Development of a genomic reference population for bovine respiratory disease in pre-weaning dairy calves using thoracic ultrasonography

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Summary

Respiratory disease has detrimental effects on the health and welfare of dairy calves and the economic viability of dairy farms, but effective strategies to reduce its incidence and severity by genetic selection are lacking. In this study, a total of 1107 Holstein calves on six Wisconsin dairy farms were assessed at 3 and 6 weeks of age using an established clinical respiratory scoring system and a novel thoracic ultrasonography system to evaluate the incidence and severity of bovine respiratory disease. Incidence rates of pneumonia in thoracic ultrasound exams, including clinical and subclinical lobar and lobular subtypes, were 16% at 3 weeks of age and 20% at 6 weeks of age. The phenotypic correlation between thoracic ultrasound scores at 3 and 6 weeks of age was 0.26. Phenotypic correlations between clinical scores and thoracic ultrasound scores were low, ranging from 0.04 to 0.20, indicating that ultrasonography provides new information about the incidence and severity of pneumonia, especially for calves with subclinical disease. This research provides a new protocol for objective assessment of bovine respiratory disease phenotypes in dairy calves. Pairs of trained evaluators can measure 50 to 100 calves per day on large farms and calf ranches, enabling rapid establishment of large genomic reference populations and facilitating the incorporation of calf health into national genomic evaluation systems.

Keywords: dairy, calf, respiratory disease, ultrasonography, genomic selection

Introduction

Bovine respiratory disease (BRD) is common cause of morbidity and mortality in U.S. dairy calves and a major contributor to pre-weaning and post-weaning death rates of 7.8 and 1.8%, respectively (NAHMS, 2007). Van Der Fels-Klerx et al. (2002) noted that BRD can impair growth and increase the mortality risk, both at the time of disease and later in life. Mahmoud et al. (2017) reported an incidence rate of 28% for BRD in Holstein-Friesian heifer calves in Germany, when recorded by farmers and veterinarians as presence or absence of disease between birth and 2 months of age. Heritability was estimated as 0.07, and significant associations were found with single nucleotide polymorphism (SNP) markers on chromosomes 8 and 19. Earlier, Henderson et al. (2011) reported a heritability estimate of 0.09 for BRD in Holstein calves in New York. Heringstad et al. (2008) reported an estimate of BRD heritability

on the underlying liability scale of 0.05 in Norwegian Red calves, but Gulliksen et al. (2009) reported that the incidence of BRD in Norwegian dairy herds is underestimated by about 40% due to underreporting by dairy farmers.

Subjectivity in evaluating the severity of clinical disease is a complicating factor, and subclinical cases of BRD are typically missed in traditional scoring systems. Keele et al. (2015) carried out a genome-wide association study (GWAS) of BRD in beef cattle by evaluating lung lesions at the time of slaughter and identified 14 SNP markers on 10 chromosomes that were significant at a 5% genome-wide error rate; these were a subset of the 85 SNP markers on 28 chromosomes that were significant at a 5% false discovery rate, and the authors concluded that the genomic footprint of lung lesions is highly complex.

An obvious challenge with the aforementioned study of lung lesions is that evaluations were carried out post-mortem. Ollivett et al. (2015) and Buczinski et al. (2015) described and validated a method for assessment of lung lesions in dairy calves based on thoracic ultrasound score (TUS). The sensitivity and specificity of TUS for detecting lung lesions were 94 and 100%, respectively, when compared with the gold standard of post-mortem evaluation.

Genomic selection offers the possibility to improve resistance to BRD, by measuring detailed phenotypes in a reference population of genotyped calves and using this information to predict the estimated breeding values (EBV) of male and female selection candidates in the national population. Selection for improved resistance to BRD is important economically, and it is a timely problem. It is well known that group-housed calves have increased risk of BRD (Cobb et al., 2014), due to more calf-to-calf contact and disease transmission, and group housing is becoming more common due to growth in the popularity of automated calf feeders. Therefore, the objective of this study was to establish a protocol for objective and efficient assessment of BRD phenotypes that will facilitate establishment of a large reference population and enable genomic selection for reduced incidence and severity of BRD in dairy calves.

Materials and methods

Four trained evaluators travelled to six Wisconsin dairy farms weekly from May through August 2017. All female Holstein calves were evaluated at 3 weeks and 6 weeks of age, in order to assess variation in BRD at different ages, as well as to identify new infections or recoveries that occurred between 3 and 6 weeks of age. In total, the data consisted of 2167 measurements on 1107 calves. Calves were evaluated for clinical respiratory scores using the system described in Table 1. Thoracic ultrasonography was performed on both the left and right sides of each calf. Approximately 100 ml of rubbing alcohol was sprayed on the calf's midsection, between the first and sixth intercostal spaces (ICS). Right middle, caudal and cranial aspects of the right cranial lobe, and left caudal and cranial aspects of the left cranial lobe were viewed and scored using an IBEX Pro scanner (E.I. Medical, Loveland, CO) with a linear rectal transducer, using the criterion described in Table 2. The IBEX Pro gain, near, and far settings were 18, 25, and 36, respectively, and data collection followed the protocol described by Ollivett et al. (2015). Classification of pneumonia phenotypes was based on the clinical and TUS scores, as described in Table 3. All clinical and TUS scores were entered into the Calf Health Scorer iPad application (University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI), and low-density genomic tests were carried out by Zoetis Genetics (Kalamazoo, MI).

