

European Union Reference Centre for Animal Welfare *Poultry SFA*



Welfare assessment during electrical stunning in rabbits

November 12th, 2024





anses







November 12th, 2024



10:00: General presentation of the EURCAW-Poultry-SFA - V. Michel
10:15: Introduction to electrical stunning in rabbits and indicators - A. Velarde
10:20: Refinement of indicators and efficiency of stunning – A. Contreras-Jodar
10:30: Methods and recommendations – A. Contreras-Jodar and V. Michel
10:45: Discussion



European Union Reference Centre for Animal Welfare *Poultry SFA*

General presentation of the EURCAW-Poultry-SFA

Virginie Michel (Coordinator, ANSES)





REGULATION (EU) 2017/625 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 15 March 2017

Art. 95 of 'The Official Controls Regulation':

"The Commission shall, by means of implementing acts, designate European Union reference centres for animal welfare that shall **support the activities of the Commission and of the Member States**"



Since October 2018



Since February 2020



*EURCAWaqua

Since June 2021

Designation mid-January 2024

JRCAW Poultry SFA



Main Target groups and objective

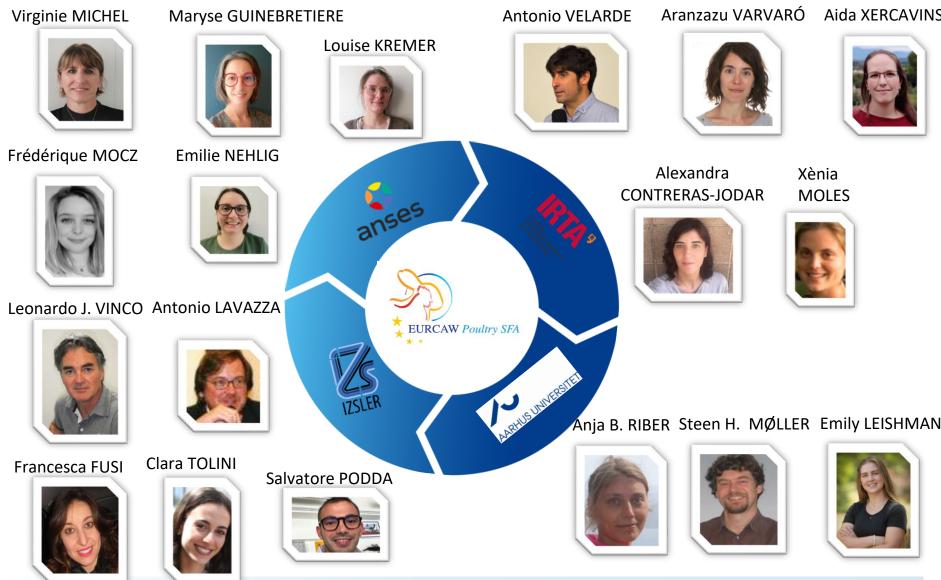
- Target groups: European Commission, Competent Authorities, National Reference Centres and 'Supporting Bodies' from MSs: science, training, communication
- **Objectives**: to support implementation of welfare legislation in a harmonized way through MSs
 - > on farms, transportation and killing
 - > poultry & other small farmed animals

What the Centre is and is not...

The Centre is	X The Centre is not
To assist Commission and MSs	To be solicited by other institutions
Able to deliver scientific and technical advice	To do risk assessment
To help implementation of legislation	To interpret legislation



EURCAW-Poultry-SFA team





Priority areas

- 1. Broiler chickens on farm
- 2. Laying hens housed in alternative housing systems
- 3. State of consciousness after stunning
- 4. Rabbits on farm, with a focus on alternative housing systems
- 5. Turkeys on farm and during transport





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Activities:

- 1. Coordinated assistance
- 2. Animal welfare indicators
- 3. Scientific and technical studies
- 4. Training courses
- 5. Disseminating research and innovations





Activity 2. Animal welfare indictors

- 1. Compile a list of legal requirements to check during official controls
- 2. Identify relevant welfare indicators to verify compliance with the legal requirements identified
- 3. Propose validated indicators and methods for assessment



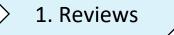
Activity 3. Scientific and technical studies

- To provide scientific and technical expertise on the official controls and the implementation of the legislation related to poultry.
- To identify the gaps of knowledge and the bottlenecks regarding legislation and formulate different topics for scientific and technical studies.
- To develop in the framework of the Centre, some scientific studies designed to answer questions raised in activity 2.
- Identify good practices





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- List of the **relevant indicators** for the assessment of consciousness of broilers and turkeys after waterbath stunning.
- Description of the considered validated indicators and associated methodology
- Identification of gap of knowledge regarding indicators.



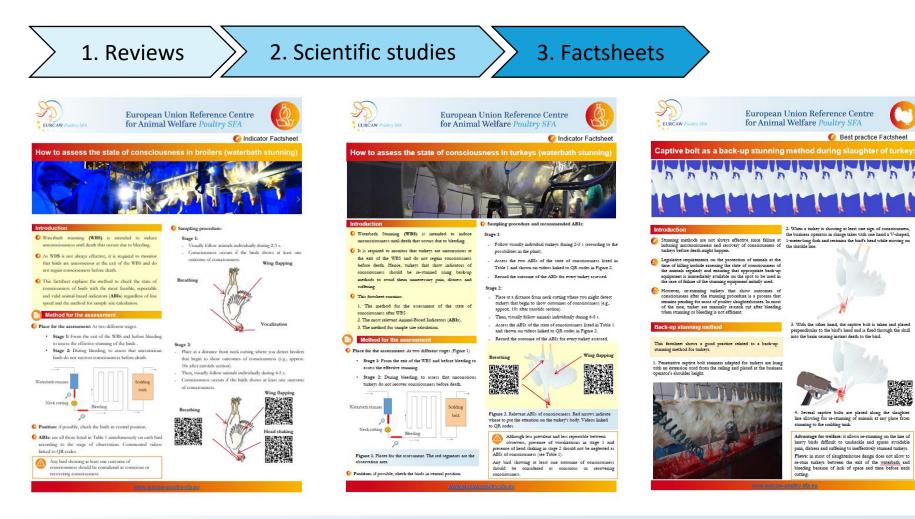


1. Reviews 2. Scientific studies

- Repeatability and feasibility of indicators of consciousness in broiler chicken after waterbath stunning and the impact of electrical key parameters on stunning efficiency.
- Repeatability and feasibility of indicators of consciousness in **turkeys** after waterbath stunning and the impact of electrical key parameters on stunning efficiency.













