The Frequency, Distribution and Effects of Antibodies, to Seven Putative Respiratory Pathogens, on Respiratory Disease and Weight Gain in Feedlot Calves in Ontario

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ABSTRACT

During 1983-85, 279 calves requiring treatment for bovine respiratory disease and 290 comparison (control) animals from 15 different groups of feedlot calves were bled on arrival and again at 28 days postarrival. Their sera were then analyzed for antibodies to seven putative respiratory pathogens. On arrival, the prevalences of indirect agglutination titers to *Pasteurella haemolytica, P. haemolytica* cytotoxin, *Mycoplasma bovis* and *M. dispar* were greater than 50%, the prevalence of titers to bovine virus diarrhea virus (BVDV) was approximately 40%, and the prevalences of titers to infectious bovine rhinotracheitis virus (IBRV), bovine respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) were all below 25%. Seroconversion during the first month after arrival occurred in more than half the calves to *P. haemolytica* cytotoxin, PIV3 and RSV. Seroconversion of agglutination titers to *P. haemolytica, Mycoplasma* and BVDV occurred in about 40% of calves, and seroconversion to IBRV was infrequent (< 5%). Initial titers were negatively correlated to subsequent titer changes within organism. Initial titers, and titer changes between organisms were essentially independent. Light calves had an increased risk of being selected for treatment for respiratory disease. Seroconversion to *P. haemolytica* cytotoxin, RSV and BVDV were predictive of respiratory disease cases, explaining approximately 69% of all respiratory disease cases in the feedlots. It was not possible to accurately predict weight gain or relapse from the serological data.

RÉSUMÉ

Cette expérience s'étalait sur la période de 1983 à 1985 et elle impliquait 15 groupes de veaux de boucherie parmi lesquels 279 nécessitèrent un traitement pour des maladies respiratoires, alors que 290 servirent de témoins. On préleva un échantillon de sang, chez tous ces veaux, à leur arrivée dans les parcs d'engraissement et 28 jours plus tard. Elle visait à rechercher des anticorps contre sept agents étiologiques possibles de maladies respiratoires, dans les échantillons précités. À leur arrivée dans les parcs d'engraissement, au-delà de 50% des veaux possédaient des anticorps sériques contre *Pasteurella haemolytica* et sa cytotoxine, *Mycoplasma bovis* et *M. dispar*; environ 400 possédaient de tels anticorps contre le virus de la diarrhée à virus bovine, alors que moins de 250 en possédaient contre le virus de la rhino-trachéite infectieuse bovine, le virus syncytial respiratoire bovin et le virus parainfluenza du type 3. Au cours du mois ultérieur à leur arrivée dans les parcs d'engraissment, au delà de 50% des veaux affichèrent une séroconversion à l'endroit de la cytotoxine de *P. haemolytica*, du virus parainfluenza du type 3 et du virus syncytial respiratoire bovin; environ 40% affichèrent aussi une séroconversion à l'endroit de *P. haemolytica*, de *Mycoplasma* et du virus de la diarrhée à virus bovine, tandis que seulement moins de 5% affichèrent une séroconversion à l'endroit du virus de la rhinotrachéite infectieuse bovine. Les titres initiaux d'anticorps contre un organisme donné n'affichèrent aucune corrélation avec leurs variations ultérieures. Les titres initiaux et ultérieurs se révélèrent complètement indépendants, d'un organisme à l'autre. Les veaux légers couraient un plus grand risque de nécessiter un traitement pour des troubles respiratoires. Une séroconversion à l'endroit de la cytotoxine de *P. haemolytica*, du virus syncytial respiratoire bovin et de celui de la diarrhée à virus bovine correspondait à des cas de maladies respiratoires et en expliqua environ 69%. Les données sérologiques ne permirent pas de prédire le gain de poids ou les rechutes.

INTRODUCTION

Bovine respiratory disease is the most common disease problem in stressed feedlot calves (1). A number of microbiological agents as well as management and environmental factors have been cited as potential causes of this disease, however, despite its multiagent, if not multivariate, causality most studies have focused on only one or two agents at a time. In some previous studies researchers have explicitly contrasted "healthy" and "sick" (respiratory disease) animals in an attempt to identify the important

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causal organisms (2,3). These studies used a combination of microbiological, serological and pathological tests to identify etiological agents or events. More recently, the serological titers to a number of putative causal agents in cases of respiratory disease were compared to titers in controls (4). The underlying assumptions and potential weaknesses of this approach were discussed and the authors concluded that the method was "useful in observational studies aimed at determining the current putative agents involved in cattle respiratory disease".

The project reported here was designed primarily to investigate the association between serological titers to *Pasteurella haemolytica* (Ph), infectious bovine rhinotracheitis virus (IBRV), bovine virus diarrhea virus (BVDV), parainfluenza virus type 3 (PIV3), respiratory syncytial virus (RSV), *Mycoplasma dispar* (Md) and *M. bovis* (Mb), and treatment for respiratory disease in Ontario feedlot calves. Secondarily, the association of these titers with relapses of respiratory disease and with weight gain during the first 28-35 days in the feedlot was examined. The results of the first year of study have been published previously (5,6).

