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## Diagnosis of respiratory disease in preweaned dairy calves using sequential thoracic ultrasonography and clinical respiratory scoring: Temporal transitions and association with growth rates

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### ABSTRACT

Bovine respiratory disease (BRD) in dairy calves is a multifactorial condition, involving environmental, host, and pathogen factors. Thoracic ultrasound scoring (TUS) has recently been validated as an accurate method of detecting BRD-related lung pathology in dairy calves. Previous studies investigating the use of TUS in preweaned dairy calves have largely been based on cross-sectional data from all-year production systems. The objectives of this longitudinal observational study were to characterize the temporal transitions in TUS scores in dairy calves from pasture-based, seasonal-calving herds using sequential examinations during the preweaning period, and to investigate the relationship between the presence and temporal pattern of BRD, diagnosed by TUS or clinical respiratory scoring (CRS), and average daily gain (ADG). In spring of 2019, 317 preweaned calves from 7 commercial dairy farms were recruited at less than 4 wk old (ranging from 1–27 d of age). Each farm was examined on at least 3 occasions at 20- to 28-d intervals and housed indoors in group or individual pens. At each visit TUS scores, CRS scores based on the University of Wisconsin Calf Respiratory Score Chart (https://www .vetmed.wisc.edu/fapm/wp-content/uploads/2020/01/ calf\_respiratory\_scoring\_chart.pdf), and live weight using a dairy breed-specific weigh band were recorded. All data were recorded by the same 2 veterinarians over the course of the study. The final data set consisted of 966 TUS and CRS scores collected from 317 calves over a period of approximately 6 wk from 7 farms. The data

were analyzed in multivariable, mixed effects, linear regression models, with separate models constructed for TUS and CRS scores. Random effects (intercepts) were included for calf, farm, and visit week. Additionally, a random slope was included for age at sampling by farm. Median farm TUS score ranged from 0 to 2.5 over the 3 visits (possible range: 0–5). The percentage of calves with a TUS score  $\geq 3$  (consolidation of the full thickness of 1 lung lobe), on each farm ranged from 0to 50%. The median CRS in calves on individual farms ranged from 1 to 3 over the 3 visits (possible range: 0-12). The percentage of calves on each farm with a CRS score  $\geq 5$  (possible range: 0–12) ranged from 0 to 26%. The TUS and CRS scores were weakly correlated. The TUS was associated with reduced ADG. Calves with TUS scores > 3 grew at 126 g/d less than unaffected calves over the 3-wk period before examination. The predicted effect on ADG was dependent on the age and duration over which the animal was affected. Calves affected later (i.e., between visits 2 and 3) had lower predicted weights at 63 d compared with calves with increased TUS scores earlier in the study period. Calves with a TUS score > 3 at each of the 3 sampling points had the lowest weight at 63 d of age. There was no association of CRS with ADG. This study showed that in contrast to CRS, higher TUS scores are associated with lower ADG, with weight loss being more pronounced in chronic cases.

**Key words:** thoracic ultrasonography, clinical respiratory score, dairy calf, average daily gain, body weight, preweaning, respiratory disease, bovine

## INTRODUCTION

Bovine respiratory disease (**BRD**) in dairy calves is a multifactorial condition, involving environmental,

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host, and pathogen factors (Dubrovsky et al., 2019). In the Republic of Ireland, BRD has been consistently identified as the most common cause (34%) of mortality in calves aged between 1 and 5 mo of age (Department of Agriculture, Food and the Marine, 2019). In this age cohort, Mannheimia haemolytica, Pasteurella multocida, Mycoplasma bovis, and bovine respiratory syncytial virus were the most common organisms identified on postmortem examination, isolated from 19, 16, 15, and 10% of all cases respectively (Department of Agriculture, Food and the Marine, 2019). Other negative effects of BRD on animal health and economics include treatment costs (Mohd-Nor et al., 2012), reduced growth rates (Cramer and Ollivett, 2019), and decreased survival to first calving (Closs and Dechow, 2017). Some of the sequelae of BRD, including decreased ADG, have been linked to reduced future cow reproductive performance (Hayes et al., 2019) and milk vield productivity (Gelsinger et al., 2016; Van de Stroet et al., 2016). Therefore, improved understanding of the diagnosis, course, and consequences of the disease is important.

