Effects of external ambient temperature at loading, journey duration and flock characteristics on the dead-on-arrival rate in broiler chickens transported to slaughter in Great Britain

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ABSTRACT Broiler mortality during transport and lairage, prior to slaughter, has negative welfare and economic implications. Knowledge of the factors affecting the dead-on-arrival (DOA) rate can help identify riskmitigating strategies. The objectives of this study were to determine the DOA rate in broiler chickens transported to slaughter in Great Britain and associated risk factors. Requested data for all loads of broilers transported to slaughter by 5 large British commercial companies on 57 randomly-selected dates in 2019 were obtained and combined with weather data extracted from the Met Office MIDAS Open database. The DOA rate was described overall and per load using summary descriptive statistics. Mixed-effects Poisson regression was used to evaluate considered flock-, journey- and weather-related risk factors. Results were reported as incidence rate ratios (IRR) and 95% confidence intervals (CI). On the selected dates, 25,476 loads transported 146,219,189 broilers to slaughter. The overall mean DOA rate was 0.08%. The median DOA rate per load was 0.06% (interquartile range 0.03-0.09%; range 0.00-17.39%). Multiple risk factors were identified including loading temperature and catch method. At relative humidity < 80%, the DOA rate was 16.89 (95% CI 15.25-18.70, P < 0.001) times higher for loads loaded in external ambient temperatures $>30.0^{\circ}$ C compared to those loaded in temperatures between 10.1° C and 15.0° C. When relative humidity was >80%, there was a 43%increase in DOA rate for loads loaded in temperatures below freezing compared to those loaded in temperatures between 10.1°C and 15.0°C (IRR 1.43, 95% CI 1.35 -1.52, P < 0.001). The DOA rate was 32% higher for loads caught mechanically compared to those caught manually (IRR 1.32, 95% CI 1.23–1.42, P < 0.001). The overall DOA rate was lower than that previously reported in Great Britain and for other European countries. Most identified risk factors had a marginal effect, however, loading temperatures >30°C substantially increased DOA rate. Internal thermal environmental conditions were not evaluated. Avoidance of loading during periods of hot weather would improve the welfare of, and reduce economic losses in, broiler chickens.

Key words: transport, mortality, broiler, risk factor, welfare

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INTRODUCTION

Approximately 1 billion broiler chickens are slaughtered each year in the United Kingdom, producing in excess of 1.6 thousand tonnes of meat (DEFRA, 2022). A potentially stressful phase in production is the transport of broilers to the slaughterhouse (Weeks et al., 2019). Broiler mortality during the transport phase, which extends from when birds are loaded to the end of lairage, has both negative welfare and economic implications (Haslam et al., 2008; Chauvin et al., 2011). In Great Britain, broiler producers are legally required to record the percentage of birds found dead-on-arrival (**DOA**) or DOA rate (Council of the European Union, 2007), with this metric recognized as a key performance indicator for broiler welfare. Published mean DOA rates vary widely from 0.10% (Norway, Kittelsen et al., 2017); 0.13% (UK, Buzdugan et al., 2021); 0.18% (France, Chauvin et al., 2011); 0.19% (Spain, Villarroel et al., 2018); 0.22% (Canada, Agriculture and Agri-Food Canada, 2017), 0.26% (Spain, Averos et al., 2020); 0.30% (Belgium, Jacobs et al., 2017); to 0.39% (Turkey, Teke,

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2019). The distribution of mortality across trailer loads usually exhibits a positive skew, with most loads experiencing a low DOA rate and a minority of loads a substantially higher rate. For this reason, median rates tend to be lower than mean rates between and within studies: 0.13% (Canada, Cockram et al., 2019); 0.16% (Spain, Averos et al., 2020); and 0.19% (Belgium, Jacobs et al., 2017).

Multiple factors have previously been shown to influence the rate of DOA in broiler chickens transported to slaughter. Commonly identified factors include ambient temperature (Caffrey et al., 2017; Teke, 2019), journey duration (Villarroel et al., 2018; Cockram et al., 2019), flock health (Chauvin et al., 2011; Cockram et al., 2019) and catch method (Chauvin et al., 2011; Mönch et al., 2020). With the majority of work exploring transportassociated broiler mortality in Great Britain performed over a decade ago, and several improvements to transport processes since, including the use of modular transport systems and better personnel training, reevaluation of risk factor associations was warranted. In addition, previous work conducted in Great Britain has rarely evaluated data from more than one producer company, considered the effect of external ambient temperature and relative humidity at loading or investigated multiple risk factors simultaneously, which may bias study findings. The purpose of this study was to use data routinely collected by multiple broiler companies to investigate the factors associated with DOA rate in broiler chickens transported to slaughter in Great Britain. We were especially interested in the effects of external ambient temperature and relative humidity at loading. Such scientific evidence is essential for informing sound regulation and policy, and ultimately ensuring a higher level of animal welfare (European Food Safety Authority, 2022).

