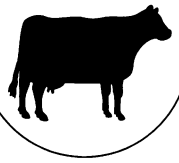

# The immunohistochemical detection of *Mycoplasma bovis* and bovine viral diarrhea virus in tissues of feedlot cattle with chronic, unresponsive respiratory disease and/or arthritis

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**Abstract** — The purpose of this study was to determine the frequency of selected pathogens in the tissues of a group of feedlot cattle with chronic disease (most often respiratory disease and/or arthritis). Samples of lung and joint tissues from 49 feedlot animals that had failed to respond to antibiotic therapy were tested by immunohistochemical staining for the antigens of *Mycoplasma bovis*, *Haemophilus somnus*, *Pasteurella (Mannheimia) hemolytica*, and bovine viral diarrhea virus (BVDV). *Mycoplasma bovis* was demonstrated in over 80% of cases, including in 45% of joints and 71% of lungs tested. *Mycoplasma bovis* was the only bacterial pathogen identified in the joints. *Haemophilus somnus* and *Pasteurella (Mannheimia) haemolytica* were found in 14% and 23% of cases, respectively, and were confined to the lungs in all instances. Infection with BVDV was demonstrated in over 40% of cases. *Mycoplasma bovis* and BVDV were the most common pathogens persisting in the tissues of these animals that had failed to respond to antibiotic therapy.

**Résumé — Détection immunohistochimique de *Mycoplasma bovis* et du virus de la diarrhée virale bovine dans des tissus de bovins en parc d'engraissement présentant une maladie respiratoire chronique ne répondant pas au traitement et/ou de l'arthrite.** Le but de cette étude était de déterminer la fréquence de pathogènes sélectionnés dans les tissus d'un groupe de bovins en parc d'engraissement présentant une maladie chronique (le plus souvent une maladie respiratoire et/ou de l'arthrite). Des échantillons de poumon et de tissus articulaires d'animaux provenant de 49 parcs qui n'avaient pas répondu à une antibiothérapie ont été testés par coloration immunohistochimique pour les antigènes de *Mycoplasma bovis*, *Haemophilus somnus*, *Pasteurella (Mannheimia) hemolytica* et le virus de la diarrhée virale bovine (VDVB). *Mycoplasma bovis* a été retrouvé dans plus de 80 % des cas dont 45 % des articulations et 71 % des poumons contrôlés. *Mycoplasma bovis* a été le seul pathogène bactérien identifié dans les articulations. *Haemophilus somnus* et *Pasteurella (Mannheimia) hemolytica* ont été retrouvés respectivement dans 14 % et 23 % des cas et confinés exclusivement aux poumons. L'infection au VDVB a été démontrée dans plus de 40 % des cas. *Mycoplasma bovis* et le VDVB étaient les pathogènes les plus fréquents à persister dans les tissus de ces animaux n'ayant pas répondu à l'antibiothérapie.

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## Introduction

Significant mortality occurs in feedlot cattle due to unresponsive disease manifesting as chronic respiratory disease, often occurring in association with polyarthritis (1,2). While a variety of pathogens have been associated with unresponsive respiratory disease in the feedlot, some studies attribute the majority of the losses to *Haemophilus somnus* (1,2). The organism that has been shown to be associated with arthritic losses in feedlot cattle is *Mycoplasma bovis* (3,4); however, there are also suggestions of a role for *Haemophilus somnus* (1,2).

The role of bovine viral diarrhea virus (BVDV) in mortality due to chronic disease in feedlot animals is uncertain. Variable numbers of cattle persistently infected with BVDV have been shown to be present in feedlot populations and may act as a source of virus for acute infections in cohort cattle (5). There is at least anecdotal evidence that cattle with either primary or persistent

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BVDV infection may be immunosuppressed and, therefore, predisposed to secondary infection with other agents (reviewed in 6).

In this study, tissues from a group of feedlot cattle that died or were killed due to chronic disease (most often respiratory disease and/or arthritis) were tested for the presence of antigens of *M. bovis*, *H. somnus*, *Pasteurella (Mannheimia) haemolytica (P./M. haemolytica)*, and BVDV. The goal was to determine which bacteria were present in the affected tissues and the frequency of BVDV in affected animals.

