The associations of viral and mycoplasmal antibody titers with respiratory disease and weight gain in feedlot calves

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Abstract — Blood samples from 32 groups of calves (n = 700) were taken on arrival and after 28–35 days at the feedlot. Eleven groups were housed in feedlots in Ontario, and 21 groups in feedlots in Alberta. Serum antibody titers to bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PIV-3), infectious bovine rhinotracheitis virus (IBRV), *Mycoplasma dispar* and *M. bovis,* plus data on bovine corona virus (BCV) from a previous study were investigated for their association with the risk of bovine respiratory disease (BRD), and with 28-day weight change, both before and after controlling for titers to *Pasteurella haemolytica* and *Haemophilus somnus.* Exposure to IBRV and *M. bovis* was infrequent, and although exposure to PIV-3 was more common, none of these agents had important associations with BRD. Higher titers to BVDV, BRSV, and BCV on arrival were associated with reduced risks of BRD and increased weight gains. However, there was some variation in these relationships and higher arrival titers to BVDV and BRSV in a subset of the calves were associated with increased risks of BRD. Titer increases to BVDV were associated with a higher risk of BRD and lower weight gains. Titer increases to BRSV were not usually associated with the occurrence of BRD, but titer increases to BRSV in a subset of calves that were vaccinated against BRSV, on arrival, were associated with an elevated risk of BRD. Of all the agents studied, BVDV had the most consistent associations with elevated risk of BRD and lower weight gains. Higher BRSV arrival titers were related to lower risk of BRD and higher weight gains; in some instances titer increases to BRSV were associated with higher BRD risk. Higher titers to BCV on arrival were related to reduced risks of BRD. Practical ways of adequately preventing the negative effects of these agents are still needed.

Résumé — Associations entre les titres d'anticorps viraux et mycoplasmiques et les maladies respiratoires et les gains de poids chez des veaux en parc d'engraissement. Des échantillons de sang provenant de 32 groupes de veaux (n = 700) ont été recuillies à l'arrivée au parc d'engraissement et 28 à 35 jours plus tard. Onze groupes étaient localisés dans des parcs d'engraissement situés en Ontario et 21 autres en Alberta. Les titres d'anticorps sériques au virus de la diarrhée bovine virale (VDBV), au virus respiratoire syncytial bovin (VRSB), au virus parainfluenza type 3 (VPI-3), au virus de la rhinotrachéite bovine infectieuse (VRBI), à *Mycoplasma dispar* et *M. bovis* ainsi que des résultats sur le corona virus bovin (CVB) provenant d'une étude antérieure ont été analysés du point de vue de leur association avec le risque de maladie respiratoire bovine (MRB) et de modification de poids à 28 jours, le tout avant et après titrage à *Pasteurella haemolytica* et *Haemophilica somnus.* Les expositions au VRBI et à M. bovis n'étaient pas fréquentes et même si l'exposition au VPI-3 était plus répandue, aucun de ces agents n'était associé de façon importante aux MRB. Des titres plus élevées au VDBV, au VRSB et au CVB à l'arrivée étaient associés à de moindres risques de MRB et à des augmentations de gains de poids. Cependant, des variations ont été notées dans ces relations et des titres plus élevés à l'arrivée au VDBV et au VRSB dans un sous-groupe de veaux ont été associés avec une augmentation de risque de MRB. Les augmentations de titres au VDBV ont été associés à un risque plus élevé de MRB et a un plus faible gain de poids. Les augmentations de titres au VRSB n'étaient pas généralement associées à l'apparition de MRB mais une augmentation des titres au VRSB dans un sous-groupe de veaux vaccinés contre le VRSB à l'arrivée était associé à un risque élevé de MRB. De tous les agents étudiés, la VDBV comportait l'association la plus constante de risque élevé de MRB et de faibles gains de poids. Des titres plus élevés au VRSB à l'arrivée étaient reliés à des risques plus faibles de MRB et à des gains de poids plus élevés de MRB. Des titres plus élevés au CVB à l'arrivée étaient reliés à de moindres risques de MRB. On attend toujours les moyens pratiques pour prévenir adéquatement les effets nuisibles de ces agents pathogènes.

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Introduction

In attempts to understand the causes of bovine respiratory disease (BRD), early multiagent experimental studies often focused on the role of one specific virus as a precursor, by exposing calves to the virus prior to experimental challenge with *Pasteurella haemolytica* (Ph). In this context, infectious bovine rhinotracheitis virus (IBRV (bovine herpesvirus 1)) was a prototype virus for producing an enhanced (synergistic) effect with Ph when exposure to IBRV preceded exposure to Ph by approximately 4 d (1). Later, parainfluenza virus type 3 (PIV-3) was shown to have similar, though lesser, properties under experimental conditions (1); other workers noted a lack of synergism from PIV-3 (2). Neither IBRV nor PIV-3 has demonstrated synergism with Ph in seroepidemiologic studies of BRD under field circumstances (3,4).

