsanguination process compared to slaughter plant 2. There are various reasons for this. Firstly, the task of performing the stunning and exsanguination on slaughter plant 1 altered between three different employees while at slaughter plant 2 it was always the same person. The individuals' personality, behaviour and skills were observed and are believed to influence the stun-to-stick interval. Secondly, the construction of the buildings has an impact on how fast exsanguination can start after stunning. At slaughter plant 1, the slaughter person has to go down a staircase, around a corner and jump over the dead cattle on the floor to reach the equipment used for hoisting the animal. This is in contrast to slaughter plant 2, where the stun box is connected to the equipment used for hoisting. This was observed to provide better and faster possibilities for the start of exsanguination after stunning.

The stun-to-stick interval for pigs did not have a significant effect on time for cardiac arrest. However, there was a 16-second difference between slaughter plant 3 and 4 where slaughter plant 4 was faster. If more data was available it is likely that a significance level would be obtained. Similar to the factors identified for exsanguination of cattle, the construction of the slaughter plant and the individuals' behaviour and skills are believed to influence the onset of exsanguination. The technical equipment provides better possibilities in slaughter plant 4 for a quicker start of exsanguination. However, at slaughter plant 4 two persons were working with hoisting the pigs by their hind legs after stunning. This enabled a higher speed, although only one person performed the exsanguination.

5.4 Definition of death in animals

Currently, there is no established definition of death in animals. Even though some studies have described death (Newhook & Blackmore 1982a; EFSA 2004) none of these descriptions have been generally accepted or adopted worldwide. Several studies have focused on investigating how animals react to external stimuli and measurements have been taken on how they perceive different situations and how it affects the expressed level of stress (Broom & Johnson 1993; Blokhuis et al. 1998). This has led to great difficulties in trying to declare what it actually means when an animal is dead.

When slaughtering animals, the actual death is unavoidable since it is the purpose of the slaughter process. Not having a definition of what actually counts as dead will lead to misunderstandings. However, the present Swedish Welfare Act, where it is stated that no other measures can be taken until the animal is dead, can only be interpreted that it has an ethical aspect which needs to be considered. Nonetheless, there is no formal definition of death in animals in Sweden. The Swedish Board of Agriculture has tried to clarify this and states that it is when all the reflexes are gone, the animal is totally relaxed and breathing has ceased. But most important is that they state that the animal is dead when the heart activity has stopped, and the animal has no pulse. Researchers found as early as in 1982 (Newhook & Blackmore) that an animal's heart continues to beat long after the EEG signal had ceased. Similar studies have also indicated that stunning with electricity in some cases lead to immediate cardiac arrest.

This leads back to the question from the introduction: when is an animal dead? In order to induce a "good death" the animal should die without pain and distress (Andrews et al. 1993) i.e. the animal should be unconsciousness and insensible to external stimuli. An existing explanation of death in animals is a physiological state in which respiration and blood circulation no longer functions. Indications of how long the heart continues to beat after stunning and exsanguination can complement the present definitions. This will provide a guarantee that the animal is completely dead and insensible for the rest of the slaughter process, both by means of absence of brain and cardiac function. To conclude, this study suggests that an animal should be determined as dead when brain activity is irreversibly lost and cardiac arrest has occurred.

The death of an animal occurs when the brain and heart stops functioning. Before death by blood loss, the stunning method which is used to induce loss of brain activity, i.e. unconsciousness and irreversible insensibility, should not cause pain or suffering for the animal. To ensure this, the main indicators for successful stunning are instantaneous absence of rhythmic breathing, absence of eye reflexes (eye movements and blinking), and absence of vocalisation. Furthermore, the animal should not show any attempt to regain an upright position if hanging downwards, or to stand up if lying down horizontally. The state of unconsciousness must be confirmed before the start of exsanguination, i.e. severance of carotid arteries, jugular veins and blood vessels. During the bleeding process, no further slaughter measures can be performed on the animal until cardiac activity is absent and cardiac arrest has occurred. This has here been shown to take approximately six minutes for cattle and three minutes for pigs. After this, death has occurred.

Severe brain hypoxia, leading to irreversible loss of brain functions, will most likely occur prior to cardiac arrest. However, it is here suggested that cardiac arrest must be achieved to ensure death, as brain functions cannot be properly monitored during the standard commercial slaughter process.

Based on this, the recommended definition of death of animals (in relation to slaughter) will be:

The death of an animal at slaughter can be established when brain functions have been lost and cardiac arrest has occurred, as a result of stunning and exsanguination.

