SCIENTIFIC OPINION

Scientific Opinion on monitoring procedures at slaughterhouses for sheep and goats[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2,3]

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ABSTRACT

This scientific opinion proposes toolboxes of welfare indicators for developing monitoring procedures at slaughterhouses for sheep and goats stunned with the head-only electrical method or slaughtered without stunning. In particular, the opinion proposes welfare indicators together with their corresponding outcomes of consciousness, unconsciousness or death. In the case of slaughter with head-only electrical stunning, the opinion proposes a toolbox to assess consciousness at three key stages of monitoring: (a) after electrical stunning and during shackling and hoisting, (b) during neck cutting and (c) during bleeding. For slaughter without stunning, another toolbox is proposed for (a) assessing unconsciousness before releasing the animals from restraint, and (b) confirming death before carcass dressing begins. Various activities—including a systematic literature review, an online survey and stakeholders' and hearing experts' meetings—were conducted to gather information about the sensitivity, specificity and feasibility of the indicators. On the basis of such information, a methodology was developed to select the most appropriate indicators to be used in the monitoring procedures. The frequency of checking differs according to the role of each person with responsibility for ensuring animal welfare. The personnel performing stunning, shackling, hoisting and/or bleeding will have to check all the animals and confirm that they are not conscious following stunning or before releasing from the restraint. For the animal welfare officer, who has the overall responsibility for animal welfare, a mathematical model for the sampling protocols is proposed, giving some allowance to set the sample size of animals to be checked at a given throughput rate (total number of animals slaughtered in the slaughterhouses) and threshold failure rate (number of potential failures—proportion of animals that are conscious after stunning). Finally, different risk factors and scenarios are proposed to define a 'normal' or a 'reinforced' monitoring protocol, according to the needs of the slaughterhouse.

KEY WORDS

stunning, slaughter, death, consciousness, welfare indicators, monitoring procedures

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SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver scientific opinions on monitoring procedures at slaughterhouses for different animal species, stunning methods and slaughter without stunning. In particular, the opinions will (i) provide indicators assessing signs of (a) consciousness, in the case of slaughter with stunning, and (b) unconsciousness and (c) death of the animals, in the case of slaughter without stunning, which have been selected based on their performance (i.e. sensitivity, specificity and feasibility of the indicator); (ii) indicate the most common risk factors and their welfare consequences to determine the circumstances of the monitoring procedures; and (iii) provide examples of sampling protocols, based on different possible scenarios.

The current opinion deals with the assessment of consciousness in small ruminants after stunning with the head-only electrical method and the assessment of unconsciousness and death during slaughter without stunning. The Working Group agreed that, although it is traditional to look for outcomes of unconsciousness in sheep following stunning, the risk of poor welfare can be detected better if sheep welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the indicators were phrased neutrally (e.g. corneal reflex) and the outcomes were phrased either suggesting unconsciousness (e.g. absence of corneal reflex) or suggesting consciousness (e.g. presence of corneal reflex). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses. A toolbox of selected indicators is proposed to check for signs of consciousness in small ruminants after head-only electrical stunning; a different toolbox of indicators is proposed for confirming unconsciousness as well as death of the animals following slaughter without stunning. Various activities (two stakeholder consultations, a systematic literature review, an online survey addressed to experts involved with monitoring welfare at slaughter) were carried out in order to obtain information on the sensitivity, specificity and feasibility of the indicators. Based on such information, the most appropriate indicators were selected and a toolbox of indicators to be used in monitoring procedures was proposed. The use of animal-based indicators is similar to the use of a diagnostic or statistical ‘test’ with either a positive or negative outcome. In the case of stunning of the animals, the major interest is to detect the undesired outcome, namely the presence of consciousness in animals. The toolbox proposes indicators and their outcomes. In the case of slaughter without stunning, the interest is to detect whether the animals become unconscious and to detect when the animal dies. However, the indicators applied for this task as well have to correctly detect animals as conscious or alive. In the case of slaughter without stunning, the toolbox proposes indicators and their outcomes.

Each of the toolboxes provides a set of recommended indicators and another set of additional indicators. The people responsible for monitoring have to choose the most appropriate set of indicators (at least two indicators) from these toolboxes according to their expertise and the available infrastructure in a slaughterhouse.

Toolboxes for head-only electrical stunning:

After head-only electrical stunning of the animals prior to slaughter the indicators should be repeatedly checked to detect signs of consciousness through the three key stages of monitoring during the slaughter process: after stunning (between the end of stunning and shackling), during neck cutting and during bleeding.

The recommended indicators in Toolbox 1 (for monitoring after stunning) are tonic/clonic seizures, breathing and the corneal or palpebral reflex. Additionally, the indicators spontaneous blinking, posture and vocalisations may be used.
For Toolbox 2 (for monitoring at neck cutting): the recommended indicators to be used are breathing, tonic/clonic seizures and muscle tone. Additionally, the corneal or palpebral reflex, spontaneous blinking and vocalisations may be used.

For Toolbox 3 (for monitoring during bleeding) the recommended indicators are breathing and muscle tone. Additionally, the corneal or palpebral reflex, spontaneous blinking and vocalisations may be used.

Toolboxes for slaughter without stunning:

In the case of slaughter without stunning, all the small ruminants should be checked until they become unconscious, before being released from the restraint; and death should be confirmed before undergoing carcass dressing. Moreover, consciousness or life in checked animals should be correctly identified. On this basis, the indicators were selected for the toolboxes.

The recommended indicators in Toolbox 4 (for monitoring unconsciousness before release from the restraint) are breathing and muscle tone. Additionally, posture and corneal or palpebral reflex may be used.

Toolbox 5 (for monitoring death before carcass dressing begins) recommends bleeding, muscle tone and pupil size as indicators to check for death.

The personnel performing stunning, shackling, hoisting and/or bleeding will have to check all the animals to rule out the presence of consciousness following head-only electrical stunning or confirm unconsciousness and death during slaughter without stunning. The person in charge of monitoring the overall animal welfare at slaughter (i.e. animal welfare officer) has to check a certain sample of slaughtered animals for approval. A mathematical model is proposed which can be used to calculate the sample size that he/she needs to check at a given throughput rate (total number of animals slaughtered in the slaughter plant) and threshold failure rate (number of potential failures—proportion of animals that are conscious after head-only electrical stunning). Finally, different risk factors and scenarios are proposed to define in addition to a ‘normal’ sampling procedure a ‘reinforced’ protocol to be used if required by particular circumstances and needs of the slaughterhouse.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Article 16 of Council Regulation (EC) No 1099/2009[4] on the protection of animals at the time of killing[5] requires slaughterhouse operators to put in place and implement monitoring procedures in order to check that their stunning processes deliver the expected results in a reliable way.

Article 16 refers to Article 5 which requires operators to carry out regular checks to ensure that animals do not present any signs of consciousness or sensibility in the period between the end of the stunning process and death.

Those checks shall be carried out on a sufficiently representative sample of animals and their frequency shall be established taking into account the outcomes of previous checks and any factors which may affect the efficiency of the stunning process.

Article 5 also requires operators, when animals are slaughtered without stunning, to carry out systematic checks to ensure that the animals do not present any signs of consciousness or sensibility before being released from restraint and do not present any sign of life before undergoing dressing or scalding.

According to Article 16(2), a monitoring procedure shall include in particular the following:

(a) indicators designed to detect signs of unconsciousness and consciousness or sensibility in the animals (before death or release from restraint, in case of slaughter without stunning, = indicators A); or indicators designed to detect the absence of signs of life in the animals slaughtered without stunning (before undergoing dressing or scalding = indicators B);

(b) criteria for determining whether the results shown by the indicators previously mentioned are satisfactory;

(c) the circumstances and/or the time when the monitoring must take place

(d) the number of animals in each sample to be checked during the monitoring.

Furthermore, Article 16 (4) specifies that: "*The frequency of the checks shall take into account the main risk factors, such as changes regarding the types or the size of animals slaughtered or personnel working patterns and shall be established so as to ensure results with a high level of confidence*."

The Commission plans to establish EU guidelines concerning monitoring procedures at slaughterhouses.

The purpose of the Commission is to provide a sort of "toolbox" for establishing monitoring procedures so that slaughterhouse operators can use scientifically based procedures which will provide them proper information on their stunning processes. The guidelines will also be used by the competent authorities in order to check that slaughterhouse operators are not using unreliable monitoring procedures.

In order to prepare these guidelines, a sound basis for checks on stunning as laid down in Articles 5 and 16 of the above-mentioned regulation is needed.

[4] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:303:0001:0030:EN:PDF

[5] OJ L 303, 18.11.2009, p. 1.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

The Commission therefore considers it opportune to request the EFSA to provide an independent view on the indicators and elements for putting in place monitoring procedures at slaughterhouses for the following methods and scope, in light of the most recent scientific developments.

- The scope of this request includes the following groups of methods/species[6]:

  (1) penetrative captive bolt for bovine animals,

  (2) head-only electrical stunning for pigs,

  (3) head-only electrical stunning for sheep and goats,

  (4) electrical waterbath for poultry (chickens and turkeys),

  (5) carbon dioxide at high concentration for pigs,

  (6) all authorised gas methods to slaughter chickens and turkeys (carbon dioxide in two phases, carbon dioxide associated with inert gases and inert gases alone).

  (7) Slaughter without stunning for bovine animals,

  (8) Slaughter without stunning for sheep and goats,

  (9) Slaughter without stunning for chickens and turkeys.

- For each group the EFSA, based on the relevant scientific basis and on indicators' performances, will provide indicators A (loss of consciousness or sensibility for all groups) or indicators B (absence of signs of life for groups 7 to 9 only) as well as the other elements of the monitoring procedure (criteria for satisfactory results in terms of animal welfare, circumstances and sampling procedure, including minimum sampling and frequency) (sampling procedures are needed only for groups 1 to 6 since checks must be systematic for groups 7 to 9).

- For that purpose, the EFSA will take into account that:

  – Indicators should be able to detect, with high level of confidence, unsatisfactory stunning/slaughtering practices within the sample observed. Hence, the EFSA should specify the criteria for selecting indicators, based on the level of sensitivity and specificity for each indicator.

  – At least two indicators are required for each process but more could be recommended.

  – Indicators will be used at slaughterhouses, which imply human (work safety, accessibility), physical (line speed, difficulties to observation, etc.) and economic (time, costs) constraints. Hence, the EFSA could indicate the possible limitations related to the measurement of each indicator.

  – Circumstances to determine the monitoring procedure have to address the risk factors most commonly associated with each group methods/species (for example the penetrative captive bolt is likely to be more sensitive to the competence of the staff than a highly mechanised method). Hence, for each groups of methods/species, the EFSA should

[6] Wording used for the stunning methods refers to Annex I to Regulation (EC) No 1099/2009.

indicate the most common risk factors and their welfare consequences to determining the circumstances of the monitoring procedure (e.g. when the staff shifts if staff is an important risk factors).

- Monitoring procedures can be dynamic instruments and different indicators and sampling procedures could be used on the same slaughter line depending on the previous results and other risk factors. Hence, based on different possible scenarios, the EFSA should provide examples of different sampling protocols (like “new line/reinforced”, “regular”, “light”) and the minimum sampling needed for indicators ‘A’ (even when results appear to be fully satisfactory).

ASSESSMENT

1. Introduction

1.1. General introduction

According to Council Regulation (EC) No 1099/2009, on the protection of the animals at the time of killing, animals must be rendered unconscious and insensible by the stunning method and they must remain so until death occurs through bleeding. One way of achieving this animal welfare requirement would be to monitor the state of consciousness and unconsciousness in animals at three key stages: (1) immediately after stunning, (2) at the time of neck cutting and (3) during bleeding until death occurs.

Within the scope of this Regulation, it is the responsibility of the food business operator (FBO) to ensure that the welfare of the animals is not compromised from the time of their arrival until they are slaughtered.

The ‘personnel’ performing stunning, shackling, hoisting and/or bleeding (hereafter referred to as the ‘personnel’) must hold a certificate of competence, awarded after training and assessment by independent organisations, attesting that they have the knowledge and skills required to recognise the signs of both effective and ineffective stunning and, in the event of a failure, re-stun the animal. In addition, the ‘personnel’ performing slaughter (neck cutting = severing carotid arteries) should also have a certificate of competence attesting that they are aware of the need, and have the skills required, not only to perform prompt and accurate slaughter, but also to perform checks for the signs of recovery of consciousness and sensibility prior to neck cutting to ensure that every animal is unconscious at the time of neck cutting. The personnel should also be able to ascertain the possibility or potential for recovery of consciousness in animals during bleeding and take action, if necessary (e.g. use a back-up stunner).

Finally, the person in charge of the overall animal welfare at slaughter (i.e. animal welfare officer) should be able to monitor the animals during the entire process, from stunning to bleeding, and ensure that they do not show any signs of consciousness and also that death occurs before further carcass dressing operations begin. Under laboratory conditions, the induction and maintenance of unconsciousness following stunning can be ascertained by recording the brain activity using electroencephalography (EEG) or electrocorticography (ECoG). The effectiveness of stunning and the duration of unconsciousness induced by the stunning method can be recognised from the unique brain state and associated EEG manifestations. When stunning-induced EEG or ECoG changes are ambiguous, abolition of somatosensory or visual evoked potentials in the brain has been used to ascertain the brain responsiveness to these external stimuli. The effectiveness of stunning and neck cutting can also be recognised under field conditions from the characteristic changes in the behaviour of animals (e.g. loss of posture), physical signs (e.g. onset of seizures, cessation of breathing, fixed eye) and from the presence or absence of response to physiological reflexes (e.g. response to an external stimulus such as blinking response to touching the cornea (corneal reflex), response to pain stimulus such as nose prick or toe pinching). In the scientific literature, these physical signs and reflexes have been referred to as indicators of unconsciousness or consciousness and used to monitor welfare at slaughter of animals (see, for example, EFSA, 2004).

At all of the key stages, monitoring is carried out to identify animals that are improperly stunned and therefore the attention is focused on the indicator of consciousness. Effectively stunned animals are expected to remain unconscious during key stages 2 and 3 until death occurs. It is thought that, for this monitoring system to be effective, it is important to define indicators (see sections 3.4 and 3.5 and the glossary), identify the pathophysiological basis of the stunning method and its relevance or appropriateness to key stages of monitoring, and also to describe how the indicator may be manifested or can be used to recognise consciousness at a particular key stage of monitoring.

The slaughter of animals without prior stunning is regulated by Article 4 (4) of Regulation (EC) No 1099/2009. Slaughter without stunning induces gradual loss of consciousness and consequently death as a result of the brain being of nutrients and oxygenated blood and onset of brain ischaemia. According to the Regulation, people performing slaughter without stunning are also required to have a certificate of competence. The Regulation also stipulates that all ruminants must be restrained mechanically during slaughter and they shall be released from the restraint only when unconsciousness has ensued. Carcass dressing shall begin after the onset of death. Therefore, it is important to define indicators that can be used to recognise unconsciousness and death following slaughter without stunning while simultaneously recognising as such any animal still conscious or alive.

1.2. Definitions

Consciousness is a state of awareness which requires the function of the brain stem and projections in the relevant cortical regions. Following everyday neurological practice (Zeman, 2001) consciousness is generally equated with the waking state and the abilities to perceive, interact and communicate with the environment and with others, which is referred to as sensibility. Consciousness is a matter of degree, and a range of conscious states extends from waking through sleep until unconsciousness is reached. For the purpose of this opinion, an animal is considered 'conscious' as long as a degree of consciousness is detected.

Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent damage to brain function and the individual is unable to perceive external stimuli (which is referred to as insensibility) and control its voluntary mobility and, therefore, respond to normal stimuli, including pain (EFSA, 2004).

For the Dialrel project (von Holleben, 2010) 'unconsciousness' is defined in a similar way to that used by anaesthesiologists: "*Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence the individual is unable to respond to normal stimuli, including pain*."

According to Regulation 1099/2009, the **sensibility** of an animal is essentially its ability to feel pain. In general, an animal can be presumed to be insensible when it does not show any reflexes or reactions to stimuli such as sound, odour, light or physical contact.

In the context of this scientific opinion, consciousness includes sensibility and unconsciousness includes insensibility.

Death is a physiological state of an animal, in which respiration and blood circulation have ceased as the respiratory and circulatory centres in the medulla oblongata are irreversibly inactive. Owing to the permanent absence of nutrients and oxygen in the brain, consciousness is irreversibly lost. In the context of application of stunning and stun/kill methods, the main clinical signs of death are absence of respiration (and no gagging), absence of pulse, and dilated pupils (EFSA, 2004).

1.3. Physiology of head-only electrical stunning

Head-only electrical stunning of animals with a current of sufficient magnitude induces immediate loss of consciousness through the induction of generalised epileptiform activity in the brain (Velarde *et al*., 2002). The neurophysiological basis of generalised epileptiform activity and associated loss of consciousness is well documented in the scientific literature (see EFSA, 2004, report for details).

Successful induction of epileptiform activity is manifested as immediate collapse of the animal, as a result of the onset of tonic seizure. During tonic seizures, the animals show tetanus (rigidly extended front legs and flexed hind legs), breathing is absent and the eyeballs might be fixed or obscured (cornea not visible owing to eyeball rotation into the socket). The fixed eyes may be obscured by the presence of wool or closed eyelids. The tonic seizure is usually followed by two episodes of clonic seizures in sheep (Velarde *et al*., 2002), which can manifest as a galloping, cantering or erratic kicking action (Gregory, 1998). Spontaneous breathing reappears shortly before the end of the first clonic phase. In contrast, the corneal reflex reappears simultaneously with or shortly after the end of the first clonic phase (Velarde *et al*., 2002). The duration of tonic seizure is influenced by several factors (e.g. category of animal, electrical stunning parameters), but usually lasts for seconds. The tonic–clonic seizures are followed by loss of muscle tone, which can be recognised from drooping ears and limp legs, especially when the animals have been shackled and hoisted on to the overhead bleeding rail. Additionally, reflexes that would require brain control are also abolished. For example, the palpebral (elicited by touching eyelashes or inner or outer canthus of the eye), corneal (elicited by touching the cornea) and pupillary (elicited by focusing a bright light into the pupil) reflexes and response to external stimuli including pain (e.g. nose prick) are also abolished during the period of unconsciousness.

Ineffective or unsuccessful head-only electrical stunning in animals can occur for various reasons (e.g. the presence of wool, lack of provision to wet the wool and skin prior to stunning, poor tong position, poor electrical contact between the stunning tongs and head of the animal, dirty or corroded stunning tongs, lack of pressure applied during stunning, slipping of tongs during stun application) and, as a consequence, the animal may not experience the generalised epileptiform activity required to achieve unconsciousness (Velarde *et al*., 2000; Gregory, 2001). This situation will lead to different behavioural manifestations and retention of reflexes, which can be recognised from the failure to collapse, the absence of tonic–clonic seizures and the presence of breathing (including laboured breathing); animals may also vocalise if the electrical application has induced pain. Ineffectively stunned animals and those recovering consciousness will show also spontaneous blinking or positive eye reflexes (palpebral, corneal and pupillary). Head righting (attempt to raise head) after stunning and body arching during bleeding are also signs of consciousness.