Initially, IBRV was thought to be an important precursor to BRD in feedlot calves (5), although, typically, the specific upper respiratory tract disease caused by the IBRV is limited to calves older than 6 mo (6). In earlier epidemiological studies, IBRV was considered to be a component cause of BRD based on evidence of its high relative frequency at gross postmortem examination and its frequent isolation from tissues (7). However, in recent field studies, there has been little evidence of IBRV exposure of feedlot calves in the first few weeks after arrival (3,4,8).

Parainfluenza virus type 3 was initially considered a more important risk factor than IBRV for BRD. It was widespread, seroconversions occurred more frequently in cases than controls (3), and in other studies, high titers to PIV-3 on arrival appeared to be protective against subsequent BRD (4). However, titers to IBRV or PIV-3 on arrival were not associated with risk of BRD in data from a bull test station (9).

Bovine viral diarrhea virus does not produce respiratory disease in its own right, but it has been incriminated as a component cause of BRD. In initial reports (10), seroconversion to BVDV was noted in 72% of calves with BRD and in only 11% of unaffected calves. In other studies, about one half of calves seroconverted to BVDV during the first 4 wk postarrival, and the seroconversions to BVDV were more common in calves with BRD than in controls (3,4). In addition, high BVDV titers on arrival were associated with better weight gains (4).

Recently, bovine respiratory syncytial virus (BRSV) has been the subject of much interest for its potential role as a cause of BRD. Seroconversions to BRSV were used to implicate the virus as a cause of an explosive herd outbreak of BRD (11), to outbreaks of BRD in young dairy calves (12), and to an outbreak of BRD in cattle on pasture (13). In earlier studies, in feedlots in Ontario, calves with low BRSV titers on arrival (3) and calves that seroconverted to BRSV (4) had increased risks of BRD. Increased weight gains and lower rates of BRD were noted in calves vaccinated against BRSV (14). Nonetheless, others (9,15,16) have stated that serum antibody to BRSV was not protective against BRD.

In the early 1990s, bovine coronavirus (BCV) was suspected of playing a role in causing BRD (17). Recent studies have indicated that animals with an antibody titer to BCV on arrival appeared to be protected against BRD within the first 28 d in the feedlot, but titer change was not as strongly related to risk of BRD (18). In support of its impact on BRD risk, each unit of titer on arrival increased the 28-day weight gain by slightly over 1 kg (18).

Mycoplasmal agents have not been identified as major causes of BRD in feedlot cattle in North America. Based on data from a vaccine trial in batches of young beef calves at one large farm, *Mycoplasma bovis* (Mbov) and *M. dispar* (Mdis) were related to outbreaks of respiratory disease (19). Despite the fact that control calves had relatively more seroconversions to Mdis than did the cases, the agent was thought to be an initiator of BRD in dairy calves (20). The potential role of mycoplasmal agents in a BRD-tenosynovitis outbreak has been described (21), but previous serologic studies in Ontario yielded equivocal results in terms of their causal importance (22).

The study reported here extends previous studies (3,4) by presenting data from an additional 32 groups of calves and, again, seeks to identify and elaborate on any associations between single and combined exposure to viral and mycoplasmal agents and the occurrence of BRD and the 28- to 35-day weight gain. In order to minimize bias caused by the effects of other causal factors, we controlled for the presence and effects of antibody to *Haemophilus somnus* (Hs) and Ph.

Materials and methods

Source of calves

Calves were 5 to 7 mo old on arrival. Calves in feedlots in Alberta may have been slightly older than those in Ontario, based on their average weights at arrival; 216 kg in Ontario and 278 kg in Alberta. The calves were examined at least twice each day for 28 d. The case definition of BRD discussed with all feedlot managers included: (a) lethargy or anorexia (lack of rumen fill); (b) signs of depression or altered respiratory pattern; (c) a rectal temperature of $\geq 40.0°C$, and (d) an absence of signs referable to other body systems.

The data were divided into 2 major sets. The first (Data Set I) contained data on calves from 19 groups, 11 (nos. 11, 12, 15–21, 33, 44) in Ontario and 8 (nos. 60–67) in Alberta. The 300 calves in groups 11, 12, and 15–21 were fed at the Advanced Agricultural Testing Feedlot in Petersburg, Ontario, during the study years 1991–92. These calves, originating from western Canada, Ontario, and Quebec, were housed under a roof with 6 to 8 calves per pen; each pen had a concrete floor front and a bedded area at the back. In group 33, 120 western Canadian calves were fed at the Elora feedlot in 1990–91. They were housed 4 to 6 calves per pen, under a roof and on a slatted floor; the pens were bedded for the first few weeks to allow the calves to acclimatize to the flooring. In group 44, 123 western Canadian calves were housed, approximately 12 calves per pen, at the Ridgetown feedlot in 1990–91. All pens were bedded with straw. These calves received a modified live virus (MLV) IBRV and PIV-3 vaccine on arrival. For groups 60–67 (n = 249), over the period

1990–91, 17 groups of calves were housed at the Advanced Research International (ARI) feedlot at Airdrie, Alberta. Two of these groups (nos. 60–61) came from sales yards in Manitoba, 4 groups (nos. 62–64, 67) came from buyers in Alberta, and 2 groups (nos. 65–66) came from Saskatchewan. Calves were housed in aggregates of approximately 32 calves (one group had only 25 calves). These calves received a MLV IBRV-PIV-3 vaccine on arrival.

