

The Association Between Serological Titers in Infectious Bovine Rhinotracheitis Virus, Bovine Virus Diarrhea Virus, Parainfluenza-3 Virus, Respiratory Syncytial Virus and Treatment for Respiratory Disease in Ontario Feedlot Calves

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ABSTRACT

A seroepidemiological study of the association between antibody titers to infectious bovine rhinotracheitis, parainfluenza-3, bovine virus diarrhea and bovine respiratory syncytial viruses, and treatment for bovine respiratory disease was conducted. A total of 322 calves from five different groups were bled on arrival, then one month later all cases (cattle treated for bovine respiratory disease) were rebled together with an equal number of controls (cattle not treated for any disease).

Titers to these viruses varied significantly from group to group. Based on seroconversion, infectious bovine rhinotracheitis virus was active in 4.4%, bovine virus diarrhea virus in 24%, parainfluenza-3 virus in 69.5% and bovine respiratory syncytial virus in 71.3% of the cattle. Cattle with low titers to infectious bovine rhinotracheitis and/or bovine respiratory syncytial viruses on arrival, were at increased risk of subsequent treatment for bovine respiratory disease. Treated cattle also had significantly greater increases to parainfluenza-3 and/or bovine virus diarrhea viruses than control calves. Treatment rates varied considerably from group to group and were not strongly correlated with weight gain in the postarrival period.

Key words: Serological, epidemiological, antibody titer, association, infectious bovine rhinotracheitis,

parainfluenza-3, bovine virus diarrhea, bovine respiratory syncytial, respiratory disease, cattle, bovine, weight gain.

RÉSUMÉ

Cette étude épizootiologique visait à déterminer la relation entre les titres d'anticorps contre les virus bovins suivants : rhino-trachéite infectieuse, parainfluenza-3, diarrhée à virus et respiratoire syncytial, et la nécessité d'un traitement pour des problèmes respiratoires. On préleva à cette fin un échantillon de sang, chez 322 veaux répartis en cinq groupes, à leur arrivée dans des parcs d'engraissement; un mois plus tard, on préleva un autre échantillon de sang chez tous ceux qui avaient reçu un traitement pour des problèmes respiratoires et chez un nombre égal de témoins.

Les titres d'anticorps contre les virus précités variaient significativement, chez les sujets des divers groupes. En se basant sur la séroconversion, on constata que le virus de la rhino-trachéite infectieuse était actif, chez 4,4% des veaux; celui de la diarrhée à virus, chez 24%; celui du parainfluenza-3, chez 69,5%; le virus respiratoire syncytial, chez 71,3%. Les veaux qui possédaient un faible titre d'anticorps contre le virus de la rhino-trachéite infectieuse et/ou celui de la diarrhée à virus, à leur arrivée dans les parcs d'engraissement, couraient un plus grand risque de nécessiter un

traitement pour des problèmes respiratoires. Les veaux qui reçurent un tel traitement affichèrent une plus grande élévation de leur titre d'anticorps contre le virus parainfluenza-3 et/ou contre celui de la diarrhée à virus, comparativement aux témoins. Le nombre de traitements varia beaucoup d'un groupe à l'autre et il ne s'avéra pas relié au gain de poids ultérieur à l'arrivée des veaux dans les parcs d'engraissement.

Mots clés : sérologie, épizootiologie, titre d'anticorps, rhino-trachéite infectieuse bovine, parainfluenza-3, diarrhée à virus bovine, virus respiratoire syncytial bovin, maladies respiratoires, bovins, gain de poids.

INTRODUCTION

Disease(s) of the respiratory tract is the most frequent and costly health problem facing the feedlot industry. The most common respiratory disease is pasteurellosis, however, because of the difficulty in differentiating the various disease entities under field conditions, the general syndrome often is called shipping fever, or bovine respiratory disease (BRD). Under field conditions, a number of factors including stress, management, and specific agents appear to form a causal web for BRD (1). Recently, Yates (2) in reviewing the vast literature on BRD stated that "It has

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been suspected for many years that synergism between viruses and pasteurallae is an important part of the etiology of bovine respiratory disease". He then reviewed the available evidence for this statement, emphasizing parainfluenza-3 (PI₃) and bovine herpesvirus 1 (i.e. infectious bovine rhinotracheitis, IBR) viruses. Both of these agents have been demonstrated, experimentally, to produce synergy with pasteurallae (3,4). The possible role of bovine virus diarrhea (BVD) and bovine respiratory syncytial (BRS) viruses was also described (2).