Effectively stunned, i.e. unconscious sheep and goats, are bled out by cutting the neck (carotid arteries and jugular veins behind the mandibles). Prompt (i.e. during tonic seizure) and accurate neck cutting of effectively stunned animals results in rapid onset of death and therefore animals do not show signs of recovery of consciousness at any key stages of monitoring. In addition, depending upon the stun to stick interval, the duration of tonic–clonic seizures may be reduced as a result onset of death. This means that, if stunning has been effective and the duration of unconsciousness induced by the stunning method is longer than the sum of time between the end of stunning and sticking (stun-to-stick interval) plus the time it takes for animal to die through blood loss, the animal will remain unconscious until death occurs. On the other hand, ineffective stunning or prolonged stun-to-stick interval and/or inappropriate/inadequate neck cutting or sticking will lead to animals showing signs of consciousness.

Inappropriate neck cutting can arise if a stab or cut is too small to completely sever both carotid arteries and jugular veins and facilitate rapid bleeding and/or if bleed-out is further impeded by the presence of wool or coagulation of blood at the stab wound (see EFSA, 2004, for details of different neck-cutting methods used in small ruminants). The incidence of recovery of consciousness in sheep and goats due to poor bleeding is not known but the possibility cannot be ruled out.

1.4. Physiology of slaughter without stunning

Slaughter without stunning does not induce immediate loss of consciousness in animals. In other words, animals are gradually rendered unconscious by the severance of carotid arteries as brain perfusion becomes insufficient to sustain normal function, eventually leading to death. The time to onset of unconsciousness and to death may vary between animals. This is because the rate of bleeding may not always be profuse or uninterrupted if severance of the carotid arteries is incomplete (poor cut) or if there is physical obstruction to the blood flow from the cut ends. These factors will lead to poor welfare, and therefore continuous and systematic monitoring of all animals slaughtered without stunning is required.

In the conscious animal, the cerebral cortex integrates posture and movement. Collapse occurring when a freely standing animal falls to the ground is the earliest indication of approaching unconsciousness after the neck cut (Blackmore, 1984; Grandin, 1994; Gregory *et al*., 2010), as it indicates that the cortex is no longer able to control postural stability (Muir, 2007). However, an animal that has collapsed after a dramatic loss of blood pressure may nevertheless have the capacity to regain consciousness as a result of the body's own counter-regulation mechanisms (von Holleben *et al*., 2010). Nevertheless, the average time to onset of unconsciousness following slaughter without stunning in sheep has been reported to be 10 seconds (Cook *et al*., 1996), The time to 90 % total blood loss after slaughter without stunning of sheep has been estimated to be 56 seconds after the neck cut (Anil *et al*., 2004).

Positive pupillary, palpebral and corneal reflexes—which are reflexes controlled by cranial nerves—assist in ascertaining the magnitude of brain dysfunction. A negative response to all reflexes suggests a good outcome of impaired midbrain or brain stem activity, and unconsciousness can be inferred, provided the muscles and afferent and efferent nerves which execute the response are still capable of working and not preoccupied with other stimuli (Gregory, 1998).

When the function of the brain stem is sufficiently impaired as a result of the blood loss, respiration will cease and the heart will also cease to function over time (Michiels, 2004; Rosen, 2004; Pallis, 1982a, b, c, d). The main clinical signs seen of death are permanent absence of respiration (and also absence of gagging) and absence of pulse.

2. Materials and methods

2.1. Indicators and criteria for selection of the indicators

The mandate requests EFSA to select:

> **Indicators A**, designed to detect signs of consciousness in the animals after stunning or, in case of slaughter without stunning, signs of unconsciousness (before release from restraint in the case of slaughter without stunning).
>
> **Indicators B**, designed to detect—in the animals slaughtered without stunning—signs of death before undergoing dressing or scalding.

For the sake of clarity and consistency, indicators checking the state of consciousness and unconsciousness or indicators checking the state of life and death in animals will be used in this opinion instead of indicators A and indicators B, as shown in Table 1.

The Working Group agreed that, although it is traditional to look for outcomes of unconsciousness in animals following stunning, the risk of poor welfare can be detected better if animal welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the indicators were phrased neutrally (e.g. posture) and the outcomes were phrased either suggesting unconsciousness (e.g. immediate collapse) or suggesting consciousness (e.g. no collapse/attempts to regain posture). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses.

Table 1: Correspondence between indicators suggested in the ToR of the mandate and indicators proposed in this scientific opinion

Species	Method	Key stage	Indicators		
			Indicators as from mandate's ToRs	**Checking state of**	**Outcome related to animal welfare**
Sheep and goats	Stunning with head-only electrical method	Key stage 1 = immediately after stunning until shackling	A	Consciousness and unconsciousness	Consciousness
		Key stage 2 = during neck cutting	A	Consciousness and unconsciousness	Consciousness
		Key stage 3 = during bleeding	A	Consciousness and unconsciousness	Consciousness
	Slaughter without stunning	Prior to release from restraint	A	Consciousness and unconsciousness	Consciousness
		Prior to dressing	B	Life and death	Life

The indicators investigated in this opinion were selected based on previous EFSA opinions (2004, 2006) and amended in Working Group discussion on the basis of feedback from (i) a stakeholder meeting at which interested parties were consulted by a questionnaire (referred to in this opinion as questionnaire 1), (ii) a systematic literature review, (iii) an online survey of experts involved in monitoring of welfare at slaughter or neck cutting in the form of a questionnaire (questionnaire 2), (iv) public consultation on the opinion on small ruminants and (v) a technical meeting with selected experts. Their suitability for inclusion in a monitoring system was determined during Working Group discussions on the basis of their sensitivity and specificity, and their feasibility for use at different key stages of the slaughter process.

2.1.1. Feasibility

The feasibility of an indicator is considered in relation to physical aspects of its assessment. These include, for example, the position of the animal relative to the assessor, the assessor's access to the animal and the line speed. Feasibility for the purpose of this opinion does not include economic aspects. It is very likely that the feasibility of assessing an indicator is influenced by the key stage of the slaughter process, i.e. after stunning, at neck cutting and during bleeding animals can be in different positions and proximity relative to the assessor, which may affect how easily the indicator can be used.

2.1.2. Sensitivity and specificity

The use of animal-based indicators is similar to the use of a diagnostic or statistical test with either a positive or negative outcome. The performance of a test (i.e. the indicator) is usually described by its sensitivity and specificity. The estimation of sensitivity and specificity requires a definition of what can be considered a positive or negative outcome of checking for an indicator. The definitions of sensitivity and specificity of indicators differ depending on whether they are used in situations where animals are slaughtered with stunning or without stunning.

2.1.2.1. Sensitivity and specificity during slaughter with stunning

When monitoring the effectiveness of the stunning, in order to safeguard animal welfare, it is of major interest to detect the animals that are not properly stunned or recover consciousness after stunning. A positive outcome of the checked indicator is that based on which the animal is considered conscious.

A negative test outcome of the indicator is that based on which the animal is considered not conscious (i.e. animal is considered unconscious).

Sensitivity is thus calculated as the number of truly conscious animals considered conscious based on the outcome of the indicator (A in Table 2) divided by the number of all conscious animals (A + C), multiplied by 100 (in short, sensitivity is the percentage of truly conscious animals that the indicator tests as conscious).

Specificity is calculated as the percentage of truly unconscious animals (B + D) that the indicator does not test conscious (D).

Table 2: Sensitivity and specificity of indicators during slaughter with stunning

Slaughter with stunning		**Truth: the animal is conscious**	
		Yes	**No**
Is the animal considered conscious, based on the outcome of the indicator?	**Yes**	A	B
	No	C	D

An indicator for slaughter with prior stunning is considered to be 100 % sensitive if it detects all the conscious animals as conscious; an indicator is considered to be 100 % specific if it detects all the unconscious animals as unconscious.

2.1.2.2. Sensitivity and specificity during slaughter without stunning

In contrast, during slaughter without stunning, all the animals are alive and conscious when neck cutting is performed and the purpose of the cut is to induce unconsciousness followed by death. Therefore, it is of major interest to detect unconsciousness and death in all animals. The use of indicators for detecting unconsciousness or death is a test with positive or negative outcome, where the positive outcome causes the animal to be considered as conscious or alive, and the negative outcome is the confirmation of unconsciousness or death, respectively.

In this case, sensitivity is calculated as the number of conscious or alive animals considered conscious or alive based on the outcome of the indicator (E in Table 3) divided by the number of conscious or alive animals (E + G), respectively, multiplied by 100 (in short, the percentage of animals truly still conscious or alive that the indicator tests conscious or alive).

Specificity is calculated as the percentage of unconscious or dead animals (F + H) that the indicator tests as unconscious or dead (H), respectively.

An indicator for slaughter without stunning is considered to be 100 % sensitive if it detects all animals still conscious or alive as conscious or alive animals. An indicator is considered to be 100 % specific if it detects unconsciousness or death in animals, when animals truly became unconscious or dead, respectively.

Table 3: Sensitivity and specificity of indicators during slaughter without stunning

Slaughter without stunning		**Truth: the animal is still conscious (alive)**	
		Yes	**No**
Is the animal considered conscious (alive), based on the outcome of the indicator?	**Yes**	E	F
	No	G	H

2.2. Establishing the ability of the indicators to detect welfare problems at slaughter

2.2.1. Stakeholder meeting and questionnaire 1

A stakeholder meeting was held on 30 January 2013 in order to inform all interested parties about this mandate. The meeting was opened to participants from all EU Member States representing research groups, FBO licensed to own premises to slaughter animals, animal welfare officers employed by the FBOs, auditing companies, the European Commission, Member State Competent Authorities, members of EFSA's Stakeholders Consultative Platform and non-governmental organisations (NGOs) with proven experience in the field of humane slaughter. The meeting was an opportunity for the experts to exchange experience and information on the animal-based indicators most commonly used to check unconsciousness and death in sheep and goats, during slaughter with or without stunning. More than 100 experts or persons claiming to be experts associated with the slaughter of animals participated in the meeting. Traditionally, animal welfare monitoring in slaughterhouses involves checking for unconsciousness or death, following the application of a stunning method. Therefore, a questionnaire on the use of animal-based indicators to check for the state of consciousness and unconsciousness or life and death at slaughter was distributed to all participants. The questionnaire asked about (i) the indicators that are mostly used and their use in combinations; (ii) the timing of the assessment of unconsciousness and death based on such indicators; (iii) the problems encountered during the assessment (feasibility of the indicators); and (iv) the reliability of the indicators based on their opinion. The participants were also asked to suggest names of experts with practical knowledge in the field of slaughter to be contacted for the following online survey (section 2.2.3).

2.2.2. Systematic literature review

A systematic literature review was conducted in order to summarise the currently available data describing the sensitivity and specificity of indicators checking the state of consciousness and unconsciousness or life and death for all stun-kill methods and species combinations (O'Connor *et al.*, in press). Traditional animal welfare monitoring in slaughterhouses involves checking for outcomes of unconsciousness, following the application of a stunning method. Therefore, in order to obtain information on sensitivity and specificity, a systematic review was conducted of studies in which outcomes of unconsciousness and outcomes of death were measured using electroencephalography (EEG). In such studies, the indicators of interest (e.g. no corneal reflex, no breathing, loss of posture) were tested against the results of EEG (e.g. a stunned animal does not show corneal reflex and its unconsciousness is confirmed by EEG).

2.2.3. Questionnaire 2 (online survey)

In addition, an online survey was launched using a questionnaire to gather subjective opinion from experts with knowledge and experience in stunning and slaughtering of animals. The survey was outsourced to an external communication company and its final technical report can be found on EFSA's website (Sellke, in press). The survey was structured on the basis of the results from the questionnaire distributed at the stakeholder meeting held on 30 January 2013 and was addressed to approximately 160 participants. In order to avoid confusion, the assessments of feasibility, sensitivity and specificity of the indicators were presented in separate sections of the questionnaire. The Panel on Animal Health and Animal Welfare of EFSA agreed that, although it is traditional to look for outcomes of unconsciousness in animals following stunning, the risk of poor welfare can be detected better if animal welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the selected indicators were phrased neutrally (e.g. posture) and the outcomes were phrased positively suggesting unconsciousness (e.g. immediate collapse) or negatively suggesting consciousness (e.g. no collapse/attempts to regain posture). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses.

Regarding feasibility, for each species and method, questions were asked on how easily the indicators are applied and checked at each key stage of the stunning and slaughter process and of the slaughter process without stunning. For each key stage the feasibility ratings were computed into a feasibility score across all respondents that weighed the proportion of ratings easy against the proportion of ratings difficult as presented in the equation below

Feasibility score = (No of ‘easy’ respondents – No of ‘difficult’ respondents)/No of all respondents

For example, in the case of data distribution of easy = 3, normal = 6 and difficult = 1, the score would be +0.2, i.e. (3 – 1)/10.

The resulting score was between +1 and –1 and covers the median rating as well as the tendency across all ratings, thus providing an overview of the distribution of the data and associated variability.

In addition, the survey asked respondents to assess the sensitivity and specificity of the indicators. This information was elicited by asking respondents to estimate, for each indicator, the proportion of truly conscious and the proportion of truly unconscious animals that would be considered conscious, based on the outcome of the indicator (i.e. A and B in Table 2). Sensitivity and specificity were estimated across all respondents using either the direct or weighted average of individual data values. The weights are provided by the uncertainty rating assigned by each respondent to every answer, which ranged between 1 and 3 (1 = ‘not sure’, 2 = ‘rather sure’, 3 = ‘very sure’). Prior to calculations, the data were closely examined for consistency and corrected according to the following rules: answers associated with uncertainty rating ‘do not know’ were excluded (e.g. 7/193 for electrical stunning); if the uncertainty rating was omitted, answers were re-set to the lowest uncertainty weight (i.e. 1 = ‘not sure’; 10/370). If a respondent’s answer to all or the priming sequence of ‘not show/respond to’ (i.e. ‘breathing’, ‘nose prick’) questions reversed the logic (i.e. “5 % of truly unconscious animals will not show eye movements”) and the same question was rather consistently answered by other respondents, then the corresponding values in the data record were reversed as ‘100 % minus rating’ (28/186). Ratings were not reversed if variability across the respondents was too large for particular indicators to conclude logical inconsistency.

2.3. Working Group discussions

The outcomes of all previous activities were assessed and discussed within the Working Group of experts developing this scientific opinion. In addition, a technical meeting with a group of external experts (five academics, two from NGOs, one representative from poultry industry, one representative from the red meat industry and two representatives from European Commission) was held on 3 September 2013. During the meeting the results obtained during the preceding activities of the Working Group were discussed, with the aim of advising the Working Group on the content of the toolboxes for captive bolt stunning and for slaughter without stunning. The experts invited to this meeting had previous access to the draft opinion, including the toolbox of indicators, and were asked to give their comments. During the meeting various presentations were given to stimulate discussion. A public consultation on the draft scientific opinion was also held during August–September 2013 (EFSA AHAW Panel, in press).

2.4. Developing the sampling protocol

In order to develop a monitoring procedure for slaughter with stunning, the mandate from the Commission requests EFSA to estimate the optimal frequency with which animals should be checked for signs of consciousness following stunning. This sampling frequency should take into account risk factors associated with the stunning procedure. For the optimal sampling fraction (or sampling frequency) to be calculated, at least two components need to be quantified: first, the highest proportion of insufficiently stunned animals that may be considered acceptable; and, second, the quantitative effects of the risk factors (individually or in combination) on the frequency of ineffective stunning.

Both components are problematic. Regarding the level of acceptability the legislation specifies that no animals should show signs of consciousness following stunning. All animals should be stunned properly, and therefore the threshold level for the acceptability of ineffective stunning is zero. The second component requires a large number of data on the interactive effects of risk factors on stunning effectiveness, given a wide range of circumstances under which animals are stunned in European abattoirs. These data are not available.

However, it is possible to model the relationship between the fraction of slaughtered animals sampled and the minimum proportion of ineffectively stunned animals that will be detectable using a certain sampling protocol. Understanding this relationship allows the risk manager (and others concerned) to relate the economic and other costs associated with a particular sample size to the benefits associated with improved detection levels (i.e. improved animal welfare).

2.4.1. The statistical background of the model

The relationship can be modelled using existing approaches for process monitoring (e.g. continuous quality assurance regarding threshold failure rate in computer chip production). Although the statistical relationship is identical to those applied in planning disease surveillance, the related terminology (e.g. design prevalence) was considered less appropriate for addressing the issue of mis-stunned animals and therefore this text adheres to the terminology of failure management. For the statistical model, we used the following parameters:

1. Threshold failure rate for proportion of mis-stunned animals. This specifies the minimum proportion of animals that are ineffectively stunned, which will still be detected by the sampling protocol.

2. Sensitivity of the indicators. As defined previously, this is the percentage of truly conscious animals detected as conscious by the indicator.

3. Slaughter population. This is the total number of animals slaughtered under the same circumstances as determined by risk factors (see Table 10). Note that the slaughter population is independent of the line speed, and can cover a period of minutes, hours or even days.

4. Sampling fraction. This is the proportion of the slaughter population which is assessed in the sampling protocol.

5. Accuracy of the sampling protocol. This is the percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if the number of ineffectively stunned animals was higher than the prescribed threshold failure rate would allow.

Please note that for the head-only electrical stunning situation, specificity is not considered for the purposes of this model, as the specificity of an indicator is not related to the risks associated with reduced welfare.[7]

Given these parameters, the details of the monitoring protocol can be calculated from Equation 1 (Cannon, 2001).

$$SF = \frac{n}{SP} \cong \frac{\left(1-(1-A)^{1/(SP\cdot FR)}\right)\cdot(SP-0.5(A(SP\cdot FR)-1)}{ISe}$$

Where:

- A = requested accuracy of the sampling protocol
- FR = standard threshold failure rate
- ISe = indicator sensitivity
- *n* = number of animals tested
- SF = sample size or sampling fraction
- SP = slaughter population

The objective was to use Equation 1 to estimate the threshold failure rate (FR) associated with a given sampling fraction. However, Equation 1 cannot be solved for the FR in an algebraic way. For this reason, it was necessary to solve the equation numerically. For this purpose, the R[8] function 'uniroot' was used.

Solving Equation 1 numerically, it was then possible to determine the minimum detectable FR associated with each SF value. The results could then be plotted in a diagram (see Figure 1). Once the relationship is formalised, it is also possible to read the results the other way round, i.e. to estimate what is the minimum SF needed to detect a given threshold FR, with a given accuracy, accounting for the indicator sensitivity and the slaughter population.

