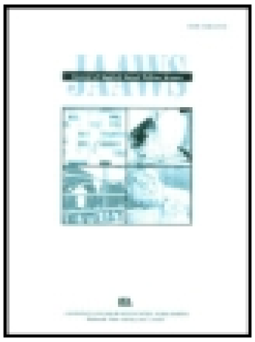

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Investigation Into the Humaneness of Slaughter Methods for Guinea Pigs (*Cavia porcelus*) in the Andean Region

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ABSTRACT
Guinea pigs (*Cavia porcelus*) are an important source of nonhuman animal protein in the Andean region of South America. Specific guidelines regarding the welfare of guinea pigs before and during slaughter have yet to be developed. This study critically assessed the humaneness of 4 different stunning/slaughter methods for guinea pigs: cervical neck dislocation ($n = 60$), electrical head-only stunning ($n = 83$), carbon dioxide (CO_2) stunning ($n = 21$), and penetrating captive bolt ($n = 10$). Following cervical neck dislocation, 97% of guinea pigs had at least 1 behavioral or cranial/spinal response. Six percent of guinea pigs were classified as mis-stunned after electrical stunning, and 1% were classified as mis-stunned after captive bolt. Increased respiratory effort was observed during CO_2 stunning. Apart from this finding, there were no other obvious behavioral responses that could be associated with suffering. Of the methods assessed, captive bolt was deemed the most humane, effective, and practical method of stunning guinea pigs. Cervical neck dislocation should not be recommended as a slaughter method for guinea pigs.

KEYWORDS
Guinea pigs; stunning; slaughter; Andean region; animal welfare

Guinea pigs have been an important source of protein in the Andean region since ancient times (Aliaga-Rodrigues, Moncayo-Galliani, Rico-Numbela, & Caycedo-Vallejo, 2009). Currently, 12 million guinea pigs are reared in Peru for meat consumption (Food and Agriculture Organization of the United States, 2014; Instituto Nacional de Estadistica e Informatica, 2012). Guinea pigs are adapted to high altitudes where other small livestock species, such as chickens and turkeys, have substantial health and production issues associated with the altitude. Recently guinea pig production has spread to other regions of the globe (Herman et al., 2014; Kouakou, Speybroeck, Assidjo, Grongnet, & Thys, 2011; Maass, Katung-Musale, Chiuri, Zozo, & Peters, 2010), where guinea pigs have been promoted as an affordable source of nonhuman animal protein and income for impoverished communities at high altitudes (Lammers, Carlson, Zdorkowski, & Honeyman, 2009).

Traditionally, slaughter by exsanguination is performed without stunning and with or without cervical neck dislocation. Cervical neck dislocation is a recommended and routinely used method for dispatching species in laboratories (American Veterinary Medical Association [AVMA] Members of the Panel on Euthanasia, 2013; *Code of practice, 1997*). However, studies on decapitation, puntilla, captive-bolt shooting into the spinal cord, and cervical neck dislocation in a range of species have

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shown that severance of the spinal cord does not always result in immediate insensibility and death (Erasmus, Lawlis, Duncan, & Widowski, 2010; Erasmus, Turner, Nykamp, & Widowski, 2010; Gregory & Wotton, 1990a; Limon, Guitian, & Gregory, 2012; Tidswell, Blackmore, & Newhook, 1986). In quadrupeds, stretching the spinal cord without causing it to break can cause transient sensory and motor impairment, with motor dysfunction ranging from inability to walk to complete paraplegia (Chang, Hung, Bleyaert, & Janetta, 1981; Gregory, 2004).

Electrical stunning is commonly used for rendering animals insensible prior to slaughter (Gregory, 2007). The passing of a sufficient electrical current across the head ("head-only") produces a reversible stun. The reported duration of insensibility after head-only stunning varies across species, with 26 ± 5 s in rabbits (Anil, Raj, & McKinstry, 1998), 26 s to 108 s in broilers (Gregory & Wotton, 1990b), and 53 s to 59 s in hens (Gregory & Wotton, 1994). Previous studies of guinea pig slaughter have suggested that electrical stunning could be a feasible and humane stunning method (Mariño Frias, 2010). However, stun parameters and the duration of insensibility have not been assessed.

Carbon dioxide (CO_2) stunning is routinely used for dispatching livestock (pigs and poultry) and species in laboratories (rodents and leporids). However, there is significant debate on the effectiveness and humanness of CO_2 stunning of rodents (Conlee, Stephens, Rowan, & King, 2004). The adverse effects of CO_2 and the time to loss of consciousness are reported as being related to the initial concentration (prefilled chamber vs. increasing concentration), concentration rise rate, and the animal species evaluated. Generally, when animals are placed directly into a high concentration of CO_2, consciousness is lost sooner and the duration of breathlessness is shorter than when placing the animal in a chamber with a rising concentration.

However, placing the animals in a prefilled chamber of a high CO_2 concentration has been reported to cause mucosa irritation and discomfort, potentially before the animal becomes unconscious. This is caused by the formation of carbonic acid when CO_2 in the chamber comes into contact with moisture, thereby stimulating nociceptors in the mucosa and producing an uncomfortable burning sensation (AVMA Members of the Panel on Euthanasia, 2013; Coates, 2001; Gregory, Raj, Audsley, & Daly, 1990). Therefore, it is considered that CO_2 is pungent to inhale and can produce a state of breathlessness (Raj & Gregory, 1996).

