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Prevalence, risk factors, and main characteristics of bruises in cattle: A meta-analysis in the American continent

Abstract

Bruises threaten the welfare of cattle because they cause pain, suffering, and stress during the pre-slaughter stage. Thus, we used meta-analyses to estimate the prevalence of carcass bruising in cattle from the American continent, determined the characteristics of the bruises, and calculated the odds ratio (OR) of risk factor categories associated with bruising. We searched electronic databases to retrieve primary studies conducted in the American continent, which reported cattle carcass bruising, analyzed the size, location, shape, and age of the bruises, and assessed risk factors for bruising. We included 46 studies from nine countries comprising 928 447 cattle carcasses with an overall prevalence of 59.5% (95% CI: 51.9-66-9) and 2.9 (range 3.4-7.4) bruises per carcass in average. The prevalence doubled from 30.7% during 1991-2000 to 64.2 % during 2011-2020. Fresh, small-sized bruises of the lowest severity and located mainly in the hindquarter were highly prevalent (37.5-66.4%). The removed meat due to bruising ranged 0.11-1.12 kg/ carcass with an accumulated weight of condemned meat between 15.6-647 kg. Among intrinsic factors, older cattle, dairy cattle, and female cattle had greater odds of bruising (OR=1.57-1.98). For extrinsic factors, the odds for bruising increased 1.4-2.2 in cattle sourced from auction markets and exposed to poor handling, suboptimal transportation conditions, and deficient facilities. In cattle from America, bruises are a concern because their prevalence is growing and 60% of cattle assessed in the studies suffered a bruise. Improving facilities and transportation conditions together with proper training of the personnel during the handling and transportation of the cattle will reduce bruising incidence.

Keywords: animal welfare; beef; carcass bruising; injuries; slaughterhouse; transportation and handling.

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Study contribution

Meta-analysis of bruises in cattle from the American continent

Bruises are among the most prevalent physical injuries occurring in cattle. In this study, we found that severity, anatomical site, and size of the bruises were the visual characteristics most frequently reported, whereas age and shape were scarcely assessed. The studies reviewed showed that bruises affected mostly the hindquarter, small-sized bruises scored, and lowest severity were highly prevalent; it revealed that most of the studies evaluated extrinsic causes for carcass bruising. We assessed the risk of bias and found substantial heterogeneity in the way the visual characteristics of the bruises were scored and classified. The results highlight the need for studies with increased methodological soundness to assess the statistical relationship between risk factors and the visual characteristics of bruises in bovine carcasses.

Introduction

Bruises are among the most prevalent physical injuries that occur in cattle during the pre-slaughter stage. A bruise –defined as superficial discolorations of tissue due to hemorrhages caused by rupture of the vascular supply and the accumulation of blood and serum at the site of the contusion–⁽¹⁾ can develop after the application of force. This type of injury leads to swelling and inflammation and causes pain and sensitivity to pressure;⁽²⁾ consequently, bruises are a serious problem due to the inflicted suffering and fear that affects the emotional state of the cattle and impairs their welfare. Once the hide is removed, bruises are easily quantifiable on the carcass; thus, they are used as a signal of impaired animal welfare during the pre-slaughter stage.⁽³⁾ Additionally, given that this stage comprises a series of activities and processes related to the handling of the cattle on the farm, during transportation, marketing, and at the slaughterhouse,⁽⁴⁾ the evaluation of carcass bruising is important because bruises provide a valuable forensic indicator for detecting several basic failures of the pre-slaughter logistic chain where bruising is most likely to occur.⁽⁵⁾

In addition to the welfare problem, carcass bruising is linked to a negative impact on cattle productivity because bruises cause considerable economic losses due to trimming and condemnation of the affected parts and the downgrading of the carcass.⁽⁶⁾ In consequence, the economic losses due to carcass bruises are a substantial problem in the meat chain and have been estimated at several million dollars annually.⁽⁷⁾ Moreover, bruises also increase the risk of meat contamination due to the higher bacterial growth induced by accumulating blood at the injured site.⁽⁸⁾ Thus, bruised meat is unsuitable for consumption.⁽⁹⁾

Worldwide, the American continent provides 34.6% of the total cattle population and some countries are the largest producers and exporters of beef cattle.⁽¹⁰⁾ In 2018, the global bovine meat output was estimated at 71.1 million tons and among the top ten producers of bovine meat were four American countries. The United States of America (USA), Brazil, Argentina, and Mexico contributed with 12.2, 9.9, 3.0, and 2.0 million tons, respectively.⁽¹¹⁾

Despite the important role of countries from America in the world production of beef, carcass bruising is still highly prevalent in several countries of the region and thus compromises cattle welfare.⁽¹²⁾ In consequence, it is fundamental to increase

our understanding of the main risk factors associated with bruising so they can be addressed in American countries and other regions of the world. Bruises originate due to extrinsic or intrinsic factors, though the country differences regarding the animal welfare legislation, the official transportation regulations and other factors such as the routes, the geographic regions, and the haulers' experience and attitude toward animal welfare, may influence the chance for bruising during transportation.^(13, 14) Extrinsic risk factors include all circumstances external to the cattle, such as handling practices in the farm, the origin of the cattle, transportation conditions, facilities at the abattoir, and handling during pre-slaughter processes.^(6, 15) In contrast, intrinsic risk factors include elements inherent to the animal and include breed, sex, age, presence of horns, fat cover, and temperament of the cattle.⁽¹⁶⁾

It is also important to detect crucial steps and suboptimal conditions during which bruises are generated in the pre-slaughter period.⁽¹⁷⁾ Characteristics such as shape, age, and the anatomical site of the bruises might be used to infer when they were sustained and the mechanism that caused them.⁽¹⁸⁾ Finally, quantifying the prevalence of bruises in American countries might be useful both to assess the magnitude of this issue and to measure further progress toward the increased welfare of the cattle. Thus, we conducted a systematic review and meta-analysis to summarize the available evidence from the American continent reporting the prevalence of bruising in cattle, the main characteristics of the bruises, and their associated risk factors.

Materials and methods

Protocol and questions addressed in the study

For this study, we developed an *a priori* protocol following the Preferred Reporting Items for Systematic reviews and Meta-analysis Protocol (PRISMA-P) statement,⁽¹⁹⁾ available online at https://osf.io/pwutm/?view_only=9f5324eed-0334ae0a71ee27c4f449111. We conducted a systematic review and meta-analysis of studies from America that reported carcass bruising data in cattle evaluated at the slaughterhouse. We addressed the following questions: 1) What is the prevalence of carcass bruising? 2) What are the main characteristics of bruises? and 3) What are the extrinsic or intrinsic risk factors associated with carcass bruising? Our study was conducted following the Cochrane guidelines⁽²⁰⁾ and reported according to the PRISMA statement.⁽²¹⁾

Eligibility criteria, information sources, and search strategy

We used the Population, Outcome, Study (POS) approach⁽²¹⁾ to define eligibility criteria for the studies (Table 1). Briefly, we included studies that met the following inclusion criteria: 1) the study reported cattle evaluated for bruising at the slaughterhouse in American countries, 2) the study reported either the prevalence of carcass bruising, the characteristics of the bruises, or assessed any risk factor for carcass bruising, 3) the studies were published in English, Portuguese, or Spanish from January 1980 to May 2019 as full-text primary peer-reviewed publications from the American continent.