Webinars:

- Repeatability and feasibility of indicators of consciousness in **broiler chicken** after waterbath stunning and the impact of electrical key parameters on stunning efficiency.
- Repeatability and feasibility of indicators of consciousness in **turkeys** after waterbath stunning and the impact of electrical key parameters on stunning efficiency.

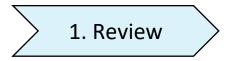
Motion video:

• Method and relevant indicators of the state of consciousness in broiler chickens





2. Controlled atmosphere stunning (CAS)



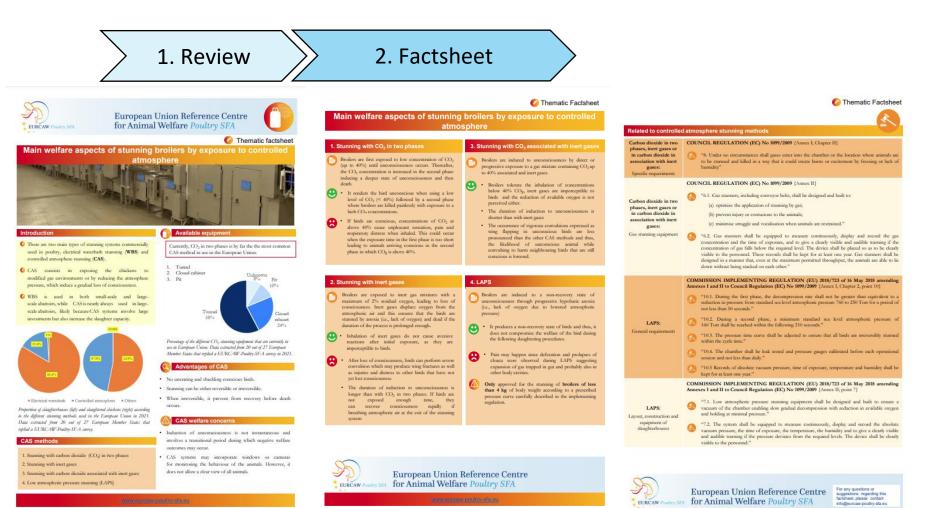
- Main welfare aspects of stunning broilers by exposure to controlled atmosphere:
 - List of CAS methods and legal requirements
 - For each CAS methods:
 - Description and available equipment
 - Positive welfare aspects
 - Regative welfare aspects



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2. Controlled atmosphere stunning (CAS)



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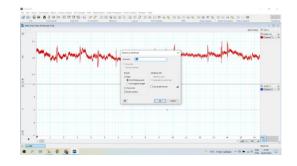
2. Controlled atmosphere stunning (CAS)



• Assessment of welfare during exposure to alternative gas mixtures to carbon dioxide in two phases in broiler chickens

Gas mixtures tested:

- 1. CO₂ in two phases (1st: 40% CO₂ (2 min); 2nd: 90% CO₂ (2 min)
- 2. 40% CO₂ + 60% N₂ (<2% O₂); 4 min
- 3. 20% CO₂ + 80% N₂ (<2% O₂); 4 min







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Activity 5. Dissemination of research findings and innovations



VIDEO



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Indicators of consciousness

Antonio Velarde (Deputy, IRTA)



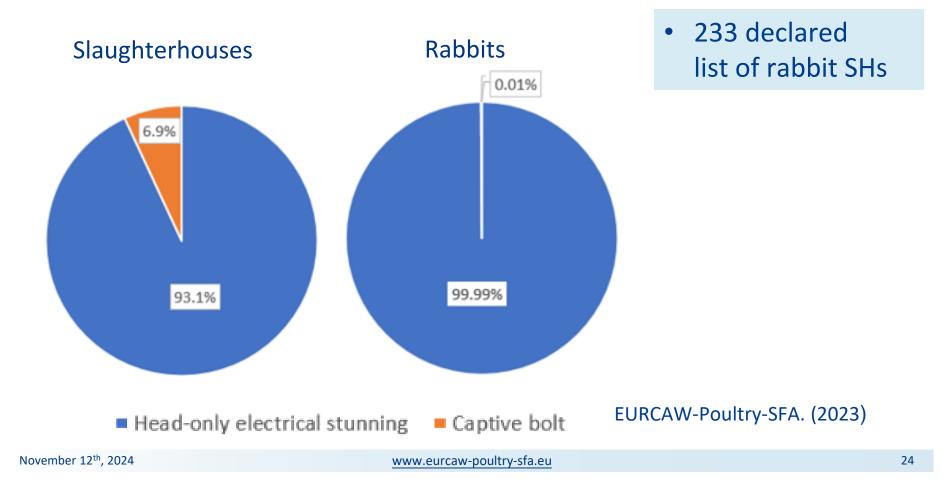




Stunning methods



1. Among the authorised stunning methods for rabbits, <u>only</u> headonly electrical stunning and captive bolt are currently in use in EU.







