Differential gene expression in peripheral leukocytes of pre-weaned dairy calves with respiratory disease

L. A. Elder, BS; H. R. Hinnant, BS; C. M. Mandella, BS; R. A. Claus-Walker, BS;
L. M. Parrish, BS; S. C. Trombetta, BS; G. S. Slanzon, PhD; C. S. McConnel, DVM, PhD

Field Disease Investigation Unit, Veterinary Clinical Sciences, Washington State University, Pullman, WA 99164

Introduction

Bovine respiratory disease (BRD) is a leading cause of calf morbidity and mortality in both beef and dairy sectors of the United States cattle industry resulting in financial losses and compromised welfare. Differential gene expression (DGE) allows detailed insight into individual immune responses and can identify enriched pathways and biomarkers that contribute to disease susceptibility and outcome. Differential expression of genes related to innate immune function has been well documented in both feedlot and post-weaned dairy cattle with experimentally induced or naturally occurring BRD. However, little is known regarding DGE in pre-weaned Holstein calves. Therefore, the aims of this study were to evaluate gene expression in peripheral leukocytes relative to BRD diagnosis, disease progression and age progression in Holstein heifer calves throughout the preweaning period.

Materials and methods

A fixed cohort study was conducted on 2 commercial dairies in central Washington. A convenience sample of Holstein heifer calves (n = 60; 30 per dairy) were enrolled at 1 week of life and serially sampled at 2-week intervals. Blood was collected via jugular venipuncture for RNA extraction, and calves were evaluated for BRD diagnosis based on clinical respiratory signs (Wisconsin Calf Health Scoring Chart) and thoracic ultrasonography (TUS). Healthy calves, which were negative for both clinical respiratory signs and TUS lesions (n = 10), and calves with lobar lung consolidation on TUS (n = 12) were selected for DGE analysis excluding individuals that had received antimicrobial treatment by farm personnel. Genes of interest (n = 19) were selected based on their involvement in inflammatory, antibacterial or antiviral innate immune pathways that have previously been shown to be differentially expressed in peripheral leukocytes of cattle with BRD. Serial samples were analyzed for each calf spanning pre-, onset- and post- BRD diagnosis and representing weeks 3-9 of life. RNA was processed by Nanostring Technologies Proof of Principal Services Laboratory in Seattle, Wash. using a custom code set and analyzed using the Nanostring system (Nanostring Technologies, Seattle, WA). This software detects probes based on a doubling of the counts relative to the median count value of the negative control and calculates log fold changes with a Benjamini-Yekutieli (B-Y) correction for multiple comparisons and estimated false discovery rate. Comparisons were evaluated between age-matched healthy and BRD calves relative to the onset and progression of disease and between healthy calves relative to week of age. Age comparisons were also evaluated in the BRD calves and the total calves irrespective of disease.

Results

No differences in gene expression were observed in the healthy vs. BRD comparisons at any point in disease progression (B-Y $P \geq 0.1$). Differential expression was, however, observed across age comparisons in the healthy calves (B-Y $P < 0.05$). The greatest difference in both magnitude and number of genes differentially expressed was observed in comparisons of week 3 vs. older ages and with increasing age difference between the samples. The pattern of increased or decreased expression of individual genes relative to age was consistent across the comparisons. These findings were also observed in age comparisons of BRD calves and age comparisons of all calves irrespective of disease.

Significance

These results suggest that factors related to age and immune system development overshadow disease impacts to influence gene expression patterns in young calves, and that immune development progresses upon a common trajectory regardless of disease during the preweaning period. This is consistent with findings in human pediatric studies and highlights the importance of understanding neonatal development independent of disease. Biomarkers of BRD identified in feedlot or post-weaned dairy cattle in the future may not be applicable to pre-weaned calves.

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