The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to bovine coronavirus and bovine viral diarrhea virus in 3 Ontario feedlots

Annette O'Connor, S. Wayne Martin, Éva Nagy, Paula Menzies, Richard Harland

Abstract

Serological evidence of previous viral exposure (titer at arrival) and current viral exposure (titer increase) during a 28-day study period, was used to determine if bovine coronavirus (BCV) or bovine viral diarrhea virus (BVDV) was associated with the occurrence of undifferentiated bovine respiratory disease (UBRD) in feedlot calves. Neutralizing antibody titers to BCV and BVDV were determined for 852 animals from 3 Ontario feedlots. Calves at 2 of the 3 feedlots (n = 753) received a modified live 4-way viral vaccine containing BVDV. On arrival at the feedlots, 90% of animals were seropositive for BCV, while 39% of animals were seropositive for BVDV. This evidence of previous exposure to both viruses was associated with reduced subsequent UBRD risk. Evidence of exposure to BCV during the study period was common, as 50% of animals showed a 16-fold or greater titer increase; however, treatment for UBRD was not associated with titer change. Although the majority of animals were vaccinated for BVDV at arrival, within a feedlot, animals treated for UBRD had larger titer increases to BVDV than non-treated animals. Based on our findings we infer that BCV was not causally related to UBRD occurrence, however consistent with other literature, BVDV may be causally related to UBRD occurrence.

Résumé

Des évidences sérologiques d'une exposition virale antérieure (titre à l'arrivée) ainsi que d'une exposition virale récente (augmentation du titre) durant la période de 28 jours que dura l'étude, furent utilisées pour déterminer si le virus corona bovin (BCV) ou le virus de diarrhée virale bovine (BVDV) étaient associés avec la présence d'une maladie respiratoire bovine non- différenciée (UBRD) chez des veaux en engraissement. Les titres d'anticorps neutralisant envers le BCV et le BVDV furent déterminés chez 852 animaux dans trois parcs d'engraissement situés en Ontario. Les veaux à 2 des 3 parcs (n = *753) reçurent un vaccin vivant modifié quadrivalent contenant le BVDV. À leur arrivée au parc d'engraissement, 90 % des animaux présentaient un titre positif pour le BCV, alors que 39 % des animaux étaient séropositifs pour le BVDV. Cette évidence d'exposition antérieure au deux virus fut associée à une réduction subséquente du risque d'UBRD. L'évidence d'une exposition au BCV durant la période d'étude était fréquente, étant donné que chez 50 % des animaux on observa une augmentation de titre de 16 fois ou plus; toutefois, aucun changement de titre ne fut associé à un traitement pour l'UBRD. Bien que la majorité des animaux furent vaccinés contre le BVDV à leur arrivée, à l'intérieur d'un parc d'engraissement, les animaux traités pour l'UBRD ont montré de plus grandes augmentation de titre envers le BVDV que les animaux non-traités. À partir des résultats il est possible de conclure qu'il n'y a pas de relation causale entre le BCV et la fréquence d'UBRD, toutefois, tel que le rapporte la littérature, une relation causale entre le BVDV et la fréquence d'UBRD a été observée.*

(Traduit par docteur Serge Messier)

Introduction

Bovine coronavirus (BCV) has been implicated as a cause of undifferentiated bovine respiratory disease (UBRD), largely because it is frequently isolated from the nasal passages of cattle with clinical respiratory disease (1–3). However, a published study on the seroepidemiology of BCV titers in feedlot cattle found that although higher antibody titers to BCV at arrival were associated statistically with a decreased subsequent risk of treatment for UBRD, there was no association between evidence of recent infection (titer increase) and the occurrence of UBRD (4). The failure to find an association between titer increase, a proxy for exposure within the 28-day study period, with UBRD occurrence suggested that BCV may not be a causative agent of UBRD. The aim of this study was to determine if evidence of current exposure to BCV was associated with increased risk of treatment for UBRD. The null hypotheses were that evidence of previous exposure was not associated with reduced UBRD risk and that evidence of current exposure was not associated

Department of Population Medicine (O'Connor, Martin, Menzies), Department of Pathobiology (Nagy), University of Guelph, Guelph, Ontario N1G 2W1; Novartis Animal Health Inc., University of Guelph Research Park, Stone Road, Guelph, Ontario N1G 2W1 (Harland).