MATERIALS AND METHODS

STUDY DESIGN

The research was conducted over a three year period from 1983 to 1985. The design of this study was to draw blood samples from calves as soon as possible after arrival, to rebleed all calves that were treated for respiratory disease (cases) at approximately 28 days postarrival, and to rebleed an equal number of randomly selected untreated (control) calves at this time. Groups of calves were housed at the Elora Research Station, or at the Ridgetown College of Agricultural Technology. The groups were differentiated on the basis that they arrived at the feedlot on different days and/or were from different sources in western Canada.

Whether a particular calf required treatment for respiratory disease was decided by the staff at the two locations, both managers having worked with feedlot cattle for many years. Calves were defined as cases if after being pulled from the pen because they appeared to be sick they had a rectal temperature $> 39.4°C$, and one or more clinical signs referable to the respiratory system. Veterinary assistance was requested for unusual or severely affected cases only. Only cattle untreated for any disease were included in the control group. Relapses were defined as 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; traceback in 1983 and 1984 revealed that only clostridial vaccines had been administered prior to arrival at the feedlot. No traceback was performed in 1985.

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 sera were frozen at -20°C until analyzed. All sera were analyzed blindly: the laboratory did not know the identity of cases or controls, nor which two sera constituted a pair. However, the laboratories were advised to analyze the sera in blocks of forty to ensure that paired sera would be tested under identical conditions.

The immune response to *P. haemolytica* biotype A, serotype 1 (Ph) surface antigens was evaluated by an indirect (antiglobulin) microagglutination test using washed formalinized bacterial antigen. Toxin neutralizing activity (Ph-cytox) was determined in a microplate colorimetric assay as the ability of serum to neutralize the toxic effect of *P. haemolytica* type 1 culture supernatant for BL-3 cells (7,8). The titer of each sample was expressed as the highest dilution which yielded at least 50% neutralization of toxicity.

For viral antibody assays, sera were tested in microtiter systems by serum neutralization (IBRV, BVDV, RSV), or by inhibition of hemagglutination (PIV3) (6).

Sera were analyzed for antibodies to *M. bovis* and *M. dispar* using the indirect hemagglutination test (5).

STATISTICAL METHODS

The antibody titers to Ph, Ph-cytox, and IBRV were coded as 0, 1, 2, 3, . . . for endpoint titers of 0, 1/2, 1/4, 1/8, etc. The titers to BVDV and RSV were coded as 0, 1, 2, 3 . . . for endpoint titers of 0, 1/3, 1/9, 1/27, etc., and the titers to PIV3 and *Mycoplasma* were coded as 0, 1, 2, 3 for titers of 0, 1/10, 1/20, 1/40 and so forth respectively. These codes correspond to the antilog of the base of the dilution factor. Since 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 exposure at arrival (based on titer at the initial sampling), and the active infection rate (based on seroconversion) during the first four to five week period (9). The optimal arrival titer to use as a cutpoint (endpoint) for discriminating between cases and controls was the titer giving the largest likelihood ratio (i.e. the quotient from dividing the odds of exposure in cases by the odds of exposure in controls) between cases and controls (10). In most instances there was little change in the likelihood ratio by titer, or the likelihood ratios were quite erratic. Hence, a cutpoint of any titer beyond the lowest dilution was taken to indicate the presence of biologically significant levels of antibody on arrival. Thus the prevalence rate was based on the proportion of animals with an antibody titer greater than a coded value of 2, except for Ph and Ph-cytox antibodies, and antibodies to Mb where the mean values of the antibody distribution (coded titers of 6, 6 and 3) were utilized as cutpoints (i.e. six or greater was positive, etc). The active infection rate was based on the proportion of animals that seroconverted (had an increase of at least two coded dilutions, — i.e. fourfold or greater — titer increase) during the first four to five week period after arrival, regardless of initial titer. (Variables denoting seroconversion are suffixed by the letters SC.)

For categorical data, the associations between initial titer, and between seroconversion, and respiratory disease in individual animals were investigated using odds ratio tech-

niques (9). Odds ratios of greater than unity imply a harmful effect, odds ratios of less than unity imply a sparing effect of exposure. In initial analyses an unconditional R x C (usually 2 x 2) table approach was used, then group effects were controlled using the Mantel-Haenszel procedure. In final analyses, dummy variables for group (GROUP) were created and forced into the logistic regression models to prevent confounding due to factors related to group and/or research farm. Similar analyses were conducted to detect differences in titers between animals treated only once and those treated two or more times (relapses) for respiratory disease. The initial weight (WT) of animals was also forced into final models after discovering it was a significant predictor of respiratory disease. The proportion of respiratory disease explained by each of the variables in the final model was determined (11).

The association between titers and weight gain was assessed using multiple least squares regression analysis (12). Control of confounding was by the use of dummy variables as for the logistic regressions.

RESULTS

The morbidity rates and number of cases and controls by group are shown in Table I. The morbidity rates varied widely from year to year, group to group, and from one research station to another. Respiratory disease accounted for over 90% of all cattle requiring treatment in the first four to five weeks postarrival, in all years.