5.5 Suggestions for future research

In order to be able to confirm the results in this study, further studies with electrocardiography (a reliable method) should be performed. Also, similar studies would be intriguing to perform on sheep and broilers. Only three slaughter plants chose to participate in this study and therefore it would be motivated to include more slaughter plants in an upcoming study. The similar experimental design as for auscultation in this study could be used. Another area that would be of interest is to investigate how long time it takes until cardiac arrest for animals that are slaughtered without prior stunning. An ethical approval would be necessary for performing such a study in Sweden. In future studies, this can be used to evaluate the possible implications this would have for the slaughter process and also for animal welfare. The last suggestion is to evaluate if there are correlations between the stun-to-stick interval and time to cardiac arrest. Lastly, an evaluation if the recommended definition of death could be used in the national legislations and around the world is necessary.

6 CONCLUSIONS

In conclusion, this study suggest that the definition of death should be based both upon loss of brain and cardiac function. It was found that it takes nearly six minutes until cardiac arrest after exsanguination following stunning in cattle and that the corresponding time in pigs is around three minutes. One important factor that affects for long the heart beats after slaughter is how soon after stunning the exsanguination starts. This is influenced mostly by the construction of the slaughter plant; some layouts allow for a timelier opportunity for the stunning person to begin exsanguination. However, it can also be influenced by the personality, skill level and behaviour of the slaughter person. Since the Swedish Welfare Act states that no further measures can be taken on the animal until death has occurred, the recommended definition in this thesis can be implemented in the legislation and be used to clarify what death means.

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Josefine
2014

In order to be irreplaceable, one must always be different.
-Coco Chanel

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APPENDIX I

The protocol used for observations on cattle and pigs (except breed and age was not registered for pigs).

DATE: **STUNNING METHOD:**

NUMBER	BREED	SEX	AGE	START OF EXSANGUINATION	CARDIAC ARREST

APPENDIX II

CATTLE

COMPARISON OF METHOD 1 AND 2

Table 4. *Mean values of time (in seconds) from start of exsanguination to cardiac arrest at slaughter plant 1, using method 1*

		Captive bolt			Gun		
Breed	**Sex**	**Mean**	**Std.**	**N**	**Mean**	**Std.**	**N**
Beef	F	251	13	5	232	38	2
	M	287	-	1	251	32	9
Dairy	F	258	42	12	201	73	3
	M	-	-	-	203	6	2

Table 5. *Mean values of time (in seconds) from start of exsanguination to cardiac arrest at slaughter plant 1, using method 2*

		Captive bolt			Gun		
Breed	**Sex**	**Mean**	**Std.**	**N**	**Mean**	**Std.**	**N**
Beef	F	478	-	1	304	18	8
	M	380	-	1	334	33	6
Dairy	F	338	31		348	59	4
	M	-	-	-	319	29	9

COMPARISON OF SLAUGHTER PLANT 1 AND 2

Table 6. *Mean values of time (in seconds) from start of exsanguination to cardiac arrest at slaughter plant 1, method 2*

		Captive bolt			Gun		
Breed	**Sex**	**Mean**	**Std.**	**n**	**Mean**	**Std.**	**N**
Beef	F	289	93	6	289	37	10
	M	334	66	2	284	53	15
Dairy	F	284	54	18	285	98	7
	M	-	-	-	298	54	11

Table 7. *Mean values of time (in seconds) from start of exsanguination to cardiac arrest at slaughter plant 2, method 2*

		Gun		
Breed	**Sex**	**Mean**	**Std.**	**N**
Beef	F	342	29	12
	M	350	36	5
Dairy	F	369	37	10
	M	305	74	12

APPENDIX III

PIGS

COMPARISON OF METHOD 1 AND 2

Table 9. *Mean values of time (in seconds) from start of exsanguination to cardiac arrest at slaughter plant 3, using method 1*

CO_2			
Sex	**Mean**	**Std.**	**n**
F	127	16	25
M	128	17	27

Table 10. *Mean values of time (in seconds) to cardiac arrest at slaughter plant 3, using method 2*

CO_2			
Sex	**Mean**	**Std.**	**n**
F	193	26	24
M	181	15	27

COMPARISON OF SLAUGHTER PLANT 3 AND 4

Table 11. *Mean values of time (in seconds) to cardiac arrest at slaughter plant 4, using method 2*

CO_2			
Sex	**Mean**	**Std.**	**n**
F	164	14	26
M	158	16	28

Mean values of time to cardiac arrest at slaughter plant 3 using method 2 are shown in table 10.

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