A Group Level Analysis of the Associations Between Antibodies to Seven Putative Pathogens and Respiratory Disease and Weight Gain in Ontario Feedlot Calves

S.W. Martin, K.G. Bateman, P.E. Shewen, S. Rosendal,
J.G. Bohac and M. Thorburn

ABSTRACT

The associations, at the group level, between serological titer to *Pasteurella haemolytica* surface antigens (Ph), *Pasteurella haemolytica* cytotoxin (Ph-cytox), infectious bovine rhinotracheitis virus (IBRV), bovine virus diarrhea virus (BVDV), parainfluenza-3 virus (PIV3), respiratory syncytial virus (RSV), *Mycoplasma dispar* (Md), *M. bovis* (Mb), and respiratory disease treatment rates, relapse rates, and 28 day weight gains were investigated in 14 groups of calves entering two feedlots during years 1983-1985, in Ontario. Based on least squares regression analyses, seroconversion rates to Mb and BVDV were predictive of increased respiratory disease rates, and seroconversion rates to Ph, Ph-cytox, Md and PIV3 were predictive of decreased weight gains. The R^2 for predicting weight gains was much higher than for morbidity rates (0.75 vs 0.47 respectively). Titer data were not predictive of relapse rates. Group level analyses were performed because calves are managed as groups (e.g. pens) in commercial feedlots. Only BVDV seroconversion rates were related to increased risk of respiratory disease at both the individual and group levels of organization. Mycoplasma may be important factors in causing respiratory disease, and their relationship to potentiating the effects of other respiratory pathogens needs further investigation.

RÉSUMÉ

Une étude ayant le groupe comme unité d'étude fut réalisée sur 14 groupes de veaux entrant dans deux parcs d'engraissement ontariens entre 1983 et 1985. Le fait que les veaux soient gérés en groupes (i.e. parcs) dans les parcs d'engraissement explique l'analyse par groupe. On a estimé, à l'aide de la régression par moindre carré, les associations entre les prévalences de positifs aux antigènes de surface à *Pasteurella haemolytica* (Ph), à sa cytotoxine (Ph-cytox), au virus de la rhinotrachéite infectieuse bovine (IBRV), au virus de la diarrhée virale bovine (BVDV), au virus parainfluenza-3 (PIV3), au virus respiratoire syncitiale (RSV), à *Mycoplasma dispar* (Md), à *M. bovis* (Mb) et les taux de traitement pour maladie respiratoire, les taux de rechute et les gains de poids à l'âge de 28 jours. Les taux de séroconversion à Mb et BVDV permettaient de prédire une augmentation du taux de maladies respiratoires. Les taux de séroconversion à Ph, Ph-cytox, Md et PIV3 permettaient de prédire une diminution des gains de poids. Les R^2 pour les modèles de gain de poids étaient meilleurs que ceux des modèles des taux de morbidité (0.75 vs 0.47 respectivement). Les modèles des taux de rechute n'étaient pas statistiquement significatifs. Seul la séroconversion à BVDV était reliée à une augmentation du risque de maladie respiratoire, autant au niveau de l'animal que du groupe. Les mycoplasmes pourraient être des facteurs importants associés aux problèmes respiratoires, par contre leurs effets favorisants sur les autres pathogènes respiratoires auraient besoin d'être étudiés. ***(Traduit par Dr Michel Bigras-Poulin).***

INTRODUCTION

Bovine respiratory disease (BRD) is a major health problem for the beef feedlot industry. Most research on this syndrome has been performed with the individual animal as the unit of concern and analysis. However, since feedlot cattle are fed, housed and managed as groups, the practical unit of concern is the group. Few observational studies of BRD, at the group level, have been reported other than a large field study of the effects of demographic and management factors on morbidity and mortality rates (1,2). The occurrence of, and by implication the importance of, specific organisms has been reported in some outbreaks of BRD (3-5).

We have reported the association between antibody titers to a number of different microorganisms and treatment for BRD, in individual beef calves (6-8). In general, light calves on arrival, and those seroconverting to *Pasteurella haemolytica* cytotoxin, respiratory syncytial virus or bovine virus diarrhea virus (BVDV) had higher treatment rates for BRD. Weight gains and relapses of BRD were not strongly related to antibody level on arrival or seroconversion to these or other common organisms. Based on serology, most infections appeared to occur independently of each other, except for BVDV infections which tended to occur concomitantly with infection by other agents (7).

