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Report

Scientific study in commercial slaughterhouses of rabbits

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September 2024

Acknowledgements

The authors would like to express their gratitude to the official veterinary services and slaughterhouses for allowing us to conduct this study at their facilities and for their selfless support.

This review can be downloaded for free at [10.5281/zenodo.13889346]

Citation : [*https://doi.org/10.5281/zenodo.13889346*]

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Co-funded by the European Union

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1 Executive Summary

This study compared the assessments of four observers on 11,540 rabbits of 38 batches from 16 different slaughterhouses on the most valid and feasible indicators of consciousness in two stages: after head-only electrical stunning immediately after stunning (i.e., tonic-clonic seizure, breathing, spontaneous blinking and vocalisation) and during bleeding (i.e., tonic-clonic seizure, breathing, spontaneous blinking, vocalisation and righting reflex). In addition, the combinations between them were calculated to further understand the association between such indicators and to propose a refined list of indicators that could be used to assess the state of consciousness of rabbits in commercial slaughterhouses. Logistic regression was also performed aimed at finding key factors that contribute to effective stunning.

Immediately after stunning, the refined list of indicators consists in tonic-clonic seizure, breathing, spontaneous blinking and vocalisations. During bleeding, the refined list includes breathing, spontaneous blinking and vocalisations. Sometimes more than one indicator of consciousness is observed in the same rabbit being presence of breathing the most frequent followed by presence of spontaneous blinking in both stages. Caution with righting reflex should be taken since this indicator appears to be poor repeatable between observers because often confused with preagonal muscle movements that can occur in brain-dead animals. Then, it should only be considered indicator of consciousness when the rabbit is breathing and/or blinking.

The range of the prevalence of rabbits with indicators of consciousness within a batch found immediately after stunning [0–15%] and during bleeding [2–93%] highlights the importance of controls. Logistic regression allowed to describe some factors that contribute to effective stunning. From greatest to least impact are: stun-to-stick interval of less than 5 s, using current above 200 mA and frequencies not above 50 Hz, and wetting the rabbits' heads. The more these key factors are present in a SH, the higher the odds of effective stunning in rabbits.

2 Introduction

This study aims to investigate the inter-observer repeatability of various animal-based indicators (ABIs) used to assess the state of consciousness in rabbits immediately after head-only electrical stunning and after neck cutting during bleeding. The ABIs considered have already been validated for assessing consciousness in commercial slaughterhouses (SHs). Additionally, this study seeks to assess the correlation between the outcomes of the ABIs, the effectiveness of stunning based on key parameters (current, frequency, voltage, and exposure time) and stun-to-stick interval used and to analyse the main factors that contribute to efficient stunning in commercial SHs in the three largest European rabbit-producing countries.

3 Methods

3.1. Selection of slaughterhouses and animals

Sixteen commercial rabbit SHs equipped with head-only electrical stunners were selected in France, Spain and Italy. Selection of the SHs was carried out together with the official veterinary services of the corresponding Member States to reflect a certain diversity in terms of size of the SH, electrical key parameters, rabbit genotype and line speed. Each SH was assigned to a number (from 1 to 16).

3.2. Description of the slaughterhouses and management

In all SHs, rabbits were head-only electrical stunned and afterwards shackled by their rear legs on the moving slaughter line. Substantial variation in age, design and layout of the SHs were observed, and the main management characteristics are described in **Table 1**.

In four out of 16 SHs, the rabbits were wet prior to stunning with the purpose of reducing electrical resistance caused by the fur and improving the stunning efficiency. Some SHs were equipped with more than one stunner used at the same time, and, in some cases, more than one operator was in charge of bleeding. In these cases, the stun-to-stick interval differed depending on how far away the stunner was from the bleeder. SH-10 used a device that stunned and immediately after automatically cut the rabbit's neck when deemed appropriate by the operator (i.e. after effective stunning assessed by tonic seizure of the animal). The bleeding procedure differed among SHs; seven SHs performed manual bleeding through ventral neck cut, seven of them did manual bleeding through lateral neck cut and one of them performed automatic ventral neck cut with one operator checking that the neck cut was appropriately performed in all rabbits.

All stunners had a digital control panel that also automatically recorded the main electrical parameters applied (i.e. the actual total current passing through the rabbit's head, voltage and frequency) and the duration of exposure to the electrical tongs. These parameters were obtained from the official veterinary service and food business operators. A summary of the electrical parameters used per batch and per SH along with the characteristics of the animals in the batch and the number of assessed rabbits is shown in Table 2. Slaughter line speed ranged from 600 to 3600 rabbits/h.

Table 1. *Main characteristics of the sixteen rabbit slaughterhouses (SH) included in the study.*

SH	Line speed, rabbits/h	Wetting heads before stunning, yes/no	Stunners in use simultaneously, n	Stun-to-stick interval according to the stunner/s, s	Bleeding method*	Bleeding cut	Number operators bleeding, n
1	800	No	2	10 and NA	M	Lateral	1
2	1500	NA	2	11 and NA	M	NA	2
3	1600	No	1	22	M	Lateral	1
4	2600	No	3	15, 10 and 8	M	Lateral	1
5	2100	No	4	36, 30, 24 and 19	M	Ventral	2
6	700	No	1	15	M	Ventral	1
7	700	Yes	1	2	M	Lateral	1
8	600	No	1	3	M	Lateral	1
9	1850	Yes	3	18, 12 and 7	M	Ventral	1
10	1400	Yes	3	0	A	Ventral	1
11	700	NA	1	3	M	Ventral	1
12	800	Yes	1	16	M	Lateral	1
13	1,700	No	3	25, 19 and 6	M	Ventral	1
14	1,920	Yes	3	33, 24 and 17	M	NA	1
15	3200	Yes	4	22, 20, 18 and 13	M	Lateral	2
16	3600	No	3	20, 13 and 5	M	Ventral	2

*Bleeding method: M (manually); A (automatically); SH: slaughterhouse; NA: data not available

3.3. Assessment of the state of consciousness

3.3.1. Observers

The stunning effectiveness was evaluated by four trained observers. Each observer (Obs) was named as letter (A to D). An additional person randomly selected the rabbits to be assessed on the shackle line and highlighted each animal with a laser pointer to ensure that all four observers were evaluating the same selected rabbit.

The stunning effectiveness was assessed on a representative sample of rabbits in each batch at two different stages along the slaughter line: stage 1) Immediately after stunning but before neck cutting, and stage 2) During bleeding (see **Figure 1**).

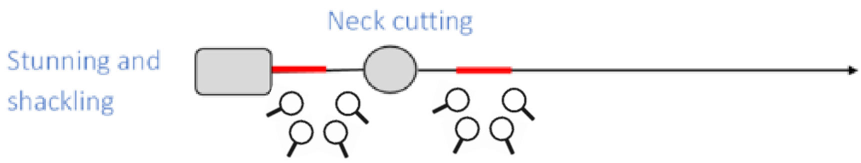

Figure 1. *Position of the four observers during the assessment of animal-based indicators of the state of consciousness after head-only stunning in rabbits. The position of the lens represents the position of the observers (i.e., immediately after stunning and during bleeding) and the red segments are the observation areas.*

The observers individually scored the ABIs within specific time frames that varied due to the SH design and visibility of the rabbits. In stage 1, the assessment was performed during 2 to 10 s post-stunning (see **Figure 2**). In stage 2, the observers were placed at a distance from the bleeder where they detected rabbits that began to show outcomes of consciousness and then, the rabbits were assessed during 6 to 15 s (**Figure 3**). Observers assessed the ABIs individually and did not discuss or disclose their assessments during the evaluation.

In SH-3 and SH-10, it was not possible to assess the state of consciousness immediately after stunning (**Figure 2**) and before bleeding (**Figure 3**), in SH-3 because there was no space available for an observer due to the design of the SH and in SH-10 because the stunner was equipped with an automatic neck cutting device after stunning.

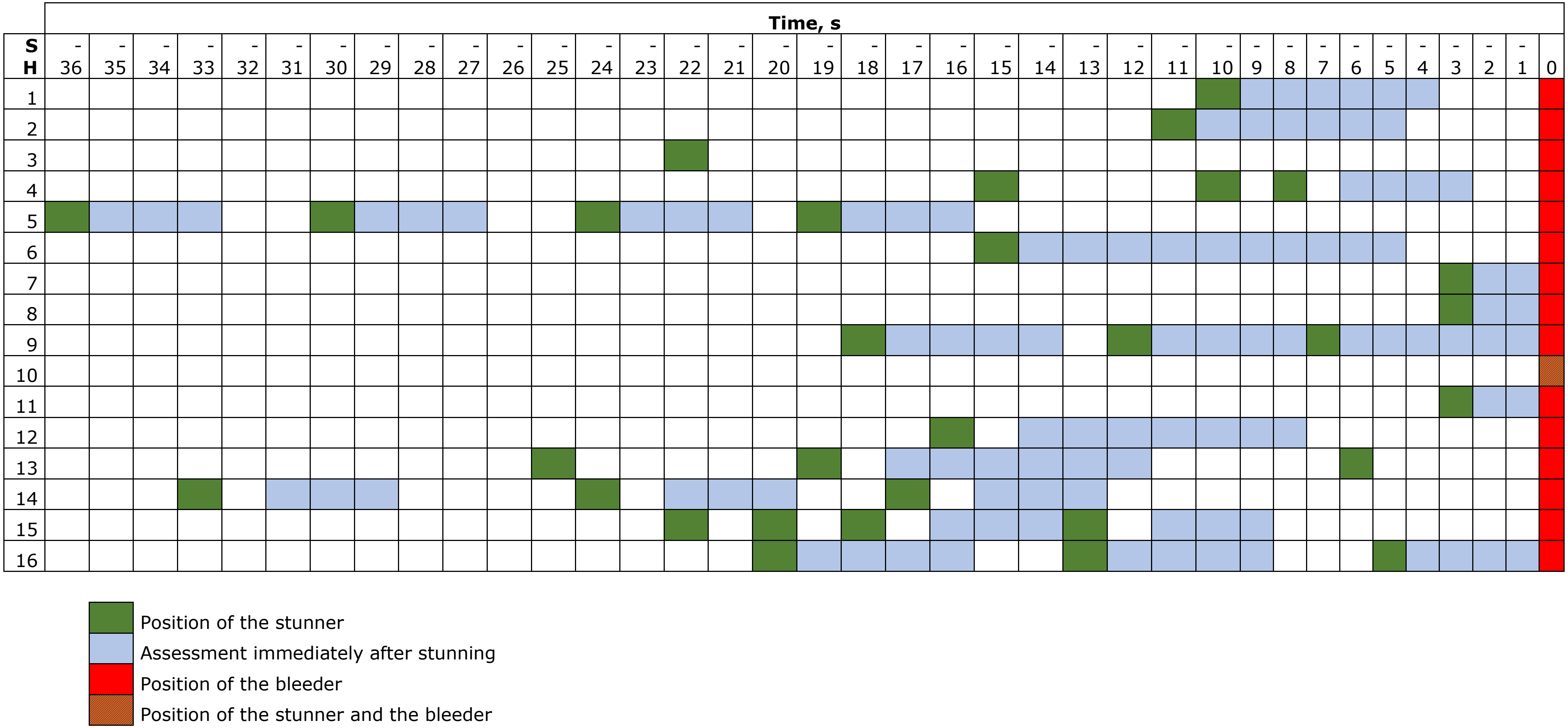

Figure 2. *Position of the stunner(s) relative to the bleeder(s), expressed as the time interval between stunning and neck cutting, and the time frame during which the state of consciousness was assessed immediately after stunning and before bleeding, according to the slaughterhouse (SH) assessed.*