## Materials and methods

The 49 animals for euthanasia were selected from among those in the "chronic pen" at a 20 000-head feedlot in central Alberta in November 1995. Inclusion of an animal in the study was determined by 2 of the authors (GKJ and EJ), based on its fulfilling one of the following criteria: 1 — there was clinical evidence for severe arthritis ("3-legged lame") and weight loss (lame calves that had maintained their body weight were not included), 2 — it was recumbent, 3 — it was dyspneic and had weight loss, and 4 — it had died during the previous night. All animals fulfilling the criteria were included and sampled.

All cases had been treated with antibiotics as follows: tilmicosin (Micotil; Provel, Eli Lilly, Scarborough, Ontario) for the 1st treatment after the onset of clinical disease, followed by 3 d with trimethoprim and sulfadoxine (Trivetrin; Schering-Plough Animal Health, Point Claire, Quebec), then 3 d with ceftiofur sodium (Exenel; Pharmacia & Upjohn, Orangeville, Ontario), and then 3 d with sulbactam and ampicillin (Synergistin; rogar/STB, Montreal, Quebec). There was usually a 1- to 5-day break between treatments.

Single tissue samples from the left lung; the affected joint synovium or joint capsule, in cases with obvious arthritis, or the right stifle synovium, in cases without obvious arthritis; and the heart in cases with obvious pericardial or myocardial lesions were collected into 10% neutral buffered formalin. The tissues were embedded in paraffin wax; serial sections from each block were stained with hematoxylin and eosin and for viral and bacterial antigens by an avidin-biotin complex immunohistochemical method (7). Duplicate sections from each block were tested for the antigen of each of the 4 pathogens (*M. bovis*, *H. somnus*, *P./M. haemolytica*, BVDV). The primary reagents used were the following: 1 — *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); 2 — *P./M. haemolytica*: monoclonal antibody P12/D6/D5 diluted 1/2000 and 1/4000 (a gift from The Veterinary Infectious Diseases Organization, Saskatoon, Saskatchewan); 3 — *H. somnus*: rabbit polyclonal antiserum diluted1/1000 and 1/2000 (a gift from The Veterinary Infectious Diseases Organization); and 4 — BVDV: monoclonal antibody 15C5 at dilutions of 1/800 and 1/1600 (a gift 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 that were tested with the substitution of an irrelevant polyclonal antiserum or monoclonal antibody. Tissues were scored as either positive or negative without knowledge of the results of histological evaluation or the other immunohistochemical stains.

**Table 1. Gross and microscopic postmortem lesions in tissues of 49 feedlot animals with chronic unresponsive disease[a]**

| | Distribution of lesions | | |
|---|---|---|---|
| | Lungs only | Joint only | Lungs and joint |
| Numbers of calves | 19/49 | 5/49 | 21/49 |

[a] 4/49 calves had no visible lesions

## Results

The gross and microscopic examination of the tissues from the selected cattle demonstrated lung lesions in 40/49 cases and arthritis in 26/49 cases. In 21/49 cases, both respiratory and joint lesions were apparent (Table 1). In 1 case, arteritis was the only apparent lesion; in 3 cases there were no visible lesions.

*Mycoplasma bovis* antigen was demonstrated in 40/49 (82%) cases, including in 35/49 (71%) lungs and in 22/49 (45%) joints. The only bacterial antigen found in the joints was *M. bovis*. In 20/49 (41%) cases, BVDV was demonstrated in the lung and/or the joint. *Mycoplasma bovis* and BVDV were found concurrently in 19/49 (39%) cases. Table 2 shows the numbers of tissues in which either *Mycoplasma bovis* or BVDV, or both *Mycoplasma bovis* and BVDV, and/or other pathogens were demonstrated.

*Haemophilus somnus* was found in 7 cases and only in the lung. There was concurrent staining for BVDV in 5/7 cases and for *M. bovis* in 5/7 cases. In 3 *H. somnus*-positive cases, both BVDV and *M. bovis* were present.

*Pasteurella (Mannheimia) haemolytica* was found in 11 cases and only in the lung. In only 1 case was *P./M. hemolytica* found in the absence of either BVDV and/or *M. bovis*. In 6/11 *P./M. hemolytica*-positive cases, BVDV was also present, and in 10/11 cases, *M. bovis* was also present. In 6/11 cases, both BVDV and *M. bovis* were demonstrated.

Among the 49 cases tested, none of the tested antigens were demonstrated in 5 cases (3 cases without visible lesions and 2 cases with respiratory lesions). Among the tissues tested in the remaining 44 cases, no antigens were demonstrated in 3/49 lungs and in 12/44 joints.