Captive-bolt stunning is used to render livestock insensible prior to slaughter. A number of low-velocity captive-bolt guns have been developed for use in poultry, neonate piglets, and rabbits. They are designed to fire a steel bolt that either penetrates or impacts the cranium and transfers the kinetic energy of the bolt to the head and brain. This causes concussion and damage to the central nervous system and results in immediate insensibility (Gregory, 2007). The recommended stunning positions vary across species mainly because of the differences in the anatomy of the head and skull.

Although captive-bolt guns have been used in small species such as rabbits (Dennis, Dong, Weisbrod, & Elchlepp, 1988), poultry (Erasmus, Lawlis, Duncan, & Widoski, 2010; Raj & O'Callaghan, 2001), piglets (Casey-Trott, Millman, Turner, Nykamp, & Widowski, 2013; Widowski, Elgie, & Lawlis, 2008), and kangaroo joeys (Sharp, McLeod, Leggett, & Gibson, 2014), the use of captive-bolt guns has not been assessed in guinea pigs. To date, there has been no published scientific study of the humaneness and effectiveness of dispatch methods for guinea pigs for human consumption. Specific guidelines regarding the welfare of guinea pigs before and during slaughter have yet to be developed.

The aims of this study were to (a) evaluate the humaneness of the current methods used for the slaughter of guinea pigs in Peru, and (b) critically assess the humaneness of three different stunning methods (electrical, CO_2, and captive bolt) while considering local needs and constraints.

Materials and methods

The study was conducted at the San Marcos University Experimental Station slaughterhouse (Veterinary Institute of Tropical and Highland Research-San Marcos University [IVITA-UNMSM, translated into English]) in Junin, Peru, from January 9 to 13, 2014.This slaughterhouse operates as a commercial guinea pig abattoir with an average of 8,000 guinea pigs slaughtered per year. The study consisted of two parts: (a) evaluation of current methods (during routine slaughter), and (b) assessment of potential alternative slaughter methods for guinea pigs (head-only electrical, CO_2, and captive-bolt stunning) on animals who were due to be slaughtered on that day (randomly assigned to one treatment). Sex and live weight of each animal were recorded prior to stunning/slaughter. Immediately following exsanguination, the carcasses were weighed prior to further processing. The study received ethical approval from the Universidad Nacional Mayor San Marcos (Peru) and the Royal Veterinary College Ethics and Welfare Committee (United Kingdom).

Current methods

The methods practiced involved cervical neck dislocation either before or after a unilateral cut to the ventral aspect of the neck without stunning. Each animal was individually restrained and slaughtered by the same operator. Method 1 involved a unilateral cut to the ventral aspect of the neck severing the left carotid artery and jugular vein followed by cervical neck dislocation (neck cut-cervical neck dislocation [NC-CND]) by extending the head 90° to the spine and pulling of the hind legs ($n = 30$). Method 2 involved cervical neck dislocation, as described, followed by the neck cut (cervical neck dislocation-neck cut [CND-NC]; $n = 30$).

Immediately following neck dislocation with both methods, the animals were assessed for behavioral and brainstem signs associated with sensibility/insensibility. The following parameters were assessed/recorded: adequate restraint of animals, the time from restraint to neck cut or dislocation (depending on the method used), number of attempts to dislocate the neck, body flaccidity, convulsions, corneal reflex, eyeball rotation, nystagmus, jaw relaxation, vocalization, rhythmic breathing, response to handling, cleat response by pressing between the digits of a hind leg (spinal reflex), and absence of righting reflex.

Immediately after slaughter, the neck cut was examined to assess the damage to the carotid arteries, jugular veins, spinal cord, trachea, and esophagus, as well as the position of neck dislocation.

Electrical stunning

Eighty-three animals were electrically stunned with a head-only 50 Hz alternating current (AC) (sinusoidal waveform) constant voltage electrical stunner with pin electrodes (Whitehead Engineering Ltd., Bath, UK) mounted on a table and activated by a foot switch.

The operator restrained each animal by holding the animal with one hand against their body and supporting the head with the free hand. Stunning electrodes were placed between the eyes and the ears, spanning the brain. During the first 5 s after stunning, the presence of rhythmic breathing, corneal reflex, and cleat response were assessed. Next the animals were immediately slaughtered with a bilateral cut to the ventral surfaces of the neck.

A subset of animals ($n = 10$ males; $n = 10$ females) were electrically stunned as described and were allowed to recover. This allowed for assessment of the duration of induced insensibility. Once the stun was assessed, the following behaviors, signs, and reflexes were recorded until the return of sensibility: onset of rhythmic breathing, corneal reflex, neck tension, and balance. Immediately after the return of three out of the four signs, the animals were electrically stunned and slaughtered by bilateral severance of the carotid arteries and jugular veins.