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[7] It should be noted that a low specificity of the indicator, although not representing an animal welfare issue, definitely represents an issue from a FBO perspective. An indicator with low specificity would more often misclassify unconscious animals as conscious. Obviously, this represent a problem from a FBO perspective as an unnecessary corrective action must be taken, entailing a waste of money and time.

[8] R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

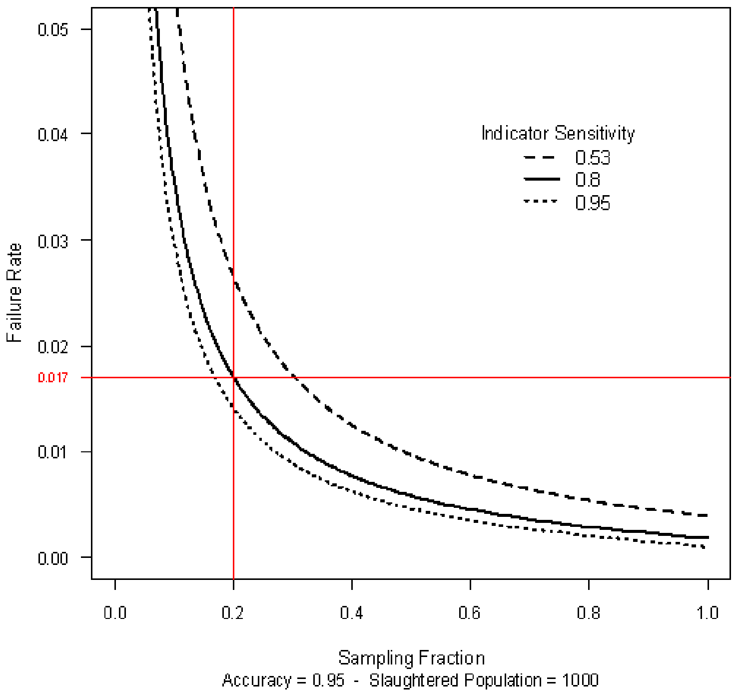


Figure 1: Example graph of the relationship between the parameters defining a sampling protocol (SF and detectable threshold FR for fixed values of accuracy (here 95 %) and slaughter population (here 1 000 animals) and varied scenarios for indicator sensitivity)

In Figure 1, a slaughter population of 1 000 animals and a required accuracy of 95 % are assumed. The red horizontal and vertical lines on the diagram form the basis for the following illustration: using an indicator with a sensitivity of 80 % (solid line), a sampling fraction of 20 % (i.e. sample size of 200 animals from a slaughter population of 1 000 animals) will be able to detect, with 95 % accuracy, a threshold failure rate of 2 % (i.e. more than 20 conscious animals out of 1 000 animals slaughtered in this example) or greater. The dotted lines illustrate how this relationship changes with indicators of varying sensitivity.

Different scenarios were considered assuming alternative model parameters for the specification of the sampling protocol. In detail the following scenarios were considered:

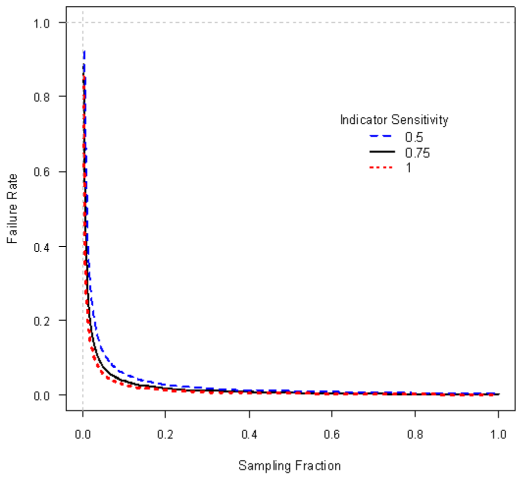
- accuracy: 0.90, 0.95, 0.99
- slaughter population: 100, 1 000, 10 000
- test sensitivity: 0.5, 0.75, 1

In order to compare the impact of these three parameters on the relationship between the threshold failure rate (FR) and the sampling fraction (SF), the other two of them were set at fixed values. Then combinations of FR and SF were evaluated, to identify those that would trigger an alarm with the required accuracy and those that would not. These critical combinations constitute the line graph exactly representing the desired accuracy level, e.g. in Figure 1. All 3 × 3 combinations were explored. Further details about the calculations can be found in the SAS Technical Report (EFSA SAS Unit, 2013).

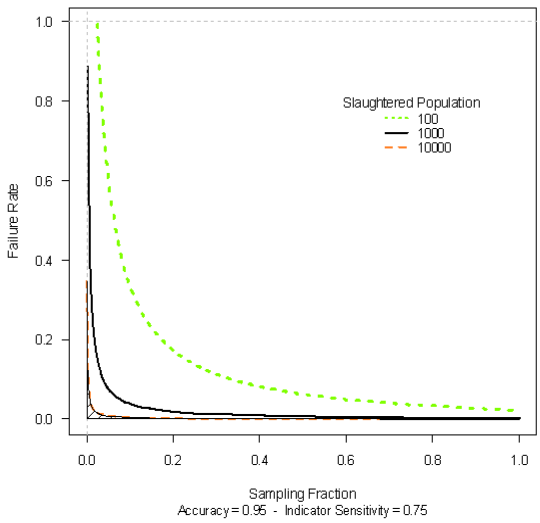
2.4.2. The resulting model for the sampling protocol

The results of the statistical modelling are summarised in Figure 2.

Using the five parameters of the model presented in Equation 1, it is possible to calculate each of them if the other four are specified. To illustrate the influence of the different parameters the full range of failure rate[9] and sampling fraction were combined with (a) the sensitivity of the indicator, (b) the slaughter population of the slaughterhouse[10] and (c) the desired accuracy of the sampling protocol,[11] whilst keeping the other two other parameters constant. The impacts of different indicator sensitivity, slaughter population and accuracy values are presented in Figure 2a, b and c.



(a) The effect of SF threshold FR for three levels of indicator sensitivity (0.5, 0,75, 1), given a slaughter population of 1 000 animals and an accuracy of 0.95

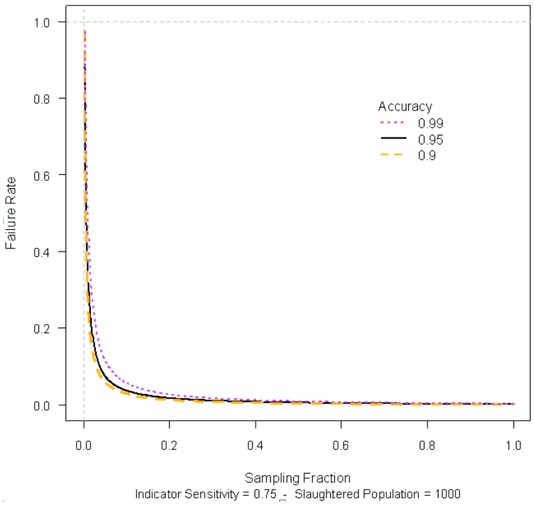


(b) The effect of SF on threshold FR for three levels of slaughter population (100, 1 000, 10 000) and accuracy (c), given an accuracy of 0.95 and indicator sensitivity of 0.75

9 Proportion of mis-stunned animals (see section 2.3.1).

10 The total number of animals being stunned during a given period according to the type of the slaughterhouse and the species slaughtered (see section 2.3.1).

11 Percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if there were more ineffectively stunned animals than the prescribed failure rate would allow (see section 2.3.1).



(c) The effect of SF on threshold FR for three levels of accuracy (0.9, 0.95, 0.99), given a slaughter population of 1 000 animals and indicator sensitivity of 0.75.

Figure 2: The effect of SF on threshold FR for three levels of indicator sensitivity (a), slaughter population (b) and accuracy (c), given a slaughter population of 1 000 animals (a, c), an accuracy of 0.95 (a, b), and indicator sensitivity of 0.75 (b, c). Each *x*–*y*-coordinate in the diagrams represents. one possible particular sampling protocol

Those sampling protocols that fall below the line describing that combination of parameters will not be able to meet the purpose of detecting if threshold FR is exceeded; those protocols above the line graph will meet the required purpose and raise an alarm.

Table 4a, b and c shows numerical examples of failure rates for three levels of indicator sensitivity, sample fraction and sampling protocol accuracy.

Table 4: The effect of SF on threshold FR for three levels of (a) indicator sensitivity, given a slaughter population of 1 000 animals and accuracy of 0.95; (b) slaughter population, given an accuracy of 0.95 and indicator sensitivity of 0.75; and (c) accuracy, given a slaughter population of 1 000 animals and indicator sensitivity of 0.75

(a) The effect of SF on threshold FR for three levels of indicator sensitivity (0.5, 0.7, 1), given a slaughter population of 1 000 animals and accuracy of 0.95

Sampling fraction	**Threshold failure rate**		
	Indicator sensitivity = 0.5	**Indicator sensitivity = 0.75**	**Indicator sensitivity = 1**
0.1	0.058	0.038	0.028
0.2	0.028	0.018	0.013
0.3	0.018	0.012	0.008
0.4	0.013	0.008	0.006
0.5	0.01	0.006	0.004
0.6	0.008	0.005	0.003
0.7	0.007	0.004	0.002
0.8	0.006	0.003	0.002
0.9	0.005	0.003	0.001
1	0.004	0.002	n.a.

(b) The effect of SF on threshold FR for three levels of slaughter population (100, 1 000, 10 000 animals), given an accuracy of 0.95 and indicator sensitivity of 0.75

Sampling fraction	**Threshold failure rate**		
	n **= 100**	***n*** **= 1 000**	***n*** **= 10 000**
0.1	0.34	0.04	0
0.2	0.17	0.02	0
0.3	0.11	0.01	0
0.4	0.08	0.01	0
0.5	0.06	0.01	0
0.6	0.05	0.01	0
0.7	0.04	0	0
0.8	0.03	0	0
0.9	0.03	0	0
1	0.02	0	0

(c) The effect of SF on threshold FR for three levels of accuracy (0.9; 0,95, 0,99), given a slaughter population of 1000 animals and indicator sensitivity of 0.75

Sampling fraction	**Threshold failure rate**		
	Accuracy = 0.9	**Accuracy = 0.95**	**Accuracy = 0.99**
0.1	0.029	0.038	0.058
0.2	0.014	0.018	0.028
0.3	0.009	0.012	0.018
0.4	0.006	0.008	0.013
0.5	0.005	0.006	0.01
0.6	0.004	0.005	0.008
0.7	0.003	0.004	0.006
0.8	0.003	0.003	0.005
0.9	0.002	0.003	0.004
1	0.002	0.002	0.003

3. Results

3.1. Results from stakeholder meeting

From the stakeholder meeting held on 30 January 2013, about 60 completed questionnaires were collected. Most of the experts provided information for more than one species and method: the total number of answers and the most used signs of unconsciousness and death in small ruminants are reported in Table 5.

Table 5: Total number of answers and the outcomes of unconsciousness and death of indicators most used for small ruminants as collected through questionnaire 1 of the stakeholder meeting

Species/method	Total No of answers	Outcome of unconsciousness of most used indicators[12]	Outcome of death of most used indicators[13]
Sheep and goat—head-only electrical stunning	32	Immediate onset of tonic seizures, followed by clonic seizures Immediate and sustained absence of rhythmic breathing Immediate collapse	
Sheep and goat—slaughter without stunning	10	No attempts to raise the head No corneal reflex No vocalisation	Absence of breathing Loss of muscle tone Permanent collapse of the animal

Experts responded that they observe the outcomes of the indicators between 10 and 30 seconds after stunning or after neck cutting. The main problem encountered in checking most of the indicators is access to the animal. Another common problem is the difficulty of evaluating the indicators in different animal categories. Several indicators are normally used by the experts to assess the state of unconsciousness and death in animals. However, there was no harmonised list of indicators, either species or method specific, or scientific rationale.

3.2. Results from systematic literature review

The comprehensive literature search identified two studies of head-only electrical stunning in sheep that reported the use of EEG-based measures of unconsciousness (Cook *et al*., 1996; Velarde *et al*., 2002). Both studies reported corneal reflex to be consistently associated with unconsciousness. Cook *et al*. (1996) found that the time to irreversible loss of consciousness was 5 to 8 seconds after neck cutting (14 to 16 seconds after stunning; standard error mean: 0.7) and reported that "*pupillary, corneal and eyelash reflexes were absent by 10 seconds after neck cutting*". Velarde *et al.* (2002) studied 25 sheep and obtained EEG data from 21and reported that all the sheep were stunned effectively, and none had a corneal eye reflex during unconsciousness. Velarde *et al*. (2002) also reported that tonic followed by clonic seizures and absence of breathing and corneal reflex occurred concurrently with unconsciousness in the 21 effectively stunned animals (Velarde *et al*., 2002). This would suggest that, following head-only electrical stunning in sheep, all unconscious animals experience tonic and clonic seizures, i.e. in this case 21 out of 21 (for the purpose of this opinion, this means 100 % specificity). This would suggest the same 100 % specificity for absence of breathing, i.e. all 21 unconscious animals were not breathing. These data are summarised in Table 6. However, the sensitivity of these indicators (number of animals tested conscious by the indicator, out of the total number of conscious animals) is unclear.

Rodriguez *et al*. (2012) assessed brain activity in eight lambs during slaughter without stunning and its correlation with heart rate and the absence of physiological reflexes. Rhythmic breathing disappeared at an average time of 44 (± 4.2) seconds after sticking (range 30–60 seconds). The corneal reflex disappeared at 116 (± 11.01) seconds (range 80–160 seconds) after sticking. Changes in brain activity occurred between 22 and 82 seconds after sticking (average 52 (± 20.2) seconds). However, no significant correlations were found between the time to decreased brain activity and either changes in the hearth rate or the absence of physiological reflexes.

Table 6 summarises the information on the characteristics of the indicators derived from the systematic review.

[12] Indicators used to check the state of consciousness and unconsciousness.
[13] Indicators used to check the state of life and death.

Table 6: Information on the characteristics of the indicators derived from the systematic review.

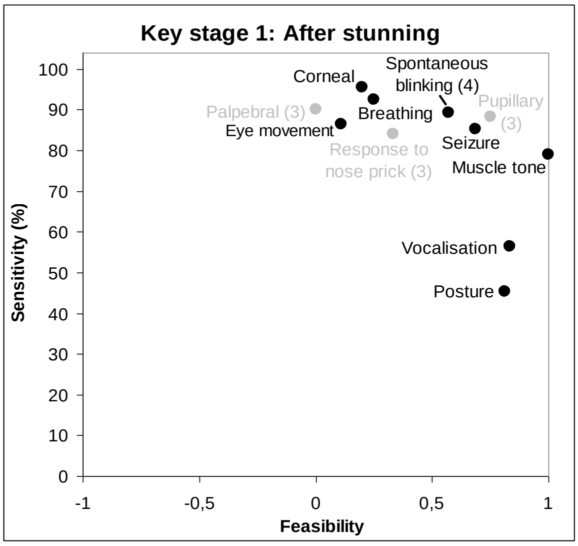
References	Species	Stun method	Outcome of unconsciousness of the indicator	Number of animals defined as unconscious	Number of animals tested unconscious by the indicator	Percentage of unconscious animals tested unconscious
Velarde *et al.*, 2002	Sheep	Head-only electrical	Immediate onset of tonic seizure	24	24	100 %
Velarde *et al.*, 2002	Sheep	Head-only electrical	No corneal reflex	24	24	100 %
Velarde *et al.*, 2002	Sheep	Head-only electrical	Immediate absence of breathing	24	24	100 %
Cook *et al.*, 1996	Sheep	Head-only electrical	Absence of eye reflex	2	2	100 %

3.3. Results from questionnaire 2

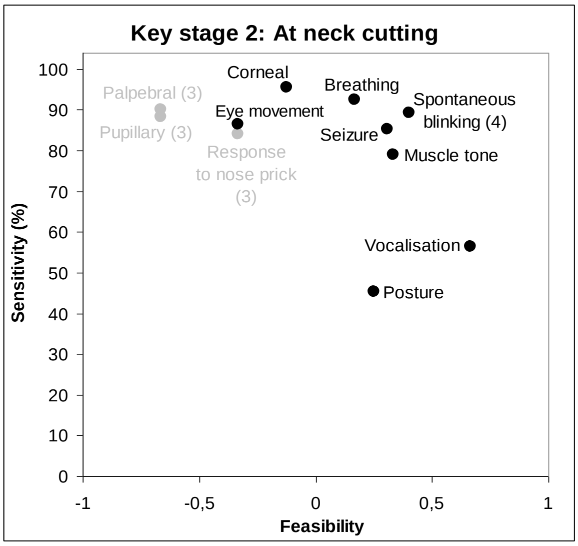
From the second questionnaire, namely the online survey, answers from around 82 experts were collected. Respondents could answer for more than one species or methods, depending upon their work experience, so the total number of completed surveys was 84.

In total, 32 respondents said that they monitor welfare of sheep and goats during stunning and slaughter. Of these, 27 respondents answered questions about head-only electrical stunning, but only 21 answered to the feasibility as well as the sensitivity/specificity question. Thus, 21 valid records are available for sheep and goats during head-only electrical stunning.

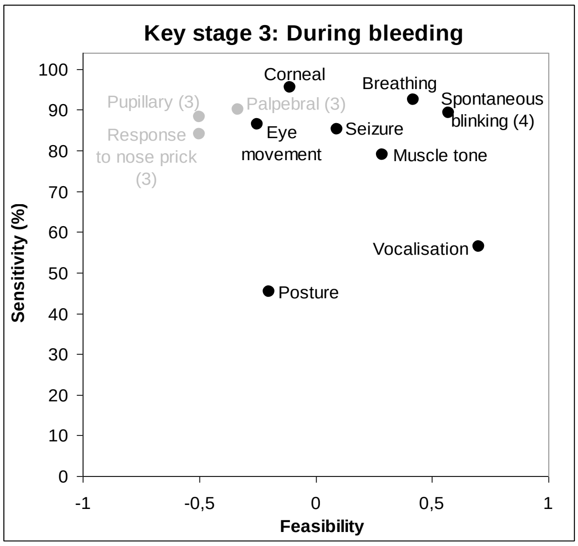
The graphs in Figure 3a, b and c combine the feasibility score and estimate of sensitivity for each indicator for head-only electrical stunning at each key stage (key stage 1 = immediately after stunning, key stage 2 = at neck cutting, key stage 3 = during bleeding). Thus, the indicators nearest the top-right corner have high sensitivity and high feasibility. In all three graphs the sensitivity value is identical but the feasibility score changes according to the respondent ratings.