Address correspondence and reprint requests to Dr. Annette O'Connor, Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa 50011-1250 USA, telephone: 515-294-5012, fax: 515-294-1072, e-mail: oconnor@iastate.edu

Received November 7, 2000. Accepted May 18, 2001.

with increased UBRD risk. The same hypotheses were tested for bovine viral diarrhea virus (BVDV). Titers to a number of other agents were controlled during the analysis to improve the validity of any inferences made about the role of BCV or BVDV in UBRD.

Materials and methods

A longitudinal observational study was conducted on 852 cattle at 3 Ontario feedlots in fall 1998. The cattle were from a variety of sources and included both western Canadian (Feedlot A) and eastern Canadian (Feedlots B and C) calves. The cattle at Feedlot C were from the University of Guelph beef cattle research farms and had received no vaccines prior to arrival. For the calves at Feedlots A and B, the farms of origin and previous vaccination history were unknown. None of the feedlots dehorned cattle or included antibiotics in the ration during the 28-day study period. The length of time from purchase to arrival at the feedlot was not known, but all animals were processed within 36 h of arrival. At the feedlot, during routine processing, the cattle were systematically assigned to 1 of 4 vaccine groups: 1) *Mannheimia haemolytica* (previously *Pasteurella haemolytica*) (Pneumo-star; Biostar Inc., Saskatoon, Saskatchewan), 2) *Haemophilus somnus* (Somnu-star; Biostar Inc.) 3) *M. haemolytica* and *H. somnus* (Somnu-star PH; Biostar Inc.), and 4) an unvaccinated control group. Subsequently, these animals were commingled. Each feedlot used multiple pens and pen information for each calf was not available. All animals at Feedlot A also received Pyramid MLV4, (Ayerst Laboratories, Saint Laurent, Quebec), while all animals at Feedlot B received Bovishield 4, (SmithKline Beecham Animal Health, Mississauga, Ontario). Animals at Feedlot C did not receive additional vaccines. At processing, the rectal temperature and body weight were recorded, animals individually identified and blood samples collected. Approximately 28 d later (± 4 d) the cattle were weighed and blood samples collected again. During the intervening days, the managers of the feedlots were asked to record cattle requiring treatment. When animals were selected for treatment, the managers recorded the rectal temperature, classified the animal's level of depression based on the criterion proposed by Perino et al (5), and recorded the clinical signs present. The reason for treatment was recorded as UBRD, unless clinical signs existed that were referable to other body systems.

Blood samples were collected in 10 mL vacuum tubes (Vacutainer; Becton Dickinson, Mississauga, Ontario), and stored in the refrigerator during the on-farm collection prior to transportation to the laboratory. All samples were transported with ice packs in styrofoam containers to the laboratory for processing. After centrifugation, the sera were pipetted into microwell plates or 1.5-mL tubes and stored at −20°C until assayed. All sera were separated and stored within 24 h of collection.

Day 0 and Day 28 serum samples were analyzed for viral neutralisation antibody titers to BCV and BVDV (É. Nagy's laboratory at University of Guelph), *M. haemolytica* leukotoxin ELISA titers (laboratory at Biostar in Saskatoon, Saskatchewan), *M. haemolytica* indirect agglutination titers (P. Shewen's laboratory at University of Guelph) and *H. somnus* ELISA titers (laboratory at Biostar Inc.). The samples were analyzed according to the methods described in previous reports (4,6,7). Alkaline phosphatase-labeled goat anti-bovine IgG (H+L) was the conjugate in the ELISA (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA).