Most studies have focused on only one or two viruses as possible causes of BRD. In the late 1960s, workers observed randomly selected cattle after their transportation from western Canada to Ontario and contrasted the characteristics, and agents, of cattle that became ill or died from BRD to those of cattle that remained healthy (5). A higher percentage of the healthy animals were positive (hemagglutination inhibition titer of 1/10 or more) for PI₃ on arrival (50% versus 30% respectively). The titers of both groups increased over the first month of observation, suggesting ongoing or active infection. Titers to PI₃ were significantly higher in calves remaining well than in sick calves, in two of the five groups; in two other groups the differences in PI₃ titers between sick and well calves varied with time, and there was no overall association with health status in these groups. In total, the percentage of calves with titers, of 1/10 or more, in well calves exceeded the percentage in sick calves. The same general findings were noted for IBR with lower prevalences on arrival and throughout the first 28 day period. On arrival, 6% of those becoming sick and 10% of those remaining well had titers to IBR, but these differences were not significant. Since, the average level of antibody to PI₃ did not differ between a relatively healthy and a highly diseased group, the authors concluded that PI₃ did not have an obvious pathogenic role in causing BRD. Infectious bovine rhinotracheitis apparently was deemed not an important factor. In subsequent studies of the same nature (6), serum antibody to PI₃ did not differ on arrival between animals that did and did not develop BRD. At

seven days, sick animals had a lower titer than well animals. There did not appear to be much active infection with PI₃ after arrival.

Other researchers have studied the role of viruses in natural outbreaks. In two studies (7,8), PI₃, IBR and BVD viruses were present in cattle with BRD, and the authors equated presence of an organism with a putative causal role. However, since the authors only reported findings on diseased animals, making inferences about the role of these agents is difficult. The need for data on "healthy" comparison (control) calves, to allow the establishment of a valid statistical association as one criterion of causation has been recognized (1,9). In one study of the possible role of BVD virus, it was noted that 90% of the treated calves and 31% of the untreated calves seroconverted to BVD after arrival at the feedlot. This established a valid association between BVD and BRD at least in that outbreak (10). In another well designed case-control study (11), the absence of BRS virus antibodies on arrival and seroconversion to BRS virus after arrival were associated with an increased risk of respiratory disease in unweaned calves in England.

This project was designed to investigate the association between serological titers to IBR, BVD, PI₃

and BRS viruses and treatment for respiratory disease in Ontario feedlot calves.

MATERIALS AND METHODS

STUDY DESIGN

The design of this study was to blood sample calves as soon as possible after arrival, to rebleed all calves that developed respiratory disease (cases), at approximately 28 days postarrival and to rebleed an equal number of untreated (control) calves at this time. Thus, most analyses are based on a case-control study design; however, since the sampling fraction of cases and controls in each group of calves was known, this feature was used to derive estimates of prevalence and incidence rates. The practical advantage of this study design was the reduced number of animals to be bled; other advantages of this design have been described (12). The control calves were further subdivided into those housed in pens with cases and those not housed in pens with cases.

Five groups of calves were included in the study, groups 1 to 3 were housed at the Elora Research Station and groups 4 and 5 were housed at the Ridgetown Agricultural College (Table I). The groups were differen-

TABLE I. The Number of Cattle by Group and their Subsequent Health Status, in a Study of Bovine Respiratory Disease, in Ontario Feedlot Cattle

	Group No.					Total
	1	2	3	4	5	
No. alive at arrival	94	89	25	76	38	322
No. treated at or before sampling	5	8	0	27	3	43
No. sera available from above cases	5	8	0	0	0	13
No. treated after sampling and ≤ 5 weeks	8	22	7	22	4	63
No. sera available from above cases	7	22	7	15	4	55
No. control sera	30	15	4	22	27	98
Total % treated for respiratory disease	13.8%	33.7%	28.0%	64.4%	18.4%	34.0%
Weight gain ^a cases	13.2	11.3	11.5	3.8	7.6	58
No. (cases)	10	22	7	15	4	
Weight gain ^a controls	13.9	8.7	11.5	6.8	7.7	85
No. (controls)	28	15	3	22	27	

^aKg of gain in first 28 days

tiated on the basis that they arrived at the feedlots on different days and/or were from different sources in Western Canada. Group 1 arrived November 1, 1983 and consisted of 32 heifers and 62 steers. These cattle were initially bled on November 3. Group 2 arrived November 5 and consisted of 41 heifers and 48 steers. These cattle were initially bled November 7. Group 3 arrived November 9 and consisted of 24 steers and one heifer. These cattle were bled November 10. Groups 1 and 2 arrived in "good condition", whereas group 3 cattle were in "poor condition", two animals were dead-on-arrival. The midpoints (median) of the respiratory disease outbreaks in these groups were four, five and two days postarrival, respectively. By utilizing ear-tag identification and/or brands, it was possible to traceback 79 group 1, 31 group 2 and 18 group 3 calves to their farms of origin. None had been vaccinated against respiratory disease.

