A. SUMMARY

During the winter of 2001-2002, two hundred and seventy seven cattle were examined at post mortem and the relevant data was collected from the feedyard and the diagnostic laboratory. Diseases caused by infectious micro-organisms were responsible for more than seventy per cent of the total mortality. The results of this project strengthen the hypothesis that livestock diseases are probably caused by a group of infectious agents working synergistically. Bovine Virus Diarrhea virus was shown to accentuate the virulence of other pathogens. While immunization for BVD in the Saskatchewan cattle herd is common, investigations such as this one show that the role of the virus in common cattle diseases continues to emerge. Efforts to describe the disease and how it is changing and to describe efficacious methods of controlling the disease should go on.
B. EXECUTIVE SUMMARY

The objective of this project was to determine the causes of mortality in cattle placed in Saskatchewan feedyards and more specifically, to examine the role of Bovine Virus Diarrhea virus (BVDV) in these cattle mortalities. Cattle that died in three feedlots within the practice of the Western College of Veterinary Medicine (WCVM) were examined “post mortem”, called a necropsy in animals. Included in the database were mortalities representative of cattle placed, fed and treated in these feedyards.

Routine necropsies were done on all suitable cattle at two feedyards and on all mortalities during three weeks of October 2001 at the third feedlot. A rudimentary history was completed. If the cadaver was frozen, too autolyzed (post mortem change), or without history, it was excluded from the data collection process. The background information needed before a necropsy was filed, included; class (calf, yearling, cow), Gender (steer, heifer, bull), number of days in lot (DOF), treatment and its duration, location at time of death (home, sick, or chronic pen) and whether the animal died or was euthanized.

The post mortem examination consisted of a dissection to reveal all internal body systems. In the majority of cases, a diagnosis as to the cause of mortality, called a gross diagnosis, could be made. If not, a series of tissues were sampled to confirm the tentative diagnosis made at the site by the pathologists of Prairie Diagnostic Services’ pathologists. In addition, a standard number of organs were routinely sampled and processed to test for the presence of the BVDV.

A total of 277 necropsies were done and all results as well as the history were entered into an electronic database. The diagnosis listed was based on the gross inspection, supplemented by any microscopic findings. The presence or absence of BVDV infection was then compared to all diagnostic categories. The causes of mortality are presented in Figure 1. Pneumonia, chronic pneumonia and arthritis caused by an organism called Mycoplasma bovis were the largest disease category. Greater than seventy one per cent of the death loss was caused by infectious agents. Evidence of BVDV was prevalent in mortalities caused by infectious agents at more than twice the level when compared to noninfectious causes, except for the category called, Haemophilosis, which is a separate category of mortality caused by the bacteria, Haemophilus somnus. Presence of the virus was especially evident in those examinations where Mycoplasma bovis was the predominant infectious agent at time of death.

Information determined by this investigation would support the conclusion that BVDV is an important pathogen amongst the group that causes death, and by extrapolation, illness in feedlot cattle. This, in spite of more vigorous immunization for BVD in Saskatchewan than ever before. Further research into the control of emerging disease syndromes like those caused by Mycoplasma bovis and amplified by BVDV would be appropriate.
Prevalence of BVDV in a selected sample of Mortalities of Feedlot Cattle

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Abstract

Necropsy examinations (n = 277) were done on bovine mortalities from three large feedlots in central Saskatchewan and a gross diagnosis was made. Tissue samples from the heart, lung, ileum and skin were collected for each necropsy and submitted for histopathological (HP) and immunohistochemical (IHC) examination for BVDV. Joint capsule and exudates were collected and submitted in selected cases.

An etiological diagnosis of mortality was made and results were categorized as follows: Bovine virus diarrhea (BVD) 7%, Interstitial pneumonia (AIP) 4%, Mycoplasma bovis associated pneumonia and/or arthritis (MBO) 28%, Digestive tract disease (DIG) 8%, Hemophilosis (HEM) 19%, Bovine respiratory disease (BRD) 17%, Others (OTH) 10%, and Unknown (UNK) 7%. The number of persistently infected (PI) animals in the study was 20, representing 7.2% of all sampled.

Immunohistochemical (IHC) examination revealed that 33.1% of all mortality were concurrently infected with BVDV, persistently infected (PI) calves accounted for 7.6% of total mortality, while 6.9% were acute BVD cases. Evidence of primary or acute BVDV in the heart, lung and ileal tissues occurred at 24.5%, 19.5%, and 16.8% respectively and at the level 12.5% in the skin. Polyarthritis was observed in 13.9% of the necropsies, of which mycoplasma was evident in 6.9%. The study was carried out between August 2001 and January 2002.