(a) Immediately after stunning



(b) At neck cutting



(c) During bleeding

Figure 3: Graphical combination of feasibility score and sensitivity resulting from questionnaire 2 for each indicator at (a) key stage 1 = immediately after stunning, (b) key stage 2 = during neck cutting or sticking and (c) key stage 3 = during bleeding

In the case of slaughter without stunning, 17 respondents answered the question on the use of the indicators, but only 12 records were valid, namely those from respondents answering both sensitivity/specificity and feasibility questions. The respondents reported the feasibility of assessing indicators prior to release from restraint and carcass dressing. The specificity of most indicators, i.e. the ability to confirm an animal becoming unconscious or dead, was estimated to be greater than 85 % throughout all indicators and was greater than 95 % in 9 out of the 12 estimates (see Tables 8 and 9).

The following graph (Figure 4) combines the feasibility score and estimates of sensitivity for indicators applied prior to release from restraint and indicators applied prior to carcass dressing.

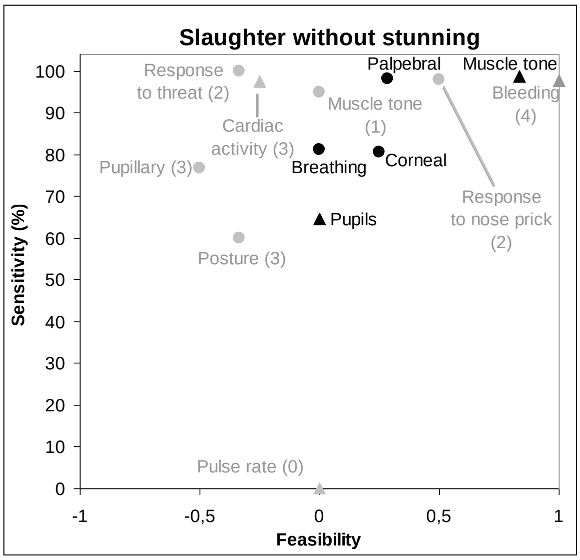


Figure 4: Graphical combination of feasibility score and sensitivity resulting from questionnaire 2 for each indicator prior to release from restraint (circles) and prior to dressing (triangles). Grey symbols/items are indicators with fewer than five data points

3.4. Description of indicators for head-only electrical stunning and overview of their performance

The combined efforts of the above activities led to the following overview of indicators and outcomes of consciousness and unconsciousness.

The following paragraphs discuss the indicators and their outcomes mentioned above in relation to their relevance in identifying consciousness at key stages of monitoring slaughter with head-only electrical stunning. Some of these outcomes occur spontaneously following stunning (e.g. tonic–clonic seizure) and/or neck cutting whereas some other outcomes will have to be intentionally provoked (e.g. corneal reflex). The Working Group agreed that the risk of poor welfare can be detected better if animal welfare monitoring is focused on detecting consciousness. The presence of certain outcomes (e.g. vocalisation) or a positive response of the animal to an applied stimulus (e.g. corneal reflex) is most relevant. In addition to this, the sensitivity, specificity and feasibility of the indicators are presented, based on information gathered in the different activities described in this opinion. Depending on all these aspects, some indicators may not be applicable to monitoring at certain key stages.

3.4.1. Posture

3.4.1.1. Description

In key stage 1 (i.e. after stunning), unconsciousness is manifested as immediate collapse of the animal and, when the current passes through the brain, if the electrical stunning is ineffective, the animal will either fail to collapse or attempt to regain posture (indication of consciousness). Animals showing these signs of ineffective electrical stunning will require immediate re-stun.

Effectively stunned sheep and goats will be shackled, hoisted and presented for neck cutting, which is key stage 2. In some slaughterhouses, sheep and goats might be neck cut while lying horizontally and before being shackled and hoisted. An unconscious animal at this stage will show tonic muscle contractions and is therefore not expected to show any changes in its posture. Hence, posture as an indicator is not applicable (n.a.) in this situation. In contrast, an animal recovering consciousness whilst hanging on the overhead shackle will attempt to regain posture, which will be manifested as arching of the neck or body; such an animal will have to be re-stunned.

An effectively stunned and stuck animal will remain unconscious during bleeding until death occurs in key stage 3 and therefore is not expected to show any change in **posture** (n.a). On the other hand, animals recovering consciousness whilst hanging on the overhead shackle and bleeding will **attempt to regain posture**, which may be **manifested as arching of the neck or body** and they will have to be re-stunned.

3.4.1.2. Feasibility

In questionnaire 2 immediate collapse was rated as an indicator that is easy or normal to assess at stunning (key stage 1) by 88 % and 6 %, respectively (*n* = 16).

3.4.1.3. Sensitivity and specificity

The positive outcome of posture is the sign of consciousness, namely the maintenance of an upright posture. Therefore, the sensitivity is the percentage of animals which maintain upright posture immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 45 % (*n* = 12). The specificity is calculated as the percentage of animals immediately losing posture, out of all truly unconscious animals. This was estimated to be 100 % (*n* = 12). The reason for the relatively low sensitivity is probably the occurrence of immediate loss of posture without loss of consciousness as a result of electro-immobilisation, which can be induced with a current lower than that necessary to induce generalised epileptiform activity in the brain.

3.4.2. Breathing

3.4.2.1. Description

In key stage 1, effective electrical stunning will lead to immediate onset of apnoea, i.e. **absence of breathing**, which can be used to monitor the effectiveness of head-only electrical stunning. Ineffective electrical stunning can be recognised from the sustained/**presence of breathing, including laboured breathing**.

In key stage 2, unconscious animals will continue to manifest apnoea. In contrast, animals recovering consciousness whilst hanging on the overhead shackle will attempt to breathe, which may begin as **regular gagging leading to resumption of breathing**; these animals will have to be re-stunned.

An effectively stunned and stuck animal will remain unconscious until death occurs in key stage 3, and therefore is expected to show apnoea. On the other hand, animals recovering consciousness whilst hanging on the overhead shackle and bleeding will attempt to breathe, which may begin as **regular gagging leading to resumption of breathing**, and they will have to be re-stunned.

3.4.2.2. Feasibility

Breathing was rated as easy or normal to assess at stunning by 40 % and 45 %, respectively (*n* = 20); at sticking by 32 % and 47 %, respectively (*n* = 19); and during bleeding by 53 % and 37 %, respectively (*n* = 19). This is probably because it may not be possible to recognise breathing in animals in the stunning box during tonic–clonic seizure, or shackled and hoisted on to the overhead rail. Also, occasional or irregular gagging may occur in unconscious animals just prior to the onset of brain death.

3.4.2.3. Sensitivity and specificity

The positive outcome of breathing is the sign of consciousness, namely the presence or resumption of breathing. Therefore, the sensitivity is the percentage of animals which show presence of breathing, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 93 %

(n = 16). The specificity is calculated as the percentage of animals showing apnoea, out of all truly unconscious animals. This was estimated to be 75 % (n = 20).

3.4.3. Tonic–clonic seizures

3.4.3.1. Description

In key stage 1, effective electrical stunning leads to the **onset of tonic seizure** immediately after collapse, which may be recognised from the occurrence of extended front legs and flexed hind legs under the body.

The tonic seizure lasts for several seconds and is followed by two clonic seizures. Tonic seizures may be present at the time of neck cutting, which is key stage 2. The tonic seizure, if present at key stage 2, will cease rapidly following sticking and, therefore, is not applicable at key stage 3.

3.4.3.2. Feasibility

From questionnaire 2, tonic seizure was rated as easy or normal to assess at stunning by 69 % and 31 % of experts, respectively (n = 16).

3.4.3.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of tonic seizure is the sign of consciousness, namely the absence of tonic seizures. Therefore, the sensitivity is the percentage of animals which do not show the onset of tonic seizures immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 85 % (n = 11). The specificity is calculated as the percentage of animals showing onset of tonic seizures, out of all truly unconscious animals. This was estimated to be 95 % (n = 16).

3.4.4. Muscle tone

3.4.4.1. Description

Effectively stunned animals will show tonic–clonic seizures followed by **loss of muscle tone**, which can be recognised from **floppy ears and relaxed jaw**. Animals which regain muscle tone may manifest **stiff (upright) ears and jaws and the righting reflex** (e.g. severe kicking, head lifting, body arching). These signs are more visible after neck cutting when the animals are hanging from the overhead rail, especially at key stage 3. Animals showing any of these signs of muscle tone must be re-stunned.

3.4.4.2. Feasibility

From questionnaire 2, muscle tone was rated as easy to assess at stunning by 100 % (n = 7), and at sticking and during bleeding by 43 % (n = 7) of experts.

3.4.4.3. Sensitivity and specificity

The positive outcome of muscle tone, namely the presence of muscle tone, is the sign of consciousness. Therefore, the sensitivity is the percentage of animals which show a certain level of muscle tone, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 79 % (n = 6). The specificity is calculated as the percentage of animals showing loss of muscle tone, out of all truly unconscious animals. This was estimated to be 86 % (n = 7).

3.4.5. Response to nose prick or ear pinch

3.4.5.1. Description

Ineffective electrical stunning and recovery of consciousness can be recognised from the **response to nose prick or ear pinch** at all key stages of monitoring. Animals showing positive response to painful stimulus at any stage must be re-stunned.

3.4.5.2. Feasibility

From questionnaire 2, response to nose prick or ear pinch was considered (*n* = 3) as easy or normal to assess by, respectively, 67 % and 0 % at stunning, 34 % and 0 % at sticking, and 0 % and 34 % during bleeding. Many of the experts reported that response to nose prick or ear pinch is difficult to assess at any of the key stage of monitoring. Lack of access to animals could be one of the reasons why response to a painful stimulus, i.e. nose prick or ear pinch, cannot be performed during bleeding.

3.4.5.3. Sensitivity and specificity

The positive outcome of the response to nose prick or ear pinch is the sign of consciousness, namely a positive response to nose prick or ear pinch. Therefore, the sensitivity is the percentage of animals which do respond to nose prick or ear pinch immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 84 % (*n* = 3). The specificity is calculated as the percentage of animals showing no response to nose prick or ear pinch, out of all truly unconscious animals. This was estimated to be 96 % (*n* = 3).

3.4.6. Vocalisation

3.4.6.1. Description

Vocalisation is expected only in conscious animals and can be used as an indicator in all key stages of monitoring. However, not all the conscious animal may vocalise, and hence absence of vocalisation does not always mean that the animal is unconscious. Animals showing vocalisation must be re-stunned. Since unconscious animals will not vocalise, this indicator is not applicable to monitoring unconsciousness.

3.4.6.2. Feasibility

From questionnaire 2, vocalisation was considered to be easy or normal to assess by, respectively, 83 % and 17 % of experts at stunning by (*n* = 12), 64 % and 9 % at sticking (*n* = 11), and 73 % and 9 % during bleeding by (*n* = 11). Some experts reported that vocalisation as difficult to assess at any of the key stages.

3.4.6.3. Sensitivity and specificity

The positive outcome of vocalisation is the sign of consciousness, namely the presence of vocalisation. Therefore, the sensitivity is the percentage of animals which do vocalise immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 56 % (*n* = 8). The specificity is calculated as the percentage of animals no vocalisation, out of all truly unconscious animals. This was estimated to be 89 % (*n* = 11). The reason for the low sensitivity is that vocalisation is a spontaneous behaviour and thus not all conscious animals, especially sheep, may vocalise.

3.4.7. Eye movements

3.4.7.1. Description

In key stage 1, effective electrical stunning will produce **fixed eyes** (eyes wide open and glassy) or eyeballs may be obscured owing to rotation into the eye socket. Animals that are not effectively stunned with an electric current or those recovering consciousness will show **eye movements**, which can be used to recognise consciousness during all the three key stages. Animals showing any eye movements must be re-stunned.

3.4.7.2. Feasibility

From questionnaire 2, eye movements were considered as easy to assess at stunning (*n* = 9) by 34 %, at sticking (*n* = 8) by 12 % and during bleeding (*n* = 8) by 25 % of the experts. Eye movements were rated as normal to assess at stunning (*n* = 9) by 44 %, at sticking (*n* = 8) by 25 % and during bleeding (*n* = 8) by 25 % of the experts. Eye movements as an indicator was also rated as difficult to assess at sticking (*n* = 8) by 37 % and during bleeding (*n* = 9) by 50 % of the experts. It may be difficult or impossible to observe eye movements at the time of sticking or bleeding because of the orientation of the animal, i.e. with the operator facing the brisket, at these two key stages.

3.4.7.3. Sensitivity and specificity

The positive outcome of eye movement is the sign of consciousness, namely the presence of eye movements. Therefore, the sensitivity is the percentage of animals which show eye movements immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 86 % (*n* = 7). The specificity is calculated as the percentage of animals showing fixed eyes, out of all truly unconscious animals. This was estimated to be 80 % (*n* = 8). Unconscious animals might show eye movement if the cranial nerves are electrically stimulated during the current application.

3.4.8. Palpebral reflex

3.4.8.1. Description

Effective electrical stunning will lead to abolition of palpebral reflex. Effectively stunned and stuck animals show **no palpebral reflex** during any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show a **positive palpebral reflex** at any key stage. Animals showing a positive palpebral reflex must be re-stunned.

3.4.8.2. Feasibility

From questionnaire 2, the palpebral reflex was rated as easy and normal to assess, respectively, at stunning (*n* = 4) by 25 % and 50 %, at sticking (*n* = 3) by 0 % and 33 %, and during bleeding (*n* = 3) by 0 % and 67 % of experts. A number of experts considered palpebral reflex as difficult at stunning (25 %), at sticking (67 %) and during bleeding (33 %).

3.4.8.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of palpebral reflex is the sign of consciousness, namely the presence of a palpebral reflex. Therefore, the sensitivity is the percentage of animals which show palpebral reflex immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 90 % (*n* = 3). The specificity is calculated as the percentage of animals showing no palpebral reflex, out of all truly unconscious animals. This was estimated to be 86 % (*n* = 4).

3.4.9. Corneal reflex

3.4.9.1. Description

Effective electrical stunning leads to abolition of corneal reflex. Effectively stunned and stuck animals show **no corneal reflex** during any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show

positive corneal reflex at any key stage. Animals showing a positive corneal reflex must be re-stunned.

3.4.9.2. Feasibility

From questionnaire 2, the corneal reflex was rated as easy to assess at stunning (*n* = 10) by 40 %, at sticking (*n* = 9) by 22 % and during bleeding (*n* = 9) by 33 % of experts. The corneal reflex was rated as normal to assess at stunning by 40 %, at sticking by 22 % and during bleeding by 33 % of experts. A number of experts also considered the corneal reflex as difficult to assess at stunning (20 %), at sticking (33 %) and during bleeding (44 %), and the reason for these high ratings could be the inaccessibility of animals.

3.4.9.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of corneal reflex is the sign of consciousness, namely the positive corneal reflex. Therefore, the sensitivity is the percentage of animals which show corneal reflex immediately after stunning, out of all conscious animals. This was estimated by questionnaire 2 respondents to be 96 % (*n* = 8). The specificity is calculated as the percentage of animals showing no corneal reflex, out of all truly unconscious animals. This was estimated to be 79 % (*n* = 10).

3.4.10. Spontaneous blinking

3.4.10.1. Description

Spontaneous blinking is expected in only conscious animals and can be used as an indicator in all key stages of monitoring. However, not all conscious animals will show spontaneous blinking, and hence the absence of blinking does not always mean that the animal is unconscious. Animals showing blinking must be re-stunned.

3.4.10.2. Feasibility

From questionnaire 2, spontaneous blinking was rated as easy to assess at stunning (*n* = 8) by 50 %, at sticking (*n* = 7) by 43 % and during bleeding (*n* = 7) by 71 % of experts. Spontaneous blinking was rated as normal to assess at stunning by 37 %, and at sticking and bleeding by 14 % of experts. It is possible that inaccessibility/lack of access to animals during neck cutting was taken into consideration by those experts who did not rate eye reflexes (palpebral and corneal reflexes and spontaneous blinking) as easy to assess.

3.4.10.3. Sensitivity and specificity

The positive outcome of blinking is the sign of consciousness, namely the presence of spontaneous blinking. Therefore, the sensitivity is the percentage of animals which show spontaneous blinking immediately after stunning, out of all conscious animals. This was estimated by questionnaire 2 respondents to be 89 % (*n* = 4). The specificity is calculated as the percentage of animals showing no spontaneous blinking, out of all unconscious animals. This was estimated to be 94 % (*n* = 4).

3.4.11. Pupillary reflex

3.4.11.1. Description

Effective electrical stunning will lead to abolition of pupillary reflex. Effectively stunned and stuck animals show **no pupillary reflex** during any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show a **positive pupillary reflex** at any key stage. Animals showing a positive pupillary reflex must be re-stunned.

3.4.11.2. Feasibility

From questionnaire 2, the pupillary reflex was rated (*n* = 4) as easy to assess at stunning by 75 %, at sticking by 0 % and during bleeding by 25 % of experts. The pupillary reflex was rated as normal to assess at stunning by 25 %, at sticking by 25 % and during bleeding by 0 % of experts. Some of the experts also considered the pupillary reflex as difficult to assess at sticking (50 %) and during bleeding (75 %). The main reason for these ratings could be the lack of access to animals.

3.4.11.3. Sensitivity and specificity

The positive outcome of pupillary reflex is the sign of consciousness, namely the presence of the pupillary reflex. Therefore, the sensitivity is the percentage of animals which show pupillary reflex immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 88 % (*n* = 3). The specificity is calculated as the percentage of animals showing no pupillary reflex, out of all truly unconscious animals. This was estimated to be 90 % (*n* = 4).

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 and the systematic literature review is presented in Table 7.