Criteria	Definition / scales used in the studies
Population	Carcasses from cattle of any age, sex, and breed that were assessed for the presence of bruises / Chilean bruising grading classification; Australian Carcass Bruise Scoring System; Harvest Audit Program Carcass Bruise Scoring System. Bruise . A traumatic lesion of variable size, shape, and severity caused by any mechanical force during the preslaughter stage and characterized by the presence of ruptured blood vessels, swelling, and accumulation of blood and serum in any anatomical site within the carcass.
¹ Outcomes	1) Prevalence of bruises . The number of bruised carcasses divided by the total number of carcasses included in the study
	2) Characteristics of the bruises Age. The age of the bruise defined according to a subjective colorimetric scale: fresh bruise, red or dark red; old bruise, bluish or dark or yellow; and very old bruise, yellowish / orange or green. Anatomical site. The location of the bruises within the carcass divided into different regions depending on the authors: forequarter or first third or front; ribs and loin or middle third; and hindquarter or last third or hips or round. ² Removed meat. The weight of the trimmed parts due to bruising / amount of bruised meat removed (kg per carcass or total). Severity. The degree of damage on the carcasses caused by the lesions, scored according to the damaged tissue: grade 1 or "S", only subcutaneous tissue; grade 2 or "M", subcutaneous tissue and muscle; and grade 3 or "O", subcutaneous tissue, muscle, and bone. Shape. The pattern or form of a bruise: linear, tramlines, circular, irregular, mottled, or comma. Size. The extent of the carcass that a bruise covers, measured in cm: small or level 1, (< 2–8 cm); medium or level 2 (9–16 cm); and large or level 3 (>16 cm)
	3) Risk factors <i>Extrinsic</i> . all circumstances external to the cattle such as vehicle type, distance traveled, transportation time, lairage time, handling, source of the cattle, loading, and stocking density. <i>Intrinsic</i> . elements inherent to the nature of the animal such as presence of horns, breed, sex, age group, fat cover, temperament/behavior of the animal, and body weight.
Study	Primary studies that were conducted in American countries and published as peer-reviewed full-text in English, Portuguese, or Spanish from 1980 to May 2019. We included cross-sectional, experiment, retrospective, survey, or case studies.

Table 1. Definitions of eligibility criteria for the studies

² Not a bruise characteristic but was considered a consequence of bruising.

Scopus, PubMed, ScienceDirect, Virtual Health Library, CAB abstracts, Web of Science, and Redalyc were consulted to find the most relevant scientific literature. The search was conducted separately to find specific studies for the prevalence, the bruise characteristics, and the risk factors. Two reviewers conducted independently the electronic database searches from April 5 through May 30, 2019. We defined a common search term for the population: (bovine OR cattle OR cow OR bull) AND (bruises OR bruising OR bruised), which was used in conjunction with search terms for the prevalence (prevalence OR incidence OR occurrence OR frequency), the characteristics of the bruises (characteristics OR traits OR size OR severity OR score OR color OR removed meat), or the risk factors (risk factor OR horns OR sex OR fat cover OR handling OR lairage OR transport OR time OR stocking density). Representative full searches per database for one reviewer are presented in the Supplementary Information (Table S1). Once the independent searches were completed, all records were downloaded and gathered into EndNote X9 (Thomson Reuters, USA).

Study selection and data extraction

Meta-analysis of bruises in cattle from the American continent

From the EndNote database, one reviewer first removed the duplicates automatically and later revised them manually. Then, the same reviewer conducted the screening process: first, based on the title, and second, based on the abstract. Two independent reviewers performed the selection for eligibility of the final studies using a standardized questionnaire based on the eligibility criteria described in the protocol.

A single reviewer extracted data from the selected studies using a predefined standardized questionnaire described in the protocol. The extracted data were registered in a spreadsheet, which included a codebook.

Assessment of the risk of bias in individual studies

To evaluate the risk of bias of individual studies, we used a modification of a method previously described.⁽²⁰⁾ The studies were rated as having a low, high, or unclear risk of bias using the following criteria: 1) appropriate definition of the population included in the study, 2) description of a case definition of a bruise, and 3) use of a standardized system for scoring carcass bruising in cattle. The results are summarized as the proportion of studies that showed a low or high risk of bias per criterion.

Summary measures and statistical data analyses

The prevalence was quantitatively summarized with a meta-analysis of proportions using the Freeman-Tukey double arcsine transformation with 95% exact confidence intervals (95% CI). Furthermore, we calculated the mean number of bruises per carcass and performed subgroup meta-analyses, first aggregating the studies from a single country and second, by decades from 1991 to 2020 to assess how the prevalence of bruises has evolved through time in America. To assess the association between risk factors categories (intrinsic or extrinsic) and carcass bruising, we performed meta-analyses to calculate the odds ratio (OR) with 95% CI independently for both factor categories. For these categories, we used all available causes from a single study and performed subgroup meta-analyses (i.e., sex, age group, and fat cover for intrinsic factors). As described elsewhere,⁽²²⁾ in the studies reporting multiple arms for a single risk factor (i.e., several transportation distances), we did not attempt to compare the different groups of a factor category. Instead, we included only two conditions, of which one was considered a "high-risk" factor that was compared to a "low-risk" factor (considered as a control/reference group). By doing this, the unit of analysis error caused by entering repeated data was avoided.

For each factor, we consulted the existing literature and defined as a "high-risk" factor the condition most likely to produce cattle bruising; for instance, in studies reporting cattle handling during transportation or at the abattoir, we defined a regular or poor handling as the "high-risk" conditions, whereas a good or appropriate handling was defined as "low-risk" conditions for bruising. A detailed list of the "low-risk" and "high-risk" conditions for each factor category are provided in the forest plots presented in the supplementary material.

For both outcomes (prevalence and OR), we defined *a priori* a random-effects model (D-L) because of the expected heterogeneity across the studies. As described elsewhere, $(^{23})$ the I^2 statistic was used to determine the proportion of variation in the effects due to variations in true effects rather than sampling error.

Meta-regression for the prevalence of carcass bruising

We performed a random-effects meta-regression analysis to determine whether the study characteristics (latitude, study design, language, gender, the percentile of the sample size, and publication year) partially explained the heterogeneity of the estimated bruise prevalence. After constructing several univariable models with each characteristic, only three significant covariates were retained: the percentile of the sample size (< 25th, 25-50th, 50-75th, and >75th) and the study design (retrospective, case-study, survey, cross-sectional or experimental) which were coded as dummy variables, and the year of publication that was used as a continuous variable.