The prevalence of initial titers, the incidence of seroconversion (active infection), and their univariate association (expressed as odds ratios) with morbidity at the individual animal level, ignoring group, are shown in Table II.

The distribution of titers on arrival in cases and controls was, in general, very similar (Table III), although significant differences in distribution of initial titers as evidenced by the chi-square statistics in Table II were noted. (These represented the points of maximum difference in titer between the cases and controls; neighboring cutpoints often were not nearly as optimal.) Thus, with the exceptions noted, it made little difference to the overall discriminating ability of initial titers what the cutpoint for declaring a titer to be biologically significant was set at. All agents except Md and IBRV had a significant relationship with health status; titers to Ph and Mb signalled an increased risk of subsequent respiratory disease treatment, titers on arrival to Ph-cytox, PIV3, BVDV and RSV were related to a decreased risk of subsequent respiratory disease treatment. Titers on arrival were quite common to all agents with the exception of IBRV, RSV and PIV3.

The distribution of titer changes in the one month period postarrival was also, in general, very similar in cases and controls (Table IV), however, significant differences in the frequency of seroconversion as evidenced by the chi-square statistics in Table II were noted. Seroconversion to Ph-cytox, PIV3 and BVDV were significantly associated with treatment for respiratory disease. Seroconversion to IBRV was infrequent, but very common to Ph-cytox, PIV3 and RSV. In general, initial titer to an organism was moderately to strongly inversely related to subsequent titer changes to that organism (Table V).

TABLE I. A Description of the Groups of Calves in a Study of theRole of Selected Agents in Bovine Respiratory Disease in Ontario Feedlots, 1983-1985

Year	Group	Morbidity Rate(%)	Number Cases	Number Controls
1983	E-1	14	12	30
	E-2	34	30	15
	E-3	28	7	4
	R-1	64	16	21
	R-2	18	4	27
1984	E-1	28	29	26
	E-2	06	3	18
	E-3	67	11	2
	E-4	21	11	14
	R-1	17	8	10
	R-2	18	10	12
	R-3	57	12	8
1985	E-1	49	34	34
	E-2	62	16	10
	R-1	55	72	59

E: Elora Research Station
R: Ridgetown College of Agricultural Technology

TABLE II. The Association Between Initial Titers and/or Seroconversion to Selected Agents of Respiratory Disease in Ontario Feedlot Calves, 1983-1985. Odds Ratios Comparing Cases (Treated) to Controls (Untreated)

Variable[c]	Initial Titers[a]	Chi-square	Seroconversion[b]	Chi-square
Ph	1.89 (75%/61%)	10.93	1.22 (46%/41%)	1.08
Ph-cytox	0.71 (41%/50%)	3.61	1.97 (71%/55%)	13.00
Mb	1.55 (74%/65%)	4.78	1.21 (47%/43%)	0.97
Md	1.01 (68%/68%)	0.00	0.86 (36%/40%)	0.52
PIV3	0.42 (11%/24%)	12.26	1.54 (67%/57%)	5.02
IBRV	1.25 (10%/ 8%)	0.33	1.57 (3%/ 2%)	0.22
BVDV	0.63 (32%/42%)	5.89	1.49 (42%/33%)	4.23
RSV	0.42 (4%/ 8%)	3.96	1.36 (61%/54%)	2.52

[a]Any titer at or beyond a dilution of two (2) except for Ph (6), Ph-cytox (6) and Mb (3) as cutpoints
[b]Any increase of two or more coded dilutions (fourfold or greater) titer increase
[c]Ph — *Pasteurella haemolytica*; Ph-cytox — *P.haemolytica* cytotoxin; Mb — *Mycoplasma bovis*; Md — *Mycoplasma dispar*; PIV3 — Parainfluenza virus type 3; IBRV — Infectious bovine rhinotracheitis virus; BVDV — Bovine virus diarrhea virus; RSV - Respiratory syncytial virus
(%) Percentage of cases/controls with initial titer at or beyond the cutpoint, or seroconversion (fourfold or greater increase in titer)

TABLE III. The Distribution of Initial Titers[a] in Respiratory Disease Cases/Controls in Ontario Feedlot Calves 1983-1985

Titer	Ph[b]	Ph-cytox	Mb	Md	IBRV	PIV3	BVDV	RSV
0	0.00/0.00	0.02/0.00	0.20/0.30	0.02/0.02	0.83/0.82	0.75/0.64	0.41/0.34	0.67/0.61
1	0.00/0.01	0.02/0.00	0.07/0.06	0.06/0.04	0.07/0.09	0.14/0.12	0.27/0.24	0.29/0.31
2	0.02/0.03	0.03/0.02	0.03/0.06	0.08/0.09	0.04/0.05	0.06/0.11	0.18/0.20	0.02/0.06
3	0.05/0.04	0.07/0.04	0.15/0.17	0.15/0.17	0.03/0.01	0.03/0.04	0.06/0.10	0.01/0.01
4	0.03/0.04	0.09/0.06	0.20/0.21	0.22/0.18	0.02/0.01	0.01/0.04	0.04/0.08	0.00/0.01
5	0.05/0.11	0.20/0.14	0.15/0.13	0.22/0.23	0.01/0.00	0.02/0.02	0.03/0.04	0.00/0.01
6	0.10/0.16	0.16/0.24	0.13/0.04	0.18/0.16	0.00/0.00	0.00/0.02	0.00/0.01	0.00/0.00
7	0.28/0.23	0.18/0.20	0.04/0.03	0.05/0.09	0.00/0.00	0.00/0.01	0.00/0.00	0.00/0.00
8	0.22/0.19	0.12/0.11	0.02/0.00	0.01/0.01	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
9	0.14/0.09	0.09/0.09	0.00/0.00	0.01/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
10	0.05/0.07	0.02/0.07	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
11	0.05/0.02	0.01/0.01	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
12	0.02/0.01	0.00/0.01	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00

