Risk factors for bruising and mortality of broilers during manual handling, module loading, transport, and lairage

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Abstract: Multiple factors can affect the risk of bruising and mortality of broilers during loading, transport, and lairage. The risk factors affecting the percentages of broilers in each load that were "dead-on-arrival" (DOA) or bruised were studied from records provided by a processing plant, by undertaking direct observations during on-farm loading and then carrying out multivariable analyses. Selected loads between 2014 and 2015 from seven producers were included in the study. The median DOA per load was 0.13% ($Q_1 = 0.06$, $Q_3 = 0.25$, $n = 212$), the median total duration from loading to unloading was 8.6 h, and the external temperature ranged from −22 to 22 °C. Although it was not possible to adequately characterise thermal conditions within each load, the analysis indicated that the main risk factors for increased mortality were in spring and winter, an increased duration between loading and end of lairage, and a period of feed withdrawal before loading longer than 6 h. The risk of mortality increased with the weight of the birds and with an increase in rearing mortality. No relationships were found between the manner in which the broilers were handled and the percentages of DOAs or bruised birds.

Key words: broilers, bruising, handling, mortality, transport.

Résumé : De multiples facteurs peuvent avoir un effet sur les risques de contusions et la mortalité des poulets à griller pendant le chargement, le transport et le temps dans les installations d'attente. Les facteurs de risque ayant un effet sur le pourcentage de poulets de chaque chargement qui étaient morts à l'arrivée (DOA — « dead-on-arrival ») ou contusionnés ont été étudiés à partir des registres fournis par l'usine de traitement, ou en observant directement lors du chargement à la ferme, puis en effectuant des analyses multivariées. Des chargements provenant de sept producteurs entre les années 2014 et 2015 ont été inclus dans cette étude. Les DOA médians par chargements étaient de 0,13 % ($Q_1 = 0,06$, $Q_3 = 0,25$, $n = 212$), la durée totale médiane du chargement au déchargement était de 8,6 heures, et la température externe variait de −22 à 22 °C. Bien qu'il n'était pas possible de caractériser adéquatement les conditions thermiques à l'intérieur de chaque chargement, l'analyse a indiqué que les principaux facteurs de risque pour la mortalité accrue se trouvaient au printemps et à l'automne, une durée accrue entre le chargement et la fin du temps d'attente, et une période de temps de plus de 6 h avec retrait de nourriture. Le risque de mortalité a augmenté avec le poids des poulets et avec une augmentation de la mortalité à l'élevage. Aucune relation n'a été trouvée entre la manière dont les poulets ont été manipulés et les pourcentages de DOA ou de poulets avec contusions. [Traduit par la Rédaction]

Mots-clés : poulets à griller, contusions, manutention, mortalité, transport.

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Introduction

In Canada, in 2017, the average mortality [dead-on-arrival (DOA)] of the 659 million broilers transported from farm to a federally inspected processing plant was 0.2% (Agriculture and Agri-Food Canada 2017). This mortality has financial and animal welfare implications (Cockram and Dulal 2018). As multiple factors affect the risk of bruising and mortality of broilers during loading, transport, and lairage (Cockram and Dulal 2018), further information is required on how these factors interact during specific environmental conditions and industry practices. Identification of factors affecting mortality and injury and their relative significance would be useful for continuous improvement in industry practices and for the formulation of codes of practice and the regulation of broiler handling and transport in Canada. Mortality in a load (DOA) is considered to be influenced by the following three main factors: (a) health status of the flock, (b) thermal stress, and (c) physical injury during catching and loading (Bayliss and Hinton 1990). Multivariable studies have identified a range of risk factors that can increase the risk of mortality. These include (a) mortality rate during rearing, method of catching, crate stocking density, and weather conditions (Chauvin et al. 2011), (b) breed, catching team, loading, and transporting during the day compared with the night, ambient temperatures ≤5 or >15 °C and increasing flock size, live weight, module stocking density, journey duration, and lairage duration (Nijdam et al. 2004), and (c) bird age and weight, catching team, journey duration and holding barn duration, and cold external temperatures (Caffrey et al. 2017). Whether a particular risk factor is identified as significant in a particular study is likely to be dependent on the range of environmental variables, for example, temperature and journey duration, the characteristics of the birds, handling, transport, and lairage procedures, and the robustness of the multivariable analyses (Cockram and Dulal 2018).

Catching and carrying a broiler inverted by its legs can cause wing flapping and struggling (Newberry and Blair 1993) and sometimes injury and death (Gregory and Austin 1992; Nijdam et al. 2006). Variation between the manner in which different catching teams handle the birds can affect DOA % (Nijdam et al. 2004) and the percentage of birds with bruising (Taylor and Helbacka 1968; Langkabel et al. 2015; Jacobs et al. 2017). Bruising is a superficial injury that occurs after trauma (Hamdy et al. 1961). However, after slaughter and defeathering, it can be difficult to differentiate bruising due to trauma from haemorrhage in muscles that can occur between rearing and processing from causes unrelated to trauma (Kranen et al. 2000; Cockram and Dulal 2018). Bruising can also occur at the plant during handling and slaughter (Gregory and Bell 1987; Gregory et al. 1989; Gregory 1994).

This study used in-barn observations of handling and multivariable analyses of processing plant data to identify risk factors for bruising and mortality, when flocks of broilers were loaded using a modular handling system and then transported to slaughter under a range of external environmental conditions and journey durations. The processing plant provided data on the percentages of loads from these flocks that were DOA, bruised, and condemned.

Materials and Methods

Study design

A cross-sectional study design was used to study risk factors that influenced the percentages of broiler chickens per trailer load (a) that were DOA, that is, found dead in the module drawers before stunning at a processing plant and (b) the percentages of birds per trailer load that were observed with leg and wing bruising after slaughter and feather removal. Potential risk factors for bruising and mortality during handling, transport, and lairage were quantified for selected flocks from records provided by the processing plant on these flocks and by undertaking direct observations during on-farm loading of these flocks. In study 1, loads from producers located in a different province from that of the processing plant were studied over a 13 mo period to enable seasonal effects to be examined in loads where the journey durations were not short. In study 2, short journeys of loads from producers located near to the processing plant were examined over a 2 mo period and compared with selected loads from study 1 that involved longer journeys conducted at the same time of year.

Selected loads of broiler chickens (number of loads = 212) transported between April 2014 and April 2015 from seven broiler producers located in one Canadian province to a processing plant in another province were included in study 1. Between 9 and 30 loads were observed during each calendar month. Loads were observed when personnel were available and when information on the time and date of the loadings were provided by the plant.

Each trailer load of broilers consisted of birds that had been raised on the same site and transported on the same date to the processing plant. Most loads consisted of broilers from the same barn and often from the same floor. Loads were observed from 13 different barns (between 4 and 33 loads per barn over the duration of the study). On some occasions, the load was composed of birds from two floors of the same barn or more than one barn. Where a load consisted of birds from more than one floor, the data for each floor were combined using a weighted average based on the proportion of birds in the load from each floor. There were between two and seven loads (median four loads) involved in a given loading event, that is, loads from the same site on the same date (total number of loading events = 55).

Table 1. Categorisation of loading arrangements.