BVDV was found in all disease categories, however this association was most evident in mortalities where an infectious cause was inferred. The association of Hemophilus somnus (p=0.8) with BVDV appears insignificant, inferring that it was not synergistic with BVDV, in comparison with BRD (p=0.03) and Mycoplasma bovis infections (p<0.01), where there is an obvious association; hence a synergy with BVDV is hypothesized. The results of this study support a synergy between bovine respiratory (BRD) and primary bovine viral diarrhea virus (BVDV). Also, this study indicates good evidence of the BVDV in heart tissue followed by lung, ileum and skin in the cases of primary infection using IHC as a diagnostic tool.

Introduction

Bovine viral diarrhea virus (BVDV) had been recognized as an important pathogen of cattle populations since Olafson et al. described gastroenteritis with severe diarrhea in dairy herds in 1946 (2).

BVDV, a single stranded RNA virus is a member of the genus Pestivirus genus of viral family Flaviviridae. Hog cholera virus and border disease virus in sheep and measles virus in humans are also members of the Pestivirus genus (2). Cythophatic and non-cythophatic are biotypes of BVDV based on pathology in cell cultures, and genotypes (types 1 and 2), based on genomic differences detected by polymerase chain reaction (PCR)
While BVDV is capable of primary infection of the bovine lung (20) evidence in literature suggests that BVDV plays a role in bovine respiratory disease (BRD) (11,12). Synergistic effects between BVDV and other respiratory viruses infectious: bovine rhinotracheitis (IBR), Parainfluenza virus (PI3) and Mannheimia hemolytica (MH) had been documented (11) Seroconversion to BVDV has been significantly associated with bovine respiratory disease (16,18). Evidence in the literature points to a role of the virus in bovine respiratory disease (12,20,21).

The practice of placing multi-sourced calves shortly after weaning by feedyards is important in the pathogenesis of BVDV infections. With most auction market derived calves the presence of a calf or calves persistently infected with BVDV initiates the spread of the virus to susceptible calves (23,24).

Among diagnostic techniques available for the detection of BVDV, immunohistochemistry (IHC) is demonstrates the ability to identify BVDV or other antigens in formalin fixed tissues. ‘Labelled’ antibodies are used to stain the virus particles in tissue section. Utilization of avidin-biotin complex results in amplification of staining reaction and allowing detection of small amounts of antigen (4,14).

Convenience of sample submission, retrospective diagnoses, ability to visualize distribution of disease agent simultaneously with histological lesion and correlate lesions with disease agent, increased sensitivity over many conventional diagnostic techniques gives IHC a superior advantage and eventual choice in diagnostic pathology (14,17,22).

The sole aim of this study was to determine the role of BVDV in feedlot mortality, using immunohistochemistry. Secondly to describe the proportion of tissue samples with evidence of the virus, and thirdly the synergy of BVDV with respiratory pathogens using immunohistochemistry.

**Materials and Methods**

**Study population**

The three feedlots enrolled in this study were all located in central Saskatchewan. Although the combined total herd capacity is about 70,000, the study mainly examined calf mortality in fall placed calves. This study was conducted during the fall and winter of 2001. Calves ranging from 7 to 10 months of age, sourced from different farms and purchased through the auction market system in Western Canada were used for the study.

The calves are processed within 24 hours after arrival at the feedlot. The procedure called processing, includes branding, unique eartag identification, an implant, a pour-on parasiticide, and an injection of vitamins A & D. In addition, the calves were given a multivalent clostridial bacterin, a combined Hemophilus somnus and Mannheimia hemolytica immunogen and a modified-live virus infectious bovine rhinotracheitis, parainfluenza and bovine viral diarrhea virus vaccine.
The calves were also injected with long acting oxytetracycline as a metaphylactic measure. In some cases a second mass treatment of long acting oxytetracycline (referred to as systematic intervention) is given 5-7 days post arrival. Febrile calves with a rectal temperature ≥ 40.3 are treated with a different broader spectrum antimicrobial.

Multi-sourced calves from different auctions and different farms were commingled and housed together in pens containing up to 300 head. The pens are managed as a single homogenous unit termed “lot” until 180-200 days in the feedlot when they are sorted as finished.