Peak current, current profile, peak voltage, stun duration, and time to reach 140 mA (minimum recommended stunning current in the European Union for rabbits; Anil et al., 1998) were recorded for all animals using a 199C Fluke Scope meter and 179 Fluke multimeter (Fluke Corporation, Everett, WA). All current and voltage recordings are root mean square values.

CO_2 stunning

Two groups of guinea pigs were stunned with a rising concentration of CO_2 (cylinder concentration of 99%) into a chamber (custom-made; Solutions for Research, Silsoe, UK) at a flow rate of 20% CO_2

($n = 9$) or 30% CO_2 ($n = 12$) of the chamber volume per minute. The total volume of the chamber was 27 L (size 30 cm × 30 cm × 30 cm), with two CO_2 sensors (COZIR Sensor, Gas Sensing Solutions Ltd., Cumbernauld, UK) positioned 50 mm and 20 mm from the bottom and perpendicular from the CO_2 inlet. The inlet tube was positioned 35 mm from the top of the chamber and delivered CO_2 at 5.4 L/min or 8.1 L/min (air flow meter, Platon, Domont, France) to obtain a fill rate of 20% or 30% of the chamber volume per minute, respectively.

A 14-mm diameter outlet for the release of displaced gas was on the opposing side to the inlet of the chamber. The CO_2 concentration was measured every second, and the results were stored on a laptop computer (Dell Inc., Austin, TX) using custom-developed software (Solutions for Research, Silsoe, UK). Data were exported to Excel (Microsoft, Redmond, WA) for data analysis.

The animals were individually placed in the chamber, with the lid firmly secured. The concentration of CO_2 was gradually increased, with a fill rate of either 20% or 30% CO_2 per minute. Prior to CO_2 filling, the animals underwent a 30-s acclimatization period, during which baseline behaviors were recorded. Video was continuously recorded for each animal from the acclimatization period, during CO_2 stunning, until removal from the chamber.

Onset and duration of spontaneous and evoked behaviors were assessed at a later date from the videos using the ethogram presented in Table 1. These data were analyzed by the same trained operator, who was blinded to the treatments and animal type. The ethogram was developed from the assessment of behavior of guinea pigs and other species during CO_2 stunning. Immediately following cardiac arrest or after negative responses to evoked stimuli, all animals were slaughtered by bilateral severance of the

Table 1. Ethogram describing behaviors evaluated during carbon dioxide stunning.

Action	Description
Animal-Mediated Behavior	
Movement and Posture	
Exploratory ambulatory behavior	Moving around the chamber calmly without trying to escape, investigating the new environment
Exploratory head movements	Inquisitive movements of the head including sniffing the surroundings
Escape behavior	Attempts to get out of the box including climbing against the walls and frantic movements
Ataxia	Gait instability and incoordination of muscle movements
Twitching	Muscle fasciculation of the head or body
Convulsions	Uncontrolled shaking of the body and leg kicking
Freezing	Animal suddenly becomes completely still and tense
Recumbency	The animal ceases to be standing and exhibits either lateral, dorsal, or sternal recumbency
Head recumbency	Loss of neck muscle tone; head is lowered and rests on the floor
Move paws against cheeks	Moving the forelimb paws against the side of the face
Face cleaning	Using paws to clean face
Chewing/jaw movements	Movements of the jaw and mouth that resemble a chewing action
Cardiorespiratory System	
Respiratory rate	The number of breaths taken per minute
Increased respiratory effort	Labored breathing, an increase in the depth of breaths taken, seen by increased movements of the thorax and/or abdomen
Nostril flare	Nares changing in diameter with inspiration and expiration
Gasping	A sharp intake of breath with the mouth open followed by exaggerated movements of the thorax and/or abdomen
Cardiac arrest[a]	Absence of a heartbeat, detected by palpation of the chest
Heave/regurgitation movement	Sequential movement of the abdomen, thorax, neck, and head in a retching-like motion; the mouth remains closed; varying severity
Other Body Systems	
Vocalization	Animal exhibits squealing or other noises
Urination	Voiding of urine
Defecation	Voiding of feces
Evoked Behaviors[a]	
Corneal reflex	Involuntary blinking of the eyelids in response to stimulation of the cornea
Righting reflex	An effort is made to regain sternal recumbency after being tipped over by tilting the induction chamber or after the animal is manually displaced; effort includes movement of legs

[a] Evaluated when guinea pig became recumbent.

carotid arteries and jugular veins. Carcasses were examined for bruises, damage to internal organs, and muscle hemorrhages.

Captive bolt

Ten animals were shot with a Dick KTBG spring-powered, captive-bolt gun (Friedr Dick GmbH & Co., Deizisau, Germany). One operator restrained the animal on a metal table, with the head held firmly against the table, while a second operator placed the captive-bolt gun on the head. The shot position was on the midline between the eyes and ears, with the objective that the bolt should penetrate the thalamus/midbrain regions. Immediately after shooting, the animals were assessed for rhythmic breathing, convulsions, corneal reflex, nystagmus, jaw relaxation, body flaccidity, response to handling, cleat response, and righting reflex.

Next the animals were immediately slaughtered by bilateral severance of the carotid arteries and jugular veins. Any animals who showed either rhythmic breathing or a corneal reflex were immediately dispatched by cervical neck dislocation, followed by a ventral neck cut. The heads of three animals were removed for postmortem examination of captive-bolt injury.

