Seroepidemiology of undifferentiated fever in feedlot calves in western Canada

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Abstract — The relationships between 4 bacterial and 3 viral antibody titers and morbidity (undifferentiated fever (UF)) and mortality were investigated in recently weaned beef calves. Blood samples from 100 animals that required treatment for UF (Cases) and 100 healthy control animals (Controls) were obtained: upon arrival at the feedlot (Arrival), at the time of selection as a Case or Control (Selection), and at approximately 33 d of the feeding period (Convalescent). Seroconversion to *Pasteurella haemolytica* antileukotoxin was associated with an increased risk of UF (OR = 2.83); however, seroconversion to bovine herpesvirus-1 G-IV glycoprotein was associated with a decreased risk of UF (OR = 0.43). Higher Arrival bovine viral diarrhea virus antibody titer was associated with a decreased risk of UF (OR = 0.83). Increases in *Mycoplasma alkalescens* antibody titer after Arrival were associated with an increased risk of UF (OR = 1.10). Higher Arrival *Haemophilus somnus* antibody titer and increases in *Haemophilus somnus* antibody titer after Arrival were both associated with a decreased risk of UF (OR = 0.76 and OR = 0.78). The odds of overall mortality (OR = 5.09) and hemophilosis mortality (OR = 11.31) in Cases were significantly ($P < 0.05$) higher than in the Controls. Higher Arrival bovine herpesvirus-1 antibody titer was associated with an increased risk of mortality (OR = 1.30). Protective immunity to *Pasteurella haemolytica* antileukotoxin, *Haemophilus somnus*, bovine herpesvirus-1 G-IV glycoprotein, bovine viral diarrhea virus, and *Mycoplasma* spp. may be necessary to reduce the occurrence of UF. Animals with UF are at an increased risk of overall and hemophilosis mortality.

Résumé — Séroépidémiologie de la fièvre indifférenciée dans des parcs d'engraissement de veaux de l'ouest du Canada. Les relations entre les titrages d'anticorps de 4 bactéries et 3 virus et la morbidité (fièvre indifférenciée (FI)) et la mortalité ont été analysées chez des veaux de boucherie récemment sevrés. Des échantillons sanguins de 100 animaux qui nécessitaient un traitement contre la FI (Cas) et 100 témoins sains (Témoins) ont été obtenus à l'arrivée au parc d'engraissement (Arrivée), au moment de la répartition dans les groupes Cas ou Témoins (Répartition) et à environ 33 j. De la période d'engraissement (Convalescent). La séroconversion à l'antileucotoxine de *Pasteurella haemolytica* était associée à une augmentation du risque de FI (RC (Rapport de cote) = 2,83); cependant, la séroconversion à la glycoprotéine G-IV du herpèsvirus bovin type 1 était associé à une diminution de risques de FI (RC = 0,43). Un plus haut titre d'anticorps contre le virus de la diarrhée bovine à l'Arrivée était associé à une diminution du risque de FI (RC = 0,83). Une augmentation du titre d'anticorps contre *Mycoplasma alkalescens* à l'Arrivée était associée avec une augmentation du risque de FI (RC = 1,10). Un plus haut titre d'anticorps contre *Haemophilus somnus* à l'Arrivée et une augmentation d'anticorps contre *Haemophilus somnus* après l'Arrivée étaient tous deux associés à une diminution du risque de FI (RC = 0,76 et RC = 0,78). Les risques de mortalité en général (RC = 5,09) et de mortalité reliée à *Haemophilus* (RC = 11,31) chez les Cas étaient significativement plus élevés ($P < 0,05$) que chez les Témoins. Un titre plus élevé d'anticorps contre l'herpèsvirus bovin type 1 à l'Arrivée à une augmentation du risque de mortalité (RC = 1,30). L'immunité protectrice contre l'antileucotoxine de *Pasteurella haemolytica*, contre *Haemophilus sommus*, contre la glycoprotéine G-IV de l'herpèsvirus bovin type 1, contre le virus de la diarrhée virale et contre *Mycoplasma* spp. pourrait être nécessaire pour réduire l'éventualité de FI. Les animaux atteints de FI ont un risque de mortalité générale et de mortalité reliée à *Haemophilus* plus élevé.

