

# Immunohistochemical study of *Hemophilus somnus*, *Mycoplasma bovis*, *Mannheimia hemolytica*, and bovine viral diarrhoea virus in death losses due to myocarditis in feedlot cattle

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**Abstract** — The purpose of this study was to determine the presence of *Hemophilus somnus*, *Mycoplasma bovis*, *Mannheimia hemolytica*, and bovine viral diarrhoea virus (BVDV) in lesional tissues of feeder calves dying with myocarditis. Tissues from the heart and lungs of 92 calves dying with myocarditis in Alberta feedlots were immunohistochemically stained for the antigens of these agents. Tissues from 44 calves dying from noninfectious causes and 35 calves dying with pneumonia were tested as controls. *Hemophilus somnus* was found in cardiac lesions in the majority of myocarditis cases (70/92). *Mycoplasma bovis* was concurrently demonstrated in the hearts of 4/92 affected calves. No bacterial pathogens were found in heart tissues from the control groups of calves. Bovine viral diarrhoea virus was demonstrated in the tissues of 4/92 myocarditis cases compared with those of 13/35 calves dying from pneumonia and 0/44 calves dying from noninfectious causes. The results demonstrate that *H. somnus* is the principle pathogen associated with myocarditis in feedlot calves and that the presence of BVDV is more common in these calves compared with calves dying of noninfectious causes. The findings also suggest that BVDV is an important pathogen in calves dying with gross postmortem lesions of pneumonia.

**Résumé** — Étude immunohistochimique sur l'implication d'*Hemophilus somnus*, de *Mycoplasma bovis*, de *Mannheimia hemolytica* et du virus de la diarrhée virale bovine sur la mortalité reliée à la myocardite chez des bovins en parc d'engraissement. Le but de cette étude était de déterminer la présence d'*Hemophilus somnus*, de *Mycoplasma bovis*, de *Mannheimia hemolytica* et du virus de la diarrhée virale bovine (VDVB) dans les lésions tissulaires de veaux à l'engraissement mourant de myocardite. Des prélèvements de cœurs et de poumons provenant de 92 veaux morts de myocardite dans des parcs d'engraissement d'Alberta ont été colorés par l'immunohistochimie pour des antigènes provenant de ces microorganismes. Des tissus de 44 veaux morts de maladies non-infectieuses et de 35 veaux morts de pneumonie ont été utilisés comme témoins. *Hemophilus somnus* a été retrouvé dans les lésions cardiaques de la majorité des cas de myocardite (70/92). *Mycoplasma bovis* était concurremment présent dans le cœur de 4 des 92 veaux atteints. Aucune bactérie pathogène n'a été retrouvée dans le tissu cardiaque des veaux des groupes témoins. Le virus de la diarrhée virale bovine a été identifié dans les tissus de 4/92 cas de myocardite en comparaison de 13/35 cas de pneumonie et de 0/44 cas non infectieux. Les résultats démontrent que *H. somnus* est le principal pathogène associé à la myocardite chez les veaux en parcs d'engraissement et que la présence du VDVB est plus répandue chez ces veaux en comparaison avec les veaux mourant de causes non-infectieuses. Ces trouvailles laissent à croire que le VDVB est un pathogène important chez les veaux décédés présentant des lésions macroscopiques post mortem de pneumonie.

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## Introduction

**S**udden death in feedlot cattle associated with multifocal myocardial infarcts, necrosis, and fibrosis was first described as a syndrome associated with septicemic infection with *Hemophilus somnus* in the late 1980s (1-3).

In spite of vaccination of incoming calves for *H. somnus* and vigorous antibiotic treatment of febrile animals, heart lesions, including myocarditis, pericarditis, and pleuritis, continue to be an important cause of calf mortality in fall-placed calves in western Canadian feedlots (4-6).

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In some studies, all losses associated with laryngitis, myocarditis, pericarditis, pleuritis, polyarthritis, and septicemia have been attributed to infection with *H. somnus* (5,6); however, this diagnosis has been presumptive, as the agent was not in all cases cultured or identified by immunohistochemistry. Recently, there has been emerging evidence that *Mycoplasma bovis*, rather than *H. somnus*, is involved in a high proportion of death losses associated with a multisystemic disease in feeder calves (7,8).

There is also increasing evidence for the importance of acute BVDV infections in feeder cattle losses. There are serological data demonstrating that high BVDV antibody titers in incoming cattle protect them from subsequent illnesses and losses (9,10) and that low BVDV antibody titers in incoming cattle (10) or seroconversion after their arrival in the feedlot is associated with increased disease (11,12). In addition, there is immunohistochemical evidence that BVDV is present in the tissues of many feeder calves dying due to chronic unresponsive pneumonia and arthritis (7,8).

The purpose of the study described in this report was to determine, using immunohistochemical stains, the presence of *H. somnus*, *M. bovis*, and *Mannheimia hemolytica* in the lesions of feeder calves dying with myocardial lesions, as well as the numbers of these calves concurrently infected with BVDV.