Table 7: Summary of information on sensitivity, specificity and feasibility of indicators and outcomes of consciousness from questionnaire 2 and the systematic review

Indicators after head-only electrical stunning	Outcomes of consciousness	Sensitivity (%)	Data (without uncertainty, average (20th, 50th and 80th percentiles	Specificity (%)	Data (without uncertainty, average (20th, 50th and 80th percentiles	Feasibility score		
						After stunning	During neck cutting	During bleed-ing
Posture [a]	Failure to collapse or attempts to regain posture	45	50 (0, 65, 94)	100	100 (100, 100, 100)	0.81	0.25	–0.20
Breathing	Presence	93	91 (80, 98, 100)	75; 100 for sheep (SR)	75 (42, 99, 100)	0.25	0.17	0.42
Tonic–clonic seizures [b]	Absence of tonic–clonic seizures	85	83 (80, 95, 100)	95; 100 for sheep (SR)	95 (95, 100, 100)	0.69	0.31	0.09
Muscle tone	Presence	79	71 (50, 83, 100)	86	84 (91, 100, 100)	1.00	0.33	0.29
Response to nose prick or ear pinch	Presence	84	83 (n.a. (*n* = 3))	96	96 (n.a. (*n* = 3))	0.33	–0.33	–0.50
Vocalisation	Presence	56	59 (38, 65, 86)	89	89 (95, 100, 100)	0.83	0.67	0.70
Eye movements	Presence	86	86 (80, 95, 100)	80	77 (58, 97, 100)	0.11	–0.33	–0.25
Palpebral reflex	Presence	90	87 (n.a. (*n* =3))	86; 100 for sheep (SR)	78 (n.a. (*n* = 4))	0.00	–0.67	–0.33
Corneal reflex	Presence	96	94 (84, 100, 100)	79; 100 for sheep (SR)	77 (68, 94, 100)	0.20	–0.13	–0.11
Spontaneous blinking	Presence	89	84 (n.a. (*n* = 4))	94	94 (92, 99, 100)	0.57	0.40	0.57
Pupillary reflex	Presence	88	83 (n.a. (*n* = 3))	90; 100 for sheep (SR)	88 (n.a. (*n* = 4))	0.75	–0.67	–0.50

(a): In questionnaire 2, posture as an indicator was referred to its outcome of unconsciousness namely ‘immediate collapse’.
(b): In questionnaire 2, the question about ‘tonic-clonic seizures’ addressed the outcome of unconsciousness, namely the ‘presence of tonic-clonic seizures’.

n.a.: not applicable because fewer than five respondents (*n*).

3.5. Description of indicators for slaughter without stunning and overview of their performance

Two separate sets of indicators and outcomes are proposed, one for the determination of unconsciousness and one for the determination of death. This is mainly because indicators of unconsciousness may not be relevant to death. In addition, Regulation 1099/2009 requires that unconsciousness be established prior to releasing animals from the restraint, while death must be established in animals prior to carcass dressing.

In the first phase of slaughter without stunning, the focus is on the determination of the onset of unconsciousness through the outcome of the tested indicators (specificity) while the monitoring focus is on animals still conscious (sensitivity). In the second phase of slaughter without stunning, the focus is on the determination of onset of death through the outcome of tested indicators suggestive of death (specificity) while the monitoring focus is on animals still alive (sensitivity).

3.5.1. Indicators prior to release from restraint

This list of indicators to be used to confirm unconsciousness is intended for use before the animal is released from the restraints. For slaughter without stunning the specific estimates of feasibility and sensitivity/specificity from questionnaire 2 have to be considered with caution because only 19 respondents were addressing this method of slaughter out of which 12 answered to the feasibility and sensitivity/specificity questions.

3.5.1.1. Posture

Description

Permanent loss of posture can be used as the earliest physical sign of the onset of unconsciousness following slaughter without stunning of an unrestrained/free-standing animal. It is inevitable that slaughter without stunning will lead to **loss of posture** provided the neck cutting was swift and bleeding was profuse and uninterrupted. **Loss of posture** (or **loss of balance**) can be used only in sheep and goats free-standing or mildly restrained in upright position. Therefore, loss of posture cannot be determined in animals that are severely restrained and/or rotated. Caution is required as animals may fall down after slaughter without stunning if the floor is slippery and they may continue to struggle to stand up again without success or simply lie down. For this reason, close and continuous observation of the animals is required to ensure that the loss of posture is a true occurrence. Some animals may exhibit loss of posture as a result of the loss of significant proportions of circulating blood volume but subsequently suffer carotid artery occlusion and recover consciousness. However, these animals may or may not attempt to regain posture. Therefore, complete and **permanent loss of posture without attempt to regain posture** can be used as an indicator of unconsciousness.

Feasibility

From questionnaire 2, loss of posture after neck cutting was rated ($n = 3$) as an easy indicator to assess by 33 % and as difficult to assess by 67 %. This is probably because loss of posture can only be assessed when the animal is free-standing or mildly restrained in the upright position. Loss of posture cannot be assessed in sheep and goats that are restrained severely and/or rotated, as they do not have freedom of movement to collapse.

Sensitivity and specificity

The negative test outcome of the indicator, i.e. the sign of unconsciousness, of the indicator ‘posture’ is the loss of posture. Therefore, the specificity is the percentage of unconscious animals which lose posture after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 100 % ($n = 4$). The sensitivity is calculated as the percentage of conscious animals that do not show collapse, out of all truly conscious animals. This was estimated to be 60 % ($n = 4$). The reason for the reduced sensitivity is that conscious animals might collapse for reasons (e.g.

slippery floor caused by the presence of blood) other than loss of consciousness. Nevertheless, the specificity is very high because an unconscious animal has to lose posture.

3.5.1.2. Muscle tone

Description

Loss of a significant proportion of circulating blood volume is also expected to result in general loss of muscle tone, resulting in a relaxed body, which can be used to recognise the onset of unconsciousness. Involuntary muscle jerks may occur occasionally. Depending upon the type and severity of restraint, **loss of tone in neck and leg muscles** could be used as an indicator of the onset of unconsciousness.

Feasibility

From questionnaire 2, muscle tone was rated as normal to assess by the only respondent (n = 1) to this part of the survey

Sensitivity and specificity

The negative outcome of the indicator 'muscle tone', i.e. the sign of unconsciousness, is the loss of muscle tone. Therefore, the specificity is the percentage of unconscious animals without muscle tone immediately after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 90 % (n = 1). The sensitivity is calculated as the percentage of conscious animals showing certain level of muscle tone, out of all conscious animals. This was estimated to be 95 % (n = 1). Consciousness is associated with certain level of muscle tone; hence, the loss of muscle tone is a highly specific sign of unconsciousness in animals. This confirms the high specificity of this indicator reported by the respondents.

3.5.1.3. Breathing

Description

Loss of consciousness following slaughter without stunning will eventually lead to cessation of rhythmic breathing. **Rhythmic breathing** can be recognised from the regular flank movement. Therefore, sustained absence of rhythmic breathing (absence of a respiratory cycle—inspiratory and expiratory movements) can be used as an indicator of the onset of unconsciousness. **Cessation of breathing** can be used as an indicator of unconsciousness in animals. However, since the trachea is also severed at the time of neck cutting at slaughter without stunning, the absence of breathing cannot be assessed from the air movement at external nostrils and will have to be confirmed by the absence of air bubbles in the liquid blood or sera accumulating at the neck wound or by the absence of any flank movements suggestive of breathing.

Feasibility

From questionnaire 2, breathing was rated (n = 6) as easy to assess by 33 % and normal to assess by 17 %. However, 33 % of experts considered breathing as difficult to assess prior to release from restraint. This is probably because breathing is difficult to recognise through the flank movements when the animal is severely restrained or rotated and cannot be ascertained through the nose if the trachea has been severed.

Sensitivity and specificity

The negative outcome of the indicator 'breathing', i.e. the sign of unconsciousness, is the immediate onset of apnoea. Therefore, the specificity is the percentage of unconscious animals which show apnoea after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 99 % (n = 5). The sensitivity is calculated as the percentage of conscious animals showing breathing, out of all animals still conscious after neck cutting. This was estimated to be 81 %

($n = 6$). The reason for the limited sensitivity is that rhythmic breathing, which is present in any animal with a functional brain stem, may not be recognised after neck cut.

3.5.1.4. Pupillary reflex

Description

The **pupillary reflex** is constriction of pupils (miosis) in response to focusing or shining a torch light at the pupils. Unconsciousness following slaughter without stunning can be determined from the **absence of the pupillary reflex**. On the other hand, animals recovering consciousness are expected to show the pupillary reflex.

Feasibility

From questionnaire 2, the pupillary reflex was rated ($n = 3$) as either normal or difficult to assess (one respondent each). The reason for these ratings is probably the lack of access to the animal and the fact that eyes are usually covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The negative outcome of the indicator 'pupillary reflex', i.e. the sign of unconsciousness, is absence of the pupillary reflex. Therefore, the specificity is the percentage of animals which do not show the pupillary reflex immediately after neck cutting out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 85 % ($n = 2$). The sensitivity is calculated as the percentage of conscious animals showing a positive pupillary reflex, out of all truly conscious animals. This was estimated to be 77 % ($n = 3$). The reason why both estimates are not closer to 100 % might be the difficulties in the assessment of this indicator under commercial conditions or the limited number of respondents.

3.5.1.5. Palpebral reflex

Description

The **palpebral reflex** is a blinking response elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Unconsciousness following slaughter without stunning can be determined from the **absence of the palpebral reflex**. On the other hand, animals recovering consciousness during bleeding are expected to show the palpebral reflex.

Feasibility

From questionnaire 2, the palpebral reflex was rated ($n = 8$) as easy to assess by 25 %, and normal to assess by 63 %. The reason for these ratings is probably the lack of access to the animal and the fact that the eyes are usually covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The negative outcome of the indicator 'palpebral reflex', i.e. the sign of unconsciousness, is absence of the palpebral reflex. Therefore, the specificity is the percentage of unconscious animals showing no palpebral reflex after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 96 % ($n = 6$). The sensitivity is calculated as the percentage of conscious animals blinking in response to stimulus of the palpebrae, out of all truly conscious animals. This was estimated to be 98 % ($n = 6$).

3.5.1.6. Corneal reflex

Description

The **corneal reflex** is a blinking response elicited by touching or tapping the cornea. Unconsciousness following slaughter without stunning can be determined from the **absence of the corneal reflex.** On the other hand, animals recovering consciousness during bleeding are expected to show the corneal reflex.

Feasibility

From questionnaire 2, the corneal reflex was rated (*n* = 9) as easy to assess by 33 % and as normal to assess by 44 %. The reason for these ratings is probably the lack of access to the animal and the fact that the eyes are usually covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The negative outcome of the indicator ‘corneal reflex’, i.e. the sign of unconsciousness, is absence of corneal reflex. Therefore, the specificity is the percentage of unconscious animals showing no corneal reflex immediately after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 99 % (*n* = 7). The sensitivity is calculated as the percentage of conscious animals showing a positive corneal reflex, out of all truly conscious animals. This was estimated to be 81 % (*n* = 6).

3.5.1.7. Response to threatening movement

Description

Conscious animals respond either by blinking or by attempting to move away from threatening movements (or clapping) of the hands close to the eye. This fear response will be abolished in unconscious animals, and therefore **response to visually threatening movements** can be used to check unconsciousness.

Feasibility

From questionnaire 2, response to threat was considered (*n* = 3) to be normal to assess by 67 % and difficult to assess by 33 %. None of the experts considered it easy to assess. The main reason might be that the head is restrained and the assessment of head movements might not be possible during this time. Other reasons include lack of access to the animal and the fact that the eyes are usually covered with blood, especially when the animals are rotated on to their backs.

Sensitivity and specificity

The negative outcome of the indicator ‘response to threatening movement’, i.e. the sign of unconsciousness, is absence of response to threatening movement. Therefore, the specificity is the percentage of unconscious animals which do not respond to threaten immediately after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 98 % (*n* = 2). The sensitivity is calculated as the percentage of conscious animals responding to threatening movement, out of all truly conscious animals. This was estimated to be 100 % (*n* = 2).

3.5.1.8. Response to nose prick or ear pinch

Description

Unconsciousness following slaughter without stunning can be determined from the **abolition of response to a painful stimulus such as nose prick** to the muzzle (area between external nostrils) **or ear pinch** with a sharp instrument. Conscious animals will show a positive response to a painful stimulus.

Feasibility

From questionnaire 2, the experts ($n = 2$) reported that response to nose prick or ear pinch is either easy (50 %) or normal (50 %) to assess.

Sensitivity and specificity

The negative outcome of the indicator 'response to nose prick or ear pinch', i.e. the sign of unconsciousness, is the absence of response to nose prick or ear pinch. Therefore, the specificity is the percentage of unconscious animals which do not respond to nose prick or ear pinch immediately after neck cutting, out of all truly unconscious animals. This was estimated by questionnaire 2 respondents to be 90 % ($n = 2$). The sensitivity is calculated as the number of conscious animals responding to nose prick or ear pinch, out of all truly conscious animals. This was estimated to be 98 % ($n = 2$).

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 and the systematic literature review is presented in Table 8.

Please note that the scores presented in Table 8 are the opinion of a small number of people (for many indicators $n < 5$, as indicated in the above paragraphs) engaged in slaughter without stunning.

Table 8: Summary of the data from questionnaire 2 regarding the specificity and feasibility of indicators applied prior to release from restraint during slaughter without stunning

Indicators to be used to detect unconsciousness (prior to release to restraint)	Outcomes of unconsciousness	Sensitivity (%)	Data (without uncertainty), no of respondents and average (20th, 50th and 80th percentiles)	Specificity (%)	Data (without uncertainty), no of respondents and average (20th, 50th and 80th percentiles)	Feasibility score (from questionnaire 2)
Posture	Permanent loss of posture	60	$n = 3$ 58 (n.a.)	100	$n = 4$ 100 (n.a.)	–0.33
Muscle tone	Loss of muscle tone	95	$n = 1$ 95 (n.a.)	90	$n = 1$ 90 (n.a.)	0.00
Breathing	Cessation of breathing	81	$n = 6$ 83 (100, 100, 100)	99	$n = 5$ 99 (99, 100, 100	0.00
Pupillary reflex	Absence of reflex	77	$n = 3$ 77 (n.a.)	85	$n = 2$ 85 (n.a.)	–0.50
Palpebral reflex	Absence of reflex	98	$n = 6$ 98 (99, 100, 100)	96	$n = 6$ 97 (100, 100, 100)	0.29
Corneal reflex	Absence of reflex	81	$n = 6$ 87 (97, 100, 100)	99	$n = 7$ 99 (100, 100, 100)	0.25
Response to threaten	Absence of response	100	$n = 2$ 100 (n.a.)	98	$n = 2$ 98 (n.a.)	–0.33
Responses to nose prick or ear pinch	Absence of response	98	$n = 2$ 98 (n.a.)	90	$n = 2$ 90 (n.a.)	0.50

n.a.: not applicable because fewer than five respondents provided data.

3.5.2. List of indicators of death for slaughter without stunning

This list of indicators of death is intended for use before the carcass dressing begins.

3.5.2.1. Pupil size

Description

Dilated pupils (midriasis) is an indicator of onset of brain death, which requires close examination of the eyes.

Feasibility

From questionnaire 2, the majority of the experts reported (n = 6) that dilated pupils are normal (67 %) to assess. Once the animal is released from the restraint, the accessibility to the eye allows assessment of the pupils.

Sensitivity and specificity

The negative outcome of the indicator 'pupil size', i.e. the sign of death, is dilated pupils. Therefore, the specificity is the percentage of dead animals which show dilated pupils immediately after neck cutting, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 97 % (n = 6). The sensitivity is calculated as the percentage of live animals without dilated pupils, out of all truly alive animals. This was estimated to be 65 % (n = 5).

The reason for the sensitivity ratings is probably the fact that the eyes are usually covered with blood, especially when the animals are rotated on to their backs.

3.5.2.2. Muscle tone

Description

Complete and irreversible loss of muscle tone leads to **relaxed body of the animal,** which can be recognised from the limp carcass, is an indicator of death.

Feasibility

From questionnaire 2, a relaxed body was considered (n = 6) to be easy or normal to assess by 83 % and 17 % of the experts. After the animal is released from the restraint, the relaxed body should be easy to assess.

Sensitivity and specificity

The negative outcome of the indicator muscle tone, i.e. the sign of death, is a relaxed body. Therefore, the specificity is the percentage of dead animals which show a relaxed body immediately after neck cutting, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 99 % (n = 6). The sensitivity is calculated as the percentage of alive animals showing certain maintenance of muscle tone out of all truly alive animals. This was estimated to be 99 % (n = 6).

3.5.2.3. Bleeding

Description

Slaughter eventually leads cessation of bleeding, with only minor dripping, from the neck cut wound, and therefore the **end of bleeding** in both carotid arteries and jugular veins can be used as the outcome of death.

Feasibility

From questionnaire 2, the end of bleeding was rated (n = 4) as easy to assess by 100 %.

Sensitivity and specificity

The negative outcome of the indicator 'bleeding', i.e. the sign of death, is the end of bleeding. Therefore, the specificity is the percentage of dead animals which stop bleeding after neck cutting, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 100 % (n = 4). The sensitivity is calculated as the percentage of live animals bleeding, out of all truly live animals. This was estimated to be 98 % (n = 4).

3.5.2.4. Cardiac activity

Description

Onset of death leads to permanent **absence of cardiac activity** (absence of heart beat), which can be ascertained using a stethoscope.

Feasibility

From questionnaire 2, cardiac activity was rated (n = 4) as difficult to assess by 50 % of experts, and as easy and normal to assess by 25 % each. Cardiac activity is normally assessed with a stethoscope, but this might not be available in the slaughterhouses.

Sensitivity and specificity

The negative outcome of the indicator 'cardiac activity', i.e. the sign of death, is the absence of heart beat. Therefore, the specificity is the percentage of dead animals without cardiac activity after killing, out of all truly dead animals. This was estimated by questionnaire 2 respondents to be 95 % (n = 3). The sensitivity is calculated as the percentage of live animals showing the presence of a heart beat, out of all truly alive animals. This was estimated to be 98 % (n = 3).

3.5.2.5. Pulse rate

Description

Onset of death leads to permanent loss of pulse. Pulse can be ascertained physically by pressing the (uncut) arteries in an extremity (e.g. femoral or coccygeal), and absence of pulse can be used to confirm death in animals.

Feasibility, sensitivity and specificity

Feasibility, sensitivity and specificity were not assessed as no response to these questions was collected from questionnaire 2.

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 is presented in Table 9.

Please note that the scores presented in Table 9 are the opinion of a small number of people (for many indicators $n < 5$, as indicated in the above paragraphs) engaged in slaughter without stunning.

Table 9: Summary of the data from questionnaire 2 and the systematic review regarding the sensitivity, specificity and feasibility of indicators and outcomes of death applied prior to dressing during slaughter without stunning

Indicators to be used (prior to carcass dressing)	Outcomes of death	Sensitivity (%)	Data (without uncertainty), no of respondents and average (20th, 50th and 80th percentiles)	Specificity (%)	Data (without uncertainty), no of respondents and average (20th, 50th and 80th percentiles)	Feasibility
Pupil size	Dilated pupils	65	*n* = 5 61 (4, 100, 100)	97	*n* = 5 96 (96, 100, 100)	0.00
muscle tone	Relaxed body	99	*n* = 6 98 (100, 100, 100)	99	*n* = 6 99 (100, 100, 100)	0,83
Bleeding	End of bleeding	98	*n* = 4 95 (n.a.)	100	*n* = 4 100 (n.a.)	1.00
Cardiac activity	Absence of cardiac activity	98	*n* = 3 97 (n.a.)	95	*n* = 3 95 (n.a.)	–0.25
Pulse rate	Absence of pulse rate	Nd	*n* = 0 n.a. (n.a.)	Nd	*n* = 0 n.a. (n.a.)	–

n.a.: not applicable owing to limited number of responses.

n.d.: not determined.