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Introduction

It has been reported that bovine respiratory disease (BRD) is the most significant feedlot disease in North America (1,2). However, a recent study has demonstrated that the term undifferentiated fever (UF) is more appropriate than BRD for describing feedlot animals that are febrile with a lack of abnormal clinical signs referable to organ systems other than the respiratory system (3). In recently weaned calves, treatment and mortality associated with UF continues to be a major animal health problem in feedlots in western Canada (3–7). Several studies have been conducted to investigate the associations between antibodies to specific viral and bacterial pathogens and UF (8–15). However, it may not be appropriate to extrapolate the results of these studies conducted in eastern Canada in the early eighties to the disease scenario that currently exists in western Canada, because the role of *Haemophilus somnus* was not evaluated. The objectives of the study reported herein were to determine the change in specific viral and bacterial antibody titer of calves in western Canada after arrival at the feedlot, to examine the association between arrival antibody titer and subsequent UF or mortality, to investigate the association between changes in antibody titer and UF or mortality, and to compare the distribution of mortality in animals with and without UF.

Materials and methods

Experimental design

A prospective case-control design was utilized in the study. Three hundred and sixteen Cases and 341 Controls were enrolled in the study from 1219 calves that arrived at the feedlot in October and November 1992. Due to financial constraints, 100 Cases and 100 Controls were randomly selected from the full data set for the determination of antibody titer. All Cases and Controls that died were included in the randomly selected subset of samples. Cases and Controls with missing samples were not eligible for selection to the 200 animal subset. Serum antibody titers to *Pasteurella haemolytica* antileukotoxin (PHAL), *Haemophilus somnus* (HS), bovine herpesvirus-1 (BHV-1), BHV-1 G-IV glycoprotein (G-IV), bovine viral diarrhea virus (BVDV), *Mycoplasma bovis* (MB), and *Mycoplasma alkalescens* (MA) were determined for all samples from the 200-animal subset. Statistical analyses were performed to examine relationships between disease status and serum antibody titer or seroconversion to these pathogens.

Trial Facilities

The trial was conducted in a commercial feedlot near Strathmore, Alberta, which has a capacity of 22 000 animals. The basic design of this feedlot is representative of standard designs used in western Canada. The animals were housed in open-air, dirt-floor pens, arranged side by side with a central feed alley and 20% porosity wood fence wind breaks. Each pen measured 50 m × 80 m.

There are 2 hospital facilities located in the feedlot. Each hospital has covered shelter for 100 animals, a hydraulic chute equipped with an individual animal scale, a chute-side computer for animal health data, and separation alleys to facilitate the return of animals to designated pens. Three open-air hospital pens are located adjacent to each hospital. Also, there is an enclosed processing facility and 6 receiving pens.

Trial animals

The animals utilized in the study were recently weaned, crossbred beef steer and bull calves purchased from auction markets throughout western Canada. Approximately 75 animals per truck were transported to the feedlot after assembly at the auction market. The animals were approximately 7 to 10 mo of age and weighed between 227 and 400 kg.

Upon arrival at the feedlot, the animals were moved through a hydraulic chute for a group of procedures known collectively as processing. All animals were ear tagged (to provide unique, individual animal identification), given an injection of vitamins A and D (Poten AD, rogar/STB, Pointe Claire-Dorval, Quebec), implanted with a progesterone-estradiol growth implant (Synovex-S, Syntex Agribusiness, Mississauga, Ontario), and vaccinated against BHV-1 and parainfluenza-3 (PI_3) virus (IBR-PI_3, Coopers Agropharm, Ajax, Ontario). In addition, each animal received a multivalent clostridial vaccine (Tasvax 7, Coopers Agropharm), a topical trichlorfon solution (8%) (Neguvon, Bayvet Division Chemagro, Etobicoke, Ontario) at the rate of 32.5 mL/100 kg body weight (BW), an intraruminal oxfendazole suspension (Synanthic 22.5%, Syntex Agribusiness), at the rate of 1 mL/50 kg BW, and tilmicosin (Micotil, Provel, Division of Eli Lilly Canada, Scarborough, Ontario) SC, at the rate of 10 mg/kg BW. Also, all bulls were castrated. Animals were assembled in 4 pens, each containing between 303 and 310 animals. In this study, it took from 1 to 3 d to fill a pen.

At approximately Day 90 of the feeding period for a given pen, all animals in the pen were implanted and vaccinated against BHV-1 and PI_3 virus.

Observation of trial animals

Experienced animal health personnel observed each pen of animals once or twice daily for "sick" animals to serve as potential Cases. Subsequent to the daily selection and removal of "sick" animals from each pen, the animal health personnel returned to that pen on that day and removed a number of "healthy" animals to serve as potential Controls. On a daily basis, within each pen, sufficient control candidates were selected to maintain a ratio of Cases to Controls of at least 1:1. Candidates for the Case and Control groups were selected daily until significant morbidity decreased (approximately Day 24 of the feeding period).