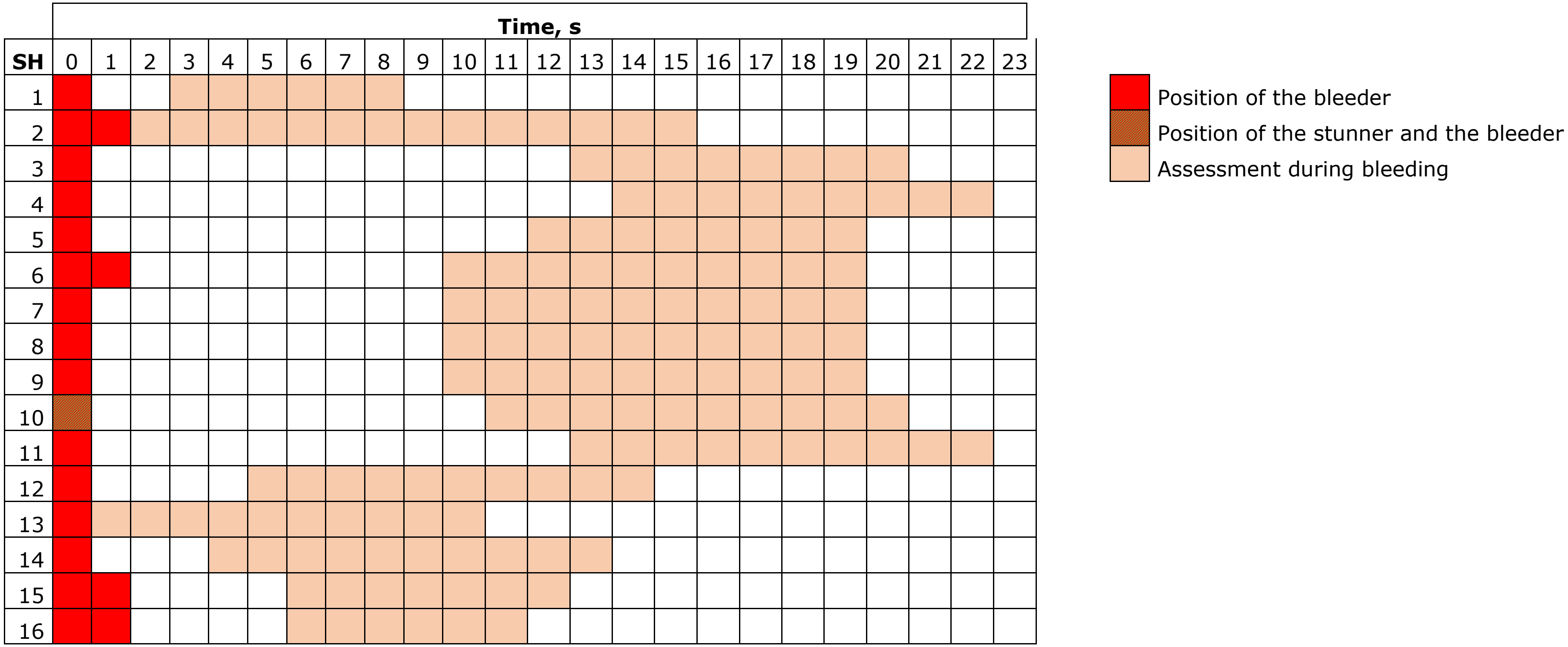

Figure 3. *Position of the bleeder(s) and the time frame during which the state of consciousness was assessed during bleeding, according to the slaughterhouse (SH) assessed.*

3.3.2 Sample assessment

All the rabbit batches slaughtered during the presence of the observers in the SH were evaluated. On each batch, samples of 50-200 rabbits were assessed before and during bleeding. This cycle was repeated until the whole batch was slaughtered, in order to obtain the biggest sample size possible.

Sometimes an observer was distracted for whatever reason (e.g., business operators passing in front of them) and could not assess the rabbit that was being indicated with the laser. In these cases, the observers made a note in their observations and the outcomes of the other observers were filtered out for repeatability assessments. A summary of the characteristics of the rabbits in the batch and the number of assessed rabbits is shown in Table 2. Although it is known that genetics affects stunning efficiency (differences in the amount of fur between genetics varies the resistance to electricity and therefore to the applied current), it was not possible to evaluate the impact of genetics its effect could be confounded with other factors such as electrical key parameters, average body weight of the batch, etc.

Table 2. *Number of batches slaughtered, category and average body weight of the rabbits in the batches evaluated for each slaughterhouse. The average electrical parameters for each of the head-only electric stunners (± standard deviation) and the exposure time of the electric tongs on the head of the rabbits (± standard deviation) are also reported.*

		Characteristics of the rabbits				Stunning parameters used			
SH	**Batch**	**Animal category**	**BW, kg/rabbit**	**No Rabbits**	**Stunner**	**Current, mA/rabbit**	**Frequency, Hz**	**Voltage, V**	**Time, ms**
1	1	M	3.00	1200	1	300±83	401	396	2440±24
					2	284±88	401	394	2340±34
	2	M	2.75	2000	1	282±78	401	397	2430±30
					2	280±84	401	397	2360±26
2	1	M	2.45	5150	1	866±122	50	182	1030±112
					2	843±125	50	183	921±120
	2	M	2.60	615	1	893±118	50	179	1042±138
					2	818±121	50	182	903±120
	3	M	NA	2050	1	812±107	50	181	920±121
					2	864±109	50	181	915±113
3	1	M	2.4	4022	1	675±94	50	277	1231±161
					2	1059±275	50	265	984±138
	2	M	2.3	10610	1	787±241	50	277	1286±597
					2	1044±286	50	267	1014±152
	3	B	4.4	120	1	753±211	50	280	3473±539

SH: slaughterhouse; n: number of rabbits in the batch; M: meat; B: breeders; BW: average body weight; NA: no data available, No: number

Continuation Table 2…

		Characteristics of the rabbits				Stunning parameters used			
SH	**Batch**	**Animal category**	**BW, kg/rabbit**	**Rabbits, n**	**Stunner**	**Current, mA/rabbit**	**Frequency, Hz**	**Voltage, V**	**Time, ms**
4	1	M	2.8	4739	1	779±229	50	302	732±111
					2	795±204	50	311	642±76
					3	1006±268	50	293	877±105
		B	4.4	276	1	1033±267	50	299	6770±292
					2	955±205	50	310	7780±236
					3	1189±348	50	290	8075±315
	2	M	2.3	1475	1	648±202	50	304	683±110
					2	926±299	50	309	657±64
					3	960±244	50	292	863±90
	3	M	2.5	6107	1	1100±252	50	297	808±69
					2	922±262	50	308	824±165
					3	797±249	50	294	772±99
	4	M	2.4	156	1	1040±259	50	289	788±106
					2	764±212	50	295	648±112
					3	695±204	50	292	725±85
5	1	M	2.4	5273	1	792	NA	350	1396
					2	713	NA	349	1324
					3	577	NA	350	1307
					4	585	NA	356	1326
	2	M	2.6	5416	1	670	NA	353	1311
					2	628	NA	352	1229
					3	576	NA	350	1369
					4	433	NA	359	1180
6	1	M	2.1	1819	1	438±128	50	180	496±253
	2	M	2.1	576	1	445±191	50	180	503±263
7	1	M	2.3	2160	1	610±70	50	145	567±121
	2	M	1.7	443	1	498±135	50	142	485±107
8	1	M	2.1	1488	1	467±175	50	210	509±152
9	1	M	2.0	1401	1	742±243	50	244	923±307
					2	679±264	50	247	935±263
					3	723±274	50	258	783±367
	2	M	1.9	1736	1	764±249	50	244	998±268
					2	606±261	50	249	907±238
					3	742±244	50	258	708±254
	3	M	ND	3080	1	742±234	50	243	938±219
					2	630±257	50	247	780±222
					3	681±231	50	258	634±193
10	1	M	2.3	5300	1	434±13	300	145	701
					2	404±100	300	158	701
	2	M	2.6	2790	1	407±97	300	156	701
					2	405±95	300	179	701
11	1	M	2.4	1320	1	138±17	150	95	400
12	1	M	2.8	1800	1	196±43	150	112	274±31
		B	4.7	50		208±38	150	116	402±17
	2	M	2.7	1904	1	183±57	150	127	386±46
		B	4.6	66		198±57	150	139	388±64

SH: slaughterhouse; n: number of rabbits in the batch; M: meat; B: breeders; BW: body weight; NA: no data available

3.3.3. Indicators for the assessment

The ABIs for the assessment of the state of consciousness immediately after stunning and during bleeding were selected based on those proposed by EFSA (2020). The selected ABIs immediately after stunning were tonic-clonic seizure, breathing, spontaneous blinking and vocalisations, while those selected during bleeding were the same with, in addition, righting reflex. The description and the outcome of consciousness and unconsciousness of these ABIs is summarized in Table 3.

***Table 3**. Description of the animal-based indicators (ABIs) and outcomes of unconsciousness and consciousness scores in head-only electrical stunned rabbits assessed in two different stages: immediately after stunning and during bleeding.*

Stage	ABIs	Outcome of unconsciousness	Outcome of consciousness
Immediately after stunning & during bleeding	Tonic-clonic seizure	Rabbit shows arched and stiff neck (*i.e.* necks appear parallel to the ground) and paws and ears held tightly close to the body. Then followed (or not) by kicking action and/or leg paddling that can be either rhythmic or erratic	General loss of muscle tone and a completely relaxed and flaccid body, with no neck tension.
	Breathing	Absence of opening of the mouth and thoracic or abdominal movements associated to cessation of inhalation and expiration. Presence of one movement is not considered as breathing.	Presence of rhythmic breathing considered as a minimum of two openings of the mouth and thoracic or abdominal muscles associated to inhalation and expiration with similar cadence.
	Spontaneous blinking	Rabbit does not open/close eyelid on its own (fast or slow) without stimulation.	Rabbit opens/closes eyelid on its own (fast or slow) without stimulation.
	Vocalisations	Absence of single or repeated short and loud shrieking (screaming).	Single or repeated shrieking (screaming).
During bleeding	Righting reflex	Absence of attempt to regain posture and/or raise the head.	Attempt to regain posture and/or raise the head.