The involvement of multiple viruses, based on seroconversion data from clinically affected animals, in

Department of Population Medicine (Martin, Bateman, Thorburn), Department of Veterinary Microbiology and Immunology (Shewen, Rosendal), Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1 and Agriculture Canada, Lethbridge, Alberta (Bohac).

Submitted July 19, 1989.

herd outbreaks of respiratory disease, has been reported recently (3). Unfortunately, in the latter study, no sera were available from healthy herd-mates. Thus, one is left to make inferences about the importance of the viral agents without data on the viral status of healthy herd-mates.

Here we report the results of investigating the association, at the group level, between serological titers to *Pasteurella haemolytica* (Ph), infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus (BVDV), parainfluenza-3 virus (PIV3), respiratory syncytial virus (RSV), *Mycoplasma dispar* (Md), *M. bovis* (Mb), and respiratory disease treatment rates, relapse rates, and 28 day weight gain in Ontario feedlot calves.

MATERIALS AND METHODS

STUDY DESIGN

The details of this study, including the results of analyzing the data at the individual calf level, are described elsewhere (6-8). Briefly, the calf groups entered the study during a three year period, from 1983 to 1985. All calves, in each group, were blood sampled as soon as possible after arrival. Thereafter, all calves that were treated for respiratory disease (cases) and a similar number of randomly selected untreated (control) calves were rebled at approximately 28 days postarrival.

There were 14 groups of calves, nine housed at the Elora Research Station, and five at the Ridgetown College of Agricultural Technology. The groups differed in that they arrived at the feedlot on different days or were from different sources in western Canada. At the Elora station calves were maintained in pens of three to four calves per pen. Calves from different sources were usually not housed in the same pen. At the Ridgetown facility, calves from different sources were housed in the same pens with six to eight calves per pen. Previous studies indicated that being housed in the same pen with a clinical case of BRD did not alter the risk of BRD in the remaining calves (9).

Calves which were removed from the pen because they appeared sick were defined as cases if they had a rectal temperature $> 39.4°C$, and one or more clinical signs such as rapid shallow breathing, depression, anorexia, and/or lack of rumen fill, and no signs directly referable to diseases of other body systems. Only untreated (for any disease) calves were included in the control group. Relapses were those cases which required treatment for respiratory disease a second time, two or more days after completing the first course of therapy. None of the calves was vaccinated against the organisms studied until after the completion of the study period.

SEROLOGICAL METHODS

The immune response to *Pasteurella haemolytica* biotype A, serotype I (Ph) surface antigens was evaluated by an indirect (antiglobulin) microagglutination test. *Pasteurella haemolytica* toxin neutralizing activity (Ph-cytox) was determined in a microplate colorimetric assay. The titer of each sample was expressed as the highest dilution which yielded at least 50% neutralization of toxicity. For viral antibody assay, sera were tested in microtiter systems by serum neutralization (IBRV, BVDV, RSV), or by inhibition of hemagglutination (PIV3). Sera were analyzed for antibodies to Mb and Md using the indirect hemagglutination test (6-8).

STATISTICAL METHODS

Reciprocal titers, on arrival, to Ph, and Ph-cytox, of 64 or greater, IBRV titers of 4 or more, titers to BVDV and RSV of 9 or greater, titers to PIV3 of 20 or greater, and *Mycoplasma* titers of 40 or greater, were considered to be positive titers. These cutpoints are similar to, but slightly higher than, those used in a recently reported study of respiratory disease in Quebec (3). Since the sampling fraction of cases and controls within each group of cattle was known, it was possible to calculate the prevalence rate of antibody on arrival (based on positive titers at the initial sampling), and the active infection rate (based on a titer increase of fourfold or more) to each of the agents during the first four to five week period (10). The names of variables are suffixed by the letters SC to denote seroconversion.

The association between prevalence rate of initial titer, and the mean rate of seroconversion, and the rates of respiratory disease were investigated using unconditional correlation coefficients, and multivariable least squares regression techniques (11). Two versions of the regression models were constructed with each of group BRD morbidity rate, and 28 day weight gain (in kg) as an outcome. In the first version of the model, the predictor data were based on prevalence rate and seroconversion data from both cases and controls. In the second version of the model only data from cases were used as predictors. The results of the second model were intended to help facilitate comparison with results in the literature, most of which are based on data from cases only.