Group 4 arrived at Ridgetown on November 18. This group was in "poor condition" on arrival and consisted of 76 steers. Group 5 arrived in "good condition" on November 22 and consisted of 38 cattle. The midpoints of the respiratory disease outbreaks in these groups were four and two days postarrival, respectively. Both groups were initially bled November 23. All cattle were traced to three farms of origin, none were vaccinated against respiratory disease.

At Elora, the calves were housed in two barns, usually in groups of four per pen. Usually, cattle from the same group were housed together. At Ridgetown, the calves were housed in one barn in groups of six per pen. The latter calves had a large size-weight range and were sorted into homogeneous groups by weight. Hence, calves from different groups frequently shared the same pen.

At Elora, the heifers were merged and subsequently rebled on December 7, the steers were merged and subsequently rebled on December 14. The Ridgetown cattle were rebled on December 22, 1983.

For purposes of this study, all cattle treated for respiratory disease, at Elora, were classified as cases. At Ridgetown, only cattle first treated after the initial sampling date were included as cases. This difference was

due to the fact that, at Elora, blood samples were obtained no more than 24 hours after treatment for the 13 cattle (Table I) requiring treatment shortly after arrival; at Ridgetown, most of the 30 cattle treated before blood sampling had been treated two to four days prior to blood sampling. Only untreated (for any disease) cattle were included in the control group. Whether or not a particular calf required treatment for respiratory disease was decided by the staff at these two locations, both managers having worked with feedlot cattle for many years. Veterinary assistance was requested for unusual or difficult cases only. Respiratory disease accounted for over 90% of all cattle requiring treatment in the first four to five weeks postarrival.

SEROLOGICAL METHODS

At the initial sampling, 60 mL of blood were obtained from the jugular vein. Forty mL of blood were obtained at the second sampling. The blood was allowed to clot at room temperature over night, and the sera were then separated and frozen at -20°C until analyzed. All sera were analyzed blindly; that is, the laboratory did not know the identity of cases or controls, nor which two sera constituted a pair.

Sera were thawed, inactivated, clarified at 3000 rpm for 30 minutes and tested in microtiter systems by serum neutralization (IBR, BVD, BRS) or by inhibition of hemagglutination (PI_3).

A standard serum neutralization (SN) test was used for the detection of antibodies to IBR, BVD and BRS viruses; a modified P37/24 method (13) was applied, supplemented by 5% guinea pig complement to detect complement-dependent IBR virus antibodies (14).

Challenge viruses used in the SN tests were: Colorado strain (IBR), NADL strain (BVD) and Q-1 strain (BRS). The challenge dose was 100 TCID_{50} of each virus.

Tests were performed on cell cultures prepared from bovine fetal organs, kidney for IBR, spleen for BVD, and used in second up to fifth subgeneration. A continuous line of bovine fetal lung cells was used for BRS virus testing.

All cultures were pretested for the

absence of noncytopathogenic BVD virus. Cells were grown in roller bottles under minimum essential medium (MEM) with Earle's salts and 10% bovine fetal calf serum (FCS). Cell suspensions were prepared by trypsin-EDTA and cells resuspended at a split ratio (i.e. 1:1) in MEM plus 20% FCS.

The total volume of reactants in each well was $150/\mu\text{L}$ composed of $50/\mu\text{L}$ of each of serum dilution, virus and cell suspension. Serial dilutions of serum (twofold for IBR, threefold for BVD and BRS) were mixed with the challenge dose of virus and incubated for one hour at room temperature (for BVD and BRS) or for 24 hours at 37°C in a CO_2 incubator (for IBR). After an incubation period for neutralization, the cell suspension was added and microplates were reincubated for an additional three days (IBR) or seven days (BVD, BRS); at that time the results were recorded.