- 62 million were slaughtered in the EU in 2022 of which Spain, France and Italy accounted for 88% of the total
- **2. Legislation** did not lay down **minimum key parameters** for rabbits (current, frequency, voltage, exposure time, stun-to-stick interval)
- **3. Variability on the recommended key parameters** found in national guidelines:
 - **Current:** >140 or > 400 mA
 - Frequency: 50 Hz
 - Voltage: 100-117 V
 - Minimum time of exposure: 0.5 3 s
 - Maximum stun-to-stick Interval: 5 20 s

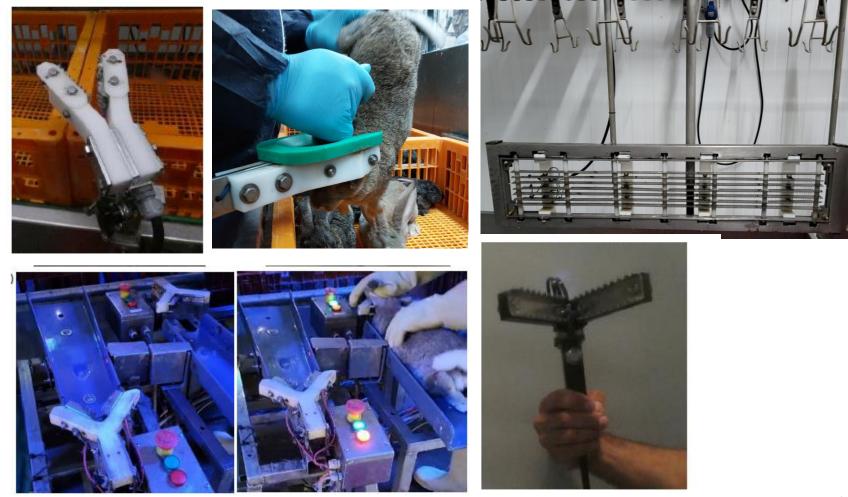
EURCAW-Poultry-SFA. (2023)



Head-only electrical stunning



5. Variability on the head-only stunning devices used



EURCAW-Poultry-SFA. (2023)



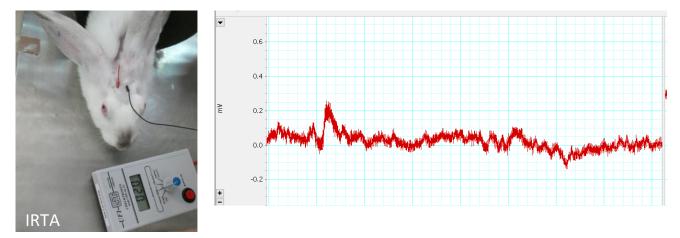
Article 5: requires operators to carry out regular checks to ensure that animals do not present any signs of consciousness or sensibility

Article 16: requires slaughterhouse operators to put in place and implement monitoring procedures.



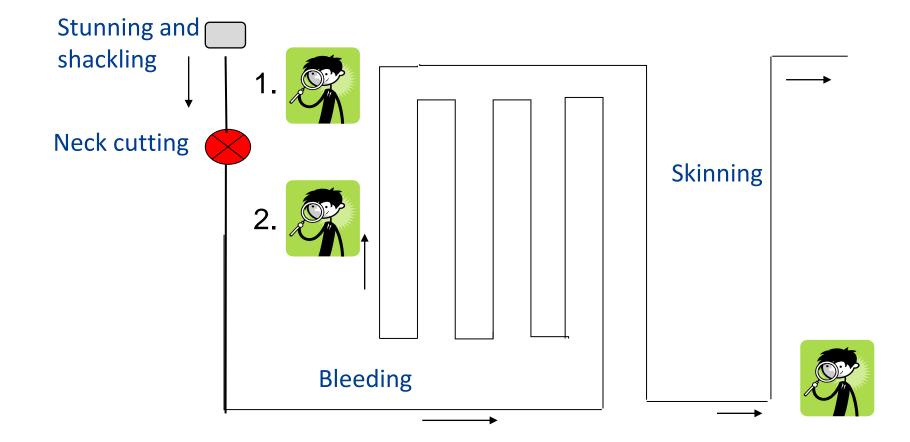


Electroencephalography (EEG)



- ✓ Animal-based indicators (ABIs):
 - **1. Behaviour** (e.g. escape attempts)
 - 2. Physical signs (e.g. onset of seizures, cessation of breathing, fixed eye)
 - **3.** Presence or absence of response to external stimulus (e.g. corneal reflex and response to pain stimulus)





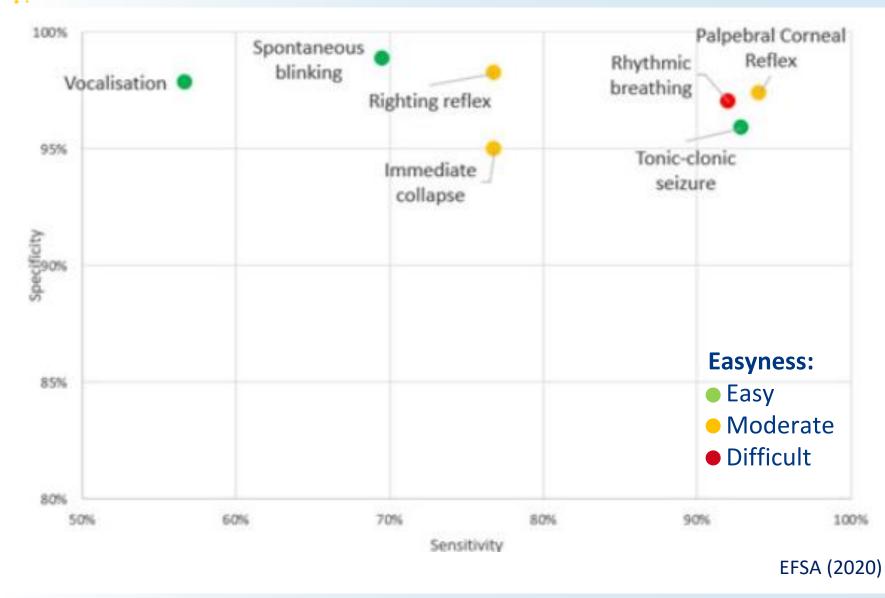


Sensitivity: is the percentage of (truly) conscious animals that are tested as conscious

	Reality						
Indicator	Conscious	Unconscious					
Conscious Corneal Reflex +	✓	Animal is unconscious, but diagnosed as conscious	Logistic problem				
Unconscious Corneal Reflex -	Animal is conscious, but diagnosed as unconscious						
Welfare problem							

EFSA (2013)

