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Cattle welfare assessment at the slaughterhouse level: Integrated risk profiles based on the animal's origin, pre-slaughter logistics, and iceberg indicators

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ABSTRACT

Detection of on farm and transport animal welfare problems at slaughterhouse level is a key issue for the meat industry; however, usually, the assessments do not include basic aspects of animal health. For that reason, it is necessary to develop an assessment method that has an integrative scope and identifies the risk profiles in animals. Therefore, the aim of the present study was to detect cattle welfare indicators that can be implemented at the slaughterhouse level and to develop integrated risk profiles based on the animal's origin, pre-slaughter logistics, and animal-based indicators. We recorded the origin, commercial category, transportation details, and horn size of 1040 cattle upon arrival at the slaughterhouse. Cattle welfare was measured based on individual scores for vocalizations, stunning shots, carcass bruises, meat pH, severe hoof injuries, and organ condemnations. To characterize operational and logistic practices from the farm to the slaughterhouse, a two-step cluster analysis was applied to the aforementioned variables (production system, cattle type, horn size, journey distance, vehicle type), which identified four clusters: small feedlot and free-range profile (C1, n = 216, 20.8 %), feedlot profile (C2, n = 193, 18.6 %), culled dairy cows profile (C3, n = 262, 25.2 %), and free-range profile (C4, n = 369, 35.5 %). The animal's diet and environmental conditions might have influenced the development of hoof disorders in C1 animals ($P = 0.023$), the proportion of animals that were re-shot was highest in C2 animals ($P = 0.033$), and C3 and C4 animals were most likely to suffer injuries such as severe bruising ($P = 0.001$). In addition, the number of stunning shots, meat pH, carcass bruises, severe hoof injuries, and liver condemnations, explained a significant variation in the incidence of various health and welfare consequences based on an animal's origin, which confirmed their importance as 'welfare iceberg' indicators. The study provided detailed data that can be included into assessment methods for the welfare of slaughter cattle, which can be tailored to specific production systems.

1. Introduction

Many slaughterhouse companies have an interest in demonstrating to internal (e.g., farmers, hauliers, and operators) and external (e.g., consumers, authorities and NGOs) stakeholders that animal welfare is important to their operations (Estévez-Moreno et al., 2021). Consequently, codes of conduct, assessment protocols, and private standards have proliferated in many meat industries (Waddock and Leigh, 2006). Currently, farm-level animal welfare assessments are the main source of information on the conditions under which animals are reared. However, the results of these assessments can be affected by the operational, physical and environmental conditions of the farm during the assessment. Additionally, such assessments are a potential biosecurity risk due to the free flow of assessors between farms. Therefore, the potential advantages of animal welfare assessments at slaughterhouse level have been recognised (Harley et al., 2012). That said, the full potential of those types of assessments has not been fully examined. There is limited evidence on the accuracy of these methods to identify welfare problems at various stages in production (Carroll et al., 2018). In addition, during pre-slaughter operations animals are exposed to a range

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of novel stimuli (Ferguson and Warner, 2008), making it difficult to identify risk factors that are a source of stress (Bourguet et al., 2010). Those conditions can reduce meat quality by causing, e.g., carcass shrinkage, higher pH, dark cutting beef (DCB), and carcass bruising (Chandra and Das, 2001); therefore, an assessment method that has practical indicators would be useful in providing information on animal welfare (Sala et al., 2019).

The animal-based welfare indicators that are included in an assessment must be valid (measure what is intended), repeatable (produce the same results for repeated observations of the same animal by the same and different observers), reliable (produce consistent results of different animals), and feasible (in terms of speed, cost, and does not compromise operating procedures) (Llonch et al., 2015). The FAWC (Farm Animal Welfare Council) advocated the use of 'iceberg' animal-based indicators to assess overall animal welfare (Heath et al., 2014; Van Staaveren et al., 2017). Iceberg indicators can provide valuable information on two important aspects of the life of production animals: welfare problems that occur during the growth of fattening animals on the farm; and recent acute or traumatic conditions that were associated with pre-slaughter operations such as transport, lairage, and slaughter (FAWC, 2009; Grandin, 2017). If it is confirmed that these indicators have the potential to provide valuable information on the state of animal welfare during the various stages of rearing, their incorporation into assessment protocols could be limited to those specific measurements, which would reduce the time needed for an assessment (Heath et al., 2014).

Animal welfare involves measuring the quality of life of a living animal and is a dynamic, adaptive, and multidimensional phenomenon (Broom, 2014) that includes biological functioning (health and appearance), mental states (ability to experience emotions and feelings), and species-specific behaviours. For that reason, a welfare assessment requires a multi-criteria approach that is based on various measures of physiological, behavioural, production, health, and meat quality indicators (Miranda-de la Lama et al., 2020). Valid indicators have been identified that indicate stress signs and operational failures in slaughterhouse conditions (vocalizations, stunning effectivity), animal health status (hoof disorders, organ condemnations), and product quality (carcass bruising and meat pH) (see Losada-Espinosa et al., 2018). Though the effects of those indicators have been reported, individually, the interactions among the indicators are not well understood, especially among those involving production system management and logistic practices. Cockram (2017) suggested that future research should include an applied animal welfare vision to large-scale epidemiological studies that examine several risk factors. Our study tests the hypothesis that interactions and clustering patterns might exist between the animal's origin and the known and potential 'iceberg' welfare indicators. Specifically, the aims of this study were 1) to identify known and novel welfare indicators that are suitable for assessments at the commercial slaughterhouse level, 2) to quantify the occurrence of vocalizations, stunning, hoof disorders, carcass bruises, partial condemnations, and meat pH at slaughter among animals of different origins (feedlot, free-range, or dairy systems) through post-mortem inspection, and 3) identify integrated risk profiles based on the animal's origin, pre-slaughter logistics, and animal-based indicators.