The four trained observers agreed beforehand on the indicators and the outcomes of consciousness and unconsciousness, the methodology of assessment, and the scoring to standardize the protocol when assessing the rabbits. Then, the four assessors were positioned where they had the best possible view of the shackled rabbits, preferably from a ventrolateral position. However, due to variation in the design and construction of the SHs, the rabbits sometimes had to be assessed from a dorsolateral position instead of ventrolateral, both immediately after stunning and during bleeding (SH-2, SH-9 and SH-13), or immediately after stunning but not during bleeding (SH-15), or only during bleeding (SH-1, SH-4, SH-5 and SH-16). This impaired the assessment of breathing since the mouths were not clearly visible. Data were recorded in a binary format: 0 if the outcome of unconsciousness was observed and 1 when an outcome of consciousness was observed. The presence of at least one indicator with outcome of consciousness may indicate that the rabbit is conscious or regaining consciousness after stunning, suggesting ineffective stunning or recovery of consciousness.

3.4. Statistical analysis

Data pre-processing, statistical analyses and plots were performed using R software v.4.1.0. (R Core Team, 2021). First, rabbits that were not assessed by all four observers were filtered out to ensure that all observations were directly comparable. For all the statistical analyses, significance was declared at $P < 0.05$.

3.4.1. Inter-observer repeatability of ABIs

The overall level of agreement between observers for each ABI was determined and expressed by the crude proportion of agreement (PoA) and the Fleiss' kappa (κ) using the "irr" package of R software (Gamer *et al*., 2019). The PoA can be misleading as it does not take into account the scores that the observers assign due to chance. Fleiss's Kappa overcomes this issue as it provides an inter-observer agreement measure between two or more observers when the variable assessed is on binomial or categorical scale. It expresses the degree to which the observed proportion of agreement among observers exceeds what would be expected if all observers made their ratings completely randomly. κ can range from −1 to +1, where 0 indicates the amount of agreement that can be expected from random chance, and 1 represents perfect agreement between the observers (McHugh, 2012). κ is a standardized value and thus is interpreted the same across multiple studies. Thus, according to Fleiss *et al*. (2003), κ can be classified as "excellent" agreement beyond chance if values are greater than 0.75; "fair to good" agreement beyond chance if values between 0.40 and 0.75 and "poor" agreement beyond chance if the values are below 0.40. However, when there is an insufficient scoring variation in the evaluated indicator (*i.e.*, low prevalence of indicators of the state of consciousness), although high agreement between observers, κ appears close to 0.

3.4.2. Prevalence and relationship among ABIs

The chi-squared % defective test was used to determine if there were statistical differences (divergence) among observers between the expected and the observed frequencies of every outcome of consciousness of the evaluated indicators. If one observer differed statistically from the others at evaluating the ABIs, the mean of the proportion of the closest evaluations or the in between value when scoring were not consistent among them were recorded. Proportions among combinations of ABIs were performed as Venn diagram considering all rabbits assessed in the present study using the "eulerr" package (Larsson, 2020).

3.4.3. Relationship between key parameters and stunning efficiency

Stunning (in)efficiency of each batch and SH was evaluated by showing the percentage of rabbits with at least one outcome of consciousness in any of the stages of the assessment: immediately after stunning and during bleeding. Chi-squared % defective test was used to determine if there were statistical differences among observers between the expected and the observed frequencies of every outcome of the indicators evaluated. If one observer differed statistically from the others at evaluating the ABIs, the mean of the proportion of the two closest evaluations or the in between value when scoring were not consistent among them were reported. Prevalence of the outcome of consciousness for each indicator within each batch was calculated and from this, the interval of confidence in the population (here in each batch) was calculated. Thus, 95% confidence interval of rabbits showing outcomes of consciousness was computed using the Wilson's formula from "epitools" package of R software (Aragon, 2020) in every batch assessed.

3.4.4. Risk factor analysis

Different variables were explored to check if they can be risk factors associated with inefficient stunning. The following variables: wetting the rabbit's head, the electrical stunning parameters, and the stun-to-stick interval (s) were evaluated using binomial logistic regression with the "MASS" package (Venables and Ripley, 2002).

For this, data was converted to a binary format. For every rabbit assessed, a 0 was recorded when no indicators of consciousness were detected, and a 1 was recorded if at least one indicator of consciousness was observed. Then, a rabbit was classified as inefficiently stunned (annotated as 1) if at least two observers recorded it as having at least one sign of consciousness; otherwise, it was classified as efficiently stunned (annotated as 0).

The variables were transformed into binary or multinomial format:

- Rabbits slaughtered in SHs that wet their heads before stunning were coded as 1; those that did not were coded as 0,
- For the electrical parameters, four groups were initially defined based on the combination of current and frequency. Various current thresholds were tested (140, 200, and 300 mA). Rabbits stunned with currents below these thresholds and a frequency of 50 Hz were categorized as "low current-low frequency." Conversely, those stunned with currents above the thresholds at 50 Hz were classified as "high current-low frequency." Rabbits exposed to both higher currents and frequencies above 50 Hz were placed in the "high current-high frequency" group. Those stunned with lower currents but higher frequencies were intended to be labeled as "low current-high frequency." However, no rabbits fell into this last category, so the model ultimately included only three electrical parameter groups.
- Stun-to-stick intervals up to 5 s were coded as 1, and those over 5 s were coded as 0.

Model selection was based on the Akaike Information Criterion (Burnham and Anderson, 2002). Then, the variables included in the model were checked for multicollinearity using the "car" package (Fox and Weisberg, 2019). Multicollinearity is considered negligible with variance inflation factors (VIFs) below 5 (O'Brien, 2007). Checking for multicollinearity ensures stable, interpretable, and reliable coefficients. Odds ratios (OR) with 95% confidence intervals (95% CI) were used to express coefficients. An OR greater than 1 with a 95% CI excluding 1, indicates that the factor has an "adverse" effect, decreasing the odds of efficient stunning. Conversely, an OR less than 1 with a 95% CI excluding 1 indicated that the factor has a "protective" effect, increasing the odds of efficient stunning.

4 Results

The ABIs were assessed on a total of 4112 rabbits immediately after stunning and 7428 during bleeding from 16 different SHs across France, Spain and Italy by four observers (A, B, C, D).

4.1. Inter-observer repeatability of the ABIs

4.1.1. Immediately after stunning

Immediately after stunning, in all SHs but in SH-3 and SH-10, four ABIs of the state consciousness were assessed: tonic-clonic seizure, breathing, spontaneous blinking and vocalisation. The average prevalence of rabbits between batches showing outcomes of consciousness, by observer and SH is shown in **Table 5**. On the other hand, the overall level of agreement between the four observers for these ABIs according to the SH is shown in **Table 6**.

4.1.1.1. Tonic-clonic seizure

Some rabbits showed absence of tonic-clonic seizure after stunning in six out of 16 SHs. The highest prevalence in a sample was found in SH-5 (27/183; 14.8%) followed by SH-4 (9/199; 4.5%), SH-9 (2/154; 1.3%), SH-16 (1/200; 0.3%), SH-14 (1/400; 0.3%) and SH1 (1/491; 0.002%) as shown in **Table 5**. Observers did not differ significantly in the detected prevalence of the absence of tonic-clonic seizure in any of the SHs evaluated ($P > 0.05$). The PoA was above 91.3% in all the SHs and the κ and its interpretation strongly differed between SHs ranging from "poor" to "excellent" agreement (Table 6). Fleiss' kappa coefficient could not be computed neither in SH-6, SH-11 nor SH-13 due to absence of scoring variation as all rabbits assessed showed presence of tonic-clonic seizure (**Table 6**).

Considering the data from the total of rabbits assessed in the present study at this stage, a mean of 1.1% (n = 4112) of the rabbits showed absence of tonic-clonic seizure with similar prevalence between observers ($P > 0.05$). The PoA among observers was 98.5% and the κ was statistically significant and interpreted as "fair to good" ($P < 0.001$; $\kappa = 0.67$; **Table 6**).

4.1.1.2. Breathing

Rabbits with rhythmic breathing were observed in two out of 16 SHs. The highest prevalence of breathing in a sample was found in SH-1 (24/491; 4.9%) followed by SH-12 (1/197; 0.5%) as shown in **Table 5**. Similarly, as in the absence of tonic-clonic seizure, the observers did not differ significantly in the detected prevalence of presence of breathing in any of the SHs evaluated ($P > 0.05$). The PoA between observers was above 93.7% in all SHs and there was divergence of κ linked to the different degree of prevalence of breathing among SHs ranging from "poor" to "fair to good" agreement (**Table 6**).

Taking into consideration all rabbits from the SHs assessed, a mean of 0.6% (n = 4112) of the animals showed presence of rhythmic breathing with similar prevalence between observers ($P > 0.05$) as shown in Table 5. The PoA among observers was 99.2% and the κ was statistically significant and interpreted as "fair to good" ($P < 0.001$; $\kappa = 0.63$; **Table 6**).

Table 5. *Count of rabbits with outcomes of consciousness immediately after head-only stunning according to the observer (A to D) and the slaughterhouse (SH) evaluated.*

		Absence of TC, %						Presence of BR, %						Presence of SB, %						Presence of VC, %					
SH	**n**	**A**	**B**	**C**	**D**	**Mean**	**P-value**	**A**	**B**	**C**	**D**	**Mean**	**P-value**	**A**	**B**	**C**	**D**	**Mean**	**P-value**	**A**	**B**	**C**	**D**	**Mean**	**P-value**
1	491	4	3	4	0	3	0.265	25	20	27	25	24	0.634	26	21	29	26	26	0.714	0	0	0	0	0	-
2	333	0	0	0	1	0	0.388	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	199	10	10	8	8	9	0.924	0	0	0	0	0	-	5	5	6	4	5	0.938	0	0	0	0	0	-
5	183	24	29	27	26	27	0.902	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
6	276	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
7	381	0	0	1	0	0	0.391	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
8	200	0	0	1	0	0	0.391	0	1	0	0	0	0.391	0	0	0	0	0	-	0	0	0	0	0	-
9	154	2	3	2	2	2	0.953	0	0	0	0	0	-	0	1	0	0	0	0.391	0	0	0	0	0	-
10	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	400	1	0	0	0	0	0.391	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
12	197	0	0	0	0	0	-	1	0	2	0	1	0.298	1	0	2	0	1	0.298	0	0	0	0	0	-
13	398	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
14	400	1	1	1	2	1	0.896	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
15	200	0	0	1	0	0	0.391	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
16	300	1	1	1	1	1	1.000	0	0	0	0	0	-	0	0	0	0	0	-	0	0	0	0	0	-
All	4112	43	47	46	40	44	0.296	26	21	29	25	25	0.728	27	22	31	26	27	0.503	0	0	0	0	0	-