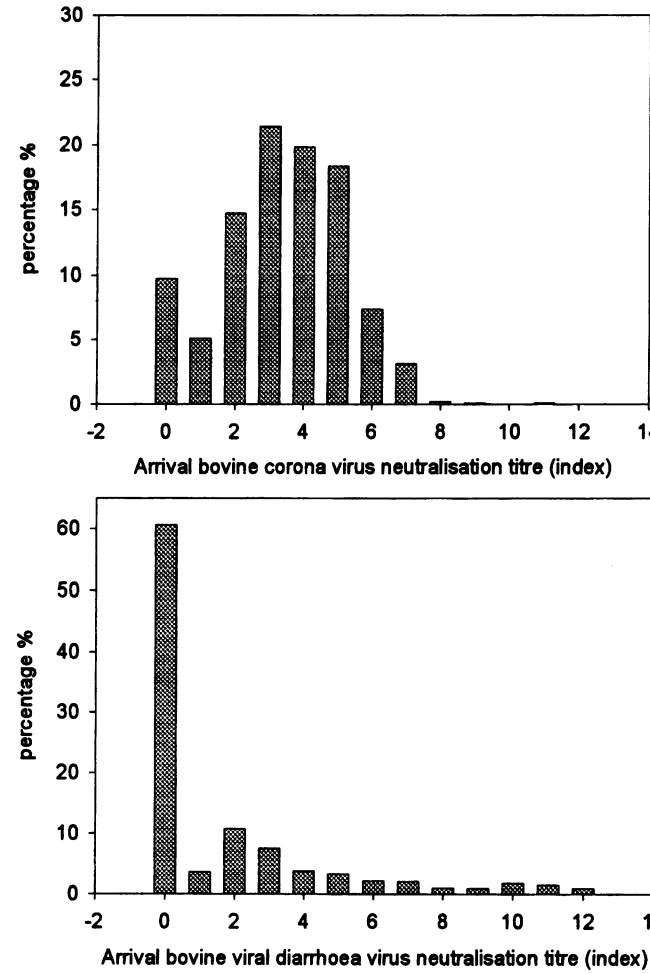

Figure 1. The frequency distribution of neutralization arrival titer for bovine coronavirus and bovine viral diarrhea virus at 3 Ontario feedlots.

For statistical analysis, the titers were transformed into an index that represented the last serum dilution (well) of the positive reaction as defined by the above techniques (8). Hereafter, the index is referred to as the titer. The initial dilution of the *M. haemolytica* leukotoxin ELISA was 1/400 and for the *H. somnus* ELISA it was 1/200. For the BCV neutralization assay the initial dilution was 1/4, while for the remaining tests the starting dilution was 1/2. Calves were categorized as seropositive or seronegative at arrival, with a titer > 0.5 considere seropositive. For all assays, a 2 titer (index) or greater increase was required for seroconversion; i.e., for 2-fold dilution tests (*M. haemolytica* leukotoxin neutralization, *M. haemolytica* indirect agglutination and BVDV neutralization titers) this represented a 4-fold increase in titer and for 4-fold dilution tests (*M. haemolytica* ELISA, *H. somnus* ELISA and BCV neutralization titers), this represented a 16-fold increase in titer.

All data analyses, unless otherwise stated, were performed by using statistical computer software (SAS v.6.12; SAS Institute Inc. Cary, North Carolina, USA). The unit of analysis was the individual animal. Descriptive analyses included the calculation of geometric means, standard deviations, and minimum and maximum values. Unconditional odds ratios (OR) were calculated to describe statistical

Table I. Descriptive statistics for bovine coronavirus (BCV) and bovine viral diarrhea virus (BVDV) neutralization titers for cattle at 3 Ontario feedlots

	Feedlot A			Feedlot B			Feedlot C		
Titers	*n*	GMT	SD	*n*	GMT	SD	*n*	GMT	SD
Day 0 BCV titer	310	3.1[a]	2.0	430	3.6[b]	1.7	99	4.7[c]	0.9
BCV titer change	309	2.2[a]	1.9	428	2.2[a]	1.9	99	1.1[b]	1.5
Day 0 BVDV titer	309	1.4[a]	2.7	429	2.4[b]	3.1	99	0.1[c]	1.3
BVDV titer change	309	3.4[a]	3.7	427	2.6[b]	2.3	99	0.005[c]	0.3

[abc] Means in the same row with the same superscript do not differ significantly at $P < 0.05$

associations between seropositivity on arrival, seroconversion, and risk of treatment.

