

## Article

# Carcass Lesion Severity and Pre-Slaughter Conditions in Heavy Pigs: A Prospective Study at a Commercial Abattoir in Northern Italy

Martina Zappaterra <sup>1,\*</sup>, Barbara Padalino <sup>1</sup>, Laura Menchetti <sup>1</sup>, Agnese Arduini <sup>1</sup>, Vincenzo Pace <sup>2</sup> and Leonardo Nanni Costa <sup>1</sup>

<sup>1</sup> Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin 46, I-40127 Bologna, Italy; barbara.padalino@unibo.it (B.P.); laura.menchetti@unibo.it (L.M.); agnese@post@hotmail.it (A.A.); leonardo.nannicosta@unibo.it (L.N.C.)

<sup>2</sup> Organizzazione Prodotto Allevatori Suini (OPAS) Pig Farmer Association, Strada Ghisiolo 57, I-46030 San Giorgio Bigarello MN, Italy; vincenzo.opas@coopgsp.it

\* Correspondence: martina.zappaterra2@unibo.it

**Abstract:** Pre-slaughter conditions and their effects on carcass quality have been largely addressed for pigs of 90–100 kg live weight, while few studies consider the effects of pre-slaughter conditions on the quality of the carcasses obtained from heavy pigs intended for Protected Designation of Origin (PDO) production. A total of 1680 heavy pigs were transported in 72 batches from a farm to a commercial abattoir on 16 different days, avoiding mixing unfamiliar animals. Slaughterhouse conditions, animal behaviors, and human–animal interactions were annotated at unloading and during the race toward the stunning cage. Carcass lesions on the rear, middle, and shoulder parts of the carcasses were scored. The prevalence of carcasses with severe lesions was 6.92%, 11.87%, and 6.83%, for the rear, middle, and shoulder parts, respectively. Among the pre-slaughter events, waiting before unloading and improper handling practices at the abattoir were the major factors affecting carcass lesion severity. Lairage pen space allowance was also found to affect severe rear and shoulder lesions, and the batches that were transported in the trailer had an increased prevalence of severe shoulder lesions. Our results suggest waiting time before unloading should be shortened as much as possible, and educational programs to train operators for more careful management of animals in the abattoir are greatly required to avoid improper animal handling practices.

**Keywords:** carcass damage; skin bruises; carcass quality; pig welfare; carcass scoring; animal handling



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## 1. Introduction

Pre-slaughter animal handling is strongly associated with ultimate pork quality [1]. Stressful events on the farm and during transport and pre-slaughter conditions have deleterious effects on the final product [2], causing abnormal enzymatic changes in post-mortem muscle-to-meat transformation and determining bruises and lesions that can penalize the quality of the carcass and cuts [1–3]. Blemishes and severe damages on the carcass skin are an economical problem for the pig production chain, as skin lesions can lead to downgrading of carcass and cuts [4,5], increased costs to remove the lesioned parts [4–6], and discarding of bruised hams from the Protected Designation of Origin (PDO) circuit [7,8]. Italy contributes to about one-third of the European meat product heritage [9], and the majority of the heavy pigs reared for PDO production are located in Northern Italy, in particular in the Po Valley. Parma and San Daniele PDO hams accounted for more than half of the total turnover generated by the Italian PDO and PGI (Protected Geographical Indication) meat products in Italy in 2019 [10].

Thighs intended for PDO ham production originate from heavy pigs slaughtered at about 150–170 kg live weights and at least 9 months of age. The higher age and maturity of heavy pigs is necessary, as older animals have a lean-to-fat balance that makes their thighs more desirable for ham production [11]. Sufficient subcutaneous fat layers and intact skin are essential features for thighs intended to undergo salting and ripening processes [11]. However, the greater slaughter weight and the more advanced age required in heavy pigs may increase the risk of fighting and lesions in heavy pig carcasses [12,13]. Furthermore, heavy pigs reared in the Po Valley are transported for short journeys (<2 h) to reach abattoirs, which may be a factor increasing stress in pigs [14] and predisposing them to aggressive interactions and injuries to the carcass.

Risk factors contributing to welfare issues and lesions in pigs of about 100 kg live weight have been extensively reported [3,7,15,16]. Despite the high economic value that PDO productions have for Italy, there is little scientific literature concerning the welfare needs of heavy pigs and the risk factors associated with carcass damage [13]. The available literature indicates that several on-farm and pre-slaughter factors are associated with an increased prevalence of carcass lesions. Factors such as reduced space allowances [17], lack of environmental enrichment and social interactions [18–20], pens with animals kept in large group sizes [21], or genetic factors [22] can trigger fights and damaging behaviors during the on-farm phase, causing tail and ear biting and injuries in various parts of the animal's body. Pre-slaughter practices comprise several stages that can result in stressful conditions for the pigs, leading to increased carcass damage [1,3,23]. Prolonged fasting [24–26], transport at high densities and with adverse microclimate [1,27], larger distances walked by the animals at unloading and stunning [28], and inappropriate handling [29] increase the prevalence of carcass lesions and blemishes while affecting muscle-to-meat transformation [30,31]. In production, one of the most common practices that can lead to carcass damage is the mixing of pigs coming from different farms and pens [32]. Mixing unfamiliar animals causes hierarchical fights, with detrimental effects on pig welfare and carcass quality [25,26,30,32,33].

Assessing carcass lesions during meat inspection in abattoirs may thus be a surveillance tool for pig welfare, as skin damage, bites, and blemishes on the carcass are markers of reduced animal welfare conditions taking place during the on-farm and pre-slaughter phases [1,5,12,15,34]. Evaluation of carcass lesions may be easily conducted during abattoir inspections [7], and the appearance and color of the lesions have been indicated as features that may be used to distinguish between fresher and older lesions. Carcass bruises and lesions with a reddish appearance are used to identify fresh skin damage [7,35] caused by transport, lairage, huddling or poor animal handling in the pre-slaughter phase [23]; on the other hand, yellowish bruises may be indicative of older lesions caused by on-farm conditions. For these reasons, it has been suggested that assessing carcass damage can integrate the measures carried out on-farm, at unloading, and lairage to create certification schemes attesting to the fact that the animals were raised, transported, and slaughtered with respect for their welfare [34].

Notwithstanding the extensive body of literature concerning pre-slaughter conditions and animal handling effects on meat quality, skin damage is a multifactorial problem [1,7], caused by the interaction of different conditions. Most of the scientific literature concerns pigs slaughtered at a commercial live weight of 90–110 kg [1,7,19,26,36], while few studies have been conducted to highlight the effects of pre-slaughter conditions in heavy pigs [12,37–39]. The hypothesis of this prospective study was that even by avoiding mixing unfamiliar animals, there are other practices in the pre-slaughter phase that can affect carcass injuries in heavy pigs. In particular, we hypothesized that animal handling and the size of the lairage pens may have an effect on the carcass damage. The aim of this prospective study is therefore to document the severity of lesions on heavy pig carcasses coming from batches where unfamiliar animals were not mixed and evaluate which conditions taking place in a commercial abattoir are contributing the most in explaining the prevalence of severe lesions.