MATERIALS AND METHODS

This study used data routinely recorded by 5 large British commercial broiler companies, the 14 processing plants they supplied and 11 Met Office weather stations. Institutional ethical approval was not required as this was a retrospective study analyzing nonpersonal data. The year 2019 was chosen as, at the start of the study, later records were unavailable through the Met Office MIDAS Open database. The main unit of observation was a trailer load. This was defined as a uniquely identified vehicle scheduled to leave a farm, at a given time, on a selected day, to transport a trailer load of broiler chickens to the slaughterhouse. A trailer load could transport birds produced in a single house (single-flock load) or multiple houses on the same farm. Broilers were transported on a vehicle formed from a tractor unit and flat-bed trailer. Trailers had a solid base and roof, solid front and rear panels with ventilation holes, and detachable side curtains. Side curtains were made of a ventilation mesh, with lighter ventilation mesh curtains, offering increased ventilation, available for use in

warmer months. In periods of very hot weather, side curtains were removed completely. Ventilation is provided by air flow through the vehicle when in motion. Broilers are loaded and transported in modules. Trailers would carry a maximum of 20 modules arranged over 2 layers. Both manual and mechanical catch methods were used to load birds into the transport modules. For manual catch, trained operatives caught birds by one or both legs. For mechanical catch, a harvesting machine moved slowly through the house, birds stepped from the house floor onto a conveyor on the harvesting machine. The birds were then conveyed and automatically dropped into the transport modules. Once a module was full it is transferred by forklift to the transport trailer.

Sample size calculations indicated that a minimum of 16,000 trailer loads were needed to detect a rate ratio of $2~{\rm or}$ more as statistically significant, at a 95% confidence level and with 80% power. This estimation was based on the following assumptions: a mean DOA rate of 0.13%(Buzdugan et al., 2021), a ratio of 1:1 or 2:1 in the number of trailer loads transported within and outside of a 5°C to 25°C temperature range, an average trailer load size of 5,000 birds and a within-flock correlation of 0.4. Given the collaborating processing plants received an estimated 28 trailer loads daily, data from 52 dates were required to achieve the minimum sample size. Study dates were chosen at random using a stratified proportional-to-size approach that accounted for decreased processing of broiler chickens on weekends. An additional five "extreme" days when the daily minimum temperature was much below 5°C or the maximum temperature much above 25°C were also selected at random and included. All trailer loads transporting birds to slaughter on the 57 randomly-selected dates throughout 2019 were included in the analysis.

Information obtained from the companies for each trailer load could be broadly categorized into three groups:

- *Flock data:* company ID, farm postcode, house ID, breed, number of birds placed, parent flock age(s), cumulative weekly and total mortality numbers
- *Trailer load data:* load date, catch age, catch method, thin or final depletion, number of birds on load, vehicle registration, reported loading completion time, reported journey duration, reported journey distance, any stoppages
- Processing plant data: processing plant postcode, unloading completion time, time in lairage, DOA number and reject number

The internal validity of the supplied data was checked, for example, numbers of birds expressed as integers, mortality numbers were cumulative, unloading time occurred after loading time. Where typographic errors, inconsistencies and extreme values were identified, the raw data were checked by the broiler companies. The supplied data were then either corrected or, if still inconsistent, set to missing. For some trailer loads, data were presented across multiple rows in the dataset (typically one row per flock contributing to the load). Where this occurred, variables were aggregated as appropriate. Individual flocks were assigned a unique identification number using a concatenate of farm postcode, house ID, number of birds placed and mortality numbers (up to 28 d), with consideration also given to the number of days between trailer loads. Trailer loads transporting multiple flocks were assigned an additional flock mix ID number reflecting the combination of flocks aboard. At all processing plants, the number of DOAs per load was recorded post gas stun. The number, therefore, represents cumulative deaths occurring during transport and in lairage. The level of rigor and temperature of the carcass was used by processing plant operatives to differentiate between gas stunned birds and DOAs. The DOA rate per trailer load was calculated as the number of DOAs on a load divided by the total number of birds on the same load, multiplied by 100. For single-flock trailer loads, flock-level mortality rates were calculated; weekly for the first 4 wk of life, the late period (between 28 d and catch) and overall. The reject rate represents the proportion of processed birds not fit for human consumption and was calculated at the flock level, that is, total number of rejects for a given flock divided by the total number of birds processed from the same flock, multiplied by 100.