Factors affecting the change in titer to BCV and BVDV were examined using mixed effects regression modelling. The exposure variable of interest was treatment for UBRD, a class variable with 2 levels (UBRD TREATMENT 1/0). Other class variables available for the analysis included the bacterial vaccine group (BVACCINE, 4 levels) and a variable representing vaccination with modified live viral vaccines (VVACCINE, 2 levels). The arrival titers to other putative causal agents were included as explanatory variables. Other continuous variables available for inclusion in the analysis were rectal temperature and weight at arrival. The variables, FEEDLOT and GROUP nested within FEEDLOT (representing the day animals were processed), were included initially in all models as random effects. The variance structure specified was simple (i.e., a different variance component for each effect specified). The calculations were performed using the method of restricted maximum likelihood (REML) within the Proc Mixed procedure in SAS v.6.12 (SAS Institute). A mixed regression model was used to examine factors affecting weight change. The explanatory variables of interest were the arrival weight and the arrival titer to the putative organisms. Because treatment for UBRD could be considered an intervening step between the association of arrival titer with weight change, models were constructed with and without the variable for treatment for UBRD. FEEDLOT and GROUP nested with FEEDLOT were initially included as random variables.

To examine the association between previous exposure and risk of UBRD, logistic regression was used. The explanatory variable of interest was the arrival titer to the putative agent. The variance structure specified was exchangeable. The calculations were performed using the generalized estimating equation approach (GEE) within the Proc GenMod procedure in SAS v.6.12 (SAS Institute).

For all models, potential confounding variables were added to, or removed from, the model based on their influence on the coefficients already present in the model, or their effect on the G statistic (logistic regression). If a variable had a non-significant P value (< 0.05), and its removal did not materially change the coefficient of the variable of interest, it was omitted. A material change in the coefficient was arbitrarily set at a 10% change in magnitude (9). After establishing a main effect model, biologically feasible interaction terms were added and assessed for their association with the outcome.

Results

Of the 852 animals 318 were at Feedlot A, 111 (35%) of these were treated, 435 animals were at Feedlot B, 54 (12%) of which were treated, and 99 animals were at Feedlot C, and only 9 (10%) were treated. Due to sample handling errors, 24 animals from the first group of cattle sampled at Feedlot B had no values for day 0 titers. At day 28, 6 animals had died and samples could not be collected from 2 other animals; these animals were all at Feedlot B. In all, 32 animals had some missing titer data. All but 9 of the 174 animals treated had a rectal temperature greater than 40°C when selected for treatment. Six of the 9 with incomplete records had a depression score of 2 (5), while for the remaining 3 animals only the clinical signs were reported.

The geometric mean titers for BCV and BVDV at arrival were 3.5 ± 1.9 and 1.2 ± 3.3, respectively (mean ± SD). Ninety percent of animals were seropositive to BCV at arrival; 82% of animals at Feedlot A, 94% at Feedlot B, and 100% at Feedlot C (Figure 1). The average titer changes for BCV and BVDV were 2.1 ± 1.9 and 3.0 ± 3.0 units, respectively. There were differences in arrival titer and titer change among feedlots for both organisms (Table I). The unconditional OR indicated that being seropositive to BCV at arrival was associated with a significant reduction in the risk of being treated (OR = 0.3, 95% CI = 0.2-0.6, n = 835). Fifty percent of animals seroconverted to BCV and the unconditional OR indicated that seroconversion was not associated with increased risk of UBRD (OR = 1.2, 95% CI = 0.8-1.6, n = 835). Thirty-nine percent of animals were seropositive to BVDV at arrival (Figure 1); 29% of animals at Feedlot A, 56% at Feedlot B, and 3% at Feedlot C. Being seropositive at arrival was associated with a decreased odds of subsequent treatment for UBRD (OR = 0.6, 95% CI = 0.4–0.8, n = 837). Forty-five percent of animals seroconverted to BVDV during the study period and the unconditional OR indicated that animals that seroconverted to BVDV were more likely to be treated (OR = 2.02, 95% CI = 1.3-2.8, n = 837).

The results of a mixed effects regression model of factors associated with the change in BCV titer are shown in Table II. The main variable affecting the change in titer was the arrival titer, the two being negatively correlated. UBRD treatment was not significantly associated with titer change at $P < 0.1$. Feedlot exerted a small effect on the model; the covariance parameter estimate for FEEDLOT was 0.02 and the residual was 1.01.