Data Set II was comprised of serological data on 370 of 1871 calves in 13 groups (nos. 71–81 and 86–87). These calves were housed at the ARI feedlot in 1988–89 and were part of a vaccine trial involving a Ph leukotoxoid (23). They originated from ranches in Alberta and received a 4-way viral (IBRV, PIV-3, BRSV and BVDV) respiratory disease vaccine on arrival.

Serologic analyses

With the exception of calves in Data Set 11, all calves were bled shortly after arrival. All calves (groups 60–67), or all cases and an equal number of unaffected controls were bled again at 28 to 35 d after arrival. The sera from calves in Data Set I were analyzed, in our Guelph laboratories, for antibody titers to Mbov, Mdis, IBRV, BVDV, PIV-3, BRSV, and BCV — the last virus listed here was the subject of a previous study (18). Mycoplasma titers (n = 337) were available only for calves in groups 33, 44, and 60–67 by using previously described methods (22). Serum samples were heat inactivated at 56°C for 30 min. All samples were tested in duplicate. For the hemagglutination inhibition (HI) test for antibodies to PIV-3 (n = 616), 2-fold serial dilutions of the sera were prepared in V-bottom, 96-well microtiter plates and mixed with 4 hemagglutination units of cell culture virus antigen in an equal volume. After incubation for 30 min at room temperature, 50 µL of fresh 0.5 % guinea pig red blood cells were added and the plates incubated for 3 h at room temperature. Known positive and negative serum controls were included in the assay. The antibody titer was the reciprocal of the mean of the dilutions at which no hemagglutination was detected.

Virus neutralization assays were used for antibodies against IBRV, BRSV, and BVDV (n = 808–885). The assays for antibodies to IBRV and BVDV were done on Madin Darby bovine kidney cells, and, to BRSV, on Georgia bovine kidney cells. Titers to BCV (n = 604) were obtained by using previously described methods (18). The cells and viruses were kindly provided by Dr. S. Carman, Animal Health Laboratory, University of Guelph. Two-fold serial dilutions of the serum samples were made in flat-bottom, 96-well tissue culture plates; mixed with an equal volume of virus containing 100 $CCID_{50}$; and incubated for 1 h at 37°C. Then the appropriate kind and number of cells were added to each well and the plates were incubated at 37°C for 4 or 5 d. Known positive and negative serum controls were included with each working batch, and virus back titration was also performed. Virus titer was calculated according to the Reed and Muench method (24). The titer was the 50% endpoint of the dilution of serum that completely inhibited the virus replication in 50% of the wells (18).

For Data Set II, viral serology was conducted on a subset of serum collected for *Pasteurella haemolytica* titers in the Alberta provincial diagnostic laboratory. Titers to IBRV were obtained by using a monolayer ELISA procedure (Wu and Dreger personal communication; method available on request). Titers to BVDV were obtained by using standard serum neutralization procedures (25) and BRSV titers were obtained by using an indirect immunofluorescence test (26).

Antibody titers to Hs (n = 702, groups 11–67) were obtained at the Veterinary Infectious Disease Organization (VIDO) using methods for the outer membrane protein (27). Serum samples were tested for indirect agglutination titer (Ph-A) and leukotoxin neutralizing titer (PhL) to Ph type A1 (n = 1074) in Dr. Shewen's laboratory (28). The titer was the reciprocal of the endpoint dilution.

Statistical analyses

Uniagent models

Statistical analyses were conducted at the calf level by using a series of logistic regression models to identify risk factors for BRD. Initial single-agent models contained the antibody titer data to each virus or mycoplasmal agent on arrival (AGENT-A) and the titer change data to that agent (AGENT-D) along with dummy variables, each denoting one group of calves, and variables denoting the vaccine history of the calves (either at the source farm or on arrival at the feedlot). This produced a within-group analysis and controlled for a myriad of effects, including year and location. At the second step of modeling, weight on arrival (WT-A) was also controlled, as it had been identified previously as a risk factor and possible confounder (4). At the third step, 2-way interaction terms between all statistically significant viral variables were created and assessed for their significance to identify whether combined agent exposure produced enhanced effects over single agent exposures. Similarly, interaction terms between antibody titer change and having received a viral vaccine were also investigated. Because of a suspected interaction between IBRV and PIV-3 titers in vaccinated calves (group 44), the associations of titer with BRD were initially modeled separately for vaccinated and unvaccinated calves. At the final step, variables reflecting antibody titer change to Ph-L and Ph-A and a variable describing antibody titer change to Hs were added to the model to control confounding and allow synergism with the viral agents to be detected, as was done earlier by Van Donkersgoed et al (16).

Subsequently, the dose-response pattern of variables with a significant association with BRD was investigated by using hierarchical dummies for the 25th, 50th, and 75th percentiles (29). This served as a check on the linearity of BRD risk to changes in titer, and a significant dose-response relationship would add credence to causal inferences.