The lack of an antemortem gold standard diagnostic test to diagnose BRD leads to difficulties in identifying true cases and in the selection of cases that would most benefit from treatment. Inaccurate diagnosis can lead to a wide variation in treatment and preventative strategies and in the effectiveness of these strategies (Karle et al., 2018). Currently, auscultation is the primary diagnostic modality used for BRD detection by veterinarians. However, auscultation lacks accuracy, with a sensitivity as low as 6% reported in one study of preweaned dairy calves (Buczinski et al., 2014). Clinical respiratory scoring (CRS) systems have been previously described (McGuirk and Peek, 2014). These systems assign a score to various parameters including rectal temperature, ear position, and the presence of a cough. However, these have also been shown to have moderate accuracy with an estimated sensitivity of 62%and specificity of 74% (Buczinski et al., 2015). The incidence of clinical respiratory illness in Irish preweaned dairy calves, using the Wisconsin CRS evaluation or a modification of this system, has previously been reported as 33 to 50% (Conneely et al., 2014; Johnston et al., 2016).

In contrast, thoracic ultrasound scoring (**TUS**) has been validated as a more accurate method of detecting lung pathology due to BRD in dairy calves, with a reported sensitivity of 79.4% and specificity of 93.9% (Buczinski et al., 2015). Thoracis ultrasound scoring offers the unique advantage that it can detect lung consolidation in cases of subclinical BRD (Buczinski et al., 2014). Previous studies investigating the use of TUS in preweaned dairy calves have largely been based on data from all-year production systems (Buczinski et al., 2015, 2018). Relatively few studies have described transitions in TUS scores over time during the preweaning period. Similarly, there have been limited longitudinal studies on the effect of TUS on ADG during the preweaning period. Of these studies, the key focus has been on the assessment of the effect of lung consolidation at any point during the preweaning period on either ADG (Cramer and Ollivett, 2019) or on future milk production (Dunn et al., 2018), rather than evaluating the effect of the timing of the lung consolidation on ADG or the temporal profile of TUS scores across different time points.

Furthermore, to our knowledge, no studies have yet been conducted examining calves in pasture-based, seasonal-calving dairy systems such as those used in the Republic of Ireland. Most calves in such systems are born in a condensed calving period (ICBF, 2019) and housed and managed in large groups, which potentially increases the risk of respiratory pathogen transmission, as well as provides comparisons with larger numbers of age-matched controls.

The objectives of this study were to characterize the changes in TUS scores over time in dairy calves from pasture-based, seasonal-calving herds during the preweaning period and to investigate the relationship between both TUS and CRS, and ADG. We hypothesized that calves with more severe or more chronic TUS or CRS scores would have reduced ADG.

## **MATERIALS AND METHODS**

### Farm and Animal Management and Recruitment

*Ethics.* This study was approved by University College Dublin Animal Research Ethics Committee (AREC-17–35-McAloon) and the Health Products Regulatory Authority (AE18982/P130).

**Farm Selection and Description.** A convenience sample of 7 herds with a history of BRD, with an estimated morbidity of greater than 10% in the previous calving season (2018), as diagnosed by an attending veterinary practitioner, were recruited for the study during the 2019 spring calving season. Farms were located across the Republic of Ireland in counties Meath, Westmeath, Cork, and Kilkenny. Of the farms selected, 4 were commercial dairy farms, 1 was a contract heiferrearing farm that reared calves for only 1 commercial dairy farm, 1 was a dairy calf-to-beef research farm that purchased dairy-beef cross-bred calves from commercial dairy farms to

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rear through to slaughter. Farms reared male, female, or both sexes spring-born dairy and crossbred dairy-beef calves. Greater detail on the numbers of milking cows and calf management is shown in Supplemental Table S1 (https://figshare.com/s/8487d3ec33b788c67c1d).