When modelling the change in BVDV titer, rather than a mixed effects model with FEEDLOT as a random effect, a fixed effects model including VVACCINE was used, as this was a herd level variable representing essentially the same data as FEEDLOT. As both variables represent the same data, one of them is redundant. The decision to include the fixed variable VVACCINE rather than the random variable FEEDLOT was based solely on the preference of the first author. The results of the regression model for the change in

Table II. The association between undifferentiated bovine respiratory disease (UBRD) and the change in bovine coronavirus (BCV) neutralization titers, controlling for covariates, during a 28-day study period in 3 Ontario feedlots

Variable	Regresssion coefficient[a]	SE	*P* value
Intercept	16.9	3.7	0.04
BCV titer	-3.5	0.9	0.00
M. haemolytica titer	0.07	0.03	0.03
H. somnus titer	0.05	0.02	0.04
Rectal temperature	−0.3	0.09	0.001
BCV titer * Rectal temperature at arrival	0.06	0.02	0.006
Treatment for UBRD	−0.12	0.09	0.12

[a] The size of the regression coefficient indicates the change in BCV titer for each unit change in the variable (i.e., each one-unit increase in BCV titer at arrival is associated with a 3.5-unit decrease in BCV titer change)

Table III. The association between undifferentiated bovine respiratory disease (UBRD) and the change in bovine viral diarrhea virus (BVDV) neutralization titers, controlling for covariates, during a 28-day study period in 3 Ontario feedlots

Variable	Regresssion coefficient[a]	SE	*P* value
Intercept	−22.2	4.6	0.00
BVDV titer	−0.4	0.03	0.00
BCV titer	0.3	0.05	0.00
Weight	0.01	0.003	0.001
Rectal temperature	0.6	0.1	0.00
Treatment for UBRD	1.2	0.2	0.00
Vaccination with BVDV (herd level variable)	3.9	0.3	0.00

[a] The size of the regression coefficient indicates the change in BVDV titer (i.e., each one-unit increase in BVDV titer at arrival is associated with a 0.4-unit titer decrease in BVDV titer change)

BVDV titer are shown in Table III. Arrival titer to BVDV was negatively correlated with change in BVDV titer. UBRD treatment was associated with BVDV titer increases. Those animals with elevated rectal temperatures and heavier animals at arrival were more likely to have larger increases in BVDV titer (Table III). VVACINE increased the titers to BVDV, but at no point in the model-building process was the interaction between VVACCINE and BVDV arrival titer significant.

When UBRD was regressed on BCV arrival titer, the coefficient was negative and significant. A negative coefficient indicates that the risk of UBRD was reduced as the arrival titer increased. For all the organisms studied, higher arrival titers tended to be sparing of disease risk, however the magnitude of the sparing effect of BCV titers was less then all the other organisms (Table IV).

The average weight at arrival was 247 ± 32 kg, and the average weight gain was 27 ± 18 kg. There was no difference in arrival weight among the feedlots but calves in Feedlot B had higher weight gains. Two multivariable models describing factors affecting the change in weight are shown in Table V. Model 1 omits the

Table IV. The association between bovine coronavirus (BCV) and bovine viral diarrhea virus (BVDV) arrival titers on the risk of being treated for undifferentiated bovine respiratory disease (UBRD), controlling for covariates, during a 28-day study on cattle from 3 Ontario feedlots

Variable		Regresssion coefficient[a]	SE	*P* value
Intercept		1.22	0.9	0.2
Vaccine	COMBINED	−0.7	0.2	0.00
	HSVACC	−0.3	0.2	0.2
	PHVACC	−0.3	0.07	0.00
	CONTROL	0.00	—	—
M. haemolytica leukotoxin ELISA		−0.2	0.04	0.00
H. somnus ELISA		−0.1	0.08	0.06
BCV		−0.08	0.04	0.03
BVDV		−0.1	0.007	0.00
Arrival weight		−0.005	0.004	0.3

[a] The exponit of the regression coefficient gives the odds ratio representing the change in UBRD risk as the factor is changed by one unit (i.e., $e^{-0.7}$ represents a 0.5 reduction in disease risk in the group receiving the combined vaccine compared to the reference (control) group)

effect of UBRD treatment, while Model 2 includes UBRD treatment as an explanatory variable. The bacterial vaccine group (BVACCINE) was not significant, nor was an interaction between the arrival titer and BVACCINE. Arrival weight was negatively associated with weight gain. Higher arrival titers to BVDV were associated with greater weight gains, even after controlling for the effects of UBRD. Animals treated for UBRD gained 15 kg less over the study period than untreated animals. FEEDLOT and GROUP effects were small; the covariance parameter estimates for FEEDLOT, GROUP and the residual were 10.6, 1.7, and 281.9, respectively.