Loading category	Floor	Module placement	No. of loads in study 1	Description
1	Lower	Inside	77	Modules moved by a forklift entering through the main barn doors
2	Upper	Inside	73	Modules moved through one of several side doors by a forklift remaining outside of the barn
3	Lower/upper	Outside	23	Modules placed outside near one of several side doors either on a stand or the ground. The birds were transferred by the handlers to one or more handlers standing outside, near the modules, who then placed the birds in the module drawers
4	Lower/upper	Mixed	38	Mixed or unclassified

For comparison, eight loading events were also observed over a 2 mo period (between February and April 2015), comprising a total of 40 loads from eight barns in broiler units located near the processing plant (study 2).

Broilers and rearing

Data were provided by the processing plant on the rearing mortality, age, and weight of the birds at the time of loading. The birds observed were from mixed-sex flocks of broilers (Ross 380, Aviagen, Huntsville, AL, USA) raised on wood shavings litter in heated and mechanically ventilated barns with automatic provision of food and water. The moisture content of the litter on the barn floor was measured based on a method described by Fairchild and Czarick (2011). After loading, samples of litter (about 100 g) were collected from eight areas of each floor (near the entrance, the middle of the floor, each corner of the floor, and underneath a set of fans) and placed in previously weighed metal foil containers. After all observations of loading had been completed, the containers were transported to the laboratory where they were stored frozen at −20 °C until analysis. Each container containing a litter sample was weighed, dried in an oven at 110 °C for 24 h, and then reweighed. The percentage of moisture concentration of the litter was calculated as [(wet litter weight − dry litter weight)/wet litter weight] × 100.

Loading

The date and timing of slaughter were determined by the processing plant, and the producers were notified of the times for food and water withdrawal before catching. In study 1, loading occurred between 1900 and 0500 (76% of loads were loaded between 2100 and 0100). For birds transported a short distance to the processing plant (study 2), loading occurred between 2000 and 1600 with 83% of loads loaded between 0100 and 1100. On some occasions, all of the birds that were determined by the catchers to be alive, fit, and of the required size were loaded, whereas on other occasions, the floor was "thinned", that is, only some of the birds were loaded,

and others left at a lower stocking density for further rearing, and these were loaded on a subsequent occasion. An experienced catching team (6–12 persons), provided by the processing plant, manually caught the birds in the barn and loaded them using a modular system. The modules (Maxiload® STD, Maxitech, Cellatica, Italy) consisted of 6, 8, or 10 plastic drawers (1.21 m × 1.29 m × 0.22 m) set in a metal frame on a metal pallet. The upper floor of a barn was loaded before the lower floor. Before the start of catching on each floor of the barn, some members of the catching team moved the birds away from the walls of the barn and towards the catching areas by walking through the flock, and then the lights were dimmed. A forklift truck moved each module in turn either onto the barn floor or immediately outside of a side door, so that the modules were evenly spaced along the length of the barn. The procedure used for loading varied between floors, barn design, biosecurity requirements associated with thinning, and availability of functional equipment. The loading arrangements were categorised as shown in Table 1.

The birds were caught by a handler who knelt down and caught each bird by both legs until 3–4 birds had been caught in each hand. The handler then carried the birds in an inverted manner to the nearest module drawer. One member of the catching team opened each drawer on the module in turn and monitored the placement of the birds by the other members of the catching team. Once the predetermined number of birds had been placed in a drawer, the arrangement of the birds in the drawer (e.g., their vertical orientation, protrusion of body parts, and dispersion) was checked and, if necessary, adjusted, and the drawer was closed. When all the drawers in a module were full, the team moved to the next module and the forklift truck moved the full module outside to a transport trailer near the barn, where the module was stacked on the flatbed of the trailer. After all of the filled modules had been stacked on a trailer, the driver adjusted the screens or tarpaulins on the trailer and then drove the loaded trailer to the processing plant. Another trailer was moved near the

Table 2. Handling categories used to characterise the manner in which birds were placed in module drawers by handlers.

Handling category	Description on how handler placed their birds into the drawer
Movement 1	Moved arm slowly and released the birds while hand was less than one bird length outside of the drawer
Movement 2	Horizontal movement of arm and released the birds while hand was at least one bird length outside of the drawer
Movement 3	Released the birds vertically while hand was at least one bird length above the drawer
Movement 4	Moved arm in a rapid curved movement and released the birds while hand was at least one bird length outside of the drawer

barn and, while the driver adjusted the empty modules in preparation for loading, the handlers usually took a break of about 30 min. Once loading of birds from a floor had been completed, the team moved to the next floor of the same or another barn on the same site.

Behavioural observations of handlers and birds during module loading

Before the study, consent to participate in the study was obtained from the handlers. They were informed of the purpose of the study and that individual confidentiality would be maintained. A pilot study was undertaken to determine what was feasible to record during loading. During catching, observation of the birds was very difficult because it was dark; the birds were handled quickly and individual handlers could not be readily identified. We found it feasible to record the manner in which the birds were placed in the module drawers and a limited number of behavioural responses of the birds during this procedure.

Three individuals undertook observations with between one and two observers per loading observation. One observer stood near the door of each floor and scored the activity level of the birds at the start of catching as follows:

- Score 1 — most of the birds were lying down, there was no movement, and the birds were not noisy.
- Score 2 — most of the birds were standing, some of the birds were moving, and the birds were not noisy.
- Score 3 — most of the birds were standing, at least half of the birds were moving, and the birds were making some noise.
- Score 4 — most of the birds were moving, there was some wing flapping and some neck extension, and the birds were very noisy.

At one point in time during catching, one observer stood near the birds and scored the response of the birds to catching as follows:

- Score 1 — most birds did not move during catching by the handler.

- Score 2 — most birds stood up during catching, but did not move more than a bird length away from the handler.
- Score 3 — during catching, most birds moved more than a bird length away from the handler and some wing flapping was present.

An observer directly and continuously monitored the loading of individual modules. Due to the speed with which modules were loaded, and the overlap between the completion of one module and the start of the next, it was not possible for one observer to observe the loading of all modules. When a single observer undertook observations, alternate modules were selected and, if two observers were involved, they selected different alternate modules. In study 1, 1368 modules were observed, the median number of modules observed per load was 8 ($Q_1 = 4$, $Q_3 = 9$, $n = 168$ loads), and in study 2, 185 modules were observed, the median number of modules observed per load was 4 ($Q_1 = 4$, $Q_3 = 8$, $n = 32$ loads). For each module observed, the time of day, the size of the module (6, 8, or 10 drawers), and the duration of loading was recorded using a Psion Organiser LZ64 (Psion Ltd., UK) and Observer software (Noldus Information Technology, Wageningen, the Netherlands).

The manner in which the birds were placed in module drawers was categorised as one of the movements shown in Table 2, and the number of times per module that a handler was observed to place each handful of birds in each manner was recorded. As it was not possible to record the movements used by all handlers to load all birds that were loaded per module, the percentages of the total number of movements (i.e., movement $1 + 2 + 3 + 4$) for each category of movement recorded per module were calculated. If one or more birds within a handful of birds were observed to flap their wings vigorously while being placed in a module drawer, this was recorded. The percentage of handfuls of birds per module that wing flapped was calculated as the percentage of the total number of movements (i.e., movement $1 + 2 + 3 + 4$) recorded per module.