*Calf Management.* Management practices were not standardized across the farms. Calves received colostrum shortly after birth. On 6 farms, calves were initially reared in single pens before being moved into straw-bedded group pens from 3 to 7 d of age. These individual pens were either in a separate calf-rearing building or shared the same air space as adult cattle. One farm continued to keep calves in individual pens until weaning. Six farms used automated feeding systems, whereas the seventh farm fed calves twice daily using bucket and teat. All farms had calf-specific group housing away from the main milking herd. Two farms used positive pressure ventilation, whereas the others relied on natural ventilation. All farmers fed reconstituted milk-replacer powder. Age of weaning and weaning protocols varied between farms. All calf veterinary medicinal treatments were recorded by the herd owner in their medicine book. Respiratory vaccination and BRD treatment protocols varied between farms.

**Calf Enrollment.** All calves (n = 317) aged between 1 and 27 d at the first visit to each farm were enrolled in the study. Calves were enrolled at this age to allow for 3 consecutive readings, at 20- to 28-d intervals to be taken before weaning, and to ensure a greater chance of examining calves before they acquired respiratory disease. An a priori sample size calculation was not specifically carried out for this study.

## **Calf Data Collection**

Data collection took place between February and May of 2019. Veterinarians examined each calf 3 times at 20- to 28-d intervals. Calves were manually restrained in the corner of a pen. Each examination included CRS, using the University of Wisconsin Calf Respiratory Score Chart (McGuirk and Peek, 2014), then TUS, using a portable linear rectal ultrasound scanner (Ollivett and Buczinski, 2016), and liveweight, using a dairy-specific weigh band. The same veterinarian (V. R.) completed all the TUS exams throughout the study period. Neither intra- nor interrater reliability was assessed as part of this study.

**Weighing.** Calf body weights were recorded using a calf weigh tape specifically designed for dairy breeds (Volac Calf Weigh Tape, Part Number 44552M, The Coburn Company, Inc.). The tape was placed around the heart girth and pulled tightly, with the calf standing square. The weight was then read from the appropriate scale on the tape. One measurement was taken from each calf per visit.

Clinical Respiratory Scoring. Using the Wisconsin scoring method as described by McGuirk and Peek (2014), evaluation of CRS was performed by the same 2 operators throughout the study. This involved examination of the calf for presence and severity of nasal and ocular discharge, ear position, presence or absence of a spontaneous or induced cough, and recording of rectal temperature. A score of 0 (normal and lowest risk of BRD) to 3 (severely abnormal and the highest risk of BRD) was assigned to each parameter, with the maximum of eye and ear score used in the total when summing to the total score. Calves were considered to be BRD-positive if they had a CRS score  $\geq 5$  (possible range 0–12).

Thoracic Ultrasound Scoring. The TUS was performed using a portable linear rectal ultrasound scanner set at a depth of 9 cm and frequency of 8.5 MHz (Easi-Scan, BCF Technology Ltd.). A couplant agent (approximately 100 mL of 70% isopropyl alcohol) was applied to the unclipped hair on both sides of the thorax. The first author (V. R.) performed all the TUS examinations. For the initial visit, the TUS was conducted in conjunction with a Diplomate of the American College of Veterinary Radiology and European College of Veterinary Diagnostic Imaging (S. H.) for training purposes. The TUS exam was performed in accordance with the technique described by Ollivett and Buczinski (2016), except that the probe was held perpendicular to the ribs, rather than parallel. This approach was used to maximize the contact of the transducer with the body wall, as the transducer footprint reduced image quality in long-axis imaging due to the narrow intercostal space in smaller calves. This method facilitated imaging of the whole lung field, as the same cross-sectional area of the thoracic wall was interrogated. The TUS exam started at intercostal space (ICS) 10 on the right-hand side at the dorsal aspect of the lung field. The probe was slid ventrally, maintaining the orientation perpendicular to the ribs, until reaching the ventral border of the lung field. The probe was then returned to the dorsal aspect of the lung field, just cranial to the footprint that was previously imaged. This process was repeated until ICS 1 was reached. The same procedure was repeated on the left-hand side of the calf, examining ICS 10 to ICS 2. Normal peripheral lung appearance was considered a hyperechoic line with reverberation artifact, corresponding to the pleuralaerated lung interface. The normal aerated lung has been reported to have some small comet tail artifacts (a thin, vertical line of reverberation artifact, originating from the pleural-lung interface). A full thickness