Definition of cases and controls

Animals deemed to be "sick" by the animal health personnel, based on subjective criteria, such as general appearance and attitude, gauntness, reluctance to move, etc, were moved from their home pen to a hospital facility. Animals with a rectal temperature ≥ 40.3°C, a lack of abnormal clinical signs referable to organ systems other than the respiratory system, and no prior treatment history for any disease, were defined as having UF and selected to the Case group for the study. These animals

were treated with an antimicrobial as per the standard feedlot protocol.

In order to identify potential Controls, animals deemed to be "healthy" by the animal health personnel, based on subjective criteria, such as general appearance and attitude, normal rumen fill, movement with intent, etc, were moved from their home pen to a hospital facility. Animals with a rectal temperature < 40.3°C, a lack of abnormal clinical signs referable to any organ system, and no previous treatment history for any disease were defined as "healthy" and selected to the Control group for the study.

Animals previously enrolled in the Control group that were subsequently identified as "sick" later in the feeding period and fulfilled the case definition for UF were reassigned to the Case group and removed from the Control group. Animals that were identified by the animal health personnel as "sick" on any given day but did not meet the minimum rectal temperature criteria (40.3°C) were not enrolled in the Control group that day. In addition, "healthy" animals selected as potential Controls that had a rectal temperature ≥ 40.3°C were treated as per the standard feedlot protocol but were not included in either the Case or Control groups. Animals in the Case group that were returned to their home pen and subsequently identified as "sick" (relapses) were not reenrolled in the study.

Animals with other diseases were diagnosed and treated as per the standard, written treatment protocols provided by the consulting veterinarians. Also, animals that were moribund or became recumbent during the feeding period were euthanized by the attending feedlot veterinarians.

Postmortem examinations

All animals that died during the feeding period were necropsied by the attending feedlot veterinarians. The cause of death was determined by the findings of the gross postmortem examination.

The gross postmortem diagnoses were categorized into those attributable to bovine respiratory disease (BRD mortality) (bronchopneumonia and fibrinous pneumonia), those attributable to *Haemophilus somnus* infection (hemophilosis mortality) (laryngitis, myocarditis, pericarditis, pleuritis, polyarthritis, and septicemia), and those attributable to causes other than BRD or hemophilosis (miscellaneous mortality) (atypical interstitial pneumonia, lymphosarcoma, and peritonitis).

Sampling

At processing, blood samples (Arrival samples) were obtained by jugular venipuncture from all 1219 animals in the study population. On a daily basis thereafter, blood samples (Selection samples) were obtained at the time of selection from animals that were enrolled in the study as Cases or Controls. At approximately Day 33 of the feeding period, blood samples (Convalescent samples) were obtained from all 1219 animals in the study population.

Serology

Whole blood samples were collected in 10-milliliter vacuum tubes (Vacutainer, Becton Dickinson, Mississauga, Ontario) and stored at 4°C for 24 h. The blood was then centrifuged at 3000 × *g* for 7 min and 1.0 mL of serum was pipetted into multiwell plates and stored frozen at −30°C until assayed. Antibody titers to PHAL, HS, BHV-1, G-IV, BVDV, MB, and MA were determined as previously described (8,16–18).

Data collection and management

Health histories for each animal, up to and including the last day of the feeding period, were printed from the mainframe computer at the feedlot and entered into a spreadsheet program (Quattro Pro 6.0, Corel Corporation, Ottawa, Ontario). Subsequently, these data were merged with the serology data to form a single data set.

The distribution of Cases and Controls by the number of days on feed when they were selected was plotted graphically for the first 24 d of the feeding period.

Statistical analysis

Analyses were performed to evaluate the change in antibody titer after arrival at the feedlot, to investigate the association between changes in antibody titer and UF or mortality, and to examine the association between arrival antibody titer and subsequent UF or mortality.

Serum antibody titers from Arrival, Selection, and Convalescent samples were used in the analyses. The magnitude of increase or decrease in antibody titer was determined by subtracting the logarithm of the Arrival antibody titer from the logarithm of the Convalescent antibody titer. Seroconversion was used as a measure of exposure to specific infectious agents and was defined as an increase in antibody titer of at least 2 logs (4-fold) between Arrival and Convalescent samples. The frequency distributions of antibody titer, log transformed antibody titer, and the change in antibody titer between Arrival and Convalescent samples were plotted to evaluate normality.