TC: tonic-clonic seizure; BR: breathing; SB: spontaneous blinking; VC: vocalisation; n: total number of rabbits assessed

Table 6. *Inter-observer proportion of agreement (PoA), Fleiss' kappa coefficient (κ) and interpretation of the animal-based indicators for the state of consciousness of head-only electrical stunned rabbits assessed immediately after stunning according to the slaughterhouse assessed.*

		Tonic seizure			Breathing			Spontaneous blinking			Vocalisation		
SH	**n**	**PoA, %**	**κ**	**P-value**	**PoA, %**	**κ**	**P-value**	**PoA, %**	**κ**	**P-value**	**PoA, %**	**κ**	**P-value**
1	491	94.7	0.127 (P)	<0.001	93.7	0.635 (FG)	<0.001	98.4	0.273 (P)	<0.001	100	*	*
2	333	99.7	0.000 (P)	0.973	100	*	*	98.8	0.130 (P)	<0.001	100	*	*
3	0	-	-	-	-	-	-	-	-	-	-	-	-
4	199	96.5	0.767 (E)	<0.001	100	*	*	96.5	0.590 (FG)	<0.001	100	*	*
5	183	91.3	0.816 (E)	<0.001	100	*	*	100	*	*	100	*	*
6	276	100	*	*	100	*	*	100	*	*	100	*	*
7	381	99.7	0.000 (P)	0.975	100	*	*	100	*	*	100	*	*
8	200	99.5	-0.001 (P)	0.965	100	*	*	100	*	*	100	*	*
9	154	99.4	0.887 (E)	<0.001	99.4	-0.002 (P)	0.961	100	*	*	100	*	*
10	0	-	-	-	-	-	-	-	-	-	-	-	-
11	200	100	*	*	100	*	*	100	*	*	100	*	*
12	397	99.7	-0.001 (P)		99.5	0.221 (P)		99.2	0.165 (P)	<0.001	100	*	*
13	398	100	*	*	100	*	*	100	*	*	100	*	*
14	400	99.8	0.799 (E)	<0.001	100	*	*	100	*	*	100	*	*
15	200	99.5	-0.001 (P)	0.965	100	*	*	100	*	*	100	*	*
16	300	97.7	0.358 (P)	<0.001	100	*	*	100	*	*	100	*	*
All	4112	98.5	0.669 (FG)	<0.001	99.2	0.631 (FG)	<0.001	99.5	0.405 (FG)	<0.001	100	*	*

* Insufficient scoring variation to calculate kappa coefficients (all indicator scores were 0). Kappa interpretation: ≥ 0.75 'excellent' (E), 0.40–0.74 'fair to good' (FG), and < 0.40 'poor' agreement (P) (Fleiss et al., 2003)

4.1.1.3. Spontaneous blinking

Rabbits with spontaneous blinking were observed in three out of 16 SHs. The highest prevalence was found in SH-1 (26/491; 5.3%) followed by SH-4 (5/199; 2.5%) and SH-12 (1/197; 0.5%) as reported in **Table 5**.

Again, the observers did not differ significantly in the detected prevalence of presence of spontaneous blinking in any of the SHs evaluated ($P > 0.05$). The PoA was above 96.5% in all SHs and there was divergence of κ among SHs ranging from "poor" to "fair to good" agreement (**Table 6**).

Taking into consideration all rabbits from the SHs assessed, a prevalence of 0.7% of the rabbits showed presence of spontaneous blinking with similar prevalence between observers ($P > 0.05$) as shown in **Table 5**. The PoA among observers was 99.5% and the κ was statistically significant and interpreted as "fair to good" ($P < 0.001$; $\kappa = 0.41$; **Table 6**).

4.1.1.4. Vocalisation

No vocalisation was heard in any of the SHs. Thus, the PoA was 100% in all SHs and the κ could not be computed.

4.1.2. During bleeding

Five ABIs were evaluated during bleeding: tonic-clonic seizure, breathing, spontaneous blinking, vocalisation and righting reflex. The prevalence of rabbits showing outcomes of consciousness by observer and SH is shown in Table 7. The overall level of agreement between the four observers according to the SH is reported in Table 8.

4.1.2.1. Tonic-clonic seizure

Rabbits with absence of tonic-clonic seizure during bleeding were observed in all SHs and the highest prevalence was found in SH-8 (89.9%) while the lowest in SH-2 (5.7%). There was uniformity on rating among observers only in eight out of the 16 SHs ($P > 0.05$; **Table 7**). The PoA ranged from 54.8 to 90.1% and the κ ranged from 0.44 to 0.72 being interpreted as "fair to good" in all SHs (**Table 8**).

Taking into consideration all rabbits from the SHs assessed, the prevalence of absence of tonic-clonic seizure was 56.3% and the prevalence according to the observer differed statistically ($P < 0.001$; **Table 7**) since Obs-A noticed around 6% less rabbits than Obs-B and Obs-C. Furthermore, the PoA among observers was 72.9% and the κ was statistically significant and interpreted as "fair to good" agreement among observers ($P < 0.001$; $\kappa = 0.70$; **Table 8**).

4.1.2.2. Breathing

Rabbits with presence of breathing during bleeding were observed in all SHs. The highest prevalence of breathing was found in SH-3 (289/398; 72.6%) and the lowest in SH-15 (25/400; 4.6%) as shown in **Table 7**. The observers differed significantly in the detected prevalence of presence of breathing in 12 out of the 16 SHs evaluated ($P < 0.001$). This is mainly because Obs-

D had less angle of visibility to properly observe both the rabbits' flanks and mouth movements to detect breathing since there was not enough space available to assess the rabbits in a dorsolateral or ventrolateral position in the SHs. Therefore, these observers had to assess the rabbits on either dorsal or ventral position according to the SH. The PoA between observers ranged from 52.3 to 97.8% whereas κ ranged from 0.43 to 0.83 and was interpreted in 12 SHs as "fair to good" and in 4 SHs as "excellent" agreement (**Table 8**).

Considering the data from all SHs, the detection of breathing differed statistically among evaluators ($P < 0.001$; **Table 7**) but the average of the closest outcomes revealed a general prevalence of 28.4%. Nonetheless, the PoA was 92.5% and the κ was statistically significant and interpreted as "excellent" agreement among observers ($P < 0.001$; $\kappa = 0.85$; **Table 8**).

4.1.2.3. Spontaneous blinking

Rabbits with spontaneous blinking during bleeding were observed in all SHs. However, the observers differed significantly in the detected prevalence in 11 out of the 16 SHs evaluated ($P < 0.001$). Similarly to the detection of breathing, Obs-D detected significantly fewer animals with spontaneous blinking due to a less favourable position for observing the rabbits' eyes than other observers. The highest prevalence of this outcome of consciousness was found in SH-3 (53/400; 13.3%) and the lowest in SH-5 (3/308; 0.8%) as shown in **Table 7**. The PoA between observers ranged from 75.8 to 99.2% whereas κ ranged from 0.28 to 0.67 and was interpreted in four SHs as "poor" and 12 SHs as "fair to good" agreement (**Table 8**).

When considering all the rabbits assessed during bleeding, the detection of spontaneous blinking differed statistically between observers ($P < 0.001$; **Table 7**) but the average of the closest outcomes revealed a prevalence of 5.7%. On the other hand, the PoA was 92.0% and the κ was statistically significant and interpreted as "fair to good" agreement among observers ($P < 0.001$; $\kappa = 0.52$; **Table 8**).

4.1.2.4. Vocalisation

Vocalisations were heard in rabbits from eight out of the 16 SHs evaluated. The observers did not differ significantly in the detected prevalence ($P > 0.05$) but in one SH (i.e. SH-15) were Obs-C heard three rabbits vocalising but the other Obs did not hear any. The highest prevalence of this outcome of consciousness was found in SH-6 (2/294; 0.7%) as shown in **Table 7**. The PoA between observers ranged from 99.1 to 100% and κ could not be computed in eight SHs due to absence of detected vocalisations. In the remaining eight SHs assessed, the κ ranged from 0.00 to 0.67 and was interpreted in six SHs as "poor" and in two SHs as "fair to good" agreement (**Table 8**).

In all the rabbits assessed during bleeding, the prevalence of rabbits vocalising was similar between observers ($P > 0.05$; **Table 7**) and the mean prevalence was 0.13%. The PoA was 99.6% and the κ was statistically significant and interpreted as "poor" agreement between observers ($P < 0.001$; $\kappa = 0.32$; **Table 8**).

Table 7. *Count of rabbits with outcomes of consciousness after bleeding in head-only stunned according to the observer (A to D) and the slaughterhouse (SH) evaluated.*

		Absence of TC, %						**Presence of BR, %**						**Presence of SB, %**					
SH	**n**	**A**	**B**	**C**	**D**	**Mean**	***P*-value**	**A**	**B**	**C**	**D**	**Mean**	***P*-value**	**A**	**B**	**C**	**D**	**Mean**	***P*-value**
1	166	23	29	28	26	27	0.815	57^{b}	68^{b}	58^{b}	24^{a}	61	<0.001	55^{b}	67^{b}	58^{b}	23^{a}	60	<0.001
2	436	20	21	33	25	25	0.213	92	96	100	75	91	0.168	85^{ab}	96^{b}	95^{ab}	66^{a}	92	0.038
3	398	342	320	337	339	335	0.139	293^{b}	276^{ab}	288^{b}	249^{a}	286	0.003	296^{b}	281^{ab}	291^{ab}	257^{a}	289	0.012
4	633	491^{ab}	510^{b}	534^{c}	451^{a}	501	<0.001	364^{b}	341^{b}	379^{b}	268^{a}	361	<0.001	360^{b}	341^{b}	379^{b}	262^{a}	360	<0.001
5	380	225^{ab}	260^{c}	238^{b}	200^{a}	232	<0.001	211^{b}	194^{ab}	200^{ab}	172^{a}	202	0.036	214^{b}	205^{ab}	204^{ab}	171^{a}	208	0.010
6	294	100	83	85	90	90	0.427	55^{b}	61^{b}	57^{b}	29^{a}	58	0.002	84^{b}	86^{b}	80^{b}	49^{a}	83	<0.001
7	574	509	509	514	513	511	0.946	38	47	39	31	39	0.313	37	44	37	30	37	0.418
8	375	336	335	339	338	337	0.961	62	53	51	46	53	0.400	25	25	21	19	23	0.735
9	395	312^{b}	303^{ab}	321^{b}	276^{a}	312	<0.001	136^{b}	130^{ab}	131^{b}	97^{a}	132	0.010	173^{b}	161^{b}	163^{b}	124^{a}	166	0.002
10	588	366a	448^{b}	349^{a}	386^{a}	367	<0.001	19^{ab}	34^{b}	28^{ab}	17^{a}	27	0.045	11	19	16	8	14	0.137
11	548	470	467	485	475	474	0.403	72	64	71	59	67	0.586	20	21	29	25	24	0.525
12	461	136^{a}	187^{b}	178^{b}	211^{b}	192	<0.001	191^{b}	181^{b}	190^{b}	142^{a}	187	0.002	180^{b}	156^{ab}	200^{c}	134^{a}	190	<0.001
13	555	98^{a}	136^{b}	147^{b}	140^{b}	141	0.002	228^{ab}	256^{b}	220^{ab}	211^{a}	235	0.038	219	231	206	194	213	0.118
14	575	256^{a}	321^{b}	334^{b}	299^{ab}	318	<0.001	190^{ab}	264^{b}	229^{b}	178^{a}	228	<0.001	252^{ab}	344^{c}	282^{b}	234^{a}	267	<0.001
15	400	94^{a}	103^{a}	139^{b}	127^{ab}	108	<0.001	32^{b}	28^{ab}	14^{a}	15^{ab}	25	0.008	39	38	23	25	31	0.061
16	650	203	184	186	192	191	0.655	46^{ab}	71^{b}	50^{ab}	39^{a}	56	0.007	40^{ab}	58^{b}	35^{ab}	30^{a}	44	0.008
All	7428	3981^{a}	4216^{b}	4247^{b}	4088^{ab}	4184	<0.001	2086^{b}	2164^{b}	2105^{b}	1652^{a}	2108	<0.001	2090^{b}	2164^{b}	2119^{b}	1651^{a}	2116	<0.001