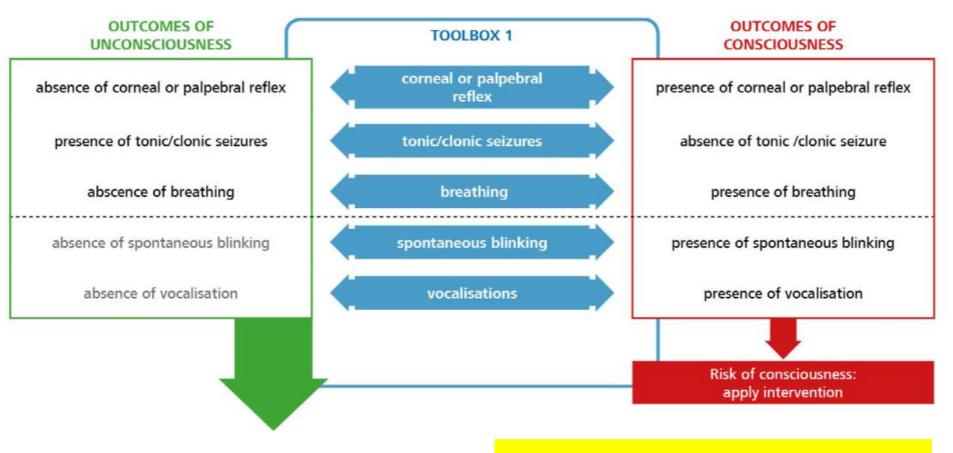
Sensitivity, specificity and easyness



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STAGE: IMMEDIATELY AFTER STUNNING



Check for outcomes of consciousness

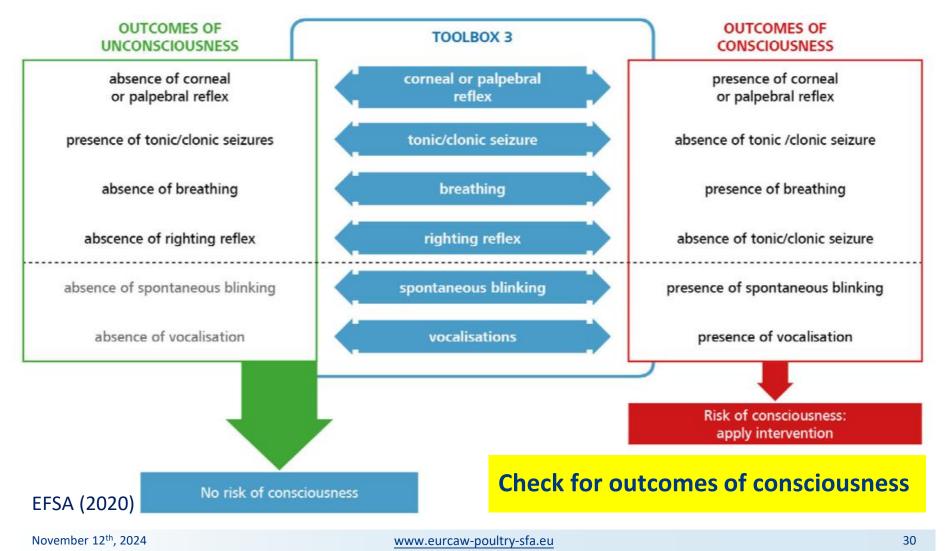
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EFSA (2020)

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STAGE: DURING BLEEDING





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Refinement of indicators and efficiency of stunning

Alexandra Contreras-Jodar

(Researcher, IRTA)







Head-only electrical stunning

- Wide variability on the recommended key parameters found in national guidelines
- 2. Heterogeneity in the indicators chosen by OVs to assess the state of consciousness in rabbits



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Scientific study



The Team:



Rabbit slaughterhouses (SHs):



16 different SHs from the 3 main rabbit producer countries in the EU





General:

Identify a refined list of indicators that can be used to assess the state of consciousness of head-only electrical stunned rabbits in commercial slaughterhouses to ensure consistency of controls and to evaluate the efficiency of induction of unconsciousness.

Specific:

- 1. Assess the **inter-observer repeatability** of the most valid and feasible indicators of consciousness according to the EFSA (2020)
- 2. Elucidate the association among the indicators
- 3. Assess efficiency of stunning
- 4. Find key factors that contribute to effective stunning





Characteristics of the slaughterhouses



SH	Speed,	Wetting	Stunners,	Stun-to-stick	Bleeding	Bleeding	Operators
	rabbits/h	heads	n	interval(s), s	method	cut	bleeding, n
1	800	No	2	10 and NA	Μ	Lateral	1
2	1500	NA	2	11 and NA	Μ	NA	2
3	1600	No	1	22	Μ	Lateral	1
4	2600	No	3	15, 10 and 8	Μ	Lateral	1
5	2100	No	4	36, 30, 24 and 19	Μ	Ventral	2
6	700	No	1	15	Μ	Ventral	1
7	700	Yes	1	2	Μ	Lateral	1
8	600	No	1	3	Μ	Lateral	1
9	1850	Yes	3	18, 12 and 7	Μ	Ventral	1
10	1400	Yes	3	<1	А	Ventral	1
11	700	NA	1	3	Μ	Ventral	1
12	800	Yes	1	16	Μ	Lateral	1
13	1700	No	3	25, 19 and 6	Μ	Ventral	1
14	1920	Yes	3	33, 24 and 17	Μ	NA	1
15	3200	Yes	4	22, 20, 18 and 13	Μ	Lateral	2
16	3600	No	3	20, 13 and 5	Μ	Ventral	2

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



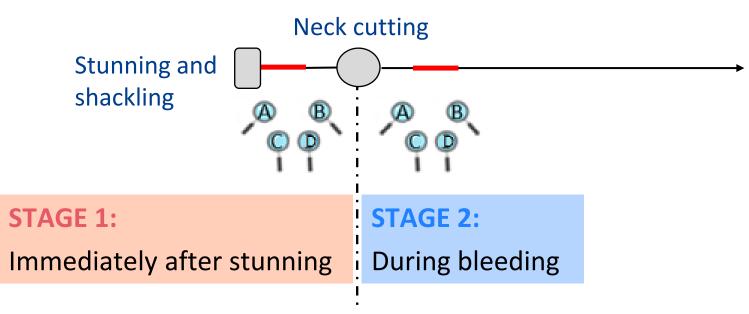


Observers:

16 slaughterhouses, 38 batches, 11,540 rabbits

4 observers 🔎

Sample assessment: Position and stages during the assessment of indicators







STAGE 1: IMMEDIATELY AFTER STUNNING

Absence of TONIC-CLONIC SEIZURE Rabbit do not show 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

Presence of SPONTANEOUS BLINKING Rabbit opens/closes eyelid on its own (fast or slow) without

stimulation.