## Materials and methods

The calves eligible for inclusion in the study were all those that died in the fall of 1999 and winter of 2000 in Alberta feedlots serviced by the veterinarians participating in the study. Five veterinary practices participated in the study, and samples were collected from 12 feedlots.

Postmortem examinations of 171 cattle found dead were conducted by the participating veterinarians; on gross diagnosis, the cases were categorized as either myocarditis ( $n = 92$ ); pneumonia ( $n = 35$ ); or a noninfectious ailment, such as bloat or atypical interstitial pneumonia ( $n = 44$ ). Myocarditis was defined as the presence of focal areas of inflammation and necrosis within the myocardium (1-3). All calves were fall-placed, recently weaned calves with initial entry weights between 250 and 300 kg. The number of days on feed at the feedlot was recorded for each case.

The following samples were harvested and placed in 10% neutral buffered formalin: a 2- × 2-cm sample of haired skin of the neck; a 2- × 2-cm sample of lung, including any consolidated tissues, obvious lesions, or both; a 1- to 2-cm sample from the wall of the right ventricle of the heart and from the left intraventricular septum of the heart; and 2 1- to 2-cm samples from the wall of the left ventricle of the heart (the latter including the papillary muscles). The participating veterinarians were also instructed to sample any area of the heart with an obvious abscess or area of inflammation that was apparent on the surface of the myocardium.

The tissue samples were embedded in paraffin wax and sectioned for immunohistochemical staining, using staining methods previously described (13). Two sections from each block were tested for antigens of the following 4 pathogens: *H. somnus*, *M. hemolytica*, *M. bovis*, and BVDV. The primary antibody reagents used in the stains

**Table 1. The numbers of days at the feedlot prior to death in groups of feeder cattle**

Group	Numbers of days at the feedlot		
	Mean	<i>s</i>	Median
Myocarditis ( $n = 92$ )	43 <sup>a</sup>	21	41
Pneumonia ( $n = 35$ )	39 <sup>a</sup>	44	18
Noninfectious ( $n = 44$ )	100 <sup>b</sup>	48	100

*s* — Standard deviation

<sup>a,b</sup>Means with different superscripts are significantly different based on a one-way analysis of variance (ANOVA) and the Tukey (HSD) comparison of means ( $P \leq 0.05$ )

were the following: *M. bovis*-polyclonal rabbit antiserum at dilutions of 1/1000 and 1/2000 (a gift from the Diagnostic Bacteriology Laboratory, Western College of Veterinary Medicine); *M. hemolytica*-monoclonal antibodies P12/D6/D5 ascites fluid diluted 1/2000 and 1/4000 (a gift from The Veterinary Infectious Diseases Organization, Saskatoon, Saskatchewan); *H. somnus*-rabbit polyclonal antiserum diluted 1/1000 and 1/2000 (a gift from The Veterinary Infectious Diseases Organization); and BVDV-monoclonal antibody 15C5 ascites fluid at dilutions of 1/800 and 1/1600 (obtained from Dr. E. Dubovi, Cornell University, Ithaca, New York, USA). Positive control sections from isolation-confirmed cases were tested concurrently with test tissues. Negative controls were tissue sections from each block tested with substitution of similar dilutions of an irrelevant polyclonal antiserum or monoclonal antibody ascites fluid. Tissues were scored, without knowledge of the gross diagnosis, as either positive or negative.

Days in the feedlot were compared for mortality categories by using a one-way analysis of variance (ANOVA) and the Tukey post hoc comparison of means test. The proportions of tissues positive for BVDV in various mortality categories were compared by using the chi-square test and Fisher exact test.

## Results

Table 1 shows the numbers of days the calves were at the feedlot prior to the death losses in each group. The losses associated with pneumonia occurred earliest in the feeding period (median number of days at the feedlot, 18), while deaths due to myocarditis occurred later (median number of days at the feedlot, 41), and the losses associated with noninfectious causes were most often later in the feeding period (median number of days at the feedlot, 100).

Tables 2 and 3 show the numbers of cases in each group in which the 4 pathogens were detected in either the heart or the lung. *Hemophilus somnus* antigen was demonstrated immunohistochemically as multiple foci of perivascular staining within the areas of necrosis in the heart tissues in the majority of cases (70/92) of myocarditis. The only other bacterium found in heart lesions was *M. bovis* (4/92 cases), in all instances concurrent with *H. somnus* infection. Bacteria were not demonstrated in heart tissues of calves dying from either pneumonia or noninfectious causes.