To identify the most appropriate stations to extract weather data from, farm and processing plant postcodes were firstly converted to latitude and longitude coordinates using FreeMapTools (https://www.free maptools.com/convert-uk-postcode-to-lat-lng.htm). The distance between each weather station and broiler farm or processing plant was then calculated using the "distm()" function in the <geosphere> package in R software. For each nearest weather station, the completeness of their hourly observation dataset for the 57 study dates was assessed. For stations with fewer than 95% of hourly observations, the next nearest station was identified, until all farms and processing plants were matched with a station where more than 95% of observations were available. For each trailer load, external ambient air temperature and relative humidity at loading (matched to the nearest hour) were retrieved from the 2019 Met Office MIDAS Open database. If data were unavailable for the nearest hour, data were obtained for the preceding hour where available. Thermal conditions within the transport modules were not measured.

The total number of farms, flocks, vehicles, trailer loads, birds and DOAs included in this study were reported by count. The number of trailer loads per farm was described using the median, interquartile range (**IQR**) and range. All trailer loads, transported on the 57 randomly-selected dates, contributed to the description of DOA rate. The mean DOA rate was reported overall and the DOA rate per trailer load summarized using the median, IQR and range.

Categorical explanatory variables (e.g., breed and catch method) were reported using counts and expressed

as a percentage of trailer loads. The distribution of continuous explanatory variables (e.g., external ambient temperature at loading and journey distance) was firstly described using the median, IQR, range and other percentiles. Due to the associations between all considered continuous explanatory variables and DOA rate being nonlinear, continuous variables were categorized for further analysis. External ambient temperature at loading (loading temperature) was converted into 8 groups using 5°C intervals. Relative humidity at loading was converted into a binary variable using a cut-off of 80% that reflects the average annual humidity for the United Kingdom. Journey time was defined as the period from when loading was completed on farm to when unloading was completed at the slaughterhouse. It is therefore the total of the time in-transit, plus any stoppages. Parent flock age was categorized into 4 groups: young (less than 32 wk), peak (32-50 wk), old (greater than 50 wk) and mixed ages. Each category for each variable was then reported by number and expressed as a percentage of trailer loads. For each variable, the DOA rate in each category was described using the median, IQR, range and other percentiles. Heat maps were generated to visually display associations between pairs of continuous variables and 2 outcomes: number of trailer loads and maximum DOA rate.

Mixed-effects Poisson regression was used to explore risk factors associated with DOA rate. For each trailer load, the number of birds DOA was offset by the natural log of the number of birds transported. To allow for the evaluation of flock demographic and health characteristics, risk factor analysis was restricted to single-flock trailer loads. Flock ID was included as a random effect to account for the transport of a single flock across multiple trailer loads and potential clustering within flock. Results are reported as incidence rate ratio (**IRR**) and 95% confidence interval (CI). Univariable models were constructed to examine crude associations between each risk factor and DOA rate separately. Variables with an associated P < 0.25, at the univariable stage, were carried forward for consideration in the multivariable model. The multivariable model was built using a manual forward selection process, starting with the model that had the lowest Akaike's Information Criterion (AIC). Variables were retained in the final model if the likelihood ratio test P was <0.05. Potential confounding was evaluated by resubmitting any variables carried forward but not included in the final model and calculating the percentage change between rate ratios for the remaining variables. Confounding was deemed to be present if the addition of a variable altered an IRR by >10% and the confounding variable retained. Plausible two-way interactions between variables retained in the final model were assessed by comparing models with and without interaction terms using the likelihood ratio test. Models including different interaction terms and numbers of interactions were compared using AIC and Bayesian Information Criterion (**BIC**). For two competing models, the most parsimonious model, that is, the simplest model with greatest explanatory predictive power, is the one with the lowest AIC and BIC. In addition, the effect size of the interaction term was considered when determining the most appropriate models for presentation. When the effect size of an interaction term was negligible, it was excluded in favor of the next simplest model with lowest AIC and BIC. All statistical analyses were performed in Stata 14 (StataCorp, College Station, TX)

RESULTS

Study Population

The study population consisted of 25,476 trailers loads of broiler chickens which came from 9,191 flocks and 725 commercial farms. The median number of trailer loads per farm was 24 (IQR 10-45; range 1 -257). Vehicle registration was unavailable for 5,984 (23.5%) trailer loads. The remaining trailer loads were transported by 288 vehicles, with a median of 47 trailer loads per vehicle (IQR 22-75; range 1-172).