We did not assess publication bias using funnel plots or Egger regression analysis, which are not considered relevant in prevalence studies. All analyses were performed using Stata 12 (StataCorp, TX, USA), and graphs were constructed using Prism 9 (GraphPad, Inc., CA, USA). A value of P < 0.05 was considered significant.

Results

Characteristics of the selected studies

We found 893 records that matched the search terms, of which only 246 records remained following duplicate removal. The screening process provided a total of 92 records that were available in full text for the eligibility assessment by two independent reviewers. For the final eligibility, we found a moderate agreement between reviewers according to a Kappa value of 0.719 (T = 6.82, P = 0) when we used the Cohen's Kappa statistic to assess the overall agreement rate between reviewers. A third reviewer corrected all the discrepancies between the independent reviewers. Finally, after applying the inclusion criteria to the full texts, 46 studies were excluded. A full list of the excluded studies and the main reasons are summarized in Table S2.

In total, 46 studies were included for the final narrative synthesis, of which 43 reported the prevalence of carcass bruising, 27 reported at least one characteristic of the bruises, and 22 assessed risk factors for carcass bruising (Figure S1). A list of the included studies is provided in Table S3 and a summary of their general characteristics is provided in Table 2.

The 46 studies involved 928447 cattle carcasses and were conducted in Argentina, Brazil, Canada, Chile, Colombia, Mexico, Paraguay, USA, and Uruguay. Brazil, USA, and Chile provided the highest number of studies (15, 10, and 8, respectively). English was the main language for publishing (Figure 1a) and most of the studies (26/46) had a cross-sectional design, whereas experimental, retrospective, and case report studies were the least frequent (Figure 1b). Of these studies, 71.7% (33/46) were published since 2010 (Figure 1c).

For the population included in the studies, the age and weight of the cattle were seldom reported, 60.8% (28/46) of the studies reported either the sex or



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Reference	Country	Sampled population	Study design	Bruised carcasses / total	Prevalence (95% CI)	Type of risk factors	Risk factors identified	Characteristics examined
¹ Bertolini et al. ⁽²⁴⁾	Brazil	Nelore cattle	Cross-sectional	199/255	78% (72.6 to 82.7)	Extrinsic	The type of transport and distance traveled	Age, anatomical sites, severity, and size
Bethancourt-Garcia et al. ⁽²⁵⁾	Brazil	Angus and Hereford culled cows and heifers (40%) and castrated steers (60%), 3-12 y, 451-467 kg of bw	Cross-sectional	39650/154100	25.7% (25.5 to 25.9	Intrinsic	Sex	Severity
² Braga et al. ⁽²⁶⁾	Brazil	NR	Cross-sectional	91/128	71.1% (62.7 to 78.2)	-	-	-
Cardoso et al. ⁽²⁷⁾	Brazil	NR	Cross-sectional	631/697	90.5% (88.1 to 92.5)	Extrinsic	Distance traveled	Anatomical site and severity
Crosi et al. ⁽²⁸⁾	Uruguay	Cattle of mixed age	Cross-sectional	457/1030	44.4 % (41.4 to 47.4)	-	-	Anatomical site, severity, and shape
da Silva et al. ⁽²⁹⁾	Brazil	NR	Experiment	285/320	89.1 % (85.2 to 92)	Extrinsic	Distance traveled	Age, anatomical site, severity, and size
de Andrade et al. ⁽³⁰⁾	Brazil	Female and male Nelore cattle, 9.5-12.5 y	Cross-sectional	83 / 88	94.3 % (87.4 to 97.5)	Extrinsic	Distance traveled and time of transportation	Anatomical site, size, and removed meat
de Andrade et al. ⁽³¹⁾	Brazil	Female and male Nelore cattle, 2.5-6.5 y	Cross-sectional	102 / 121	84.3 % (76.8 to 89.7)	Extrinsic	Distance traveled	Age, removed meat, and size
de Assis et al. ⁽³²⁾	Brazil	NR	Survey	1 280 / 13 000	9.8% (9.3 to 10.4)	-	-	-
³ de Freslon et al. ⁽³³⁾	Chile	Male black and red Friesian cattle, 2 y, 450 kg of bw	Cross-sectional	30 / 41	73.2% (58.1 to 84.3)	-	-	-
Eastwood et al. ⁽³⁴⁾	USA	NR	Survey	9478 / 24366	38.9 % (38.3 to 39.5)	-	-	-
Gallo et al. ⁽³⁵⁾	Chile	NR	Retrospective	8829/114666	7.8 % (7.6 to 7.9)	-	-	-
⁴ Gallo et al. ⁽³⁶⁾	Chile	Female and male Hereford and Angus cattle, 400 kg of bw	Experiment	24 / 40	60% (44.6 to 73.7)	Extrinsic	Resting period during transportation	Severity
Gallo et al. ⁽³⁷⁾	Chile	Male Friesian steers, 2 years, 447–438 kg of bw	Experiment	NR / 139	NE	-	-	Severity

Table 2. Summary of the 46 studies included in the systematic review and meta-analysis

Systematic review & meta-analyses DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2022.1088

Reference	Country	Sampled population	Study design	Bruised carcasses / total	Prevalence (95% CI)	Type of risk factors	Risk factors identified	Characteristics examined
Garcia et al. ⁽³⁸⁾	USA	NR	Survey	3 335 / 9 475	35.2 % (34.2 to 39.5)	-	-	-
Godoy et al. ⁽³⁹⁾	Chile	NR	Cross-sectional	418/4517	9.3% (8.4 to 10.1)	Extrinsic	Distance traveled	Anatomical site, removed meat, and severity
Goldhawk et al. ⁽⁴⁰⁾	Canada	Female cattle, 688 kg of bw	Cross-sectional	529/627	84.4% (81.3 to 87)	-	-	Severity
Harris et al. ⁽⁴¹⁾	USA	NR	Survey	2899/4651	62.3 % (60.9 to 63.7)	Intrinsic	Sex	-
Huertas et al. ⁽⁴²⁾	Uruguay	NR	Survey	9106/15168	60% (59.3 to 60.8)	-	-	Severity
Huertas et al. ⁽⁴³⁾	Uruguay	European breed cattle, 450 kg of bw	Cross-sectional	7360/8132	90.5% (89.9 to 91.1)	-	-	Anatomical site and severity
⁵ Lee et al. ⁽⁴⁴⁾	USA	Male and female Holstein or beef cattle	Cross-sectional	6725/9860	68.2% (67.3 to 69.1)	-	-	Anatomical site and size
Lorenzen et al. ⁽⁴⁵⁾	USA	NR	Survey	14505/37002	39.2 % (38.7 to 39.7)	-	-	-
McKeith et al. ⁽⁴⁶⁾	USA	NR	Survey	4 177 / 18 159	23 % (22.4 to 23.6)	-	-	-
McKenna et al. ⁽⁴⁷⁾	USA	NR	Survey	20360/43595	46.7 % (46.2 to 47.2)	-	-	-
Mendonça et al. (48)	Brazil	Female and Male British breed and zebu cattle, 442-461 kg of bw	Cross-sectional	2405/4438	54.2 % (52.7 to 55.7)	Mixture	Sex, time of transportation, handling, and type of transport	-
Mendonça et al. (49)	Brazil	Zebu or Taurine cattle, cull females, bulls, oxen, and cows	Experiment	NR/2520	NE	-	-	Anatomical site and removed meat
Mendonça et al. (50)	Brazil	Mixed breed oxen (55%) and cull cows (45%), 442-461 kg of bw	Cross-sectional	1977/4611	42.9% (41.4 to 44.3)	Mixture	Sex, distance traveled, time of transportation, and type of transport	Anatomical site
Menezes et al. ⁽⁵¹⁾	Brazil	Steers (37.8 %) and cows (62.2 %)	Cross-sectional	NR/246	NE	-	-	Anatomical site and removed meat