[a]The data indicate the proportion of cases/controls at each initial titer
[b]Abbreviations as in Table II

Table VI describes the results of a multivariable logistic analysis of the data. It should be noted that initial titers to Ph-cytox were not associated with treatment after control of GROUP, whereas initial IBRV titers became significant after control of GROUP and other agent titers. Seroconversion to RSV became significant after control of GROUP. Initial titers to Ph and IBRV increased the risk of subsequent respiratory disease, initial PIV3 and BVDV titers decreased the subsequent risk of respiratory disease. Seroconversion to Ph-cytox and RSV were associated with an increased risk of respiratory disease. A subsequent analysis in which GROUP, WT, and all initial titer information were forced into the model, and the variables describing seroconversion allowed to enter in a stepwise manner produced essentially the same model.

In order to detect the presence of interaction, all possible two-way cross product terms involving all main effect variables in the model were created. No interaction terms were created with GROUP. GROUP, WT, all initial titer variables, Ph-cytoxSC and RSVSC were forced into the model and then interaction terms were available for entry in a stepwise manner. None of the interaction terms was significant.

The prevalence of initial titers and seroconversion in the cases for the variables in the final model are shown in Table II. Based on their distribution and association with treatment for respiratory disease, from the final model, the population attributable fraction for Ph was 43%, for Ph-cytoxSC 36%, for RSVSC 23%, and 37% for the absence of initial PIV3 titers.

Correlation coefficients between the initial titer and/or titer change of one organism to another indicated no strong ($r > 0.3$) correlations. More specifically, for the variables in the final model, 65% of calves with Ph titers on arrival seroconverted to Ph-cytox, 60% of those without Ph titers also seroconverted to Ph-cytox; 56% of calves with Ph titers seroconverted to RSV virus, 60% of those without Ph titers seroconverted to RSV; 17% of calves with Ph titers had PIV3 titers on arrival, 20% of those without Ph titers had PIV3 titers on arrival. None of these differences approached statistical significance.

A logistic model was created with only seroconversion data available for

TABLE IV. The Distribution of Titer Change[a] in Respiratory Disease Cases/Controls in Ontario Feedlot Calves, 1983-1985

Titer Change	Ph[b]	Ph-cytox	Mb	Md	IBRV	PIV3	BVDV	RSV
-5	0.04/0.03	0.00/0.01	0.00/0.01	0.00/0.01	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
-4	0.05/0.01	0.01/0.00	0.01/0.01	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.01	0.00/0.00
-3	0.03/0.03	0.01/0.02	0.01/0.02	0.02/0.03	0.01/0.00	0.00/0.02	0.00/0.02	0.00/0.00
-2	0.07/0.07	0.04/0.04	0.04/0.02*	0.02/0.02*	0.03/0.05	0.02/0.05	0.03/0.07	0.00/0.00
-1	0.07/0.12	0.05/0.10	0.11/0.08	0.11/0.10	0.12/0.10	0.03/0.06	0.15/0.19	0.03/0.05
0	0.10/0.15	0.06/0.12	0.21/0.24	0.20/0.19	0.80/0.81	0.12/0.10	0.25/0.29	0.11/0.14
1	0.18/0.18*	0.12/0.15	0.15/0.20	0.28/0.25	0.02/0.02*	0.16/0.20	0.15/0.10*	0.25/0.27
2	0.19/0.14	0.17/0.19	0.21/0.21	0.20/0.19	0.00/0.00	0.34/0.32*	0.15/0.11	0.30/0.27*
3	0.14/0.10	0.13/0.12*	0.13/0.09	0.08/0.09	0.00/0.01	0.12/0.10	0.15/0.13	0.23/0.17
4	0.07/0.11	0.14/0.10	0.08/0.07	0.05/0.07	0.01/0.00	0.10/0.08	0.08/0.06	0.07/0.07
5	0.04/0.04	0.11/0.08	0.04/0.03	0.03/0.02	0.00/0.00	0.07/0.03	0.04/0.02	0.01/0.02
6	0.01/0.01	0.07/0.04	0.02/0.01	0.01/0.02	0.00/0.01	0.01/0.01	0.00/0.00	0.00/0.00
7	0.01/0.01	0.08/0.03	0.00/0.01	0.00/0.01	0.00/0.00	0.01/0.01	0.00/0.00	0.00/0.00

[a]The data indicate the proportion of cases/controls at each titer change
[b]Abbreviations as in Table II
"*"indicates the optimal titer change for differentiating cases from controls