Antibody titers were evaluated over time by performing analyses of variance (ANOVA) on the change in log antibody titer from Arrival to Convalescent samples and ANOVA for repeated measures using the Arrival, Selection, and Convalescent samples. In these analyses, differences associated with the following factors were controlled for: pen, days on feed at the time of selection as a Case or Control, and Arrival weight (19–21). Preliminary examination of the data showed that distributions of BHV-1, G-IV, BVDV, and MA antibody titers and their log transformations were not normally distributed. Therefore, data for these variables were analyzed after rank transformation. Least square means (LSMeans) were calculated at each sampling point when ANOVA models were significant ($P < 0.05$). Separate analyses were performed for change in antibody titer to PHAL, HS, BHV-1, G-IV, BVDV, MB and MA.

In general, the methods used to examine the associations between Arrival antibody titer or changes in antibody titer with UF or mortality were similar. Independent variables were evaluated in univariate logistic regression models to evaluate the simple relationships between each pair of predictor and outcome variables (22). Cross tabulation of disease status with seroconversion to each infectious agent was performed.

Table 1. Summary of antibody titers from the arrival, selection, and convalescent samples[a]

Variable	Case					Control					
	Min[b]	Q1	Med	Q3	Max	Min[b]	Q1	Med	Q3	Max	*P*-value
Pasteurella haemolytica antileukotoxin[c] ($\times 10^3$)											
Arrival	< 1	5	12	40	1292	< 1	4	14	118	1634	0.198
Selection	< 1	19	60	200	1474	< 1	10	40	243	1430	0.263
Convalescent	< 1	20	75	314	1328	< 1	19	75	245	1634	0.231
Haemophilus somnus[c] ($\times 10^3$)											
Arrival	< 1	4	19	57	1096	< 1	6	37	227	1634	0.144
Selection	< 1	10	26	168	1634	< 1	10	39	218	1599	0.705
Convalescent	< 1	18	51	175	1077	< 1	45	127	434	1634	0.006
Bovine herpesvirus-1[d]											
Arrival	1	1	1	1	7718	1	1	1	1	15 201	0.378
Selection	1	1	47	306	26 343	1	1	69	600	29 506	0.338
Convalescent	1	1	146	583	39 801	1	66	226	1097	10 201	0.097
Bovine herpesvirus-1 G-IV glycoprotein[de]											
Arrival	1	1	1	1	11 298	1	1	1	1	1339	NA
Selection	1	1	1	1	1898	1	1	1	1	5945	NA
Convalescent	1	1	1	1	3115	1	1	1	63	2022	NA
Bovine viral diarrhea virus[d]											
Arrival	2	2	16	64	16 000	2	2	16	1 000	16 000	0.010
Selection	2	2	16	64	16 000	2	2	64	1 024	16 000	0.001
Convalescent	2	161	1024	4000	16 000	2	16	1024	10 000	16 000	0.655
Mycoplasma bovis[c]											
Arrival	2	8	16	32	8 192	1	8	16	32	512	0.149
Selection	4	16	64	128	8 192	1	16	32	64	2 048	0.001
Convalescent	4	64	128	256	8 192	8	64	128	256	8 192	0.237
Mycoplasma alkalescens[d]											
Arrival	1	1	1	16	256	1	1	1	32	512	0.575
Selection	1	1	16	64	512	1	1	1	32	8 192	0.035
Convalescent	1	1	32	128	8 192	1	1	8	128	8 192	0.087

[a]Arrival weight and days on feed before selection were included as variables in all analyses.
[b]Min is the minimum of the antibody titers. Q1 is the limit of the first quartile of the antibody titers. Med is the median of the antibody titers. Q3 is the limit of the third quartile of the antibody titers. Max is the maximum of the antibody titers.
[c]Comparisons were made using log transformed data.
[d]Comparisons were made using rank transformed data.
[e]The Arrival, Selection, and Convalescent bovine herpesvirus-1 G-IV glycoprotein antibody titers were not compared between Cases and Controls because the G-IV antibody titer did not show significant ($P \geq 0.05$) change over time, difference between Cases and Controls, or interaction between the change over time in Cases and Controls in the repeated measures of analysis of variance.

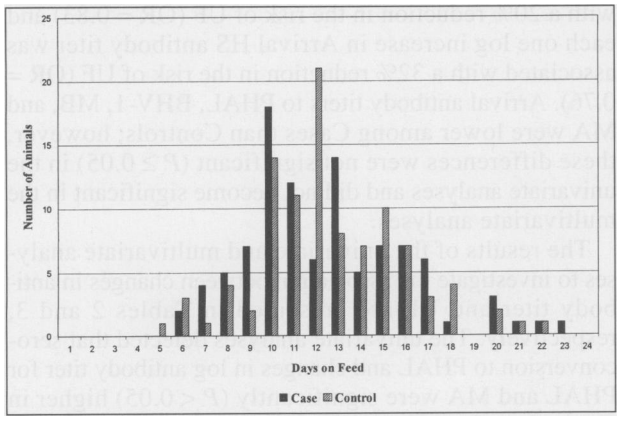

Figure 1. The distribution of Case and Control animals by number of days on feed when selected.