2. Material and methods

The study was carried out between March and July 2018 at a Federal Inspected Type (FIT or TIF in Spanish) slaughter plant in Malaga, Durango, Mexico (24°09′37.8″N 104°30′19.3″W), which was in compliance with the stipulations of the Official Mexican Norms that establishes the sanitary, safety, and animal welfare requirements for the slaughtering, processing, storage, import and export of all meat and meat products (NOM-008-ZOO-1994; NOM-009-ZOO-1994; NOM-033-ZOO-1995; NOM-194-SSA1–2004). The study area has a semi-arid climate, a mean annual temperature of 19 °C, a mean annual rainfall of 500 mm, and was at approximately 1885 m above sea level. The slaughterhouse was chosen because of the homogeneity in the type of animals slaughtered (at least 85 % of the animals slaughtered were *Bos taurus*), an infrastructure and operational quality that was at international standards, and its strategic geographical location from which it processed animals from grazing (free-range), feedlots, and dairy farms coming from three widespread ecosystems in Mexico (i.e., semi-arid, valleys, and mountain range). Permission to conduct the study was granted by the Institutional Subcommittee for the Care and Use of Experimental Animals in the Faculty of Veterinary Medicine, National Autonomous University of Mexico (Protocol Number DC-2018/2–11).

2.1. Study description

Ante-mortem and post-mortem assessments were implemented as a cross-sectional study to assess the stunning stage (vocalizations and number of stunning shots), hoof health, carcass pH, occurrence of bruises, and organ condemnation in cattle from the feedlot, free-range, and dairy systems that entered the slaughter chain through standard schedules. Data were collected from 1040 commercial cattle that had a median (90 % confidence interval) live weight of 477 kg (467.0–484.0), of which 362 came from feedlots (Hereford, Charolais, Limousine, and Angus commercial crossbreds), 414 came from free-range systems (Wagyu or British and Continental crossbred animals, with up to one half *Bos indicus* influence), and 264 came from intensive dairy systems (Holstein breed). Of the cattle assessed, 52.2 % (543/1040) were males. Among commercial categories, 8.5 % (n = 88) of the livestock were bullocks (castrated or intact males, 1–2 years of age), 29.4 % (n = 306) were young bulls (castrated or intact males, 2–5 years of age), 14.3 % (n = 149) were old bulls (castrated or intact males, >5 years of age), 2.4 % (n = 25) were heifers (females 1–2 years of age), 9.8 % (n = 102) were young cows (females 2–5 years of age), and 35.6 % (n = 370) were old cows (females >5 years of age). Information on journey distance (1−50 km, 51−100 km, 101−150 km, 151−200 km, or > 200 km), type of livestock vehicle [small trailer (3 tons), gooseneck trailer (10 tons), or potbelly trailer (30−50 tons)], animal origin (feedlot, free-range, or dairy system), and cattle type (steer, young bull, old bull, heifer, young cow, old cow) were obtained from the Veterinary Office of SENASICA (Mexican animal health authority) at the slaughterhouse. The study used a procedure that identified the location of each individual animal from the farm to the refrigeration chamber, which allowed us to identify any predisposing factors for injuries and carcass defects (Losada-Espinosa et al., 2021).

2.1.1. Slaughterhouse conditions

The slaughterhouse operated from Monday to Friday (0830−1500 h) and had a slaughter capacity of 9000 heads/month. The concrete unloading ramps (19 °), which had nonslip floors that were as wide as the livestock trailers (6.0 m wide), were connected through a curved metal race (3.0 m wide) to a lairage area that contained 24 pens (6.5 m wide x7 m long), which had nonslip concrete floors and were covered by Polyshade™ (high-density polyethylene screen) or galvanized sheet metal roofing (16 and 8 pens, respectively). In the slaughterhouse, animals from different livestock trucks were not mixed, and each group of animals was housed in a separate pen. During lairage, the animals had access to water *ad-libitum*, and food was not provided. A concrete passageway led from the lairage area to three parallel single-file races that had a single-file race in the last 10 m before the stun box. The floors were slatted concrete and had metal bars between the races. A stockperson drove the animals manually into the stun box using his body, hands, and various tools (mainly an electric goad). The slaughterhouse had a hydraulic, vertically sliding tailgate at the entrance of the box. The stunning box (2 m long x 1.5 wide x1.8 m high) had an automatic head fixation system, and its surface was stainless steel and did not have a non-skid floor. One side of the stun box had a guillotine door that made the animal to fall out of the box after stunning, which was facilitated by a slight slope of the floor. The slaughterhouse used a standard, pneumatically powered, penetrating captive bolt gun (model STUN-BP1, FREUND®) and, occasionally, a handheld powder-loaded device. The stockpersons always worked the animals from outside the race or box. Normally, one person worked each animal in the stun box. After being stunned, the animals were suspended by a hind leg, bled, and transferred to the production line to begin the process of removing the head, feet, skin, viscera, and the splitting of the carcass (Losada-Espinosa et al., 2021).

2.2. Stunning stage

The scoring method for vocalizations was the same as that used by Grandin (2001). Each animal was classified as either a vocalizer (audible sound emitted from mouth and or nasal cavities) or a silent animal. All animals (n = 1040) were scored as they were moved into the stunning box. Vocalizations were scored when the animals were moved through the race into the stun box and during stunning. The acoustical features of vocalizations and the number of vocalizations per animal were not recorded. Animals that vocalized in the forcing pen and in the stunning box were counted once, only. Observations were made from the handler's catwalk near the entrance of the stunning box, where the stunning box and the forcing pen could be observed. Two observers assessed the number of stunning shots. Cattle origin (feedlot, free-range, or dairy breeds), cattle type (bullock, young bull, old bull, heifer, young cow, old cow), and the number of times each animal was shot at the stunning box were recorded.