For each load, the times that food and water were withdrawn before the start of loading, the times of the start and end of loading, the number of birds placed

per drawer, the number of birds per load, and the ambient temperature in the barn at the start of loading were provided by the processing plant. The speed of loading was calculated as the number of birds loaded onto a trailer/number of catchers/duration of loading (birds catcher^{-1} h^{-1}). The module stocking density was calculated as the number of birds put into each module drawer and the weight (kg) of birds per drawer (No. of birds × weight of birds). Except for after thinning, at the end of loading, the number of live birds that had been rejected by the handlers (e.g., too small) and the number of dead birds observed per floor were recorded.

Weather data for the end of loading were obtained from historical data recorded at the nearest Government of Canada weather station (Government of Canada 2017). Precipitation was categorised as dry, rain, or snow. The external temperature was categorised as cold <0 °C, medium 0–10 °C, or warm >10 °C, and relative humidity was categorised as low ≤90% or high >90%.

Journey to processing plant

The trailer consisted of a flat-bed with a solid front, back, and roof. For study 1, the processing plant provided information on the temperature (during loading, the journey, and while waiting for unloading at the processing plant) that was recorded from up to four sensors placed near the roof of each trailer. Unfortunately, not all sensors were operational. Data cleaning to provide valid data to calculate a mean trailer temperature from at least three readings from the four sensors for each journey was undertaken. Recordings were only used from sensors that before loading recorded a temperature that was not more than 5 °C different from the external temperature and within 5 °C of the average obtained from the other sensors. The information provided on the tarpaulin position during the journey was categorised as closed on both sides of the trailer or open/mixed (left or right side of the trailer in a different position, position changed during the journey or partially open). The driver had the option of opening or closing three ventilation doors on the front and the back of the trailer. The position of these ventilation openings were categorised as position 1 — all open for all of the journey/top front and back top open and back left and right sides open or partially open; position 2 — top front and back top open and back left and right sides closed or partially open or adjusted during journey, or mixed; position 3 — top front and back top partially open or adjusted during journey and back left and right sides closed/all closed for all of the journey. The number and duration of stops made during the journey, and the condition of the birds (dry or wet) were recorded by the driver.

Lairage

After the load had arrived at the processing plant, the trailer was moved into the lairage area. In the lairage, the modules were removed from the trailer and restacked. Mechanical fans and heaters were used in the lairage to adjust the temperature and ventilation. From information provided by the processing plant, the mean lairage temperature for each load was calculated. The percentage maximum capacity of the use of fans in the lairage was recorded as a mean of the following percentages: % of intake fans used + % of extractor fans used + % maximum speed of intake fans used + % maximum speed of extractor fans used. At the required time, each module was then moved onto an automatic handling system for transfer into the slaughter area.

Slaughter

Each drawer was removed from the module and moved along a conveyor belt. Each drawer was examined by processing plant personnel and DOA birds were removed from the drawer and counted. The conveyor belt then moved each drawer to a carbon dioxide stunning chamber (Maxitech, Cellatica, Italy). After stunning, the birds were removed from the drawers, inverted and placed on a shackle line. The birds were then slaughtered by exsanguination. The carcases were processed by de-feathering and removal of the head, feet, and viscera. When possible, a sample of 100 birds from each load was visually examined by the processing plant quality control team, and the number of birds with leg and (or) wing bruising recorded. The following condemnation data provided by the processing plant were used to provide an assessment of the health status of the birds: percentage of load condemned, percentage of load with abdominal oedema, and percentage of load with a liver condition (CFIA 2017).

Statistical analysis

One load in study 1 had an abnormally high DOA of 6.59%. The driver report for that load indicated that there had been an unusually long stop during the journey caused by a Department of Transport inspection of the load (total duration stopped during the journey was 0.85 h compared with a mean duration of only 0.33 h). This one load was excluded from subsequent analysis as it was a clear "outlier". The DOA values were not normally distributed as they were skewed by a large number of loads with very low DOA % values. As the conditions related to loads from study 1 and those from study 2 were different, separate analyses of risk factors for bruising and mortality were conducted.

In study 1, a series of risk factors were considered, which may have influenced the risk of mortality, as reflected in the percent DOA values reported at the processing plant. The associations between the DOA values recorded for each load and the potential risk factors were investigated using a multilevel linear mixed model. As the percent DOA values were not normally distributed, a natural logarithmic transformation was applied. To generate an outcome variable with skewness close to

Fig. 1. Histogram indicating the frequency distribution of loads with differing levels of percentage dead-on-arrival (DOA) in study 1 and study 2.
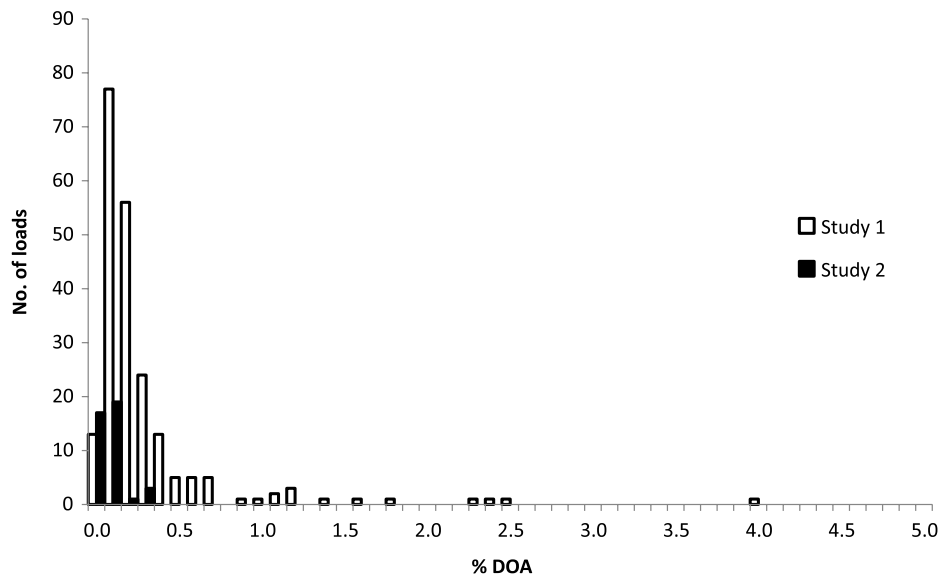
zero, the transformation ln(DOA + k) (with k = 0.011) was applied. In addition to helping meet the required model assumptions, this transformation allowed for the retention of loads which had a reported DOA percentage value of zero.

For study 1, the data had a hierarchical structure with each load (n = 212) belonging to an event (n = 55) which consisted of all loads from the same location on a given day. Each event was associated with a specific producer (n = 7), but this potential third level grouping was dealt with by treating this factor as a fixed effect. Likelihood ratio tests were used to assess the significance of the random effect, and intraclass correlation coefficient (ICC) values were reported. Associations between the outcome variable (percent DOA) and 16 risk factors were assessed using unconditional linear mixed models, with six factors and one interaction term demonstrating an association (P < 0.2) being retained for potential inclusion in the final model. The linearity of all continuous predictors was assessed using plots of the residuals against explanatory variables together with lowess smoothed plots; with the application of a suitable transformation where variables were found to be nonlinear. Categorical predictors were similarly assessed using unconditional models that included the random effect of event.