TC: tonic-clonic seizure; BR: breathing; SB: spontaneous blinking; VC: vocalisation; RR: righting reflex; n: number of total rabbits assessed
a–c = Values with different superscripts within the same row differ among observers by chance ($P < 0.05$).

Continuation Table 7…

		Presence of VC, %						Presence of RR, %					
SH	**n**	**A**	**B**	**C**	**D**	**Mean**	**P-value**	**A**	**B**	**C**	**D**	**Mean**	**P-value**
1	166	0	0	0	0	0	-	7	5	4	7	6	0.749
2	436	0	0	0	0	0	-	3	5	2	6	4	0.471
3	398	1	0	1	0	1	0.572	43	33	29	35	35	0.354
4	633	0	0	0	0	0	-	37	37	47	28	37	0.161
5	380	0	0	0	0	0	-	50^{b}	37^{b}	52^{b}	18^{a}	46	<0.001
6	294	4	2	1	1	2	0.388	5^{c}	1^{b}	0^{a}	0^{a}	0	0.010
7	574	0	0	0	0	0	-	0	0	0	0	0	-
8	375	0	0	0	0	0	-	4	2	6	3	4	0.502
9	395	0	0	2	0	0	0.111	13^{ab}	15^{ab}	28^{b}	8^{a}	12	0.003
10	588	0	0	0	0	0	-	1	0	0	0	0	0.391
11	548	1	0	1	1	1	0.801	4	3	7	3	4	0.466
12	461	2	2	0	3	2	0.436	3	7	4	9	6	0.261
13	555	0	0	0	0	0	-	0	2	4	1	2	0.171
14	575	3	0	1	3	2	0.276	8^{a}	37^{b}	42^{b}	24^{b}	34	<0.001
15	400	0^{a}	0^{a}	3^{b}	0^{a}	0	0.029	11	11	13	16	13	0.716
16	650	1	0	5	2	2	0.071	47^{b}	28^{ab}	29^{ab}	18^{a}	25	0.002
All	7428	12	4	14	10	10	0.132	236^{b}	223^{ab}	267^{b}	176^{a}	242	<0.001

n: number of rabbits
a–c = Values with different superscripts within the same row differ among observers by chance ($P < 0.05$).

Table 8. *Inter-observer proportion of agreement (PoA), Fleiss' kappa coefficient (κ) and interpretation of the animal-based indicators for the state of consciousness of head-only electrical stunned rabbits assessed after bleeding according to the slaughterhouse assessed.*

		Tonic seizure			Breathing			Spontaneous blinking			Vocalisations			Righting reflex		
SH	**n**	**PoA, %**	**κ**	**P-value**	**PoA, %**	**κ**	**P-value**	**PoA, %**	**κ**	**P-value**	**PoA, %**	**κ**	**P-value**	**PoA, %**	**κ**	**P-value**
1	166	74.7	0.491 (FG)	<0.001	56.6	0.432 (FG)	<0.001	86.7	0.522 (FG)	<0.001	100	*	*	94.0	0.531 (FG)	<0.001
2	436	87.8	0.436 (FG)	<0.001	79.1	0.628 (FG)	<0.001	94.3	0.569 (FG)	<0.001	100	*	*	98.4	0.484 (FG)	<0.001
3	398	81.4	0.628 (FG)	<0.001	75.1	0.670 (FG)	<0.001	97.7	0.279 (P)	<0.001	99.7	0.332 (P)	<0.001	86.7	0.543 (FG)	<0.001
4	633	65.9	0.454 (FG)	<0.001	68.6	0.658 (FG)	<0.001	95.3	0.476 (FG)	<0.001	100	*	*	91.0	0.544 (FG)	<0.001
5	387	54.8	0.485 (FG)	<0.001	68.2	0.651 (FG)	<0.001	99.2	0.581 (FG)	<0.001	100	*	*	79.1	0.355 (FG)	<0.001
6	294	64.3	0.556 (FG)	<0.001	61.2	0.434 (FG)	<0.001	94.9	0.305 (P)	<0.001	98.0	0.090 (P)	<0.001	98.0	-0.005 (P)	0.829
7	574	90.1	0.718 (FG)	<0.001	96.2	0.827 (E)	<0.001	99.7	0.666 (FG)	<0.001	100	*	*	100	*	*
8	375	89.1	0.658 (FG)	<0.001	96.0	0.811 (E)	<0.001	97.9	0.375 (P)	<0.001	100	*	*	98.7	0.663 (FG)	<0.001
9	395	62.8	0.426 (FG)	<0.001	69.1	0.644 (FG)	<0.001	91.9	0.277 (P)	<0.001	99.5	-0.001 (P)	0.951	90.1	0.316 (P)	<0.001
10	588	61.7	0.500 (FG)	<0.001	97.8	0.728 (FG)	<0.001	99.3	0.531 (FG)	<0.001	100	*	*	99.8	0.000 (P)	0.980
11	548	79.0	0.524 (FG)	<0.001	92.5	0.807 (E)	<0.001	87.2	0.623 (FG)	<0.001	99.6	0.666 (FG)	<0.001	96.7	0.392 (P)	<0.001
12	461	60.3	0.533 (FG)	<0.001	60.1	0.519 (FG)	<0.001	86.1	0.488 (FG)	<0.001	99.1	0.379 (P)	<0.001	95.9	0.111 (P)	<0.001
13	555	74.1	0.606 (FG)	<0.001	68.8	0.629 (FG)	<0.001	95.3	0.418 (FG)	<0.001	100	*	*	98.7	-0.003 (P)	0.855
14	575	67.1	0.642 (FG)	<0.001	52.3	0.479 (FG)	<0.001	76.2	0.461 (FG)	<0.001	99.5	0.475 (FG)	<0.001	90.6	0.428 (FG)	<0.001
15	400	67.5	0.577 (FG)	<0.001	86.2	0.495 (FG)	<0.001	75.8	0.516 (FG)	<0.001	99.2	-0.002 (P)	0.927	92.5	0.359 (P)	<0.001
16	650	78.6	0.724 (FG)	<0.001	89.2	0.501 (FG)	<0.001	92.6	0.478 (FG)	<0.001	99.1	0.248 (P)	<0.001	90.2	0.415 (P)	<0.001
All	7428	72.9	0.701 (FG)	<0.001	92.5	0.807 (E)	<0.001	92.0	0.522 (FG)	<0.001	99.6	0.316 (P)	<0.001	94.0	0.448 (P)	<0.001

* Insufficient scoring variation to calculate kappa coefficients (all indicator scores were 0). Kappa interpretation: ≥ 0.75 'excellent' (E), 0.40–0.74 'fair to good' (FG), and < 0.40 'poor' agreement (P) (Fleiss *et al.*, 2003).

4.1.2.5. Righting reflex

Rabbits showing righting reflex were observed in 14 out of the 16 SHs evaluated. The observers differed significantly in the detected prevalence in 12 SHs (P > 0.05). The highest prevalence of this outcome of consciousness was found in SH-5 (46/380; 12.1%) as shown in **Table 7**. The PoA between observers ranged from 86.7 to 100% and κ could not be computed in one SHs due to absence of detected righting reflex. In the remaining 15 SHs assessed, the κ ranged from 0.00 to 0.66 and was interpreted in eight SHs as "poor" and in seven SHs as "fair to good" agreement (**Table 8**).

As a general result, the prevalence of rabbits with righting reflex differed statistically between observers (*P* < 0.001; **Table 7**) but the average of the closest outcomes revealed a prevalence of 3.3%. The PoA was 94.0% and the κ was statistically significant and interpreted as "poor" agreement between observers (P < 0.001; κ = 0.45; **Table 8**).

4.2. Relationship among ABIs

4.2.1. Immediately after stunning

The proportions of rabbits showing outcomes of consciousness and the combinations of ABIs for the same rabbit is shown as a Venn diagram (**Figure 4**).

Absence of tonic-clonic seizure was the most frequent indicator followed by presence of breathing and spontaneous blinking. Vocalisation was considered absent in all rabbits assessed. Combinations of more than one outcome of consciousness included absence of tonic seizure and presence of breathing or spontaneous blinking (**Figure 4A**). No rabbit showed simultaneously the three outcomes of consciousness.