Statistical analysis

All statistical analyses were carried out using the statistical package Stata, Version 11 (Stata Corporation, College Station, TX). Descriptive statistics were performed on all variables evaluating brain and spinal function for each of the different methods assessed.

Electrical stunning

Current profiles were categorized in four typologies based on Gregory (2001) and following visual exploration (poor initial contact, climbing satisfactory, interrupted current, and failure to maintain current flow). The extent to which current profiles had effects on (a) peak current and (b) time to reach 140 mA (the minimum current suggested in rabbits) was assessed using a student t test (Anil et al., 1998).

Animals who had a positive cleat response, positive corneal reflex, or rhythmic breathing immediately after stunning were classified as mis-stunned. The extent to which peak current had an effect on the stun (appropriate stun vs. mis-stun) was assessed using a student's t test.

For the subset of animals who were allowed to recover, the extent to which sex was associated with time to recovery was assessed using a t test. Multivariate lineal regression was performed to adjust for the potential effect of current received and animal weight.

CO_2

Descriptive statistics were obtained for each of the spontaneous and evoked behaviors (Table 1) stratified by CO_2 flow-rate groups (20% or 30% per minute). Fisher's exact test and student's t test were used to compare presence/absence and the difference of mean times to onset of each behavior between the two groups. Kaplan Meier survival curves were used to describe the time to cessation of thoracic respiratory movements following initiation of gas filling the chamber. The effect of the CO_2 rising concentration flow rate in the chamber was assessed using a Cox proportional hazard model. Lineal, quadratic, and cubic models were tested for best fit to assess the relationship between respiratory rate and CO_2 concentration. Visual inspection, adjusted R^2 score, and a likelihood ratio test were used to determine which model best fit the data. Multivariate analysis was performed to adjust for the potential effect of CO_2 group and sex.

Table 2. Behavioral and cranial/spinal responses after NC-CND and CND-NC.

Parameter	NC-CND ($n = 30$) number (%)	CND-NC ($n = 30$) number (%)	p value
Absence of body flaccidity	6 (20)	14 (47)	.028
Convulsions	7 (23)	12 (40)	.089
Corneal reflex	12 (40)	9 (30)	.417
Jaw tension	14 (47)	18 (60)	.301
Nystagmus	0 (—)	3 (10)	.076
Rhythmic breathing	0 (—)	0 (—)	—
Response to handling	4 (13)	13 (43)	.010
Cleat response	17 (57)	17 (57)	1
Fail to collapse	0 (—)	0 (—)	—

Note. NC-CND = neck cut followed by cervical neck dislocation; CND-NC = cervical neck dislocation followed by neck cut.

Results

Current methods

Sixty animals were evaluated using the current methods: 30 animals with NC-CND (mean live weight, 1,072 ± 194 g) and 30 with CND-NC (mean live weight, 1,046 ± 184 g; Table 2). Thirteen (43%) and 4 (13%) guinea pigs in the CND-NC and NC-CND groups, respectively, responded to handling ($p = .010$). Body flaccidity was absent in 6 (20%) and 14 (47%) animals in the NC-CND and CND-NC groups, respectively ($p = .028$). Almost half (47%) of the animals who received the NC-CND and more than half (60%) who received CND-NC presented with jaw tension after their respective treatments. Twelve (40%) and 9 (30%) animals in the NC-CND and CND-NC groups, respectively, had positive corneal reflexes, and more than half of the animals had positive cleat responses during both methods. In addition, 27 (90%) animals in the NC-CND group and 29 (97%) animals in the CND-NC group had at least one behavioral or cranial/spinal response.

All animals in both treatment groups had at least the spinal cord partially severed. However, it was not possible to assess in detail the extent of the damage in each animal due to the importance of retaining the connection between the head and the carcass during processing.

Electrical stunning

Eighty-five guinea pigs (40 females and 46 males) were evaluated after head-only electric stunning (mean live weight, 1,098 ± 219 g), with 2 (2%) animals being classified as mis-stunned. Both animals had rhythmic breathing. In addition, 3 out of 80 had a positive cleat response. None of them presented with a corneal reflex. Mean peak current and voltage were 583 ± 116 mA and 117 ± 2 V, respectively. The time to reach the peak current was 1.2 s to 9.9 s ($M = 4.9 \pm 1.4$ s; Table 3).

Four current profiles were identified (Figure 1): climbing satisfactory ($n = 59$; 68%), poor initial contact ($n = 22$; 26%), interrupted current ($n = 7$; 8%), and failure to maintain current flow ($n = 9$; 11%). Climbing satisfactory and poor initial contact were recorded as being mutually exclusive; however, each could occur in the same animal along with interrupted current or failure to maintain contact. Time to reach peak current was significantly quicker when a climbing-satisfactory profile was

Table 3. Electric stunning parameters in all the animals stunned.

