

Association of bovine leukemia virus and *Mycobacterium avium* subsp *paratuberculosis* with shedding of Shiga toxin-producing *Escherichia coli* (STEC)

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Introduction

Cattle are a reservoir of Shiga toxin-producing *Escherichia coli* (STEC), which is an important cause of foodborne illness and hemolytic uremic syndrome (HUS) in children. Bovine leukemia virus (BLV) is a retrovirus that causes enzootic bovine leukosis. Most animals infected with BLV never develop clinical signs, but 30% of BLV carriers will develop a persistent lymphocytosis, and < 5% will develop malignant lymphosarcoma. Johne's disease (JD) is caused by *Mycobacterium avium* subsp *paratuberculosis* (MAP), and cattle generally become infected with MAP at a very early age (< 30 days old), although clinical disease does not typically manifest itself until 3-5 years of age. Recent studies have estimated the herd prevalence of BLV and JD in Michigan dairy herds to be approximately 85% and 50%, respectively. Both diseases are chronic in nature and can have a negative impact on the health and productivity of cattle from both clinical and subclinical effects. Because of their chronic and potentially debilitating nature, secondary health issues are often associated with BLV and JD. Our hypothesis was that cattle infected with BLV and JD were more likely to shed STEC than were uninfected cattle. Our rationale for this hypothesis was that BLV affects the immune system of cattle, which might favor the colonization and shedding of STEC. In the case of JD, the alteration of normal intestinal function and structure could potentially favor the colonization and shedding of STEC in infected cattle. Our objective was to determine whether cattle that test positive for JD and BLV could be a potential target population for the implementation of control strategies to reduce STEC contamination in the human food chain.

Materials and Methods

A cross-sectional population of 1,100 cattle from 11 herds (6 dairy and 5 beef feedlot) in Michigan were sampled for STEC, BLV, and JD. These types of herds were chosen because they are closest to the public food supply. The herds were sampled during the spring and

summer months of 2011 or 2012. A fecal grab specimen from each animal was collected for STEC culture following enrichment in gram-negative (GN) broth overnight and plating on STEC CHROMagar. Immunomagnetic separation specific for STEC O157 was also used for each fecal sample followed by culture on STEC CHROMagar. Multiplex PCR for *stx1*, *stx2*, and *eaeA* was used to screen suspect colonies and STEC confirmation. Antibody detection ELISA assays specific for BLV and MAP (Bovine Leukemia Virus Antibody Test Kit by VMRD and MAP Antibody Test Kit by Prionics) were used to screen serum from each animal. The serum was collected at the same time the fecal grab specimen was collected. Whole blood samples were collected from 513 of 1,100 animals for quantification of the percentage of lymphocytes, monocytes, and neutrophils by use of flow cytometry. Data were analyzed with the SAS statistical program. The strength of the association between STEC shedding and BLV and JD status were examined with the chi-square or Fisher's exact test, and t tests were used to analyze continuous variables. Logistic regression was used to adjust for factors associated with infection.

Results

All herds were positive for STEC, and the within-herd prevalence for the herds ranged from 8.2% to 53.7%. Beef cattle had a higher risk of being STEC-positive than did dairy cattle (OR, 1.76; 95% confidence interval, 1.26 to 2.47). Only 2 of the 11 herds were negative for BLV; the within-herd BLV prevalence ranged from 0% to 79.21%. Dairy cattle more frequently tested positive for BLV than did beef cattle (OR, 8.47; 95% confidence interval, 5.88 to 12.21). Only 3 herds had no JD-test positive cattle, and the within-herd prevalence of JD ranged from 0% to 7.92%.

Neither BLV nor JD status was associated with STEC status on the basis of results of univariate analyses or when herd was included in the model by means of Proc Genmod and Proc Glimmix. When BLV and JD status were modeled as continuous variables on the basis

of the strength of the ELISA results, there was still no association between BLV or JD and STEC status.

In the subset of cattle for which the white blood cell differential was analyzed, there was no association between STEC status and the percentage of lymphocytes, percentage of neutrophils, or the lymphocyte-to-monocyte ratio, even when BLV status, JD status, and herd were controlled in the model. The percentage of lymphocytes did not differ significantly ($P = 0.21$) between BLV-positive and BLV-negative cattle; however, the percentage of neutrophils ($P = 0.03$) and the lymphocyte-to-monocyte ratio did differ significantly between BLV-positive and BLV-negative cattle. Logistic regression results indicated that only the lymphocyte-to-monocyte ratio was significantly associated with BLV status.

Significance

It is important to develop intervention strategies to reduce the risk of STEC contamination of the human food chain. We explored the possibility that infection with immune suppressive diseases such as BLV and JD was associated with STEC shedding. Results indicated no association between BLV or JD status and the likelihood that an individual animal was shedding STEC. Although controlling both BLV and JD is important for overall herd health and productivity, there does not appear to be a benefit for targeting either BLV- or JD-positive cattle for STEC control measures.