Dead-on-Arrival Rate

Of the 146,219,189 birds transported to slaughter, 118,604 were recorded DOA. This equates to an overall mean DOA rate of 0.08%. A positive skew was observed in the distribution of DOA rate per trailer load, presented in Figure 1. The median DOA rate per trailer load was 0.06% (IQR 0.03-0.09%; range 0.00-17.39%). No deaths were observed for 2,848 (11.2%) loads. The number of loads with a DOA rate greater than 1% was 102 (0.4%).

Trailer Load Characteristics

The majority of trailer loads consisted of birds caught from single flocks (n = 23,799; 93.4%). Mechanical catch methods were uncommonly employed, with 23,838 (93.6%) trailer loads caught manually. Final depletion accounted for 18,686 (73.4%) trailer loads. Continuous trailer load variables are summarized in Table 1.

The median distance between the most appropriate weather station and farm was 13.7km (IQR 8.8 -19.0 km, range 0.2-40.4 km). The median external ambient temperature at loading was 10.2°C. The minimum and maximum external ambient temperatures at loading were -9.3°C and 36.3°C, respectively.

Flock characteristics were described using data from 22,820 single-flock trailer loads. These birds came from 8,470 flocks and 723 farms. Excluded were 979 apparently single-flock trailer loads where the total number of birds processed was greater than the number placed. Ross 308 was the most common breed, transported on 18,472 (81.0%) single-flock loads. Cobbs and JA Hubbards were transported on 3,892 (17.1%) and 465 (2.0%) single-flock loads, respectively. The majority of single-flock loads carried birds produced by a parent flock of

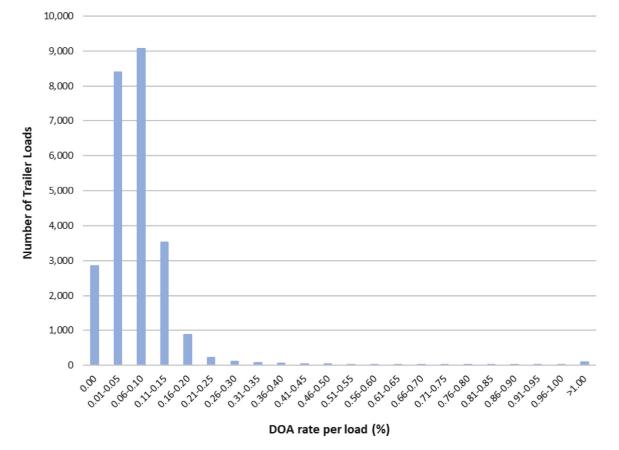


Figure 1. Distribution of dead-on-arrival rate for 25,476 trailer loads of broiler chickens transported to slaughter in 2019.

Table 1. Summary of selected continuous variables for 25,476 trailer loads of broiler chickens transported to slaughter in 2019.

	Smallest value	Percentile									Lanmoot
Variable		1%	5%	10%	25%	50% (median)	75%	90%	95%	99%	Largest value
Number of birds per load	48	1,092	2,875	4,224	4,978	5,760	6,600	7,480	8,460	9,385	15,696
Reported journey time (min) ^a	5	14	25	30	45	70	105	148	170	210	365
Reported journey distance (km) ^a	2.3	8.1	12.7	15.8	27.4	52.6	90.1	138.3	169.0	209.2	419.1
Time in lairage (min)	0	0	0	26	60	93	128	168	197	264	1,177
External ambient temperature at loading (°C)	-9.3	-3.6	-0.3	1.8	5.8	10.2	14.9	18.4	21.1	28.4	36.3
Relative humidity at loading (%)	26.8	40.6	49.2	56.6	71.4	85.0	93.4	97.7	98.9	100.0	100.0

 $^{\mathrm{a}}\mathrm{Restricted}$ to 22,744 trailer loads travelling at an average speed between 20 and 120 km/h.

peak age only (n = 11,837; 53.7%). The median catch age was 38 d (IQR 35–39 d; range 21–76 d). The median 7-d mortality rate was 1.43% (IQR 1.02–2.05%, range 0.02–17.62%). The median late period mortality rate was 0.80% (IQR 0.52–1.24%, range 0.00–34.12%). Full descriptive statistics and univariable associations for single-flock trailer loads are available in Supplementary Tables S1 (flock characteristics), S2 (journey duration and distance), and S3 (external ambient temperature and relative humidity at loading).

Associations Between Reported Journey Time, Distance and Maximum DOA Rate

The associations between reported journey time and distance and 1) the number of trailer loads, and 2) maximum DOA rate are displayed in Figure 2. In Figure 2A, each colored square represented an observed combination of journey time and duration. The shade of the square represents the number of trailer loads transported at that specific combination of time and distance, with darker shades representing greater numbers of loads. Reported journey time and distance were strongly correlated. The reported journey time and distance for the majority of loads was less than 200 min and 100 km, respectively.