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Reference	Country	Sampled population	Study design	Bruised carcasses / total	Prevalence (95% CI)	Type of risk factors	Risk factors identified	Characteristics examined
Miranda-de la Lama et al. ⁽⁵²⁾	Mexico	Male mixed breed cattle, 1-2 years of age, 450 kg of bw	Cross-sectional	1 143 / 1 236	92.5 % (90.9 to 93.8)	-	-	-
Moreira et al. ⁽⁵³⁾	Brazil	Male Nelore cattle, 2.5-3 y	Cross-sectional	414/624	66.3 % (62.5 to 69.9)	Extrinsic	Distance traveled	-
Petroni et al. ⁽⁵⁴⁾	Brazil	NR	Cross-sectional	880/898	98% (96.9 to 98.7)	Extrinsic	Distance traveled	Anatomical site, removed meat, and severity
⁶ Rebagliati et al. ⁽⁵⁵⁾	Argentina	NR	Survey	3549/9343	38% (37 to 39)	Mixture	Sex, distance traveled, and source of the cattle	Anatomical site, removed meat, and severity
Rezac et al. ⁽⁵⁶⁾	USA	NR	Survey	790/1461	54.1 % (51.5 to 56.6)	-	-	-
Roeber et al. ⁽⁵⁷⁾	USA	NR	Survey	4725/5679	83.2 % (82.2 to 84.2)	-	-	-
Romero et al. ⁽⁵⁸⁾	Colombia	Females and male <i>Bos</i> <i>indicus</i> and <i>B. taurus</i> cattle, 436 kg of bw	Cross-sectional	1929/2288	84.3 % (82.8 to 85.7)	-	-	Anatomical site, severity, and size
Romero et al. ⁽⁵⁹⁾	Colombia	Female and male Zebu breeds cattle, 1-3 y, 455 kg of bw	Cross-sectional	442/1179	37.5% (34.8 to 40.3)	Mixture	Sex and source of the cattle	Anatomical site, size, severity, and shape
Sanchez-Perez et al. ⁽⁶⁰⁾	Mexico	NR	Cross-sectional	335/442	75.8% (71.6 to 79.6)	Mixture	Sex, distance traveled, and age group	Anatomical site, size, severity, and shape
Sornas et al. ⁽⁶¹⁾	Brazil	NR	Retrospective	26 155 / 253 583	10.3 % (10.2 to 10.4)	-	-	-
Strappini et al. ⁽⁶²⁾	Chile	NR	Retrospective	15586/127838	12.2% (12.0 to 12.4)	Mixture	Sex, fat cover, lairage time, and source of the cattle	Severity
Strappini et al. ⁽⁶³⁾	Chile	Female dairy type cattle	Cross-sectional	238/258	92.2 % (88.3 to 94.9)	Extrinsic	Source of the cattle	Age, severity, shape, and size
Strappini et al. ⁽⁶⁴⁾	Chile	Female black and red Friesian cattle	Cross-sectional	37/52	71.2% (57.7 to 81.7)	-	-	Age, anatomical site, severity, shape, and size
Tuninetti et al. ⁽⁶⁵⁾	Argentina	Female and male Brangus and Bradford cattle, 488 kg of bw	Cross-sectional	299/300	99.7 % (98.1 to 99.9)	-	-	-

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Reference	Country	Sampled population	Study design	Bruised carcasses / total	Prevalence (95% CI)	Type of risk factors	Risk factors identified	Characteristics examined
Van Donkersgoed et al. ⁽⁶⁶⁾	Canada	NR	Survey	10952/20281	54 % (53.5 to 54.7)	Intrinsic	Sex	-
Van Donkersgoed et al. ⁽⁶⁷⁾	Canada	NR	Survey	20322/26054	78% (77.5 to 78.5)	-	-	-
Vega-Britez et al. ⁽⁶⁸⁾	Paraguay	NR	Cross-sectional	127/652	19.5% (16.6 to 22.7)	Extrinsic	Distance traveled	-
⁷ Youngers et al. ⁽⁶⁹⁾	USA	Male and female Holstein and beef breed cattle	Case study	2370/4287	55.3 % (52.8 to 56.8)	-	-	Anatomical site and severity

NR=non-reported in the study, NE=non-estimated, y=years of age, bw=body weight

¹ Results from experiment II are reported in this study.

² Results from the fifth evaluation are reported in this study.
 ³ The study reports only recent bruises with a bright red or dark red color.

⁴ This study compares four schemes of the time traveled in two seasons of the year (cold, autumn-winter; hot, spring-summer).

⁵ The study reports the average prevalence as a percentage for 75 lots (131 heads per lot) assessed in the study.

⁶ The data correspond to abattoir 1.

⁷ The data correspond to 27 lots with an average number of 159 animals per lot.