## 2. Materials and Methods

### 2.1. Animals, Pre-Slaughter and Slaughter Conditions

Data were collected in a commercial processing plant located in Northern Italy from a total of 1680 crossbred heavy pigs (Duroc × (Landrace × Large White)). The pigs were reared in the same farm until a final live weight of about 160 kg ( $164.9 \pm 5.6$  kg) at 9 months of age. Then, the animals were transported to the same slaughterhouse on 16 dates, between January and June 2014. Pigs were transported in the same truck (Carrozzeria Pezzaioli, Montichiari, Italy), consisting of a lorry and a trailer, each one equipped with three hydraulic decks, as described in Arduini et al. [38]. Each deck was loaded with 23/24 pigs (a batch), for a total of about 140 pigs being transported on each date. After a fasting period of 12 h, pigs were loaded into trucks and transported to the commercial abattoir. The loading procedures started at 6:00 a.m. and lasted about 45 min; during this time, pigs from the same pen were loaded on the truck using a mobile ramp (width 0.7 m, length 6.0 m, with 1.0 m solid side walls and adjustable height) avoiding mixing between unfamiliar animals of different batches. All decks had almost the same dimensions (bottom deck = 17.40 m<sup>2</sup>; middle deck = 15.60 m<sup>2</sup>; upper deck = 15.20 m<sup>2</sup>). The vehicles traveled on secondary roads at an average speed of 30 km/h, taking approximately  $31 \pm 5$  min to arrive from the farm to the abattoir. At the plant, in some cases, the vehicle was made to wait before starting the unloading phases. This waiting time before unloading was measured by the operators. The unloading procedures lasted about 12 min for all vehicles. After unloading, pigs were weighed and driven to lairage pens, where they were allowed to rest for about 30 min. The 23/24 pigs loaded on each deck were kept together in the same lairage pens, avoiding mixing unfamiliar animals of different batches. The abattoir had 17 lairage pens, located in the same area, at the entrance of the abattoir, in the dirty area. The size of the 17 lairage pens was measured with a laser meter (DTAPE DT50, Vorstik, Shenzhen Hanmer Precision Technology Co.,Ltd., Shenzhen, China). Lairage pens had different sizes, while the pen door had the same dimension for all pens. In addition to lairage pen dimensions, the distance between each lairage pen door and the entrance of the passageway toward the stunning area was also measured with the same laser meter. Corridors and lairage pens had a concrete floor. After the rest (lasting about 30 min), the pigs were showered and moved through a single passageway toward the stunning area. The animals were handled and slaughtered in compliance with European rules on the protection of animals during transport and at slaughter (Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009). All slaughter procedures were monitored by the veterinary team appointed by the Italian Ministry of Health.

A trained operator observed and noted the animal behaviors and events at two different checkpoints: at unloading (T1, from the truck ramp to the lairage pens), and when the pigs were moved towards the stunning area (T2, from the lairage pen door to the entrance of the stunning area). The events and type of observations were adapted from [36,40], following the protocol in Table 1. The data sheet used to annotate events is reported as File S1 in the Supplementary Material. A graphical representation of the experimental design and of the variables annotated at the two checkpoints is reported in Figure 1.

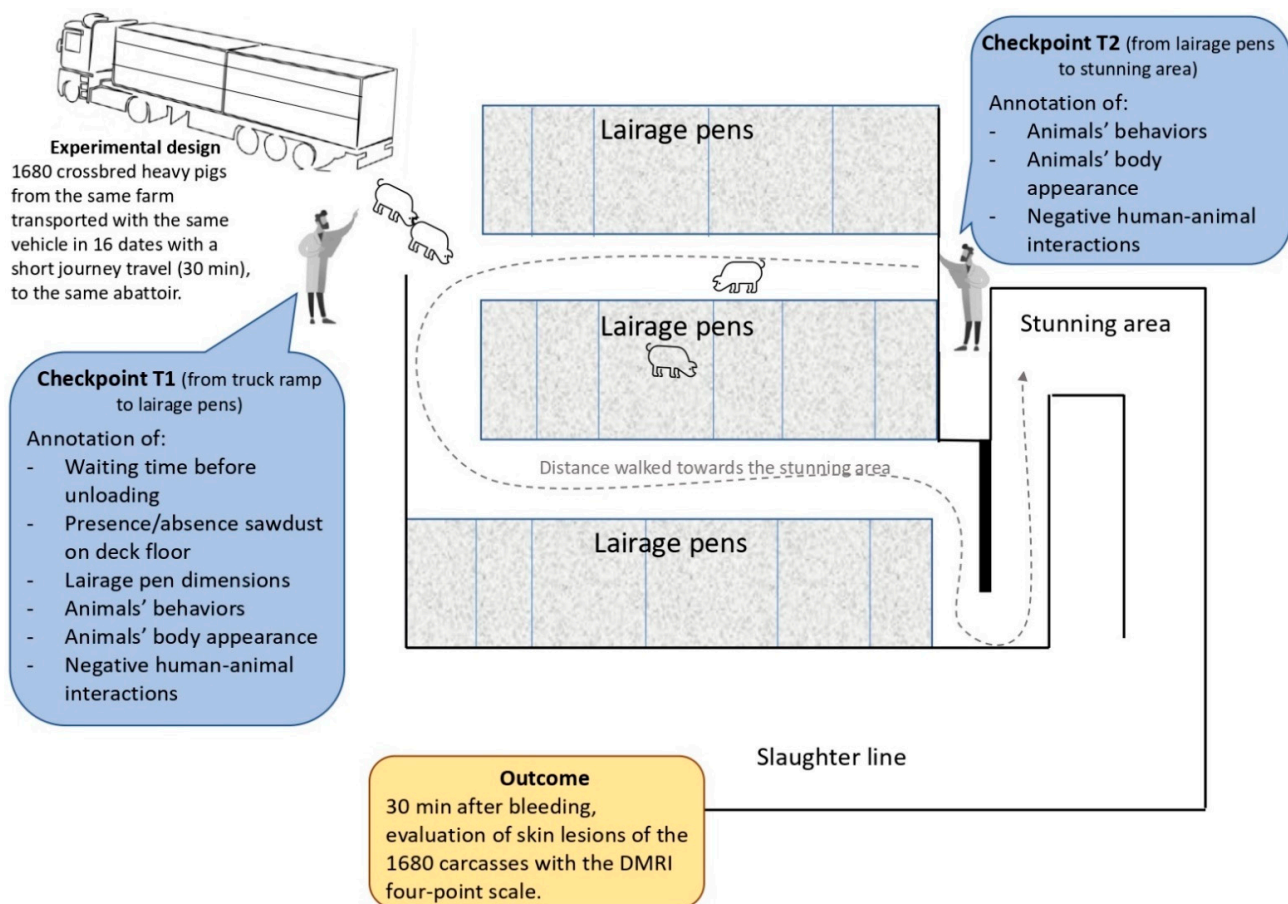
Electric stunning was performed with head-only electrodes applied manually between the eyes and the base of the ears, with an intensity of 1.3 A and a voltage of 170 V. Pigs were then horizontally exsanguinated for 3 min, hanged up by the left leg for 10 min, and then were immersed in a scalding tank for 5 min at 62 °C. Hairs were then manually removed and carcasses were evaluated for skin damage by a trained operator. In the slaughter line, 30 min after bleeding, the carcasses were categorized by a trained operator on a four-point scale following the Danish Meat Research Institute (DMRI) scale [8]. The carcasses were evaluated for their skin damages in three different regions of the carcasses: (i) rear (leg and posterior); (ii) middle (middle and side part of the trunk); (iii) shoulder (shoulder/neck and head). The four-point scale used is reported in Table S1.

The skin damages evaluated for classifying carcasses were those with a reddish appearance, while the yellow ones were not considered for this study, following [7].

After their evaluation, carcasses were then eviscerated, split, sectioned in different marketing pieces, and stored in chilling rooms for 24 h.

**Table 1.** Protocol used to annotate events, animal behaviors and skin discoloration of the pigs at unloading (T1) and when the pigs were moved towards the stunning area (T2).