In Figure 2B, the color of each square reflects the highest DOA rate observed for the trailer loads whose journey parameters correspond to the specific combination of journey time and distance as determined by the square's location. For the majority of combinations, the maximum DOA rate was lower than 0.06% indicated by the light squares. The loads exhibiting higher maximum DOA rates, represented by the darker squares, occurred on shorter journeys. White areas of the plot reflect combinations of journey time and distance for which no observations were available.

Associations Between External Ambient Temperature and Relative Humidity at Loading and Maximum DOA Rate

Associations between external ambient temperature and relative humidity at loading and 1) the number of trailer loads, and 2) maximum DOA rate are presented in Figure 3. On Figure 3A, the darker squares indicate that the majority of trailer loads had a loading temperature between 5°C and 25°C and relative humidity >80%. The combinations of loading temperature and humidity with the higher maximum DOA rates, indicated by the darker shades in Figure 3B, occurred at temperatures >30°C and when relative humidity was below 60%.

Risk Factor Analysis

The final multivariable model exploring factors associated with DOA rate in 17,179 single-flock trailer loads transported to slaughter in 2019 is presented in Table 2.

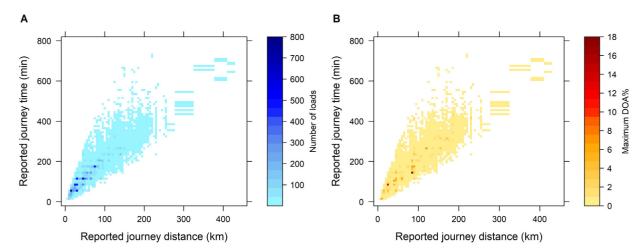


Figure 2. Heat maps showing distribution of the number of trailer loads (A) and the maximum dead-on-arrival rate (B) by reported journey time and distance for 22,820 single-flock trailer loads of broiler chickens transported to slaughter in 2019.

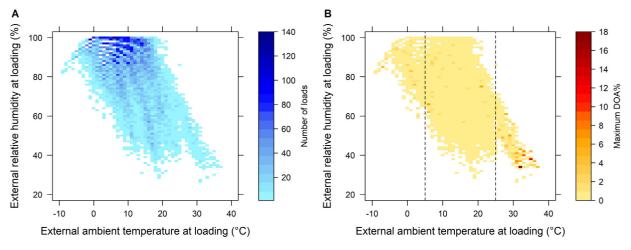


Figure 3. Heat maps showing the distribution of the number of trailer loads (A) and the maximum dead-on-arrival rate (B) by external ambient temperature and relative humidity at loading in 22,820 single-flock trailer loads of broiler chickens transported to slaughter in 2019.

Table 2. Final multivariable mixed-effects Poisson regression results for risk factors associated with dead-on-arrival rate in 17,179 single-flock trailer loads of broiler chickens transported to slaughter in 2019. Flock ID was included as a random effect (n = 6,603).

Risk factor	IRR	Wald P-value	Lower 95 CI	Upper 95 CI	LRT <i>P</i> -value	Risk factor	IRR	Wald <i>P</i> -value	Lower 95 CI	Upper 95 CI	LRT <i>P</i> -value	
Loading temperature					< 0.001	Loading temperature					< 0.001	
$Humidity \leq 80\%$						Humidity > 80%						
<0°C	1.14	0.451	0.81	1.60		<0°C	1.43	< 0.001	1.35	1.52		
0°C-5.0°C	1.14	0.001	1.05	1.23		0°C-5.0°C	1.05	0.029	1.01	1.11		
5.1°C-10.0°C	1.21	< 0.001	1.15	1.27		$5.1^{\circ}C - 10.0^{\circ}C$	1.02	0.392	0.98	1.06		
10.1°C-15.0°C	1.00					10.1°C-15.0°C	1.00					
15.1°C-20.0°C	0.98	0.490	0.94	1.03		$15.1^{\circ}C-20.0^{\circ}C$	0.94	0.033	0.89	1.00		
$20.1^{\circ}C-25.0^{\circ}C$	1.20	< 0.001	1.13	1.29		$20.1^{\circ}C - 25.0^{\circ}C$	2.02	< 0.001	1.65	2.46		
25.1°C-30.0°C	3.93	< 0.001	3.60	4.28		$25.1^{\circ}C - 30.0^{\circ}C$	-	no observations in sample				
>30.0°C	16.89	< 0.001	15.25	18.70		>30.0°C	-	no observations in sample				
Reported journey time					0.004					-		
≤60 min	1.00											
>60 min	1.06	0.004	1.02	1.11								
Reported journey					< 0.001							
distance												
≤50 km	1.00											
>50 km	1.13	< 0.001	1.08	1.18								
Catch method					< 0.001							
Manual	1.00											
Mechanical	1.32	< 0.001	1.23	1.42								
Thin or depletion					< 0.001							
Deplete	1.00											
Thin	1.09	< 0.001	1.06	1.13								
Time in lairage					0.002							
≤90 min	1.00											
>90 min	1.07	0.002	1.05	1.10								
Breed					< 0.001							
Ross	1.00											
JA	0.27	< 0.001	0.19	0.40								
Cobb	0.94	0.015	0.90	0.99								
Catch age					< 0.001							
≤38 d	1.00											
>38 d	1.15	< 0.001	1.11	1.20								
Parent flock age					0.003							
Young	1.01	0.629	0.97	1.06								
Peak	1.00											
Old	1.04	0.116	0.99	1.09								
Mixed ages	1.13	< 0.001	1.06	1.21								
7-d mortality					0.079							
≤1.50%	1.00											
>1.50%	0.97	0.078	0.93	1.00								
Late period mortality					< 0.001							
≤0.80%	1.00											
>0.80%	1.26	< 0.001	1.22	1.31								
between flock variance	0.41		0.39	0.43								