TABLE V. The Correlation Coefficient Between Initial and Final Titer to Putative Agents of Respiratory Disease in Feedlot Calves, 1983-1985

Variables	Year		
	1983	1984	1985
Ph[a]	-0.66	-0.55	-0.61
Ph-cytox	-0.65	-0.68	-0.71
Mb	-0.44	-0.49	-0.56
Md	-0.59	-0.43	-0.52
IBRV	-0.45	-0.43	-0.67
PIV3	-0.77	-0.78	-0.62
BVDV	-0.54	-0.42	-0.56
RSV	-0.61	-0.58	-0.40

[a]Abbreviations as in Table II

entry to the model. After controlling group effects and weight, Ph-cytoxSC, RSVSC and BVDVSC were entered in that order. PIV3SC was significant until Ph-cytoxSC and RSVSC had entered the model. The final coefficients were 0.70, 0.50, and 0.41 for Ph-cytoxSC, RSVSC and BVDVSC respectively, which translate to odds ratios of 2.01, 1.65 and 1.51. At a model sensitivity of 72%, the specificity was 60%. Based on this model, the population attributable fraction for each of these organisms was 36%, 24% and 15% for Ph-cytoxSC, RSVSC and BVDVSC respectively. The population attributable fraction for all three organisms was 69%.

With regard to differences between calves which relapsed, and the cases requiring only one treatment series, only IBRV was significant when GROUP effects were ignored (Table VII). Using logistic regression with GROUP effects controlled, Ph, Ph-cytox, IBRV, PhSC and weight were significantly related to the outcome. WT entered the model first, followed by IBRV and Ph. Respiratory syncytial virus then entered but was removed due to colinearity. The coefficients for variables in the final model, ignoring GROUP coefficients, were: WT (0.029), IBRV (0.24) and Ph (-0.43). The coefficient for RSV was negative. The minimum number of prediction errors were made at a specificity of 100% and a sensitivity of 4%. At a sensitivity of 72% the model had a specificity of 63%. The population attributable fraction for IBRV was 2.1% and for Ph it was 8.3%.

With regard to weight gain, treatment for respiratory disease had a more statistically significant impact than any of the titer variables, treated calves gaining 2.71 kg less than untreated calves. Calves with a coded titer of 2 or more to BVDV, on arrival, gained 2.1 kg more than other calves. Although only significant at the 10% level, calves with initial coded titers of 2 or more to PIV3 gained 1.8 kg less than other calves. The same variables were selected when the weight gain was calculated on a per day in feedlot basis. The variable reflecting treatment for respiratory disease did not seem to prevent other variables denoting titer from entering the regression model.

DISCUSSION

In this study, we compared the distribution of antibody titers on arrival, and the distribution of seroconversion in the postarrival period, with treatment for bovine respiratory disease in the hopes of identifying a few very important respiratory pathogens. The calves studied were typical of feedlot calves arriving in Ontario from western Canada. However, since calves at only two research stations were included in the study, caution should be taken when extrapolating results to the general population of feedlot calves.

The presence of antibody on arrival was taken to indicate either persistent colostral titer or, more likely, exposure to the agent prior to arrival. Other workers have reported that some calves have significant titers at the farm of origin, however there is a dramatic increase in the proportion of calves with titers after they leave the farm and are transported to the feedlot and/or mixed at auction markets (13). In general, since titers begin to appear from one week to two weeks postinfection, and most calves in this study would have been removed from their source farms at least ten days prior to being sampled after arrival, there was ample opportunity for antibodies to agents experienced after leaving the source farm to appear. Seroconversion after arrival was taken to indicate active infection with the agent in the period from shortly before arrival up to two to three weeks postarrival. In accordance with epidemiological principles (9,14), only those agents having a statistical relationship with health status were deemed to be possible determinants of health status. Since management and other non-agent factors specific to a group of calves could influence the antibody response and the percentage of the group requiring treatment for bovine respiratory disease, the effects of GROUP were controlled, when possible, to prevent confounding. Previous publications based on the first year's data provide more detailed information on group to group differences (5,6).

Numerous serological results have been reported for putative agents of respiratory disease from both observational and experimental studies. However if one accepts that the

TABLE VI. The Result of Stepwise Logistic Regression Analysis of Initial Titers and Seroconversion to Putative Pathogens of Respiratory Disease in Ontario Feedlot Calves, 1983-1985

Step No.	Variables Added to Model (Coefficient from final model)	Variables Available in Order of Significance, $p < 0.05$
0	GROUP	Ph[a], Ph-cytoxSC, WT, BVDV, PIV3, RSVSC, PIV3SC, BVDVSC
1	Ph (0.88)	Ph-cytoxSC, WT, PIV3, BVDV, RSVSC, PIV3SC, BVDVSC
2	Ph-cytoxSC (0.70)	WT, RSVSC, PIV3, BVDV, PIV3SC, BVDVSC, RSV
3	WT (-0.012)	RSVSC, BVDV, PIV3, BVDVSC, PIV3SC
4	RSVSC (-0.48)	PIV3, BVDV
5	PIV3 (-0.55)	