Type of Event	Event	Description	Checkpoint
Animal	Falling	The pig loses balance and touch the ground with a shoulder, thigh, trunk or another part of the body that is not one leg [41].	T1, T2
Animal	Slipping	The pig loses balance without a part of the body touching the floor [41].	T1, T2
Animal	Reluctance to move	The pig refuses to walk and stops without moving the head or the body for more than 2 s [41].	T1, T2
Animal	Turning back	The pig turns away and moves in the direction opposite of the desired [41].	T1, T2
Animal	Jumping	The pig does a sudden movement and jumps.	T1, T2
Animal	Elimination	The pig defecates and/or urinates.	T1, T2
Animal	Vocalization	The pig vocalizes.	T1, T2
Animal	Panting	The pig breaths loudly and fast with mouth open.	T1, T2
Animal	Wounds on body	The pig has wounds on the body [41].	T1
Animal	Hematoma	The pig has one or more hematomas.	T1
Animal	Discoloration	The pig shows skin discoloration.	T1
Animal	Ear discoloration	The pig shows skin discoloration on ears.	T2
Animal	Thigh discoloration	The pig shows skin discoloration on thighs.	T2
Animal	Back discoloration	The pig shows skin discoloration on the back part of the trunk.	T2
Animal	Side discoloration	The pig shows skin discoloration on the side part of the trunk.	T2
Animal	Lameness	The pig fails to walk normally. It can vary in severity, from reduced ability to inability to bear weight [41].	T1
Animal	Shivering	The pig trembles. Slow or irregular vibration of a body part or the whole body [41].	T1
Animal	Overlapping	The pig mounts another one with its front legs on the back of the other.	T1
Animal	Pushes	The pig presses the head on the body of another pig lying or walking ahead.	T2
Animal	Huddling	More pigs huddle and create a group.	T1, T2
Human–animal	Hit with stick	An operator beats the pig with a stick [40].	T1
Human–animal	Hit with plastic tube	An operator beats the pig with a plastic tube [40].	T1, T2
Human–animal	Hit with rubber stick	An operator beats the pig with a rubber stick [40].	T1
Human–animal	Poke with electric goad	An operator touches the pig with the electric goad [40].	T1
Human–animal	Shouts	An operator shouts at animals to encourage them proceeding.	T1, T2



**Figure 1.** Graphical representation of the experimental design. The checkpoints (T1 and T2) used to annotate the animal behaviors, body appearance and negative human–animal interactions are reported on a simplified plan of the lairage and stunning area of the commercial abattoir located in Northern Italy.

## 2.2. Data Management and Statistical Analyses

### 2.2.1. Data Management and Descriptive Statistics

Batches (23/24 pigs) were considered as the experimental unit. The events observed at T1 and T2 were transformed into relative frequencies and expressed in % of events on the total number of pigs in each batch. Four new variables were also created: the total number of animal events at T1 (obtained by summing all the animal events annotated at T1); the total number of animal events at T2 (obtained by summing all the animal events annotated at T2); the total number of human–animal events at T1 (obtained by summing all the human–animal events at T1); the total number of human–animal events at T2 (obtained by summing all the human–animals events at T2). The dimensions of the lairage pens were used to calculate the total area of the pen and the space allowance per pig of the batch. The live weight of the pigs in each batch was summed and used to calculate the stocking density of the lairage pen for each batch of pigs. Furthermore, the number of carcasses classified for each point of the DMRI carcass lesion scale was expressed as % of total carcasses in the batch.

Descriptive statistics (mean, standard deviation, minimum and maximum values) were performed for the lairage pens dimensions, the % of the annotated events, and the % of carcasses classified in each of the four points for the three anatomical locations (rear, middle, front). To identify possible shared patterns of variability between the frequency of events and carcass lesions, a Spearman correlation analysis was performed using the % of events and carcasses as a dataset. Spearman correlation analysis was performed using *Hmisc* package in the R environment [42]. The obtained correlation coefficients  $r$  and

relative  $p$ -values were used to evaluate collinearity between the frequency of the events. Two events were considered collinear when their  $r$  was  $\geq 0.95$ .

For the subsequent use in regression models, the carcasses classified with the four-point scale were grouped into two groups for each of the three anatomical locations, following the recommendations of Faucitano [3], who indicated the scores 3 and 4 as unacceptable: (i) the moderate lesion group, obtained by adding for each anatomical location the number of carcasses classified as 1 and 2 of the DMRI scale in each batch, and then the prevalence of moderate lesions in each anatomical location was determined for each batch as a percentage of the sum of scores 1 and 2 on the total carcasses of the batch; (ii) the severe lesion group, obtained by adding for each anatomical location the number of carcasses classified as 3 and 4 of the DMRI scale in each batch; then the prevalence of severe lesions in each anatomical location was determined for each batch as a percentage of the sum of scores 3 and 4 on the total carcasses of the batch.

### 2.2.2. Single- and Multivariable Regression Analysis

A regression analysis was performed to identify the possible risk factors and signs of fear and distress associated with the prevalence of carcasses with severe lesions in rear, middle, and shoulder. A separate regression analysis was performed for the three anatomical locations with the aim of identifying the possible distinct risk factors associated with the occurrence of severe lesions in the rear, middle and front regions of the pig carcasses.

First, for each anatomical location, the prevalence of carcasses with severe lesions was used as a dependent variable in single-variable quasi-Poisson regression models. The latter included as independent variables whether each batch was transported in the lorry or trailer (dichotomous variable), the hydraulic decks (3 levels), the lairage pen dimensions and space allowance, the pen stocking density, the pen distance from the stunning area, the time elapsed between unloading and bleeding, and the % of events annotated for each batch. The single-variable quasi-Poisson regression models were performed to identify the predictive variables to be included in the stepwise backward selection and find the final multivariable model for the prevalence of severe lesion carcasses for each anatomical location. Results of the single-variable quasi-Poisson regression models are reported as estimates and  $p$ -values. The independent variables that showed a  $p < 0.25$  were considered in the stepwise backward selection for their inclusion in the final multivariable model. Due to the collinearity between the frequency of hit with plastic tube and the operator shouts at T2, if those independent variables were significant in single-variable models, only the hit with plastic tube was further considered in the backward selection step.

A stepwise backward elimination procedure was indeed conducted for the % of carcasses showing an unacceptable quality in each anatomical location to test the combined effect of the independent variables. The independent variables were removed until all variables in the final model had a  $p < 0.05$ . The final multivariable models resulting from the stepwise backward selection are presented as estimates and  $p$ -values. The estimates and  $p$ -values of the variables entering in the final multivariable models were then used to graphically present the standardized estimate effects with forest plots. The scripts used to perform the univariate logistic and stepwise multiple regressions were a combination of functions in the packages *nlme*, *lsmeans*, *lmttest*, *stats* and *car*; forest plots were obtained using a combination of functions in the packages *sjPlot*, *sjlabelled*, *sjmisc*, and *ggplot2*. All the aforementioned packages were implemented in the R environment [42].

### 2.2.3. Regression Tree Analysis

A regression tree analysis was used in order to identify the independent variables that effected the greatest variability observed among batches in the prevalence of carcasses with severe lesions in the rear, middle and shoulder. The explanatory variables used for single- and multivariable regression analysis were the same, except for the frequency of skin discoloration. None of the observations concerning skin discoloration were included in the regression tree analysis, as we were more interested in estimating which independent

variables were the most important risk factors associated with the prevalence of severe lesion carcasses. Skin discoloration may instead be considered an early marker indicative of carcass lesions. Regression tree analysis was performed following [43], using packages *rsample*, *dplyr*, *rpart*, *rpart.plot*, *ipred*, and *caret* in the R environment [42].