4. Discussion

4.1. Introduction

As previously described, this scientific opinion proposes welfare indicators to be used for monitoring during the slaughtering process of sheep and goats. In order to allow effective monitoring, the animals must be able to express behaviours and reflexes associated with consciousness. Consequently, procedures, processes or treatments that could mask the expression of such behaviours (such as electrical immobilisation or electrical stimulation) should not be used prior to confirmation of death in animals. Owing to the low number of scientific publication involving simultaneous assessment of EEG indicators of unconsciousness and welfare indicators (such as physical reactions and reflexes), the systematic literature review was not very productive and, therefore, much of the information for the selection of the indicators comes from questionnaire 2, which was especially aimed at obtaining estimated values for their sensitivity, specificity and feasibility. The indicators proposed in the toolboxes were selected based on sensitivity, specificity and feasibility as derived from various activities and on an expert consultation process (public consultation and technical meeting with experts from interested parties on 3 September 2013). Similarly, the model proposed for the sampling protocols was discussed with interested parties. The description of indicators in sections 3.4 and 3.5 also contains some basic information about elicitation of reflexes and responses and how to use the indicators. This is particularly relevant for indicators that warrant evoking a response from the animals (e.g. the corneal reflex). A short description of the physiology and elicitation of the indicators or evoking a conscious response is also presented in the glossary.

Indicators additional to those recommended in the toolboxes can also be used if considered necessary.

It should also be noted that the sample size, i.e. respondents to the questionnaire, was small and mainly from small to medium size slaughterhouses; nevertheless it indicates the existing knowledge, understanding and skill levels.

The outcomes of questionnaire 2 and the systematic review were discussed with external hearing experts on a meeting held on 3 September 2012. During the meeting, consensus was achieved on a set of recommended indicators to be included for each toolbox. Furthermore, for each toolbox, additional indicators were identified which can be used, but have lower sensitivity or feasibility, and are therefore not sufficient by themselves. In addition, they argued that skill levels in slaughterhouses and the feasibility of assessing the indicators may vary from slaughterhouse to slaughterhouse and therefore the toolbox should have more indicators. The external experts also felt that provision of indicators alone is not helpful in the decision making, and therefore a flowchart should be considered.

The outcomes of questionnaire 2 and discussion with hearing experts suggested that the reason for the low sensitivity and specificity ratings given to some outcomes of consciousness could be that the overall practice is to look at the outcomes of unconsciousness, which is the expected outcome of stunning, rather than detection of consciousness as a poor welfare outcome. Misconceptions with regard to the physiological basis of indicators were also inferred. These misconceptions need to be eliminated to harmonise welfare monitoring in slaughterhouses. It is also suggested that the sensitivity and specificity of these indicators would improve as people acquire relevant knowledge, skill and experience in assessing them. The feasibility scores reported in this opinion are also based upon limitations of the existing infrastructure, which is not necessarily designed and constructed with welfare monitoring as a priority. Therefore, it is suggested that the feasibility of monitoring these indicators would also improve if welfare monitoring is taken into consideration during the design, layout and construction of a new, or following structural change to existing, slaughterhouses.

The monitoring procedures are intended for use by the FBO in order to prevent negative welfare outcomes for the animals. The FBO, as a licence holder of a slaughterhouse, and employees with responsibility for animal welfare, including those designated as animal welfare officers, should undergo proper training and assessment of competence in welfare monitoring before licences are granted. For this to occur, any training, assessment and certification programmes implemented by the Member States should include welfare monitoring, and the contents of such education/training courses should be harmonised. Within the scope of Regulation (EC) 1099/2009, standard operating procedures should be implemented by the FBO and Member States/Competent Authorities should develop guides to good practice. These instruments should include welfare monitoring protocols/procedures for all key stages.

In addition, the regulation requires that the personnel handling, stunning or bleeding have a certificate of competence, and awarding of such certificate should also include monitoring animal welfare.

4.2. Monitoring procedures for head-only electrical stunning

4.2.1. Combination of selected indicators (the 'toolboxes')

For the creation of the toolboxes of indicators to be used in the monitoring procedures, indicators and their outcomes were selected by the working group members based on their knowledge regarding the validity, feasibility and indicator sensitivity. The specificity is not relevant for the toolbox considered to address potential welfare issues using consciousness as outcome (see section 2.1.2).

Indicators with high sensitivity and feasibility ratings in the questionnaire were selected for the toolbox. Some additional indicators that were given relatively lower ratings for sensitivity or feasibility were also included because the hearing experts and the Working Group thought that some of these indicators, such as eye movement in electrically stunned sheep and goats, might have good feasibility (ease of use) in slaughterhouses. The experts of the Panel on Animal Health and Welfare also agreed that indicators given low sensitivity and specificity at present by the respondents to the questionnaire might have potential for improvement in the future through education, training and assessment of personnel with responsibility for monitoring and ensuring welfare at slaughter (i.e. award of the Certificate of Competence). Similarly, indicators with low feasibility at present could be improved by changes in the design and layout or changes to existing practice. It was also thought that the toolbox should contain practical guidance with regard to recognition of consciousness and the decision-making process.

Indicators can be used either in parallel or in series. If two or more indicators are used in series, the second indicator is checked conditional on the outcome of the first indicator applied; if two or more indicators are used in parallel, they are performed simultaneously and therefore the animal is considered conscious when at least one of the indicators is positive.

For the purpose of detecting conscious animals in the slaughterline, indicators should be used in parallel. Indicators from the toolbox must be checked simultaneously on each sampled animal. To rule out consciousness, it is necessary that none of the indicators selected from the toolbox shows the outcome of consciousness. In practice, however, action may already have been taken, if there is evidence of consciousness, before all indicators have been checked.

When applying more than one indicator, it seems reasonable to expect an increase in the probability of detecting conscious animals, i.e. higher overall sensitivity of the monitoring protocol. If the outcomes of the checked indicators are independent of each other, then the overall sensitivity indeed increases. However, this possible increase in sensitivity would be reduced if the outcomes of the indicators are correlated, e.g. because of a common physiological basis or the checking procedure itself. The exact quantification of this correlation is not yet possible owing to a lack of scientific information. But it can be shown that the combined sensitivity of the two or more indicators is at least equal to the highest sensitivity of either or any alone (Gardner *et al*., 2000). Therefore, and in the absence of a quantified correlation between indicator outcomes, it is recommended that more than one indicator be used for monitoring but that highest sensitivity of the selected indicators be considered when planning the required sample size. This approach may lead to an oversampling, which, on the other hand, is in line with the precautionary principle needed to protect the welfare of animals.

4.2.1.1 Toolbox for key stage 1 (Toolbox 1 = immediately after stunning)

This opinion recommends the following indicators (and their outcomes of consciousness) for inclusion in the toolbox at key stage 1: tonic/clonic seizures, breathing and the corneal or palpebral reflex (these are presented above the dashed line in the flow chart). Additional indicators—spontaneous blinking, posture and vocalisation– are also proposed (these are presented below the dashed line in the flow chart), but their sensitivity or feasibility is lower and they should not be relied upon solely.

The reasons for this approach are presented in the following paragraphs.

<u>Recommended indicators (above the dashed line in the flow chart)</u>

Tonic/clonic seizures

The scientific rationale is that tonic–clonic seizures occur as a consequence of the induction of generalised epileptiform activity in the brain. This indicator is routinely used in small ruminant slaughterhouses during key stage 1 and, therefore, was considered to be highly sensitive, specific and feasible in questionnaire 2.

Breathing

This indicator is feasible and highly sensitive according to questionnaire 2 and can be used during key stage 1.

Corneal or palpebral reflex

The presence of the corneal or palpebral reflex was considered as a sensitive, specific and feasible indicator at key stage 1 by the respondents to questionnaire 2. A positive response should be taken as a clear sign of consciousness warranting intervention (re-stunning).

Additional indicators (below the dashed line in the flow chart)

Spontaneous blinking

The presence of spontaneous blinking was considered as a highly sensitive and specific indicator by the respondents to questionnaire 2. This indicator was also rated highly feasible during key stage 1. The presence of spontaneous blinking should be taken as a clear sign of consciousness warranting intervention (re-stunning).

Posture

The respondents to questionnaire 2 gave a low rating for the sensitivity but a very high rating for specificity. The low sensitivity is probably because immediate collapse may occur in conscious animals as a result of electro-immobilisation, for example. Nevertheless, immediate collapse as the outcome suggestive of unconsciousness is routinely used in small ruminant slaughterhouses to recognise effective head-only electrical stunning at key stage 1.

Vocalisations

Vocalisation was considered as a highly feasible indicator, although not very sensitive because not all the conscious animals may vocalise (e.g. electrically immobilised animals will remain conscious but may not vocalise). However, the presence of vocalisations such as grunting is a clear sign of consciousness at key stage 1 and warrants intervention (re-stunning).

Indicators not considered in the flow chart

The following indicators were not considered in the flow chart because of their low sensitivity or feasibility, e.g. limited or no access to the animal (see section 3.4): muscle tone, eye movements, response to nose prick or ear pinch and pupillary reflex.

4.2.1.2 Toolbox for key stage 2 (Toolbox 2 = during neck cutting or sticking)

This opinion recommends that the following indicators be included in the toolbox at key stage 2: breathing, tonic/clonic seizures and muscle tone (these are presented above the dashed line in the flow chart). In addition, the corneal or palpebral reflex, spontaneous blinking and vocalisations may also be used (these are presented above the dashed line in the flow chart).

The reasons for this are as follows.

Breathing

This indicator is feasible and highly sensitive according to questionnaire 2 and can be used at key stage 2.

Tonic/clonic seizures

This indicator was considered highly feasible during key stage 2 by the respondents to questionnaire 2 as tonic–clonic seizures would be present at sticking if the stun-to-stick interval is short.

Muscle tone

This indicator is highly sensitive and sufficiently feasible at key stage 2 according to questionnaire 2. Recovery of different degrees of muscle tone may manifest as leg kicking, the head-righting reflex, attempts to raise the head and arching of the back, and these signs can be used to recognise consciousness. The righting reflex and attempts to raise the head can be used to recognise consciousness in effectively stunned animals any time after the termination of tonic–clonic seizures.

Additional indicators (below the dashed line in the flow chart)

Corneal or palpebral reflex

According to questionnaire 2, the corneal and palpebral reflexes are highly sensitive but very low on feasibility. The experts felt that corneal and palpebral reflexes can be used at key stage 2 in slaughterhouses, in combination with other indicators.

Spontaneous blinking

The presence of spontaneous blinking was considered as a highly sensitive but not so specific indicator by the respondents to questionnaire 2. This indicator was highly feasible during key stage 2. The presence of spontaneous blinking should be taken as a clear sign of consciousness warranting intervention (re-stunning).

Vocalisations

Vocalisation was considered as a highly feasible indicator at key stage 2, although not very sensitive because not all conscious animals may vocalise (e.g. electrically immobilised animals will remain conscious but may not vocalise). However, the presence of vocalisations such as grunting is a clear sign of consciousness warranting intervention (re-stunning).

Indicators not considered in the flow chart

The following indicators were not included in the flow chart because of their low sensitivity or feasibility, owing to limited or no access to the animal (see section 3.4): eye movements, posture, response to nose prick or ear pinch, pupillary reflex, response to nose prick and ear pinch.

4.2.1.3 Toolbox for key stage 3 (Toolbox 3 = during bleeding).

This opinion proposes that the following indicators be included in the toolbox at key stage 3: breathing and muscle tone (these are presented above the dashed line in the flow chart). In addition, the corneal and palpebral reflexes, spontaneous blinking and vocalisations may also be used (these are presented above the dashed line in the flow chart).

The reasons for this are as follows:.

Recommended indicators

Breathing

This indicator is feasible at key stage 3 and highly sensitive according to questionnaire 2.

Muscle tone

This indicator is feasible at key stage 3 and highly sensitive according to questionnaire 2. Recovery of different degrees of muscle tone may manifest as leg kicking, the head-righting reflex and arching of the back, and these signs can be used to recognise consciousness. When electrically stunned animals are bled out during the tonic phase, the loss of muscle tone after the clonic activity during bleeding would confirm the persistence of unconsciousness in animals.

Additional indicators (below the dashed line in the flow chart)

Corneal or palpebral reflex

The respondents to questionnaire 2 rated the corneal and palpebral reflexes as highly sensitive but very low on feasibility during key stage 3.

Spontaneous blinking

The presence of spontaneous blinking was considered a highly sensitive and specific indicator by the respondents to questionnaire 2. This indicator was rated as highly feasible during key stage 3. The presence of spontaneous blinking should be taken as a clear sign of consciousness warranting intervention (re-stunning).

Vocalisations

Vocalisation was considered as a highly feasible indicator at key stage 3, although not very sensitive because not all conscious animals may vocalise (e.g. electrically immobilised animals will remain conscious but may not vocalise). However, the presence of vocalisations such as grunting is a clear sign of consciousness warranting intervention (re-stunning).

Indicators not considered in the flow chart

The following indicators were not recommended and are not included in the flow chart because of their low sensitivity and/or feasibility owing to limited access to the animal (see section 3.4): tonic/clonic seizures, posture, eye movements, pupillary reflex, response to nose prick and ear pinch.

4.2.2. Flow chart for the use of the toolbox indicators at slaughter with head-only electrical stunning

A flow chart was designed to support the understanding of the use of the indicators and is shown in Figure 5. Please refer to the section 3.3 for the definitions and selection process of the indicators and refer to section 3.4 and Table 7 for the sensitivity of each indicator (which is used to calculate the sample size). Please refer to the SAS Technical Report (EFSA SAS Unit, 2013) for further details on the practical calculation of the sample size.

The flow chart in Figure 5 illustrates this opinion's recommendations regarding the three key stages of monitoring, the recommended outcomes of consciousness or unconsciousness and the course of action to be taken when outcomes of consciousness are detected in sheep and goats following head-only electrical stunning. Following the stun, and prior to shackling (key stage 1), it is recommended that the top indicators listed above the dashed line in blue Toolbox 1 be used to recognise consciousness. The indicators below the dashed line can also be used to check for signs of consciousness, but their sensitivity or feasibility are low and they should not be relied upon solely. If the animal shows any of the signs of consciousness (red box), then appropriate intervention should be applied.

If no indicator suggests that the animal is conscious, i.e. all performed checks resulted in outcomes of unconsciousness (green box), then the animal can be shackled and bled out by a neck cut or chest stick. In Toolbox 2, recommended indicators are presented above the dashed line, and these can be used to check for consciousness at key stage 2. There are additional indicators below the dashed line in Toolbox 2, and these may also be used to check for outcomes of consciousness, but with low sensitivity or feasibility. If the animal shows any of the outcomes of consciousness (red box), then appropriate intervention should be applied.

If no indicator suggests consciousness, i.e. all performed checks resulted in outcomes of unconsciousness (green box), then the animal should continue to be checked during bleeding (key stage 3).

The blue Toolbox 3 recommends indicators to be used to check for consciousness. If any one outcome of these indicators suggests consciousness (red box), then appropriate intervention should be applied.. If no indicator presented in Toolbox 3 suggests consciousness, i.e. all performed checks resulted in outcomes of unconsciousness (green box), then it can be concluded there is no risk of regained consciousness.

Of the recommended indicators above the dashed line, a minimum of two indicators relevant to each key stage should be employed for an effective monitoring of the process.

Please note that, in the case of those indicators which rely on the animal manifesting certain behaviour suggestive of consciousness (e.g. spontaneous blinking, vocalisations), the outcomes of unconsciousness are presented in grey as a reminder of limited predictive value of the indicator, i.e. the percentage of non-vocalising animals that are truly unconscious, out of all non-vocalising animals). Nevertheless, the outcome of consciousness suggests that the animal is conscious and is a "warning signal" requiring an intervention.

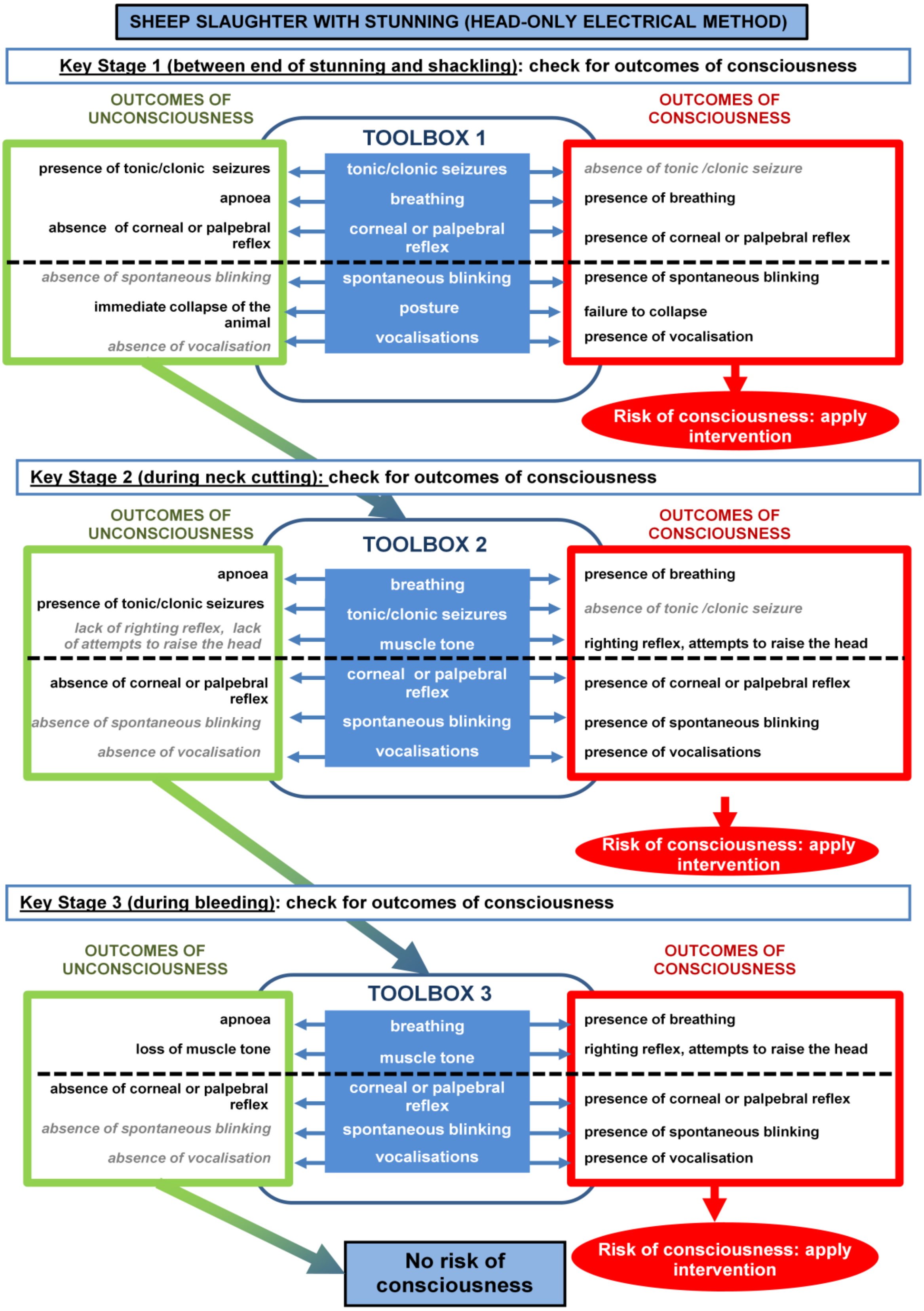


Figure 5: Toolbox of indicators that are considered suitable to be used for detection of conscious animals at each key stage of the procedure of head-only electrical stunning in sheep and goats

4.2.3. Sampling protocol for head-only electrical stunning

Independent of the sampling protocol specified in section 3.2.3 and discussed below, but in line with the duties of the personnel, who should process only unconscious animals, all animals (SF 100 %) should be monitored to prevent poor welfare outcomes. The indicators suggested in the flow chart are aimed at achieving effective monitoring of welfare of the animals by all personnel involved in stunning and slaughter.