Multiagent models

This process was repeated again including titer on arrival and titer change variables for the 4 viruses (BRSV, BVDV, IBRV, PIV-3) and retaining those with a significant association with BRD occurrence. Then, data on BCV from a previous study (18) were added and, finally, data on Mdis and Mbov (this approach was

Table 1. Titers, on arrival (−A), and titer change between arrival and day 28 (−D) to viral and mycoplasmal agents in calves in feedlots in Ontario and Alberta that were not vaccinated against these agents

	First		Third	
Variable	Mean	Quartile	Median	Quartile
Calves not treated for bovine respiratory disease				
BCV-A	5.5	2	6	8
BCV-D	3.3	0	3	6
BRSV-A	3.3	2	3	4.5
BRSV-D	3.8	2.5	4	5.5
BVDV-A	1.4	0	0	2
BVDV-D	1.7	0	1.5	3
IBRV-A	0.1	0	0	0
IBRV-D	0.3	0	0	0
PIV-3-A	3.6	3	4	4
PIV-3-D	1.2	0	1	2
Mbov-A	1.1	0	1	2
Mbov-D	−0.1	−1	0	1
Mdis-A	7.7	6	8	9
Mdis-D	0.4	−2	1	2
Calves treated for bovine respiratory disease				
BCV-A	5.6	2	6	8
BCV-D	3.2	0	2	6
BRSV-A	3.1	1.5	3	4.3
BRSV-D	4.8	3.5	5	6
BVDV-A	1.0	0	0	1
BVDV-D	2.2	0	2	4
IBRV-A	0.0	0	0	0
IBRV-D	0.2	0	0	0
PIV-3-A	3.8	3	4	5
PIV-3-D	1.3	0	1	2
Mbov-A	1.3	0	1	2
Mbov-D	−0.2	−1	0	0
Mdis-A	6.8	5	7	9
Mdis-D	0.7	−1	0	3

BCV — Bovine corona virus; BRSV — Bovine respiratory syncytial virus; BVDV — Bovine viral diarrhea virus; IBRV — Infectious bovine rhinotracheitis virus; PIV-3 — Parainfluenza virus type 3; Mbov — *Mycoplasma bovis;* Mdis — *Mycoplasma dispar*

taken, because there were progressively fewer animals with titer data for these last 2 agents) were added to the models.

Weight gain

Least squares regression was used to model 28-day weight gain; the procedure was similar to that for BRD, except that weight on arrival (WT-A) was forced into all models. The effect of BRD on weight gain was also assessed.

For Data Set II, there were no serologic data on PIV-3, Hs, Mbov, or Mdis, and there were no weight data. Also, because these calves were all vaccinated and the viral titers were analyzed at a different laboratory, these data were analyzed separately by using the same general model building procedure as outlined above for Data Set I.

Results

The case-control sampling format used in this study does not lead directly to the determination of BRD incidence; however, most groups had in excess of 30% of calves treated for BRD. Calves from auctions had earlier postarrival treatments than did calves from ranches, and calves in feedlots in Ontario were treated approximately 2 d earlier than were calves in feedlots in Alberta (7 vs 9 median days to first treatment, respectively).

Table 2. Coefficients[a] of association between individual viral and mycoplasmal agent titers, on arrival (−A), and titer change between arrival and day 28 (−D), with bovine respiratory disease in calves in feedlots in Ontario and Alberta (Data Set I)

	Factors controlled			
Agent of interest	Group	Group Wt-A	Group Wt-A PhL-D PhA-D	Group Wt-A PhL-D PhA-D Hs-D
	$n = 616$	$n = 616$	$n = 609$	$n = 586$
BRSV-A	−0.18	−0.18[b]	−0.16[b]	−0.16[b]
BRSV-D	0.05[b]	0.06	0.05	0.04
BVDV-A	−0.18[b]	−0.15[b]	−0.13[b]	−0.11[b]
BVDV-D	0.11[b]	0.11[b]	0.12[b]	0.12[b]
IBRV-A	−0.18	−0.25	−0.13	−0.49
IBRV-D	0.14	0.03	0.04	0.05
PIV-3-A	0.06	0.07	0.08	0.07
PIV-3-D	0.05	0.06	0.06	0.06
	$n = 337$	$n = 337$	$n = 337$	$n = 337$
Mbov-A	0.11	0.11	0.10	0.10
Mbov-D	0.02	0.02	0.01	0.01
Mdis-A	−0.18[b]	−0.17[b]	−0.14[b]	−0.14[b]
Mdis-D	−0.02	−0.02	−0.02	−0.02

Group — Dummy variables used to enter and control for 'group' effects; Wt — Weight; PhL — *Pasteurella haemolytica* leukotoxin titer; PhA — *Pasteurella haemolytica;* agglutination titer; Hs — *Haemophilus somnus* titer.
[a]The logistic regression coefficients are from models with no interaction terms. The antilog of these coefficients is the odds ratio ($e^{-1.5} = 0.86$, meaning the risk of BRD is reduced by 0.86 times for each unit increase in titer)
[b]Significant at $P \leq 0.5$
See Table 1 for viral and mycoplasmal codes

In general, the BRSV titers on arrival were higher in calves housed in Alberta (Data Sets I and II, data not shown). The BVDV titers, on arrival, analyzed by the Alberta laboratory were significantly higher and less variable than the BVDV titers assessed by the Guelph laboratory (including titers for the calves housed in Alberta). The IBRV titers, on arrival, were very low in all instances but could not be directly compared because of marked differences in the tests used and the titer scales. Titers to PIV-3, on arrival, were higher in calves from western Canada (including western calves housed in Ontario).