Differences in rates were analyzed by using Fisher's exact test, and odds ratios and 95% confidence intervals were calculated (23). Preliminary evaluations suggested that risk factors might differ among pens. However, analysis of pen specific data was not substantially different from analysis of all data. Multivariate logistic regression models were constructed by using the serological variables and Arrival weight, Arrival rectal temperature, days on feed at the time of selection as a Case or Control, and pen. Variables representing Arrival antibody titer were allowed to enter models with variables representing change in antibody titer or seroconversion. Moreover, the variable representing the Arrival antibody titer to a specific agent was forced into models whenever the change in antibody titer or seroconversion for that agent was included. The Arrival antibody titer variable was then removed, if it was not statistically significant ($P \geq 0.05$). Age can be a nonspecific measure of previous exposure to pathogens. As a result, Arrival weight was used as a proxy for age and forced into all final multivariate logistic regression models. Arrival weight was removed from the models if it was not

Table 2. Summary of odds ratios for undifferentiated fever, univariate analyses

Variable	Odds Ratio	95% CI[a]	*P*-value
Arrival temperature (per 0.1°C)	1.00	0.97–1.03	0.930
Arrival weight (per 100 lb./45.5 kg)	1.02	0.68–1.53	0.930
Days on feed before selection (per d)	0.98	0.91–1.07	0.660
Pasteurella haemolytica antileukotoxin			
Arrival antibody titer (per log)	0.89	0.78–1.02	0.096
Change in log antibody titer[b]	1.20	1.04–1.38	0.010
Seroconversion	2.46	1.36–4.47	0.003
Haemophilus somnus			
Arrival antibody titer (per log)	0.88	0.77–1.00	0.058
Change in log antibody titer[b]	0.93	0.81–1.06	0.260
Seroconversion	0.70	0.38–1.30	0.270
Bovine herpesvirus-1			
Arrival antibody titer (per log)	0.97	0.86–1.09	0.600
Change in log antibody titer[b]	0.96	0.88–1.04	0.340
Seroconversion	0.97	0.53–1.77	0.920
Bovine herpesvirus-1 G-IV glycoprotein			
Arrival antibody titer (per log)	1.09	0.91–1.32	0.350
Change in log antibody titer[b]	0.87	0.78–0.98	0.020
Seroconversion	0.41	0.20–0.84	0.020
Bovine viral diarrhea virus			
Arrival antibody titer (per log)	0.86	0.77–0.95	0.003
Change in log antibody titer[b]	1.08	1.00–1.17	0.050
Seroconversion	1.79	1.01–3.18	0.045
Mycoplasma bovis			
Arrival antibody titer (per log)	1.17	0.99–1.38	0.060
Change in log antibody titer[b]	0.95	0.83–1.08	0.440
Seroconversion	1.02	0.49–2.12	0.960
Mycoplasma alkalescens			
Arrival antibody titer (per log)	0.97	0.88–1.08	0.610
Change in log antibody titer[b]	1.06	0.98–1.14	0.130
Seroconversion	1.34	0.75–2.38	0.320

[a]95% CI is the 95% confidence interval calculated for each odds ratio
[b]The change in log antibody titer is from the Arrival to Convalescent samples

statistically significant ($P \geq 0.05$). Variables representing Arrival rectal temperature and the number of days on feed at the time of selection as a Case or Control were similarly forced into final models and removed if not significant ($P \geq 0.05$). Odds ratios and their 95% confidence intervals (95% CI) were determined for variables from logistic regression models.

The overall, BRD, hemophilosis, and miscellaneous mortality rates of animals with and without UF (using data from all 316 Cases and 341 Controls) were compared by using Fisher's exact test, and odds ratios and 95% confidence intervals were calculated (20).

Results

The distribution of Cases and Controls by the number of days on feed when selected is shown in Figure 1.

The Arrival, Selection, and Convalescent antibody titers are shown in Table 1. The analyses to determine the change in antibody titer after arrival at the feedlot demonstrated that Convalescent antibody titers of all infectious agents were significantly ($P < 0.05$) higher than the Arrival antibody titers.