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area of consolidation in a lung lobe would present as a hypoechoic area deep to the pleural-lung interface, superficial to more normal lung which would elicit a reverberation artifact. The entire lung field was examined on each calf and assigned a score from 0 to 5 as previously described by Ollivett and Buczinski (2016; 0 = normal aerated lung with no consolidation and none to few comet tail artifacts; 1 = diffuse small comet tailartifacts without consolidation; 2 = isolated areas ofconsolidation larger than 1 cm, but not involving the full thickness of the lobe; 3 = consolidation of the fullthickness of 2 lung lobes; 5 = consolidation of the fullthickness of 3 or more lung lobes). A TUS score  $\geq 3$  is considered positive for BRD.

**Data Analysis.** Records collected on paper were entered in Microsoft Excel (Microsoft Office Professional Plus 2013). Next, animal records for each farm were downloaded from the Irish Cattle Breeding Federation (https://www.icbf.com/?page\_id=14233) database. The date of birth for each calf was matched to the records collected from the farm visits.

The data were then imported into R version 3.6.2 (https://www.r-project.org/) for further data processing and statistical analysis. First, calves with 2 or fewer recordings (n = 77) were excluded from further analysis. For calves with 2 or more recordings, additional variables were created for each visit. Current age was calculated in days using the interval between date of birth and the visit date. Visit interval was calculated as the time between the present visit and the previous visit. Visit week was calculated as the week of the year in which the visit took place, and week of birth was calculated in the same way. Growth rate was calculated as the difference in weight between the current visit and the previous visit, divided by the time interval in days between the 2. All data manipulation was conducted using the "dplyr" package in R (Wickham et al., 2020). After the creation of these variables, all first visit data were deleted from the data set. Treatment was not included in any of the analyses.

Statistical Analysis. Two separate models were created to examine the association between TUS or CRS and ADG (dependent variable), accounting for farm, age of the calf, interval between successive assessments, previous weight, and visit week. Continuous data were first visualized using histograms to ensure they were normally distributed. Ordinal data (CRS and TUS scores) were treated as categorical data. For both CRS and TUS, several forms of the variable were created. First, all scores were individually included; in the rest, different permutations of adjacent categories were amalgamated (e.g.,  $\leq 1, 2, 3, \geq 4$ ). Only the form of the variable resulting in the best model fit, as determined by the Akaike Information Criterion (AIC), was selected for analysis. Finally, the shape of the association between each variable and ADG was visualized using scatter plots. Where a nonlinear relationship appeared to exist, the variable was offered to the model in both a linear and second order polynomial term with the version with the lowest AIC being used in the final model.

All variables were first screened in a univariate analysis. Variables with a P-value < 0.2 were offered to the subsequent multivariable analysis. For this, a random effects model with random intercepts for calf, farm and visit week, a fixed effect of current age, and random slope of current age by herd was first constructed. Before the addition of each variable to the model, the correlation between that variable and the rest of the fixed effects in the model was calculated. When 2 variables were highly correlated (>0.8), only 1 variable was chosen for inclusion in the model. The decision over which variable to include was determined based on the variable with the lowest AIC value. Variables were added to the model in order of their univariate *P*-value with variables with the lowest P-value added first. After the addition of each variable, the P-values for the remaining variables were recalculated, and variables with P > 0.05 were excluded from the model. Separate models were created for TUS scores and CRS scores. Mixed effects models were run in R using the "lme4" package. The *P*-values were manually calculated from the estimates and standard errors as  $P = \exp(-0.717 \times$  $Z - 0.416 \times Z^2$ ), where Z equals the effect estimate divided by the standard error (Altman and Bland, 2011). All 2-way interactions between the final variables in the model were assessed with significant interactions (P <(0.05) included in the final model. The model fit was assessed by predicting on the full data set, computing the coefficient of determination  $(\mathbf{R}^2)$  between real and predicted values, as well as the mean absolute error. Variance partitioning coefficients were calculated as

$$\frac{\sigma_i^2}{\sigma_i^2 + \sigma_j^2 + \sigma_k^2 + \sigma_e^2},$$

where  $\sigma_i^2$  is the variance at the calf level,  $\sigma_j^2$  is the variance at the sampling week level,  $\sigma_k^2$  is the variance at the herd level, and  $\sigma_e^2$  is the residual variance.