Parameter (unit)	n	Mean ± SD	Range
Peak current (mA)	83	583 ± 116	340–860
Time to peak (seconds)	83	5 ± 1	1–10
Stun duration (seconds)	83	7 ± 1	5–11
Time to 140 mA (milliseconds)	78	93 ± 57	16–384
Time to 240 mA (milliseconds)	83	306 ± 285	48–1496
Time to 400 mA (seconds)	81	1.6 ± 2	0.06–8.6

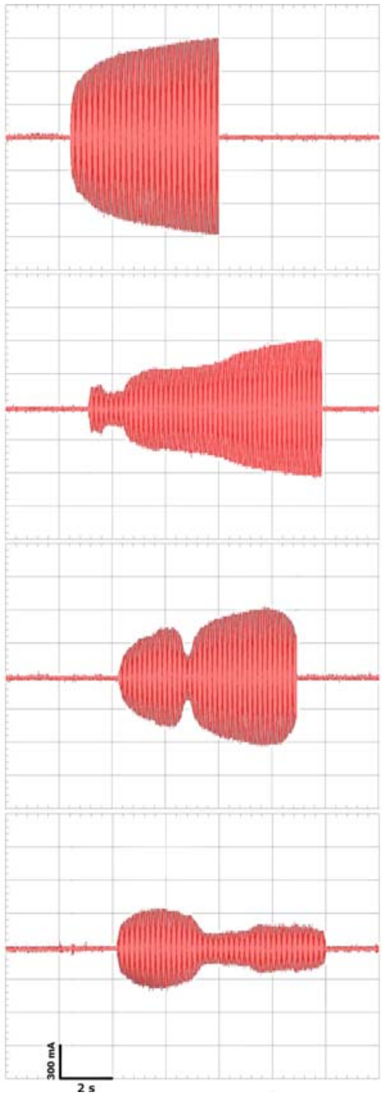

Figure 1. Current profiles identified (top to bottom): (1) climbing satisfactory; (2) poor initial contact; (3) interrupted current; (4) failure to maintain current flow.

achieved ($p < .001$). There was no significant difference in peak current reached between animals who were mis-stunned and those who were properly stunned ($p = .65$).

The four behavioral parameters measured in a subset of animals to assess the duration of insensibility were highly correlated (data not shown). The mean time to onset of rhythmic breathing

Table 4. Time between electric stunning and reappearance of recovery sign.

	Females ($n = 10$)			Males ($n = 10$)			
Parameter	*n*	Mean ± *SE*	Range	*N*	Mean ± *SE*	Range	*p* value
Rhythmic breathing	8	45 ± 3	30–51	10	34 ± 3	20–54	.02
Corneal reflex	8	40 ± 4	26–58	8	35 ± 4	22–49	.40
Neck tension	9	39 ± 3	27–63	9	34 ± 4	25–57	.30
Balance	7	47 ± 5	30–69	4	32 ± 3	26–40	.04
Peak current (mA)	10	540 ± 39	340–700	8	655 ± 31	520–820	.04
Live weight (g)	10	1,278 ± 60	1,046–1,666	10	922 ± 22	850–1,054	< .001

Note. Peak current and live weight by sex (time in seconds).

Table 5. Effect of peak current received and live weight on sex.

Variable	Coefficient	95% CI	p value
Peak current	−0.001	−0.003 to 0.001	.34
Live weight	−0.002	−0.003 to 0.009	< .001
Constant	4.41	2.01 to 6.81	< .001

Note. $R^2 = .63$.

and balance was shorter in males than females ($p = .02$ and $p = .04$, respectively; Table 4). However, there was no significant difference in the mean duration of insensibility between sexes (females, 47 ± 3.5 s; males, 39 ± 2.9 s; $p = .09$). Peak current reached in the subset was 340 mA to 820 mA ($M = 591 \pm 121$ mA), and the mean peak voltage was 119 ± 2 V (range = 115–122 V).

The duration of induced insensibility was 30 s to 69 s ($M = 43 \pm 11$ s). There was a significant difference in the peak current reached between males and females ($p = .04$), with males receiving, on average, higher current levels than females (Table 4). Meanwhile, females were, on average, heavier than males ($p < .001$). After adjusting peak current for the animal live weight, the effect of current becomes nonsignificant (Table 5), suggesting that live weight was the main determining factor for duration of insensibility (Table 5).

CO_2 stunning

Twenty-one animals were evaluated during CO_2 stunning. Nine animals (7 males and 2 females; mean live weight = 993 ± 218 g) were stunned with a CO_2 flow rate of 20% per minute and 12 animals (8 males and 4 females; mean live weight = 1,109 ± 236 g) were stunned with a flow rate of 30% CO_2 per minute. Time to onset of the spontaneous and evoked behaviors is presented in Table 6.

Mean time to onset of increased respiratory effort occurred earlier in the 30% CO_2 flow-rate group compared with the 20% CO_2 flow-rate group ($p = .02$). In both groups, the onset of increased respiratory effort occurred before head recumbency ($M = 62 \pm 11$ s in the 20% flow-rate group; $M = 60 \pm 8$ s in the 30% CO_2 flow-rate group). There was a significant difference in evoked behaviors between the two groups (negative righting reflex, $p = .01$; negative corneal reflex, $p = .004$).