The results of the univariate and multivariate analyses to investigate the association between arrival antibody titer and subsequent UF are summarized in Tables 2 and 3, respectively. Arrival BVDV ($P = 0.003$) and HS ($P = 0.058$) antibody titers were lower among Cases than Controls. In the multivariate analyses, each one log increase in Arrival BVDV antibody titer was associated with a 20% reduction in the risk of UF (OR = 0.83) and each one log increase in Arrival HS antibody titer was associated with a 32% reduction in the risk of UF (OR = 0.76). Arrival antibody titers to PHAL, BHV-1, MB, and MA were lower among Cases than Controls; however, these differences were not significant ($P \geq 0.05$) in the univariate analyses and did not become significant in the multivariate analyses.

The results of the univariate and multivariate analyses to investigate the association between changes in antibody titer and UF are presented in Tables 2 and 3, respectively. The univariate analyses detected that seroconversion to PHAL and changes in log antibody titer for PHAL and MA were significantly ($P < 0.05$) higher in Cases than Controls. As a result, the risk of UF in the multivariate models was greater among animals that seroconverted to PHAL when compared with animals that did not seroconvert (OR = 2.83). In addition, each one log increase in MA antibody titer from the Arrival to Convalescent samples was on average associated with a

Table 3. Summary of odds ratios for undifferentiated fever, multivariate analyses

Variable	Odds Ratio	95% CI[a]	*P*-value
Pasteurella haemolytica antileukotoxin			
Seroconversion	2.83	1.44–5.57	0.003
Haemophilus somnus			
Arrival antibody titer (per log)	0.76	0.62–0.92	0.005
Haemophilus somnus			
Change in log antibody titer[b]	0.78	0.64–0.94	0.010
Bovine herpesvirus-1 G-IV glycoprotein			
Seroconverison	0.43	0.19–0.99	0.047
Bovine viral diarrhea virus			
Arrival antibody titer (per log)	0.83	0.74–0.94	0.003
Mycoplasma alkalescens			
Change in log antibody titer[b]	1.10	1.01–1.20	0.022

[a]95% CI is the 95% confidence interval calculated for each odds ratio
[b]The change in log antibody titer is from the arrival to convalescent samples

Table 4. Summary of odds ratios for mortality, univariate analyses

Variable	Odds Ratio	95% CI[a]	*P*-value
Arrival temperature (per 0.1°C)	1.00	0.96–1.05	0.910
Arrival weight (per 100 lb./45.5 kg)	0.93	0.45–1.92	0.840
Days on feed at selection (per d)	1.06	0.92–1.22	0.410
Pasteurella haemolytica antileukotoxin			
Arrival antibody titer (per log)	0.88	0.68–1.14	0.320
Change in log antibody titer[b]	0.98	0.70–1.35	0.880
Seroconversion	1.05	0.31–3.53	0.940
Haemophilus somnus			
Arrival antibody titer (per log)	0.93	0.72–1.21	0.610
Change in log antibody titer[b]	1.01	0.76–1.34	0.960
Seroconversion	0.51	0.10–2.52	0.410
Bovine herpesvirus-1			
Arrival antibody titer (per log)	1.30	1.07–1.58	0.010
Change in log antibody titer[b]	0.89	0.74–1.06	0.710
Seroconversion	0.65	0.19–2.24	0.580
Bovine herpesvirus-1 G-IV glycoprotein			
Arrival antibody titer (per log)	0.89	0.62–1.29	0.540
Change in log antibody titer[b]	0.99	0.78–1.25	0.920
Seroconversion	1.27	0.25–6.60	0.770
Bovine viral diarrhea virus			
Arrival antibody titer (per log)	0.88	0.69–1.13	0.330
Change in log antibody titer[b]	1.03	0.88–1.21	0.710
Seroconversion	1.44	0.39–5.30	0.580
Mycoplasma bovis			
Arrival antibody titer (per log)	0.96	0.71–1.31	0.790
Change in log antibody titer[b]	1.25	0.93–1.68	0.140
Seroconversion	2.75	0.33–22.89	0.350
Mycoplasma alkalescens			
Arrival antibody titer (per log)	1.14	0.94–1.38	0.190
Change in log antibody titer[b]	1.00	0.85–1.18	0.990
Seroconversion	0.65	0.19–2.21	0.490

[a]95% CI is the 95% confidence interval calculated for each odds ratio
[b]The change in log antibody titer is from the arrival to convalescent samples

10% increase in the risk of UF (OR = 1.10). Conversely, the univariate analyses detected that changes in log antibody titer for HS and G-IV were significantly ($P < 0.05$) lower (and seroconversion to HS and G-IV tended to be lower) in Cases than Controls. The risk of UF in the multivariate models was less among animals that seroconverted to G-IV when compared with animals that did not seroconvert (OR = 0.43). Also, each one log increase in HS antibody titer from the Arrival to Convalescent samples was associated with a 30% reduction in the risk of UF (OR = 0.78).