Table 1. Clinical respiratory scoring system used to evaluate Holstein heifer calves at 3 and 6 weeks of age.

	Score			
	0	1	2	3
Rectal temperature	100-100.9	101-101.9	102-102.9	≥ 103
Cough	None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge	Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral, cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
Eye score	Normal	Small amount of ocular discharge	Moderate amount of ocular discharge	Heavy ocular discharge
Ear score	Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop

Table 2. Thoracic ultrasound scoring system, where consolidations are measured in the dorsal-ventral plane. The category 2JB (just barely) is a modification of the Ollivett et al. (2015) scoring system that acknowledges differences of less than 1 cm in lung consolidation

	Thoracic Ultrasound Category	Thoracic Ultrasound Score
Normal	Normal	0
Comet-tail artifacts imaged as vertical hyperechoic lines emanating from the pleural surface without hypoechoic consolidations	Very Mild	1
< 1 cm of hypoechoic consolidation lacking the hyperechoic line of the pleura and reverberation artifact in the area of the lesion	Mild	2JB
≥ 1 cm of hypoechoic consolidation	Moderate	2
1 lobe of hypoechoic consolidation	Moderate	3
2 lobes of hypoechoic consolidation	Severe	4
≥ 3 lobes of hypoechoic consolidation	Severe	5

Table 3. Protocol for assignment of overall bovine respiratory disease scores for genomic analysis, based on clinical respiratory scores and thoracic ultrasound scores.

	Clinical Respiratory Score	Thoracic Ultrasound Score	Overall Bovine Respiratory Disease Score
Healthy	< 2 scores ≥ 2	≤ 2JB	1
Upper Respiratory Tract Infection	≥ 2 scores ≥ 2	≤ 2JB	2
Subclinical Lobular Pneumonia	< 2 scores ≥ 2	2	3
Subclinical Lobar Pneumonia	< 2 scores ≥ 2	≥ 3	4
Clinical Lobular Pneumonia	≥ 2 scores ≥ 2	2	5
Clinical Lobar Pneumonia	≥ 2 scores ≥ 2	≥ 3	6

Grouping of clinical respiratory scores is based on the number of categories in which a calf receives a score of 2 or greater. For example, a calf that is scored as 2 for temperature and 3 for cough, when combined with a TUS of 2, will be considered as subclinical lobular pneumonia and assigned an overall respiratory disease score of 3 for genomic analyses.

Results

Correlations and descriptive statistics (means and standard deviations) across all herds, as well as correlations between clinical scores and TUS are presented in Table 4, whereas overall respiratory disease scores and the corresponding percentages are shown in Table 5.

Table 4. Means and standard deviations (SD) for clinical score, as well as Pearson correlations of clinical scores with thoracic ultrasound scores, at 3 and 6 weeks of age.

	Means (SD) of Clinical Scores		Pearson Correlations with Thoracic Ultrasound Scores	
	3-Week	6-Week	3-Week	6-Week
Attitude	0.04 (0.21)	0.04 (0.21)	0.09	0.08
Nose	0.05 (0.23)	0.09 (0.33)	0.04	0.14
Eye	0.07 (0.27)	0.11 (0.34)	0.07	0.06
Ear	0.06 (0.29)	0.07 (0.29)	0.07	0.07
Cough	0.12 (0.45)	0.16 (0.53)	0.17	0.18
Temperature	1.92 (0.68)	1.71 (0.73)	0.11	0.08
Total Respiratory Score	2.23 (1.02)	2.15 (1.18)	0.19	0.20

The correlation between TUS measured at 3 and 6 weeks of age was 0.26, indicating moderate repeatability. Correlations between clinical scores and TUS were small, indicating that significant variation exists in lung consolidation due to clinical and subclinical BRD beyond that which can be assessed using a conventional clinical respiratory scoring system.

The overall incidence rates for pneumonia at 3 and 6 weeks of age, based on the aforementioned combination of clinical scores and TUS, were 16.0% and 20.2%, respectively, as shown in Table 5.

Table 5. Overall percentages of calves in each pneumonia category, at 3 and 6 weeks of age, based on overall respiratory disease scores derived by combining clinical scores with thoracic ultrasonography.

	3-Week Incidence Rate (%)	6-Week Incidence Rate (%)
Healthy	81.1	77.1
Upper Respiratory Tract Infection	2.9	2.7
Subclinical Lobular Pneumonia	11.9	12.5
Subclinical Lobar Pneumonia	2.6	5.3
Clinical Lobular Pneumonia	0.8	1.4
Clinical Lobar Pneumonia	0.7	1.0

Conclusions

A protocol for objective and efficient assessment of BRD phenotypes in dairy calves is provided, based on combining clinical scores with thoracic ultrasonography. Pairs of trained evaluators can assign clinical scores and carry out ultrasound scans on 50 to 100 calves per day on large dairy farms or calf ranches. Evaluation of calves at two time points allows the detection of differences in recovery rate, while decreasing the incidence of type II errors (missed cases of clinical or subclinical disease). This protocol will facilitate rapid establishment of large genomic reference populations for the major dairy cattle breeds, with the objective increasing resistance to bovine respiratory disease during the pre-weaning period and reducing the severity of lung damage and its impact on future performance.

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