In Data Set I, the group of calves (#44) that was vaccinated against IBRV and PIV-3 on arrival had no titer increases to IBRV and lower titer increases to PIV-3, after arrival, than did calves in other groups. The summary data on titers in unvaccinated calves, in Data Set I, are shown in Table 1. Based on titer increases, after arrival, there was evidence of recent infection with BCV, BRSV, and BVDV, but little activity for IBRV, PIV-3, Mbov, and Mdis. Calves with titers of greater than 2 were taken as positive for all viruses, and titers greater than 3 for mycoplasma were regarded as positive. Among calves not treated for BRD, the proportions of calves that were positive serologically on arrival were

Table 3. Coefficients of association[a], from logistic regression models, between 2 or more viral and mycoplasmal agent titers, on arrival (−A), and titer change between arrival and day 28 (−D), and bovine respiratory disease in calves in feedlots in Ontario and Alberta (Data Set I)

	Factors controlled					
Agents in model	Group Wt-A	Group Wt-A PhL-D PhA-D HS-D	Group Wt-A	Group Wt-A PhL-D PhA-D HS-D	Group Wt-A	Group Wt-A PhL-D PhA-D HS-D
	Base model		BCV data added		Mdis and Mbov data added	
BVDV-A	−0.15[b]	−0.12[b]	−0.14[b]	−0.12[b]	−0.12	−0.10
BVDV-D	0.10[b]	0.11[b]	0.11[b]	0.13[b]	0.70[b]	0.18[b]
BRSV-A	−0.17[b]	−0.15[b]	−0.12	−0.11	−0.18[b]	−0.19[b]
BRSV-D	0.06	0.05	0.11[b]	0.09	0.06	0.05
BCV-A	NA	NA	−0.19[b]	−0.18[b]	−0.07	−0.08
BCV-D	NA	NA	−0.07	−0.09	−0.04	−0.08
Mdis-A	NA	NA	NA	NA	−0.21[b]	−0.19[b]
Mdis-D	NA	NA	NA	NA	−0.07	−0.07

See Tables 1 and 2 for viral and mycoplasmal codes
[a]The antilog of these coefficients is the odds ratio ($e^{-1} = 1.14$, meaning the risk of BRD is increased by 1.14 times for each unit increase in titer difference)
[b]Significant at $P \leq 0.05$

BRSV, 60%; BVDV, 24%; IBRV, 0.8%; PIV-3, 87%; BCV, 83%; Mbov, 5%; and Mdis, 93%. Based on titer increases of at least 2 dilutions (4-fold titer increase), the seroconversion rates in unvaccinated calves were BRSV, 86%; BVDV, 50%; IBRV, 6%; PIV-3, 40%; BCV, 61%; Mbov, 14%; and Mdis, 35%.

In Data Set II, in which all calves were vaccinated against all viruses, except BCV, on arrival, among calves not treated for BRD, the proportions of calves that were positive for the virus(es) on arrival were BRSV, 100%; BVDV, 29%; and IBRV, 6%. Based on titer increases of at least 2 dilutions (or 4-fold increase), the seroconversion rates were BRSV, 53%; BVDV, 53%; and IBRV, 3%. The BRSV, BVDV, and IBRV titers increased by 28%, 73%, and 22%, respectively, over their arrival values by Days 28 to 35 (data not shown).

Uniagent associations with BRD

Data Set I

For IBRV and PIV-3, neither titer on arrival nor titer changes after arrival was associated with the risk of BRD, and there was no interaction of these titers with prior vaccination (in group 44 calves). Titer increase to BRSV in the first 28 d after arrival was associated with an elevated risk of BRD; after control of arrival weight and other factors (Table 2), higher BRSV titers on arrival were associated with a decreased risk of subsequent BRD, and titer change became nonsignificant. For BVDV, a higher titer on arrival consistently reduced the risk of subsequent BRD, and increases in BVDV titer in the first 28 d after arrival consistently were associated with an increased risk of BRD. For Mdis, based on samples from 337 calves, higher titers on arrival were associated with a decreased risk of subsequent BRD; titer change was not associated with BRD occurrence. Titers to Mbov were not associated with BRD. All the associations were relatively stable regardless of the other agent-variables included in the model (Table 2).

The uniagent analyses were repeated to study the dose-response associations between organism titer and BRD risk by using hierarchical dummies (see Table 1 for actual values). For BVDV titer on arrival, any titer (> 0) appeared to be protective; for titer change, BVDV titer increases beyond 3 dilutions were associated with an increased risk of BRD. With both variables in the model, both retained their significance. Calves with low BRSV titers on arrival, had no apparent protection against BRD, but calves with titers beyond 4.5 units had a decreased risk of BRD. For BRSV titer change, calves with titer increases beyond 2.5 units had an increased risk of BRD, and higher titer increases tended to be related to even higher risks of BRD. For Mdis titer on arrival, calves with mid level titers (8 units) had a decreased risk of BRD, but there was little indication of a linear dose-response relationship.