The final multivariable, linear mixed model was fitted to 206 loads (there was incomplete data for six loads) using restricted maximum likelihood estimation with the modelled results being reported on the natural log scale. Sets of marginal predictions were created from the model; these were back-transformed to illustrate estimates of the effect of interest on the outcome measure (DOA %). Graphs demonstrating these effects were generated to indicate the relationship between the

predictor at different levels on the percent DOA, with vertical error bars indicating the 95% confidence intervals.

For study 2, comparisons were made using Mann–Whitney tests between loads transported a short distance to the processing plant with those transported from another province to the processing plant. As they were conducted during approximately the same time of year, the last eight loading events from study 1 consisting of 40 loads were selected for comparison with the eight loading events and 40 loads observed in study 2.

Approval for the project

Approval for this study was obtained from the University of Prince Edward Island's (UPEI) Animal Care Committee and Research Ethics Board. The work was undertaken within a confidentiality agreement between UPEI and the processing plant.

Results

DOA distribution

Figure 1 shows the frequency distribution of DOA % from loads in study 1 and study 2. In study 1, the median DOA % per load was 0.125 (n = 212, Q_1 = 0.06, Q_3 = 0.25, range 0–3.92). In study 2, the median DOA % per load was 0.019 (n = 40, Q_1 = 0, Q_3 = 0.06, range 0–0.29).

Study 1: Risk factors for DOA
Descriptive statistics and unconditional associations
Broilers and rearing

Most of the variability in DOA values was at the level of the load rather than at the event or producer levels. Table 3 provides descriptive statistics on the weight and age of broilers, litter moisture, and health measurements. In unconditional associations, the DOA increased

Table 3. Descriptive statistics on the weight and age of broilers, litter moisture, and health measurements.

Variables	No. of loads	Minimum	Q_1	Median	Q_3	Maximum
Bird						
Weight (kg)	212	1.66	2.14	2.22	2.43	2.72
Age (d)	212	30	35	36	38	39
Health (%)						
Rearing mortality	212	0.74	2.90	3.22	4.86	23.9
Birds found dead at end of loading	157	0.01	0.06	0.11	0.16	0.81
Live birds rejected during loading	157	0.00	0.02	0.07	0.14	0.34
Litter moisture	176	22	32	36	42	59
Total condemnations	212	0.09	0.45	0.65	0.90	3.29
Condemnations due to abdominal oedema	205	0.00	0.07	0.14	0.24	1.08
Condemnations due to liver condition	205	0.00	0.01	0.02	0.03	0.53

Table 4. Descriptive statistics for variables related to loading.

Variables	No. of loads	Minimum	Q_1	Median	Q_3	Maximum
Duration of feed withdrawal before loading (h)	211	0.08	2.75	3.95	5.52	9.57
Duration of water withdrawal before loading (h)	210	0.05	0.98	2.00	3.83	7.33
Duration of loading (h)	212	0.57	0.92	1.08	1.23	2.25
Speed of loading (No. birds catcher^{-1} h^{-1})	211	146	643	751	887	1,445
Speed of module loading (birds s^{-1})	162	0.96	1.85	2.09	2.39	3.17
Stocking density						
(No. birds per drawer)	212	28	32	35	36	40
(Weight kilogram per drawer)	212	64.6	74.1	77.0	79.5	84.3
No. of birds loaded per trailer	212	3,292	6,010	6,600	6,840	8,320
Barn temperature (°C)	194	0.0	7.3	15.6	19.9	28.1
External temperature (°C)	211	−22.6	−2.2	6.1	12.4	22.3
Difference between barn and external temperature (°C)	193	−3.2	5.9	8.2	12.9	27.2
External relative humidity (%)	211	41	80	88	95	100
External wind speed (km h^{-1})	211	1	8	12	18	36
Trailer temperature (°C)	64	−14.7	6.6	11.5	15.1	22.4

significantly with bird age ($P = 0.001$) and weight ($P < 0.001$). Increased rearing mortality and increased condemnations due to abdominal oedema were also associated with significantly increased DOA values. The estimated percentage of litter moisture content had no significant effect on DOA.

Loading

Descriptive statistics for variables related to loading are shown in Table 4. When considered as continuous variables in unconditional associations, there were no significant effects of duration without feed or water before loading on the DOA. However, when the duration of feed withdrawal before loading was categorised as either ≤6 or >6 h, there was an increased mortality risk with the longer duration of feed withdrawal. In unconditional associations, a longer duration of loading increased the mortality risk ($P = 0.03$). Increased speed of loading (No. of birds catcher^{-1} h^{-1}) decreased the mortality risk ($P = 0.002$). The loading arrangements did not

have a significant effect on DOA, nor did the manner in which birds were handled and loaded (Table 5). Increased stocking density (weight of birds per drawer) increased the mortality risk ($P = 0.01$). When the external temperature at the end of loading was >10 °C, and the relative humidity was ≤90%, the mortality risk was lower than at colder temperatures ($P = 0.02$). Season had a significant ($P = 0.001$) effect on DOA in that during summer the mortality risk was lower compared with the winter.

Journey

Descriptive statistics for trailer temperature and journey duration are shown in Table 6. In unconditional associations, there was a trend for increased mortality risk with journey duration ($P = 0.06$) and duration of stops during the journey ($P = 0.07$). When the tarpaulin was closed on both the left and right sides of the trailer, the mortality risk increased ($P = 0.01$) compared with when it was not completely closed. The position of the ventilation openings at the front and back of the vehicle

Table 5. Effect of loading category on bird handling, dead-on-arrival (DOA %), and % bruising.

Loading category		1			2			3			4		
Floor		Lower			Upper			Lower/upper			Lower/upper		
Module placement		Inside			Inside			Outside			Mixed		
No. of loads		77			73			23			38		
		Q_1	Median	Q_3	Q_1	Median	Q_3	Q_1	Median	Q_3	Q_1	Median	Q_3
Score of bird activity at start of catching[a]		1	1	2	1	1	2	1	1	2	1	1	2
Score of bird response to catching[b]		1	1	1	1	1	1	1	1	1	1	1	1
Speed (No. of birds catcher^{-1} h^{-1})		638	730	853	422	790	926	457	686	859	569	683	808
Manner in which birds were	Movement 1[c]	78.1	89.6	96.9	79.3	85.3	93.3	82.3	94.2	98.9	84.1	89.8	96.5
placed in module	Movement 2[d]	2.7	5.2	14.7	3.0	6.7	10.2	1.0	2.3	7.4	3.0	5.5	11.2
(% of the total number of	Movement 3[e]	0	0.5	3.8	0.8	2.3	8.1	0	0	4.2	0	0.5	1.7
movements observed)	Movement 4[f]	0	0	3.1	0.2	2.6	5.3	0	0	0.6	0	0.6	1.9
Wing flapped[g] (% of the total number of movements observed)		2.6	6.4	9.1	4.2	6.0	8.9	1.8	6.0	9.5	3.9	6.1	9.3
DOA (%)		0.06	0.14	0.26	0.06	0.14	0.27	0.04	0.06	0.14	0.08	0.15	0.30
Wing bruising (%)		1	2	4	1	2	3	1	2	4	1	2	4
Leg bruising (%)		0	0	0	0	0	0	0	0	0	0	0	0

[a]Score 1 — most of the birds were lying down, there was no movement, and the birds were not noisy; Score 2 — most of the birds were standing, some of the birds were moving, and the birds were not noisy; Score 3 — most of the birds were standing, at least half of the birds were moving, and the birds were making some noise; Score 4 — most of the birds were moving, there was some wing flapping and some neck extension, and the birds were very noisy.