### 3. Results

#### 3.1. Description of Pre-Slaughter Conditions and Observations, and Prevalence of Carcass Skin Lesions

The vehicles transporting the pigs to the abattoir waited between 4 min and 29 min, with an average waiting before unloading of  $12.33 \pm 8.26$  min. Lairage pen dimensions were variable; width ranged from 1.67 m to 5.67 m (average width  $3.37 \pm 1.50$  m), length from 5 to 6 m (average length  $5.52 \pm 0.44$  m), and areas from  $8.33 \text{ m}^2$  to  $34 \text{ m}^2$  (average area  $18.80 \pm 9.30 \text{ m}^2$ ). Lairage pen density ranged from 107.22 to  $452.18 \text{ kg/m}^2$  (average density  $252.82 \pm 104.71 \text{ kg/m}^2$ ), and pen space allowance per pig was highly variable, from  $0.36 \text{ m}^2$  to  $1.48 \text{ m}^2$  per animal ( $0.81 \pm 0.40 \text{ m}^2$  on average per pig). Depending on the lairage pen, the animals had to walk different distances to reach the stunning area. The closest lairage pen was located at 2.33 m from the stunning area entrance, while the farthest was at 47.33 m (average distance  $19.99 \pm 14.03$  m).

An overview of the relative frequency of events annotated at unloading (T1) and from the lairage pen door to the entrance of the stunning area (T2) is presented in Table 2. At T1, pigs expressed more frequently turning back behavior and vocalization, followed by overlapping, huddling, panting, slipping, and discoloration. Out of the 72 batches, 15 showed a frequency of turning back above 10%, and only 26 batches did not show animals expressing this behavior. Vocalization and slipping were also behaviors frequently expressed in pigs at T1, with 45 and 44 batches showing, respectively, at least one pig vocalizing or slipping. Huddling was observed in 27 out of the 72 batches, and 23 batches had at least one animal that fell during unloading procedures (T1). Discoloration was noticed in 19 batches, with four batches having more than 10% of pigs showing discoloration. Panting was noticed in 13 batches, six of which were the batches transported in June. Shivering was extremely rare, with just three batches having an animal expressing this behavior. Elimination has never been observed at T1. The health status of the animals at T1 was in general good, with no pigs showing wounds and only two batches having a pig with a hematoma and a pig with lameness. At T1, to encourage pigs to move towards lairage pens, operators used shouts in 36 batches out of the 72 and in 25 batches hit the pigs with a rubber stick or with a plastic tube.

At T2, almost all behaviors and events were more frequently expressed compared to T1. Out of the 72 batches, 59 had at least one animal turning back, 49 had at least one pig showing reluctance to move, 23 batches had at least one pig jumping, 19 batches had at least one pig pushing against the others, and 18 had huddling events. Panting at T2 was expressed in 7 batches out of the 72, distributed over five different dates. Discoloration in the different body regions was frequently observed; out of the 72 batches, 52 had at least one animal with thigh discoloration, 49 had animals with side discoloration, 45 had pigs with back discoloration, and 36 had at least one animal with ear discoloration. An increased frequency of negative human–animal interactions was also observed at T2, with 64 batches out of the 72 having at least one pig hit by an operator with a plastic tube. The same 64 batches also had operators using shouts to encourage pigs to move towards the stunning area.

**Table 2.** Descriptive statistics (mean, median, standard deviation, minimum and maximum) for the frequency of animal and human–animal events annotated at unloading (T1) the frequency of animal and human–animal events annotated from the lairage pen door to the entrance of the stunning area (T2), and for the prevalence of carcass skin lesions.

Variables	Mean	Median	S.D.	Min	Max
<b>Frequency of events annotated at T1</b>					
Animal events:					
Falling (%)	1.73	0.00	2.76	0.00	8.70
Slipping (%)	3.59	4.17	3.72	0.00	17.39
Turning back (%)	6.46	4.35	7.75	0.00	34.78
Reluctance to move (%)	2.79	0.00	3.45	0.00	13.04
Overlapping (%)	1.97	0.00	4.09	0.00	21.74
Jumping (%)	0.12	0.00	0.69	0.00	4.17
Huddling (%)	2.21	0.00	3.91	0.00	21.74
Elimination (%)	0.00	0.00	0.00	0.00	0.00
Vocalization (%)	9.04	4.35	12.53	0.00	60.87
Panting (%)	1.72	0.00	4.53	0.00	21.74
Wounds on body (%)	0.00	0.00	0.00	0.00	0.00
Hematoma (%)	0.06	0.00	0.51	0.00	4.35
Discoloration (%)	1.96	0.00	3.93	0.00	17.39
Shivering (%)	0.18	0.00	0.87	0.00	4.35
Lameness (%)	0.06	0.00	0.51	0.00	4.35
Total number of animal events at T1 (N)	7.43	7.00	4.54	2.00	32.00
Human–animal events:					
Hit with stick (%)	0.71	0.00	2.04	0.00	8.70
Hit with plastic tube (%)	1.91	0.00	2.87	0.00	8.70
Poke with electric goad (%)	0.36	0.00	1.19	0.00	4.35
Shouts (%)	2.38	2.00	2.58	0.00	8.70
Hit with rubber stick (%)	1.61	0.00	2.35	0.00	9.09
Total number of human–animal events at T1 (N)	1.62	1.00	1.66	0.00	6.00
<b>Frequency of events annotated at T2</b>					
Animal events:					
Falling (%)	0.06	0.00	0.49	0.00	4.17
Slipping (%)	1.01	0.00	3.45	0.00	26.09
Turning back (%)	12.02	8.70	10.67	0.00	40.91
Reluctance to move (%)	6.87	4.55	7.77	0.00	44.00
Pushes (%)	1.94	0.00	4.03	0.00	20.00
Jumping (%)	2.03	0.00	3.47	0.00	13.64
Huddling (%)	1.71	0.00	3.79	0.00	17.39
Elimination (%)	0.36	0.00	1.41	0.00	8.70
Vocalization (%)	7.31	8.33	4.90	0.00	13.04
Panting (%)	0.66	0.00	2.48	0.00	17.39
Side discoloration (%)	9.54	8.51	8.87	0.00	30.43
Ear discoloration (%)	3.94	2.00	5.21	0.00	25.00
Thigh discoloration (%)	11.15	8.89	10.65	0.00	43.48
Back discoloration (%)	11.32	8.70	12.22	0.00	47.83
Human–animal events:					
Hit with plastic tube (%)	7.61	8.70	3.67	0.00	13.04
Shouts (%)	7.50	8.70	3.71	0.00	13.04
Total number of human–animal events at T2 (N)	7.03	7.00	3.22	0.00	12.00
<b>Skin lesion score</b>					
Rear					
1 (%)	66.86	65.22	24.19	13.04	100.00
2 (%)	26.22	25.00	18.93	0.00	70.83
3 (%)	6.03	4.26	7.65	0.00	30.43
4 (%)	0.89	0.00	2.48	0.00	13.04
Moderate lesions (1 + 2)	93.08	95.65	8.79	65.22	100.00
Severe lesions (3 + 4)	6.92	4.35	8.79	0.00	34.78



Table 2. Cont.