4.2.3.1. Risk factors and welfare consequences

The final welfare consequence of failed head-only electrical stunning is the risk of conscious or not fully unconscious animals being shackled, stuck or dressed. This risk needs to be reduced to zero, by ensuring proper stunning routines and monitoring of stun efficacy.

In order to develop a monitoring protocol, the mandate from the Commission requests EFSA to estimate the optimal frequency with which animals should be checked for signs of consciousness following stunning.

This frequency should take into account risk factors associated with the stunning procedure.

The most common risk factors involved in the welfare of animals during slaughter are listed in Table 10. They have been linked to two categories: those risk factors that affect the quality of the stun and those that affect the quality of the assessment.

The two types of risk factors have a different effect on the sampling protocol.

Risk factors that reduce the quality of the stun.

When the quality of the stun is reduced, the probability of an animal not being properly stunned increases. This will increase the number of conscious animals which are presented to the operator for checking, i.e. increased failure rate. The model based sampling procedure developed in Chapter 2 is designed to detect any increase of this proportion of mis-stunned animals: in particular, the system will detect at least one conscious animal as soon as the overall proportion of poorly stunned animals exceeds the set failure rate. Therefore, in case of risk factors affecting the quality of the stun, the frequency of sampling does not have to be increased even though the number of animals that are mis-stunned increases. These risk factors do not necessitate any change in the sampling fraction.

Risk factors that reduce the sensitivity of the indicators used

Factors reducing the effectiveness of the assessment of consciousness will increase the likelihood that conscious animals are processed as if they were unconscious. This, of course, is an undesirable situation from an animal welfare point of view. If we deal with the indicators as if they were a diagnostic test, the ‘effectiveness’ of an indicator is expressed by the sensitivity, i.e. the probability of correctly classifying a truly conscious animal as conscious. It is intuitive that the lower this probability (i.e. the sensitivity of the indicator), the greater the number of animals that have to be tested in order to achieve a consistent level of confidence. This relationship is quantified through the model developed in Chapter 2.

The quantification of these sensitivity values is based on the knowledge and experience of a pool of stakeholders who were asked to complete questionnaire 2 (see section 3.3). Therefore, the resulting figures have to be referred to as ‘regular’ or ‘average’ for the situation. As a consequence, it is plausible to assume that under certain circumstances or ‘risk factors’ (e.g. the employment of new personnel) the same indicator may perform worse than under regular circumstances. Quantitatively speaking, when dealing with these different conditions, the sensitivity reference values may no longer hold; thus, the sample size required under these circumstances will be larger. These risk factors will therefore affect the monitoring procedure, because they alter the sensitivity of the indicator.

Table 10: Risk factors to animal welfare associated with head-only electrical stunning of sheep and goats

Component	Risk factor	Risk of poor stunning*	Risk of poor assessment *
STAFF	Competence (e.g. poor tong position)	√	√
	experience	√	√
	Fatigue	√	√
EQUIPMENT	maintenance	√	
	Features (e.g. poor electrical contact between the stunning tongs and head of the animal, dirty or corroded stunning tongs)	√	
	Presence of records of maintenance (e.g. cleaning)	√	
RECORDS OF THE CHECKS	Conformity in the past	√	√
ANIMALS	Increased resistance to the flow of current across the head due to the presence of wool or fleece on the head; Presence of horns interfering with the tong application	√	√
	Category/breed/temperament	√	√
ESTABLISHMENT	Line speed	√	√

*: The choice of risk category is based on expert opinion only.

4.2.3.2. Different scenarios for the sampling protocols

The risk factors described in the previous paragraph may require changes to the sampling protocol applied in the slaughter house. Three levels of sampling can be identified: standard, reinforced and light (also referred to in the literature as normal, tightened and reduced inspections).

<u>‘Standard’ sampling protocol</u>

The standard operating procedure for slaughter of small ruminants will involve a sampling fraction of 100 % by slaughterhouse personnel, as the operators check each animal for indicators of consciousness immediately after stunning, before sticking and during bleeding. In addition to this, the animal welfare officer will sample a fraction of all animals to monitor the effectiveness of the process, and will correct the operator or other aspects of the stunning process if necessary. The fraction sampled by the welfare officer can be calculated by the model, and is dependent on the indicator sensitivity, the slaughtered population, the maximum allowed threshold failure rate and the required accuracy, as described previously.

The larger the chosen slaughter population, or the higher the threshold failure rate, the lower the resulting sampling fraction will be. This means that the number of animals between two consecutively tested animals becomes larger. For example, if we take a required accuracy of 95 %, and an indicator with a sensitivity of 90 %, then the following calculation illustrates the effects of a risk manager’s decision regarding threshold failure rate and slaughter population. Given a slaughter population of number of animals killed on one day (e.g. 500 animals), and a threshold failure rate of 0.01, the sampling fraction will be 50 %. Therefore one in every two animals will need to be monitored. However, if the slaughter population is set at one working week (at the same daily throughput, so 2 500 animals), then the sampling fraction will be 13 %: so one in every eight animals. An appropriate decision on the criterion for defining a slaughter population and threshold failure rate would therefore help in achieving the requirements of the legislation on animal welfare at slaughter.

It goes without saying that the sampling protocol itself should not be a reason to delay the procedure. If slaughterhouse personnel identify a mis-stunned animal, they should take immediate remedial action. Subsequently, the personnel have to identify the reason for the poor stun and take remedial action. They should then inform the FBO or animal welfare officer.

If the animal welfare officer identifies a mis-stunned animal during execution of the sampling procedure, her or she should take remedial action and instigate the reinforced sampling protocol.

'Reinforced' sampling protocol

If one of the above-mentioned risk factors is present, which suggests a reduction in the sensitivity of the indicator applied by the personnel, the welfare officer will need to implement the back-up sampling. This can be done by concentrating the sampling efforts in a shorter time following the introduction of the risk factor, until the risk is identified and rectified. The degree to which the sampling needs to be increased is determined by the incurred reduction in indicator sensitivity. However, because the reduction in indicator sensitivity is not known, a pragmatic approach is required. This is to test all animals during a period represented by one-tenth of the slaughtered population. For example, if the slaughtered population as referred to in the standard sampling protocol was set to 200 animals, then for the time until the next 20 animals are processed, i.e. one-tenth of the slaughter population, all animals have to be retested by back-up sampling.

'Light' sampling protocol

There are no circumstances under which the sampling frequency (sample fraction) of the welfare officer can be relaxed, as a reduction in the sampling fraction will immediately reduce the accuracy by which a given excess threshold failure rate may be detected with the monitoring protocol (the other factors of the model, slaughtered population and test sensitivity, being unchanged).

4.3. Monitoring procedures for slaughter without stunning

4.3.1. Combination of selected indicators (the 'toolboxes')

As explained in section 3.3.1, since, in the case of slaughter without stunning, unconsciousness and death are induced gradually, indicators checking the state of unconsciousness and death were selected based on their specificity to detect unconscious animals out of all unconscious animals and preferably based on their sensitivity to detect animals still truly conscious as conscious. The sensitivity of the indicators was considered together with the feasibility of each stage. Here, the specificity—the detected number of unconscious animals out of all unconscious animals—would be less relevant for the purpose of monitoring welfare as no further processing can occur as long the outcome of the checked indicator suggests consciousness, regardless whether that outcome is true or false.

As explained in section 4.2.1, with the purpose of detecting unconscious and dead animals in the slaughterline, indicators can be used in parallel.

4.3.1.1. Toolbox for indicators prior release from restraint for slaughter without stunning (Toolbox 4)

This opinion recommends the following indicators for inclusion in the Toolbox: breathing and muscle tone. Additional indicators—posture, corneal or palpebral reflex—are also proposed, but they should not be relied upon solely.

The reasons for this are as follows.

Recommended indicators (above the dashed line in the flow chart)

Breathing

Breathing is considered a sensitive and reasonably feasible indicator at key stage 1 during slaughter without stunning according to questionnaire 2, and the cessation of breathing can be used to confirm unconsciousness prior release from restraint.

Muscle tone

Muscle tone is considered a highly sensitive and reasonably feasible indicator at key stage 1 during slaughter without stunning according to questionnaire 2. Loss of muscle tone can be used to confirm unconsciousness prior release from restraint.

Additional indicators (below the dashed line in the flow chart)

Posture

Posture is considered a reasonably specific for unconsciousness, less sensitive for consciousness but low in feasibility at key stage 1 during slaughter without stunning when the animal is restrained.

Corneal or palpebral reflex

The corneal and palpebral reflexes are considered sensitive, highly specific and reasonably feasible at key stage 1 during slaughter without stunning. In animals rotated on to their back, the head might be covered in blood and the corneal reflex might be more difficult to check. The presence of corneal and palpebral reflexes should be used as a warning signal to check other indicators prior to release from the restraint. This indicator was rated as very specific in confirming unconsciousness.

Indicators not considered in the flow chart

The following indicators were not recommended and are not included in the flow chart because of their low sensitivity or their low feasibility (see section 3.5) owing to the limited movement of, or difficulty of accessing, restrained animals: pupillary reflex, response to threat and responses to nose prick or ear pinch.

4.3.1.2. Toolbox for indicators prior to dressing for slaughter without stunning (Toolbox 5)

This opinion proposes the following indicators to be included in the toolbox for the assessment of death in slaughter without stunning: bleeding, muscle tone and pupil size.

The reasons for this are as follows.

Bleeding

The indicator bleeding is very sensitive and feasible at key stage 2 during slaughter without stunning. The indicator was rated as very specific in confirming death of the animal by cessation of bleeding.

Muscle tone

The indicator muscle tone is highly sensitive and highly feasible at key stage 2 during slaughter without stunning. The indicator was rated as very specific in confirming death of the animal by a fully relaxed body

Pupil size

The indicator ‘pupil size’ is reasonably sensitive and reasonably feasible indicator at key stage 2 during slaughter without stunning. The indicator was rated as specific in confirming death of the animal by dilated pupils.

Indicators not considered in the flow chart

The following indicators were not recommended and are not included in the flowchart because of their low performance or their low feasibility (see paragraph 3.5) owing to the limited access to the animal: cardiac activity and pulse rate.

4.3.2. Flow chart for the use of the toolbox indicators for slaughter without stunning

A flow chart was designed to support understanding the use of the indicators.

The flow chart in Figure 6 illustrates this opinion's recommendations regarding the key stages of monitoring, important outcomes of consciousness or unconsciousness and the course of action to be taken when outcomes of consciousness and life are detected in sheep and goats slaughtered without stunning. Following neck cutting and prior to release from restraint, it is recommended that the two indicators listed above the dashed line in blue Toolbox 4 be checked. The indicators below the dashed line may also be checked, but they may become difficult to ascertain under certain conditions (severe restraint and rotation). If the indicators suggest that the animal is still conscious (red box) and bleeding, than the animals should not be released from the restraint. Please refer to section 3.5 for the definitions and selection process of the indicators.

If all the indicators suggest unconsciousness (green box), then the animal can be released from the restraint. Prior to dressing, the indicators in blue Toolbox 5 should be checked. If all indicators show outcomes presented in the green box, then the animal can be assumed to be dead.

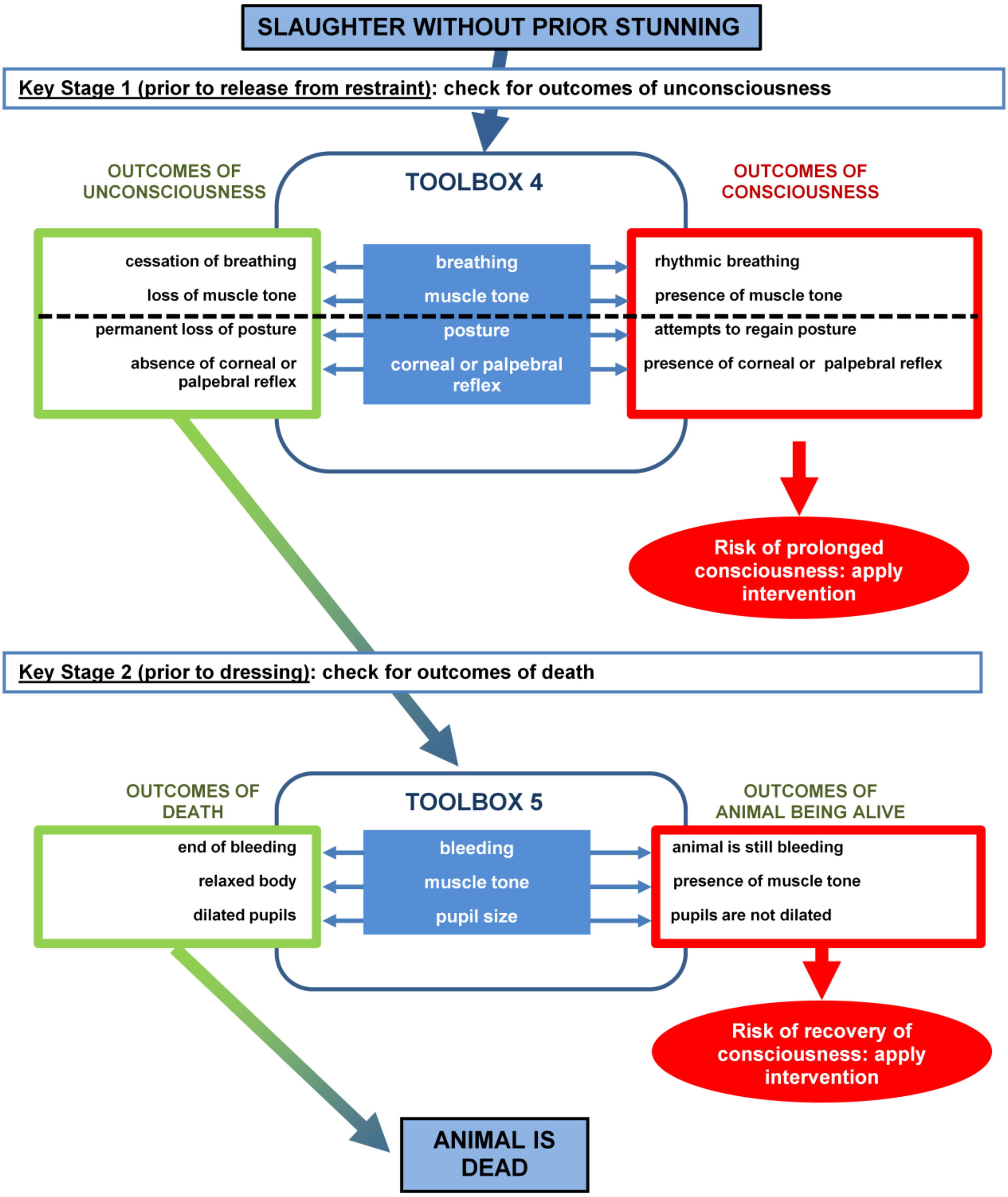


Figure 6: Toolbox of indicators and their outcomes as applicable prior to release from restraint and indicators and outcomes as applicable prior to dressing for slaughter without stunning in sheep and goats that are considered suitable to be used for confirmation of animals becoming unconscious and dead as well as detection of animals still conscious or alive.

4.3.3. Sampling protocol for slaughter without stunning

According to Regulation (EC) 1099/2009, when small ruminants are killed without prior stunning, persons responsible for slaughtering shall carry out systematic checks to ensure that the animals present signs of unconsciousness before being released from restraint and signs of death before undergoing dressing or scalding. Therefore, the personnel responsible for slaughtering should carry out the monitoring in all animals slaughtered without stunning.

4.3.3.1. Risk factors and welfare consequences

The legislation requires the inspection frequency of animals being slaughtered without stunning to be 100 %. Although several aspects of the neck cutting procedure are likely to affect the time to unconsciousness or death, their presence or absence does not affect the frequency of inspection, nor any further actions required by slaughter personnel.

Risk factors affecting the quality of neck cutting have been described in the DIALREL report (von Holleben *et al*., 2010). The restraining device, including both the body and head restrainers, might affect the assessment feasibility of unconsciousness. The design of the head restraint must not obscure the front of the head and should also allow good access to the eyes to check the corneal (or palpebral) reflex, and to the trachea to assess breathing.

CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS

1) From the stakeholder meeting it was learned that several indicators are currently used by experts to assess unconsciousness and death in animals. However, there is no harmonised list of indicators, either species or method specific, nor is there a scientific rationale. This highlights the need to develop a scientifically based set of indicators and monitoring protocols.

2) The systematic literature review revealed that no study explicitly has reported the sensitivity and specificity of the indicators in unconscious animals—as determined by brain activity measured using electroencephalography (EEG). Therefore, there is a scarcity of scientific publications reporting correlation between unconsciousness or death ascertained by EEG and the behavioural and physiological indicators to detect unconsciousness and death that could be used in slaughterhouse conditions.

3) The feasibility of monitoring any welfare indicator may vary depending upon the design and layout of the slaughter plant. Therefore, the feasibility of monitoring these indicators can be improved if welfare monitoring is taken into consideration during the design, layout and construction of a new, or following structural change to existing, slaughterhouses.

4) Stakeholders need to be aware that this opinion provides a methodology and scientifically valid approach to determining the sample size and sampling protocols. In this regard, the sensitivity, specificity and feasibility of indicators that are relevant to the skill level and facilities of the slaughterhouse should be ascertained and used in estimating appropriate sample size and protocols.