A microtest hemagglutination inhibition test was conducted for PI_3 virus inhibiting antibodies (15). Heated sera were pretreated with kaolin to remove nonspecific inhibitors and diluted phosphate buffered saline (PBS) in twofold series from 1:10-1:640. Four hemagglutinating units of shipping fever strain PI_3 virus ($25/\mu\text{L}$) were mixed with diluted sera ($25/\mu\text{L}$) and incubated for one hour at room temperature. Then, 0.4% washed bovine red blood cells in PBS were added ($50 \mu\text{L}$) and the plates reincubated for one to two hours at room temperature. The test was evaluated after reincubation and again after staying over-night in the refrigerator.

STATISTICAL METHODS

The serological titers to IBR were coded as 0, 1, 2, 3, . . . for endpoint titers of 1, 1/2, 1/4, 1/8 etc. The serological titers to BVD and BRS virus were coded as 0, 1, 2, 3 . . . for endpoint titers of 0, 1/3, 1/9, 1/27 etc, and the titers to PI_3 were coded as 0, 1, 2, 3 for titers of 0, 1/10, 1/20, 1/40 respectively. Any titer above zero was assumed to represent exposure to the specific virus. As mentioned, because the sampling fraction of cases and controls within each group of cattle was known, it was possible to calculate estimates of the prevalence rate of antibodies at arrival (i.e. at the initial

TABLE IIa. The Distribution of Initial Titers and of Seroconversion (SC)^a to IBR and BVD Viruses in Treated (Cases) and Untreated (Controls), Ontario Feedlot Calves 1983

Titer ^b	IBR				Titer	BVD			
	Cases		Controls			Cases		Controls	
	Initial Sample	SC	Initial Sample	SC		Initial Sample	SC	Initial Sample	SC
0 (0)	58	5	75	2	0 (0)	29	12	36	7
1 (2)	3	0	11	0	1 (3)	15	4	21	12
2 (4)	1	0	5	0	2 (9)	15	2	23	1
3 (8)	0	0	1	0	3 (27)	4	0	10	0
4 (16)	1	0	1	0	4 (81)	0	0	5	0
5 (32)	0	0	0	0	5 (243)	0	0	0	0
6 (64)	0	0	1	0					
7 (128)	0	0	1	0					
No. Animals	63		95			63		95	

^aA fourfold or greater increase in titer

^bCoded and () reciprocal of end-point titer

^cCases were animals requiring treatment for respiratory disease; controls were randomly selected from animals not receiving any treatment for disease

sampling), and the incidence rate of infection during the first four to five week period. The prevalence rate was based on the proportion of animals with an antibody titer greater than zero on arrival. The incidence rate was based on the proportion of animals with initial titers of zero that developed titers during the first four to five week period after arrival.

The association between titers to the above viruses and respiratory disease were investigated using two-way analysis of variance; treatment effects were analyzed on a within-group basis (16). The association between titers and weight gain, during the first four weeks, on a within group basis, was evaluated using stepwise multiple regression (16).

The association between initial titer, and between seroconversion, and respiratory disease were investigated using odds ratio techniques. Group effects were controlled using the Mantel-Haenszel procedure, or the log odds ratio method (12).

RESULTS

Serology was completed on between 153 and 163 of the 166 samples, depending on the virus assay. The distribution of antibody titers on arrival, and the number of calves seroconverting after arrival are shown in Tables IIa and IIb. In general, both the prevalence rate and incidence rate of infection with IBR virus was low. On arrival, the prevalence rate of antibodies (titers > 0) to IBR virus was 18.3%. Seven (12%) of 58 treated calves, and six (8%) of 75 untreated calves had titers to IBR on the second, but not the initial sample. The overall incidence rate of infection with IBR virus was estimated to be approximately 10%, but this varied from 0% in groups 4 and 5 to over 50% in group 3. All seven seroconversions occurred in cattle with no initial titer. On arrival, the prevalence rate of antibodies to BVD virus was 55.8%. Thirteen (45%) of 29 treated calves, and eight (22%) of 36 untreated calves had titers to BVD on the second, but not on the initial sample. About one-third of the cattle with titers of less than nine seroconverted to BVD virus. The overall incidence rate of infection with BVD

TABLE IIb. The Distribution of Initial Titers and Seroconversion (SC)^a to PI₃ and BRS Viruses in Treated (Cases) and Untreated (Controls), Ontario Feedlot Calves 1983