Mortalities occurring in all pens were necropsied within 24 hours by the attending veterinarian and a gross diagnosis was made. Formalin fixed tissue samples were selectively collected from the heart, lung, ileum and skin for presence of BVDV. If a gross diagnosis was not made, more tissues (e.g. brain, abomasum, rumen, liver) were submitted as indicated by gross lesions and/or clinical signs.

The criteria used for selection of tissues were absence of autolysis, class of animal and inconvenience. The tissue samples were submitted to Prairie Diagnostic Laboratory at the Western College of Veterinary Medicine for HP and IHC to determine the cause of death. The diagnosis of Mycoplasma bovis seen during gross necropsy was only confirmed by histology and not IHC or cultures.

Statistical analysis

Detailed information about the necropsied animal including: the feedlot of origin, identification, sex, class, days on feed, date of necropsy and treatments were recorded on a standardized form. Specific pathology observed for each body system was also recorded on the form, which was used to create a database using Statistix 7 Analytical Software™.

RESULTS

The results of gross necropsies performed on (277) mortalities are presented in Figure 2. The association between gross and laboratory diagnosis made is shown in Figure 3. The agreement between the two diagnostic methods was 79.4%. Fall placed calves represented 85% of the total proportion of animals necropsied, Figure 4. Mortality peaked during the first 45 days on feed.

Persistently infected (PI) calves were 7.6% of total mortality in the study. When a diagnosis of PI with BVDV was made, the cause of mortality is illustrated in Figure 5. When the PI calves were excluded, the association between BVDV positive (IHC) samples and reasons for mortality is presented in Figure 6.

Mortality occurring in PI calves was not entirely due to mucosal disease (50%), Mycoplasma bovis also accounted for 20% of deaths in PI calves, and bovine respiratory disease caused 10% of the mortality. In addition, BVD was the leading cause of death.
followed by Mycoplasma bovis associated mortality and BRD when PI calves were excluded (Figure 7). Mycoplasma bovis were evident in 62% of joint exudates with polyarthritis.

The heart tissues at 24.5% yielded more BVD IHC positive tissues (Figure 8), compared with the lung at 19.5%, ileum at 16.8% and the skin at 12.5%. Of significance was generalized myocardial necrosis (25.6%), and Purkinje cell necrosis (10.1%).

Using IHC, concurrent Mycoplasma bovis infection and BVD was most prevalent in all samples examined, Figure 7. Acute Interstitial pneumonia occurred over a longer period of time in comparison with all other causes of death in the study. BVD IHC positive tissue samples were also concurrent with other diagnoses, Figure 6.

When mortality was arbitrarily classified as infectious and non-infectious causes of BVD tissue examined prevalence in tissue of various mortality categories was examined BRD was significant at (33.3%) p=0.03, BVD at (44.4%) p=0.04, and Mycoplasma bovis at (37.0%) p<0.01. However, mortality called hemophilosis was only significant at (17.0%) p=0.8, thus indicating a strong association between the presence of BVDV. Thus BVDV was associated with most diagnoses, yet it was most significant in mortalities where an infectious cause was inferred (Table 2). Existing “background noise” of BVDV related mortalities in all categories (Figure 6), especially the non-infectious causes, is not significant.

DISCUSSION

This study was designed to “snapshot” BVD prevalence in feedyard mortality, especially in fall placed calves. It should be in cognizance that the “fall placed calves” are a sampling cohort within the entire population of thousands of animals involved in this study. Fall placed calves by convention are born in the spring, and if PI, has not yet been exposed to the CP-BVDV.

Several unreported histopathological lesions were observed in the tissue materials submitted from the field necropsies. The unique pathological features seen in this study includes: necrosis of Purkinje fibres (10.1%) and generalized myocardial necrosis at 25.6%, in addition encephalitis associated with BVDV positive IHC, all these has not been published before in any literature. The generalized myocardial and purkinje cell necrosis have not yet been noted in our diagnostic laboratory before. The occasional calf with clinical central nervous system signs and histopathological evidence of a non suppurative meningoencephalitis that is BVD IHC positive was observed for the first time in this group of necropsy examinations.

Mucosal disease mortalities accounted for 50% of the mortalities in PI calves, followed by Mycoplasma bovis and bovine respiratory disease (BRD) as seen in Figure 5. Incidentally this same trend was repeated when the PI calves were excluded (Figure 7), BVD was the leading cause of mortality followed by Mycoplasma bovis and then bovine
respiratory disease. This is a unique feature of this study and has not been reported in literature.