2.3. Hoof disorders

Assessments were made on one front and one hind limb of each animal (n = 1040). Given the practical problems of sampling all limbs, and the order of amputation for each limb (front left, front right, hind left, hind right), the limbs from the left flank of each animal, which were the first and third limbs to be removed, were assessed. At the time that the animals arrived at the slaughterhouse, the official veterinarian assigned a number to each animal which was written on the animal's back and was used to identify it in the stunning box. Once stunned, the animal was hoisted and bled. Immediately after bleeding started, the operating personnel removed the front left limb and hind left limb at the tarsal-metatarsal joint. One assistant collected the portion of the limbs between the hock and claw. Each limb was evaluated for general and interdigital cleaning and, thereafter, the limb was submerged in water and surface organic matter was removed by a brush. Subsequently, the claw was placed on a flat surface and inspected as follows: 1) verification of conformation (heel height, wall length, interdigital opening, and presence of growth defects as asymmetrical or corkscrew hoof, scissor hoof, overgrown hoof, and chronic laminitis), 2) integrity of the skin on the metatarsals and metacarpals (skin wounds above the coronary band), 3) inspection of the wall, 4) inspection of the sole, and 5) inspection of the heel and for evidence of white line disease (Bautista-Fernández et al., 2021).

2.4. pH measurements

To measure carcass pH 24 h post-mortem (pH24) of the *M. longissimus*, we used a digital pH meter that had a penetration probe (Hanna Instruments, H199163, Woonsocket, Rhode Island, USA), which was inserted into a small incision on the left side of the carcass ($12/13^{th}$ rib interface). After every five samples, two standard buffer solutions at pH 7.0 and 4.0 were used to re-calibrate the pH meter at the temperature of the operation room (4 °C). The pH was the mean of the readings at the two sites. Carcasses that had a pH24 greater or equal to 6.0 were classified as dark cutting (DCB). Meat of normal quality has a pH24 < 6.0.

2.5. Bruising assessment

The protocol for the carcasses post-mortem was based on one modified from Strappini et al. (2012). The 1040 entire carcasses (hanging by both hind legs) were evaluated by one researcher trained for a month prior to the start of the study. The recording of the bruises was carried out in the cooling chamber as the half carcasses arrived to rest for 24 h. For the evaluation of each animal, the identification of the two half carcasses per animal was always considered, according to the slaughterhouse traceability numeral. A bruise was a lesion in which tissues had been crushed leading to a rupture of the vascular supply and an accumulation of blood and serum, without discontinuity of the skin (Capper, 2001). If bruises in the assessed carcass were present, the number of bruises per carcass and the number of bruises per anatomical site were recorded. The location, size, severity, and shape of each bruise was recorded. The carcass was partitioned into the following seven anatomical sections: 1 = neck, 2 = front leg, 3 = thoracic and abdominal wall, 4 = hind leg, 5 = *Tuber isquiadicum* and its muscular insertions (butt/pin), 6 = *Tuber coxae* and its muscular insertions (hip), and 7 = loin. The size of the bruise was assessed based on its diameter as follows: small = 5 cm, medium = 10 cm, large = 15 cm, extra-large = 20 cm. The severity of the bruise was rated as follows: grade 1 = subcutaneous tissue affected, grade 2 = as grade 1, but with muscle tissue affected, grade 3 = as grades 1 and 2, but with the presence of broken bones.

2.6. Condemnations

All animals were subjected to macroscopic meat inspection involving separate inspections of the carcass, the plucks, and the intestines. The official veterinarian designated by the SENASICA performed the post-mortem inspection of the animals, which followed the protocols established in the NOM-009-ZOO-1994 regulation and the International Regional Organization for Agricultural Health meat inspection manual OIRSA (Organismo Internacional Regional de Sanidad Agropecuaria) (2016). The inspectors have received extensive training in meat inspection, disease identification and pathology of farm animals, allowing them to accurately identify pathological lesions with a small margin of error (Ninios et al., 2014). Following applicable regulation (NOM-009-ZOO-1994), evisceration was performed within 30 min from the moment the animal was stunned and bled. Any carcass that had an injury was sent to the retention rail for examination by the official veterinarian, and the viscera and head that corresponded to that carcass were identified for a thorough inspection and could not be washed or cut before the final assessment. Post-mortem examinations involved visual inspection, palpation and systematic incision of each carcass and visceral organs. In Mexico, any carcasses, viscera, and other animal-derived products that are deemed unfit for human consumption and may only be utilized for industrial purposes are considered as a condemnation. Lungs, liver, heart, udder, and intestines were selected for this study because they were among the most commonly reported portion condemnations by inspectors throughout the study period and represent potential animal and public health concerns (Alton et al., 2012). In the slaughterhouse assessed it was not necessary to determine the degree of injury intensity. The occurrence of abnormalities in the lungs (abscess, edema, pneumonia, haemorrhage, adherence, traumatic reticuloperitonitis -TRP-), liver (abscess, Fasciola hepatica, jaundice, telangiectasia, haemorrhage, TRP, calcification, adherence, cirrhosis), heart (edema, adherence, jaundice, TRP, calcification), udder (mastitis, brucellosis, abscess, fibrosis, haemorrhage), and intestines (TRP, gastrointestinal parasites, edema, abscess) were recorded daily on standardized forms. One of the authors of this study was present at each stage of the inspection.