During the adaptation period, most animals displayed exploratory behavior; this behavior was absent during CO_2 stunning. A third of the animals in the 30% CO_2 flow-rate group displayed nostril flaring. Only one animal showed escape-like behavior, and this behavior only occurred once. None of the animals in either treatment group presented with excessive salivation, ataxia, convulsions, or freezing behavior (Table 7). The hazard ratio of ceasing breathing was 1.2 higher in guinea pigs stunned with a flow rate of 20% CO_2 (95% CI [1.01, 1.3]; $p = .03$; Figure 2).

Table 6. Time (in seconds) to onset and conclusion of spontaneous and evoked behaviors observed during carbon dioxide (CO_2) exposure, stratified by flow rate.

	20% CO_2 per minute			30% CO_2 per minute			
Parameter	n	Mean ± SE	Range	N	Mean ± SE	Range	p value
Onset increase respiratory effort	9	19.7 ± 1.7	11–25	12	14.8 ± 1.2	9–21	.02
Onset heaving	9	22.3 ± 1.8	13–28	12	29.9 ± 3.0	8–45	.06
Time to head recumbent	9	81.3 ± 3.5	67–99	12	74.3 ± 2.6	59–87	.11
Finish heaving	9	82.9 ± 3.9	60–102	12	78.1 ± 4.1	63–110	.41
Onset gasping	9	85.3 ± 3.9	60–104	12	75.9 ± 5.9	28–113	.23
Negative righting reflex	8	173.5 ± 28.1	118–356	12	109.9 ± 4.4	91–134	.01
Negative corneal reflex	8	239.4 ± 16.4	187–331	12	185.2 ± 7.8	152–227	.004
Time to cease breathing	8	335.4 ± 18.0	232–405	9	287.8 ± 10.5	240–334	.03
Duration of heaving	9	60.6 ± 3.8	38–75	12	48.2 ± 6.2	25–91	.13

Table 7. Number and percentage of animals presenting spontaneous and evoked behaviors while in the chamber, stratified by CO_2 flow rate.

Animal-mediated behavior	20% (n = 9) Number animals (%)	30% (n = 12) Number animals (%)	p value
Movements and posture			
Exploratory head movements	5 (56)	9 (75)	.31
Twitching	3 (33)	3 (25)	.52
Exploratory (ambulatory) behavior[a]	2 (22)	7 (58)	.1
Move paws against cheeks and/or face washing	2 (22)	2 (17)	.59
Chewing/jaw movement	0	2 (17)	.31
Body tremor	2 (22)	10 (8)	.38
Escape behavior	1 (11)	0	.23
Convulsions	0	0	—
Freezing	0	0	—
Ataxia	0	0	—
Respiratory system			
Nostril flare	1 (11)	4 (33)	.25
Other systems			
Urination	2 (22)	6 (50)	.57
Defecation	2 (22)	10 (8)	.38
Excess salivation	0	0	—

[a] Once carbon dioxide (CO_2) is introduced.

The data were fitted with a cubic model to assess the relationship between respiratory rate and CO_2 concentration (R^2 = .89; Figure 3). The respiratory rate was predicted by the following equation:

$$\text{Respiratory rate} = 130.359 - 8.377 \times [CO_2] + 0.219 \times [CO_2]^2 - 0.002 \times [CO_2]^3 + \text{error}$$

Fill-rate group and sex were not significant (p = .7 and p = .4, respectively) and therefore were not considered in the final model.

Captive bolt

Ten animals were shot with a spring-powered captive-bolt gun. Only one animal presented nystagmus, rhythmic breathing, cleat response, and righting reflex (Table 8). The shot in this animal was misplaced, with the animal being shot at the back of the head with the bolt angling left laterally and only superficially damaging the left occipital lobe. No other animals showed any signs of sensibility or return of sensibility after captive-bolt shooting. The depth of penetration was 10 mm to 13 mm.

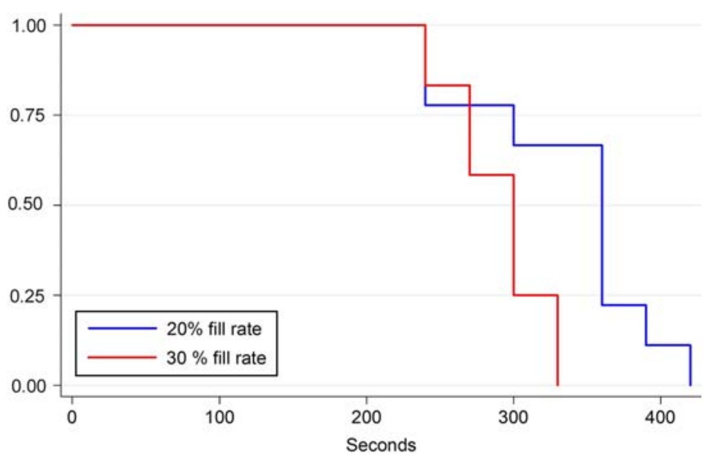

Figure 2. Kaplan-Meier survival curves for time to cessation of breathing according to carbon dioxide (CO_2) concentration used for stunning (20% and 30% per minute). Time at risk was from 30 s to 7 min (420 s). Y-axis indicates cumulative survival rate.

