

Effects of an innate immune stimulant on dairy calf respiratory health, *Salmonella* shedding and nasopharyngeal cytokine gene expression

G.M. Jakes,¹ BS; L.D. Sommers,² BS; J.N. Gotte,² BS; J. De Seve,² MS, BS; T.J. Applegate,² DVM, DACVIM; S. Dow,² DVM, PhD; S.M. Raabis,² DVM, PhD, DACVIM

¹Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80521

²Department of Clinical Sciences, Colorado State University, Fort Collins, CO 80521

Introduction

Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in dairy calves. Alternative treatment strategies are needed to reduce antimicrobial use. Innate immune stimulation using toll-like receptor (TLR) agonists has emerged as a strategy to reduce BRD incidence, but the prophylactic effects of this treatment have not been evaluated in pre-weaned dairy calves. Calves that develop enteric disease may be at a higher risk for BRD; however, evidence of direct links between disease processes is limited. *Salmonella* is an important enteric pathogen in dairy calves, but the impact of *Salmonella* infection on the risk of BRD development has not been determined. The objectives of this study were to evaluate the effects of an innate immune stimulant on dairy calf respiratory health, *Salmonella* shedding and cytokine gene expression.

Materials and methods

Fifty pre-weaned Holstein calves were assigned to one of 2 intranasal dosing groups administered at 1, 2 and 3 weeks of age. Control: 2 mL sucrose diluent (n = 25); LTC: 0.1 mL liposome TLR complex (LTC) in 1.9 mL sucrose diluent (n = 25). Clinical and ultrasound scores were collected twice per week using the University of Wisconsin-Madison calf health scoring system up to 10 weeks of age. Deep nasopharyngeal swabs (DNPS) were collected on each calf pretreatment, and at 2, 3, 4 and 8 weeks of age. Cytokine gene expression from DNPS was evaluated using RT-qPCR for the bovine cytokines IFN- γ and MCP-1 on a subset of calves (n = 6), with fold change in gene expression measured relative to pretreatment baseline. Fecal screening for *Salmonella* was performed using a standard enrichment protocol followed by PCR amplification for the *invA* gene. Clinical data were analyzed in R (4.3.1) for the following outcomes: prevalence of total respiratory score > 4, prevalence of ultrasound score \geq 2, and incidence of *Salmonella* shedding by treatment.

Results

There were no differences between treatment groups on the odds of developing clinical or subclinical respiratory disease based on respiratory score and lung ultrasound ($P = 0.74$ and 0.32 , respectively). Incidence of subclinical respiratory disease was higher than clinical disease presentation in this population. MCP-1 mean fold change in gene expression was 1.61 in the LTC calves, while it did not change from baseline in CON calves. IFN- γ gene expression did not differ between treatments. *Salmonella* shedding was observed in 22% of calves, though a *Salmonella* positive status did not impact the odds of developing respiratory disease during the study ($P = 0.78$).

Significance

These results suggest that while calves exhibited some increased proinflammatory immune gene expression following LTC treatment, innate immune stimulation may not improve respiratory disease risk in healthy pre-weaned calves. *Salmonella* shedding was identified in pre-weaned dairy calves; however, links to BRD risk were not identified in this study.