2.7. Statistical analysis

Data were entered into Microsoft Excel (Microsoft Corporation,

2010) and analysed with the IMB© SPSS software 22 version. Prevalence was defined as the proportion of animals that had an occurrence of each animal-based indicator (vocalizations, stunning, pH, bruises, severe front and hind limb injuries, organ condemnation), and was calculated as the indicator frequency divided by the total sample (n = 1040). To identify their distribution and to detect outliers, univariate analyses were performed for each of the variables included in the study. To identify logistic profiles (types or groups) based on production system type, cattle type, horn size, journey distance, and vehicle type, a cluster analysis was performed. Various combinations of the variables were assessed in several cluster analyses, which were accepted or rejected based on the overall silhouette measure of cohesion and separation. The two-step method was used as the conglomeration method. The log-likelihood distance measure was applied for clustering and Schwarz's Bayesian Criterion was used to select the optimal number of clusters (Miranda-de la Lama et al., 2020). A cluster number was assigned to each group and a dummy variable called "cluster membership" was created to identify the logistic profile group. Once the clusters were defined, they were characterized based on their orientation toward performance in the logistic chain. To identify the significant variables that allowed discrimination among clusters, Chi-square test was used. In subsequent analyses, Chi-square and Kruskal-Wallis Tests were used to identify significant differences between logistic profiles on a set of additional cattle welfare (vocalizations, number of stunning shots, carcass bruises, meat pH) and health variables (hoof disorders, organ condemnations) (both qualitative and quantitative) that were not used in the cluster analysis. If those tests indicated a significant relationship, a post-hoc test was performed using the adjusted standardized residuals method and the Mann-Whitney *U* test for qualitative and quantitative variables, respectively (Estevez-Moreno et al., 2019). Differences were considered statistically significant at $P < 0.05$.

3. Results

Table 1 shows the distribution of the animals based on production system of origin, horn size, commercial category, journey distance, and vehicle type. Among the 1040 animals, 36.9 % vocalized, 32.1 % received >1 stunning shot, 23.8 % had a pH ≥ 6, 41 % had severe bruising (grade 2 or 3), and 43.9 % had severe lesions on at least one limb (32.3 % presented severe lesions in the front limb and 23.8 % presented severe lesions in the hind limb). Most (79.8 %) of the animals did not present any condemnation in the organs evaluated. Of the 244 condemned organs (n = 210 animals), 72.5 % were liver, 12.3 % were lungs, 8.2 % were heart, 5.3 % were udders, and 1.6 % were intestines.

Table 1
Distribution of the animals included in the current study (n = 1040).

Variable	Category	Frequency	Percentage
Journey distance	1–50 km	374	36.0
	51–100 km	125	12.0
	101–150 km	335	32.2
	151–200 km	32	3.1
	>200 km	174	16.7
Vehicle type	Small trailer (3 Tons)	470	45.2
	Gooseneck (10 Tons)	255	24.5
	Potbelly (30–50 Tons)	315	30.3
Production system	Feedlot	362	34.8
	Free-range	414	39.8
	Dairy	264	25.4
Cattle type	Steer	88	8.5
	Young bull	306	29.4
	Old bull	149	14.3
	Heifer	25	2.4
	Young cow	74	7.1
	Old cow	398	38.3
Horn size	No horns	333	32.0
	1–8 cm	166	16.0
	9–16 cm	135	13.0
	>16 cm	406	39.0

3.1. Integrated risk profiles: Animal origin and pre-slaughter logistics

The cluster analysis identified four main logistic profile groups (C1, C2, C3, C4; Table 2). All of the variables associated with production system, commercial category, and logistic practices differed significantly ($P < 0.001$) among groups. Significant differences in multiple variables associated with either operational or logistic practices, and cattle welfare and health indicators were used to characterize each group. C1 mostly consisted of animals from feedlot systems and or had been extensively raised (free-range). The presence of bullocks (18.4 %) and young bulls (38.2 %), as well as young heifers (8.0 %) and young cows (14.2 %) that had medium horns stood out. Most of the animals in that group had been transported in gooseneck trailers, that had made either short (1–100 km) or long (151–200km) journeys. In C2, 98.4 % of the animals were either bullocks or young bulls without horns, reared in feedlot systems, that travelled long (>200 km) distances in a trailer ($P < 0.001$). C3 consisted of young and old cows that had small horns and had come from dairy systems. Most of the cows in that group had been transported in gooseneck trailers (46.2 %) or potbelly trailers (39.3 %) in which they travelled intermediate (101–150 km) distances. In C4, 99.7 % of the animals were mature cows and old bulls that had long horns (46.6 %), had been extensively raised (free-range), and travelled short distances (1–100 km) in small trailers.

3.2. Integrated risk profiles: Cattle welfare indicators

Table 3 shows the cattle profiles associated with the animal-based indicators. Vocalizations did not differ significantly ($P > 0.05$) among the clusters. The proportion of animals that received >1 shot in C2 (39.4

Table 2
Integrated risk profiles of cattle slaughtered at Mexico and their association with pre-slaughter logistics (n = 1040).