Discussion

Exposure to BCV prior to arrival was extremely common, with 90% of animals being seropositive at arrival. This, in addition to reports from other authors, demonstrates that BCV is a ubiquitous organism in Canadian cattle populations (10,11). If titer changes to BCV indicate recent infection, then exposure to BCV during the early feedlot period was also common, as demonstrated by large antibody titer increases from day 0 to day 28. However, based on not finding an association of titer change with UBRD in the multiple regression model, a finding also reported previously by Martin et al (4), we would suggest that BCV was not related to an increased risk of UBRD. Bovine coronavirus was not associated with weight gain either. Other researchers have suggested that an association exists between BCV and respiratory disease occurrence, based on the observation that the organism was frequently isolated from animals with respiratory disease (1–3). However, these studies did not examine the prevalence of BCV in control animals, and hence are of limited value for causal inferences.

The unconditional association of seropositivity at arrival with improved health during the feedlot period, and the reduced risk

Table V. The association between bovine coronavirus (BCV) and bovine viral diarrhea virus (BVDV) arrival titers and weight change, controlling for covariates, during a 28-day study period in 3 Ontario feedlots

	Model 1			Model 2		
Variable	Regression coefficient	SE	*P* value	Regression coefficient	SE	*P* value
Intercept	26.0	5.9	0.04	35.2	5.7	0.02
M. haemolytica ELISA titer	1.9	0.5	0.000	1.5	0.5	0.004
H. somnus ELISA titer	1.4	0.4	0.001	1.1	0.4	0.006
BCV titer	0.5	0.4	0.2	0.3	0.3	0.4
BVDV titer	0.8	0.2	0.000	0.6	0.2	0.007
Arrival weight	−0.04	0.02	0.04	−0.05	0.02	0.008
Treatment for UBRD	—	—	—	−15.0	1.6	0.00

[a] The size of the regression coefficient indicates the change in weight for each one-unit change in the variable (i.e., each one-unit increase in BVDV arrival titer at arrival is associated with a 0.8 kg increase in weight gain)

predicted by higher titers at arrival in the multiple regression model, was not taken as strong evidence for BCV having a causal role in UBRD. Largely, this is because of the lack of supporting evidence that potential active infection (shown by titer increase) was associated with treatment. The association between arrival titer and reduced disease risk does not necessarily imply that the protection was BCV specific. Rather, this relationship could be interpreted as evidence of "a healthy animal." That is, calves experience widespread exposure to BCV, probably from birth, and the better the calf can respond to that exposure, as well as to exposure to other agents, the better its general level of health. In contrast, a failure to respond to that exposure would be taken as evidence of an unhealthy, or high-risk calf. The hypothesis that titers to BCV may represent a proxy for a healthy calf, rather than implicate the agent as a cause of disease, has been previously suggested by Ganaba et al (10). They suggested that although BCV was statistically associated with respiratory disease in calves, "it is possible that this lack of seroreaction in some calves could be an indicator of their incapacity to respond immunologically as efficaciously as other calves" (10). Further support for this is that higher arrival titers to all of the agents investigated in this study were sparing for subsequent UBRD. This "healthy animal" effect may explain why sparing associations between arrival titers to putative agents and UBRD risk are reported frequently without corresponding evidence that current exposure is associated with UBRD (12–14). With respect to BCV, there was also a lack of association between BCV antibody titers at arrival and improved weight gains. In a recent paper by Storz et al (15), discussing the causal role of BCV in UBRD in an outbreak of UBRD, calves with fatal UBRD (n = 26) tended to have lower hemagglutination titers than a group of well calves (n = 18). These fatal cases also tended to show smaller or no change in titer to BCV, between day 0 and day 5, while the healthy animals had larger titer changes. In the same study, BCV could be cultured from the nasal secretions 80% of fatal cases at arrival (n = 21) and none of the controls. *Mannheimia haemolytica* could be cultured from nasal secretions of 7% of the fatal cases (n = 2) and none of the controls on arrival; by day 5, 96% of the fatal cases were BCV-positive (n = 25), and 65% of the cases were *Mannheimia haemolytica*-positive (n = 17). At necropsy *Mannheimia haemolytica* was isolated from the lungs of 25 animals and BCV was isolated from 18 of the 26 fatal cases. If we apply our criteria for evidence of a causal relationship, i.e. placing little causal inference on measurements of exposure made prior to disease, and increased weight to measures of exposure that occur concurrent with disease occurrence, then the larger change in the prevalence of culture positive *Mannheimia haemolytica* nasal secretions from day 0 to day 5 in the fatal cases (7 to 65%) compared to BCV (80% to 96%), concurrent with the onset of fatal disease gives greater credence to the idea that the outbreak was causally related to *Mannheimia haemolytica* rather than BCV. The low titers in the group of fatal animals could suggest that these animals were "not healthy" at arrival rather than lacking BCV-specific protection.