In the univariate analyses, the change in log antibody titer to BVDV and seroconversion to BVDV were significantly ($P < 0.05$) higher in Cases than Controls. However, when Arrival BVDV antibody titer entered the multivariate models, these associations were no longer

Table 5. Mortality summary for the 316 Cases and 341 Controls

Variable	Case (*n*)	Control (*n*)	Odds Ratio	95% CI[a]	*P*-value
Overall mortality[b]	18	4	5.09	1.60–17.97	0.001
BRD mortality[c]	5	2	2.83	0.48–21.17	0.264
Hemophilosis mortality[d]	10	1	11.31	1.49–237.54	0.004
Miscellaneous mortality[e]	3	1	3.39	0.31–85.06	0.347

[a]95% CI is the 95% confidence interval calculated for each odds ratio
[b]Overall mortality is mortality attributable to all causes
[c]BRD mortality is mortality attributable to bovine respiratory disease (bronchopneumonia and fibrinous pneumonia)
[d]Hemophilosis mortality is mortality attributable to *Haemophilus somnus* infection (laryngitis, myocarditis, pericarditis, pleuritis, polyarthritis, and septicemia)
[e]Miscellaneous mortality is mortality attributable to causes other than BRD or hemophilosis (atypical interstitial pneumonia, lymphosarcoma, and peritonitis)

significant ($P \geq 0.05$). There were no significant ($P \geq 0.05$) differences in the change in log antibody titer for BHV-1 or MB between Cases and Controls. Differences in seroconversion rates were noted among different pens. In one pen, seroconversion to PHAL, BVDV, and MA were significantly ($P < 0.05$) higher in Cases than Controls.

The univariate analyses to investigate the association between arrival antibody titer or changes in antibody titer and mortality are presented in Table 4. Arrival BHV-1 antibody titer was significantly ($P < 0.05$) associated with the risk of mortality. Each one log increase in Arrival BHV-1 antibody titer was associated with a 30% increase in the risk of mortality (OR = 1.30). There were no other significant associations between Arrival antibody titers or changes in antibody titer and mortality.

The mortality summary of animals with and without UF is presented in Table 5. The odds of overall mortality (OR = 5.09) and hemophilosis mortality (OR = 11.31) in Cases were significantly ($P < 0.05$) higher than in Controls.

Discussion

The results of this study indicate that feedlot calves in western Canada are commonly exposed to PHAL, HS, BHV-1, G-IV, BVDV, MB, and MA in the early feeding period. With the exception of BHV-1 and G-IV, these findings are consistent with findings previously reported from feedlot calves in Ontario (9–13,17). In the current study, the activity of BHV-1 may be due to animals being vaccinated with a modified-live BHV-1 vaccine upon arrival at the feedlot to prevent infectious bovine rhinotracheitis.

The association of PHAL seroconversion with morbidity provides additional evidence that pasteurellosis is an important component of UF (10–13,24). Previous studies have found no difference in the activity of PHAL antibody titer profiles between treated and untreated animals (9,25). This inconsistency may be explained by differences in the case definition for disease. Supportive information regarding the role of PHAL in UF is that vaccination with PHAL has been shown to reduce the occurrence of disease (18,26–28).

The increased risk of morbidity associated with increases in MA antibody titer has been previously described with other *Mycoplasma* spp. (8). These findings are consistent with the isolation of *Mycoplasma* spp. from the lung tissue of diseased animals (10,29,30) and the reduction in respiratory disease with vaccination against *Mycoplasma* spp. (31,32). However, the former vaccine study (31) was conducted in neonatal calves and utilized respiratory syncytial and parainfluenza-3 viruses in the vaccine, and the latter study (32) was conducted under experimental challenge conditions. There are no field trials confirming these results. Thus, the role of *Mycoplasma* spp. as a primary or secondary pathogen in UF is not fully understood (10,33).

The sparing effect of G-IV seroconversion in naturally occurring disease challenge is consistent with previously published experimental models pertaining to the protective immunity of antibodies to G-IV glycoprotein (34–36). These findings may support the use of G-IV subunit vaccines for the reduction of UF in feedlot calves. In this study, G-IV seroconversion may have been due to a response to the BHV-1 vaccine administered at processing or natural exposure during the feeding period; however, the reason for the seroconversion cannot be determined from the results of this study. It would have been interesting to exclude BHV-1 vaccination from the processing procedures, but the economic risks of a BHV-1 outbreak superceded the exclusion of BHV-1 vaccination from the study design.