Variable	C1 (N = 216)	C2 (N = 193)	C3 (N = 262)	C4 (N = 369)	P-value
Production system (%)					
Feedlot	76.9 $^{(+)}$	98.5 $^{(-)}$	0.0 $^{(+)}$	0.0 $^{(-)}$	
Free range	23.1 $^{(+)}$	1.6 $^{(-)}$	0.0 $^{(-)}$	99.7 $^{(+)}$	< 0.001
Dairy	0.0 $^{(-)}$	0.0 $^{(-)}$	100.0 $^{(+)}$	0.3 $^{(-)}$	
Cattle type (%)					
Bullock	18.4 $^{(+)}$	17.1 $^{(+)}$	6.1	0.8 $^{(-)}$	
Young bull	38.2 $^{(+)}$	83.0 $^{(+)}$	6.1 $^{(-)}$	13.0 $^{(-)}$	
Old bull	3.8 $^{(-)}$	0.0 $^{(-)}$	2.3 $^{(-)}$	35.8 $^{(+)}$	< 0.001
Heifer	8.0 $^{(+)}$	0.0 $^{(-)}$	3.1	0.8 $^{(-)}$	
Young cow	14.2 $^{(+)}$	0.0 $^{(-)}$	17.9 $^{(+)}$	7.3 $^{(-)}$	
Old cow	17.5 $^{(-)}$	0.0 $^{(-)}$	65.3 $^{(+)}$	42.3 $^{(+)}$	
Horn size (%)					
No horns	28.7	36.3 $^{(+)}$	30.5	32.2	
1–8cm	15.7	14.0	23.3 $^{(+)}$	11.9 $^{(-)}$	< 0.001
9–16cm	18.1 $^{(+)}$	16.6	11.5	9.2 $^{(-)}$	
>16cm	37.5	33.2	34.7	46.6 $^{(+)}$	
Journey distance (%)					
1–50km	45.8 $^{(+)}$	3.6 $^{(-)}$	5.0 $^{(-)}$	68.8 $^{(+)}$	
51–100 km	28.8 $^{(+)}$	0.0 $^{(-)}$	1.5 $^{(-)}$	15.7 $^{(+)}$	
101–150km	15.1	11.4	92.8 $^{(+)}$	10.6	< 0.001
151–200 km	9.4 $^{(+)}$	0.0 $^{(-)}$	0.4 $^{(-)}$	3.0	
>200 km	0.9 $^{(-)}$	85.0 $^{(+)}$	0.4 $^{(-)}$	1.9 $^{(-)}$	
Vehicle type (%)					
Small trailer (3 t)	27.8 $^{(-)}$	0.0 $^{(-)}$	14.5 $^{(-)}$	100.0 $^{(+)}$	
Gooseneck (10 t)	63.2 $^{(+)}$	0.0	46.2 $^{(+)}$	0.0	< 0.001
Potbelly (30–50 t)	9.0 $^{(-)}$	100.0 $^{(+)}$	39.3 $^{(+)}$	0.0 $^{(-)}$	

NB: *P*-values correspond to Chi-square test, $P < 0.05$ denotes statistically significant differences. (+) or (−) indicate that the observed value is higher or lower than the expected theoretical value according to adjusted standardized residuals.

Table 3
Integrated risk profiles of cattle slaughtered in Mexico based on animal-based welfare indicators (n = 1040), with four clusters.

Variable	C1 (N = 216)	C2 (N = 193)	C3 (N = 262)	C4 (N = 369)	*P*-value
Vocalizations (yes) (%)[b]	38.9	38.9	35.8	38.5	NS
Stunning (No. of shots) (%)[b]					
One shot	64.6	60.6 (−)	71.4	71.1	0.033
>1 shot	35.4	39.4 (+)	28.6	29.0	
pH (%)[b]					
< 6.0	70.8 (−)	80.3(+)	72.1(−)	80.2	0.012
≥ 6.0	29.2 (+)	19.7 (−)	27.9 (+)	19.8	
Carcass bruises					
Prevalence of severe bruises (%)[b]	35.2 (−)	35.2 (−)	47.0 (+)	43.1	0.017
No. of bruises (median, 90 % CI)[a]	0.0 (0.0–0.0) [a]	0.0 (0.0–0.0) [a]	0.0 (0.0–1.0) [b]	0.0 (0.0–0.0) [a]	0.001
Hooves					
Prevalence of severe front or hind limb injuries (%)[b]	52.8 (+)	42.5	43.5	39.8	0.023
Prevalence of severe front limb injuries (%)[b]	41.2 (+)	30.1	32.8	27.9	0.009
Prevalence of severe hind limb injuries (%)[b]	31.9 (+)	22.8	22.8	22.0	0.043

NB: *P*-values correspond to Kruskal-Wallis (a) and Chi-square (b) tests, $P < 0.05$ denotes statistically significant differences. NS: no significant. (+) or (−) indicate that the observed value is higher or lower than the expected theoretical value according to adjusted standardized residuals. [a,b,c] Different letters indicate significant differences (P < 0.05) between clusters according to the Mann-Whitney *U* test

%) differed significantly (P = 0.033) from that of the other groups. Animals in C1 (29.2 %) and in C3 (27.9 %) had a pH ≥ 6 (*P* = 0.012), and animals from dairy systems (C3) had the highest prevalence (47.0 %) of severe bruising (P = 0.017). A total of 43.5 % of C1 animals had severe hoof lesions in either front or hind limbs (*P* = 0.023).

Table 4
Integrated risk profiles of cattle slaughtered in Mexico based on animal-based health indicators (n = 1040), with four clusters.

Variable	C1 (N = 216)	C2 (N = 193)	C3 (N = 262)	C 4 (N = 369)	*P*-value
Liver condemnations (%)					
No condemnation	84.3	88.6 (+)	85.1	77.8 (−)	
Abscess	4.2 (+)	3.1	2.7	1.1 (−)	
Liver fluke (Fasciola hepatica)	9.7 (−)	7.8 (−)	11.1 (−)	18.2 (+)	0.005
Jaundice	0.5	0.5	0.4	1.1 (+)	
Other pathologies	1.4	0.0	0.8	1.9	
Other organs condemnations (%)					
Lungs	2.8	4.2	3.1	2.1	NS
Hearth	0.9	1.6	1.5	3.0	NS
Udder	0.5	0.0	1.9	1.9	NS
Intestines	0.5	0.0	0.4	0.5	NS

NB: *P*-values correspond to Chi-square test, $P < 0.05$ denotes statistically significant differences. NS: no significant. (+) or (−) indicate that the observed value is higher or lower than the expected theoretical value according to adjusted standardized residuals.