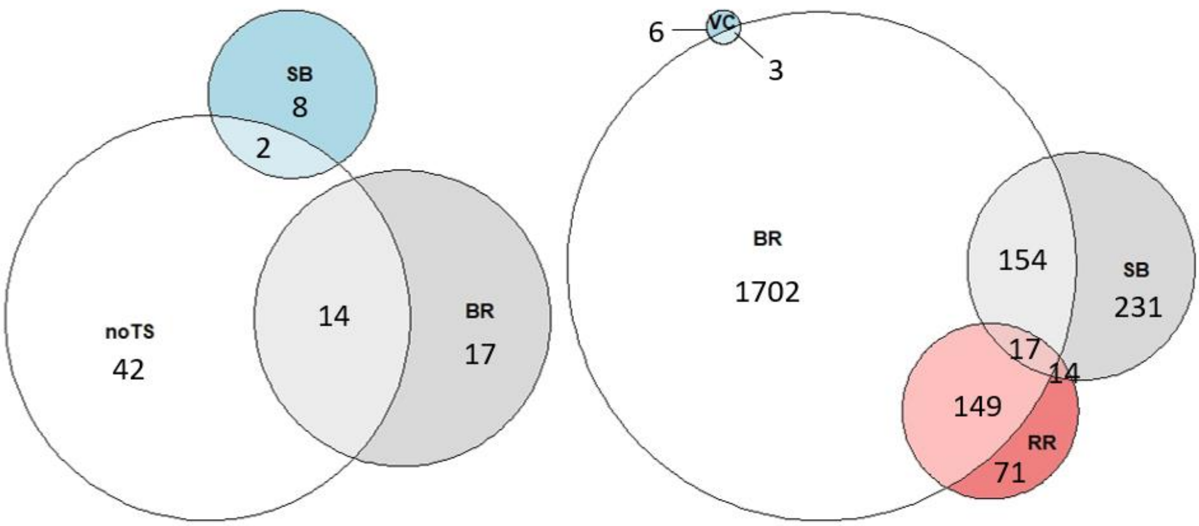

Figure 4. *Venn diagram of the outcomes of consciousness observed in the animal-based indicator assessed after head-only electrical stunned rabbits A) immediately after stunning and B) during bleeding. Outcomes of consciousness are: no TS: absence of tonic-clonic seizure; BR: presence of breathing; SB: presence of spontaneous blinking; VC: presence of vocalisation; RR: presence of righting reflex. Numbers specify the total amount of rabbits showing each outcome of consciousness or combinations of outcome of consciousness from a total of 4112 rabbits assessed immediately after stunning and 7428 during bleeding.*

4.2.2. During bleeding

The proportions of rabbits showing outcomes of consciousness and their combinations at individual level is shown as Venn diagram in **Figure 4B**. This diagram showed that presence of breathing was the most frequent outcome of consciousness observed, followed by presence of spontaneous blinking and righting reflex and to a lesser extent, vocalisations. Furthermore, some rabbits showed simultaneously two of the four indicators of consciousness evaluated. The most frequent combinations were the presence of breathing and spontaneous blinking and presence of breathing and righting reflex. Other combinations found but less observed included presence of spontaneous blinking and righting reflex and presence of breathing and vocalisations. On the other hand, some rabbits were also observed with three of the four indicators of consciousness assessed simultaneously, the combination observed being the presence of breathing, spontaneous blinking and righting reflex.

4.3. Relationship between key parameters and stunning efficiency

Risk factors related to management conditions at slaughter known to be linked to ineffective stunning were analysed. These factors included not wetting the rabbits' head prior to stunning, the number of stunners, and long stun-to-stick intervals. Additionally, combinations of electrical parameters applied to batches of different characteristics, such as genetics and average body weight of the batch, were examined. The mean prevalence and 95% confidence interval of the closest outcomes between observers regarding failure to induce unconsciousness in rabbits (i.e. observations made immediately after stunning) and the prevalence of rabbits regaining consciousness during bleeding (i.e. observations made during bleeding) were calculated. In addition, the management procedures and key stunning parameters per SH and batch are shown in **Table 9**.

4.3.1. Immediately after stunning

All rabbits were effectively stunned in SH-6, SH-7, SH-8, SH-11, SH-13 and SH-15 (in all batches assessed) as no rabbit showed outcomes of consciousness (**Table 9**). Meanwhile, SH-5 showed the highest prevalence of failure at inducing unconsciousness with 15.3% ([8.9 - 18.4] 95% CI) of rabbits showing at least one ABI in batch 1. The layout of SH-3 and the design of the stunner that automatically bled the rabbits in SH-10 did not allow to assess the state of consciousness Immediately after stunning and before bleeding and therefore, no information could be provided on them.

4.3.2. During bleeding

In all the 16 SHs, each batch had some rabbits with at least one outcome of consciousness. The prevalence of rabbits showing outcomes of consciousness varied greatly between batches and SHs. The lowest prevalence during bleeding was observed in SH-10 (batch 1: 2.3% [0.6 - 7.9%] 95% CI; batch 2: 2.6% [1.5 - 4.4%] 95% CI) while the highest in SH-3 (batch 1: 92.9% [77.4 - 98.0%] 95% CI; batch 2: 71.4% [66.5 - 75.7%] 95% CI) as shown in **Table 9**.

Table 9. *Mean prevalence of rabbits with at least one outcome of consciousness immediately after head-only electrical stunning and during bleeding and 95% confidence interval (CI) according to the number of rabbits assessed per batch (n) and slaughterhouse (SH: 1 to 16).*

					Head-only electrical stunning parameters				Immediately after stunning			During bleeding		
SH	**Wet, Yes/no**	**Stunners, n**	**Stun-to-stick interval, s [min, max]**	**Batch**	**Current, mA**	**Frequency, Hz**	**Voltage, V**	**Time, ms**	**Rabbits, n**	**Mean, %¥**	**95% CI**	**Rabbits, n**	**Mean, %¥**	**95% CI**
1	No	2	[NA, 10]	1	291±86	401	395±31	2380±306	193	13.0	[8.9 - 18.4]	51	51.0	[37.7 - 64.1]
1	No	2	[NA, 10]	2	281±81	401	397±0	2391±283	298	0.7	[0.2 - 2.3]	115	37.4	[29.1 - 46.5]
2	NA	2	[NA, 11]	1	855±124	50	182±3	978±128	200	0.5	[0.1 - 2.8]	197	20.3	[15.3 - 26.5]
2	NA	2	[NA, 11]	2	854±125	50	181±3	972±147	33	0.0	[0.0 - 10.4]	50	16.0	[8.3 - 28.5]
2	NA	2	[NA, 11]	3	836±111	50	181±3	918±117	100	1.0	[0.2 - 5.5]	189	23.8	[18.3 - 30.4]
3	No	1	22	1	875±284	50	270±7	1102±194	0	-	-	28	92.9	[77.4 - 98.0]
3	No	1	22	2	913±294	50	272±7	1153±460	0	-	-	370	71.4	[66.5 - 75.7]
4	No	3	[8, 15]	1	860±257	50	302±8	750±138	0	-	-	325	61.2	[55.8 - 66.4]
4	No	3	[8, 15]	2	858±287	50	301±8	739±129	0	-	-	16	37.5	[18.5 - 61.4]
4	No	3	[8, 15]	3	939±283	50	299±7	801±120	71	0.0	[0.0 - 5.1]	-	-	-
4	No	3	[8, 15]	4	834±271	50	292±10	721±117	128	10.2	[6.0 - 16.6]	292	54.5	[48.7 - 60.1]
5	No	4	[19, 36]	1	667	NA	351	1338	6	0.0	[0.0 - 39.0]	192	51.0	[44.0 - 58.0]
5	No	4	[19, 36]	2	577	NA	354	1272	177	15.3	[10.7 - 21.3]	188	55.3	[48.2 - 62.3]
6	No	1	15	1	437±128	50	180±0	495±258	148	0.0	[0.0 - 2.5]	140	25.7	[19.2 - 33.5]
6	No	1	15	2	447±135	50	180±0	503±261	128	0.0	[0.0 - 2.9]	154	29.9	[23.2 - 37.5]
7	NA	2	2	1	610±171	50	145±4	567±121	381	0.0	[0.0 - 0.1]	440	4.3	[2.8 - 6.6]
7	NA	2	2	2	498±135	50	143±4	485±107	0	-	-	134	14.9	[9.9 - 21.9]
8	No	1	3	1	467±175	50	210±4	509±152	200	0.0	[0.0 - 1.9]	375	7.2	[5.0 - 10.3]
9	Yes	3	[7, 18]	1	723±254	50	936±316	250±8	0	-	-	41	39.0	[25.7 - 54.3]
9	Yes	3	[7, 18]	2	708±258	50	843±270	251±8	12	0.0	[0.0 - 24.3]	156	51.9	[44.1 - 59.6]
9	Yes	3	[7, 18]	3	696±244	50	739±239	251±8	142	1.4	[0.4 - 5.0]	198	35.9	[29.5 - 42.7]
10	Yes	3	0	1	411±90	300	156±69	669±136	0	-	-	88	2.3	[0.6 - 7.9]
10	Yes	3	0	2	406±96	300	166±87	665±141	0	-	-	500	2.6	[1.5 - 4.4]

¥: mean prevalence of the closest evaluations between four observers. NA: not available

Continuation Table 9…

					Head-only electrical stunning parameters				Immediately after stunning			During bleeding		
SH	**Wet, Yes/no**	**Stunners, n**	**Stun-to-stick interval, s [min, max]**	**Batch**	**Current, mA**	**Frequency, Hz**	**Voltage, V**	**Time, ms**	**Rabbits, n**	**Mean, %¥**	**95% CI**	**Rabbits, n**	**Mean, %¥**	**95% CI**
11	NA	1	3	1	138±17	150	95±34	NA	200	0.0	[0.0 - 2.8]	548	18.1	[15.1 - 21.5]
12	Yes	1	16	1	196±43	150	113±56	399±31	200	0.5	[0.0 - 2.8]	147	43.5	[35.8 - 51.6]
12	Yes	1	16	2	184±57	150	127±74	386±47	197	0.0	[0.0 - 1.9]	314	40.1	[34.9 - 45.6]
13	No	3	[6, 25]	1	126±26	250	107±38	700±0	200	0.0	[0.0 - 2.8]	122	43.4	[35.0 - 52.3]
13	No	3	[6, 25]	2	133±22	250	97±33	700±0	198	0.0	[0.0 - 1.9]	433	38.6	[34.1 - 43.2]
14	Yes	3	[17, 33]	1	170±38	250	162±34	NA	200	0.5	[0.0 - 2.8]	5	100	[56.1 - 100]
14	Yes	3	[17, 33]	2	173±39	250	159±35	NA	0	-	-	386	54.9	[49.9 - 59.8]
14	Yes	3	[17, 33]	3	177±39	250	155±36	NA	200	0.0	[0.0 - 2.8]	184	60.9	[53.7 - 67.6]
15	Yes	4	[13, 22]	1	257±65	50	116±5	419±119	200	0.0	[0.0 - 2.8]	200	25.5	[20.0 - 32.0]
15	Yes	4	[13, 22]	2	262±69	50	117±5	461±131	0	-	-	200	21.5	[16.4 - 27.7]
16	No	3	[5, 20]	1	609±176	50	207±4	449±99	21	0.0	[0.0 - 15.5]	0	-	-
16	No	3	[5, 20]	2	566±161	50	209±6	437±94	279	0.4	[0.0 - 2.0]	319	15.7	[12.1 - 20.1]
16	No	3	[5, 20]	3	529±150	50	209±5	424±95	0	-	-	197	12.7	[8.7 - 18.1]
16	No	3	[5, 20]	4	557±154	50	209±5	444±92	0	-	-	36	16.7	[7.9 - 31.9]
16	No	3	[5, 20]	5	508±131	50	211±3	424±88	0	-	-	98	7.1	[2.8 - 12.7]

¥: mean prevalence of the closest evaluations between four observers
NA: not available

4.4. Risk factor analysis

Multicollinearity was negligible among the potential factors influencing efficient stunning as all VIFs were below 1.16.