The sparing effects of higher Arrival HS antibody titer and increases in HS antibody titer after arrival on the occurrence of UF are logical, given that hemophilosis has become the most significant cause of mortality in beef calves in western Canada (3,4,7,37–39). This is consistent with results reported with enzootic pneumonia in young beef calves (16) and feedlot calves in western Canada (37). Similar associations have not been investigated in calves arriving at the feedlot in eastern Canada (9–11). However, the sparing effect of HS bacterins has been previously described in feedlot calves (14,40,41) and young beef calves (16).

The association of lower Arrival BVDV antibody titer with an increased risk of UF has been previously described with BVDV seroconversion (10,11,15). It has been postulated that these associations may be due to the immunosuppressive effects of BVDV, demonstrated in experimental models (42). However, there is a lack of substantive evidence to support this hypothesis in naturally occurring UF in feedlot calves (43). Interestingly, in the current study, Control animals expressed seroconversion to BVDV without evidence of

UF. These data are consistent with studies that have demonstrated that BVDV seroconversion is common in populations of feedlot calves where UF occurs (11,44). Conversely, it has been shown that UF can occur in the virtual absence of BVDV seroconversion (40). As a result, the role of BVDV in UF is not fully understood. Due to the association of lower Arrival BVDV antibody titer with an increased risk of UF, large scale, properly designed field studies evaluating the efficacy of BVDV vaccines in feedlot calves are required.

The odds of overall mortality and hemophilosis mortality were higher in Cases than in Controls. The increased odds of overall mortality in Cases is not surprising; however, the observation that the odds of hemophilosis mortality was also higher in Cases than in Controls has not been previously documented. These data provide additional evidence for the use of the term UF, as opposed to BRD or hemophilosis, for animals that are febrile with a lack of clinical signs referable to organ systems other than the respiratory system.

The increased risk of mortality with higher Arrival BHV-1 antibody titer may have been due to the production of nonprotective antibodies in previously infected animals or other synergistic causes of death following initial infection with BHV-1. An alternative explanation for the increased risk of mortality with higher Arrival BHV-1 antibody titer is type I error. Because all of the mortalities that occurred in the 316 Case and 341 Control animals were included in the 200-sample subset (as opposed to a random sample), the mortality analyses were potentially biased towards identifying serological associations that may not be true. However, the inclusion of all of the mortalities that occurred in the 316 Case and 341 Control animals in the 200-sample subset reduced the likelihood of type II error (the likelihood of type II error still existed because of the low number of mortalities). Serologic determinations for all 316 Case and 341 Control animals were not performed for economic reasons.

In this study, animals previously selected to the Control group that were subsequently identified as "sick" later in the feeding period and fulfilled the case definition for UF were reassigned to the Case group and removed from the Control group. This approach does not exactly conform with classical case-control methodology. However, in this study, the investigators made a conscious decision to deviate from classical design, because it would bias the findings of the study toward the null. This is due to the fact that the factors associated with becoming a Control would also be the same factors associated with becoming a Case. The investigators wanted to make sure that timing of selection did not prevent animals destined to remain healthy throughout the feeding period from being in the Control group and animals destined to have UF from being in the Case group. This was further emphasized in the design by the fact that UF could only occur once in each animal (multiple occurrences in the same animal were not allowed).

In the statistical analyses, the change in log antibody titer from Arrival to Convalescent samples and the occurrence of seroconversion were used as independent variables and allowed to simultaneously enter models. While seroconversion denotes a minimum defined increase in specific antibody titer among individual animals, it does not account for the biological relevance of the magnitude of increase detected. Thus, it may be more appropriate to measure the absolute change in antibody titer. In addition, variables representing Arrival antibody titer were allowed to enter models with variables representing change in antibody titer or seroconversion. This was due to the fact that the magnitude of serum antibody titer may have significantly affected the severity of infection. As a result, the amount of viral and bacterial replication and exposure and the likelihood that animals would have demonstrated a significant increase in specific antibody titer may have been affected.

The results of this study indicate that animals with UF are at an increased risk of overall and hemophilosis mortality. Furthermore, the serological evidence indicates that protective immunity to PHAL, HS, G-IV, BVDV, and *Mycoplasma* spp. may be necessary to reduce the occurrence of UF in feedlot calves in western Canada. A considerable number of vaccines for these antigens have been developed; however, with the exception of PHAL and G-IV, the apparent ability of these vaccines to confer protective immunity for UF is limited or unknown. Finally, additional research efforts to develop and evaluate more efficacious vaccines for HS, BVDV, and *Mycoplasma* spp. may be necessary to reduce the occurrence of UF in feedlot calves in western Canada.

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