3.3. Integrated risk profiles: Cattle health indicators

Table 4 shows the cattle profiles associated with the health indicators assessed in this study. The liver was the only organ for which condemnations differ significantly (P = 0.005) among clusters (17 %). In C2, 88.6 % of animals had no liver condemnations, 4.2 % of C1 animals had liver abscesses, and 18.2 % of C4 animals had *Fasciola hepatica* (*P* = 0.005). Old cows and bulls that had been raised in extensive systems (C4) tended to have ‘other pathologies’ in the liver, including telangiectasia, haemorrhage, reticuloperitonitis, calcifications, adhesions, and cirrhosis.

4. Discussion

This study aimed to identify cattle welfare indicators that could be implemented at the slaughterhouse and to create integrated risk profiles based on the animal origin, pre-slaughter logistics transport, and animal-based indicators. Our study is among the first to integrate this knowledge. Throughout the chain, there was a clear effect of production system, cattle type, vehicle type, and journey distance on those indicators. Given those associations, we identified four main logistic profile types: C1, C2, C3, and C4. We will first present the logistic profiles identified, followed by their interactions with the welfare and health indicators evaluated.

4.1. Integrated risk profiles: Animal origin and pre-slaughter logistics

Feedlot and free-range beef systems are quite common in the arid and semiarid regions of Mexico, where there has been a long tradition of extensive systems, rather than feedlots. Yet, increased domestic demand for grain-fed beef has generated growth in the feedlot sector (Valadez-Noriega et al., 2020). In our study, the C2 profile was typical of a feedlot dedicated to the production of beef from young males or lots to be exported to the United States for finishing. Although C1 was associated with the presence of animals from confined systems, it had characteristics that distinguished it, e.g., the presence of young females (22 %) and some old animals (21 %), which suggests that these might have been feedlots dedicated to finish cull cows or some finished/cull cattle extensively raised. C4 could be considered to have a profile that is representative of the region of our study, and this cluster consisted of animals that came from extensive beef-only or dual-purpose productions, which are very common in the region. Although it might not be obvious why dairy systems are included in a discussion of beef production, in many areas of the world most meat production is a ‘by-product’ of milk production systems (Herring, 2014), which is true in Mexico. For example, the C3 profile comprised animals that came exclusively from dairy systems in the *Comarca Lagunera* region or communities that have had a tradition of cheese production (Mennonites). Cattle horn size was associated with conventional management practices in each type of production system. Slaughterhouses should have specific protocols for handling horned animals because they can cause lesions and contusions in other animals and can pose a risk to handlers and veterinarians during routine management practices (Losada-Espinosa et al., 2020).

In our study, vehicle type and production system of origin were strongly correlated. Potbelly trailer was the main means of transport for C2 animals (large feedlot cattle) and some of the C3 animals (dairy cattle). This type of trailer is divided into two parallel decks, the lower one being straight with a drop just past the rear tires of the truck and before the rear axle, thus dividing the trailer into the rear, belly, nose, deck and doghouse, with internal ramps for easy access (Schuetze et al., 2017). To a certain extent, it was expected that animals that travelled long distances and or that had a high live weight would be transported in that type of vehicle, primarily because of its high load capacity, which reduces the per-head cost of transport. These types of trucks have been criticised because they lack ventilation controls and, therefore, have great potential to affect animal welfare and health, particularly, in extreme weather conditions (Schwartzkopf-Genswein et al., 2012; Theurer et al., 2013). Although it is widely accepted that the physical condition and fitness to be transported is better in feeder cattle compared to cull cows, it is important to consider the animal welfare risk of this type of truck (Edwards-Callaway and Calvo-Lorenzo, 2020). In C3, a portion was characterized by a modern intensive production scheme affiliated with a milk-industrialization structure. Their logistics have been adapted to handle a large number of animals, including the transport of cull dairy cows in potbelly trailers, because these systems have large herds.

In our study, transportation by small (3 t capacity) and gooseneck trailers (10 t capacity) mainly were used for production systems that had small herds. The difference between C4 and C2 in the relationship between vehicle type and journey distance was very marked (C4 – small trailer/short journey distance; C2 – potbelly trailer/long journey distance). Short journeys are under less government control; therefore, the use of secondary vehicles that carry fewer animals is a widespread practice (Pulido et al., 2019). C1 and C4 comprised production systems that were near the slaughterhouse and which specialized in meat production for the domestic market. Common in both clusters was the mixing of animals from different commercial categories and different farms, which produced heterogeneous cattle lots. In some cases, the vehicles to transport those animals seemed somewhat improvised.

In northern Mexico, Mennonite cheese has become part of the region's identity and, typically, this product comes from relatively small production systems that are managed almost exclusively by owners and their families, which were significantly represented in C3. Often, cull cows were shipped from farms in small groups and, quite possibly, experienced delays and were mixed with other classes of animals before the truck was fully loaded. The animals in C3 were not a uniform group and tended to differ in age, parity, and type of clinical findings (see Dahl-Pedersen et al., 2018). In C3, the proportion of animals that travelled in potbelly or gooseneck trailers were similar; therefore, it is important to investigate whether the effects on the welfare and health indicators were because of the animal's origin (e.g., large/small herds, specialized/family labour), vehicle size and design, truck driver efficiency, or slaughterhouse management protocols. Systematic documentation of lorry driver ID, transportation lorry type, and slaughterhouse staff present per shift would facilitate identification of the source of welfare problems that become apparent (Knock and Carroll, 2019).