Presence of 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.

Presence of VOCALISATIONS Single or repeated shrieking (screaming).





STAGE 2: DURING BLEEDING

Absence of TONIC-CLONIC SEIZURE

Rabbit do not show 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

Presence of SPONTANEOUS BLINKING Rabbit opens/closes eyelid on its own (fast or slow) without stimulation.

Presence of RIGHTING REFLEX Attempt to regain posture and/or raise the head.

Presence of 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.

Presence of VOCALISATIONS Single or repeated shrieking (screaming).



- a) Inter-observer repeatability of ABIs
 - 1. Crude proportion of agreement (PoA): % of agreement



Can be misleading as it does not take into account the scores that the observers assign due to chance

2. Fleiss' kappa (κ): degree to which the observed proportion of agreement among observers exceeds what would be expected if all observers made their ratings completely randomly. Ranges from -1 to 1.

According to Fleiss et al. (2003):

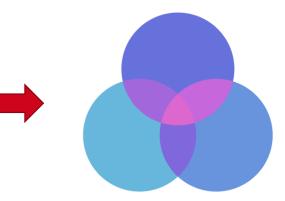
- $\kappa > 0.75$: **Excellent** agreement beyond chance
- 0.40 < κ > 0.75: **Fair to good** agreement beyond chance
- κ < 0.40: Poor agreement beyond chance

 $\kappa \approx 0$ when there is an insufficient scoring variation (i.e., low prevalence of outcomes of consciousness) despite high agreement between observers



b) Association between the observed ABIs

 Proportions among combinations of ABIs: displayed as Venn diagram



c) Relationship between electrical parameters and stunning efficiency

Compare the effectiveness of stunning among the different combination of electrical key parameters through:

• **Prevalence**: % of rabbits showing at least one outcome of consciousness

d) Key factors that contribute to effective stunning

Logistic regression

Stage 1: Immediately after stunning **Stage 2:** During bleeding

ltem	All	
Tonic-clonic seizure		
Agreement, %	98.5	
κ interpretation	Fair to good	
Breathing		
Agreement, %	99.2	
κ interpretation	Fair to good	
Spontaneous blinking		
PoA, %	99.5	
κ interpretation	Fair to good	
Vocalisation		
Agreement, %	100	V
κ interpretation	*	

 κ not able to be computed. No scoring variation.

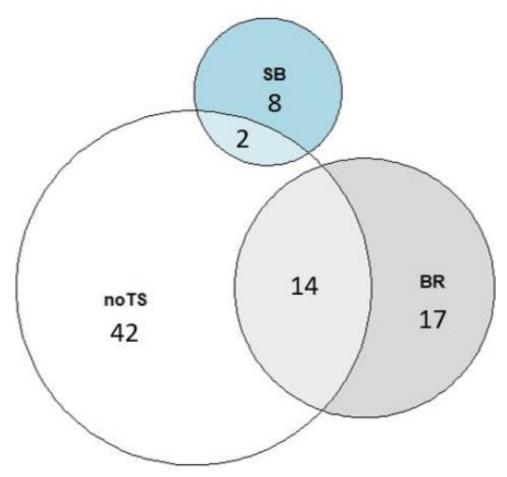
ltem	All	
Tonic-clonic seizure		
Agreement, %	72.9	
κ interpretation	Fair to good	
Breathing		
Agreement, %	92.5	
κ interpretation	Excellent	
Spontaneous blinking		
Agreement, %	92.0	
κ interpretation	Fair to good	
Vocalisation		
Agreement, %	99.6	
κ interpretation	Poor	X
Righting reflex		•
Agreement, %	94.0	~
κ interpretation	Poor	×

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Results: Association between ABIs

Stage 1: Immediately after stunning



Rabbits assessed: 4,112

Indicators were:

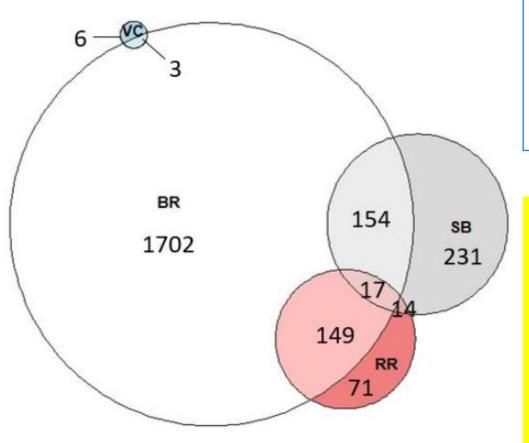
no TS: absence of tonic-clonic seizureBR: presence of breathingSB: presence of spontaneous blinkingVC: presence of vocalizations

No vocalisations



Results: Association between ABIs

Stage 2: During bleeding



Rabbits assessed: 7,428

Indicators were:

BR: presence of breathingSB: presence of spontaneous blinkingVC: presence of vocalizationsRR: presence of righting reflex

- Absence of tonic-clonic seizure at this stage does not imply consciousness
- RR only when the rabbit breath or blink
- RR is often confused with preagonal muscle movements that can occur in brain-dead animals