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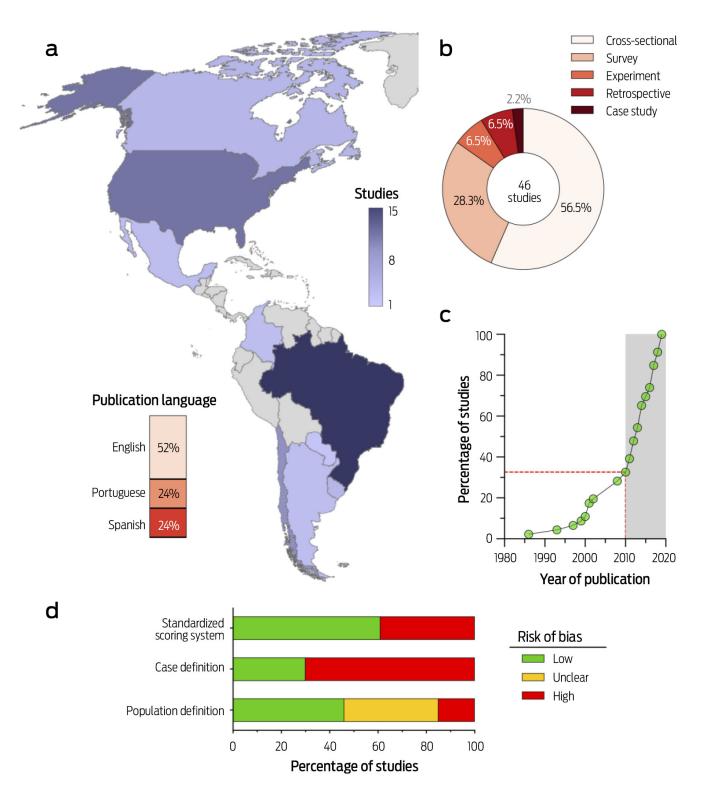


Figure 1. a) Distribution of studies per country, **b)** The percentage of publications according to the design of the study, **c)** Accumulation of studies per year, **d)** Summary of the risk of bias assessment for the 46 studies reviewed. The insert in A shows the percentage of studies according to the language of publication.

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the commercial categories, and the specific breed evaluated was reported only in 20/46 studies. Given that the characteristics of the population were unevenly reported across studies, the prevalence of bruising was not estimated according to the different types of cattle.

Risk of bias assessment

Among the 46 included studies, 30.4 to 60.9% were judged as having a low risk of bias for the three criteria defined in our study. In total, 69.6% (32/46) and 39.1% (18/46) of the studies did not report a case definition of a bruise or use a standardized system for scoring bruises. Consequently, these two judgments were the main issues that caused a high risk of bias (Figure Id). Of the studies, 39.1% (18/46) had an unclear risk of bias for the inclusion of a population definition. In Figure S2, we summarize the risk of bias assessment per study.

Prevalence of carcass bruising in cattle from America

The estimated pooled prevalence of carcass bruising was 59.5% (95% CI: 51.9 to 66.9) with a significant proportion of variation attributable to heterogeneity across the 43 studies (($I^2 = 99.9\%$, P = 0; Figure 2a). AThis heterogeneity was also observable at the national level (Figure S3), although the overall estimated prevalence per country was either moderate (19.5–40.5% in Paraguay, Chile, and Argentina) or high (> 50-89%, in the other countries) (Table 3).

The subgroup meta-analysis revealed a trend toward increased values across decades: prevalence doubled up from 30.7% (3.9 to 68.6) during 1991–2000 to 64.2% (55 to 72.9) in 2011–2020, though the number of studies did also increase substantially in recent decades (Figure S4). Finally, the meta-regression analyses revealed that the estimation of the prevalence was lower as the sample size increased, whereas the year of publication and the level of evidence of the studies were positively associated with higher values of prevalence (Figure 2b).

A total of 20 studies reported the number of bruises per carcass, from which we calculated an overall mean value of 2.9 (SD, \pm 1.4) bruises per carcass. There was a variation as the studies from Argentina and Colombia showed relatively higher values of 4.7 and 3.9 bruises per carcass, whereas in the studies conducted in Paraguay and Chile we found lower values of 2.4 and 2.3 (Table 3).

Main characteristics of bruises reported in studies in America

In total, 27 studies reported at least one characteristic of the bruises (Table 2). It is worth mentioning that for each category within a single characteristic, not all the studies reported the full set of categories either because the authors did not find bruises with all categories, or because the authors focused on a particular category of interest. Consequently, it is not expected that the prevalence across the categories sum up to 100% for each characteristic. Among the five studies that reported the age of bruises, the prevalence of fresh and old bruises was 57.7% (31.6 to 83.8) and 52.2% (26.6 to 78.2), respectively. Nineteen studies reported the anatomical location of the bruises, among which the hindquarter showed the highest prevalence (37.5%, 24.1 to 50.9) and the forequarter the lowest (17.1%, 10.8 to

Meta-analysis of bruises in cattle from the American continent

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a			Prevalence	b		•			
Study	Country	ı.	D-L, Random (95% Cl)			•		0	
Gallo (1999)	CHILE		7.75 (7.60, 7.91)	-	89.			0	0
Godoy (1986)	CHILE		9.25 (8.42, 10.14)					0	
de Assis (2011)	BRAZIL		9.85 (9.34, 10.37)		œ			0	0
Sornas (2016)	BRAZIL		10.31 (10.20, 10.43)		B	0		0	0
Strappini (2010)	CHILE		12.19 (12.01, 12.37)			۰		0	0
Vega-Britez (2019)	PARAGUAY	+	19.48 (16.51, 22.73)		4	0		8	Q
McKeith (2012)	USA		23.00 (22.39, 23.62)						\bigcirc
Bethancourt (2019)	BRAZIL		25.73 (25.51, 25.95)		~				\bigcirc
Garcia (2008)	USA		35.20 (34.24, 36.17)					0	\bigcirc
Romero (2013)	COLOMBIA	-	37.49 (34.72, 40.32)					0	G
Rebagliati (2008)	ARGENTINA		37.99 (37.00, 38.98)		•-[1	
Eastwood (2017)	USA		38.90 (38.29, 39.51)		<25th	25-501	:h	50-75th	>75th
Lorenzen (1993)	USA		39.20 (38.70, 39.70)	Л	Lower		Percentile o	f 🛌	Higher
Mendonça (2019)	BRAZIL		42.88 (41.44, 44.32)	Higher	sample size		the sample si		sample size
Crosi (2015)	URUGUAY	-	44.37 (41.31, 47.46)	Ξ					
McKenna (2002)	USA		46.70 (46.23, 47.17)	▲				:	
Van Donkersgoed (2001)	CANADA		54.00 (53.31, 54.69)					٢	
Rezac (2014)	USA	-	54.07 (51.48, 56.65)		φ		0	•	
Mendonca (2018)	BRAZIL	-	54.19 (52.71, 55.67)	a Ce	w -		0		
Youngers (2017)	USA	-	55.28 (53.78, 56.78)	sing				0	
Gallo (2001)	CHILE		60.00 (43.33, 75.14)	eva rui	φ		8		
Huertas (2010)	URUGUAY		60.03 (59.25, 60.82)	a d s	S	0	0	0	
Harris (2017)	USA		 62.33 (60.92, 63.73) 	cas			0	•	
Moreira (2014)	BRAZIL			nat	4		8	۰	
Lee (2017)	USA		■ 68.20 (67.28, 69.12)	Estimated prevalence of carcass bruising					
Braga (2014)	BRAZIL		71.09 (62.42, 78.76)	ш	N -	-	0		
Strappini (2013)	CHILE	-	71.15 (56.92, 82.87)		B		0	0	
de Freslon (2014)	CHILE	-	73.17 (57.06, 85.78)				-		
Sanchez-Perez (2019)	MEXICO		— 75.79 (71.52, 79.71)		• 1,	1	1	ļ	
Van Donkersgoed (1997)	CANADA		78.00 (77.49, 78.50)	Lower	Retrospective	Case-study	Survey	Cross-sectiona	al Experimer
Bertoloni (2012)	BRAZIL			ГŌ	Lower level		Design of		Higher level
Roeber (2001)	USA		83.20 (82.20, 84.17)		of evidence		the study		of evidence
de Andrade (2008b)	BRAZIL								
Romero (2011)	COLOMBIA		84.31 (82.75, 85.78)						· ·
Goldhawk (2015)	CANADA							•••	. 0
da Silva (2014)	BRAZIL		= 89.06 (85.12, 92.26)		æ -		0	· 0	
Huertas (2018)	URUGUAY		90.51 (89.85, 91.14)				0		
Cardoso (2011)	BRAZIL		90.53 (88.11, 92.60)						. 0
Strappini (2012)	CHILE				œ –			0	00
Miranda-de la Lama (2012)	MEXICO		 92.48 (90.86, 93.88) 		ES				
de Andrade (2008a)	BRAZIL		94.32 (87.24, 98.13)		4		0	0	° °
Petroni (2013)	BRAZIL		98.00 (96.85, 98.81)			\bigcirc		8 *	
Tuninetti (2017)	ARGENTINA		99.67 (98.16, 99.99)					0	()
Overall (I ² = 99.98%, p = 0.	00)	<	59.52 (51.96, 66.86)		ci -			\frown	\frown
						0	\bigcirc	G	\bigcirc
	(0 25 50	75 100		• - [
		Prevalen			1980	1990	2000	2010	2020
		Fievalelli			Older	🔶 Ye	ear of publica	tion —	Newer