**Model Predictions.** To illustrate the effects of the models, a series of predictions were made. First, growth rates were predicted from the final model across the range of ages in the data set with a fixed previous weight. Next, weights over time were predicted for a series of calves, each following one of several scenarios: an unaffected calf with a TUS score <2 at each of the

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Table	<b>1.</b> Hy	pothetical	scenarios	used	$_{\mathrm{to}}$	illustrate	model	results'
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Scenario	Simulated TUS score at 21 d	Simulated TUS score at 42 d	Simulated TUS score at 63 d
Unaffected Early-affected–recovered Mid-affected–recovered Late-affected Chronic		$ \begin{array}{c} \leq 1 \\ 2 \\ \geq 3 \\ \leq 1 \\ \geq 3 \end{array} $	$ \begin{array}{c} \leq 1 \\ \leq 1 \\ 2 \\ \geq 3 \\ \geq 3 \end{array} $

<sup>1</sup>Each scenario predicts the weight of the calf according to a thoracic ultrasound scoring (TUS) score at each of the 3 observation windows (21 d, 42 d, and 63 d). For example, the "unaffected" scenario predicts the weight of calf over time that had a TUS score  $\leq 1$  at each observation window. In contrast, the "late affected" scenario predicts the weight of the calf that scored  $\leq 1$  at the first 2 observation windows, but  $\geq 3$  at the final observation window.

3 observation points; an "early-recovered" calf with a TUS score of 3 at 21 d of age, a score of 2 at 42 d, and a score of 0 or 1 at 63 d of age; a "mid-recovered" calf with a TUS score of 3 at 42 d of age; a "late-affected" calf with a TUS of 3 at 63 d of age; and finally a "chronic" calf with a TUS of 3 at each of the 3 observation stages. The prediction scenarios are shown in Table 1.

For these predictions, the weight at the previous observation and the ADG, predicted from the final model, was used to predict the weight at each observation. For the first observation window, a birth weight was predicted by fitting a separate model to weight data by days of age, basis splines were used to model time, and random effects for visit week, calf, and farm were included. The intercept of the resulting model was used as the birth weight.

### RESULTS

### **Descriptive Statistics**

The final data set consisted of 955 TUS scores and 951 CRS scores collected from 317 calves over a period of approximately 6 wk from 7 farms. Farms A, B, C, D, E, F, and G contributed 14, 41, 70, 37, 82, 31, and 44 calves, respectively. Any calves that did not have 3 data collections were excluded from the study. Five calves died either between the first and second visit (n = 2) or between the second and third visit (n = 3).

Descriptive results are shown in Table 2 and Figures 1 and 2, including the median TUS score over the 3 visits, the proportion of calves with TUS scores over 2 across the 3 visits, median CRS scores over the 3 visits, the proportion of calves with CRS scores including and over 5 over the 3 visits, and the ADG between visits 1 and 2 and visits 2 and 3.

The median farm TUS score ranged from 0 to 2.5 over the 3 visits (possible range 0-5). The percentage of calves with a TUS score  $\geq 3$  on each farm ranged from 0 to 50%. Median CRS in calves on individual farms ranged from 1 to 3 over the 3 visits (possible range 0-12). The percentage of calves on each farm with a CRS score > 5 ranged from 1 to 29%. The TUS scores and CRS scores were weakly correlated (Spearman's correlation = 0.10). Transitions between TUS scores over time are shown in Figure 3. Each calf is represented as a single line within the stream fields between the bars and can be followed over the course of the 3 visits, demonstrating how their TUS scores changed in relation to their score at the previous visit. The bars represent the number of calves with TUS scores 0, 1, 2and  $\geq 3$  at each of the 3 visits.