RESULTS

The overall mortality rate was less than one percent, and was too low for meaningful analyses at the group level.

The average BRD morbidity rate was $34 \pm 21\%$ (SD). There were large variations in morbidity rate, weight gain, and prevalence proportion of titers from group to group (Table I). Prevalence rates of titers on arrival to most agents were moderately high, except for IBRV and RSV. The prevalence of BVDV titers had a significant positive correlation with IBRV titer prevalence. Parainfluenza-3 virus titer prevalence rates had a significant negative correlation with BRD morbidity rates. *Pasteurella haemolytica* cytotoxin titer prevalence rates had a significant negative correlation with weight gain. Infectious bovine rhinotracheitis virus titer prevalence had a significant positive correlation with weight gain.

The group rates of morbidity, seroconversion, and weight gain are shown in Table II. There was considerable variation in the extent of seroconversion from group to group. Seroconversion rates to Mb and BVDV had a significant positive correlation with BRD morbidity rates. Seroconversion rates to Ph had a significant negative correlation with weight gain. The seroconversion rates were greater than 30% for Md and BVDV, and greater than 50% for the remaining agents, except for IBRV at 4%.

TABLE I. Prevalence rates of initial titers[a], morbidity rates and weight gains, by group, in Ontario feedlot calves, 1983-1985

Group	Morbidity rate/n	Weight gain	Ph	Ph-cytox	Mb	Md	IBRV	PIV3	BVDV	RSV
831	0.14/ 94	0.97	0.36	0.68	0.00	0.52	0.16	0.16	0.41	0.15
832	0.34/ 89	0.67	0.33	0.30	0.07	0.36	0.04	0.10	0.20	0.00
833	0.28/ 25	0.75	0.20	0.12	0.52	0.30	0.40	0.48	0.44	0.48
834	0.64/ 76	0.29	0.62	0.43	0.02	0.07	0.06	0.11	0.47	0.24
835	0.18/ 38	0.62	0.61	0.56	0.03	0.20	0.00	0.67	0.36	0.09
841	0.28/104	0.88	0.73	0.23	1.00	0.96	0.04	0.26	0.41	0.03
842	0.06/ 52	1.11	0.52	0.32	1.00	1.00	0.31	0.22	0.33	0.06
843	0.67/ 18	0.88	0.06	0.00	1.00	0.87	0.27	0.07	0.30	0.00
844	0.21/ 52	1.04	0.28	0.10	0.92	0.98	0.14	0.32	0.49	0.08
845	0.17/ 48	0.47	0.23	0.71	1.00	1.00	0.09	0.02	0.32	0.02
846	0.18/ 56	0.04	0.40	0.88	0.90	0.98	0.02	0.32	0.33	0.00
851	0.49/ 70	1.06	0.96	0.56	0.98	0.91	0.08	0.17	0.36	0.02
852	0.62/ 26	0.46	0.88	0.50	0.96	0.92	0.04	0.09	0.37	0.00
853	0.55/131	0.32	0.92	0.54	1.00	0.74	0.09	0.09	0.44	0.02
Avg	0.34	0.73	0.51	0.42	0.67	0.70	0.12	0.22	0.37	0.09
SD	0.21	0.28	0.28	0.26	0.44	0.34	0.12	0.18	0.08	0.13
"r" Morbidity		-0.17	0.33	-0.24	0.09	-0.14	-0.12	-0.46	0.09	-0.08
"r" Weight gain			-0.15	-0.43	0.20	0.31	0.49	0.04	0.01	-0.02

[a]See text STATISTICAL METHODS for titers considered positive

"r" Morbidity: indicates the correlation, at the group level, between the initial titer and the subsequent morbidity rate. "r" Weight gain: indicates the correlation with weight gain per day. (For significance at the 5% level, with 13 degrees of freedom, a correlation coefficient of $> |0.51|$ is necessary, $> |0.4|$ at the 10% level).

Ph: *Pasteurella haemolytica* agglutinins
Ph-cytox: *Pasteurella haemolytica* leukotoxin
IBRV: infectious bovine rhinotracheitis virus
BVDV: bovine virus diarrhea virus
PIV3: parainfluenza-3 virus
RSV: respiratory syncytial virus
Md: *Mycoplasma dispar*
Mb: *Mycoplasma bovis*
28 day weight gain in kg

The correlation coefficients between seroconversion rates, at the group level, are shown in Table III. Seroconversion to RSV had a significant correlation with seroconversion to a number of other organisms, but not in a consistent manner (three were positive and three were negative). Although not shown, group average initial titer to an organism was usually negatively correlated with the subsequent group seroconversion rate to that organism.