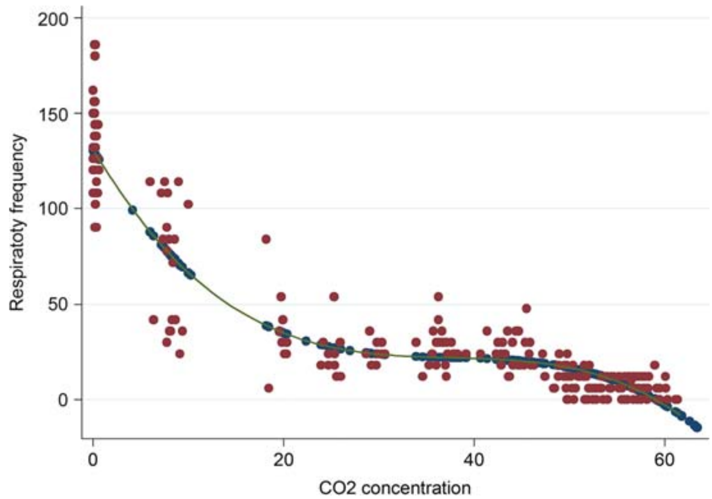

Figure 3. Scatter plot representing carbon dioxide (CO_2) concentration and mean respiratory frequency after CO_2 is turned on and throughout the period in the box. Blue dots represent fitted data and red dots are observed data.

Discussion

Neck dislocation

The Code of Practice for Humane Killing recommends dislocation of the neck as an effective method of dispatching rodents up to 500 g (*Code of practice*, 1997), while the AVMA guidelines consider cervical dislocation as an acceptable method of dispatching animals weighing < 200 g (AVMA Members of the Panel on Euthanasia, 2013). However, for heavier animals, the large muscle mass in the cervical region makes neck dislocation difficult to perform proficiently, thereby increasing the risk for failing to completely sever the spinal cord.

In this study, the minimum live weight was 796 g. Forty percent of the animals in the NC-CND group and 30% in the CND-ND groups had positive corneal reflex; 47% and 60%, respectively, presented jaw tension; and more than half of the animals had a positive cleat response after both treatments. Although all animals in both treatment groups had the spinal cord at least partially severed, the presence of cranial and spinal responses suggests that ascending and descending spinal pathways were at least partially intact and functional in most animals.

These findings confirm that in practice, it is difficult to induce complete insensibility and eliminate brainstem reflexes when using cervical neck dislocation. Therefore, cervical neck dislocation should not

Table 8. Number and percentage of animals presenting behavioral and cranial/spinal responses, using spring-powered captive bolt ($n = 10$).

Parameter	Number animals (%)
Absence of body flaccidity	3 (30)
Convulsions	2 (20)
Corneal reflex	1 (10)
Jaw tension	0
Nystagmus	1 (10)
Rhythmic breathing	1 (10)
Response to handling	0
Cleat response	1 (10)
Righting reflex	1 (10)

be recommended as a dispatch method for guinea pigs for meat production, which raises the question of whether it should be considered an effective and humane method for dispatching guinea pigs in the laboratory.

Electrical stunning

Two of the 85 animals in the study had rhythmic breathing immediately after the stun. In both cases, the peak current was below the overall mean peak current, and in 1 animal, the current profile was categorized as being interrupted and failing to maintain contact. Similarly, in 2 of the 3 animals who had a positive cleat response, the current profile identified was poor initial contact and a peak current below the mean.

The application of an adequate current across the brain is essential for ensuring animals are rendered immediately insensible when using head-only electrical stunning. The reduced area of contact at the surface of the head can result in heating effects enhancing the buildup of carbon on the electrodes and therefore increasing the resistance (Sparrey & Wotton, 1997). In addition, the amount of fur on the guinea pigs' heads might have hindered appropriate contact in some animals. As a result, in some animals, the delivery of electrical current to the head and brain was insufficient to stun.

The minimum recommended stunning current for rabbits is 140 mA (Anil et al., 1998). A previous study in guinea pigs showed that the higher voltages produced a better stun (Mariño Frias, 2010). However, that study did not report the current delivered to the head. Wotton and O'Callaghan (2002) found that the magnitude of the applied voltage was positively correlated with the current delivered, and high initial voltages are important for breaking down the initial high impedance of biological tissues. However, it is the current delivered to the head and not voltage alone that is responsible for inducing grand mal epileptic activity within the brain and the resulting period of insensibility (Gregory, 2007).

Head-only electrical stunning usually produces a stun that is reversible; therefore, the animals need to undergo a subsequent dispatching procedure soon after stunning to ensure that they do not recover. Previous studies with broilers have shown an increase in the duration of insensibility when the current delivered to the head increased (Gregory & Wotton, 1990b; Raj & O'Callaghan, 2004); however, this effect was not observed in hens (Gregory & Wotton, 1994).

In this study, live weight was the main factor associated with the duration of insensibility, with heavier animals having a longer period of insensibility. Potentially, the increased soft tissue over the skull in heavier animals could have resulted in improved electrical contact with the pin electrodes as they depressed or pierced the skin, thereby reducing the impedance of the head to current flow. The period of induced insensibility varies between species. In this study, the first signs of potential recovery appeared at 20 s (one animal showing rhythmic breathing).