[a]Abbreviations as in Table II. The suffix SC indicates seroconversion

Note: IBRV (0.90) and BVDV (-0.48) added to the model subsequent to step 5

: Optimal Model Sensitivity = 72%

: Optimal Model Specificity = 66%

TABLE VII. The Association Between Initial Titers and/or Seroconversion to Selected Agents and of Respiratory Disease in Ontario Feedlot Calves, 1983-1985. Odds Ratios Comparing Relapsed to Single Treatment Cases

Variable[c]	Initial Titers[a]	P Value	Seroconversion[b]	P Value
Ph	0.89 (72%)	0.82	1.75 (59%)	0.17
Ph-cytox	0.73 (34%)	0.55	0.75 (65%)	0.52
Mb	2.06 (85%)	0.24	0.95 (46%)	1.00
Md	1.62 (77%)	0.38	1.58 (46%)	0.29
PIV3	1.01 (12%)	0.60	0.77 (62%)	0.52
IBRV	3.21 (23%)	0.03	1.39 (4%)	0.56
BVDV	0.95 (31%)	1.00	1.22 (46%)	0.68
RSV	0.42 (0%)	0.60	0.79 (56%)	0.67

[a]Any titer beyond the minimum test dilution (2) except for Ph (7), Ph-cytox (6) and Mb (3) as cutpoints

[b]Any fourfold or greater titer increase

[c]Abbreviations as in Table II

(%) Percentage of relapse cases with initial titer at or beyond the cutpoint, or seroconversion (fourfold or greater increase in titer)

antibody response may indicate nothing other than exposure to the agent, as opposed to immunity, it may be futile to attempt to compare actual titer levels from one study to another. Further, given that the immune response may be a two-edged sword, that "protective antibody levels" are agent-dose dependent, and that there is a lack of test standardization, it is even more difficult to compare, quantitatively, the effects of specific titer levels, particularly when challenge dose may differ widely. Thus only qualitative comparisons with the literature, principally from field studies, will be made. In the absence of firm quantitative data on the subject, we assume that the sensitivity and specificity of the tests are sufficient to provide high predictive values for most of the agents in this study.

In previous publications, the senior author has discussed the scale of measurement of titer and initially concluded that using the coded titers was the appropriate method (14). Since that time, our belief is that too many statistically significant, but possibly biologically trivial, associations were being identified. Thus in this paper we report the presence of "biologically significant" titers on arrival and of seroconversion. A variety of titers have been reported as biologically significant hence our approach was to identify the titer, on arrival, which best differentiated subsequent cases from controls. However, because the distributions of initial titer were very similar in cases and controls, with only relatively small (albeit statistically significant) differences in their means, one specified titer was nearly as good as another at differentiating cases from controls. Thus, arbitrary cutpoints, usually coded titers of two, were used. The choice of cutpoint had a greater influence on the prevalence of "significant" titers on arrival than on our ability to distinguish subsequent cases from controls.

Based on the results of the multivariable logistic model of initial titers and seroconversions, titers to Ph and IBRV on arrival were harmful, whereas titers to PIV3 and BVDV were sparing. Given the high frequency and levels of titer to Ph on arrival, it is reasonable to assume that the animals had been recently exposed to large numbers of *P. haemolytica*. Given the low prevalence of titers to IBRV on arrival, and the fact that the majority of titers remained stable or decreased thereafter, the majority of initial IBRV titers may reflect colostral antibodies. On the other hand, the fact that antibodies to IBRV on arrival appeared to increase the risk of subsequent respiratory disease suggests that the antibodies may have reflected active infection. In any event, IBRV could not contribute greatly to the occurrence of respiratory disease given its low frequency of occurrence.

Titers to PIV3 or BVDV on arrival appeared to protect against subsequent treatment for respiratory disease. Whether this straightforward interpretation is correct, however, is unknown. The reader should consider that initial titers and seroconversion were reasonably highly negatively correlated. Partly as a result of this, there was no occasion when both the initial titer and seroconversion for an organism entered the same model. Also, in the logistic model with seroconversion data only, BVDVSC was significant in the final model and PIV3SC was not significant until after Ph-cytoxSC and RSVSC entered the model. Thus, if one puts the most weight on the results of the "seroconversion model", having a titer on arrival was a reasonable proxy for not seroconverting, both of which were associated with a reduced risk of respiratory disease. Use of the "seroconversion model" results may be preferable because of its simplicity and straightforward interpretation, despite the fact that the model is not as good at discriminating between cases and controls as the model containing initial titer data. Ph-cytoxSC, RSVSC and BVDVSC were indicators of increased rates of treatment for respiratory disease; this ordering reflects both their strength of association (as measured by odds ratios) and the proportion of disease attributable to each. In total, active infection with these organisms appears to explain up to 69% of respiratory disease. The individual population attributable fractions sum to more than 69%, due to the fact that the agents are probably components of the same sufficient cause of respiratory disease.