Results: Stunning efficiency

SH	Wet, Yes/No	Stunners, n	Stun-to-stick interval, s [min, max]	Batch	Current, mA	Frequency, Hz	Voltage, V	Time, ms
1	No 2	2	[NA, 10]	1	291±86	401	395±31	2380±306
				2	281±81	401	397±0	2391±283
2	NA	2	[NA, 11]	1	855±124	50	182±3	978±128
				2	854±125	50	181±3	972±147
				3	836±111	50	181±3	918±117
3	3 No 1	1	22	1	875±284	50	270±7	1102±194
				2	913±294	50	272±7	1153±460
4	4 No 3	3	[8, 15]	1	860±257	50	302±8	750±138
				2	858±287	50	301±8	739±129
				3	939±283	50	299±7	801±120
				4	834±271	50	292±10	721±117
5	5 No 4	4	[19, 36]	1	667	NA	351	1338
				2	577	NA	354	1272
6	6 No 1	1	15	1	437±128	50	180±0	495±258
				2	447±135	50	180±0	503±261
7	NA	2	2	1	610±171	50	145±4	567±121
				2	498±135	50	143±4	485±107
8	No	1	3	1	467±175	50	210±4	509±152



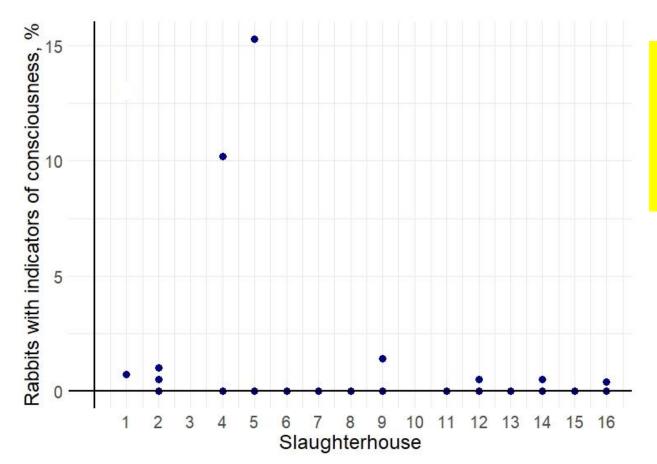
Results: Stunning efficiency

SH	Wet, Yes/No	Stunners, n	Stun-to-stick interval, s [min, max]	Batch	Current, mA	Frequency, Hz	Voltage, V	Time, ms	
9	9 Yes 3	[7, 18]	1	723±254	50	936±316	250±8		
				2	708±258	50	843±270	251±8	
				3	696±244	50	739±239	251±8	
10	Yes	3	0	1	411±90	300	156±69	669±136	
				2	406±96	300	166±87	665±141	
11	NA	1	3	1	138±17	150	95±34	NA	
12	Yes	1	16	1	196±43	150	113±56	399±31	
				2	184±57	150	127±74	386±47	
13	No	3	[6, 25]	1	126±26	250	107±38	700±0	
				2	133±22	250	97±33	700±0	
14	Yes	3	3	[17, 33]	1	170±38	250	162±34	NA
				2	173±39	250	159±35	NA	
				3	177±39	250	155±36	NA	
15	Yes	4	[13, 22]	1	257±65	50	116±5	419±119	
15	Yes	4	[13, 22]	2	262±69	50	117±5	461±131	
16	No	3	[5, 20]	1	609±176	50	207±4	449±99	
16	No	3	[5, 20]	2	566±161	50	209±6	437±94	
16	No	3	[5, 20]	3	529±150	50	209±5	424±95	
16	No	3	[5, 20]	4	557±154	50	209±5	444±92	
16	No	3	[5, 20]	5	508±131	50	211±3	424±88	
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STAGE 1: IMMEDIATELY AFTER STUNNING



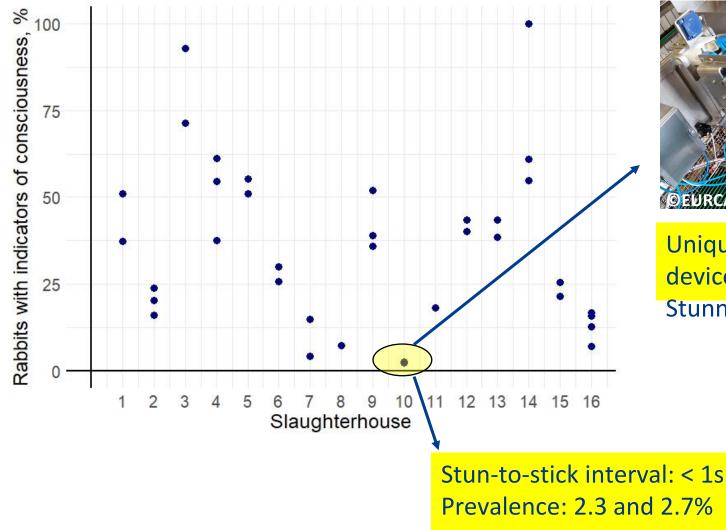
Efficient induction to unconsciousness was observed in some of the batches assessed (15 out of 25 batches)



Results: Stunning efficiency



STAGE 2: DURING BLEEDING





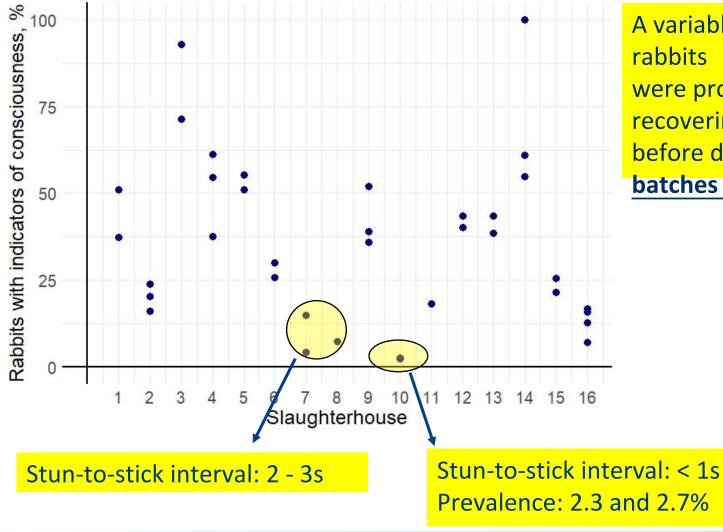
Unique stunning device Stunner-bleeder



Results: Stunning efficiency



STAGE 2: DURING BLEEDING



A variable prevalence of rabbits were progressively recovering consciousness before death <u>in ALL</u> batches from ALL SHs



Results: Key factors that contribute to effective

stunning

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

Predictors	Odds	95 % confidence	P-value	
Fredictors	Ratios	interval	r-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	

Protective factors:

- Stun-to-stick interval below 5 s had the largest effect, reducing the odds by 92% (OR = 0.08)
- The use of high current and low frequency (> 200mA and 50Hz) showed reductions of 58% (OR = 0.42).
- Wetting the rabbit's head reduced the odds by 34% (OR = 0.66).