[b]Score 1 — most birds did not move during catching by the handler; Score 2 — most birds stood up during catching, but did not move more than a bird length away from the handler; Score 3 — during catching, most birds moved more than a bird length away from the handler, and some wing flapping was present.

[c]Moved arm slowly and released the birds while hand was less than one bird length outside of the drawer.

[d]Horizontal movement of arm and released the birds while hand was at least one bird length outside of the drawer.

[e]Released the birds vertically while hand was at least one bird length above the drawer.

[f]Moved arm in a rapid curved movement and released the birds while hand was at least one bird length outside of the drawer.

[g]One or more birds within a handful of birds flapped their wings vigorously while being placed in a module drawer.

Table 6. Descriptive statistics for variables related to the journey.

Variables	No. of loads	Minimum	Q_1	Median	Q_3	Maximum
Journey duration (h)	207	4.33	5.42	5.83	6.25	8.67
Total duration of stops during journey (h)	192	0.07	0.20	0.33	0.50	0.50
Trailer temperature (°C)	88	−9.4	9.5	13.2	15.9	23.0

Table 7. Trailer ventilation configuration during the journey and environmental conditions.

Position of tarpaulin	Not fully closed			Fully closed		
Ventilation (position of doors)	All open/top open and back not closed	Top open and back not fully open	All closed/top not fully open and back closed	All open/top open and back not closed	Top open and back not fully open	All closed/top not fully open and back closed
No. of loads	74	26	5	6	34	52
Percentage of loads	38	13	3	3	17	26
Median trailer temperature (°C)	17.0	11.4	8.9	13.4	9.5	3.9
External temperature (°C)	14.2	8.7	4.8	12.0	0.9	−6.5
External relative humidity (%)	87	94	91	69	91	87
Precipitation (% loads in this arrangement with wet conditions, rain or snow)	18	31	0	17	41	27
Condition of birds (% loads in this arrangement with wet birds)	3	15	0	0	0	4

Table 8. Descriptive statistics for lairage.

Variables	No. of loads	Minimum	Q_1	Median	Q_3	Maximum
Lairage temperature (°C)	211	11.2	15.2	16.0	16.9	26.8
Fan use (% of maximum capacity)	211	13	38	50	67	100
Duration (h)						
Lairage	201	0	0.83	1.59	2.52	5.12
Total loading + journey+ lairage	206	6.23	7.75	8.62	9.47	11.70
Total without water	204	7.27	9.96	10.99	12.13	14.96
Total without food	205	7.35	11.97	12.63	13.73	17.14

did not significantly affect the DOA. The mortality risk decreased with increased temperature within the trailer ($P = 0.01$). Table 7 shows details regarding the trailer ventilation configuration during the journey and the environmental conditions experienced during the journey.

Lairage

Descriptive statistics on lairage temperature, fan use, and lairage duration are shown in Table 8. In unconditional associations, there was no significant effect of duration in the lairage or temperature within the lairage on DOA. In univariate analysis, increased fan use appeared to be associated with decreased mortality risk ($P = 0.05$), but this effect did not remain when other variables were considered. The total duration of loading, journey, and lairage significantly increased the mortality

risk ($P = 0.018$), but there were no significant effects of the total durations without feed or water (i.e., the period from preloading withdrawal to slaughter).

Multiple variable model of risk factors affecting DOA

Although in the univariate analysis, they were both identified as significant factors affecting DOA %, bird age and weight were unsurprisingly highly correlated. Due to this collinearity, the relative effects of including either bird age or bird weight were examined. The inclusion of weight rather than age provided a better model fit (lower Akaike information criterion score), and therefore, weight was selected for inclusion in the multiple variable model. When measurements of health status were considered in combination with other factors in the multiple variable model, the best

Table 9. Multilevel linear mixed model examining risk factors for % dead-on-arrival.

Variables	Coefficient	SE	P	95% CI	
Bird weight (kg)	1.325	0.4133	0.001	0.515	2.135
Rearing mortality (%)	0.120	0.0466	0.010	0.029	0.212
Duration of feed withdrawal before loading >6 h	0.399	0.1634	0.015	0.079	0.719
Speed of loading (No. of birds catcher^{-1} h^{-1})	−0.001	0.0004	0.013	−0.002	−0.0002
Season (ref = winter)					
Fall	3.627	1.477	0.014	0.732	6.523
Spring	1.101	1.452	0.448	−1.744	3.946
Summer	3.035	1.471	0.039	0.153	5.918
Total duration of loading + journey + lairage (h)	0.488	0.1333	0.000	0.227	0.750
Total duration × season					
Fall	−0.452	0.1647	0.006	−0.775	−0.130
Spring	−0.138	0.1635	0.400	−0.458	0.183
Summer	−0.425	0.1654	0.010	−0.750	0.101
Constant	−8.778	1.6084	0.000	−11.930	−5.625

Note: Number of loads = 206. Number of events = 54 (Number of loads per event ranged from 2 to 7). Wald χ^2 = 75.69; P = 0.000. SE, standard error; CI, confidence interval.

Fig. 2. Frequency distribution of percentage dead-on-arrival (DOA) values (after natural logarithm transformation) in loads from study 1 only, also indicating the best normal fit.
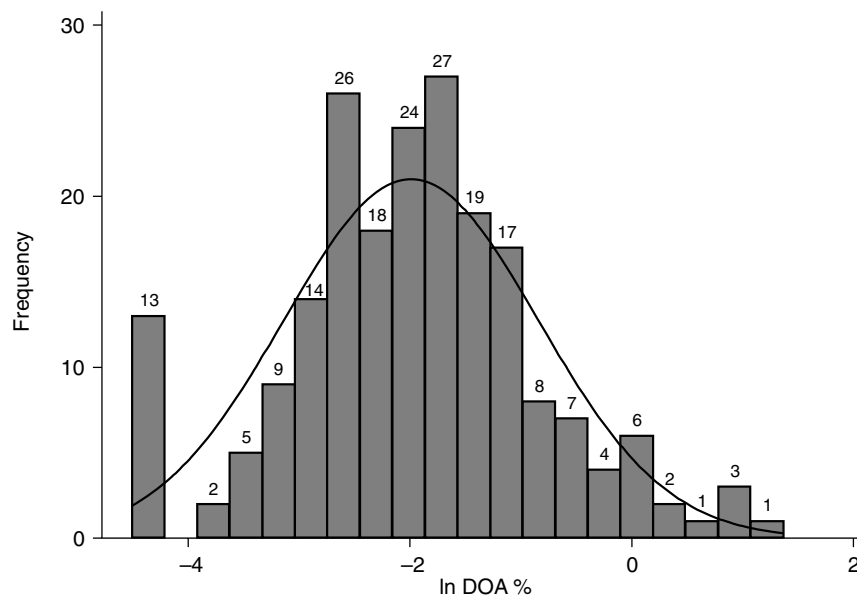
fit was obtained by including rearing mortality, rather than the percentage of the load with abdominal oedema or the overall percentage of the load that was condemned.