Titer ^b	PI ₃				Titer	BRS			
	Cases		Controls			Cases		Controls	
	Initial Sample	SC	Initial Sample	SC		Initial Sample	SC	Initial Sample	SC
0 (0)	44	44	46	45	0 (0)	32	28	30	29
1 (10)	10	7	19	10	1 (3)	25	19	47	35
2 (20)	3	1	7	1	2 (9)	3	1	11	8
3 (40)	1	0	7	0	3 (27)	2	0	2	1
4 (80)	1	0	4	1	4 (81)	0	0	2	0
5 (160)	3	0	4	0	5 (283)	1	0	2	0
6 (320)	0	0	5	0	6 (849)	0	0	0	0
7 (640)	0	0	3	0					
No. Animals	62		95			63		94	

^aA fourfold or greater increase in titer

^bCoded and () reciprocal of end-point titer

^cCases were animals requiring treatment for respiratory disease; controls were randomly selected from animals not receiving any treatment for disease

virus was estimated to be 19.1%, with most of the new infections in groups 2 and 4. On arrival, the prevalence rate of antibodies to PI₃ virus was 39.1%. All of the calves with no titer to PI₃ virus on arrival had detectable titers on the second sampling, giving an incidence rate of 100%; in fact, all but one of these calves seroconverted. On arrival, the prevalence rate of antibodies to BRS virus was 62.1%. Thirty of 94 control calves had no titer on arrival, all had titers of one or more on the second sample (29 seroconverted). Thirty-two of 63 treated calves had no titer on arrival, all had titers of one or more on the second sample (28 seroconverted). Based on the titer increases, the estimated incidence rate of infection was 100%.

Seroconversion (a fourfold titer increase) was taken as indicative of active infection and on this basis, the active infection rates were 4.4%, 24%, 69.5% and 71.3% for IBR, BVD, PI₃ and BRS viruses respectively (Table III). All of the active infection with IBR was localized to cattle at the Elora station, particularly group 3. Most of the BVD infection activity occurred in group 4 cattle at the Ridgetown station. Active infection with both PI₃ and BRS virus was widespread in all groups, with the exception of a lower PI₃ virus activity in group 5 cattle and a low BRS virus activity in group 3 cattle. On a group level, there was little association between active infection rates and the percentage of cattle receiving treatment for BRD.

The serum titers to IBR, BVD, PI₃ and BRS virus are shown in Tables IVa and IVb. Differences between cattle groups in first and second titers, and titer changes, were significant except for IBR titer change and initial BVD titers. After removing, analytically, these group to group differences, treated calves had significantly lower initial IBR titers, significantly higher second IBR titers and significantly greater IBR titer changes than nontreated calves. The titer changes to IBR were not consistent across groups (*p* of interaction = 0.02). There was no significant difference in the first or second BVD titers between treated and untreated calves, although there were significantly greater titer increases (or smaller decreases) to BVD between treated and untreated

TABLE III. The Active Infection Rates^a, with IBR, BVD, PI₃ and BRS Virus in Five Groups of Ontario Feedlot Calves

Group	IBR	BVD	PI ₃	BRS	Treatment Rate ^b
1	4.0%	1.2%	56.3%	68.8%	13.8%
2	3.4%	23.3%	79.1%	68.9%	33.7%
3	30.0%	30.0%	52.0%	22.0%	28.0%
4	0.0%	52.1%	84.0%	80.9%	64.4%
5	0.0%	6.4%	34.9%	91.1%	18.4%
Average	4.4%	24.0%	69.5%	71.3%	34.0%

^aBased on seroconversion, i.e. > fourfold increase in titer

^bTotal % treated for respiratory disease

calves. There was no significant difference in the first or second PI₃ titers between treated and untreated calves, however treated calves had a significantly greater (*p* = 0.06) titer increase, to PI₃, than untreated calves. Calves that were subsequently treated had significantly lower BRS virus titers on arrival than nontreated calves, their second sample titers did not differ. Titer changes were usually marginally, but nonsignificantly, higher in treated than nontreated calves.

The association between the existence of an initial titer and subsequent treatment is shown in Table V. Based

on the crude odds ratio, calves with no titer to IBR, PI₃ or BRS virus on arrival had a significantly increased risk of subsequent treatment of 3.1, 2.6 and 2.4 times respectively. However, most of the association between PI₃ and BRS virus titers and treatment disappeared when the effect of group differences was removed using the Mantel-Haenszel procedure. (The large group to group variation in titers was noted previously, Table IVa, IVb.) Contrasting initial titers of 0 or 1 versus 2 or more reduced the strength of association and level of significance for all four viruses. Not having a titer to BVD on arrival did not appear to

TABLE IVa. Average Serological Titers to IBR and BVD Viruses in Ontario Feedlot Calves Classified by Treatment for Undifferentiated Bovine Respiratory Disease