Figure 2. a) Forest plot of the 43 studies included in the meta-analysis for estimating the prevalence of carcass bruising in cattle from America, **b)** results of meta-regression analyses of the prevalence of carcass bruising according to the percentile of the sample size (upper panel), the design of the study (middle panel), and publication year (bottom panel). Please note the contrasting effect of the covariates on the estimated values for prevalence.

Country	Studies	Bruised / examined carcasses	Pooled prevalence (95 % CI)	Mean number of bruises per carcass (± SD, studies)
TOTAL	43	225 291/925 542	59.5 (51.9 to 66.9)	2.9 (±1.4, 20)
Argentina	2	3848/9643	40.5 (39.5 to 41.5)	4.7 (±3.7, 2)
Brazil	13	74 152/432 863	64.2 (54.5 to 73.4)	2.8 (±0.6, 9)
Canada	3	31803/46962	72.9 (53.3 to 88.7)	NR
Chile	7	25225/247412	36.1 (30.4 to 42.1)	2.3 (±0.9, 4)
Colombia	2	2 371/3 467	70.1 (68.5 to 71.6)	3.9 (±1.8, 2)
Mexico	2	1 478/1 678	88.9 (87.3 to 90.3)	NR
Paraguay	1	127/652	19.5 (16.5 to 22.7)	2.4 (±0, 1)
Uruguay	3	16923/24330	66.9 (38.1 to 90.1)	2.9 (±1.2, 2)
USA	10	69364/158535	50.8 (41.4 to 60.1)	NR

Table 3. Summary of the estimated prevalence of carcass bruising from studies aggregated at the national level

NR = non-reported

23.3). The prevalence of bruising of the second third (middle region/loin) and the hips were similar (28.9%, 18.1 to 39.8 and 28.3%, 11.9 to 68.5, respectively) (Figure 3a).

Bruises scored as the lowest severity (Grade I, affecting only subcutaneous tissue) were highly prevalent and varied broadly from 19.8% to 97.1% according to 17/21 studies in which we found a mean prevalence of 66.4% (56.3 to 76.6). In contrast, bruises scored either as Grade II (affecting subcutaneous and muscular tissue) or III (affecting bone as well) were less prevalent (26.8%, 18.3 to 35.2 and 6.5%, 1.8 to 14.9, respectively). Irregular- and circular-shaped bruises were the most prevalent forms found in the studies (56.8%, 11.6 to 85.3 and 54.9%, 4.9 to 74.1, respectively).

In contrast, the prevalence of mottled, tramline, and linear shapes was lower (range 2.5% to 11.1%). Small-sized (2–8 cm) and medium-sized (9–16 cm) bruises were highly prevalent (44.1%, 30.6 to 57.6 and 36.1%, 25.9 to 46.7, respectively) compared with large- (> 16 cm) sized bruises (17.3%, 8.4 to 26.3) (Figure 3a). Removed meat due to bruising was assessed in seven studies, among which four studies reported values ranging from 15.6 to 647.1 kg of removed meat for the total number of carcasses evaluated during the study. Six studies that reported the mean amount of removed meat per carcass showed broad heterogeneity (range, 0.11–1.12 kg per carcass).

Main extrinsic or intrinsic risk factors associated with carcass bruising

We included 22 studies that reported a combination of intrinsic factors (12 studies) and extrinsic factors (19 studies) for carcass bruising (Table 2). From these 22 studies, we extracted 57 comparisons of low-risk vs high-risk conditions; although we found heterogeneity for some comparisons made between studies, these were subgrouped in the meta-analysis according to the specific causes to provide pooled estimations for each cause (details are summarized in Figures S5 and S6).

10

H

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1

100

Factor

OR (95% Cl)

5.8 (0.5 - 71.8)

1.9 (1.7 - 2.3)

1.6 (1.4 - 1.9)

1.6 (1.1 - 2.1)

1.9 (1.5 - 2.4)

OR (95% Cl)

3.4 (0.6 - 19.6)

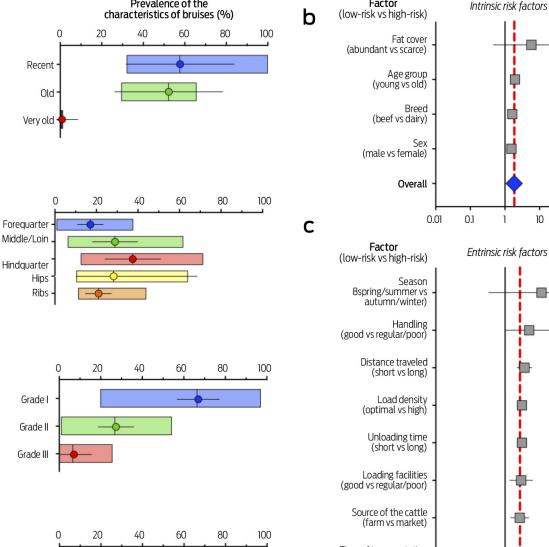
2.2 (1.03 - 4.9)

