Investigation of the Role of Bovine Viral Diarrhea Virus (BVDV) in Undifferentiated Fever of Feedlot Cattle

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Introduction

The undifferentiated fever (UF)/bovine respiratory disease (BRD) complex continues to be the single most important infectious disease entity in beef feedlot production. Current management practices have focused on successfully managing this disease complex through the use of prophylactic and therapeutic antimicrobial strategies, as well as vaccination programs targeting common viral and bacterial etiologic agents of feedlot UF/BRD described in the veterinary literature. In spite of these preventive and control strategies, there are substantial amounts of concrete and circumstantial epidemiologic, pathologic, serologic, and immunologic evidence that BVDV infection continues to play an important role in UF/BRD in commercial feedlot production, even though BVDV vaccination programs are in use.

The purpose of the project reported herein was to improve our understanding of the role that BVDV plays in the pathogenesis of pen-level UF/BRD. The specific objectives of this study were: to describe the frequency and character (Type I or Type II) of BVDV infections both persistent infection (PI) and acute infection (AI)) at feedlot arrival and at the time of initial UF diagnosis; to investigate the effects of BVDV PI animals on pen-level animal health and feedlot performance; to investigate the effect of BVDV viremia on subsequent animal health outcome; and to describe the frequency of BVDV identification in postmortem tissue samples using immunohistochemistry

Materials and Methods

A prospective longitudinal study design was used, with population-based, morbidity-based and mortality-based BVDV testing surveys conducted on the study animals. Pens of suitable candidate animals at three commercial feedlots in Alberta were selected for enrollment based on site and laboratory logistical constraints. All animals were vaccinated upon arrival to the feedlot with a commercial, modified-live viral vaccine, contain-ing Types I and II BVDV and again later in the feeding period with a commercial, modified-live viral vaccine containing Type I BVDV. In addition to the standard processing procedures performed at each feedlot, a citrated whole blood sample and a skin biopsy were collected from all study animals at processing (population-based BVDV testing survey to identify BVDV PI and AI animals at the time of feedlot arrival). Subsequently, a second citrated whole blood sample was collected from animals in pens enrolled on the study at the time of initial diagnosis of UF or no fever (NF) during the first 30 days of the feeding period (morbidity-based BVDV testing survey to identify BVDV AI animals at the time of initial morbidity diagnosis). Finally, skin (obtained from the outer edge of the ear), lung, heart, ileum and synovium samples were collected from each animal at the time of gross postmortem examination by an FHMS veterinarian (mortality-based BVDV testing survey to identify BVDV in tissues of dead animals). Sixteen pens (ten pens at one site and six pens at a second site) were enrolled in the fall of 2003 and nine pens (five pens at one site and four pens at a second site) were enrolled in the fall of 2004.

Citrated whole blood samples for both the population-based and morbidity-based surveys collected from the animals in pens enrolled on the study, were shipped to the Western College of Veterinary Medicine (WCVM) for polymerase chain reaction (PCR) testing to detect animals with BVDV PI or BVDV AI. An RNA extraction method was used to isolate RNA from the blood cells of each sample. The extracts of five animals were pooled and an RT-PCR method was used to identify positive pools, and then subsequently to identify the infected individual animal within each positive pool. The method distinguished among BVDV types (Ia, Ib, and II) viruses and results were reported as Type I or Type II BVDV.

The skin biopsies collected at the time of feedlot arrival were frozen in a cryoprotectant storage and transport media and retained at FHMS. Skin samples from animals that tested positive by PCR analysis of the citrated whole blood samples were retrieved and shipped to the WCVM for BVDV IHC testing to differ-

entiate between BVDV PI and BVDV AI.

The postmortem samples collected by FHMS veterinarians from animals that died during the study were placed in formalin and sent to the WCVM for BVDV IHC testing. At the WCVM, formalinized samples were trimmed and processed into paraffin-embedded tissue blocks. Serial sections of each tissue block were immunohistochemically stained for the gp48 protein of BVDV using monoclonal antibody 15C5 and an avidin-biotin complex immuno-peroxidase method. Stained tissues were examined by light microscopy and scored as negative to 3+, relative to the staining in tissues from a known BVDV PI infected positive control animal tested concurrently with each group of tissues tested.

Animal health information during the entire feeding period describing initial treatment of animals diagnosed with UF Animals pulled for respiratory sign including depression and a rectal temperature $\geq 105.0°$ F ($> 40.5° $ C) subsequent to arrival) and NF a rectal temperature < $105.0°$ F ($\leq 40.5° $ C) subsequent to arrival), as well as the gross postmortem diagnoses assigned by FHMS veterinarians were extracted from the chute-side animal health data collection system (Feedlot Health Animal Record Management© (FHARM), FHMS, Okotoks, Alberta), collated, and verified. Feedlot performance data from each site-specific feedlot administrative software package were entered into an electronic spreadsheet (Microsoft Office Excel 2003) and verified. Finally, the BVDV testing surveys, animal health, and feedlot performance data were merged to form complete pen-level and individual animal-level data sets.

In the pen-level data set, pens were categorized by PI status as follows: PI Pens included pens that had at least one animal with a BVDV PI and Non-PI Pens included pens that did not have an animal with a BVDV PI. In addition, pens were categorized as having evidence of Type I or Type II BVDV viremia based on the PCR results from the arrival samples. At the individual animal level, the arrival and morbidity PCR results were used to categorize individual animals as having evidence of Type I or Type II BVDV viremia.

The data were analyzed using an analytic software program (SAS™ for Windows, Release 9.1, SAS Institute Inc, Cary, North Carolina). In the pen-level data, animal health variables were compared between the PI Pens and the Non-PI Pens using log linear modeling techniques, controlling for clustering of disease and lack of independence created by grouped management of cattle using generalized estimating equations. The feedlot performance variables were compared between the PI Pens and the Non-PI Pens using least squares analysis of variance for site and pen PI status effects.