The rate of seroconversion to BVDV in the vaccinated animals in this study was very similar to the 40 to 50% seroconversion rates in unvaccinated animals usually reported (12,13,16). Because the cattle used in this study were vaccinated at the feedlot level, rather than at the individual animal level, where analysis occurred, it was not possible to make causal inferences about BVDV vaccination. However, we were able to test for an association between UBRD treatment and BVDV titer change, and, based on this, make inferences about the causal role of BVDV. With respect to BVDV, larger titer increases were associated with increased disease risk. Given that the majority of animals were naïve at arrival to BVDV it may be hypothesized that natural exposure after vaccination resulted in both increased titers due to an anamnestic response and an increased risk of UBRD treatment. Because any titer change as a result of UBRD treatment might differ between vaccinated and unvaccinated cattle, it would have been preferable to examine interactions between vaccination and UBRD treatment on titer change. This was not possible in this study; however, the results of a number of other studies are in agreement with our findings and support the view that high titers to BVDV at arrival are protective against UBRD (12–14). Given the literature available suggesting that BVDV is causally related to UBRD and the association between exposure and disease occurrence reported here, this sparing effect may be organism-specific protection rather than just the healthy animal effect posited for BCV. The association between higher arrival titers to BVDV and larger weight gains also support the hypothesis that BVDV was causally related to UBRD. We have no explanation as to why calves with higher arrival titers to BCV would have larger BVDV titer increases.

With respect to study design and its impact on results, the sensitivity and specificity of the diagnostic process to determine disease status and the quantitative characteristics of the laboratory tests to determine exposure status were unknown. However, provided any errors were non-differential, and we have no reason to suspect that they were differential, the study results would be biased toward the null hypothesis (17–20). Future seroepidemiological studies may benefit by reducing the sampling interval to 2 wk to reduce the likelihood of exposure status misclassification. Furthermore, exposure to the organisms was measured by the presence of circulating antibodies, and there was no mechanism to determine if these resulted from pulmonary or gastrointestinal tract exposure. For organisms that are commensal and only pneumopathogenic in particular situations, such as *M. haemolytica*, associating pulmonary exposure with UBRD occurrence may be a more relevant study goal (21). However, at least with respect to BVDV, where the mechanism of causality may not involve direct lung pathology, the association between pulmonary exposure to BVDV and UBRD occurrence may not be of particular importance (22).

In summary, the results of this and other studies support the view that current BCV infection is not associated with an increased risk of treatment for UBRD, thus we infer that BCV does not cause UBRD. In contrast, given that evidence of previous exposure to BVDV predicts a lower risk of UBRD treatment, and that UBRD treatment is associated with increased titer changes to BVDV, we infer that BVDV may play a causal role as a component of the UBRD complex. These findings are consistent with other literature about BVDV and UBRD (23).

Acknowledgments

This work was supported by a research grant from the Ontario Cattlemen's Association and the Ontario Ministry of Agriculture, Food and Rural Affairs. The help of Mr. Mike Buis, the staff at The Elora Beef Research Centre, and the staff at Advanced Agricultural Testing is gratefully acknowledged. The technical assistance of Paul Huber and Betty Ann McBey is greatly appreciated.