1.9 (1.5 - 2.4)

1.8 (1.7 - 1.8)

1.7 (1.5 - 2.1)

1.7 (1.2 - 2.5)



Prevalence of the

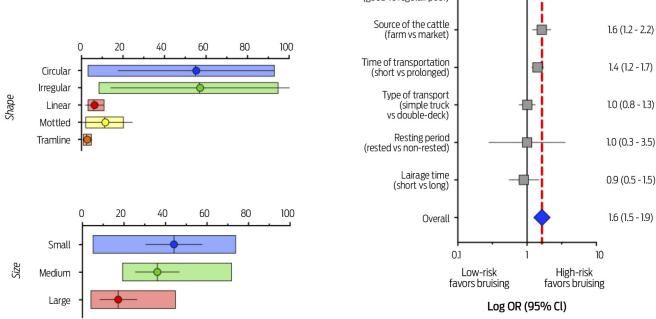


Figure 3. a) Floating bars showing the prevalence and 95% CI per category of each characteristic of the bruises and forest plots according to subgroup meta-analysis, b) intrinsic risk factors, c) extrinsic risk factors for carcass bruising. Please note that it is not expected that the prevalence of the characteristics of the bruises sum up to 100% across the categories because not all the studies reported the full set of categories for each characteristic.

a

Age

Anatomical site

Severity

The 12 studies that assessed intrinsic risk factors included 16 comparisons distributed in the following subgroups (*n*, low-risk vs high-risk): sex (10, male vs female), age group (3, young vs old), fat cover (2, presence vs reduced/absence), and breed (1, beef vs dairy). Pooled data showed a significant association between the presence of high-risk conditions of intrinsic factors and carcass bruising (OR = 1.86, 95% CI: 1.5 to 2.4) with 99.3% of variation attributable to heterogeneity (I², P = 0; Figure S5). The subgroup meta-analysis demonstrated that older cattle, dairy cattle, and female cattle (all considered as high-risk conditions) were 1.98 (1.7 to 2.3), 1.64 (1.4 to 1.9), and 1.57 (1.1 to 2.2) times more likely to get bruised than younger cattle, beef cattle, and male cattle, respectively (Figure 3b). Likewise, the presence of fat cover was associated with carcass bruising.

The 19 studies that assessed extrinsic risk factors included 41 comparisons according to the following subgroups (*n*, low-risk vs high-risk): distance traveled (13, short vs long), source of the cattle (4, farm vs auction market), load density (4, optimal vs high), the time of transportation (4, short vs prolonged), type of transport (4, simple truck vs double-deck trailer), handling (3, good vs regular/poor), lairage time (3, short vs prolonged), loading facilities (2, good vs regular/poor), season of the year (2, spring/summer vs autumn/winter), unloading time (1, short vs long), and a resting period during transportation (1, rested vs non-rested). The pooled analysis indicated that cattle exposed to high-risk conditions of extrinsic factors had greater odds for carcass bruising than cattle evaluated in low-risk conditions (OR = 1.64, 1.5 to 1.9).

We observed a significant proportion of variation attributable to heterogeneity $(I^2 = 98.5\%, P = 0; Figure S6)$. Subgroup meta-analysis revealed that cattle exposed to high-risk conditions such as regular/poor handling, long distances traveled, high loading densities, regular/poor loading facilities, cattle from auction markets, and prolonged time of transportation increased the odds for carcass bruising between 1.4 and 2.2 times compared to cattle exposed to low-risk conditions (Figure 3c and S6). In contrast, cattle kept for a long lairage time, cattle transported without resting, cattle transported in double-deck trailers, and cattle slaughtered during autumn/ winter had no increased chance of carcass bruising compared to cattle exposed to low-risk conditions for these extrinsic factors.

Discussion

In our meta-analysis, we found that except for Argentina, Chile, and Paraguay, the remaining countries had estimated values higher than 50%. Such a trend represents a concern given that the American continent produces and exports high volumes of beef cattle.⁽¹¹⁾ Besides, there was substantial heterogeneity in the prevalence of bruising at the national level. Factors such as handling practices, transportation conditions, and quality/safety of the abattoir facilities both between and within countries could partially explain the observed heterogeneity.^(15, 70) Likewise, this variability might be associated with country-specific geographic conditions (distances, mountains, roads), their socioeconomic indicators, and the existing legislation, because all of these contribute to different set-ups that might differentially influence the incidence of carcass bruising.^(71, 72) We did not find any previous meta-analysis studies on the prevalence of carcass bruising in America. Hence, our estimations might be used as a reference to measure further progress toward increased welfare of cattle during the pre-slaughter stages within the region. Our meta-analyses demonstrated that the prevalence of bruising showed a trend toward higher values across decades. Such a result seems like contradictory because there is a growing global awareness of the need to increase cattle welfare in conjunction with an increased interest in understanding human-animal interactions to implement adequate handling of productive farm animals.^(73, 74)

In consequence, the increase in the incidence of bruising questions whether the efforts to promote improved-handling practices aimed at increasing animal welfare have not been successful enough to reduce the problem in several countries from America.⁽¹²⁾ Besides, given that bruises are preventable, research is still needed to unveil the exact factors that are failing to reduce this issue. Furthermore, bruises are a reflex of the pre-slaughter handling practices to which cattle are submitted; thus, their presence evidences the degree of negligence of both the animal handlers and the producers.⁽⁷⁵⁾ In consequence, the lack of proper training of the workers that causes rough handling and poor animal welfare during stunning,⁽⁷⁶⁾ in conjunction with equipment problems that cause collisions with the structures at the slaughterhouse⁽⁷⁷⁾ could be synergistically participating to increase the incidence of carcass bruising in several countries from the American continent.