Variables	Mean	Median	S.D.	Min	Max
Middle					
1 (%)	52.05	47.92	27.77	8.70	100.00
2 (%)	36.58	39.13	19.71	0.00	73.91
3 (%)	10.06	6.44	10.46	0.00	39.13
4 (%)	1.31	0.00	3.41	0.00	16.67
Moderate lesions (1 + 2)	88.63	91.49	12.07	52.17	100.00
Severe lesions (3 + 4)	11.37	8.51	12.07	0.00	47.83
Shoulder					
1 (%)	72.94	78.26	21.52	21.74	100.00
2 (%)	20.23	17.39	14.31	0.00	56.52
3 (%)	4.94	0.00	7.63	0.00	30.43
4 (%)	1.90	0.00	3.97	0.00	16.67
Moderate lesions (1 + 2)	93.17	98.00	10.38	60.87	100.00
Severe lesions (3 + 4)	6.83	2.00	10.38	0.00	39.13

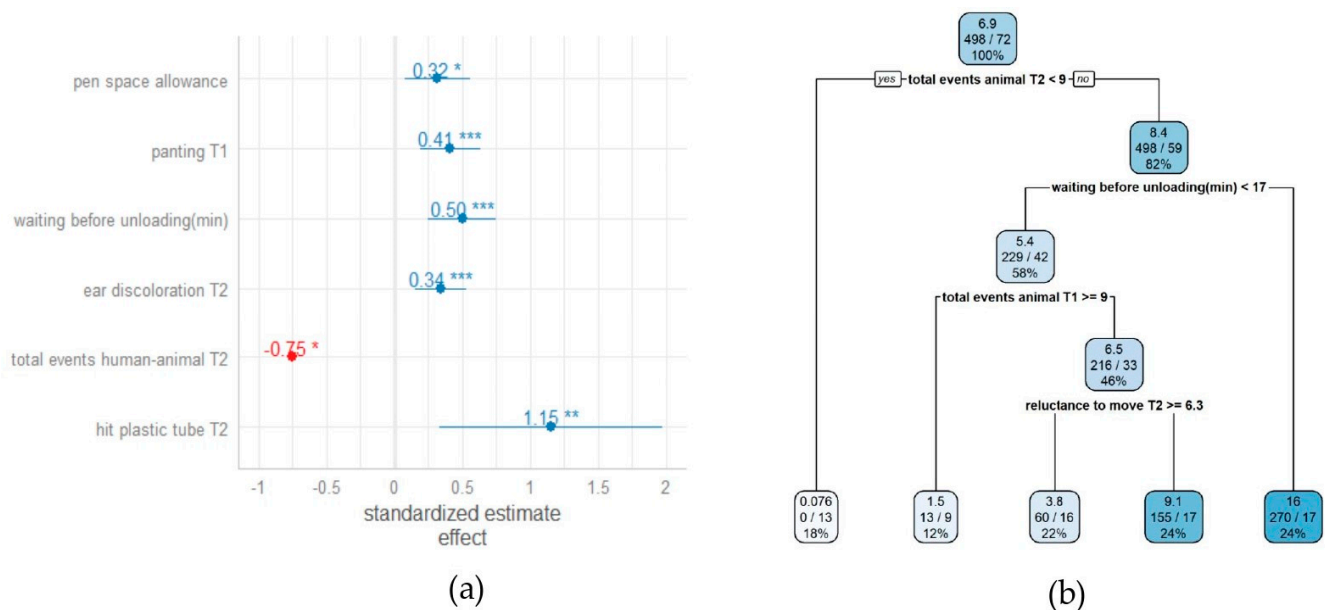
Table 2 also shows the descriptive statistics of the prevalence of carcasses scored with the DMRI scale for skin lesion severity. On average, severe lesions (classes 3 and 4) for the rear and shoulder regions were observed in about 6% of the carcasses in the 72; a higher prevalence was observed for trunk lesions, with severe lesions in the middle region exceeding on average 11% of the carcasses in each batch. Out of the 72 batches, 41 had at least one carcass with severe lesions in the rear region, 17 of which had a prevalence of carcasses with severe rear lesions above 10%. The number batches with at least one carcass with severe middle lesions was 57, 27 of which had more than 10% of carcasses with severe middle lesions. Thirty-six batches showed at least one carcass with severe shoulder lesions, and 16 of them had more than 10% of carcasses with severe shoulder lesions.

Correlations among all variables annotated at T1 and T2 and the prevalence of carcasses scored with the DMRI scale are reported in File S2 in the Supplementary Material. The results of the correlation analysis indicated that batches having more carcasses with the rear region scored as 1 tended to also have more carcasses with superficial lesions in the middle and shoulder regions. On the other hand, batches that had a higher prevalence of carcasses scored as 2 or 3 for the three regions were also those with higher frequencies of events such as pigs falling and overlapping at T1; with more animals showing ear, side, back, and thigh discoloration at T2; with more pigs vocalizing at T2; and with a higher frequency of negative human–animal interactions at T2 (both hit with plastic tube and shouts, as those two events were highly correlated, with  $r = 0.98$ ,  $p < 0.001$ ).

### 3.2. Association between Pre-Slaughter Conditions and Severe Lesions in the Rear Region of Heavy Pig Carcasses

The results of the univariable models for the prevalence of severe rear lesions are reported in Table S2. In particular, the prevalence of severe rear lesions was associated with longer waiting before unloading ( $b = 0.058$ ,  $p = 0.0004$ ), increased total number of human–animal events at T1 ( $b = 0.066$ ,  $p = 0.009$ ), ear discoloration at T2 ( $b = 0.071$ ,  $p = 0.001$ ), back discoloration at T2 ( $b = 0.028$ ,  $p = 0.009$ ), shouts at T2 ( $b = 0.116$ ,  $p = 0.014$ ), hit with plastic tube at T2 ( $b = 0.109$ ,  $p = 0.023$ ), falling at T2 ( $b = 0.356$ ,  $p = 0.021$ ).

The results of the final multivariable regression model for the prevalence of severe lesion in the rear region of the carcass are graphically displayed with a forest plot in Figure 2a. The  $x$ -axis reports the standardized estimate effect of the factors included in the final multivariable regression model after stepwise backward selection. An increased prevalence in rear lesion severity was related to batches that waited longer before being unloaded ( $p = 0.0002$ ) and were placed in lairage pens with higher space allowance per animal ( $p = 0.010$ ), with higher percentages of pigs showing panting at T1 ( $p = 0.0004$ ), with more animals displaying ear discoloration at T2 ( $p = 0.0006$ ), and with more frequent negative human–animal interactions at T2 (hit with plastic tube and shouts;  $p = 0.003$ ).



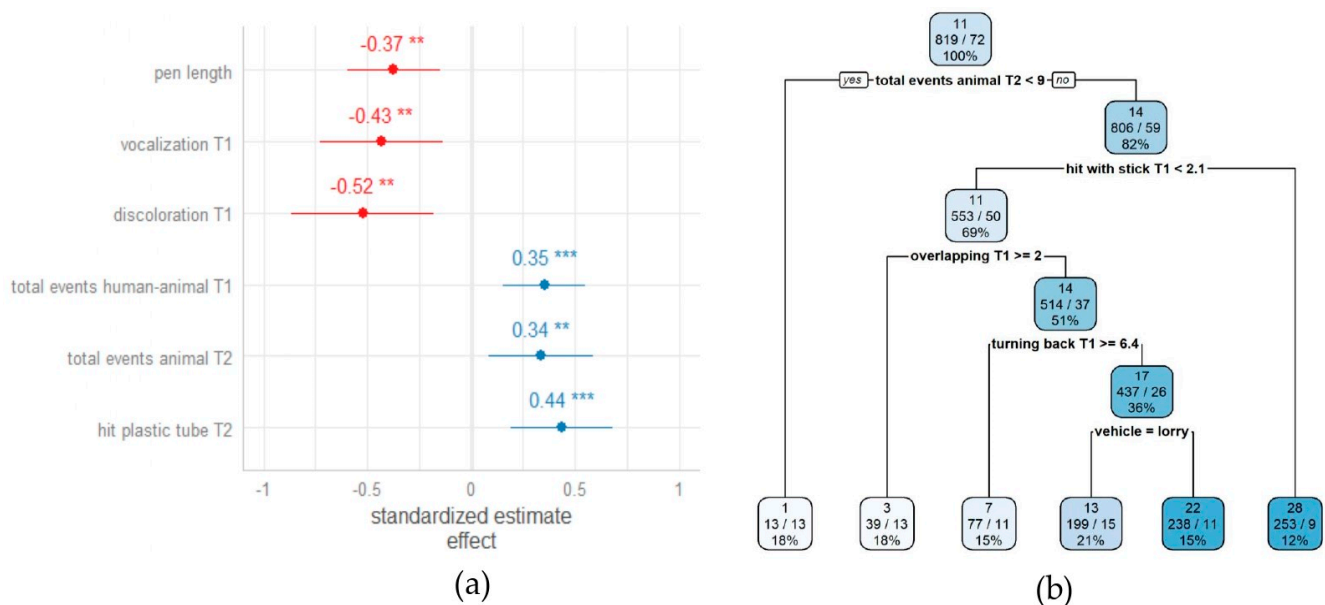
**Figure 2.** Association between pre-slaughter conditions and severe lesions in the rear region of heavy pig carcasses: (a) forest plot and (b) regression tree plot. In forest plot (a), \* stands for  $p < 0.05$ , \*\* for  $p < 0.01$ , and \*\*\* for  $p < 0.001$ .