In a stepwise regression of BRD morbidity rate (as a proportion) on titers, the final equation was:

$$\text{Morbidity rate} = -0.03 + 0.46\ \text{MbSC} + 0.55\ \text{BVDVSC}$$

with an R^2 of 0.47. MbSC entered, at $p = 0.08$, before BVDVSC. Both were significant at $p < 0.05$ in the final model. Examination of residuals revealed that the assumptions of least squares regression were met (11). A second analysis was performed in which the prevalence of Mb and BVDV, on arrival, were forced into the equation. However, neither added significantly to the model. (Analyses were also conducted using arcsine transformed morbidity rates and the results were very similar.) Weight on arrival did not approach significance in any model. When data from only cases were used as predictors the final model was:

$$\text{Morbidity rate} = 0.15 + 0.36\ \text{PIV3SC}$$

with an R^2 of 0.26. PIV3SC was significant at $p = 0.06$.

The mean BRD relapse rate was $12 \pm 9.3\%$ (SD). Ph, Ph-cytox, and

TABLE II. Prevalence rates of seroconversion[a], morbidity rates and weight gains, by group, in Ontario feedlot calves, 1983-1985

Group	Morbidity rate/n	Weight gain	PhSC	Ph-cytoxSC	MbSC	MdSC	IBRVSC	PIV3SC	BVDVSC	RSVSC
831	0.14/ 94	0.97	0.49	0.37	0.79	0.33	0.04	0.60	0.01	0.75
832	0.34/ 89	0.67	0.42	0.53	0.74	0.29	0.04	0.91	0.24	0.73
833	0.28/ 25	0.75	0.52	1.00	0.48	0.22	0.30	0.52	0.30	0.23
834	0.64/ 76	0.29	0.12	0.36	0.50	0.81	0.00	0.82	0.51	0.81
835	0.18/ 38	0.62	0.27	0.58	0.20	0.59	0.00	0.36	0.17	0.94
841	0.28/104	0.88	0.19	0.79	0.15	0.27	0.00	0.60	0.57	0.37
842	0.06/ 52	1.11	0.31	0.53	0.12	0.29	0.06	0.65	0.29	0.41
843	0.67/ 18	0.88	0.12	0.64	0.67	0.20	0.00	0.70	0.13	0.57
844	0.21/ 52	1.04	0.08	0.56	0.61	0.47	0.00	0.69	0.29	0.43
845	0.17/ 48	0.47	0.77	0.30	0.09	0.19	0.00	0.69	0.09	0.13
846	0.18/ 56	0.04	0.71	0.67	0.22	0.33	0.10	0.55	0.29	0.35
851	0.49/ 70	1.06	0.41	0.57	0.43	0.36	0.02	0.45	0.68	0.34
852	0.62/ 26	0.46	0.68	0.66	0.76	0.38	0.00	0.23	0.42	0.58
853	0.55/131	0.32	0.62	0.75	0.45	0.41	0.00	0.68	0.36	0.59
Avg	0.34	0.73	0.41	0.59	0.44	0.37	0.04	0.60	0.31	0.52
SD	0.21	0.28	0.23	0.18	0.25	0.17	0.08	0.18	0.19	0.23
"r" Morbidity		-0.17	-0.14	0.13	0.48	0.27	-0.27	0.01	0.42	0.26
"r" Weight gain			-0.47	-0.02	0.11	-0.34	-0.01	-0.09	0.03	-0.19

[a]SC: Fourfold or greater increase in titer

"r" Morbidity: indicates the correlation, at the group level, between seroconversion and the morbidity rate. "r" Weight gain: indicates the correlation with weight gain per day. (For significance at the 5% level, with 13 degrees of freedom, a correlation coefficient of > 0.51 is necessary)

Abbreviations are as in Table I. The suffix SC denotes seroconversion

TABLE III. Group level correlation coefficients[a] between seroconversion to putative respiratory pathogens in Ontario feedlot calves, 1983-1985

Variable	PhSC	Ph-cytoxSC	MbSC	MdSC	IBRVSC	PIV3SC
PhSC						
Ph-cytoxSC						
MbSC						
MdSC	-0.7		0.6			
IBRVSC		0.5				
BVDVSC						
PIV3SC	-0.4		0.7	0.4		
RSVSC	-0.5	-0.4	0.5	0.8	-0.3	0.4

[a]Only correlation coefficients greater than | 0.3 | are shown
For significance at $p < 0.1$, coefficients must be greater than | 0.4 |
Abbreviations are as in previous tables. The suffix SC denotes seroconversion

BVDV titers were most strongly correlated with relapse rate, but none of the coefficients approached significance ($p > 0.15$).