Abbreviation: LRT, likelihood ratio test.

Multiple risk factors were identified and there was evidence of statistical interaction between loading temperature and humidity (P < 0.001). To demonstrate this significant interaction, the effects of loading temperature on DOA rate are presented separately for trailer loads loaded at relative humidity $\leq 80\%$ and $\geq 80\%$.

Loading temperatures $>30^{\circ}$ C had the strongest effect on DOA rate. At relative humidity $\leq 80\%$, the DOA rate was 16.89 (95% CI 15.25-18.70, P < 0.001) times higher for loads loaded in temperatures >30.0°C, 3.93 (95% CI 3.60-4.28, P < 0.001) times higher for loads loaded in temperatures between 25.1° C and 30.0° C and 1.20 (95%CI 1.13–1.29, P < 0.001) times higher for loads loaded in temperatures between 20.1–25°C compared to those loaded in external ambient temperatures between 10.1° C and 15.0°C. At relative humidity >80%, the DOA rate was 2.02 (95% CI: 1.65–2.46, P < 0.001) times higher for loads loaded in temperatures between 20.1°C and 25° C, and 1.43 (95% CI 1.35-1.52, P < 0.001) for loads loaded in temperatures below freezing compared to those loaded in external ambient temperatures between 10.1° C and 15.0° C. When relative humidity was < 80%. the rate of DOA for loads loaded in temperatures below freezing was not statistically different to the rate observed for those loaded in temperatures between 10.1° C and 15.0° C (P = 0.45).

Both reported journey time and distance were associated with DOA rate. For journeys more than 60 min in duration, the DOA rate was 6% higher than for journeys ≤ 60 min in duration (IRR 1.06, 95% CI 1.02–1.11, P = 0.004). For trailer loads travelling more than 50 km, the DOA rate was 13% higher than that for journeys ≤ 50 km in length (IRR 1.13, 95% CI 1.08–1.18, P < 0.001).

Catch method also influenced DOA rate, with trailer loads caught using mechanical methods experiencing a 32% increase in DOA rate compared to those caught manually (IRR 1.32, 95% CI 1.23–1.42, P < 0.001). The rate of DOA was greater for trailer loads transporting thinned birds compared to those transporting birds undergoing final depletion (IRR 1.09, 95% CI 1.06–1.13, P < 0.001). Trailer loads kept in lairage for more than 90 min had a 7% increase in DOA rate, compared to loads processed within 90 min of arrival at the processing plant (IRR 1.07, 95% CI 1.05–1.10, P = 0.002).

Apart from 7-d mortality rate, all flock characteristics influenced DOA rate. Breed had the strongest effect, with DOA rates 73% lower in JA Hubbards compared to Ross 308s (IRR 0.27, 95% CI 0.19–0.40, P < 0.001). The difference between Cobb and Ross broilers was much less pronounced, with a 6% lower DOA rate seen in Cobbs (IRR 0.94, 95% CI 0.90–0.99, P = 0.015). Older catch age was associated with increased rate of DOA, with loads transporting broilers >38 d having a 15% higher rate of DOA compared to those transporting broilers \leq 38 d (IRR 1.15, 95% CI 1.11–1.20, P < 0.001). Parent flock age did not influence DOA when all parent flocks came from the same age group, however, loads with parent flocks of mixed ages had a 13% higher rate of DOA compared to those from parent flocks of peak age only (IRR 1.13, 95% CI 1.06–1.21, P < 0.001). An increased rate of DOA was also observed for loads transporting flocks with above median late period mortality. The rate of DOA was 26% higher for loads transporting flocks with a late period mortality >0.8% compared to loads transporting flocks with a late period mortality >0.8% (IRR 1.26, 95% CI 1.22–1.31, P < 0.001).