5) The level of competence of the staff influences the feasibility, sensitivity and specificity of the indicators. Therefore, lack of knowledge and understanding of physiological basis of indicators may have contributed to some ratings being low on sensitivity, specificity and feasibility by the respondents of the questionnaires.

6) Sampling protocols suggested in this opinion are based on sensitivity assessment for indicators involving expert survey because there are no (or few) controlled studies under laboratory conditions which determine the sensitivity of the indicators based on correlation with the EEG parameters.

7) In a slaughterhouse, consciousness, unconsciousness and death of the animals are checked throughout the process by two different categories of operators: (i) the ‘personnel’, namely the person(s) performing pre-slaughter handling, stunning, shackling, hoisting and/or bleeding, and (ii) the animal welfare officer, the person responsible for overall animal welfare at slaughter.

CONCLUSIONS ON HEAD-ONLY ELECTRICAL STUNNING IN SHEEP AND GOATS

8) To reduce welfare risks due to poor stunning, it is important to detect the animals that are not properly stunned or recover consciousness after stunning. Therefore, it is most important to check periodically indicators with high sensitivity and feasibility in detecting conscious animals.

9) For detecting consciousness in sheep and goats after head-only electrical stunning, the sensitivity of the indicators (ability of an indicator to detect conscious animals as conscious) is relevant for animal welfare whereas specificity (ability of an indicator to

detect unconscious animals as unconscious) is more related to the logistics (personnel have to re-stun the animal).

10) Since unconsciousness should be confirmed from the stunning application until death, this opinion recognises three key stages for monitoring welfare at slaughter: (i) immediately after stunning (between end of stunning and shackling), (ii) during neck cutting (sticking) and (iii) during bleeding.

11) The opinion concludes that a set of indicators (a minimum of two indicators) to be used to detect conscious animals following head-only electrical stunning should consist of:

Key stage 1 (between end of stunning and shackling): tonic/clonic seizures, breathing, corneal or palpebral reflex. Additional indicators—spontaneous blinking, posture and vocalisations—are also proposed, but they should not be relied upon solely.

Key stage 2 (during neck cutting): breathing, tonic/clonic seizures and muscle tone. Additionally, the corneal and palpebral reflex, spontaneous blinking and vocalisations may be used.

Key stage 3 (during bleeding): breathing and muscle tone. In addition, the corneal or palpebral reflex, spontaneous blinking and vocalisations may be used.

12) In order to develop sampling protocols for monitoring consciousness in electrically stunned sheep and goats, indicator(s) sensitivity, threshold failure rate (i.e. tolerance level) for acceptable proportion of mis-stunning, the size of the slaughter population, the sampling frequency (i.e. sample fraction) and the desired accuracy of the sampling protocol are required.

13) In electrical stunning, there are two types of risk factors: (i) those associated to stun quality and (ii) those associated to the quality of the monitoring. Only the latter have an effect on the sampling protocol.

14) Risk factors related to the quality of monitoring may require changes to the sampling protocol applied in the slaughterhouse, from a ‘standard’ to a ‘reinforced’ sampling protocol.

CONCLUSIONS ON SHEEP AND GOATS SLAUGHTER WITHOUT STUNNING

15) In the case of slaughter without stunning, it is important to detect the onset of unconsciousness prior to release from restraint, and death prior to dressing, in all animals while assuring identification of conscious and alive animals as such. Therefore, it is most important to routinely check indicators that have high feasibility and both high specificity and sensitivity in detecting conscious and live animals, respectively.

16) During slaughter without stunning, the restraining device, and the presence of blood in the eyes, might affect the feasibility of monitoring the welfare indicators and, as a consequence, the ability to detect unconsciousness.

17) In small ruminants rotated on to their back for the purpose of neck cutting, the monitoring of some of the welfare indicators is not feasible, as the animal’s head and body are severely restrained and the head is also covered with blood. The experts reported this to be a risk factor leading to reduced feasibility. This concerns eye reflexes, loss of posture and response to nose prick or ear pinch.

18) For monitoring in sheep and goats slaughtered without stunning, the sensitivity of an indicator (ability to detect conscious animals as conscious) is relevant for animal welfare whereas specificity (the ability of an indicator to detect unconscious animals as unconscious) is more related to the logistics (the personnel of the slaughterhouse have to wait longer before releasing the animals from restraint).

19) For monitoring in sheep and goats slaughtered without stunning, the sensitivity of an indicator (the ability to detect alive animals as alive) is relevant for animal welfare whereas specificity (ability of an indicator to detect dead animals as dead) is related to the logistics (the personnel of the slaughterhouse have to wait longer before performing carcass dressing).

20) The opinion concludes that the indicators to be used to detect unconscious animals prior to release from restraint following slaughtering without stunning are breathing and muscle tone. In addition, posture and the corneal and palpebral reflexes may also be used.

21) The opinion concludes that that the indicators to be used to detect dead animals prior to carcass dressing following slaughtering without stunning are bleeding, muscle tone and pupil size.

22) In slaughter without stunning, there are two types of risk factors: (i) those associated with neck cutting quality and (ii) those associated with the quality of the monitoring. However, none of them affect the sampling protocols since all animals have to be checked as required in Regulation (EC) 1099/2009.

RECOMMENDATIONS

GENERAL RECOMMENDATIONS

1) A scientifically based and harmonised set of indicators for use in standard operating procedures in slaughterhouses as well as in monitoring protocols is needed.

2) Further scientific studies should be carried out to reveal the correlation between the state of unconsciousness and death—as measured by brain activity using electroencephalography—and the behavioural and physiological indicators used to detect unconsciousness and death in order to collect valid information on indicator sensitivity and specificity.

3) In a controlled laboratory conditions the sensitivity of the indicators should be determined by correlation to EEG parameters, according to the “Guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing” (EFSA AHAW Panel, 2013).

4) The level of competence of slaughterhouse staff, which determines the feasibility, sensitivity and specificity of the indicators, should be improved through harmonised education, training and assessment throughout the EU. Until such time as any improvement in sensitivity or specificity resulting from personnel training is objectively demonstrated, the values given in this opinion for calculating the sample size should be considered as a minimum requirement.

5) The procedure of approval of the design, layout and construction of a new slaughterhouse, or of a structural change to existing slaughterhouses, should include as a criterion the feasibility of welfare monitoring throughout the slaughtering process.

6) The animal welfare officer should monitor the effectiveness of the entire stunning and slaughter process, and correct the personnel behaviour or other aspects of the slaughter process if necessary.

7) Since unconsciousness should be confirmed from the stunning application until death, this opinion also suggests to check that the animal is not conscious at each of the three key stages: (i) immediately after stunning (between end of stunning and shackling), (ii) during neck cutting (sticking) and (iii) during bleeding.

RECOMMENDATIONS ON HEAD-ONLY ELECTRICAL STUNNING IN SHEEP AND GOATS

8) During slaughter with stunning, indicators to detect conscious animals should be used to recognise failures (i.e. poor welfare) and apply intervention.

9) A toolbox composed of the following indicators should be checked to determine consciousness of animals after stunning at all three key stages of the process, to ensure that animals remain unconscious until death occurs.

Key stage 1 (between end of stunning and shackling): tonic/clonic seizures, breathing, corneal or palpebral reflex. Additional indicators—spontaneous blinking, posture and vocalisations—are also proposed, but they should not be relied upon solely.

Key stage 2 (during neck cutting): breathing, tonic/clonic seizures and muscle tone. Additionally, the corneal and palpebral reflexes, spontaneous blinking and vocalisations may be used.

Key stage 3 (during bleeding): breathing and muscle tone. In addition, the corneal or palpebral reflex, spontaneous blinking and vocalisations may be used.

10) In order to develop sampling protocols for monitoring consciousness in small ruminants head-only electrical stunning:

- The ‘personnel’ of the slaughterhouse should sample 100 % of the animals immediately after stunning, during neck cutting and during bleeding.

- The animal welfare officer should periodically sample the slaughter population that can be calculated by the statistical model proposed in this opinion (here referred to as the ‘standard’ sampling protocol). This fraction is dependent on the test sensitivity, the slaughtered population, the maximum allowed threshold failure rate and the required accuracy.

11) In head-only electrical stunning, the ‘standard’ monitoring protocol should be reinforced (here referred to as the ‘reinforced’ sampling protocol) when a conscious animal is detected, or when a risk factor affecting the quality of the monitoring is identified, until the risk is identified and rectified. All animals should be tested during a period represented by one-tenth of the slaughtered population.

12) It is recommended that the animal welfare officer should not reduce the sampling frequency (sample fraction), as a reduction in sampling fraction ((here referred to as the ‘light’ sampling protocol) will immediately reduce the accuracy of the monitoring protocol.

13) Of the recommended indicators above the dashed line in the flow chart, a minimum of two indicators relevant to each key stage should be employed for an effective monitoring of the process.

14) In order to allow effective monitoring, the animals must be able to express behaviours and reflexes associated with consciousness. Consequently, procedures, processes or treatments that could mask the expression of such behaviours (such as electrical immobilisation or electrical stimulation) should not be used prior to confirmation of death in animals.

RECOMMENDATIONS ON SHEEP AND GOAT SLAUGHTER WITHOUT STUNNING

15) The design of the head restraint must not obscure the front of the head and should also allow good access to the eyes to check the corneal (or palpebral) reflex, and to the trachea to assess breathing.

16) Animals should be observed closely for the signs of occlusion and appropriate intervention must be applied immediately.

17) Severe restraint and rotation of animals on to their back for the purpose of neck cutting is not conducive to effective welfare monitoring using key indicators (eye reflexes and response to nose prick or ear pinch) as the animals' heads are covered with blood, and therefore this should be discouraged or phased out.

18) According to Council Regulation (EC) 1099/2009, all ruminants must be mechanically restrained for the purpose of slaughter without stunning and unconsciousness should be established before releasing them from the restraint, and death should be confirmed before carcass dressing begins. Therefore, a toolbox of indicators for the determination of unconsciousness and death was presented in the flow chart, and these should be used during slaughter without stunning. From the toolbox on sheep and goats after slaughter without stunning, it is recommended that breathing, muscle tone, posture and the corneal or palpebral reflex should be checked. Their outcomes of unconsciousness should be confirmed before the animal can be released from the restraint. Bleeding, muscle tone and pupil size should be checked and their outcomes of death should be confirmed before the animal can be further processed.

19) For slaughter without stunning, 100 % of the animals need to be assessed for unconsciousness and death by checking appropriate indicators i.e. those in Toolboxes 4 and 5 respectively. The animal welfare officer should confirm unconsciousness and death of the animals as well as the skill and aptitude of the operator in checking the indicators.

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GLOSSARY

GLOSSARY FOCUSED ON KEYWORDS

Outcome of a welfare indicator: the result of check performed using an indicator based on which the animal is considered conscious or unconscious, or alive or dead.

Welfare indicator: an observation used to obtain information on an animal’s state of consciousness/unconsciousness or life/death. In this opinion, all indicators are animal-based observations.

GLOSSARY FOCUSED ON THE MODEL

Accuracy of the sampling protocol: the percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if there were more ineffectively stunned animals than the prescribed threshold failure rate would allow. This corresponds to confidence level in freedom from disease methodologies.

Sampling fraction: the proportion of the slaughter population which is assessed in the sampling protocol.

Sensitivity of the indicators: as defined previously, this is the percentage of truly conscious animals detected as conscious by the indicator. This corresponds to diagnostic test sensitivity in freedom from disease methodologies.

Slaughter population: a group of animals slaughtered under the same circumstances as determined by risk factors (see Table 10).

Threshold failure rate for proportion of mis-stunned animals: the minimum proportion of animals that are ineffectively stunned, which will still be detected by the sampling protocol. This corresponds to design prevalence in freedom from disease methodologies.

GLOSSARY FOCUSED ON INDICATORS

DESCRIPTIONS/DEFINITIONS OF THE INDICATORS FOR HEAD-ONLY ELECTRICAL STUNNING IN SHEEP AND GOATS

Breathing: effective electrical stunning will result in immediate onset of apnoea (absence of breathing). Ineffectively stunned animals and those recovering consciousness will start to breathe in a pattern commonly referred to as rhythmic breathing, which may begin as regular gagging and involves a respiratory cycle of inspiration and expiration. Rhythmic breathing can be recognised from the regular flank and/or mouth and nostrils movement. Recovery of breathing, if not visible through these movements, can be checked by holding a small mirror in front of the nostrils or mouth to look for the appearance of condensation due to expiration of moist air.

Corneal reflex: the corneal reflex is elicited by touching or tapping the cornea. Ineffectively stunned animals and those recovering consciousness will blink in response to the stimulus. Unconscious animals may also intermittently show a positive corneal reflex.

Eye movement: eye movements and the position of the eyeball can be recognised from close examination of the eyes after stunning. Correctly stunned animals will show fixed eyes, and this can be recognised from wide open and glassy eyes with clearly visible iris/cornea in the middle. Eyeballs may be obscured in some animals owing to rotation into the eye socket following effective head-only electrical stunning. Ineffectively stunned animals and those recovering consciousness will show eye movements.

Muscle tone: head-only electrically stunned animals will show general loss of muscle tone after the termination of tonic–clonic seizures coinciding with the recovery of breathing and the corneal reflex if not previously stuck. Loss of muscle tone can be recognised from the completely relaxed legs, floppy ears and tail, and relaxed jaws with protruding tongue. Ineffectively stunned animals and those recovering consciousness will show a righting reflex and attempts to raise the head.

Palpebral reflex: the palpebral reflex is elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Correctly stunned animals will not show a palpebral reflex. Ineffectively stunned animals and those recovering consciousness will blink in response to the stimulus.

Posture: effective head-only electrical stunning will result in immediate collapse or loss of posture in animals that are not restrained or prevented from doing so. Ineffectively stunned animals, on the other hand, will fail to collapse or attempt to regain posture after collapse.

Pupillary reflex: the pupillary reflex can be elicited by focusing/shining a torch light at the pupils. Correctly stunned animals will not show a pupillary reflex. Ineffectively stunned animals will show pupillary constriction (miosis) in response to light.

Responses to a nose prick or ear pinch: response to a painful stimulus such as a pin prick to the muzzle (area between external nostrils) or the ear with a sharp instrument indicates consciousness following stunning using an electric current.

Spontaneous blinking: conscious animals may show spontaneous blinking and therefore this sign can be used to recognise ineffective stunning or recovery of consciousness after electrical stunning. However, not all the conscious animals may show spontaneous blinking.

Tonic–clonic seizures: effective head-only electrical stunning leads to the onset of tonic–clonic seizures soon after immediate collapse of the animal. The tonic seizure, which may be recognised from the tetanus, lasts for several seconds and is followed by clonic seizures lasting for seconds and leading to loss of muscle tone.

Vocalisation: conscious animals may vocalise, and therefore purposeful vocalisation can be used to recognise ineffective stunning or recovery of consciousness after electrical stunning. However, not all conscious animals may vocalise.

DEFINITIONS OF THE INDICATORS PRIOR TO RELEASE FROM RESTRAINT FOR SLAUGHTER WITHOUT STUNNING IN SHEEP AND GOATS

Breathing: loss of consciousness following slaughter without stunning will eventually lead to cessation of rhythmic breathing. Rhythmic breathing can be recognised from the regular flank movement. Therefore, sustained absence of rhythmic breathing (absence of respiratory cycle—inspiratory and expiratory movements) can be used as an indicator of the onset of unconsciousness. The trachea is severed during slaughter without stunning, and therefore signs of rhythmic breathing cannot be observed at the nostrils or mouth. Cessation of breathing can be indicated by the absence of air bubbles in the liquid blood or sera accumulating at the neck wound or by the absence of any flank movements suggestive of breathing.

Corneal reflex: the corneal reflex is elicited by touching or tapping the cornea. Permanent loss of the corneal reflex can be used as an indicator of unconsciousness.

Fixed eyes: permanent loss of consciousness following slaughter without stunning will lead to fixed eyes, and this can be recognised from wide-open and glassy eyes with clearly visible iris/cornea in the middle.

Muscle tone: unconscious animals will show general loss of muscle tone, and this can be recognised from the completely relaxed legs, floppy ears and tail, and relaxed jaws with protruding tongue.

No full eyeball rotation: unconscious animals have fixed eyes and therefore do not show rotation of the eyeballs.

Nystagmus: unconscious animals have fixed eyes and therefore do not show nystagmus, i.e. no spontaneous, rapid, side-to-side (twitching) movements of the eyeballs.

Palpebral reflex: the palpebral reflex is elicited by touching or tapping a finger on the inner/outer eye canthus or eye lashes. Permanent loss of palpebral reflex can be used as an indicator of unconsciousness.

Posture: permanent loss of posture can be used as the earliest physical sign of the onset of unconsciousness following slaughter without stunning of an unrestrained/free-standing animal. An animal that has lost consciousness irreversibly will make no attempt to regain posture. Loss of posture cannot be determined in animals that are severely restrained and/or rotated. Conscious animals may lose posture if the floor is slippery and may or may not attempt to regain posture.

Pupil size: dilated pupils (midriasis) are an indication of death.

Pupillary reflex: the pupillary reflex can be elicited by focusing/shining a torch light at the pupils. Live and conscious animals will show pupillary constriction (miosis) in response to light. Unconscious animals will not show a positive pupillary reflex.

Responses to a nose prick or ear pinch: the absence of response to a painful stimulus such as a pin prick to the muzzle (area between external nostrils) or the ear with a sharp instrument indicates unconsciousness following stunning.

Response to being threatened: conscious animals respond either by blinking or by attempting to move away from threatening movements (or clapping) of the hands close to the eye. This fear response will be abolished in unconscious animals and therefore response to visually threatening movements can be used to check unconsciousness.

Spontaneous blinking: unconscious animals do not blink.

Vocalisation: conscious animals may vocalise, and therefore purposeful vocalisation can be used to recognise ineffective stunning or recovery of consciousness. During slaughter without stunning, the trachea is also severed and therefore animals cannot vocalise; however, blood enters the trachea and movement of air through the blood or serum generates bubbling and gurgling noises that may misconstrued as vocalisation.

Definitions of the indicators prior to dressing for slaughter without stunning in sheep and goats

Cardiac activity: the onset of death leads to permanent absence of cardiac activity (absence of heart beat), which can be ascertained using a stethoscope where possible.

End of bleeding: slaughter eventually leads cessation of bleeding, with only minor dripping, from the neck cut wound and therefore end of bleeding can be used as an indicator of death.

Pulse rate: the onset of death leads to permanent loss of pulse, which can be ascertained physically by pressing the arteries in an extremity where possible.

Pupil size: dilated pupils (midriasis) are an indication of death.

Relaxed carcass: the onset of death leads to a complete and irreversible loss of muscle tone, which can be recognised from the limp carcass.