Data Set II

In the second set of calf groups (groups 71–87), meaningful titers to IBRV on arrival were present in only 6% of calves; subsequently, 86% of calves had no titer increase (only 3% seroconverted). For BVDV, the average titer on arrival was 2.2 units and the average increase to day 28 was 1.6 units. For BRSV, the average titer on arrival was 4.9 units and the average increase after arrival was 1 unit.

In Data Set II calves, only BVDV-A and BVDV-D titers were significantly related to BRD, both with coefficients of approximately b = 0.78 (odds ratio of 2.18). When the Ph-L and Ph-A titer change variables were added, BVDV titer on arrival and titer increase remained significant (odds ratios of 2.86 and 3.74, respectively). The BRSV and IBRV titers were not significant predictors of BRD. Viral vaccination of calves at the source farms, 3 wk prior to arrival, increased the titer change to IBRV after arrival; however, prior vaccination was not a significant predictor of (did not prevent) BRD and there

was no interaction between titer and prior vaccination. Vaccination at the farm had no significant impact on any of the other agent titers.

Multiagent associations with BRD

Data Set I

When 2 or more of the agents were placed in the same model, the relationships stayed similar to the uniagent results in most instances (Table 3). Translating the regression coefficients into odds ratios, each unit increase in titer to BVDV on arrival decreased the risk of subsequent BRD treatment by 0.9 times, each unit of titer increase after arrival was associated with an increased risk of BRD of about 1.14 times. Each unit increase in titer to BRSV on arrival reduced the risk of subsequent BRD by about 0.9 times (significant in 4 of 6 models). In the model with BCV, but not mycoplasma, each unit increase in BCV titer on arrival was associated with a decreased risk of BRD of approximately 0.8 times; BCV titer increases were not associated with BRD. Titers to BCV were not significant in the model containing mycoplasma; however, this model had data on the smallest number of calves, and the magnitude of the coefficients was also reduced considerably from the previous model. In general, however, the associations of BVDV and BRSV with BRD remained relatively stable in terms of magnitude, regardless of the model.

Data Set II

Titers to BVDV remained significant after control of Ph titer changes, but not with BRSV in the model. The BRSV titers were not significant by themselves but with BVDV variables in the model they became significant (BRSV-A odds ratio = 2.11; BRSV-D odds ratio = 1.54). Both BRSV titers became nonsignificant when Ph was controlled.

Weight data

Controlling for group and arrival weight, higher BRSV titers on arrival were associated with an increased weight gain, but this became nonsignificant when other factors were controlled (Table 4). Higher titer to BVDV on arrival was associated with increased weight gain, and a subsequent BVDV titer increase was associated with decreased weight gain; both became nonsignificant when BRD was controlled. Titers to IBRV on arrival were associated with increased weight gains until BRD was controlled. Titer increases to IBRV were associated with weight gain decreases. Titers to PIV-3 were not associated with weight gain. Titers for Mbov and Mdis on arrival were not associated with weight gain, but titer increases to these agents had a negative impact on weight gain.

With titer data on multiple agents present (Table 5), all significant associations of titer on arrival with weight gain were positive, and all significant associations of titer change with weight gain were negative. Basically the coefficients were relatively stable regardless of the other variables present in the model, except that controlling for BRD made the significant associations between BVDV and weight gain nonsignificant. Titers to BRSV were not associated with weight gain. Titer increases to IBRV, Mdis, and Mbov were consistently associated with lower weight gains, even when BRD was controlled (data not shown).

Table 4. Coefficients[a] of association, from least squares regression, of individual viral and mycoplasmal agent titers, on arrival (−A), and titer change, between arrival and Day 28 (−D)), with 28-day weight gain in calves in feedlots in Ontario and Alberta

	Factors controlled		
Agent of interest	Group Wt-A	Group Wt-A PhL-D PhA-D Hs-D	Group Wt-A PhL-D PhA-D Hs-D BRD
BRSV-A	0.68[b]	0.54	0.32
BRSV-D	−0.09	−0.06	0.03
BVDV-A	0.69[c]	0.52[b]	0.39
BVDV-D	−0.47[b]	−0.48[b]	−0.31
IBRV-A	3.09[b]	2.53[b]	2.38
IBRV-D[1]	−0.83[b]	−0.90[c]	−0.82[b]
PIV-3-A	0.08	0.09	0.19
PIV-3-D	0.07	−0.08	−0.01
Mbov-A	−0.5	−0.44	−0.34
Mbov-D	−0.91[b]	0.90[b]	−0.89[c]
Mdis-A	0.13	−0.06	−0.18
Mdis-D	−1.15[b]	−1.02[b]	−1.08[c]

BRD — Bovine respiratory disease
[a]Coefficient gives the change in weight gain (kg) for each unit change in titer or titer difference
[b]Significant at $P \leq 0.10$
[c]Significant at $P \leq 0.05$
See Tables 1 and 2 for viral, mycoplasmal, and weight codes

Discussion

There is much in the literature to suggest that exposure to the viral agents associated with BRD occurs widely and within a short time period during transportation and in the first few weeks after arrival in the feedlot. Twenty-nine percent of calves on ranches in Colorado had titers to BRSV, 36% had been exposed prior to feedlot entry, and 59% seroconverted while in the feedlot (30). Titers to PIV-3 occurred in 34% of feedlot cattle and BVDV titers were present in 22% of feedlot cattle (30). With the exception of IBRV and *M. bovis*, exposure to the agents studied here was very common in these feedlot calves. For the other agents, if titers were low on arrival, the level of seroconversion after arrival increased the prevalence of antibodies at 28 d postarrival to very high levels.