Experimentally, a biological interaction (synergism) has been demonstrated, for example between IBRV and *P. haemolytica* (15) and between PIV3 and *P. haemolytica* (16), in producing respiratory disease. We attempted to identify this in our data by using cross-product terms, however, there was no evidence of interaction. Thus, we conclude that the effects of multiple organisms on the risk of respiratory disease are additive in the natural logarithm scale (this is the additive logistic model). In some calf groups, antibody titers to an agent appeared to be harmful, in other calf groups sparing. However, since we had no biological explanation for this phenomenon, we did not include interaction terms involving GROUP in our models.

An interesting and potentially important ancillary finding of this study is that although, within an organism, initial titer and titer change were correlated, initial titer and titer change between organisms were virtually independent. That is, initial titer, or titer response, to one organism did not appear to be influenced by the titer or response to another organism. In particular, Ph and Ph-cytox titers appeared to be independent suggesting that many animals respond only to the surface antigens of *P. haemolytica*, others respond only to the cytotoxin, still others respond to both; the proportion of animals in the latter category being the proportion expected by chance alone. Since all *Pasteurella* produce cytotoxin, this may explain how an animal could respond only to the cytotoxin and not the surface antigen of type A1.

Thomson *et al* compared stressed cattle that were febrile ($\geq$ 104.5°F) and had three days of elevated plasma fibrinogen (these were designated as sick) to controls ("well") (2). Sick calves had significantly higher *P. haemolytica* counts from nasal swabs in only one of five groups. Most cattle had titers to *P. haemolytica*, on arrival and these titers increased subsequently, particularly in the first seven days. Although not statistically significant, a lower percentage of sick cattle had titers during the first two weeks than well cattle. Parainfluenza virus-3 titers were moderately common (30-50% prevalence), IBRV titers were infrequent (6-10%) on arrival, and both increased subsequently, PIV3 more so than IBRV. Although not significant, fewer sick calves had PIV3 titers than well calves throughout the 28 day period. In a later study, Thomson *et al* relaxed the sick criteria and four animals previously classified as well were classified as sick (3). These eight groups of calves plus six new groups of calves were again investigated by contrasting selected parameters in sick and well calves. It was noted that sick animals had lower *P. haemolytica* titers at day 1 than well animals; but this difference was not significant at day 7. Antibody titers to PIV3 did not differ between sick and well animals; IBRV titers were not evaluated. These viral results agree with the findings of the current study, however our findings for *Pasteurella* are opposite to this earlier work.

More recently, Shewen and Wilkie evaluated Ph and Ph-cytox titers in feedlot cattle (7,8). *Pasteurella haemolytica* titers increased after arrival, and calves dying of fibrinous pneumonia had lower Ph and Ph-cytox titers than those dying of other causes. Confer *et al* demonstrated a protective effect of naturally derived titers to *P. haemolytica* at the time of experimental challenge (17). As mentioned, in the current study, Ph titers on arrival increased the risk of subsequent treatment for respiratory disease, Ph-cytox titers reduced that risk. Active infection with *P. haemolytica* type A1 or cytotoxin producing organisms occurred more frequently in cases than controls.

With regard to viruses, the prevalence and incidence in feedlot calves remain quantitatively uncertain. Yates *et al* reported on three viruses in 62 calves from one ranch (18). Infectious bovine rhinotreacheitis virus and BVDV titers were absent at the first sampling, PIV3 titers were present in 10 of 62 calves. Although subsequently vaccinated with an intranasal IBRV-PIV3 vaccine, a maximum of 15 of 62 developed titers to PIV3 and 29 of 62 to IBRV before what was presumed to be natural infection with IBRV resulting in titers in 59 of the 62 calves. BVDV titers became widespread and were associated, temporally, with the occurrence of respiratory disease. None of the organisms studied, including isolation of *Mycoplasmas*, was indicative of the health status of lung tissue.

Gillete and Smith sampled calves at the farm of origin, at the auction market, and cases (unfortunately without controls) were sampled at the feedlot (13). Most calves had RSV titers ($>$ 16) at the farm, however titers increased at the auction sampling and in cases at the feedlot. Between 46 and 71% of the calves seroconverted to RSV. In 1976, 40% of the calves had titers to BVDV at the source farm; this increased to 80% in cases at the feedlot. In 1977, 25% had BVDV titers at the farm of origin and minimal seroconversion occurred subsequently.

In the current study, interpretation of the titer data on arrival was difficult because for some (Ph, Mb and IBRV) it appeared to increase the risk for subsequent respiratory disease treatment, whereas for others (Ph-cytox, Md, PIV3, BVDV and RSV) it appeared to decrease the risk. This apparent contradiction persisted in the results of the logistic model (Table VI). Based on the logistic model using only seroconversion data, RSV, BVDV and perhaps PIV3, are important viruses in the respiratory disease complex. As noted previously, IBRV infection was not widespread in these cattle groups, and therefore was apparently not important as a respiratory pathogen. Infectious bovine rhinotracheitis virus was frequently isolated from dead calves in the Bruce County study (19). Many of these may have been of vaccinal origin, however there appeared to be an association of IBRV infection with respiratory disease in unvaccinated calves also. No other Canadian field studies implicate IBRV as a frequent component of the postarrival respiratory disease complex, although its role in experimentally induced respiratory disease has been well documented (15).