The average weight gain per day during the first four to five weeks postarrival was 0.73 ± 0.28 (SD) kg. Weight gain and BRD morbidity rates were negatively correlated (r = -0. 17). Initial weight (236.1 kg) and weight gain were also negatively correlated (r = -0.37).

With weight gain per day as the outcome and using data from both cases and controls as predictors the final model was:

Weight gain = 2.51 -1.11 PhSC
-1.39 MdSC -0.78 PIV3SC -0.58 Ph-cytoxSC

with an R^2 of 0.75. (The variables are listed in order of entry to the model. At entry their significance levels were p = 0.09, 0.02, 0.13, and 0.08 respectively. All were significant at p = 0.05, except for Ph-cytoxSC at p = 0.08, in the final model.) Initial weight was not a significant predictor of group level weight gains in the multivariate model. If data from only cases were used as predictors the final model was:

Weight gain = 0.14 + 0.94
Ph-cytoxSC -1.07 PhSC + 0.58 MdSC

with an R^2 of 0.79. All variables were significant at $p < 0.05$ in the final model. Note the change in sign, that is direction of both the Ph-cytox and the MdSC effects from the previous model. Only the negative effect of Ph was consistent across models.

DISCUSSION

In this study, we investigated the statistical relationship between titers and health status, at the group level, as a first step in making causal inferences about the role of putative pathogens of BRD. Most causal theory applies to the individual animal level (10). In the case of serological data, epidemiological inferences about causation, at the individual level, are based on the comparison of titer status between healthy and affected animals. At the group level, epidemiological inferences about the role of agents are based on a monotonal relationship between the "dose" (incidence/prevalence) of the agent and the "response" (level of disease, or weight gain) in the group. Thus, we compared the prevalence rate of antibody titers on arrival, and the mean seroconversion rate in the postarrival period, with group treatment rates for BRD, relapse rates and group weight gains. As mentioned in the introduction, group level analyses were deemed appropriate because in commercial feedlots calves are housed and managed as groups, not as individuals. Consistent with our previous papers (6-8), when interpreting the results of the regression models, the presence of antibody on arrival was taken to indicate either persistent colostral titer or, more likely, exposure to the agent just prior to arrival. Seroconversion after arrival was taken to indicate active infection with the agent most likely in the period from shortly before arrival up to two to three weeks postarrival. From epidemiological principles (10), only those agents (titers) having a statistical relationship with treatment rates, or weight gain, were deemed to be possible determinants of these outcomes.

The 14 groups of calves in this study were qualitatively typical of feedlot calves arriving in Ontario from western Canada. However, since calves at only two research stations were observed, caution should be taken when making inferences about the prevalence rates and levels of seroconversion to these agents in the general population of feedlot calves. The fact that data from only a small number of groups were available also limited the power of the study to detect biologically significant associations between the titers and the outcomes. Finally, the validity of the models may be questioned since there were more variables available for selection than cases, and no single variable was significant at $p < 0.05$. Only combinations of variables allowed this level of significance to be achieved. On the other hand, the data set is the largest to date to investigate what agents influence BRD, and weight gains, at the group level; hence, we believe it useful to share the data and our interpretations of the results.

Unfortunately, since most causal theory is directed towards individuals, we have no precedents for interpreting analytic results from two levels of organization simultaneously. This is a particular problem when the results of analyses differ between the levels. The problems with interpreting ecological regressions have been discussed (12) but do not materially assist us. Hence, we are speculating when attempting to explain our findings.

In terms of factors predicting the level of morbidity, BVDV was the only agent common to models based on both individual (8) and group data. Others have alluded to the potential importance of BVDV (3-5,8), although the actual role of this virus in BRD pathogenesis remains disputed. In a recent report arising from respiratory disease outbreak investigations in Quebec (3), BVDV was reported to be the "most frequent viral agent" (based on seroconversion frequency) in calves with respiratory disease. No samples were taken from healthy calves.