In both the pen-level and individual animal-level data, frequency distributions and descriptive statistics were calculated and cross-tabulations were used to evaluate simple associations between the arrival and morbidity BVDV viremia variables and the subsequent animal health outcome variables. Generalized linear modeling techniques were used to evaluate the complex associations between the arrival and morbidity BVDV viremia variables and the subsequent animal health outcome variables, controlling for feedlot, initial weight effects, and intra-pen clustering of animal health events.

Results

Thirteen PI animals (0.18%) were detected in nine of 25 pens enrolled on the study. Eleven of the PI animals had a Type I strain of BVDV, one of the PI animals had a Type II strain of BVDV and one of the PI animals had an undetermined strain of BVDV that was lost during laboratory follow up. Death occurred prior to slaughter in 8/13 (61.50%) PI animals (three mucosal disease, one peritonitis, and four BRD).

With respect to the effect of animals with a BVDV PI on pen-level outcome variables, the BVDV/enteritis mortality rate was significantly ($P < 0.05$) higher in the pens containing animals with a BVDV PI (PI Pens) than in pens not containing animals with a BVDV PI (Non-PI Pens). However, there were no significant ($P \geq 0.05$) differences in any of the other morbidity or mortality variables studied, and the general trend was for numerically improved animal health outcomes in PI Pens as compared to Non-PI Pens. With respect to feedlot performance, there were no significant ($P \geq 0.05$) differences in average daily gain (ADG) or the dry matter intake to gain ratio (DM:G) between PI Pens and Non-PI Pens. However, there was a numerical trend toward 1-2% improvements in ADG and DM:G in Non-PI Pens as compared to PI Pens. Results did not show a strong impact of animals with a BVDV PI on pen-level animal health and feedlot performance variables in commercial feedlot production in western Canada.

With respect to arrival BVDV infections (both PI and AI), the occurrence of BVDV viremia was low at feedlot arrival (0.41% of animals were viremic with Type I BVDV at feedlot arrival and 0.27% of animals were viremic for Type II BVDV at feedlot arrival). However, there was a significant ($P < 0.05$) association between type-specific BVDV viremia and pen-level morbidity and mortality outcomes. Overall mortality and infectious disease mortality rates were significantly ($P < 0.05$) higher in pens categorized as positive for Type I BVDV infection at feedlot arrival as compared to negative pens. In addition, initial UF treatment and initial NF treatment rates were numerically ($P \geq 0.05$) higher in pens categorized as positive for Type I BVDV at feedlot arrival as compared to negative pens. Conversely, overall mortality rates were significantly ($P < 0.05$) lower; and infectious mortality, initial UF treatment and initial NF

treatment rates were numerically ($P \geq 0.05$) lower in pens categorized as positive for Type II BVDV infection at feedlot arrival as compared to negative pens.

At the individual animal level, acute BVDV infections, defined as BVDV viremia either at the time of arrival or at the time of initial diagnosis of UF or NF, were associated with a significantly ($P < 0.05$) increased risk of overall mortality and infectious disease mortality. However, these associations were more consistently observed with Type I BVDV than with Type II BVDV. Note that Type I BVDV viremia was only detected at the time of initial diagnosis in 4.00% of UF cases and 2.55% of NF cases. Similarly, Type II BVDV viraemia was only detected at the time of initial diagnosis in 2.50% of UF cases and 1.70% of NF cases. In the mortality-based BVDV testing survey, evidence of BVDV infection was only found in 5.56% of non-PI animals.

Significance

In summary, the results of this study are in general agreement with previous seroepidemiologic work that has demonstrated highly variable correlations between pen-level evidence of BVDV infection and animal health outcome. However, the differences observed between Type I BVDV viremia and Type II BVDV viremia on pen-level morbidity and mortality and the effect of acute BVDV infections on the risk of individual animal mortality have not been previously described. Based on the overall findings of this study, BVDV infection in non-PI animals has occurred less frequently than BVDV infection in previously studies. This observation may have been a result of the BVDV vaccination program used in the study.

Bovine Respiratory Syncytial Virus, Bovine Coronavirus, and Bovine Viral Diarrhea Virus Diagnosis by PCR Testing of Nasal Swabs: Comparison with Cell Culture Procedures

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Introduction

Bovine respiratory diseases (BRD) also includes "shipping fever" among its several disease manifestations. "Shipping Fever" may be caused by *Mannheimia haemolytica, Pasteurella multocida* and occasionally *Histophilus somni,* as well as viral agents including infectious bovine rhinotracheitis virus (bovine herpesvirus-1 [BHV-1], bovine viral diarrhea viruses (BVDV1a, BVDV1b, BVDV2a), bovine parainfluenza-3 virus (PI-3V) and bovine respiratory syncytial virus (BRSV). These viral antigens are in the viral vaccines used to control BRD. These viruses may cause respiratory tract infection and disease but often predispose the cattle to

the bacterial pathogens. Additional viruses associated with BRD include bovine coronavirus (BCV) and bovine adenoviruses (BAV) with multiple serotypes.

Diagnosis of viral infections has traditionally used viral isolation in cell culture and serologic testing (antibody levels) to detect active infection (rising antibody levels). These procedures require considerable time for the results, especially cell culture isolation (days to weeks for multiple passages). Serology requires the host response post infection (3-4 weeks for acute to convalescent samples), plus the antibody test performance. Use of cell culture systems for viral diagnostics is useful for selected viruses, yet some viruses may not be readily identified in cell culture or may require unique cell cul-