Figure 2b reports the regression tree plot displaying the nodes affecting the most the variability observed among batches in the prevalence of carcasses with severe lesions in the rear region. Each rectangle reports the sum of the prevalence of carcasses with severe rear lesions for the batches remaining at each split step and the percentage of batches remaining at each split. Four variables remained in the classification obtained with regression tree analysis, generating four splitting nodes. The overall variability in the prevalence of severe rear lesions throughout batches was initially split on the total number of animal events at T2. The batches showing fewer than nine animal events at T2 (falling, slipping, turning back, huddling, discoloration in the different body regions, etc.) were those having a lower prevalence of severe rear lesions (13 batches). Among the batches showing more than nine animal events at T2, those with animals that waited more than 17 min before being unloaded exhibited higher prevalences of severe rear lesions (17 batches).

### 3.3. Association between Pre-Slaughter Conditions and Severe Lesions in the Middle Region of Heavy Pig Carcasses

The results of the univariable models for the prevalence of severe middle lesions are reported in Table S3. The prevalence of severe middle lesions was associated with increased frequencies of hit with stick at T1 ( $b = 0.152$ ,  $p = 0.0002$ ), higher frequencies of falling at T1 ( $b = 0.102$ ,  $p = 0.010$ ), the presence of sawing on the transport deck ( $p = 0.031$ ), longer waiting before unloading ( $b = 0.060$ ,  $p = 0.005$ ), and larger total number of human-animal events at T1 ( $b = 0.356$ ,  $p = 0.021$ ). Among the variables annotated at T2, increased prevalence of severe middle lesions was associated with larger total number of animal events at T2 ( $b = 0.135$ ,  $p = 0.0009$ ), increased frequencies of shouts ( $b = 0.132$ ,  $p = 0.0006$ ) and hit with plastic tube ( $b = 0.129$ ,  $p = 0.001$ ), higher percentages of animals showing back ( $b = 0.026$ ,  $p = 0.005$ ), ear ( $b = 0.049$ ,  $p = 0.013$ ), and side discoloration ( $b = 0.029$ ,  $p = 0.035$ ).

The results of the final multivariable regression model for the prevalence of severe lesions in the middle region of the carcass are graphically displayed with a forest plot in Figure 3a. An increased prevalence in middle lesions severity was related to batches that had experienced more negative human-animal interactions at T1 ( $p = 0.0008$ ), more animal events at T2 ( $p = 0.008$ ), and whose pigs received more hits with plastic tube at T2 ( $p = 0.0006$ ).



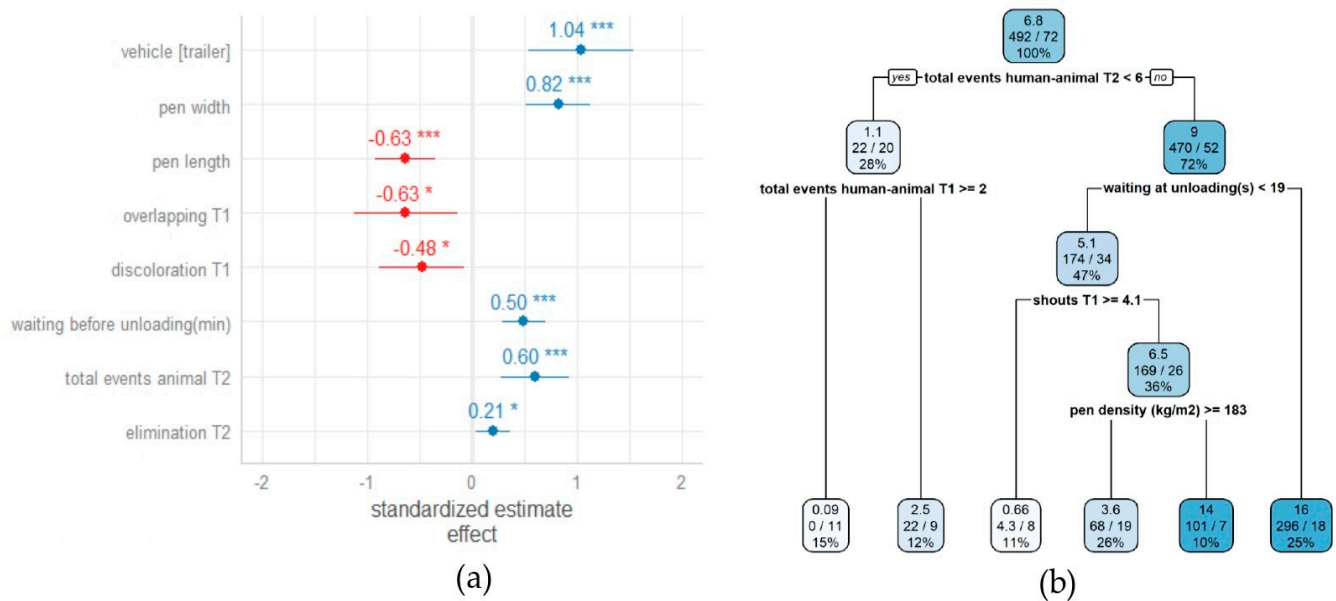
**Figure 3.** Association between pre-slaughter conditions and severe lesions in the middle region of heavy pig carcasses: (a) forest plot and (b) regression tree plot. In forest plot (a), \*\* stands for  $p < 0.01$ , and \*\*\* for  $p < 0.001$ .

Figure 3b reports the regression tree plot displaying the nodes most significantly affecting the variability observed among batches in the frequency of carcasses with severe lesions in the middle region (middle severe lesions). Five variables remained in the classification obtained with regression tree analysis generating five splitting nodes. The overall variability in the prevalence of severe middle lesions throughout batches was initially split on the total number of animal events at T2. The batches showing fewer than nine animal events at T2 (falling, slipping, turning back, huddling, discoloration in the different body regions, etc.) were those having a lower prevalence of severe middle lesions (13 batches). Among the batches showing more than nine animal events at T2, those with animals that experienced more frequent hit with stick at T1 ( $>2.1\%$ ) also had higher prevalences of severe middle lesions (nine batches). Of interest is also the fifth splitting node with the vehicle variable: batches transported in the trailer also had more carcasses with severe middle lesions (11 batches) compared to those transported in the lorry (15 batches).

### 3.4. Association between Pre-Slaughter Conditions and Severe Lesions in the Front Region of Heavy Pig Carcasses

The results of the univariable models for the prevalence of severe shoulder lesions are reported in Table S4. The prevalence of severe shoulder lesions was associated with increased frequencies of hit with stick at T1 ( $b = 0.158$ ,  $p = 0.006$ ), longer waiting before unloading ( $b = 0.052$ ,  $p = 0.014$ ), the transport in the trailer ( $b = 0.772$ ,  $p = 0.038$ ), ear discoloration at T2 ( $b = 0.065$ ,  $p = 0.013$ ), larger total number of animal events at T2 ( $b = 0.123$ ,  $p = 0.035$ ), and increased frequencies of shouts ( $b = 0.128$ ,  $p = 0.019$ ) and hit with plastic tube at T2 ( $b = 0.125$ ,  $p = 0.025$ ).