Factors influencing the efficiency of head-only electrical stunning in rabbits is shown in Table 10. Specifically, a stun-to-stick interval below 5 s had the largest effect, reducing the odds by 92% (OR = 0.08), followed by wetting the rabbit's head with a 34% reduction (OR = 0.66). The combination of high current and low frequency (> 200mA and 50Hz) and high current and high frequency electrical parameters (> 200mA and > 50Hz) showed reductions of 58% (OR = 0.42) and 39% (OR = 0.61), respectively. The intercept, with an OR of 1.81, indicates that when the stun-to-stick interval is longer than 5 s, lateral cut is performed, the head of the rabbits are not wet when stunning and the electrical parameters are considered suboptimal (i.e. < 200 mA and > 50 Hz), the odds of inefficient stunning are 1.81 times higher compared to when none of these "protective" factors are present.

***Table 10**. Factors influencing the efficiency of head-only electrical stunning in rabbits.*

Predictors	**Odds Ratios**	**95 % confidence interval**	**P-value**
(Intercept)	1.81	1.53 – 2.15	<0.001
Stun-to-stick interval < 5 s	0.08	0.11 – 0.30	<0.001
Wetting the rabbit's head	0.66	0.57 – 0.77	<0.001
Electrical parameters			
> 200mA and 50Hz	0.42	0.30 – 0.58	<0.001
> 200mA and > 50Hz	0.61	0.52 – 0.71	<0.001

5 Discussion

One of the aims of the study was to gain insight into the inter-observer repeatability of valid and feasible ABIs of the state of consciousness after head-only electrical stunning in rabbits. In addition, to report the prevalence of failure to induce and maintain unconsciousness in commercial slaughterhouses.

This study compared the assessment of four observers on 11,540 rabbits of 38 batches from 16 different SHs and different key stunning parameters applied from the three main rabbit producer countries in the EU-27. Even though SHs were not *per se* randomly sampled, they represent quite a variety in terms of slaughter capacities, equipment designs, key electrical parameters combinations, stun-to-stick interval and line speed. Furthermore these 16 SHs alone slaughter the majority of rabbits reared in the EU.

Regarding the observers, all of them were well trained, conducted a similar study in broiler chickens (Contreras-Jodar et al., 2022), and turkeys (Contreras-Jodar et al., 2023) and agreed on the definition of the ABIs before the assessments. The number of observers was kept to the maximum number possible with the intention of causing minimum disturbance to the operators and to each other with the objective to have a clear view for the assessment of the ABIs. For this purpose, they were placed side by side assessing the same rabbits over the same span of time. Nevertheless, in certain SHs, one or even two observers had difficulties in assessing the state of consciousness of the rabbits due to lack of sufficient space to be able to choose where to stand to have a clearer view of the animals.

5.1. Inter-observer repeatability of the ABIs

Inter-observer repeatability of the ABIs was analysed per individual assessed using the combination of PoA and κ. On the one hand, high PoA may suggest that there is a high agreement among observers. However, it may happen that the agreement is high because the outcome of consciousness is rarely or hardly ever observed (e.g., vocalisations) and the observers agree when nothing is perceived. On the contrary, the agreement is lower in the outcomes of consciousness that are more frequently observed (e.g., absence of tonic seizure, presence of breathing). On the other hand, the κ interpretation slightly varied according to the SH assessed for most of the indicators. It happened because κ is strongly influenced by the prevalence of rabbits showing outcomes of consciousness. The lower the prevalence, the lower may be the κ although sometimes, when an outcome of consciousness is not detected in any rabbit within a sample, the κ cannot be even computed. These results suggest that these are cases in which the calculation of PoA does not give much information *per se*. The same occurs when paying attention to the κ found. However, the combination of PoA and κ does allow to get a general overview of the goodness of an ABI in terms of inter-observer repeatability.

Immediately after stunning, the most repeatable indicators are tonic-clonic seizure, followed by breathing and spontaneous blinking while vocalisation was artificially highly repeatable since it was never heard in any SH.

During bleeding, the most repeatable ABI was breathing, followed by tonic-clonic seizure and spontaneous blinking, while righting reflex and vocalisations were the least repeatable. Despite being the most repeatable, breathing had three main sources of variation in scoring. First, there was hesitation at considering presence of breathing in some SHs where rabbits showed very shallow depth of flank movements accompanied or not with rapid muzzle movements. Second, evaluating breathing was more challenging in batches of dark-furred rabbits compared to white-furred rabbits. Third, presence of breathing was considered when a minimum of two thoracic or abdominal muscle movements associated with breathing were observed. Some rabbits performed the second thoracic or abdominal muscle movements at the end of the observation span, raising doubts on whether it occurred just before or after the established time limit. This highlights the importance of determining an optimal observation period during which more accurate outcomes of consciousness can be observed in a slaughterhouse. The best position for assessing breathing is ventrolateral, as dorsal or lateral positions may underestimate the prevalence of breathing in rabbits.

It should be noted that the inter-observer repeatability of spontaneous blinking was underestimated in some SHs, affecting the overall PoA and κ values for this indicator. This is because in some SHs, the space available for four observers to have a clear view for detecting spontaneous blinking throughout the assessment period was suboptimal. While two observers could assess spontaneous blinking relatively easily, one or even two of the other observers sometimes reported difficulty in accurately assessing this ABI due to a lack of a proper viewing angle to clearly observe the rabbits' eyes. Furthermore, although not quantified, it is quite common to find a proportion of rabbits with sealed eyelids due to burns from incorrect electrode placement, potentially underestimating the prevalence of rabbits that would spontaneously blink as a sign of consciousness.

Righting reflex had poor repeatability between assessors, as it might be confounded with preagonal muscle movements. These episodes are similar to a tonic phase with some muscle tremor and sometimes in combination with righting of the head. Therefore, the evaluators found it difficult to differentiate from the righting reflex as voluntary attempts to recover consciousness.

Vocalisation was the least repeatable indicator of consciousness since it was hardly ever heard and when it seemed to occur there was no consensus among the observers. This was because when the observers heard vocalisations, they were not able to identify which rabbit it came from and, moreover, this also depended on the hearing ability of the evaluator. In addition, it is important to highlight that the noise level in the SHs is likely to impair its assessment. This is because the vocalisations in bled rabbits are not high-pitched or loud and therefore, not clearly detectable unless the assessor is within a few centimetres of the animal's head, but this was not the case in the assessments carried out in the present study. In contrast, rabbits that were stunned but for whatever reason escaped from the bleeder, once they regained consciousness, high pitch vocalisations were clearly detected for all at a distance despite the different hearing abilities and the ambient noise of the SH and, therefore, this indicator should not be neglected despite being poorly repeatable at this stage.

5.2. Relationship among ABIs

Effective electrical stunning is known to induce epileptiform activity in the brain (Anil et al., 2000; Velarde et al., 2002), manifesting as tonic-clonic seizure, cessation of breathing, and absence of physical and behavioural reflexes. In the present study, a total of 58 out of 4112 rabbits showed flaccid muscle tonicity after the electrical stunning instead of tonic-clonic seizure. According to Anil et al. (1998), some of these animals may exhibit normal EEG patters and are therefore considered conscious, while others may display epileptic EEG patterns, indicating unconsciousness. Therefore, effective induction of unconsciousness is only confirmed by the observation of tonic-clonic seizure immediately after stunning and the presence of flaccidity is not indicative of unconsciousness. Furthermore, 17 animals out of 4112 showed rhythmic breathing and 8 spontaneous blinking after or during the tonic-clonic seizure and before neck cutting. The presence of these indicators does not allow to consider them effectively stunned.

During bleeding, the presence of tonic-clonic seizure indicates that the rabbit is still unconscious and usually it means that the stun-to-stick interval was short (with the tonic-clonic phase still persisting), reducing the likelihood of regaining consciousness. However, the absence of these seizures does not imply consciousness, as the rabbit may have already experienced them shortly before. A rabbit is considered regaining consciousness only when it shows absence of tonic-clonic seizure followed by the presence of at least one of these indicators of consciousness: breathing, spontaneous blinking, and vocalisations.

Some indicators of consciousness were sometimes observed simultaneously in the same rabbit. The most common combinations were presence of breathing and spontaneous blinking, and presence of breathing and righting reflex. A triple combination of presence of breathing, spontaneous blinking, and the righting reflex was also noted. It seems that when a rabbit starts breathing, it is more likely to spontaneously blink or attempt to regain posture (righting reflex) later on. Nevertheless, some rabbits exhibited spontaneously blinking without breathing or righting reflex, indicating that the order of appearance of the outcomes of consciousness varied. Rabbits that breathe and/or blink spontaneously and show righting reflex, are rabbits considered to be conscious. Being conscious during the slaughter process means the animal is aware and can feel pain, fear, and distress.

On the other hand, rabbits that are scored as showing a righting reflex but do not breathe or blink spontaneously cannot be considered conscious, and it is more likely to be what is known as Lazarus' sign described in humans (Urasaki et al., 1992) as well as in slaughtered animals (EFSA, 2004). The Lazarus sign, named after the biblical man who rose from the dead, refers to spinal reflexes and automatisms observed in individuals with apparent brain death. These reflexes in humans can include spontaneous head turning or shaking, neck-arm flexion, neck-hip flexion, neck-abdominal flexion, arm extension, and elbow and finger flexion that mimic voluntary grasping or clasping. The Lazarus sign is a reflex mediated by a reflex arc neural pathway which passes via the spinal column but not through the brain. As a consequence, the movement is possible in brain-dead patients. The reflex is often preceded by slight shivering motions. When the rabbit is bleeding out, the lack of oxygen can cause dysfunction in the central nervous system, but spinal reflexes can remain active for a time. They include movements such as muscle contractions or spasms, which can resemble the righting reflex in rabbits.

5.3. Relationship between key parameters and stunning efficiency

Effective stunning is achieved when a rabbit is rendered unconscious and remains in this state during bleeding until death occurs. To ensure this, the state of consciousness was assessed immediately after stunning and during bleeding.

One of the objectives of the present study was to compare the efficiency of stunning across different SHs and key parameters (i.e. current, voltage, frequency, time of exposure and stun-to-stick interval) when using a head-only electrical device on rabbits. However, numerous other factors can influence the effectiveness of stunning in rabbits. These factors include animal characteristics (e.g. genetics, body weight), operator management practices (e.g. wetting the rabbits' head prior to stunning, regular maintenance and cleaning of the electrical equipment, staff fatigue) and the type of bleeding (ventral or lateral).