In considering factors during loading that were candidates during the univariate analyses; speed of loading (No. of birds catcher^{-1} h^{-1}) but not stocking density remained significant in the multiple variable model. The external temperature and relative humidity at the time of loading did not make a meaningful contribution to the model. Unfortunately, there were insufficient trailer temperature recordings to include this factor in a multiple variable model. Tarpaulin position during the journey was explored, but it was no longer a

significant factor (P = 0.08) and was dropped from the model. When included within a multiple variable model, fan use during lairage was also no longer a significant factor and was dropped. The total duration of loading, journey, and lairage was a better predictor of DOA than considering the duration of any of the individual preslaughter stages. Biologically relevant interactions were examined, and a significant interaction was found between season and total duration from loading to slaughter. The final model is shown in Table 9.

Figure 2 illustrates the distribution of DOA values following natural logarithmic transformation. The final multilevel linear mixed model identified that DOA

Fig. 3. Predictive margins with 95% confidence interval (A) illustrating the effect of bird weight on dead-on-arrival (DOA %). The other predictors were set as follows: rearing mortality = 3.5%, duration of feed withdrawal before loading = <6 h, speed of loading = 750 birds catcher^{-1} h^{-1}, and total duration = 8.6 h; (B) illustrating the effect of rearing mortality on DOA %. The other predictors were set as follows: bird weight = 2.1 kg, duration of feed withdrawal before loading = <6 h, speed of loading = 750 birds catcher^{-1} h^{-1}, and total duration = 8.6 h; (C) illustrating the effect of speed of loading on DOA %. The other predictors were set as follows: bird weight = 2.1 kg, rearing mortality = 3.5%, duration of feed withdrawal before loading = <6 h, and total duration = 8.6 h.

A   B   C
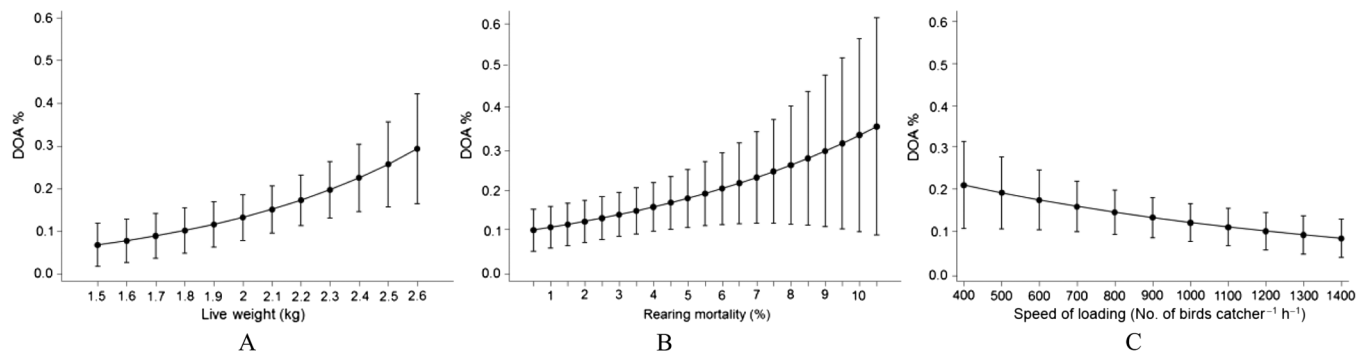
Fig. 4. Predictive margins illustrating the effect of total duration from loading until the end of lairage as this interacts with season on dead-on-arrival (DOA %).
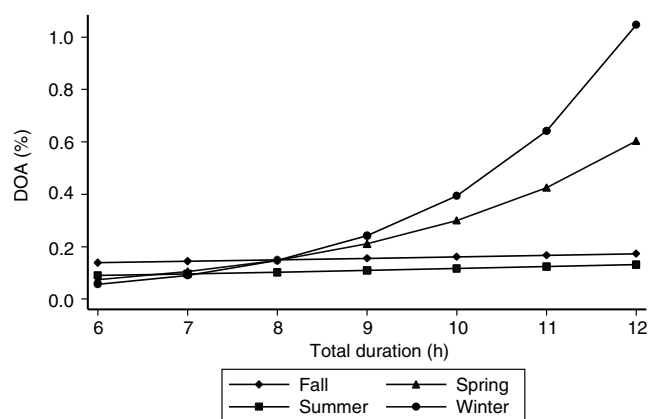
increased with increased bird weight and with increased rearing mortality, and decreased with increased speed of loading. The DOA was greater when the duration of feed withdrawal was >6 h. During winter and spring, the DOA increased with the total duration from loading until slaughter. Predictive margins for the effects of weight (Fig. 3A), rearing mortality (Fig. 3B), and speed of loading (Fig. 3C) on the DOA were graphed based on the model shown in Table 9 to more clearly illustrate the predicted effect of changes in these factors. Predictive margins showing the interaction between season and total duration from loading to slaughter on the predicted DOA value are shown in Fig. 4.

**Study 1: Risk factors affecting bruising**

The frequencies of bruising per load for wing and leg injuries are shown in Figs. 5A and 5B. As can be seen from Fig. 5B, there were virtually no loads for which even modest levels of leg injuries were reported; as such, this outcome variable was not considered any further. There was no significant relationship between the DOA % and the wing bruising percentage per load. There were no significant relationships between the wing bruising percentage per load and weight of the birds, speed of loading, manner of placement of the birds into the modules or wing flapping during placement of the birds in the modules. There was also no significant effect of loading category on the wing bruising percentage per load.

Study 2: Risk factors for DOA

Comparisons between loads transported on short journeys in study 2 and equivalent loads from study 1 transported on longer journeys are shown in Supplementary Tables S1–S4[1]. Many aspects of the loads from study 1 were similar to those from study 2 (Supplementary Table S1[1]), but due to the scheduling of the time of slaughter, the times of day when loading took place were different between study 2 and study 1. In study 2, 65% of the loads were loaded between 0600 and 1600, whereas none of the loads selected from study 1 started loading between 0600 and 1600. The broilers in study 2 were slightly older but lighter than those from study 1, and the health status (based on rearing mortality and condemnations) of the loads in study 2 was considerably better than those in the loads selected from study 1 (Supplementary Table S2[1]), with the exception of condemnations due to a liver condition which was very low

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[1]Supplementary Tables S1–S4 are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjas-2018-0032.

Fig. 5. Frequency distribution illustrating the number of loads in study 1 which recorded various levels of (A) wing bruising (percentage of load) and (B) leg bruising (percentage of load).