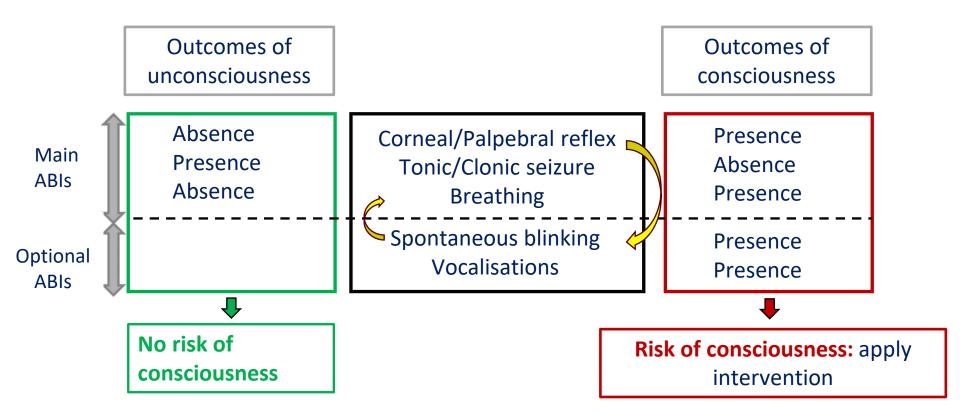
Conclusions

1. 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.

2. This study identified **the most relevant** (i.e., valid, feasible and repeatable) **and prevalent ABIs** that should be used for assessing the state of consciousness in commercial slaughterhouses during official inspections.



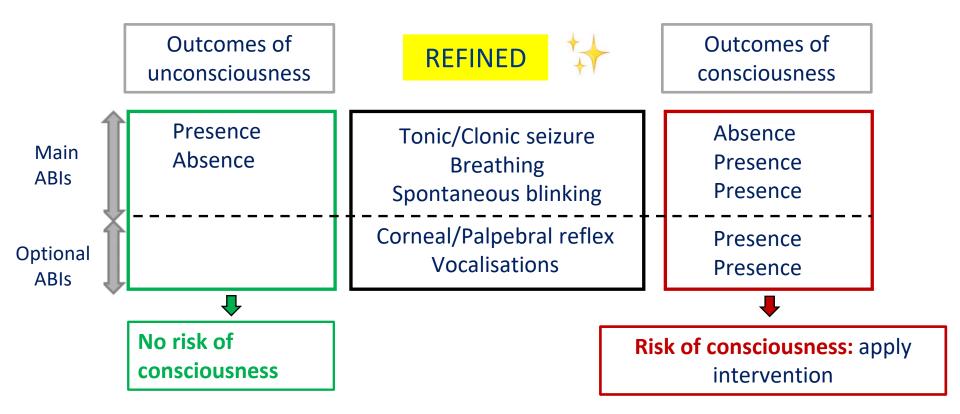
STAGE 1: IMMEDIATELY AFTER STUNNING



Toolbox: EFSA (2020)



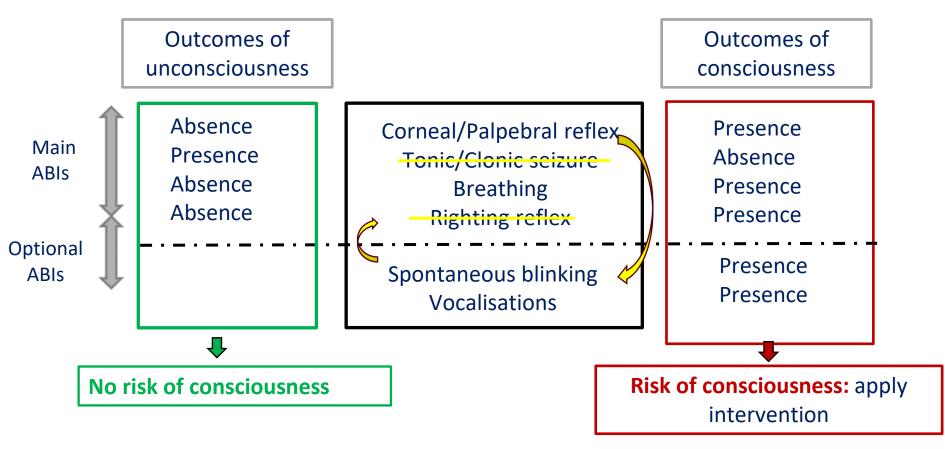
STAGE 1: IMMEDIATELY AFTER STUNNING





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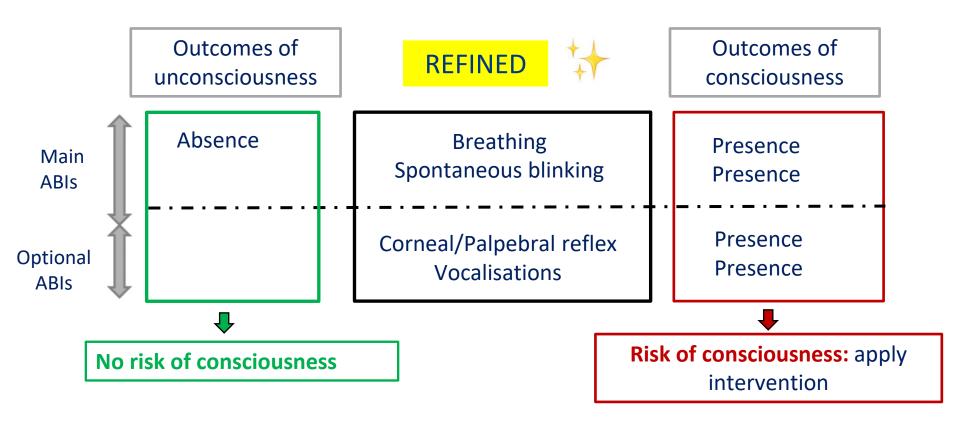
STAGE 2: DURING BLEEDING







STAGE 2: DURING BLEEDING





Conclusions

1. 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.