In addition to the ethical concern caused by bruising, there is a harmful effect both on the productivity of cattle and the meat quality.⁽⁴⁾ Several studies have estimated the negative impact on the meat industry caused by bruises in American countries. In Uruguay,⁽⁷⁾ a survey estimated that nearly 2 million tons of high-quality meat representing approximately US8 billion are lost each year due to bruising, whereas a survey from Argentina found that nearly US 12.5 million was lost due to bruising.⁽⁵⁵⁾ In North America, the Canadian beef quality audits have estimated losses between US 4.3 and US 10.5 million,^(66, 67) and in the USA the financial losses due to bruising in cattle ascent to US 22.4 million per year.⁽⁷⁸⁾

Despite this compelling evidence, the incidence of bruises is not only high but also increasing in several American countries and shows a trend toward increasing. In countries where the producers are paid after the trimmed meat is removed, the profit for the farmers is reduced;⁽⁷⁾ therefore, when the producer must pay for the losses, there is a strong incentive to reduce the causes for bruising. On the contrary, when the cost of the bruises is transferred from the producer to the industrial processor, there is no motivation to reduce them.⁽⁷⁹⁾

Given that bruises affect animal welfare and all stakeholders in the meat chain, from the producer to the consumer, several actions are needed to tackle this complex problem in the American continent, for instance: 1) abattoir management should provide both rewards and fines to reduce bruises, 2) employees and stock people should be trained to have a positive attitude towards animals,⁽¹⁸⁾ and 3) the awareness of the stakeholders must be increased by enforcing regulations and legislation aimed at improving both animal welfare and meat quality.⁽⁸⁰⁾ However, studies comparing current national regulations, awareness, and legislation regarding animal welfare are needed for the different American countries.⁽⁷¹⁾

Our summary of evidence showed that bruises affected mostly the hindquarter of the carcasses, and small-sized bruises affecting only subcutaneous tissues were highly prevalent. These types of bruises are usually related to both abattoir facilities (blows with infrastructure, protruding edges, and falls during the stunning) and improper handling (hitting, poking, and pricking with driving aids) by untrained people.⁽⁸¹⁾ The higher frequency of fresh bruises found in our review indicates that they probably occurred shortly before slaughter,⁽⁷⁷⁾ especially during the handling of the animals at the abattoir.⁽⁸²⁾

Therefore, assessing the age of the bruises is fundamental to identify steps at risk⁽⁸³⁾ during which injury prevention should be focused to improve animal welfare conditions, especially during transportation to the abattoir, lairage time, and cattle handling before stunning. Despite the relevance of the shape of the bruises to infer their possible causes,⁽⁸³⁾ a limited number of the reviewed studies reported this outcome. Our review showed that circular- or irregular-shaped bruises were the most prevalent forms. Besides, the shape of the bruise might be useful to detect human-inflicted bruises caused by inappropriate handling from the personnel⁽⁸⁴⁾ or to determine whether the contusions were caused by the interaction with other animals or due to deficient facilities at the abattoir.

Our results demonstrated an association between bruising and the presence of high-risk conditions of both intrinsic and extrinsic factors and confirm that carcass bruising is a multifactorial issue⁽⁸⁵⁾ associated with a complex combination of these factors.⁽⁸⁶⁾ For intrinsic risk factors, female cattle, older age groups, the breed, and the amount of fat cover were associated with bruising. Previous reports have shown a greater incidence of bruises in female cattle and that the lower economical value of mature and old animals might be associated with the incidence of bruising caused by the extra handling and prolonged transportation from livestock markets.^(86, 87)

With respect to the breed, the increased frame size of dairy cattle prone them to experience more traumatic events causing bruises,⁽⁴⁴⁾ whereas animals with a poor body condition score and thus lacking fat coverage are most likely to get bruised.⁽⁸⁸⁾ Overall, these results suggest that intrinsic factors are an important cause for bruising and should be considered when designing strategies to reduce their effect on animal welfare during the pre-slaughter stage of the cattle.⁽⁸⁹⁾ Unfortunately, the reviewed studies did not report comparisons for the presence of horns, the temperament of the cattle, or behavioral responses whereas for some causes there was a limited number of studies. In consequence, to increase the strength of the evidence, more research focused on animal factors is required.

We also found that extrinsic factors were associated with bruising. For instance, the source of the cattle was associated with bruising because of longer transportation, rough handling, poor quality of the facilities, and increased handling when animals pass through livestock markets increase the chance of getting bruised.^(87, 90) Transportation conditions such as long distances traveled, high load densities, and prolonged times of transportation increased the odds for bruising, though we found no effect for the inclusion of a resting period during the journey or the type of vehicle used. Previous studies have demonstrated an increased chance for bruising when cattle are transported at moderate or high load densities⁽⁸⁵⁾ and that exposing cattle to long-distance journeys causes stress, fear, and fatigue that impair animal welfare and increase the frequency of injuries.^(91, 92)

With respect to the handling at the slaughterhouse and the quality of the facilities, we found that cattle exposed to a prolonged unloading time and poor/regular quality of the loading facilities had a higher incidence of bruises. However, we did not find a significant effect of a prolonged lairage time, even though it has been previously associated with bruising.⁽⁹³⁾ Additionally, equipment problems that cause collisions with the structures also increase the incidence of bruises;⁽⁷⁷⁾ therefore, animal welfare could be improved through appropriate training of animal handlers in conjunction with the improvement of the facilities in American countries. For instance, avoiding the overloading of the crowd pen, elimination of electric prods and visual distractions, secure footing to avoid slips, round corners and curved chutes, covered open sides on the squeeze chute, rubber strips on the sidebars, and optimum pressure of the restraining apparatus.⁽⁹⁴⁾

Limitations

Our study is not devoid of limitations: 1) according to the GRADE approach to rate the overall quality of evidence,⁽⁹⁵⁾ we found a moderate level of quality: the true prevalence of bruising is probably close to our estimation, though with possible substantial difference; 2) to avoid bias from the inclusion of unpublished results, we only included published peer-reviewed publications, which could have limited the number of studies included in the meta-analysis; and 3) only 9/35 American countries were included and for some countries the number of studies was limited; therefore, it is possible that the estimations might be biased and do not represent the current figure for some countries.

Conclusions

Our results showed that the prevalence of cattle carcass bruising is high in American countries, though with great national heterogeneity, and we found a high average of poly-contused carcasses that further aggravates the animal welfare in the region. Various risk factors are associated with bruising incidence, and some occur simultaneously during the pre-slaughter period; thus, these should be controlled to prevent and reduce unnecessary pain and suffering caused by bruising.

The high prevalence of fresh bruises indicated that these injuries possibly are inflicted during the 24 h before slaughter when animals are handled from the farm to the abattoir. Also, the high prevalence of small-sized bruises affecting subcutaneous tissues and located mainly in the hindquarter of the carcasses indicate that these lesions are related to deficient abattoir facilities and improper handling and thus it might also be a priority to provide training and increase awareness of animal welfare to all stakeholders.

Data availability

All data and material are available from the corresponding author upon request.

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Conflicts of interest

Daniel Diaz is member of the editorial board of Veterinaria México OA. Following the current journal's policies in this regard, he only participated as author during the editorial process of this submission. Moreover, all authors agreed to publish the peer review process along with the article.

Author contributions

Conceptualization: JNSP, BJFL, AMP, ACS, CG, DD and HDR Data curation and Methodology: JNSP, BJFL, DZV and DD Formal analysis, Software and Visualization: DD Investigation: JNSP, BJFL, and DZV Project administration and Supervision: DD and HDR Resources: AMP, JRR, JCRE, JJPL and HDR Writing-original draft: JNSP, BJFL, DD and HDR Writing-review and editing: JRR, AMP, ACS, CG

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