The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to *Haemophilus somnus* and *Mannheimia haemolytica* at 3 Ontario feedlots

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Abstract

The association between exposure to *Haemophilus somnus* and *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*) and the risk of undifferentiated bovine respiratory disease (UBRD) was investigated using serological evidence of exposure coupled with a factorial design vaccine field trial. Measures of previous exposure (titer at arrival) and current exposure (titer increase in the study period) to these agents were used. The vaccine field trial involved systematic allocation of animals into groups that received either a *M. haemolytica* vaccine, an *H. somnus* vaccine, a combined *M. haemolytica* and *H. somnus* vaccine, and an unvaccinated control group. Serum was collected from the 852 animals enrolled to determine titers to *H. somnus*, *M. haemolytica*, bovine coronavirus and bovine viral diarrhea virus. Vaccination with *H. somnus* in combination with *M. haemolytica* and with *M. haemolytica* alone reduced the risk of UBRD. The odds ratio for vaccination with *H. somnus* alone and UBRD risk suggested some sparing effect, but the 95% confidence limits included unity. There was no association between serological evidence of concurrent exposure to *M. haemolytica* and UBRD occurrence. There was an association between titer change to *H. somnus* and UBRD risk. However, the association changed with time of BRD treatment; animals diagnosed and treated for UBRD on or after day 10 showed little evidence of exposure to *H. somnus*, despite evidence of natural *H. somnus* exposure in the unvaccinated group. The association between titer change to *H. somnus* and UBRD occurrence seen in this study may be a consequence of prolonged exposure to antibiotics, rather than a causal association.

Résumé

La relation entre une exposition à Haemophilus somnus *et* Mannheimia haemolytica *et le risque de maladie respiratoire bovine non-différenciée (UBRD) fut étudiée en examinant les évidences sérologiques d'exposition combinées avec un essai clinique de vaccination. Les mesures d'exposition antérieure (titre à l'arrivée) et d'exposition actuelle (augmentation du titre durant la période d'étude) envers les agents considérés furent utilisées. L'essai clinique de vaccination impliquait la répartition des animaux dans un des groupes suivants : vaccin contre* M. haemolytica, *vaccin contre* H. somnus, *vaccin combiné contre* M. haemolytica *et* H. somnus, *groupe témoin non-vacciné. Du sérum fut prélevé à partir des 852 animaux de l'étude afin de déterminer les titres envers* H. somnus, M. haemolytica, *le virus corona bovin et le virus de la diarrhée virale bovine. La vaccination à l'aide du vaccin combiné* H. somnus-M. haemolytica, *de même qu'avec le vaccin contre* M. haemolytica *a permis de réduire le risque d'UBRD. L'indice de cote pour la vaccination contre seulement* H. somnus *et le risque d'UBRD laisse supposer un effet protecteur. Aucune relation entre une évidence sérologique d'exposition à* M. haemolytica *et la présence d'UBRD ne fut notée. Toutefois, la relation changea en fonction du temps de traitement du BRD; les animaux chez lesquels un diagnostic fut posé et un traitement pour l'UBRD entrepris au jour 10 ou plus tard présentèrent peu d'évidence d'une exposition à* H. somnus *malgré l'évidence d'une exposition naturelle à* H. somnus *chez les animaux témoins non vaccinés. La relation entre un changement de titre envers H. somnus et la fréquence d'UBRD observée dans la présente étude pourrait être une conséquence d'une exposition prolongée aux antibiotiques plutôt qu'une évidence d'une association causale.*

(Traduit par docteur Serge Messier)

Introduction

Authors of recent studies examining the association between titer changes to *H. somnus* and undifferentiated bovine respiratory disease (UBRD) occurrence reported that animals diagnosed and treated for UBRD had smaller titer increases, or larger titer decreases, than animals not diagnosed with UBRD (1,2). This suggests that either *H. somnus* is not causally related to UBRD or that the immune response, reflected in the titer to the agent, does not behave in the manner traditionally anticipated for infectious agents of disease. The

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

Table I. Number and characteristics of calves enrolled in a study of UBRD at 3 Ontario feedlots in 1998

	Feedlot A	Feedlot B	Feedlot C
No. enrolled	318	435	99
No. of processing days	4	4	1
Implants at processing	No	Yes	No
Antibiotic used at arrival	Oxytetracycline	Oxytetracycline (< 40°C) Tilmicosin (> 40°C)	None
Modified live 4-way vaccine at processing	Yes[d]	Yes[e]	No
Sex	Mixed (276 F/42 M)	Male	Male
Rectal temp at arrival; °C (mean ± SD)	40.4 ± 0.9[a]	39.9 ± 0.7[b]	39.4 ± 0.4[c]
Range of temp °C	4.2	3.7	2.9
Weight at arrival; kg (mean ± SD)	235.0 ± 34.9[a]	247.2 ± 20.2[a]	283.4 ± 37.8[a]
Range of weight (kg)	208	129	219
Housing type	Open sided, concrete floor barn, multiple pens	Open sided, concrete floor barn, multiple pens	Open sided, concrete floor barn, multiple pens
Mingled with cattle not on *H. somnus* trial during the study	Yes	No	Yes