### Model Results

Results of the final multivariable model investigating the association between TUS and ADG are shown in Table 3. Both previous weight and current age were

Table 2. Descriptive statistics of thoracic ultrasound scores	(TUS	), clinica	l respiratory	scores	(CRS)	), and ADG	for	each f	farm
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Farm	Number of calves sampled	Median TUS score	Proportion of TUS $\geq 2$	Median CRS score	Proportion of CRS $\geq 5$	ADG (kg/d) between visits 1 and 2	ADG (kg/d) between visits 2 and 3
A	14	2.5	0.52	3	0.26	0.70	0.40
В	40	1	0.13	2	0.10	0.38	0.66
С	68	1	0.12	2	0.00	0.47	0.80
D	37	0	0.10	2	0.06	0.33	0.36
Ε	81	1	0.26	3	0.18	0.02	0.28
F	32	0	0.02	1.5	0.00	0.34	0.62
G	45	1	0.14	2	0.07	0.63	0.53

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significant as second order polynomial terms before trialing interactions. When included as interactions, there was a significant interaction between the squared term of the previous weight and both the linear and quadratic components of the current age variable. The visit interval between examinations was also significant.

A range of TUS variables were trialed in the final model. The best model fit was achieved when scores 0 and 1 and scores 3 and 4 were amalgamated into the same category (i.e., scores 0 and 1, score 2, score 3+). When accounting for the TUS score at the current visit, the TUS score at the previous visit was not found to be significant. Calves with a TUS score 3+ at any time point had a reduced ADG, with these calves growing 0.126 kg/d less than their counterparts (P =0.03). The final TUS model had an R<sup>2</sup> of 0.48 with a mean absolute error of 0.18 kg/d. The variance partition coefficients for the variance partitioned into the individual calf, visit week, farm, and residual levels were <0.01, 0.15, 0.61, and 0.25 respectively. There was no association between positive CRS and growth rate in our study (P = 0.10).

### Model Predictions

The results of the predicted scenarios are shown in Figure 4. Calves with score 0 for both TUS and CRS were predicted to grow to 85 kg by 63 d of age. Calves that were either affected (i.e., TUS 3+) early and recovered, or were affected late and recovered, had a 63-d weight that was approximately 2 kg lighter than unaffected calves. Calves that were affected in the



Figure 1. Distribution of thoracic ultrasound (TUS) scores among 317 calves each examined 3 times at 20- to 28-d intervals. Mean age (SD) at each visit was 16.1 (10.3), 36.1 (8.7), and 57.0 (7.8), respectively.

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late window were approximately 2.5 kg lighter. However, the largest effect was predicted for calves that were chronically affected with a score of 3 or higher throughout the preweaning period, with these calves predicted to weigh approximately 7 kg less at 63 d than unaffected calves.

### DISCUSSION

The objectives of this study were to characterize the temporal transitions in TUS scores in dairy calves over the course of the preweaning period, and to investigate the relationship between the presence and temporal pattern of BRD, diagnosed by TUS and CRS, and growth rate. To the authors' knowledge, this is the first longitudinal study throughout the preweaning period

to assess the association of TUS with ADG in springborn dairy calves in a seasonal-calving system. Previous studies have focused on a calf's first BRD event (Cramer and Ollivett, 2019) or TUS at 1 point during the preweaning phase (Ollivett et al., 2014) and were based on all-year dairy production systems. In seasonalcalving herds, a high proportion of similar-aged calves of relatively uniform genetics and weight are available at a defined period of the calendar year, reducing the potential for confounding due to external variables that may change with time over the course of the year. Furthermore, in seasonally calving dairy herds, calf birth weights and growth rates might be expected to be lower due to differences in genetics between larger North American Holstein-Friesians and British-Friesian and Channel Island breeds.



Figure 2. Distribution of clinical respiratory (CRS) scores among 317 calves each examined 3 times at 20- to 28-d intervals. Mean age (SD) at each visit was 16.1 (10.3), 36.1 (8.7), and 57.0 (7.8), respectively.