In contrast to BVDV, *M. bovis* was selected only in the group level model of BRD morbidity rates. This result may indicate that Mb has an effect at the group but not at the individual level, or it may reflect the effects of statistical "control" in the two levels of analyses. For example, in our previous

individual level analyses, the effects of group, and all factors related to group, were controlled (i.e. blocked or removed) by the use of dummy variables (8). This procedure gives more importance to source (i.e. group) than Mb, and the Mb titers which were significant unconditionally, lost that significance after control of group effects in the logistic models (8). We take this to indicate that the level of Mb and/or the effects of Mb were related to the source groups. In the current group level analyses, any relationship of Mb to source (i.e. group) was not removed, thus allowing the effects of Mb to appear. It may be that Mb, which is a common organism in these calves, is insufficient as a cause of BRD, but it may be a component of a number of sufficient causes with other organisms. In these circumstances, the less frequent members of the sufficient causes may appear more important, statistically, than the more prevalent components (13). Thus it is reasonable to postulate an umbrella of Mb which, although harmless by itself, enhances the ability of other organisms to cause BRD. The possible role of *Mycoplasma* spp as a causal factor in BRD, has been mentioned before (14).

Unlike in the individual level analyses (8), weight on arrival was not a good predictor of subsequent morbidity rate at the group level. Respiratory syncytial virus and *P. haemolytica* also were predictors of respiratory disease in individuals but not at the group level. No explanations for this lack of significance are apparent, although the lack of association between *Pasteurella* and BRD at the group level is surprising given the accepted causal role(s) of this organism. Obviously, other organisms, and other factors beside microorganisms, play an important role in the frequency of BRD. Further studies on the effects of mycoplasma on BRD, and their relationship with *Pasteurella* in producing respiratory disease are warranted.

With regard to weight gain, there were dramatic differences in the results of the individual and group level analyses. The ability to predict weight gain in the individual level analyses was very poor, with morbidity, BVDV and PIV3 viruses selected. In the group level results, Ph, Ph-cytox, Md and PIV3 were predictive of group weight gains with an R^2 of 75%. Confounding (distortion or suppression, 15) of Ph-cytoxSC and PIV3SC effects were present as they were not significant until the effects of Ph and Md were controlled. Morbidity was negatively, but not strongly related to group weight gains as in individual level studies (16). *Mycoplasma dispar* seroconversion was predictive of decreased weight gains. The reason for the differences in "explained variance" between the individual and group level analyses, are unknown but may well include those cited above for differences in the morbidity models. Here the sufficient causes are for poor weight gain, not morbidity. As with the morbidity models, the control of differences among groups in the individual level analyses would remove variability in weight gains related to "source" (i.e. group) as well as the effects of microorganisms, that were related to group or weight gains. Presumably the negative effects of these agents on weight gain are mediated through tissue and functional changes that do not produce clinical illness. The increased R^2 also may indicate that at the group level it is preferable to use the more accurately measured weight gain, rather than the more subjectively defined morbidity level, as a measure of the impact of these respiratory pathogens. This may also relate to the design of field trials to assess vaccine efficacy; that is, it might be preferable to use weight gain rather than morbidity (or mortality) as an outcome. By measuring weight gain, including data on calves that die, one may obtain a more valid view of the impact of these organisms than solely measuring morbidity and mortality.

In terms of the approach to creating regression models, models using only data from cases were included to allow comparison of results with data in the literature. From an epidemiological perspective, data from cases only are insufficient for purposes of making causal inferences. In this study, these "case only" models differed dramatically from the theoretically correct models based on data from both cases and controls. In addition, it was difficult to make meaningful comparisons with the literature, because the organisms investigated and/or the study design differed from those in this study. For example, in one outbreak of BRD, seven of nine sampled (sick) calves in a herd, with 50% BRD morbidity, seroconverted to PIV3. In a second herd, seven of 90 calves that were suspected to have BRD seroconverted to PIV3 and failed to gain weight until 56 days postarrival (4). In another outbreak of BRD involving three groups of calves, five of 90, 19 of 60, and two of 38 calves with BRD were studied. Five animals seroconverted to BVDV, four to IBRV, and three to PIV3. The IBRV activity appeared to be restricted to just one of the three, separately housed, groups (5). In the recent report from Quebec, BVDV seroconversion was present in more than half of the cases, however the morbidity rates for each group were not stated (3). No common thread of relationship of titers to disease rates or weight gain emerges from these examples.