Alongside interaction between external ambient temperature and relative humidity at loading (when the adverse effects of higher temperature were exacerbated under conditions of higher humidity), there was also evidence of interaction between loading temperature and late period mortality, and loading temperature and journev duration. Rather than presenting a complex 4-way interaction model, which would be difficult to interpret, the presented model was stratified and the results presented separately for when late period mortality was $\leq 0.8\%$ (Supplementary Table S4a), late period mortality was >0.8% (Supplementary Table S4b), journey time was $\leq 60 \min$ (Supplementary Table S5a) and journey time was >60 min (Supplementary Table S5b). All models showed similar trends and point estimates, however, some associations were no longer statistically significant due to the smaller sample size in these stratified models decreasing the power to detect subtle differences in rates as statistically significant. The key points to note are that the detrimental effects of loading temperatures >30.0°C when relative humidity $\leq 80\%$, and of loading temperatures between 20.1°C and 25.0°C when relative humidity >80%, were more pronounced for flocks with above median late period mortality. On longer journeys, the detrimental effects of mechanical catch were more pronounced, with the rate ratio for mechanical catch being 1.31 (DOA rate 31% greater than for manual catch) on journeys $\leq 60 \text{ min and } 2.43 \text{ (DOA rate})$ 143% times greater than for manual catching) on journevs >60 min.

DISCUSSION

To date, this is the largest study of mortality rates on arrival at the slaughterhouse in broiler chickens produced on British farms. The overall mean DOA rate was 0.08%, which equates to 8 in 10,000 birds. Several risk factors were shown to influence DOA rate including external ambient temperature and relative humidity at loading, catch method and breed. Knowledge of these risk factors can help identify practices which aim to reduce losses during transport and improve broiler welfare.

The overall mean and median DOA rates were lower than those previously reported for the United Kingdom and other European countries. UK studies have reported a mean DOA rate between 0.12 and 0.13% (Haslam et al., 2008; Buzdugan et al., 2021). From this work, it is not possible to determine, categorically, the reasons for the lower DOA rate observed, however, it probably reflects the UK broiler sector's continuous drive to improve welfare in transport and husbandry. Sampling bias should also be considered, although this is likely negligible, given the broiler companies contributing data to this project represent over 90% of UK broiler production and study dates were randomly selected throughout 2019.

Consistent with previous work, this study identified several risk factors for DOA. External environmental conditions greatly influenced DOA rate, with loading temperatures >30.0°C substantially increasing the percentage DOA. This finding is similar to other studies that demonstrated an increased mortality rate when broilers were loaded in external ambient temperatures >18.0°C (Warriss et al., 2005) and transported during the warmer summer months (Vecerek et al., 2006). This study also identified a small increase in mortality when relative humidity was >80% and external ambient temperature below freezing. Cold temperatures at loading and in-transit have previously been shown to increase broiler mortality in Canada (Caffrey et al., 2017) and the Netherlands (Nijdam et al., 2004). Heat and cold stress are recognized as major causes of broiler mortality; accounting for up to 40% of deaths (Ritz et al., 2005).

The effects of higher loading temperatures on longer journeys, under conditions of high humidity, were stronger in flocks with above average late period mortality. Late period mortality is likely an indicator of overall flock health. Poor flock health is known to predispose to DOA (Whiting et al., 2007; Chauvin et al., 2011; Kittelsen et al., 2015; Kittelsen et al., 2017); with pre-existing health conditions thought to account for 25% of DOAs (Ritz et al., 2005). As birds in poor health are more vulnerable to environmental extremes (Mitchell and Kettlewell, 2009), it stands to reason that at higher temperatures, poorer health flocks exhibit a higher rate of mortality compared to healthier flocks better able to cope with the stresses of catching and transport in extreme conditions.

One of the main limitations of this study is that loading temperature and relative humidity will not accurately reflect the thermal conditions experienced by broiler chickens in the transport modules. Measurement of internal environmental factors was neither practical nor feasible for this study, however, future work would benefit from the additional evaluation of internal ambient temperature and water vapor density in-transit. Careful consideration needs to be given to the placement of probes within the trailer, as deaths do not occur uniformly throughout a load and center in the thermal core (Mitchell and Kettlewell, 1998). Another weakness of this study is external ambient temperature and relative humidity were measured at the most appropriate weather station and not on farm. This may have led to measurement error, however, this is likely to have had little effect, as for the majority of farms were located only a short distance from the weather station.