Group	Case	IBR			BVD		
		First Sample	Second Sample	Titer Change	First Sample	Second Sample	Titer Change
1	Yes	0.33	1.00	0.64	1.17	0.73	-0.36
	No	0.72	0.47	-0.24	1.21	0.17	-1.03
2	Yes	0.07	0.35	0.29	0.72	1.10	0.29
	No	0.20	0.20	0.00	0.73	1.33	0.60
3	Yes	0.71	3.29	2.57	1.14	2.00	0.86
	No	2.25	1.50	-0.75	1.00	1.00	0.00
4	Yes	0.20	0.07	-0.13	1.00	2.73	1.73
	No	0.27	0.05	-0.23	1.59	3.14	1.55
5	Yes	0.00	0.00	0.00	0.50	2.00	1.50
	No	0.08	0.00	-0.08	1.31	0.74	-0.65
Treatment ^a difference		-0.44	0.50	0.93	-0.26	0.44	0.70
Analysis of Variance Results (Observed <i>p</i> levels)							
Source of variation							
Group		0.00	0.00	0.30	0.20	0.00	0.00
Treatment within group		0.03	0.05	0.00	0.20	0.10	0.03
Interaction ^b		0.30	0.30	0.02	0.60	0.10	0.20

^aAverage case titer minus average control titer

^bIf significant, the treatment difference was not constant across groups

TABLE IVb. Average Serological Titers to PI₃ and BRS Viruses in Feedlot Calves Classified by Treatment for Undifferentiated Bovine Respiratory Disease

Group	Case	PI ₃			BRS		
		First Sample	Second Sample	Titer Change	First Sample	Second Sample	Titer Change
1	Yes	0.64	3.09	2.70	0.58	3.11	2.56
	No	0.69	2.77	2.00	0.93	2.90	2.03
2	Yes	0.21	4.45	4.32	0.17	2.76	2.64
	No	0.40	4.13	3.73	0.40	2.79	2.36
3	Yes	1.14	3.14	2.00	1.71	2.50	0.50
	No	1.75	3.75	2.00	2.50	3.25	0.75
4	Yes	0.27	4.47	4.20	1.19	3.81	2.63
	No	1.32	3.55	2.23	1.38	3.91	2.52
5	Yes	3.75	5.25	1.50	0.25	3.50	3.25
	No	3.08	3.71	0.58	0.81	3.71	2.92
Treatment ^a difference		-0.25	0.50	0.84	-0.42	-0.18	0.20

Source of variation	Analysis of Variance Results (Observed p levels)					
Group	0.00	0.00	0.00	0.00	0.00	0.00
Treatment within group	0.40	0.10	0.06	0.03	0.40	0.40
Interaction ^b	0.40	0.40	0.60	0.80	0.70	0.90

^aAverage case titer minus average control titer

^bIf significant, the treatment difference was not constant across groups

alter the risk of subsequent treatment.

Overall, seroconversion, to these four viruses, was not associated with treatment in these calves (Table VI). For IBR, the odds ratio (adjusted for group to group differences) reflecting the risk of treatment in calves which seroconverted to the risk in those that did not was 4.0 with 95% confidence

limits of 0.66 to 12.2. For BVD, PI₃ and BRS virus the odds ratios were 1.17 (0.5 - 2.8), 1.51 (0.6 - 3.7) and 0.87 (0.39 - 1.92), respectively.

There were significant differences in initial titer and subsequent treatment time for IBR and between IBR titer change, BVD titer change, initial BRS virus titer and time of treatment (data

not shown). However, with the exception of the decrease in titer change with an increasing number of days to subsequent treatment after initial bleeding, for BVD, there were no obvious patterns to these differences.

Being housed with animals that were treated for respiratory disease was not related to titer change for IBR, BVD or BRS viruses in control calves. Untreated calves housed with cases had a greater titer increase to PI₃ than untreated calves in pens with no treated calves (data not shown).

As indicated in Table I, weight gain data were available in 58/68 = 85.3% of cases and in 85/98 = 86.7% of controls. At the group level, there was no association between treatment for respiratory disease and weight gain. At the individual level and after controlling for group differences in weight gain, neither IBR nor BVD titer was related to weight gain. Each unit increase in PI₃ titer change was related to a decrease of about 0.5 kg of weight gain (b = -0.45, p ≤ 0.01). In addition, each unit increase in BRS virus titer change was related to a smaller (b = -0.38) decrease in weight gain, but this was significant only at p = 0.10.