The time to hypoxemia-induced brain failure following bleeding was not assessed in this study, so the required duration of unconsciousness cannot be estimated. However, the results do suggest that the period of induced insensibility in guinea pigs is relatively short. Therefore, exsanguination should occur as soon as possible after head-only electrical stunning to minimize the potential for recovery during the bleeding period.

If head-only electrical stunning is to be adopted, the initial cost of the stunner, the cost of electricity, and the fact that blackouts are common in countries where guinea pigs are kept for human consumption should be considered before adopting this method. The blackouts could be addressed by using battery-powered electrical stunning systems.

CO_2 stunning

Initial CO_2 concentration and fill rate of the chamber have been reported as important factors for respiratory distress and time to loss of consciousness (Gerritzen, Lambooij, Hillebrand, Lankhaar, & Pieterse, 2000; Velarde et al., 2007). In this study, increased respiratory effort and heaving were observed before head recumbency in both groups, suggesting that there was a period of respiratory distress before loss of consciousness. CO_2 is a potent activator of hyperventilation and respiratory distress (Raj & Gregory, 1995). The mean time to the end of respiratory distress and cessation of breathing was significantly shorter in the 30% CO_2 flow-rate group ($p = .03$). Lee and Weary (2007) found aversion to CO_2 in rats at a concentration of 17% to 20%; the authors suggested the avoidance was in response to severe dyspnea.

Adverse reactions such as seizures, convulsive chewing, nasal hemorrhage, sero-sanguineous nasal discharge, and excessive salivation have been reported in mice and rats prior to death from exposure to CO_2 (Conlee et al., 2004) and are commonly used as indicators of mucosal irritation and discomfort. In mink, convulsions during CO_2 euthanasia have been reported after they become recumbent and when all movements (including respiration) have ceased (Enggaard-Hansen, Creutzberg, & Simonsen, 1991).

In this study, none of the animals convulsed; however, a third of the animals had nostril flaring in the 30% CO_2 flow-rate group. A number of animals in the study urinated and defecated during CO_2 stunning. Previous studies have shown urination and defecation as signs of distress. However, caution must be taken with this interpretation, as alternatively, the animals could have urinated and defecated due to relaxation of sympathetic and parasympathetic tone (Conlee et al., 2004).

Although there were some signs of respiratory distress during CO_2 stunning, the overall lack of negative behavioral responses and signs of suffering suggests that the distress associated with CO_2 stunning prior to insensibility was less than the distress reported in other rodent species. Based on these results, CO_2 stunning would likely represent an improvement in terms of welfare over the currently used methods of neck dislocation and unilateral neck cut. However, due to the prolonged time to complete cessation of respiratory activity at the tested flow rates and the lack of recycling of the gas, it would be impractical and costly for slaughterhouses to use a similar single-animal system in Peru today. A more practical approach would be batch stunning of animals either with recycling CO_2 or lowering batches into a prefilled chamber.

Spring-powered captive bolt

Of the 10 animals shot with the spring-powered captive bolt, only one failed to be rendered immediately insensible. Postmortem examination revealed that the bolt only superficially damaged the left occipital lobe, suggesting that the cause of incomplete concussion was due to misplacement of the shot. Gibson et al. (2012, 2015) reported that for sheep and alpacas, a shot with a penetrative captive bolt that damages the thalamus and brainstem is essential in ensuring irrecoverable insensibility (Gibson et al., 2012, 2015).

The mean peak velocities of spring-powered, captive-bolt guns are significantly lower than the cartridge-powered guns, and the maximum kinetic energy delivered is only 5.01 J (Sharp et al., 2014). With low-velocity/kinetic energy captive-bolt guns, it is thought that the direct focal damage to specific brain regions rather than concussive trauma produces brain dysfunction and loss of sensibility. Contrary to results found in kangaroo in-pouch young (Sharp et al., 2014), the spring-powered captive bolt was sufficient to cause immediate insensibility in adult guinea pigs.

Stunning using the nonpenetrative captive bolt has been demonstrated to be a humane stunning method for rabbits, turkeys, and piglets (Casey-Trott et al., 2013; Dennis et al., 1988; Erasmus et al., 2010). In the current study, although only 10 animals were assessed, the results suggest that the spring-powered captive bolt is an effective method for rendering guinea pigs insensible prior to slaughter. If this method is adopted, it would be essential that the guns are routinely inspected, tested for performance, and calibrated. Over time, the performance of the springs and rubber absorbers in spring-powered captive-bolt guns can degrade due to normal wear and tear.

Conclusion

This study is the first detailed assessment of the humaneness of five different stunning/slaughter methods for guinea pigs processed for human consumption. Of the methods examined, the spring-powered captive bolt appeared to be the most humane, effective, and practical method of stunning guinea pigs for small-scale subsistence production. Cervical neck dislocation with a neck cut prior to or after dislocation should not be recommended as a slaughter technique. These results have direct relevance to all countries where guinea pigs are used as a source of protein (in South America and elsewhere) and for the dispatching of guinea pigs in the laboratory.

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