Despite the limitations of this study, the results can still be used to make practical recommendations to the industry. One simple and feasible proposal to decrease the DOA rate is to ameliorate the negative effects of high temperatures by encouraging and supporting the

catching and processing of birds earlier in the day or at night, especially when hot weather is predicted. This would require the co-operation of abattoirs and for slaughter capacity at night or during the early morning to be adjusted during these periods. Catching earlier in the day may also have the added benefit of reducing rejections, with injury and bruising less common in broilers caught and transported at night (Nijdam et al., 2004). All broilers transported in the current study were transported using a modular system. Although the effect of module stocking density on DOA rate was not explicitly considered in this study, it was routine practice for the collaborating producers to reduce module stocking densities during periods of hot weather. If high temperatures cannot be avoided, it is possible that further reduction in module stocking density during hot weather, particularly within the thermal core of the trailer, may be beneficial and warrants investigation.

Catching is the process that transfers birds from the poultry house to the transport module. Higher rates of DOA were observed for loads caught mechanically rather than manually: an observation that is consistent with several studies of broiler mortality (Nijdam et al., 2004, Vizzier-Thaxton et al., 2006, Chauvin et al., 2011, Mönch et al., 2020). Mechanical catching is thought to be less stressful (Delezie et al., 2006; Weeks et al., 2019), however, evidence that it reduces bird injury rates is conflicting and the model of harvester used may be influential (Mönch et al., 2020). Although relatively few flocks were caught mechanically in the current study, the association with DOA rate was strong. The greater rate of DOA may be explained by anecdotal reports of mechanical harvesters also inadvertently collecting any dead birds present in the shed during the catch. However, this would not explain the further relative increase in DOA rate that occurred with longer journey times in mechanically-caught birds. This observation warrants further investigation. Measurements of bird injury rates and mortality after catching but prior to transportation would be particularly informative. Manual catch method (Kittelsen et al., 2018) and catch team (Caffrey et al., 2017) are also known to influence DOA rate and warrant consideration in future studies of broiler mortality.

A substantially lower rate of DOA was observed in the slower-growing JA Hubbards compared to Ross 308 broilers. JA Hubbards may be less susceptible to heat stress due to genetic differences; with many studies demonstrating greater robustness and health in slow-growing breeds (Rayner et al., 2020; Abeyesinghe et al., 2021; Baxter et al., 2021). While this work suggests that a move away from production of conventional fast-growing Ross 308s towards the use of slower-growing JA Hubbards may be beneficial, as only 2% of study trailer loads transported JA Hubbards, there is a moderate degree of uncertainty surrounding the effect size. Purpose-designed studies, that can account for the confounding effects of the factors considered in this study and other potentially influential management practices such as module stocking density, would provide further insight into the effect of breed on DOA rate. In addition,

the sustainability of producing slow-growing broilers remains debatable (Chan et al., 2022) and warrants further evaluation before recommendations concerning breed types can be made.

Alongside the limitations mentioned above, the data included in this study were not specifically collected for the purpose of this project and there were areas where errors seemed likely. For example, it was noteworthy that over 2,700 loads had a calculated average journey speed of <20 km/h or >120 km/h. We would expect such low or high average speeds to occur relatively rarely, and so loads with these extreme values were excluded from analysis of the effects of journey speed. It would be beneficial for future monitoring to develop more accurate recording systems for recording this measure. In most cases, we were reliant on route plans and expected loading completion times when estimating journey parameters. Whilst it is likely these planned measures were a reasonable proxy for the true values, it is possible their use may have introduced some error. Weather data may also have been incorrectly estimated when loading was completed ahead of, or after, the expected completion time. In addition, the use of secondary data limited risk factor analysis to factors for which data were available. Future work would benefit from the collection and analysis of additional data relating to other potentially influential factors not considered in this work such as vehicle design, pretransport management, bird weights and lairage conditions. Although this information was lacking, we believe our results are still highly applicable to the British broiler industry as the companies contributing data to this project are responsible for 90% of broiler production in Great Britain.

In conclusion, the mortality rate in broilers transported to slaughter, was lower than previously reported and multi-factorial in nature. Many risk factors only had a marginal effect on DOA rate, however, both high external ambient temperature at loading and breed exhibited strong effects. Avoiding the loading and transport of broiler chickens during hot weather would help limit economic losses and improve bird welfare.

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DISCLOSURES

Y.M. Chang reports financial support was provided by British Poultry Council. C. D. Parker reports a relationship with British Poultry Council that includes: consulting or advisory.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j. psj.2023.102634.

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