Reference

1. Storz J, Purdy C, Lin XBM, et al. Isolation of respiratory bovine coronavirus, other cytocidal virus, and *Pasteurella* spp. from cattle involved in two natural outbreaks of shipping fever. J Am Vet Med Assoc 2000;216:1599–1604.
2. Carman PS, Hazlett MJ. Bovine coronavirus infection in Ontario, 1990–1991. Can Vet J 1992;33:812–814.
3. Storz J, Stine L, Liem A, Anderson GA. Coronavirus isolation from nasal swab samples in cattle with signs of respiratory tract disease after shipping. J Am Vet Med Assoc 1996;208: 1452–1455.
4. Martin SW, Nagy E, Shewen PE, Harland RJ. The association of titers to bovine coronavirus with treatment for bovine respiratory disease and weight gain in feedlot calves. Can J Vet Res 1998;62: 257–261.
5. Perino LJ, Apley MD. Clinical trial design in feedlots. Vet Clin North Am Food Anim Pract 1998;14:343–366.
6. Shewen PE, Wilkie BN. Vaccination of calves with leukotoxin culture supernatant from *Pasteurella haemolytica*. Can J Vet Res 1988;52:30–36.
7. Martin SW, Harland RJ, Bateman KG, Nagy E. The association of titers to *Haemophilus somnus*, and other putative pathogens, with the occurrence of bovine respiratory disease and weight gain in feedlot calves. Can J Vet Res 1998;62:262–267.
8. Thrusfield M. Veterinary Epidemiology. Don Mills, Ontario: Blackwell Science Ltd, 1995.
9. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993;138:923–936.
10. Ganaba R, Belanger D, Dea S, Bigras-Poulin M. A seroepidemiological study of the importance in cow-calf pairs of respiratory and enteric viruses in beef operations from northwestern Quebec. Can J Vet Res 1995;59:26–33.
11. Crouch CF, Acres SD. Prevalence of rotavirus and coronavirus antigens in the feces of normal cows. Can J Comp Med 1984;48: 340–342.
12. Martin SW, Bateman KG, Shewen PE, Rosendal S, Bohac JE. The frequency, distribution and effects of antibodies, to 7 putative respiratory pathogens, on respiratory disease and weight gain in feedlot calves in Ontario. Can J Vet Res 1989;53:355–362.
13. Martin SW, Nagy E, Armstrong D, Rosendal S. The associations of viral and myocplasmal antibody titers with respiratory disease and weight gain in feedlot calves. Can Vet J 1999;40:560–567.
14. Booker CW, Guichon PT, Jim GK, Schunicht OC, Harland RJ, Morley PS. Seroepidemiology of undifferentiated fever in feedlot calves in western Canada. Can Vet J 1999;40:40–48.
15. Storz J, Lin XiaoQing, Purdy CW, et al. Coronavirus and *Pasteurella* infections in bovine shipping fever pneumonia and Evans criteria for causation. J Clin Microbiol 2000;38:3291–3298.
16. Taylor LF, van Donkersgoed J, Dubovi EJ, et al. The prevalence of bovine viral diarrhea virus infection in a population of feedlot calves in western Canada. Can J Vet Res 1995;59:87–93.
17. Kleinbaum DG, Kupper LL, Morganstern H. Epidemiological Research, Principals and Quantitative Methods. New York: Von Nostrand Reinhold International Company Ltd, 1982.
18. Greenland S. The effect of misclassification in the presence of covariates. Am J Epidemiol 1980;112:564–569.
19. Marshall JR, Hastrup JL. Mismeasurement and resonance of strong confounders: uncorrelated errors. Am J Epidemiol 1996;143:1069–1078.
20. Jacobson RH. Validation of serological assays for diagnosis of infectious diseases. Rev Sci Tech Off Int Epiz 1998;17:469–486.
21. Potgieter LN. Bovine respiratory tract disease caused by bovine viral diarrhea virus. Vet Clin North Am Food Anim Pract 1997; 13:471–481.
22. Potgieter LN. Immunology of bovine viral diarrhea virus. Vet Clin North Am Food Anim Pract 1995;11:501–520.
23. Martin SW, Bohac JG. The association between serological titers in infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, parainfluenza-3 virus, respiratory syncytial virus and treatment for respiratory disease in Ontario feedlot calves. Can J Vet Res 1986;50:351–358.