Despite the importance of studying the impact of these infectious agents under field conditions, the feedlot environment makes it very difficult to draw meaningful valid inferences. The key features relating to the difficulties of interpreting the effects of arrival titer (or titer changes) include: 1) antibodies may be merely markers for exposure to an agent, not a signal of protection from the effects of exposure (for simplicity, we further assume that all exposed and no unexposed animals respond by producing antibodies and that we have a test that has a sensitivity and specificity both equal to 100%); 2) the magnitude of any antibody response depends on the challenge dose (including natural

Table 5. Least squares regression coefficients[a] between 2 or more viral and mycoplasmal agent titers (on arrival (−A) and titer change between arrival and Day 28 (−D)) and 28-day weight gain in calves in feedlots in Ontario and Alberta

Factors controlled								
Agents in model	Group Wt-A	Group Wt-A PhL-D PhA-D Hs-D	Group Wt-A	Group Wt-A PhL-D PhA-D Hs-D	Group Wt-A	Group Wt-A PhL-D PhA-D Hs-D	Group Wt-A	Group Wt-A PhL-D PhA-D Hs-D
	Base Model		BCV data added		IBRV data added		Mdis and Mbov data added	
BVDV-A	0.70[b]	0.53[b]	0.68[b]	0.56[b]	0.70[c]	0.58[c]	0.39	0.25
BVDV-D	−0.44	−0.45[c]	−0.36[c]	−0.42	−0.34	−0.39	−0.57[c]	−0.58[c]
BRSV-A	0.63	0.51[c]	0	0.04	0.05	0.09	0.37	0.47
BRSV-D	−0.08	−0.05	−0.52[c]	−0.52	−0.49[c]	−0.38	−0.14	0
BCV-A	NA	NA	1.39[b]	1.37[b]	1.41[c]	1.39[b]	1.09[b]	1.17[b]
BCV-D	NA	NA	0.24	0.31	0.25	0.32	0.02	0.23
IBRV-A	NA	NA	NA	NA	2.45[c]	2.19	2.40	2.02
IBRV-D	NA	NA	NA	NA	−1.04[b]	−1.05[b]	−1.09	−1.13[b]
Mbov-A	NA	NA	NA	NA	NA	NA	−0.02	0.02
Mbov-D	NA	NA	NA	NA	NA	NA	−0.64[c]	−0.68[c]
Mdis-A	NA	NA	NA	NA	NA	NA	−0.15	−0.33
Mdis-D	NA	NA	NA	NA	NA	NA	−0.85[b]	−0.79[b]

NA — Not available
[a]Coefficient gives the change in weight gain (kg) for each unit change in titer or titer difference
[b]Significant at $P \leq 0.05$
[c]Significant at $P \leq 0.10$
See Tables 1 and 2 for viral and mycoplasmal codes

challenge vs vaccination), time since challenge, and the physiological state (including the immunity level) of the calf when exposed; 3) the short period of risk for BRD relative to the time taken for antibody production (a detectable antibody response may take a few days to a week or more, and in many instances the highest risk period for BRD may be included in this "nondetectable response" time frame); and 4) the antibody response to multiple agents is moderately to highly correlated.

Some of these difficulties were documented earlier by other workers (16); BRSV vaccinated calves had higher titers by Day 40 after arrival than did nonvaccinated calves, but during the peak BRD risk period (between arrival and Day 21) calves not treated for BRD had (or tended to have) higher titers than treated calves. By Day 40, the calves with BRD had the highest titers. Thus, at any point in time, it is very difficult to differentiate a titer (or titer change) as a signal of being protective (a cause of "nontreatment") or as a signal of an effect (a result of experiencing BRD caused by that agent).

Given the presumed causal model that viral and mycoplasmal agents are precursors of bacterial infection in many cases of BRD, inclusion of data on the bacterial titer status of calves can drastically alter the apparent effect of the viral and mycoplasmal agents. In this setting, the bacterial titers are intervening variables between the viral and mycoplasmal agents and BRD. To the extent that the associations of the viral and mycoplasmal agents with BRD did not change with the addition of the Ph and Hs variables, the inference drawn is that these agents are directly related to BRD (all coefficients decreased slightly in magnitude, but all significant variables remained significant after the bacterial variables were added; Table 2) and not merely precursors for bacterial pathogens of BRD. That some of the viral and mycoplasmal titer associations with weight gain became nonsignificant when BRD occurrence was added to the model (specifically BVDV and IBRV; Table 4) indicates that the effects of the agents on weight gain are largely mediated through BRD.