In some instances, calves dying due to myocarditis had concurrent lung infections with *H. somnus* (5/92 cases), *M. hemolytica* (1/92 cases), or *M. bovis* (6/92 cases). In calves dying from pneumonia, *M. hemolytica* was detected

**Table 2. Immunohistochemical demonstration of *Hemophilus somnus*, *Mycoplasma bovis*, and *Mannheimia hemolytica* in heart and lung tissues of feeder cattle dying in feedlots**

Group	<i>H. somnus</i>		<i>M. hemolytica</i>		<i>M. bovis</i>	
	Heart	Lung	Heart	Lung	Heart	Lung
Myocarditis (n = 92)	70	5	0	1	4	6
Pneumonia (n = 35)	0	9	0	17	0	9
Noninfectious (n = 44)	0	0	0	0	0	0

**Table 3. Immunohistochemical demonstration of bovine viral diarrhoea virus in heart and lung tissues of feeder cattle dying in feedlots**

Group	Tissue	
	Heart	Lung
Myocarditis (n = 92)	4 (4.3%)	4 (4.3%)
Pneumonia (n = 35)	13 (37.1%)	13 (37.1%)

Proportions of positive tissues were significantly different between disease groups ( $P < 0.0001$ , Odds Ratio = 13.0)

in the lungs in about half (17/35) the cases. The other bacteria (*H. somnus* and *M. bovis*) were also detected in the lungs in a substantial number of cases (9/35 in each instance). In 5 cases, there was concurrent detection of *M. bovis* and *M. hemolytica* in the lungs of calves dying with pneumonia (data not shown). No bacteria were detected in the lungs of calves dying of noninfectious causes.

Bovine viral diarrhoea virus was found in 4/92 cases of calves dying due to myocarditis. This is a significantly increased rate compared with the absence of detection of this virus in 44 animals dying of noninfectious causes ( $P < 0.0001$ ). The most compelling association with BVDV was demonstrated in the cases dying with pneumonia (13/35) compared with those cases with noninfectious deaths ( $P < 0.0001$ ). Bovine viral diarrhoea virus was detected significantly more often in cases dying with pneumonia than in cases dying due to myocarditis (Odds ratio = 13.00,  $P < 0.0001$ ).

## Discussion

*Hemophilus somnus* was shown to be associated with the lesions in the heart tissues of the majority of feeder cattle dying due to myocarditis. This is in agreement with several previous reports (1–6). While *M. bovis* is increasingly recognized as having an important role in feeder calf disease and deaths due to chronic pneumonia and polyarthritis (7,8,11,14), in the present study, this bacterium was found in only a minority of the myocarditis cases, and in all instances, it was coincident with *H. somnus* infection, suggesting that *M. bovis* is not a primary causal pathogen of this syndrome.

In most cases of fatal *H. somnus*-associated myocarditis, there was no evidence for the presence of the bacterium in the lung tissue examined. Given the propensity of the agent for dissemination to multiple tissues, the reason for the apparent preferential myocardial localization is unclear. Other studies have suggested that there has been a shift in the manifestations of the *H. somnus*-associated disease to favor an increased occurrence of the myocardial form alone (15,16). While the current study did not address the

distribution of the agent throughout the tissues of affected calves, the findings support these observations, as only 5/70 calves dying with *H. somnus*-associated myocardial lesions had evidence for pulmonary infection.

For more than a decade, evidence has been accumulating that increased risk of disease in feedlot cattle may be associated with acute BVDV infections. Emerging from the present study is additional evidence for an association of BVDV with feeder cattle losses. Bovine viral diarrhoea virus was found more frequently in cattle dying from either myocarditis or pneumonia than in calves dying of noninfectious causes. While cattle dying of noninfectious causes died later in the feeding period than did those dying with either myocarditis or pneumonia, this fact is unlikely to have impacted on the absence of BVDV in the tissues, since previous studies have demonstrated the highest proportions of BVDV in chronically diseased animals, which often have been over 60 d or more in the feeding period (7,8).

The association of the presence of BVDV with myocarditis losses was not as strong as that found with pneumonia losses and with chronic death losses previously reported (7,8); nevertheless, there is evidence that this virus may be increasing the numbers of losses due to *H. somnus*-induced myocarditis. Other studies have shown that the level of *H. somnus*-specific serum antibody in the calf on entry to the feedlot is important in protecting it against subsequent disease (6,9). The current study did not examine the effects of the level of specific immunity; however, the association of *H. somnus* losses with the immunosuppressive BVDV also supports the suggestion that the immune status of the animal may be important.

In the current study, the strongest association with BVDV was found in animals dying due to pneumonia. This finding extends the data presented in previous reports (7,8) of the association of BVDV with feeder calf death losses due to chronic unresponsive disease (usually associated with *M. bovis*) occurring late in the feeding period. In the current study, the majority of losses due to pneumonia occurred early in the feeding period (median number of days at the feedlot, 18) and the infectious agent demonstrated most often was *M. hemolytica*. The findings support previous serological studies demonstrating the importance of BVDV immunity in the protection of feedlot calves from disease and death (9–12) and reemphasizes the need to address improved BVDV immunity in calves entering feedlots.

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