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Variable	Estimate	SE	<i>P</i> -value
Intercept	0.360	0.088	0.000
TUS 0 or 1	Referent		
TUS 2	-0.010	0.031	0.763
TUS 3+	-0.126	0.054	0.020
Previous weight	0.005	0.644	0.994
Previous weight <sup>2</sup>	-0.513	0.504	0.314
Interval 20 d	Referent		
Interval 21 d	0.355	0.073	0.000
Interval 22 d	0.041	0.066	0.542
Interval 23 d	-0.051	0.111	0.661
Interval 25 d	0.357	0.205	0.080
Interval 28 d	0.290	0.103	0.005
Current age	2.160	0.916	0.018
Current $age^2$	-0.797	0.409	0.051
Previous weight $\times$ current age	3.111	15.219	0.849
Previous weight <sup>2</sup> $\times$ current age	-27.448	10.917	0.012
Previous weight $\times$ current age <sup>2</sup>	11.846	8.289	0.153
$Previous weight^2 \times current age^2$	9.497	4.361	0.029

**Table 3.** Results of final mixed effects linear regression model investigating the association between thoracic ultrasound scores (TUS) and ADG, accounting for age, previous weight, and interval between scans

This study examined the temporal dynamics in TUS score at 3 time points during the preweaning period. Although animals were only monitored during the preweaning period, it was evident that the timing and duration of elevated TUS scores (score 3 and

above) had a negative effect on ADG. The prevalence of lung consolidation (TUS  $\geq$  3) ranged from 0 to 50% of calves during the preweaning period across the 7 herds, with a median within-herd prevalence of 10%. Farm-to-farm variation is common. A Canadian study



Figure 3. An alluvial plot showing the transitions in thoracic ultrasound scores (TUS) over time. The vertical block columns represent each visit 1, 2, and 3 at 20- to 28-d intervals. The colors in each block correlate to a TUS score depicted in the key at the bottom of the figure. Individual calves are represented by individual lines passing between the visit blocks. Calves can be tracked over the study period with the TUS score at each visit point.

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using TUS found the median within-herd prevalence of lung consolidation across 39 farms was 8%, with a range of 0 to between 85 and 90%. (Buczinski et al., 2018). More recently, a Belgian study of 60 dairy and beef farms found an average herd-level prevalence of lung lesions of 41% (range 0–100%; van Leenen et al., 2020). This highlights the farm-to-farm variability of BRD prevalence, most likely affected by a combination of nutritional, housing, and animal factors.

Due to multiple ultrasound examinations of the same animals, it was possible to follow an individual animal's progression throughout the preweaning phase, with regard to both TUS and ADG. This has previously been analyzed in weaned beef cattle (Cuevas-Gómez et al., 2020). Although most calves in our study (80%) had a TUS score of 0 or 1 during the whole preweaning period, some individuals (20%) did increase in score. However, it is apparent that although a very small percentage of calves remained at higher TUS scores (>3) throughout the duration of the study (2%), some calves were able to reduce their TUS score to a score that is not associated with a positive BRD result when further scans were undertaken. A proportion of calves on 2 farms would have been treated with antimicrobials during this period. However, treatment records were



Figure 4. Model predictions of calf weights associated with different temporal patterns (as outlined in Table 1) of thoracic ultrasound score at 3 different time points (21 d, 42 d, and 63 d) during the preweaning period. Horizontal lines represent the predicted calf weights at 63 d under each of the scenarios.

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not available for these farms that might have helped determine the effect of treatment on recovery and ADG.

There is limited research regarding the effects of lung consolidation diagnosed by thoracic ultrasonography in dairy calves with respect to analyzing subsequent growth and production affects. Cramer and Ollivett (2019) demonstrated that preweaned calves with lung consolidation detected during the preweaning period grew at 0.12 kg/d less than unaffected calves. Other studies have shown the effects of lung consolidation diagnosed with TUS to have an increased risk of removal from the herd before calving, a lower pregnancy rate, and reduced milk production (Adams and Buczinski, 2016; Teixeira et al., 2017; Dunn et al., 2018).