The association of Hemophilus somnus (p=0.8) with BVDV appears weak, inferring that it is not synergistic with BVDV, in comparison with BRD (p=0.03) and Mycoplasma bovis (p<0.01) (18). BVDV would seem synergistic with other pathogens, however causality cannot simply be inferred and the exact mechanisms of this synergism are unknown. This may be explored in subsequent research.

Using immunohistochemistry BVDV involvement with Mycoplasma bovis has been described by Campbell et al (19). Haines et al, demonstrated BVDV by IHC in 40% of cases of chronic respiratory disease with or without arthritis (14,15). Shahriar et al also found BVDV in 64% cases of Mycoplasma bovis (22). Pollock also found out that cases from the chronic pen with a high titre to BVDV were 4.5 times more likely to have polyarthritis (Pollock, in press). BVDV involvements with Mycoplasma bovis infections have been reported by others (15,22). In this study, we found BVDV to be more associated with all causes of infectious mortality except Haemophilus somnus.

Using IHC, we were able to demonstrate 38% (Figure 6) of BVD positive samples in this study also had Mycoplasma bovis infection. Mycoplasma was evident in 62% of joint exudates collected in this study. Samples with simultaneous BVDV and Mycoplasma have been repeatedly treated for BRD or unthriftness (15,25)

BVDV infection was more associated with infectious than non infectious causes in all samples examined by IHC (Figure 6), categories of mortality due to Mycoplasma bovis and Bovine respiratory disease (BRD) with concurrent BVD positive samples only ranks second to those caused entirely by BVD (11,15,16,18, 21)

Glycoprotein E2 (gp53) is of interest because this is the area where nucleotide and amino acid sequence vary significantly between different BVDV isolates and is thought to be responsible for antigenic variation and virulence in BVDV isolates (3,5,8,13). BVD epidemics that occurred in the mid nineties revealed that there is a degree of stability in BVDV genome: especially in region of the viral genome coding for glycoprotein E2 (gp53) viral proteins (8). The same glycoprotein E2 is also thought to be responsible for mediating replication of BVDV and the production of neutralizing antibodies during natural infection or vaccination (3,5,8,13)

Corapi et al, Donis et al and Dubovi et al in separate publications have suggested the existence of antigenic differences in and within BVDV biotypes (6,7,9). Bolin et al has reported difference in virulence between two non-cytopathic BVDV (3). Fulton et al also reported these differences, identified the genotype and categorized the identified biotypes (15). The above evidence supports the variability of BVDV genome and the diversity of virulence. We postulate that BVDV is ubiquitous and varied in it’s manifestations and given the reported genome instability and antigenic drift can expect it to remain a leading pathogen and speculate it may avoid immune mechanisms provoked by convectional vaccination
Further research should continue to investigate the role of BVDV in the etiological evidence of microorganism that our animals are subjected to upon arrival at Western Canada feedyards. This study illustrates the need to control BVDV infections in feedyards. The methods employed to control immunocompetent and immunotolerant BVDV infections are not the same.

Primary infections or those in immunocompetent calves may be prevented by immunization with the appropriate vaccine prior to exposure. However, prevention of immunotolerant calves is directed at the cows on before calves are conceived, born and placed in feedyards. Identification of immunotolerant or those persistently infected with BVDV at the feedyard would allow separate or culling of those calves contributing to the infectious process at the feedyard. Therefore a quick method to identify PI calves at arrival would be a prospect most worthy of further study.
Figure 1  Degree of autolysis in all tissue samples harvested from necropsy examinations completed

Figure 2  Causes of mortality based on gross only necropsy in study group of animals.
Figure 3  Etiology and frequency of occurrence of mortality based on gross necropsy and immunohistochemical diagnosis.

Figure 4  Category of animals examined by necropsy in the study.
Figure 5  Causes of mortality in BVD persistently infected calves (n=20)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal</td>
<td>50%</td>
</tr>
<tr>
<td>BRD</td>
<td>10%</td>
</tr>
<tr>
<td>CPPS</td>
<td>20%</td>
</tr>
<tr>
<td>Digestive</td>
<td>5%</td>
</tr>
<tr>
<td>H. somnis</td>
<td>5%</td>
</tr>
<tr>
<td>Other</td>
<td>5%</td>
</tr>
<tr>
<td>Unknown</td>
<td>5%</td>
</tr>
</tbody>
</table>

Figure 6  Distribution of BVD + Samples within Mortality Categories

(PI calves excluded)
Figure 7  Distribution of BVD + Samples within Mortality Categories (PI calves excluded)

Figure 8  Proportion of BVD IHC Positive Results by Tissue Location
Table 1  Characteristics of the feedlots used in this study