The results showed that, despite the divergence in the applied electrical parameters and exposure time, the induction of unconsciousness was effective in almost all batches evaluated in the different SHs. However, in some batches the effectiveness in inducing unconsciousness was abnormally low (e.g., SH-1 batch 1, SH-4 batch 4, SH-5 batch 2). According to our observations, poor electrical contact was the main cause of rabbits showing outcomes of consciousness immediately after stunning. This occurs when the electrodes are not replaced due to wear and/or when trapped fur or carbonized debris remain in the stunning electrodes and are not regularly removed. In one of the SHs visited, the fur trapped in the device not only impaired the electrical contact, causing the failure to induce unconsciousness in some rabbits, but also a small fire due to burning of the trapped fur. In none of the SHs with low stunning efficiency, the head of the animals was wetted. Rabbits with absence of tonic-clonic seizure and/or presence of breathing and/or spontaneous blinking are at high risk of experiencing pain, distress and suffering when shackled, being bled and during bleeding.

It should be highlighted that some rabbits received pre-stun shocks due to incorrect contact between the rabbit's head and the stunner electrodes which is very painful for conscious animals. In these animals, muscle tonicity did not occur, and operators repeated contact of the animal with the stunner until tonicity occurred as a sign of unconsciousness. Only then that the rabbits were shackled on the SH line.

In this study, the prevalence of rabbits that were not effectively rendered unconscious was recorded from the moment they were shackled. As a result, cases where rabbits were not successfully rendered unconscious on the first attempt and needed to be re-exposed to the electrical stunning device were not specifically identified. Therefore, there is no estimation of the frequency of re-stunning before shackling.

Although unconsciousness was effectively induced in almost all rabbits across all batches (4035 out of 4112, 98.1%), the prevalence of rabbits regaining consciousness varied strongly, ranging from 2.3% to 92.9% depending on the batch and the SH assessed. The lowest prevalence was found in SH-10 (batch 1: 2.3% [0.6 - 7.9 %]; batch 2: 2.6% [1.5 - 4.4 %]). This was likely because the stunner they used not only wet the rabbit's head and stunned but also bled immediately after stunning if the operator pressed a button (once the operator noticed the tonic seizure), resulting in an extremely short stun-to-stick interval. The type of cut was ventral, allowing for a more rapid death by exsanguination and reduced the chance of regaining consciousness before death. The stunning device used in this SH was unique and offered by far the best results, as rabbits died so quickly that they exhibited only muscle tonicity without progressing to clonic seizure. However one drawback of this system is that it does not provide a means to verify the efficiency of stunning (except by the presence of tonic seizure) before bleeding, which could potentially lead to the risk of bleeding conscious rabbits.

After SH-10, the SH with the lowest prevalence of rabbits regaining consciousness in a batch was SH-7. SH-7 was equipped with a unique stunner, different from the commonly observed ones. It featured an electrified grill mounted on the wall, where the operator would make contact with the rabbit's head, then hang it on the shackle line, and another operator would bleed the animal. Due to SH-7's low throughput capacity, there was only one operator stunning and hanging and one for bleeding the rabbits, resulting in a very short stun-to-stick interval (approximately 2 s). The electrical parameters at SH-7 seemed appropriate, providing high intensities and low frequencies to ensure sufficient unconsciousness time for the rabbits to die without regaining consciousness. However, variation was observed in the prevalence of rabbits regaining consciousness between batches (batch 1: 4.3% [2.8 - 6.6]; batch 2: 14.9% [9.9 - 21.9]). Although both batches were of the same genetics, the differences might be attributed to the variance in average body weight between batches (batch 1: 2.3 kg; batch 2: 1.7 kg). The lighter rabbits received a lower electrical intensity (batch 1: 610±171 mA/rabbit; batch 2: 498±135 mA/rabbit). Although it cannot be assured, this might be explained by the fact that the electrical tongs were designed for heavier rabbits (with larger heads), not pressing the head of smaller rabbits, leading to impaired electrical contact. On the other hand, in this SH, the rabbits were wetted with a pressurized hose controlled by an operator while still in stacked transport containers. Consequently, it cannot be ensured that all rabbits had their heads wetted, as likely only those positioned closest to the container openings were wetted. In addition, the type of cut was lateral rather than ventral, which could lead to a longer time to death and, therefore, a greater chance of the rabbits regaining consciousness before death.

In SH-16, the prevalence found ranged between 7.1 to 15.7 % according to the batch assessed. The SH-16 was equipped with three stunners, so three operators were in charge of stunning rabbits while two operators bled the rabbits on the line. The stunners consisted in electrical tongs that were fixed in a channel-shaped support. The rabbits were not wetted before stunning. The stun-to-stick interval varied considerably (from 5 to 20 s) depending on the distance between each stunner and the bleeding operator. In this context, rabbits stunned with the stunner closest to the bleeding operator likely had a lower risk of regaining consciousness compared to those stunned farther away, due to the shorter stun-to-stick interval. Despite the long stun-to-stick interval for most of the rabbits, the prevalence of rabbits regaining consciousness was unexpectedly lower than in other SHs with similar characteristics. This may be attributed to the apparently optimal electrical parameters (high current: > 400 mA/rabbit, low frequency: 50 Hz), which likely ensured a prolonged period of unconsciousness. Additionally, the ventral cuts performed are presumed to increase bleeding speed and shorten the time to death, thereby reducing the likelihood of rabbits regaining consciousness before death.

For the rest of the SHs assessed, slaughtered batches had a prevalence between 20% and 92.9% of rabbits showing at least one indicator of consciousness (in SH-1, SH-2, SH-3, SH-4, SH-5, SH-6, SH-9, SH-12, SH-13, SH-14, SH-15).

5.4. Risk factor analysis

The risk of rabbits showing at least one indicator of consciousness during bleeding is significantly influenced by several factors, with the stun-to-stick interval being the most critical. Intervals exceeding 5 s and not wetting the rabbits' head all exacerbate this risk. It is well-established that the higher the current and the lower the frequency applied in electrical stunning, the more efficient is the stunning method in inducing and maintaining unconsciousness (EFSA, 2004). The European Commission (2017) cites thresholds of over 140 mA and over 400 mA for rabbits, while the model selected in the present study considers a current below 200 mA as increasing the odds of ineffective stunning. It is important to note that the more risk factors are present in a SH, the higher the odds of ineffective stunning in rabbits.

6 Conclusions and recommendations

Our study held between 2023 and 2024, showed that in commercial rabbit slaughterhouses across the European Union, there is considerable variability in slaughterhouse designs, slaughter capacities, rabbit management practices, types of head-only electrical stunning devices used, electrical parameters applied, duration of head exposure to electrical tongs, stun-to-stick intervals, and type of neck cuts used.

We assessed the welfare of head-only electrically stunned rabbits using animal-based indicators of consciousness in two key stages, immediately after stunning to evaluate the efficiency of induction of unconsciousness, and during the bleeding process to assess maintenance of unconsciousness

<u>Immediately after stunning:</u>

- The most relevant indicators are tonic-clonic seizure, breathing, spontaneous blinking.
- Vocalisations were discarded since they were never heard at this stage. Nevertheless, if vocalisation is detected, it should not be neglected.
- Presence of tonic-clonic seizure is crucial to confirm that a rabbit has been effectively stunned and rendered unconscious.
- Rabbits that do not exhibit tonic-clonic seizure and/or display rhythmic breathing and/or spontaneous blinking are at high risk of experiencing pain, fear and distress when shackled, bled, and during the bleeding process.

<u>During bleeding:</u>

- The most relevant indicators are breathing, spontaneous blinking, vocalisations.
- At this stage, the absence of tonic-clonic seizure does not imply consciousness. The presence of tonic-clonic seizures indicates that the rabbit remains unconscious and usually it is observed when the stun-to-stick interval is short.
- Vocalisations were not repeatable between observers because it is difficult to determine which animal vocalised. Although vocalisations were rare, it is recommended not to overlook them when detected.
- The righting reflex was not repeatable between observers because often confused with the Lazarus signs—reflex-like behaviours that can occur in brain-dead animals. Thus, the righting reflex should only be considered an indicator of consciousness when it is accompanied by breathing and/or spontaneous blinking.
- The most observed combinations are presence of breathing and spontaneous blinking and presence of breathing and righting reflex.

Although unconsciousness is effectively induced in nearly all rabbits, indicators of consciousness are frequently observed after neck-cutting, suggesting that a variable but significant proportion of rabbits are progressively recovering consciousness before death in almost all slaughterhouses.

Key factors ranked in order of their contribution to effective stunning from greatest to least are: stun-to-stick interval of less than 5 s, using current above 200 mA and frequencies not above 50 Hz, and wetting the rabbits' heads. The more these key factors are present in a SH, the higher the odds of effective stunning in rabbits.

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EURCAW-Poultry-SFA is one of the four European Union Reference Centres for Animal Welfare. It focuses on poultry and other small farmed animals welfare and legislation, and covers the entire life cycle from hatch/birth to the end of life. EURCAW-Poultry-SFA's main objective is to scientifically and technically support the European Commission and Member States for implementation of welfare legislation. This includes:

- Directive 98/58/EC concerning the protection of animals kept on farms;
- Regulations 1/2005/EC and 1099/2009/EC concerning their protection during transport and slaughter;
- Directive 1999/74/EC laying down minimum standards for the protection of laying hens;
- Directive 2007/43/EC laying down minimum rules for the protection of chickens kept for meat production.

Partners

EURCAW-Poultry-SFA receives funding from DG SANTE of the European Commission and represents a collaboration between the following four partner institutions:

- ANSES, France
- IRTA, Spain
- ANIVET, AU, Denmark
- IZSLER, Italy

Funded by the European Union. Views and opinions expressed are however those of the EURCAW only and do not necessarily reflect those of the European Union or HaDEA. Neither the European Union nor the granting authority can be held responsible for them.

Activities of EURCAW-Poultry-SFA

- Coordinated Assistance
  Providing support, networking and Questions to EURCAW;
- Welfare indicators, Assessment & Good Practices
  Identifying animal welfare indicators, including animal based, management based and resource-based indicators, that can be used to verify compliance with the EU legislation;
- Scientific and technical studies
  Preparing Scientific Reviews of knowledge on welfare topics, identify research needs and perform scientific and technical studies to fill the gaps of knowledge;
- Training
  Reviewing existing training activities and developing new training materials, webinars and knowledge pills for official inspectors and competent authorities;
- Communication and Dissemination
  Increasing awareness of our outputs via the website, and newsletter.

Website and contact

EURCAW-Poultry-SFA's website offers relevant and actual information to support enforcement of poultry and other small farmed animals' welfare legislation.

We offer a 'Questions to EURCAW' service for official inspectors, policy workers, and other personnel providing advice or support for official controls of poultry and other small farmed animals welfare in the EU. For more information go to the Q2E webform available online [here](https://survey.anses.fr/SurveyServer/s/DSL/Queryw) or https://survey.anses.fr/SurveyServer/s/DSL/Queryw. All Q2E answers are available online.

Co-funded by the European Union
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