Clearly, there remains much to be learned about respiratory disease and the effects of multiple putative pathogens on ill-health and productivity. In an attempt to understand BRD, it seems sensible to accept that infections involving multiple agents are the rule rather than the exception. Debate may occur about the relative importance of each agent, about the causal structure, and about the best way to break the causal chain/web. However there should be little doubt that many putative pathogens are active in both individuals and groups during BRD outbreaks, and their effects may go far beyond morbidity and mortality. The apparent effects of these agents should be interpreted in the light of the outbreak situation and the presence of other, noninfectious, causes of respiratory disease. Until their role is clearly understood, we believe there is much benefit to be gained from studying the presence and activity of microorganisms in "healthy" herd-mates, not just in diseased individuals, and in using groups, such as pens, as the unit of analysis when possible.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Shelley James for her assistance in

blood sampling, Mr. Tim Lesnick for his help with computing and data analysis, and the Managers and staff of the Elora and Ridgetown beef feedlots for their assistance. The financial support of the Ontario Ministry of Agriculture and Food, Agriculture Canada, and the Ontario Cattlemen's Association is gratefully acknowledged.

REFERENCES

1. **MARTIN SW.** Factors influencing morbidity and mortality in feedlot calves in Ontario. Vet Clin North Am [Large Anim Pract] 1983: 575-586.
2. **MARTIN SW, MEEK AH, DAVIS DG, THOMSON RG, JOHNSON JA, LOPEZ A, STEPHENS L, CURTIS RA, PRESCOTT JF, ROSENDAL S, SAVAN M, ZUBAIDY AJ, BOLTON MR.** Factors associated with mortality in feedlot cattle: the Bruce County beef cattle project. Can J Comp Med 1980; 44: 1-10.
3. **RICHER L, MAROIS P, LAMONTAGNE L.** Association of bovine viral diarrhea virus with multiple viral infections in bovine respiratory disease outbreaks. Can Vet J 1988; 29: 713-717.
4. **SWEAT RL.** Isolation of myxovirus parainfluenza 3 from cattle with respiratory diseases. J Am Vet Med Assoc 1967; 150: 172-177.
5. **ROSENQUIST BD, ENGLISH JE, JOHNSON DW, LOAN RW.** Mixed viral etiology of a shipping fever epizootic in cattle. Am J Vet Res 1970; 31: 989-994.
6. **ROSENDAL S, MARTIN SW.** The association between serological evidence of mycoplasma infection and respiratory disease in feedlot calves. Can J Vet Res 1986; 50: 179-183.
7. **MARTIN SW, BOHAC JG.** 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. Can J Vet Res 1986; 50: 351-358.
8. **MARTIN SW, BATEMAN KG, SHEWEN PE, ROSENDAL S, BOHAC JG.** The frequency, distribution and effects of antibodies to seven putative respiratory pathogens on respiratory disease and weight gain in feedlot calves in Ontario. Can J Vet Res 1989; 53: 355-362.
9. **MARTIN SW, DARLINGTON G, BATEMAN K, HOLT J.** Undifferentiated bovine respiratory disease (shipping fever): is it communicable? Prev Vet Med 1988; 6: 27-35.
10. **MARTIN SW, MEEK AH, WILLEBERG P.** Veterinary Epidemiology: Principles and Methods. Iowa State Press, 1987.
11. **DRAPER N, SMITH H.** Applied Regression Analysis, 2nd ed. Toronto: John Wiley & Sons, 1981.
12. **MORGENSTERN H.** Uses of ecologic analysis in epidemiologic research. Am J Public Health 1982; 72: 1336-1337.
13. **ROTHMAN KV.** Causes. Am J Epidemiol 1976; 104: 587-592.
14. **LANGFORD EV.** Comments on "Update on pasteurellosis in young cattle". Can Vet J 1980; 21: 265.
15. **SUSSER M.** Causal Thinking in the Health Sciences: Concepts and Strategies in Epidemiology. Oxford University Press, 1973.
16. **BATEMAN KG.** Undifferentiated bovine respiratory disease: An evaluation of therapy. MSc Thesis, University of Guelph, 1988.