A

B
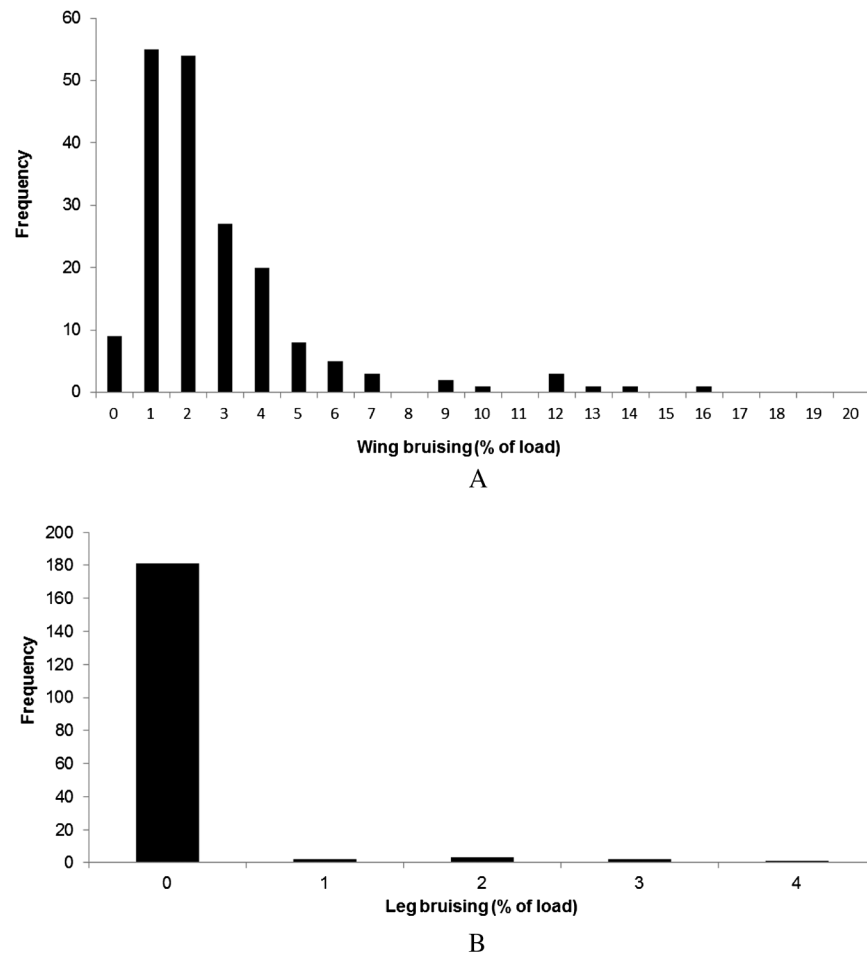
across both studies. Another difference was that because most loading took place during the night in study 1, the external temperature at the end of loading was colder for the loads in study 1 than for those in study 2 (Supplementary Table S3[1]). There was a clear difference in the journey duration and the DOA % between loads from study 2 and those from study 1 (Supplementary Table S4[1]). A number of potential risk factors, based on the model for study 1, did not show significant differences between studies. For example, the speed of loading was similar, whereas the duration without feed prior to loading was higher in study 2. However, we know from the interaction term in the multivariable model from study 1 that total duration time was a particularly serious risk factor in the winter and spring when these comparative loads were collected, with the risk beginning to climb steeply from a total duration of around 9 h. No loads from study 2 experienced this length of total duration from loading to lairage, but it was a relatively common occurrence (~25%) for the loads taken from study 1. Although the data sets were too small to carry out formal modelling, it would appear that the reduced journey and

total duration times together with lighter birds and better overall health status were the main reasons for the significant reduction seen in the percentage DOA values in study 2 compared with those in study 1.

Discussion

As in previous studies (Nijdam et al. 2004; Chauvin et al. 2011; Caffrey et al. 2017), there were multiple risk factors that influenced the DOA % in a load. The median DOA % for broilers in study 1 was lower than the mean DOA % reported for all poultry slaughtered in Canadian federally inspected plants (0.22%) and in those slaughtered in Atlantic Canada (0.23%) in 2014 (Agriculture and Agri-Food Canada 2017). The DOA % reported in most studies in Europe is about 0.2% (Cockram and Dulal 2018). Although we were dependent on the processing plant to facilitate the on-farm observations and to provide data, we received excellent assistance, all data were readily provided, and we had no reason to question the representativeness of the data collected. There are likely to be several environmental and management factors that influenced the lower than average DOA % found

in the current study. Within the locations studied in study 1, the circumstances associated with the rearing, loading, and transport of the birds during specific loading events were more important risk factors for mortality than those associated with consistent differences between producers. This suggests that producer issues such as the quality of management and facilities were of less importance than either health issues that occurred during the rearing of specific batches and variation associated with loading, transport, and lairage. Although the wetness of the litter can provide a useful indication of the quality of the management of the housing conditions, and if broilers are loaded wet due to poor litter they are at increased risk of hypothermia in cold conditions (Hunter et al. 1999; Cockram and Dulal 2018), there was no effect of litter dry matter percentage on the risk of DOA.

As shown previously (Drain et al. 2007; Whiting et al. 2007), the percentage rearing mortality affected the DOA %. At a similar DOA % to that reported in study 1, Hunter et al. (2001) reported that 71% of DOA birds examined were considered to have had pre-existing pathological conditions that increased their risk of mortality. If there were major health problems during rearing, and if some of the affected birds died this will have been recorded in the percentage rearing mortality. Some of the birds that survived may have been weakened and (or) still had pathology that affected their physiological ability to respond to the challenges of handling and transport to the extent that they were more likely to die during handling and transport than healthy birds. As previously reported by Lupo et al. (2009), in study 1, there was a significant relationship ($P = 0.021$) between the percentage mortality during rearing and the percentage of broilers condemned after slaughter, as not fit for human consumption. Although Lupo et al. (2009) reported a significant relationship between the total percentage of condemnations and the DOA %, this relationship was not found in study 1. However, there was a significant univariate relationship between the percentage of condemnations due to abdominal oedema and the DOA %. It was not possible to include both rearing mortality and abdominal oedema in the final multiple variable model, but abdominal oedema was likely an important factor that affected the DOA %. A greater prevalence of abdominal oedema has been identified in DOA broilers than in those that survived transport and were subsequently slaughtered (Nijdam et al. 2006). In older and heavier birds, heart and lung size in proportion to the total body weight decreases (Havenstein et al. 2003), and this requires the heart to work harder to maintain effective blood circulation throughout the body. If the heart starts to fail (chronic congestive heart failure), fluid collects in the lungs and abdomen (ascites) causing respiratory difficulties and an increased risk of mortality during transport (Wideman 2001). In study 1, heavier birds had an increased risk of mortality. The

significant effects of age and weight of the birds were consistent with previous studies (Nijdam et al. 2004; Drain et al. 2007; Whiting et al. 2007; Haslam et al. 2008; Chauvin et al. 2011).