In the present study, calves were assessed from as early as the first day of life and followed through for a period of at least 6 wk. This allowed assessment of the effect and duration of the lung consolidation at particular time points in these preweaned calves, as well as its effects on ADG. The ADG of dairy calves was not constant over the preweaning period, but varied according to the age of the calf. In the first 3 wk of the study, the predicted ADG for unaffected calves was 0.48 kg/d. Afterward, predicted ADG followed a quadratic relationship with age of the unaffected calf, increasing to a maximum of 0.87 kg/d at 54 d of age and decreasing thereafter. Predicted ADG for calves with TUS  $\geq$ 3 was 126 g/d less than that of unaffected calves across the preweaning period. The ADG was dependent upon when calves were diagnosed with elevated TUS scores. Calves affected later (i.e., between visits 2 and 3) had lower predicted weight at weaning at 9 wk than those that had increased TUS scores earlier and successfully recovered (Figure 2). This was due to calves having higher predicted growth rates as they increased in age and calves affected later having less time to recover until weaning time compared with those affected earlier, which may then have had compensatory growth (Johnson et al., 2018). Therefore, a disruption in feed intake and growth secondary to BRD would have a proportionately higher effect in these older calves.

There was no association between positive CRS and growth rate in our study (P = 0.10). However, the direction of the association was negative, indicating a trend toward lower growth rates with higher CRS scores. A previous study found that CRS+ (when at least 2 areas including nose, eyes, ears, cough, and rectal temperature scored  $\geq 2$ ) calves had lower ADG compared with CRS- (CRS less than 5) calves (0.74 vs. 0.84, respectively, P = 0.04; Cramer and Ollivett, 2019). However, the magnitude of the effect was small in our study and may have been underpowered with respect to detecting associations between CRS and growth rate given this small effect size.

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An a priori sample size was not carried out for this specific study. However, a retrospective simulation demonstrated a power of approximately 0.95, with a sample size of 320 calves and an ADG reduction of 24%, based on our final estimated effect size of 0.126 kg/d divided by the mean ADG for the entire sample (0.50 kg/d). However, when the effect size was reduced to 10%, the estimated power for a sample of 320 calves was approximately 0.35.

In this study, TUS and CRS results were found to be weakly correlated (r = 0.10). This has been a common finding in other similar studies and this may be due to the fact that the 2 methods assess different aspects of BRD, concentrating on either upper or lower respiratory disease (Cramer and Ollivett, 2019). One previous study in calves with induced *Pasteurella multocida* pneumonia indicated that CRS and TUS scoring were poorly correlated, with calves showing severe clinical respiratory signs having minimal ultrasound lesions (Reinhold et al., 2002). This suggests that using CRS in combination with TUS decreases specificity when evaluating BRD, but that by combining the 2 methods, the sensitivity is increased (Buczinski et al., 2014).

There were some limitations to this study. The first is that although farms were selected as previously having BRD issues, the proportion of calves with BRD as diagnosed by CRS and TUS over the observation period was still relatively low (22 and 12%, respectively) during our observation period. This might be explained by changes in housing and husbandry that were instigated before the period of data collection. On 2 farms, metaphylactic treatment of all calves for BRD was instigated with in-feed antimicrobials between visits and most likely resulted in reduced clinical and subclinical disease. Management practices on farms varied, including housing, bedding material, milk-replacer brands, and the quantity of milk being fed. Our analysis included herd (and individual identification) as a random effect in the regression model. Therefore, final effect estimates are corrected for unmeasured differences in farm management. However, if these farm management practices were systematically different to the overall population of dairy herds in the country, this would limit the external validity of the study.

We chose to use a dairy breed-specific weigh band rather than a weigh bridge and scales for practicality purposes and for biosecurity between farms. This method introduced greater measurement error (Wangchuk et al., 2018). One study has shown that weigh bands can overestimate the weight of cattle by 35.3% (Machila et al., 2008). However, these errors are expected to be normally distributed around 0; therefore, they would not have biased our effect estimates, but may have affected absolute estimated BW values. Finally, farms were

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not randomly selected and were a convenience sample; therefore, they may not be truly representative of the overall population of dairy herds in Ireland.

### CONCLUSIONS

Average daily gain of dairy calves was associated with TUS score (but not CRS). The magnitude of this effect was dependent on the age and duration over which the animal was affected. Calves with TUS scores of 3 or more grew approximately 126 g/d less compared with unaffected calves.

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