In 4 of the submitted cases, there were obvious gross lesions associated with the heart, from which samples were submitted and tested for the antigens of the 4 pathogens (data not shown). In 3/4 cases, the lesion was a fibrinous pericarditis and there was immunohistochemical staining for *M. bovis* antigens. In 1/4 cases, the lesion was focal myocardial necrosis and there was immunohistochemical staining for *H. somnus*. Bovine viral diarrhea virus was demonstrated in 2/4 cases with cardiac lesions, concurrently with *M. bovis* in one and *H. somnus* in the other.

**Table 2. Immunohistochemical demonstration of antigens of *Mycoplasma bovis*, *Haemophilus somnus*, *Pasteurella (Mannheimia) haemolytica*, and bovine viral diarrhea virus (BVDV) in the lung and/or joint tissues of 49 feedlot animals with chronic disease**

| | Antigen location (number of animals) | | | |
|---|---|---|---|---|
| Antigen | Lung only | Joint only | Lung and joint | Total (%) |
| *M. bovis* | 18 | 5 | 17 | 40/49 (82%) |
| *H. somnus* | 7 | 0 | 0 | 7/49 (14%) |
| *P./M. haemolytica* | 11 | 0 | 0 | 11/49 (23%) |
| BVDV | 5 | 4 | 11 | 20/49 (41%) |
| *M. bovis* + BVDV | 12 | 2 | 5 | 19/49 (39%) |
| *H. somnus* + BVDV | 5 | 0 | 0 | 5/49 (10%) |
| *P./M. haemolytica* + BVDV | 6 | 0 | 0 | 6/49 (12%) |
| *H. somnus* + *M. bovis* | 5 | 0 | 0 | 5/49 (10%) |
| *P./M. haemolytica* + *M. bovis* | 10 | 0 | 0 | 10/49 (20%) |
| *P./M. haemolytica* + *H. somnus* | 1 | 0 | 0 | 1/49 (2%) |
| *M. bovis* + *P./M. haemolytica* + BVDV | 6 | 0 | 0 | 6/49 (12%) |
| *M. bovis* + *H. somnus* + BVDV | 3 | 0 | 0 | 3/49 (6%) |
| All 4 pathogens | 1 | 0 | 0 | 1/49 (2%) |
| No pathogens | 3 | 12 | 5 | 5/49 (10%) |

*M. bovis* — *Mycoplasma bovis*; *H. somnus* — *Haemophilus somnus*; *P./M. haemolytica* — *Pasteurella (Mannheimia) haemolytica*; BVDV — bovine viral diarrhea virus

## Discussion

In this study, *M. bovis* was the most common bacterium demonstrable in a group of feedlot cattle with chronic unresponsive disease (most often pneumonia and/or arthritis). The association of *M. bovis* with feedlot calf arthritis has been reported in other recent investigations (4). Studies in western Canada reported circumstantial evidence for *H. somnus* in cattle with chronic unresponsive arthritis; however, the presence of that bacterium was not confirmed by microbiological or immunohistochemical methods in most instances (1,2).

The frequent presence of *M. bovis* in lung tissue is in agreement with studies reporting serological evidence for an association between mycoplasma antibodies and risk of respiratory disease in eastern Canada (8,9). In some reports of feedlot mortality due to chronic respiratory disease, *H. somnus* has been assumed to be the prevalent pathogen in western Canadian feedlots (1,2). However, in the current study, *H. somnus* antigens were demonstrated only in a minority of the lungs and were found only in cases in which *M. bovis* and/or BVDV were concurrently demonstrated.

In this study, all of the animals that died or were killed due to chronic disease had been treated extensively with antibiotics. This treatment strategy likely affected the presence of the various bacteria, and it is possible that if these same animals had been tested earlier in the course of their illness, different bacteria might have been present. This study presents a static picture of the bacterial antigens persisting in the animals at the end of the process and suggests that the antimicrobial program was ineffective against *Mycoplasma* spp. The findings suggest that confirmation of cause-specific mortality may be necessary in cases of unresponsive disease in feedlot cattle to accurately assess treatment and preventative success. The high proportion of cattle with 2 or more bacteria present (32%) also suggests that any single bacterial pathogen may not always be the primary etiologic agent that predisposes to unresponsive disease.