Manual catching, handling, and loading of birds have the potential to cause trauma that can result in injury and sometimes death (Gregory and Austin 1992; Nijdam et al. 2006). An important finding in this study was that no relationships were found between the type of handling during loading and the DOA % and the percentage wing bruising. Although different loading arrangements affected how close the modules could be placed to the birds and, therefore, the duration that the birds were carried and whether the birds had to be passed to another handler located outside of the barn, the loading arrangements did not have a significant effect on DOA %. In addition, the manner in which birds were loaded did not affect DOA %. This might have been due to inadequate numbers of observations of modules in some loads and not appropriately characterising the types of handling that could have caused injury. In addition, it is possible that the presence of external observers could have modified the behaviour of the handlers. However, each catching team contained a supervisor responsible for the conduct of the handlers, and the handlers soon became used to the presence of the observers and did not appear to modify their behaviour during the 13 mo of the study. In this study, there was no evidence that problems associated with inappropriate handling increased the risk of mortality. As variation has been reported between catching teams in the risk of broiler mortality and injury (Taylor and Helbacka 1968; Nijdam et al. 2004; Langkabel et al. 2015), this suggests that the catching team observed handled the birds in a competent manner. When loading was undertaken efficiently, and the speed of loading was high, the loading was undertaken quicker, and the risk of mortality was lower. Minimizing the duration that a partially loaded trailer is stationary without adequate ventilation is beneficial in reducing the risk of mortality (De Koning et al. 1987). Although there are few detailed comparative studies, modular systems have been reported to reduce the DOA % compared with crates (Bayliss and Hinton 1990). Using modules to load broilers rather than crates results in less damage to the birds (De Koning et al. 1987). Unloading and handling at the processing plant are also facilitated by the use of modules (Bayliss and Hinton 1990).

As in most other studies on broiler DOAs, the seasonal effect suggested that environmental conditions experienced by the birds affected the risk of mortality. Univariate analysis showed that if the external temperature at the end of loading was >10 °C, and the relative humidity was ≤90%, the mortality risk was lower than at colder temperatures. The DOA % has been reported to increase when the external temperature decreases to ≤5 °C (Nijdam et al. 2004; Vecerek et al. 2016). The

birds in some loads had to experience challenging cold external temperatures (i.e., −22 °C), and these cold temperatures would have required the birds to respond behaviourally and physiologically to avoid hypothermia (Dadgar et al. 2010; Knezacek et al. 2010). However, the highest external temperature recorded at the end of loading was only 22 °C. Unless the humidity was high, there was high stocking density and inadequate ventilation, this maximum external temperature in itself would not have posed significant challenges to the broilers (Mitchell and Kettlewell 1998). However, the external temperature and humidity during a journey do not necessarily provide a good indication of the environmental conditions experienced by the broilers within the module on the trailer (Knezacek et al. 2010; Burlinguette et al. 2012). Unfortunately for many loads, the temperature recordings provided from sensors placed along the roof of the trailer did not provide a good profile of the temperature conditions within the trailer during the journey, and there was no measure of humidity within the trailer. From the temperature recordings that were available, it would appear that some birds could have been exposed to low temperatures that placed the birds at risk of hypothermia, but the maximum temperature recorded in the trailer would probably not have resulted in hyperthermia. In addition to the external temperature, the stocking density and number of birds within the trailer, the temperature within the trailer would be expected to have been influenced by the degree of ventilation within the trailer.

When the trailer was stationary, the ventilation would be considerably lower than that when the vehicle was moving (Dalley et al. 1996; Hoxey et al. 1996). Reduced ventilation within the trailer was probably an important factor responsible for the tendency for an increased risk of mortality that was associated with periods when the vehicle was stationary during the journey from the farm to the plant. Another important factor that reduces ventilation within the trailer is the use of tarpaulins along the sides of the trailer to protect the birds on the outside of the load from cold temperature, wind chill, and precipitation. In a closed or partially closed ventilation configuration, internal temperature and humidity can rise at one or more locations within the vehicle (Kettlewell et al. 1993; Mitchell and Kettlewell 1998; Burlinguette et al. 2012). In this study, there was no indication that the temperature within the trailers increased as a result of fully closed tarpaulins to the extent that would have caused hyperthermia.

On arrival at the plant, the modular system permits the birds to be unloaded from the trailer. The subsequent arrangement of the modules within the lairage facilitates ventilation and avoids the extremes of temperature that can occur within containers kept in a lairage (Hunter et al. 1998; Warriss et al. 1999). The temperature conditions within the lairage were never sufficiently cold to have put the birds at risk of hypothermia. For most loads, the temperature within the lairage was unlikely to have put the birds at risk of hyperthermia. During periods of extreme high external temperature, the temperature in the lairage approached conditions that might have posed a risk of hyperthermia (Quinn et al. 1998), and at these times, the lairage fans would have been working to maximum capacity. There was a marginal association between maximum fan use in the lairage and an increased risk of mortality, but this potential influence was not present when other variables were considered within the analysis. The durations that most loads were kept in the lairage were shorter than those identified by Chauvin et al. (2011) as posing an increased risk of mortality.

Under the conditions in this study, when the total duration from the start of loading to the end of lairage was less than about 9 h, there was no effect of total duration on DOA %. In summer and fall, there was no effect of total durations from the start of loading to the end of lairage of up to about 12 h on DOA %. However, in winter and spring, when the total duration from the start of loading to the end of lairage was longer than about 9 h, there was a significant increase in the risk of DOA with each increased hour. Effects of journey duration on the DOA % have been reported by Warriss et al. (1992), Nijdam et al. (2004), Chauvin et al. (2011), and Caffrey et al. (2017). Although the total duration without feed was not significantly related to the DOA %, one possibility for the seasonal effect of duration on DOA % is that, as a result of the colder external temperature during winter and spring, some of the birds needed to utilise body energy reserves to attempt to avoid hypothermia. During the journey, these reserves in some birds may have been insufficient to enable them to maintain body temperature, and as the total duration from loading to unloading increased, there could have been increased deaths due to hypothermia. The effects of fasting are greater during cold conditions (Vosmerova et al. 2010). During cold exposure, fasted birds show greater reductions in blood glucose concentration and liver glycogen concentration than those kept at thermoneutral temperatures, and they are at an increased risk of hypothermia (Dadgar et al. 2011, 2012). Feed withdrawal before loading is undertaken to allow sufficient time for the digestive tract to empty and reduce the risk of carcass contamination. Too long period of feed withdrawal can be detrimental due to the development of a negative energy balance (Nijdam et al. 2005) and a decreased ability to cope with cold temperatures (Berman and Snapir 1965). Christensen et al. (2012) found that fasting broilers at 24 °C, for 6 h caused a decreased body temperature. This might explain why there was an increased risk of mortality when the feed withdrawal period before loading was >6 h.

The aim of study 2 was to examine the effect of journey duration on the risk of mortality by comparing loads from broiler units near to the processing plant

with those located further away. The 40 loads in study 2 had a median journey duration of 0.66 h to the processing plant, whereas the 40 equivalent loads from study 1 had a journey duration of 6.02 h. There was a clear difference in the journey durations and the DOA % between these two groups. Although an attempt was made to keep factors other than journey duration similar between the two groups, there were some other important differences between the two groups that also likely influenced the mortality risk. Although the DOA % in loads from study 2 was considerably lower than that in loads from study 1 and journey duration was likely the most important factor influencing this difference, other differences, such as health status, external temperature, and age and weight, may have contributed to this difference.

With the caveat that it was not possible to adequately characterise thermal conditions within each load, in conditions that resulted in a DOA % per load lower than the regional and national statistics, the main risk factors for increased mortality during transport were an increased duration between loading and the end of lairage (especially during winter) and a period of feed withdrawal before loading of greater than 6 h. The risk of mortality increased with the weight of the birds (over the range 1.7–2.7 kg) and increased with the percentage rearing mortality. No relationships were found between the manner in which the broilers were handled and the percentages of DOA or bruised birds.

Acknowledgements

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