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total necropsied</td>
<td>76</td>
<td>150</td>
<td>56</td>
</tr>
<tr>
<td>Feedlot capacity</td>
<td>20,000</td>
<td>12,000</td>
<td>40,000</td>
</tr>
<tr>
<td>BVD vaccination</td>
<td>Selected</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>Selected</td>
<td>Selected</td>
<td>No</td>
</tr>
<tr>
<td>Oxytetracycline LA</td>
<td>Yes</td>
<td>Yes, twice</td>
<td>Yes</td>
</tr>
<tr>
<td>PI</td>
<td>1</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2  BVD prevalence in tissues of various mortality categories

<table>
<thead>
<tr>
<th></th>
<th>Infectious %</th>
<th>Non-Infectious %</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRD</td>
<td>33.3</td>
<td>15.3</td>
<td>0.03</td>
</tr>
<tr>
<td>BVD</td>
<td>44.4</td>
<td>15.3</td>
<td>0.04</td>
</tr>
<tr>
<td>MBO</td>
<td>37.0</td>
<td>15.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HEM</td>
<td>17.0</td>
<td>15.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>
REFERENCES


D. PERSONNEL

Dr Niyi Olaloki was employed for three months to manage the data collected. Information had been collected in the field and Dr Olaloki was tasked with entering, correcting and analyzing this data.
Mycoplasma bovis associated disease in weaned Canadian beef calves

Commonly, calves at 6-7 months of age are weaned from late September until mid November, are sold through an extensive auction market system and are confined on western Canadian feedyards or farms. Immediately on arrival or shortly thereafter, calves are processed (vaccinated, implanted, identified) and in many cases metaphylactically treated with a sustained action antimicrobial. Within a week of arrival, attending stockpersons begin to treat calves that look sick (a measure of depression) and have a high fever (>40°C). While a high proportion (by convention, should be > 90%) of these calves are treated successfully, some calves must be retreated within 3 days to 2 weeks. Often it is at this time, the livestock attendants notice these calves are lame, Figure 1. Upon close inspection, swollen joints (usually a stifle or a hock) are easily visible.

In greater than half the lame calves, over the next several weeks the disease process gets worse. If one joint is visibly affected, closer examination usually reveals evidence of the disease in many joints (1); hence, polyarthritis is appropriate. By the time this calf has been on the premises 5-6 weeks, the calf has lost weight, the calf has a great deal of difficulty walking or getting up and may not even be able to rest comfortably in sternal recumbency.

When these calves are examined at this time, either clinically or at necropsy, there is a swelling of the joints and around the joints which usually means a septic arthritis, periartthritis and/or tendionsynovitis. There usually is a similar destructive process occurring in the lungs (2, 3, 4), which may or may not be audible on thoracic auscultation. Supportive laboratory effort has shown the main organism causing the pathology is Mycoplasma bovis (1, 2, 3, 4, 6). In a high number of the cases, Mycoplasma sp would appear to be assisted or exacerbated by an acute Bovine Virus Diarrhea Virus (BVDV) infection (4, 6), in a mechanism not yet fully understood.
The diagnosis of *Mycoplasma bovis* infection is usually made at necropsy, but initially clinicians may be asked to examine calves that are not responding to routine therapy. *Mycoplasma bovis* will remain in the bronchoalveolar lavage after antimicrobial treatment, even if other pathogens like *Mannheimia hemolytica*, *Pasteurella multocida* and *Haemophilus somnus* are markedly reduced (7, 8). When a treatment failure for respiratory disease is accompanied by an intractable lameness that increases in severity and *Mycoplasma sp* are isolated either from the respiratory tract or joint aspirates, the diagnosis is considered definitive. *Mycoplasma sp* have always been considered part of the respiratory microbial flora of the bovine and it is only recently that studies have begun to describe the prevalence of *Mycoplasma bovis* in the cattle population (9) and its significance (3, 4, 6, 10, 11).

The most important aspect of this disease, often referred to as the Chronic Pneumonia and Polyarthritis Syndrome (CPPS), is the severity of the disease in some individuals. In some feedyards this creates considerable frustration because the nursing care to maintain these calves becomes prohibitive. In spite of heroic specific and supportive therapy by animal attendants and their veterinarians, many of these calves must be euthanized, Figure 2.


Figure 1. Calves may appear bright and alert but quickly present with weight loss, lameness, and swollen joints.
Figure 2. Stock attendants are ever vigilant to ensure that calves are always able to get up and access feed and water.