2. This study identified **the most relevant** (i.e., valid, feasible and repeatable) **and prevalent ABIs** that should be used for assessing the state of consciousness in commercial slaughterhouses during official inspections.

3. 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 all slaughterhouses**.

This highlights the importance of controls!!!



4. **Key factors** ranked in order of their contribution to effective stunning from greatest to least are:

- i. stun-to-stick interval of less than 5 s,
- ii. using current above 200 mA and frequencies not above 50 Hz, and
- iii. wetting the rabbits' heads.

The more these key factors are present in a SH, the higher the likelihood of effective stunning in rabbits.





European Commission

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Methods and recommendations

Alexandra Contreras (IRTA) & Virginie Michel (ANSES)



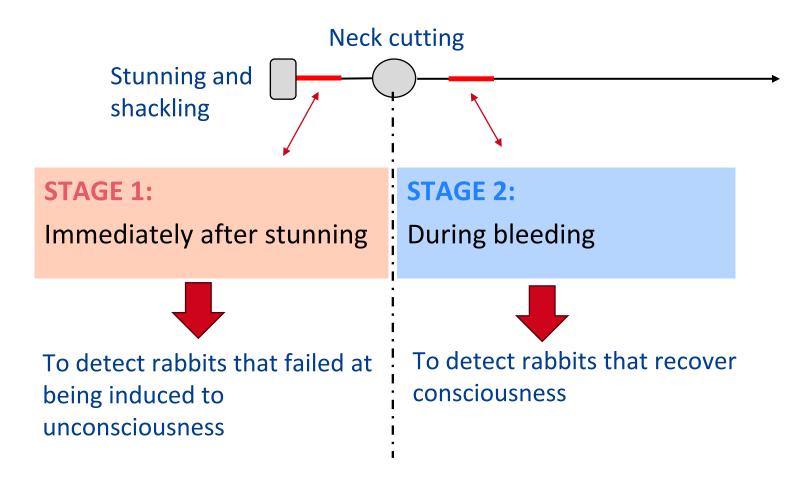


Designated by the EU Commission





1. Position





Method of assessment

Observe each rabbit individually for a few seconds while they are in a ventral-lateral position in both stages, when feasible









STAGE 1: IMMEDIATELY AFTER STUNNING

Absence of TONIC-CLONIC SEIZURE Rabbit do not show arched and stiff neck (*i.e.* necks appear parallel to the ground) and paws and ears held tightly close to the body followed (or not) by kicking action and/or leg paddling that can be either rhythmic or erratic

Presence of SPONTANEOUS BLINKING Rabbit opens/closes eyelid on its

own (fast or slow) without stimulation.

Presence of 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.

Presence of VOCALISATIONS Single or repeated shrieking (screaming).





STAGE 2: DURING BLEEDING

Presence of SPONTANEOUS BLINKING Rabbit opens/closes eyelid on its own (fast or slow) without stimulation. Presence of 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.

Presence of VOCALISATIONS Single or repeated shrieking (screaming).



Indicators of consciousness

STAGE 1: IMMEDIATELY AFTER STUNNING

- Absence of <u>Tonic-clonic seizure</u>
- Presence of Breathing
- Presence of Spontaneous blinking
- Presence of Vocalizations



STAGE 2: DURING BLEEDING

- Presence of Breathing
- Presence of Spontaneous blinking
- Presence of Vocalizations

The presence of at least one indicator indicates the rabbit is conscious or regaining consciousness



Key factors for effective stunning:

- 1. Wetting the rabbits' heads prior stunning
- 2. Stun-to-stick interval of less than 5 s: The shorter the better!!!
- 3. Current >200 mA and frequencies =50 Hz: The higher current the better!!!
- 4. Remove regularly the accumulated fur and debris

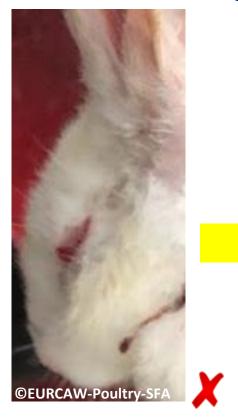




Recommendations

Use eye protectors to prevent burns: Adaptations for breeders:

electric stunning models that Use safeguard the rabbit's eyes from contact ineffective stunning. with the electrical tongs.





Breeders are more to prone

- Key electrical parameters. Ο
- The size of electrical tongs \bigcirc should be adapted.

Improvements on the wetting systems are needed



Recommendations

Stunner - bleeder device:



Pros:

- Wets the rabbits' heads just prior stunning and not during lairage
- Bleed the rabbit once the operator feel the tonic seizure and pushes a button
- Stun-to-stick interval < 1 s

Cons:

• Assessment of the state of consciousness is not possible immediately after stunning and before bleeding

Resulted in the lowest prevalence of rabbits regaining consciousness (2.3 – 2.7%). Better outcomes may be achieved by setting the stunner at 50 Hz.



Sampling method

Regulation 1099/2009



Article 5.1. Those checks shall be carried out on a sufficiently representative sample of animals and their frequency shall be established taking into account the outcome of previous checks and any factors which may affect the efficiency of the stunning process.

• To decide the frequency of the sampling: every batch of rabbits? Some batches?

At least 2 batches a day, at different period of the day (Anses, 2021)

- Random sampling in the batch will ensure representativity
- Sample of the batch according to «factors which may affect the efficiency of the stunning process"
- Sample size: depends on your objective: assess a prevalence of rabbits showing indicators of consciousness? Monitor consciousness through time? both? Consecutively (Anses, 2021)

Excel for sample size: Online calculator tool



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Thank you for your attention



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