Since calves that relapse have reduced weight gains and are more likely to die than calves that respond to the first treatment (20), we investigated the predictive ability of titer information for relapses. As in the case versus control models, IBRV titers on arrival appeared harmful. However in this model, Ph titers on arrival appeared to be sparing. We note again that PhSC would have entered the model had not Ph titer data been available, and PhSC would have been associated with an increased risk of relapse.

Titers explained only a small percentage of the variation in weight gain; BVDV titers on arrival (again a proxy for not seroconverting to BVDV) appeared to increase weight gains. Smaller calves were at increased risk of respiratory disease treatment, but larger calves tended to relapse. An explanation of these effects is not apparent.

In conclusion, based on this study, we believe there is a lack of serological evidence to support an etiological role

for *M. bovis* or *M. dispar*, the results of the first year of study and our unconditional statistics notwithstanding (5). Of the other organisms studied, *P. haemolytica* type A1, particularly the leukotoxin produced by *Pasteurella* species, plays a predominant role in the respiratory disease complex. Active infection with BVDV and/or RSV appears harmful, and these two viruses and *Pasteurella* may explain almost 60% of respiratory disease occurrence. Active infection with PIV3 may also be harmful. Indeed, infection with many potential pathogens is common in feedlot calves, but many of the infected calves are not treated for respiratory disease. Infectious bovine rhinotracheitis virus, although associated with initial treatment and relapses, was infrequent in these calves and hence was not considered an important pathogen under the conditions of this study. More studies on the natural history of these organisms and the host responses to infection using advanced diagnostic procedures, as well as serology, may shed useful light on the sufficient causes of this complex syndrome.

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REFERENCES

1. **MARTIN SW.** Factors influencing morbidity and mortality in feedlot calves in Ontario. Vet Clin North Am (Large Anim Pract) 1983: 575-86.
2. **THOMSON RG, BENSON ML, SAVAN M.** Pneumonic pasteurellosis of cattle: microbiology and immunology. Can J Comp Med 1969; 33: 194-206.
3. **THOMSON RG, CHANDER S, SAVAN M, FOX ML.** Investigation of factors of probable significance in the pathogenesis of pneumonic pasteurellosis in cattle. Can J Comp Med 1975; 39: 194-207.
4. **PRITCHARD DG, EDWARD S, MORZARIA SP, ANDREWS AH, PETERS AR, GILMOUR NJL.** Case-control studies in the evaluation of serological data from respiratory disease outbreaks in cattle. Proc Soc Vet Epidemiol Prev Med 1983.
5. **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.
6. **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.
7. **SHEWEN PE, WILKIE BN.** Antibody titers to *Pasteurella haemolytica* A1 in Ontario beef cattle. Can J Comp Med 1982; 46: 354-356.
8. **SHEWEN PE, WILKIE BN.** *Pasteurella haemolytica* cytotoxin neutralizing activity in sera from Ontario beef cattle. Can J Comp Med 1983; 47: 497-498.
9. **KLEINBAUM DG, KUPPER LL, MORGENSTERN H.** Epidemiologic Research. Belmont, California: Lifetime Learning Publications, Wadsworth, Inc., 1982.
10. **SACKETT DL, HAYNES RB, TUGWELL P.** Clinical Epidemiology; A Basic Science for Clinical Medicine. Toronto: Little, Brown Co., 1985.
11. **BRUZZI P, GREEN SB, BYAR DP, BRINTON LA, SCHAIRER C.** Estimating the population attributable risk for multiple risk factors using case-control data. Am J Epidemiol 1985; 122: 904-914.
12. **DRAPER N, SMITH H.** Applied Regression Analysis, 2nd ed. Toronto: John Wiley & Sons, 1981.
13. **GILLETTE KG, SMITH PC.** Respiratory syncytial virus infection in transported calves. Am J Vet Res 1985; 46: 2596-2600.
14. **MARTIN SW.** Analysis and interpretation of biologic data: a seroepidemiologic study of respiratory disease. Proc 4th Int Sym Vet Epidemiol Econ. Singapore Vet Assoc 1986.
15. **JERICHO KWF, BABIUK LA.** The effect of dose, route and virulence of bovine herpesvirus 1 vaccine on experimental respiratory disease in cattle. Can J Comp Med 1983; 47: 133-139.
16. **JERICHO KWF, DARCEL CLeQ, LANGFORD EV.** Respiratory disease in calves produced with aerosols of parainfluenza-3 virus and *Pasteurella haemolytica*. Can J Comp Med 1982; 46: 293-301.
17. **CONFER AW, PANCIERA RJ, FULTON RW.** Effect of prior natural exposure to *Pasteurella haemolytica* on resistance to experimental bovine pneumonic pasteurellosis. Am J Vet Res 1984; 45: 2622-2624.
18. **YATES WDG, KINGSCOTE BF, BRADLEY JA, MITCHELL D.** The relationship of serology and nasal microbiology to pulmonary lesions in feedlot cattle. Can J Comp Med 1983; 47: 375-378.
19. **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.
20. **BATEMAN KG.** Undifferentiated bovine respiratory disease: an evaluation of antimicrobial therapy. MSc Thesis, University of Guelph, 1988.