The results of the final multivariable regression model for the prevalence of severe lesions in the front region of the carcass are graphically displayed with a forest plot in Figure 4a. An increased prevalence in shoulder lesion severity was related to batches that were transported in the trailer ( $p = 0.0001$ ), waited longer before being unloaded ( $p < 0.0001$ ), rested in lairage pens with greater width ( $p < 0.0001$ ), and had experienced more animal events at T2 ( $p = 0.0004$ ). Those batches also had more pigs displaying elimination at T2 ( $p = 0.015$ ).



**Figure 4.** Association between pre-slaughter conditions and severe lesions in the front region (shoulder) of heavy pig carcasses: (a) forest plot and (b) regression tree plot. In forest plot (a), \* stands for  $p < 0.05$ , and \*\*\* for  $p < 0.001$ .

Figure 4b reports the regression tree plot displaying the nodes affecting the most the variability observed among batches in the frequency of carcasses with severe lesions in the front region (shoulder severe lesions). Five variables remained in the classification obtained with regression tree analysis generating 5 splitting nodes. The overall variability in the prevalence of severe shoulder lesions throughout batches was initially split on the total number of human–animal events at T2. The batches showing fewer than six human–animal negative interactions at T2 were those having a lower prevalence of severe shoulder lesions (20 batches). Among the batches showing more than six human–animal events at T2, those that waited longer (more than 19 min) before being unloaded had the most severe lesions in the front region of the carcass (18 batches). Lairage pen density was also shown to be an important variable for explaining shoulder lesion severity, with the batches that had a density lower than or equal to 183 kg/m<sup>2</sup> being the ones with high prevalences of severe lesions in the shoulder (7 batches).

#### 4. Discussion

This prospective study documented the prevalence of severe lesions in the rear, middle, and shoulder regions of carcasses obtained from heavy pigs slaughtered at a commercial abattoir and evaluated the associations between carcass severe lesions and pre-slaughter conditions. Longer waiting before unloading and improper handling practices at the abattoir were two of the major factors that were found to affect carcass lesion severity the most. Lairage pen space allowance was also found to affect rear and shoulder severe lesions, and the batches that were transported in the trailer had an increased prevalence of shoulder severe lesions compared with those transported in the lorry. Our results supported the hypothesis that besides mixing unfamiliar pigs, other pre-slaughter factors can have a major effect on carcass damage. The approach employed in the present study considered the rear, middle and front parts of the carcass separately, in agreement with the approach used in other studies [7]. This approach was selected as lesions in different anatomical locations can cause economic losses of different entities. In heavy pigs, severe lesions in the rear part of the carcass may be more detrimental, as defects and lesions which make trimming necessary cause the exclusion of the damaged thighs from the PDO circuit [44]. Therefore, the high economic losses caused by damaged pig carcasses [1–3] may further

increase when bruises are located on thighs intended for PDO ham production, as the value of PDO hams is 20 to 40% higher than that of non-PDO [12].

The average prevalence of severe lesions in the three anatomical regions was overall higher than the prevalence reported in the scientific literature. The prevalence of rear severe lesions identified in the present study (i.e., 6.92%) was higher than what was observed in Driessen et al. [7] (2.56%) in pigs slaughtered at about 106 kg live weight. In the present study, the percentage of unacceptable severe lesions in the rear region of heavy pig carcasses may also have been greater (i.e., 33.14%), considering that the PDO ham circuit requires thighs with intact skin, and hams coming from the rear scored as 2 are often discarded. Due to practical constraints, however, the fate of the thighs obtained from the carcasses considered in the present study remains unknown. Comparing the prevalence of severe lesions in the middle (i.e., 11.87%) and shoulder regions (i.e., 6.83%) found in our study with those reported in the literature [7,45], it is clear that severe lesions were much more frequent in our sample. This result seems to confirm that heavy pigs have features that increase the risk of damaged carcasses [12,13], and must be therefore handled taking into consideration the increased risks posed by the heavier bodyweight. This point is also of vital importance, considering the fact that a survey on consumer perceptions showed that the final consumer expects from PDO products not only better organoleptic quality but also greater attention to aspects concerning animal welfare [46].

Several associations between the pre-slaughter conditions and events annotated at the abattoir and the prevalence of severe lesions were identified. A higher prevalence of severe lesions in the rear region of heavy pig carcasses was noticed for the batches subjected to longer waiting time before unloading, more space allowance in lairage pens, and more frequent hitting with a stick when moved towards the stunning area. Other factors associated with increased severity in rear lesions were the higher percentage of panting animals at unloading (T1) and the higher prevalence of ear discoloration in pigs at T2. A longer stop on arrival, and therefore a longer wait before the pigs are unloaded from the truck to be taken to the lairage pens, is one of the main risk factors for carcass injuries [47,48]. During this period, pigs may engage in fights or cause lesions to themselves due to the stress experienced during transport [47,48]. Furthermore, longer waits can be responsible for increased fatigue in pigs [47,48], as also confirmed by the higher percentage of panting animals annotated at T1. Panting behavior in livestock is expressed when animals try to cope with heat stress [49,50] or prolonged stressful situations causing physical efforts and muscle exercise [51,52]. In the present study, panting can also be due to the stress caused by the short journey. Short journeys lasting less than 2 h were indeed associated with increased fatigue in pigs, which were more difficult to handle in abattoirs [14] and showed higher concentrations of cortisol and lactate in exsanguination blood [53,54]. These results can be related to the fact that pigs transported for such short journeys do not have sufficient time to recover from the stress of loading and are not able to get used to the vehicle environment before being unloaded. In addition to long waiting before unloading, another risk factor identified in this study is represented by lairage boxes with greater space allowance per pig. Similar results were reported by Guàrdia et al. [30] in pigs transported for short journeys (1 h). In those pigs, higher transport densities and thus less space allowance per animal were associated with decreased prevalence of low-quality pork. Petrolli et al. [55] indicated an association between the presence of larger stalls and a higher prevalence of carcass injuries, suggesting, however, that this is linked to the fact that larger stalls favor the mixing of unfamiliar animals, coming from different pens or farms. The presence of admixture between unfamiliar animals increases the possibility of hierarchical fights among pigs, increasing carcass injuries [1,55]. However, in our study, each lairage pen consisted of the same number of pigs (i.e., 23 or 24 pigs per group), and we avoided mixing animals from different batches. Despite that, pigs with larger space allowances in lairage pens may, however, engage in fights. This result was unexpected but may be explained by the fact that larger space allows more animals to move around, possibly causing more disturbance and aggression, in particular when animals are transported for short journeys (<2 h) and are

not able to recover from the loading and transport stress before being conducted to lairage pens. It is possible that the greater ear discoloration noticed at T2 in batches that also had more carcasses with severe rear lesions may have been caused by increased fighting in lairage pens. When fighting, pigs tend to target in particular the head, neck, and ear [56]. Fatigued and stressed animals are harder to move and handle in the abattoirs [14], which may have further increased the frequency of operators hitting the animals to make them enter the stunning area. However, painful stimuli such as the use of electric goads or hitting animals with a stick must be avoided [57], as they lead to more blemishes and lesions on the carcass [58]. In the present study, we observed that operators in some cases used plastic sticks and shouts to encourage pigs to move forward. The batches with animals that experienced more negative interactions with the operators were also those that had the highest prevalence of rear severe lesions, strongly supporting the need for more careful animal handling practices in abattoirs.