In large part, because of the vaccination practices conducted on the calves in Data Set II, only the data on titers on arrival are comparable with the data in Data Set I. Thus the discussion of titer change will focus on the 19 groups of calves in Data Set I, unless otherwise noted. Interestingly, vaccination of the one group of calves on arrival (group 44 in Data Set I) seemed to reduce the titer increases after arrival, but had no significant impact on the risk of BRD (although it could be argued that calves in this group would have had a different level of BRD without the vaccination). In Data Set II, vaccination of calves in 3 groups at the farm of origin 3 wk prior to arrival had little impact on titers on arrival, and except for IBRV, did not influence the titer increase thereafter. In Data Set II, there were larger titer increases to IBRV in vaccinated calves, which is contrary to the effects in group 44, so overall conclusions about the effect of prior vaccination on titer changes are difficult to make. Nonetheless, vaccination at the farm of origin was not related to the risk of BRD. Other studies have supported the use of vaccines against most of these agents (excluding BCV), based on either titer response alone (31) or superior protection in vaccinated vs unvaccinated calves (14,32). However, the results of this latter trial (14) are suspect, because it was based on group allocation of vaccination and housing, but analyzed (incorrectly) at the individual level. In most instances, the calves used in experimental studies are seronegative for the agents at the start of the experiment,

which makes valid comparison to titer changes after arrival at a feedlot, where many calves are already positive, difficult to make. Titer increases after arrival are invariably negatively related to titer on arrival.

In Data Set I, titers to BRSV, BVDV, BCV, and Mdis on arrival were protective against subsequent BRD in virtually all models. In addition, seroconversion to BVDV was consistently associated with the occurrence of BRD. Thus, we speculate that increasing the titers to these 4 agents on arrival, perhaps through a complete vaccination schedule, would reduce the subsequent level of BRD. Of the 4 agents, BVDV might be the most important to control, as it was the only agent in which seroconversion was consistently associated with BRD. The observations on high BRSV titers on arrival being protective and seroconversions to BVDV being harmful were reported in our previous studies (3,4).

Similar to our findings with respect to BRD, a relatively high titer to all viruses on arrival, except PIV-3 and perhaps BRSV, was associated with increased weight gains over the first 28-day period. Seroconversion to all of the agents, except PIV-3, was associated with decreased weight gains. Previous reports have indicated that higher titers to BCV on arrival were associated with higher 28-day weight gains (18). Thus, efforts to protect calves against these organisms should be continued, although the frequency of IBRV and Mbov exposures are so low that they are likely unimportant during this period.

In Data Set II, because of the manner in which the viral vaccines were administered, we cannot assess their impact on BRD. Certainly, there can be a wide variation in response of calves to vaccination with different components, albeit with the same agent (31,33). In another study (9), vaccination prior to arrival increased titers to IBRV and PIV-3, and protected calves against BRD, but titer increases and protection were not seen for BVDV. Vaccination on arrival against BRSV did not affect BRD risk in this study (9). It is interesting, however, that the coefficients describing the relationships between antibody titer and BRD or weight gain in the current study were not biased by the fact of vaccination, nor was the relationship of titer with BRD altered in magnitude by prior vaccination of calves (no significant interactions). One might have expected a titer increase in unvaccinated calves to have a different strength or direction of association with BRD or weight gain than that observed in vaccinated calves, although a high titer on arrival seems to be protective regardless of whether it arises from a natural infection or from vaccination. Overall, the effects of vaccination on titer change and protection remain unclear. Titers to IBRV and BRSV increased very little (relative increases of 28% and 22%, respectively), whereas larger increases to BVDV (73%) were observed. In Data Set I, in unvaccinated cattle, these percentages were 300%, 121%, and 60%, respectively.

The results for BVDV-A and BRSV-A in Data Set II differed from those in Data Set I in that higher titers, on arrival, signaled an increased risk of disease. The fact that these calves were vaccinated against these agents at arrival should not have biased the arrival titer associations with BRD. No obvious explanations for these discordances are forthcoming. The associations involving titer increase were similar to those in Data Set I.

Overall, our findings are supportive of the thesis that BCV, BVDV, BRSV, and perhaps Mdis, can play a role in causing BRD (34). Ensuring that the case definition of BRD is valid is very difficult; however, any misclassification of BRD would reduce the significance (increase P values) and strength (odds ratios) of our findings. The 28-day weight gain data are viewed as a method of validating the findings with respect to BRD, as well as being an independent evaluation of the potential impact of the agents on the general health status of feedlot calves. Based on our data, we suggest that IBRV and Mbov are not widespread pathogens, at least in the early postarrival period, and that PIV-3, although widespread, is not a major pathogen in terms of BRD. However, because increasing titers after arrival were associated with decreased weight gain, it would be useful to continue trying to provide adequate protection on arrival to all agents, except perhaps PIV-3.

One suggestion for clarifying these issues is to ensure that in future clinical trials of vaccines using individuals as the experimental unit, serologic studies be conducted, wherein calves are sampled every 2 wk for the first 4 wk in the feedlot. One would wish to examine the serologic responses in those that were treated for BRD compared with those that were not, while examining for interaction between titer and BRD depending on the vaccine status. More specifically, the occurrence and timing of vaccination relative to arrival, the arrival titer, and the occurrence (and timing relative to arrival) of BRD, all need to be considered in a multifactorial setting and analytic model.

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