DISCUSSION

The results of this study clearly indicate that all four viruses were present, active, associated with respiratory disease, and for PI₃ and BRS were associated with weight gain in these feedlot calves. However, since only serological evidence of infection is available, before a detailed discussion of the results, it is instructive to note the limitations of the data. A weakness of this and virtually all serological studies of BRD to date is that the epidemiological sensitivity and specificity of the tests is unknown. [Sensitivity is the percentage of recently or currently (relative to time of sampling) infected animals that give a positive titer, specificity is the percentage of nonrecently and non-currently infected animals that give a negative titer.] Certainly, the standard neutralization test for IBR has been found to be less than 100% sensitive but the modified test rivals the

TABLE V. The Association between Initial IBR, BVD, PI₃ and BRS Serum Titers, and Treatment for Undifferentiated Bovine Respiratory Disease

Virus	Initial Titer	Case	Control	Crude Odds Ratio ^a (Chi-Square)	Adjusted ^b Odds Ratio (Chi-Square)
IBR	0	58	75	3.1 (3.96)	3.1 (3.57)
	≥ 1	5	20		
		63	95		
BVD	0	29	36	1.4 (0.81)	1.4 (0.45)
	≥ 1	34	59		
		63	95		
PI ₃	0	44	46	2.6 (6.91)	1.6 (1.26)
	≥ 1	18	49		
		62	95		
BRS	0	36	35	2.4 (6.08)	1.6 (0.95)
	≥ 1	18	64		
		64	99		

^aAn odds ratio of 1 indicates equal viral titer (as dichotomized here) in cases and controls, an odds ratio greater than 1 indicates higher titers in cases than controls, an odds ratio of less than 1 indicates lower titers in cases than controls

^bAdjusted for group effects by Mantel-Haenszel procedure (12)

TABLE VI. Odds Ratios Between Seroconversion^a (Active Infection Rates) to IBR, BVD, PI₃, BRS Viruses and Undifferentiated Bovine Respiratory Disease in Ontario Feedlot Calves

Group	IBR	BVD	PI ₃	BRS	No. Cases
1	2.64	0.00	1.07	0.86	12
2	0.00	0.55	2.15	1.38	30
3	2.25	2.25	1.33	0.50	7
4	0.00	1.14	6.53 ^b	1.53	15
5	0.00	1.92	0.57	0.00	4
Summary odds ratio ^c	4.01	1.16	1.62	1.21	
Summary chi-square ^c	1.14	0.02	0.89	0.07	

^aBased on a fourfold or greater increase in titer

^bSignificant at $p < 0.10$

^cThe Mantel-Haenszel method was used to summarize the odds ratios (12)

sensitivity of the ELISA test (13). Part of this lack of sensitivity may reflect the fact that maternal antibody prevents a serological response to infection, although the immune system is primed by the exposure. For BVD virus, exposure of the calf *in utero* can produce an infected seronegative animal, thus giving rise to false negatives (17) in serological testing. In most but not all cases, a serological response to IBR virus will occur by seven days in calves devoid of maternal antibodies (17). Young calves and those receiving a lower challenge have a reduced antibody response to IBR. In one experiment, all five exposed calves did not seroconvert following a large intranasal exposure to IBR virus until 18 days later. A rule of thumb for elevated serum antibody levels is that 10-14 days are required (> 14 for PI₃ virus (cited in 18), and 9-15 for BRS virus (19)). For the viruses studied in this project, a fair assessment, in the absence of quantitative data, is that false positives are of minimal importance, but that false negatives may be of some concern. By considering any titer greater than zero as indicative of infection (current or previous), the sensitivity was maximized. However, the specificity may have been decreased. The usual approach of using a fourfold increase in titer as indicative of active infection may be conservative but also may reflect an

optimum balance of sensitivity and specificity. Notwithstanding these errors, by keeping the laboratory blind to the identity of individual animals the likelihood of biased results is reduced. Also, any lack of sensitivity or specificity would bias the odds ratio (measure of association) toward one, its null value. Hence the true association may be stronger than the statistical analyses indicated.

Another consideration is that respiratory disease was detected by the farm managers rather than veterinarians. This could increase the classification error for BRD; however, both managers are well versed in detecting BRD. Questionable cases were referred to veterinarians for diagnosis and cattle treated for diseases other than BRD were excluded from this study. In addition, sick cattle at Elora are regularly examined by veterinarians to validate the diagnoses and ensure correct therapy. Although precise diagnoses (e.g. to differentiate viral from bacterial pneumonia) would have allowed more detailed analyses, the detection of BRD was deemed appropriate for this study.