A high frequency of BVDV was demonstrated in the selected cattle (greater than 40%). While, in the absence of testing tissues from a series of control animals, it is not possible to know the significance of this detection of BVDV, the high frequency in this selected group of cattle suggests that either persistent or primary BVDV infection may be a predisposing factor to the development of chronic unresponsive arthritis and/or pneumonia. Further, the presence of this agent may have been underestimated due to the limited number of tissues examined. Additional studies examining skin for detection of persistently infected calves (10) and Peyer's patches for detection of primary BVDV (11) may be necessary to accurately assess the prevalence of BVDV in feedlot losses. Rates of persistent infection with BVDV were previously reported to be as low as < 0.1% in cattle entering a typical western Canadian feedlot (5), which suggests that many of the animals detected in the present study might be primary infections. This assumption is supported by serological studies in which high incoming titers to BVDV decreased the risk of undifferentiated fever (12) and seroconversion to BVDV was associated with an increased risk of respiratory disease (9) in feedlot cattle. The high proportion of cases in which BVDV might, arguably, be predisposing to chronic bacterial infections suggests that control of chronic disease in the feedlot might require rapid identification and removal of persistently infected animals and/or effective vaccination to protect them from primary BVDV infection.

Lesions in the hearts of feeder cattle are usually assumed to be associated with *H. somnus* (1,2). In this study, heart tissues were studied in only 4 of the affected animals, with *M. bovis* being found in 3, *H. somnus* in 1, and BVDV concurrently in 2, which suggests that further study for the involvement of other pathogens might be informative.

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## Answers to Quiz Corner/Les réponses du test éclair

1. e — Normal toucans and cockatoos commonly harbor *E. coli*.
   *e — Les toucans et les cacatoès hébergent communément* E. coli.
2. d — Antinuclear antibodies are present in patients with systemic lupus erythematosus. One of the complications associated with this disorder is development of immune complexes and subsequent type-III hypersensitivity reaction in the glomeruli, which ultimately results in glomerulonephritis and kidney failure.
   *d — Les anticorps antinucléaires sont présents chez les patients qui souffrent de lupus érythémateux disséminé. Une des complications associées à ce problème est le développement de complexes immuns et d'une réaction d'hypersensibilité de type III subséquente dans les glomérules, qui conduisent ultimement à la glomérulonéphrite et à l'insuffisance rénale.*
3. d — Antibiotics may alter the gastrointestinal flora, leading to bacterial overgrowth and toxin production.
   *d — Les antibiotiques peuvent altérer la flore gastro-intestinale, menant à la prolifération bactérienne et à la production de toxines.*
4. c — Body clearance is directly proportional to volume of distribution; therefore, drug X has a smaller volume of distribution than drug Y.
   *c — La clairance corporelle est directement proportionnelle au volume de distribution. Ainsi, le médicament X a un plus petit volume de distribution que le médicament Y.*
5. d — Lack of fever differentiates ethylene glycol from dinitrophenol toxicosis.
   *d — L'absence de fièvre distingue l'empoisonnement par l'éthylène glycol de celui par le dinitrophénol.*
6. a — Heifers generally have a 20-day cycle. Therefore the chances are 1 in 20 (5%) that a heifer will be in heat on any given day. With 100 heifers, that means 5 would be expected to be in heat.
   *a — Les génisses ont généralement un cycle de 20 jours. Ainsi, les probabilités sont de 1 sur 20 (5 %) pour qu'une génisse soit en chaleur à n'importe quel jour. Avec 100 génisses, cela signifie que 5 génisses devraient être en chaleur.*
7. b — Heinz bodies are typically observed in the red blood cells of animals with toxic anemia.
   *b — Les corps de Heinz sont typiquement observés dans les globules rouges des animaux qui souffrent d'anémie toxique.*
8. e — The QT internal is the summation of ventricular depolarization and repolarization.
   *e — L'intervalle QT est la somme de la dépolarisation et de la repolarisation des ventricules.*
9. a — Larger stem sizes reflect forage maturity, high fiber levels, and lower digestibility and available energy.
   *a — Des tiges plus grosses témoignent de la maturité du fourrage, du taux élevé de fibres ainsi que d'une digestibilité et d'une énergie disponible plus faibles.*
10. c — Aminoglycosides are highly polar compounds and, consequently, are poorly absorbed PO and cross biologic membranes poorly.
    *c — Les aminoglycosides sont des composés fortement polaires et, conséquemment, ils sont faiblement absorbés p.o. et franchissent difficilement les membranes biologiques.*