4.2. Integrated risk profiles: Cattle welfare indicators

Vocalisations can be indicative of the emotional state of the animal (Briefer, 2012). Unlike many physiological measurements, documenting vocalizations does not require physical interaction with the animal and, therefore, they can be a non-invasive measure of stress (Green et al., 2020). Measuring vocalizations at the time of stunning might be useful for detecting deficient personnel training, extremely excited cattle, inefficient gun calibration, lack of maintenance, or the excessive pressure of the head brace (Grandin, 2001). In our study, however, that indicator did not indicate clear differences between the profiles. Even though vocalizations are an effective indicator of poor welfare at the slaughterhouse, in the present study it did not show a relationship to either transport or farm of origin. Also, the information it provided was unclear and did not always provide a direct inference about the cause of a welfare problem. However, in general, the evaluated slaughterhouse had an extremely high percentage of cattle vocalising. According to a review of studies conducted in several abattoirs, the occurrence of more than 5% vocalisers can be indicative of handling and equipment problems (Grandin, 2001). On the other hand, observers should not conclude that a procedure is not painful to an individual animal, simply because it did not vocalize during the procedure (Rushen et al., 2008). In any case, our results show that classifying animals simply as either a vocaliser or a non-vocaliser might not help to develop robust vocal welfare indicators at the slaughterhouse. Because vocalizations are representative of short-lived emotional changes, classifying animals as vocal vs. non-vocal across a long period of time can be challenging.

In our study, the number of stunning shots distinguished the different production systems. It is widely recognised that stunning is a critical point during slaughter operations (Gibson et al., 2019). Audits and standards required by major buyers of meat have greatly improved conditions in the United States (Grandin, 2017); however, in Mexico, there is no consensus on a standard for assessing stunning and the procedure is unregulated (Miranda-de la Lama et al., 2012). For many years, the acceptable proportion of animals stunned effectively in USA slaughterhouses was 95 % (Grandin, 2010), but it was raised in 2017 (96 %) (Edwards-Callaway and Calvo-Lorenzo, 2020). If that efficiency rate is used as a reference point, none of the four clusters met those standards, and the proportion of animals' re-shot was higher in C2. Poor effective stunning rates might have been related to the lack of maintenance of the stunning equipment, deficiencies in the training of stunner operators (Hultgren et al., 2014), floor type within the chute, and head-restraint device features (Muñoz et al., 2012). The effectiveness of stunning is highly dependent on the previous handling of the animals (Romero et al., 2017). Specifically, for C2 cattle, phenotype characteristics (e.g., musculature, shape, size, weight, ticker skull), and animal age might have had an influence (Njisane and Muchenje, 2013). Trends in those data could be used to identify high/low risk categories of animals for double stunning shots, to quantify the influence of the operator, and aid in the development of guidelines for stunner operators, and the permanent maintenance of the stunning equipment.

In commercial terms, it is widely accepted that the ultimate pH is a reference indicator of meat quality (Terlouw, 2015). This is because the pH allows to infer the stress experienced by the animals during pre-slaughter operations, because it includes muscle energy stores and metabolic routes (Losada-Espinosa et al., 2018). In our study, pH_{24} helped to identify differences between clusters; specifically, C1 and C3. Culled dairy cows, certain feedlot cattle, and old animals extensively raised had a greater likelihood of presenting dark cutting. The presence of females (Mahmood et al., 2019), animal breed (ease of handling) (Voisinet et al., 1997), susceptibility to heat stress (Gonzalez-Rivas et al., 2020), mixing with unfamiliar animals in transport to the slaughterhouse (Schwartzkopf-Genswein et al., 2012), slaughter season (extreme temperatures, food availability) (Scanga et al., 1998), and severe bruising (Vimiso and Muchenje, 2013) are some of the factors that might cause animals in C1 and C3 to have $pH_{24} \geq 6$. DCB is a multi-factor phenomenon that is influenced by on-farm, off-farm, and animal issues, and our study identified patterns associated with this condition (Ponnampalam et al., 2017).

Bruises can indicate welfare problems that occur in road transport, traffic accidents, loading/unloading, and stunning of livestock, which can be used to assess cattle welfare at the slaughterhouse level (Miranda-de la Lama, 2013). Traditionally, the study of bruises in cattle carcasses is focused on reporting prevalence by anatomical area, severity, colour and shape. This approach helps to understand the damage to the carcass but does not usually provide more information about profiles of occurrence of bruising (Miranda-de la Lama et al., 2021). In our study, the assessment of severe bruises identified a cluster that was associated with animals that were in severely deteriorated physical condition (C3) and indicated a relationship between bruising and cattle age (C4). The high prevalence of bruising reported in our study underlines the importance of including a bruising score in any assessment of cattle welfare. It is necessary, however, to have an efficient measurement system that is easy to use in commercial slaughterhouses. Bruises are a basic indicator for identifying areas where improvements can be made that effect the welfare of the most susceptible animals. There is evidence that there is a progressive loss of empathy by farmers towards sick or old animals because they involve economic and time losses (Losada-Espinosa et al., 2020). The type of care omission found in our study emphasizes the need to implement awareness and training programs for stockpersons.

The identification and prevalence of lame cattle are among the main factors evaluated in third-party welfare audit programs (Coetzee et al., 2017). Beef cattle can suffer lameness; however, hoof problems and impaired mobility have been relatively little studied compared to dairy cattle (Edwards-Callaway et al., 2017). Our study indicated a high prevalence of severe hoof injuries in the cattle population, particularly, among C1 animals, possibly because of diets high in carbohydrates, which are used to finish the fattening of the animals (feedlots) (LokeshBabu et al., 2018). In addition, a rapid increase in body weight places pressure on the base of the developing claws that, coupled with low physical activity, can affect claw health and might be an overlooked cause of claw pathologies (Pauler et al., 2020). Extensively raised cattle are reared and fattened in challenging climates and locations; therefore, it is difficult to perform routine claw inspections and trimming, which can be a predisposing factor for claw disorders (Álvarez et al., 2017). Severe hoof injuries are an indicator of the welfare state of the animals and the impact that these problems can have on cull rates and cattle longevity (Bruijnis et al., 2012; Alvergnas et al., 2019). The inclusion of severe injuries in a program for monitoring animal welfare at the slaughterhouse level might provide basis for identifying methods that either support or drive different risk management strategies that can be adopted by farmers and the beef industry (Bautista-Fernández et al., 2021).