A final consideration is the method of statistical analysis in which average titers of cases and controls, within each cattle group, were used to make inferences about the relationship between serological titer and requiring treatment for respiratory disease. Traditionally, most researchers have

used the individual titer, and/or seroconversion, for this purpose, and because of this, the authors have presented much of the data and results in this format. The analysis of variance approach does not rely on a particular titer, or titer change being deemed biologically significant, but rather uses all of the available information on titer and titer change and in this sense is more powerful than the traditional approach. This feature is highlighted in that, using the traditional approach no titer level was found to be appropriate for discriminating between cases and controls and seroconversion was poorly associated with respiratory disease status. Yet, many significant associations were noted using the analysis of variance approach. Previous reports (5,6,12) have utilized this method of analysis, hence this study is not precedent setting in this regard. One reason for the divergent results between the statistical methods is that large titer increases occur principally in those with low initial titers and although most titer increases exceeded the fourfold level, the differences in titer between cases and controls was usually less than one dilution. These differences are more likely to be detected using the analysis of variance method, and this method is also useful for a multivariate study of titer changes and will be used (in the form of least squares and logistic regression) in future studies by the authors.

In general, the authors imply that the presence of antibody indicates current or recent exposure to the virus, but does not indicate resistance. If one assumes that all animals were capable of mounting an equivalent immune response, then low titers on arrival would indicate more recent exposure, or a lower level of exposure, than a high titer. In either case, a significant titer on arrival was likely a result of recent or current infection and thus a high titer would not likely reflect protection, even if circulating antibody titers were indicative of protection. Since the general tendency was for subsequently treated calves to have low viral titers on arrival, and since many of these same calves evidenced subsequent titer increases suggestive of active infection, it is likely that infection beginning at about the time

of shipment and/or weaning poses the most serious risk of clinical disease. Further, since the titer increases of treated calves usually were larger than those in control calves, a suppressed ability to mount an antibody response could not be blamed for the clinical disease.

With regard to the specific viruses, the prevalence rate of IBR virus on arrival was low and subsequent IBR activity was mainly localized to the cattle at Elora, particularly group 3. A low titer on arrival, and increasing titers after arrival both increased the risk of clinical disease.

Initial titers to BVD virus and subsequent BVD virus activity (seroconversions) were more widespread than IBR activity. However, the relationship of this activity to treatment for BRD is unclear. In group 4, although there was a high frequency of BVD activity, this activity increased the risk of clinical disease only slightly. In groups 2 and 3, with nearly equal BVD virus activity, active infection appeared to decrease the risk of disease in group 2 cattle, and increase the risk in group 3 cattle. Nonetheless, titer increase to BVD virus was significantly related to BRD.

Titers to PI₃ virus on arrival and its subsequent activity were widespread. The moderate to high prevalence rate of PI₃ antibodies, and the lower prevalence rate of IBR antibodies in Ontario feedlot calves has been noted in previous studies (5,6). In the current study, PI₃ virus titer on arrival did not appear to influence the risk of BRD, but thereafter, titer increases were significantly greater ($p=0.06$) in cases than controls in a similar manner to BVD virus titers. Unlike BVD however, PI₃ activity was more predictable in its relationship to BRD. In groups 2 and 4, PI₃ virus activity (seroconversion) was very common and in both instances, this increased the risk of clinical disease, significantly so in group 4 cattle. Increasing PI₃ titers were also associated with decreased weight gains. Although treatment for respiratory disease and weight gains were independent (poorly correlated), the dual negative effects of PI₃ virus activity suggest that such activity may have importance for the feedlot cattle owner.

Bovine respiratory syncytial virus antibodies were prevalent on arrival and subsequent activity was also widespread; in excess of 80% of the Ridgetown cattle experienced active infection. However, active infection was not strongly associated with clinical disease; in three of the five groups the activity was associated with no, or a decreased, risk of clinical disease. Nonetheless, BRS virus may play a causal role in BRD, since low titers on arrival were predictive of increased risk of subsequent treatment for BRD. Bovine respiratory syncytial infection may be of practical importance to the feedlot cattle owner because low titers on arrival were related to decreased weight gains after arrival.

In conclusion, the results of this study suggest a possible role for IBR, PI₃, BVD and BRS viruses, as causes of BRD. Ongoing studies of the occurrence and effects of these viruses will include multivariate analyses designed to elucidate the independent and joint effects of these viruses on BRD and weight gain.

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