A higher prevalence of severe lesions in the middle region of heavy pig carcasses was noticed for the batches subjected to more frequent improper handling events at unloading (T1) and during the movement of pigs towards the stunning area (T2) and experiencing more animal events at T1. The improper handling practices may have caused the higher number of total animal events at T2 noticed with the regression tree analysis for severe middle lesions. Total animal events are indeed the total number of events, such as pigs falling, slipping, being reluctant to move, turning back, jumping, huddling, and pushing other animals. Those events are behaviors expressed in particular when pigs are experiencing fear and distress [59,60]. Events such as slipping and falling are associated with wounds and lesions on the pigs' bodies, which finally result in damaged carcasses. A consistent body of literature outlines the association existing between animal handling, stressed animals, and low-quality meat and carcasses. Brandt et al. observed a positive correlation between handling (with pigs slipping and falling) in the race towards the stunning area and the plasma concentration of glucose and lactate in blood gathered at exsanguination [61]. Similar results have also been found by other authors [29,62,63], suggesting that those events, coupled with the fasting period applied before slaughter, can cause stress and wounds on pigs' bodies, ultimately resulting in lower pork quality and damaged carcasses. Handling practices are of primary importance in reducing stress in animals transported to the abattoir, and despite the negative welfare and meat quality implications of improper handling practices (such as the use of electric goads and sticks or tubes), operators often continue using those tools to manage movements of pigs at loading, unloading and in the race toward the stunning area. Correa et al. [64] tested the effect of different handling devices, comparing the effects on meat quality and animal welfare of the use of an electric goad or board and paddles for moving the pigs at the abattoir. The authors clearly found the use of electric goads had a detrimental effect on pork quality and carcass damage [64]. Our results thus confirm those reported in the literature, and strongly emphasize the need to apply more careful handling practices at unloading and in the abattoir in order to protect both the welfare of the animals and the quality of the carcass.

A higher prevalence of severe lesions in the shoulder of heavy pig carcasses was noticed for the batches transported in the trailer, subjected to a longer waiting time before unloading and lairage in wider pens. The effects of waiting time before loading and pen space allowance on carcass lesions have already been discussed. This association seems to confirm the hypothesis that longer waiting and wider lairage pens may have caused more fights among the pigs within each batch, as lesions on the neck and head are typically caused by fights and aggressions between pigs [55]. Concerning the effect of the trailer identified in the present study, several authors have already investigated the effects of vehicle design on pig welfare and carcass lesions [1,27,38,65]. Our results are in agreement with evidence reported in previous studies, highlighting that pigs transported in the rear region of the vehicle are more likely to lose balance, fall, or get trampled, resulting in a higher prevalence of lesioned carcasses [66,67]. Furthermore, higher stress levels were observed in pigs transported in a twin-axle trailer when compared with pigs transported

in a fixed body truck [68]. The association we found between trailer transport and shoulder severe lesions may thus be the effect of the lesions and physical stress caused by the necessity of the animals to keep the standing position and balance while coping with the vibrations and movements of the trailer. Compared to the lorry, which is directly bound with the driver's cabin, the trailer may have increased the animals' instability, resulting in more falls and an increased likelihood of injury in the front region of the animals' bodies. Some of the effects we observed in carcasses from pigs transported in the trailer may also be due to higher vibrations, which could be reduced by a right driving style and trucks with improved suspension systems, as suggested in the study by Dalla Costa et al. [69].

Our results increase the knowledge about the prevalence of carcass damage in heavy pigs and suggest possible factors associated with the severity of lesions in the rear, middle and front region of the carcass. The present research completes the observations reported in our previous study, in which we highlighted the effect of environmental parameters such as temperature and humidity on the surface temperature of pigs and on the skin damage score, which appeared to increase with increasing Temperature Humidity Index [38]. The results obtained should, however, be considered in the light of some limitations. The present study considered lesions to the posterior carcass region, and due to practical constraints, it was not possible to follow the thighs obtained from the considered carcasses nor obtain information about their fate in the PDO circuit. It could therefore be of interest for future studies to consider more specifically the percentage of hams discarded from the PDO circuit, and to associate this prevalence with pre-slaughter conditions. Furthermore, in the present research, we considered animals from a single farm and slaughtered in an abattoir. This choice was due to the fact that we wanted to eliminate the possible effect of different farms so that we could only evaluate the effects of the pre-slaughter factors taking place in the abattoir. It will therefore be important to perform further studies taking into account animals from different farms and slaughtered in different abattoirs. Another point that should be considered is that we did not perform a visual evaluation of the animals before they were transported to the abattoir or during transportation, which makes it difficult to understand with certainty the source of the injuries caused in the 24 h prior to slaughter. Future studies should also consider events and animals' interactions during loading and transport. Finally, animal behaviors and events were noted by an operator that noted what was visible from the observation points. In order to avoid causing any problems to routine activities during the unloading and handling phases, it was not possible to have multiple observation points with different operators. This point may have limited, in some cases, the accuracy of the observations. Notwithstanding these limitations, this is the first study investigating the pre-slaughter factors associated with the severity of lesions in the rear, middle, and shoulder regions of heavy pig carcasses.

## 5. Conclusions

Overall, this prospective study documented that waiting before unloading, improper handling operations, and lairage pen space allowance were the major factors associated with a higher prevalence of severe lesions in heavy pig carcasses. It is, therefore, essential to shorten as much as possible the time spent by animals waiting to be unloaded and moved toward the stunning area, in order to avoid fights and other events that could lead to welfare issues and damaged carcasses. Additionally, pre-slaughter animal handling is of great importance, and abattoirs should reinforce educational programs to train operators for better and more careful management of animals in the abattoir. Furthermore, providing animals with a larger space allowance during a short lairage time (30 min) may pose additional risks to animal welfare and carcass quality, as larger space allows fatigued and stressed animals to move around, possibly causing more disturbance and aggression. Our results confirm that even by avoiding mixing unfamiliar animals, there are other risk factors that can affect the welfare of heavy pigs at the abattoir, causing detrimental effects on the quality of the carcass. Such factors must be taken into consideration by the abattoirs supplying cuts for the PDO ham circuit.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app12031078/s1>, File S1: the data sheet used to annotate events; File S2: Results of the pairwise correlations among all variables annotated at T1 and T2 and the prevalence of carcasses scored with the DMRI scale. Correlation coefficients are reported in the first sheet, and the relative *p*-values are reported in the second sheet; Table S1: Description of the four-point scale used to classify heavy pig carcasses; Table S2: Associations between the dependent variable of the prevalence of carcasses with rear severe lesions and the independent variables of pen conditions and pre-slaughter events at unloading (T1) and before stunning (T2); Table S3: Associations between the dependent variable of the prevalence of carcasses with middle severe lesions and the independent variables of pen conditions and pre-slaughter events at unloading (T1) and before stunning (T2); Table S4: Associations between the dependent variable of the prevalence of carcasses with shoulder severe lesions and the independent variables of pen conditions and pre-slaughter events at unloading (T1) and before stunning (T2).

**Author Contributions:** Conceptualization, A.A. and L.N.C.; methodology, B.P. and L.N.C.; formal analysis, M.Z. and L.M.; investigation, A.A., V.P., B.P., L.M., M.Z. and L.N.C.; resources, V.P.; data curation, A.A., L.N.C. and M.Z.; writing—original draft preparation, M.Z. and L.N.C.; writing—review and editing, B.P., L.M. and L.N.C.; visualization, M.Z.; supervision, L.N.C.; project administration, L.N.C.; funding acquisition, L.N.C. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The observations annotated for the present study involved animals transported to a commercial abattoir and intended for human consumption. No pigs were killed specifically for the study. Before the experiment began, the procedure was explained to the abattoir staff. No tissue or any other sample was collected; therefore, according to the EU legislation DL n. 26, 24/03/2014, there was no need for the approval of the experimental protocol by the Italian Health Ministry.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

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