4.3. Integrated risk profiles: Cattle health indicators

Slaughterhouse data can provide valuable evidence on the incidence and epidemiology of animal diseases, including zoonotic disease outbreaks; however, these data have been under-utilized in animal welfare science. The contribution of culling to disease-related losses is high, and half of the herd removals occur involuntarily and prematurely because of health disorders (Beaudeau et al., 2000). In our study, health problems and animal origin were strongly correlated. If this information were evaluated, improvements could be made to enhance the welfare of the animals to be slaughtered. Acute and chronic acidosis, conditions that follow ingestion of excessive amounts of readily fermented carbohydrates, are common production problems for ruminants fed diets that are high in concentrate (Losada-Espinosa et al., 2018). The term “rumenitis-liver abscess complex” is commonly used because of the strong correlation between the incidence of ruminal pathology and liver abscesses. Ruminal pathology can occur at all ages in all types of livestock, but abscesses that have an economically substantial impact occur in fattening cattle, especially (Tadepalli et al., 2009). In our study, C1 had the highest proportion of animals that had liver abscesses. Probably, animals from feedlot systems increased the prevalence of liver condemnations because of the high incidence of abscesses in this cluster. Studies in North America have shown that the prevalence of liver abscesses has increased in Holstein-type steers (and their crosses), probably, because of an increase in the number of feeding days (Reinhardt and Hubbert, 2015). Although C2, which had a profile that was more typical of feedlot, might have been expected to have more animals that had abscesses, this was not so. Apparently, cattle breed and nutritional management on the farm of origin have significant effects.

C4 animals had a high prevalence of *Fasciola hepatica* and a tendency to present other liver pathologies (telangiectasia, haemorrhage, TRP, calcification, adhesions, cirrhosis). Fascioliasis, a food-borne trematodiasis, has become a major public health concern because of the increasing number of human cases reported worldwide (Barbosa et al., 2019). The disease has caused large economic losses for livestock producers and food industries worldwide that are associated with decreases in meat and milk production, and with livers that are rejected for consumption that, in some slaughterhouses, has reached 50 % (Almeida da Costa et al., 2019; Barbosa et al., 2019). In our study, 18.2 % of C4 animals were infected with that parasite, which might have been influenced by the management practices implemented in the region (herds raised in extensive systems were constantly grazing) and with the longevity of some cattle breeds in these systems (Innocent et al., 2017). In that context, it is important to identify this situation as a simultaneous bias in which it is possible to underestimate the effects of fascioliasis in populations of older animals that have a high prevalence and an ‘ideal weight for slaughter’ (Mazeri et al., 2017; Almeida da Costa et al., 2019).

Contrary to what was expected, and even though it was the cluster that had the most udder condemnations, only 2% of the animals presented some type of damage or pathology in this organ. Bascom and Young (1998) reported that in high producing Holstein dairy herds, farmers were more reluctant to identify mastitis as a reason for culling. If the animals in C3 came from high-production herds they might have been under better management protocols, which resulted in better udder health and reduced culling because of mastitis. In the same study, farmers that had non-Holstein herds identified mastitis and low production as reasons for culling at a significantly higher frequency than did farmers that had the highest producing Holstein herds which suggests, perhaps, that certain dual-purpose cattle or breeds other than Holstein might have contributed to udder condemnations in C3. In our study, the condemnation data were evaluated for the specified organs, only; however, it is important to be aware of other pathologies/conditions that might be more common in dairy cattle. C3 and C4 had the highest number of vulnerable animals. The animals were old, usually with a deteriorated body condition and others health problems. For those animals, calm handling and management in separate groups should be used.

Meat inspection at slaughterhouse level is a highly regulated and demanding activity in terms of time and veterinary staff to perform, and has low detection sensitivity. However, several studies have assessed and recommended the use of risk-based surveillance to improve meat inspection sensitivity (Dupuy et al., 2014). The procedure involves implementing more surveillance resources in those animals that present a high risk of infection or other health conditions. Data identified in our study could be used to identify, upon arrival at the slaughterhouse, the types of animals that are at high or low risk. That evidence should provide a better understanding of the epidemiological and animal welfare conditions and help to identify the factors that influence the level of risk and, therefore, the implementation of risk-based approaches (Laranjo-González et al., 2016). For that reason, it is essential to have consistent surveillance systems, protracted data collection, and the measuring of multiple indicators, concurrently.

5. Conclusions

Our results suggest that the indicators assessed are suitable for assessment at commercial slaughterhouse level. Those animal-based indicators reflected a marked effect of the production system of origin and the pre-slaughter logistics to which animals were exposed. The prevalence of double stunning shots, severe bruising, claw disorders, and liver condemnations in the cattle population was high. Cluster analyses identified four profiles: small feedlot and free-range profile (C1), feedlot profile (C2), cull dairy cows’ profile (C3), and free-range profile (C4). Those profiles were defined by the production system, cattle type, journey distance, and vehicle type. The number of stunning shots, meat pH, carcass bruises, severe hoof injuries, and liver condemnations were indicators that explained a significant proportion of the variation in the prevalence of various welfare and health outcomes based on animal origin, which confirmed their importance and/or potential as iceberg indicators. The associations between risk profiles and livestock health and welfare issues identified in this study provide useful information to improve pre-slaughter operations. Finally, our study shows that it is feasible to use a series of animal welfare indicators with differential sensitivity capable of identifying specific welfare problems in the animals sampled.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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