[a,b,c] Means in the same row with the same superscript ([a, b, c]) do not differ significantly at $P < 0.05$

[d] Pyramid 4MLV, Ayerst Laboratories, Saint Laurent, Quebec

[e] Bovishield 4, SmithKline Beecham Animal Health, Mississauga, Ontario

objective of the current study was to determine if evidence of recent exposure to *H. somnus* was associated with UBRD occurrence. The null hypothesis was that the change in *H. somnus* titer would not be related, statistically, to UBRD treatment, and, by implication, that *H. somnus* was not a cause of UBRD. The relationship between titer change to *H. somnus* and the timing of UBRD treatment was also examined. The associations between titers to *M. haemolytica* and UBRD occurrence and timing were examined in a similar manner. Vaccination was used to identify the response of feedlot calves to an artificial challenge with *H. somnus* and *M. haemolytica* antigens under the same environmental circumstances.

Materials and methods

The animal management and serological analysis are outlined in detail in an accompanying paper in this issue, but are summarized in Table I. All animals were processed within 36 h of arrival. At the feedlot, during routine processing, the cattle were systematically assigned to 1 of 4 vaccine groups: 1) *Mannheimia haemolytica* (Pneumo-star; Biostar Inc., Saskatoon, Saskatchewan), 2) *Haemophilus somnus* (Somnu-star; Biostar Inc.) 3) *M. haemolytica* and *H. somnus* (Somnu-star PH, Biostar Inc.), and 4) an unvaccinated control group. Subsequently, these animals were commingled.

All data analyses, unless otherwise stated, were performed using statistical computer software (SAS v.6.12; SAS Institute Inc., Cary, North Carolina, USA). The unit of analysis was the individual animal. For all analyses, the results of the serological assays were transformed into an index representing the well number of the last positive reaction, as defined by the test/technique, and corresponded to the negative log of the dilution factor (1,3–5). Hereafter, the index will be referred to as the titer. Animals with no reaction were recorded as having a titer of 0. The change in titer was calculated as the difference between transformed titers at arrival and day 28. Calves were categorized as seropositive or seronegative at arrival, any titer > 0.5 being deemed as seropositive. Seroconversion denoted an increase in titer (index) greater than 2, i.e. 2-fold dilution tests required a 4-fold increase in titer and 4-fold dilution tests required a 16-fold increase in titer for seroconversion.

To facilitate examining the data, an initial stratified analysis was conducted and calves were classified according to whether they received treatment for UBRD (yes = 1; no = 0) and the time of treatment for UBRD (UBRD TIME — 3 levels: untreated, treated during the first 9 d post arrival, or treated on or after the 10th day post-arrival). Within the strata, titer changes were examined. These strata were included as explanatory variables in the regression modelling. Other class variables included feedlot (FEEDLOT — 3 levels), calf group defined by day of processing (GROUP — 9 levels) and vaccine group (VACCINE — 4 levels).

A logistic regression model was built to examine the association of arrival titers to the risk of UBRD. Explanatory variables of interest were the arrival titers to *M. haemolytica* and *H. somnus*; covariates included the vaccine group, and bovine coronavirus and bovine viral diarrhea virus arrival titers. Feedlot was included as a fixed effect.

Mixed effects regression modelling was used to determine factors affecting the change in titer. The explanatory variable of interest was either UBRD treatment or UBRD TIME. The explanatory variables were forced into the initial models, as was the arrival titer to the organism being modelled, vaccine group and an interaction between the arrival titer and vaccination. Potential confounding variables included the arrival titers to the other putative causal agents, an interaction between vaccine group and the arrival titers of the other putative causal organisms, arrival weight, and arrival rectal temperature. Confounding variables were added or removed from the model and the resulting effect on the coefficients of the variables of interest examined. If the change in the point estimate of the variables involving UBRD treatment or UBRD TIME was not greater than 10%, then the potential confounding variable was not considered to be a source of confounding and was excluded from the final model

Table II. Distribution[a] of treatments for undifferentiated bovine respiratory disease, by feedlot and vaccine group, at 3 Ontario feedlots in 1998

Vaccine	Times treated	Feedlot A $n = 318$	Feedlot B $n = 435$	Feedlot C $n = 99$	Total
H. somnus and *M. haemolytica* vaccine[b]	0	58	97	22	177 (83%)
	1	14	8	2	24 (11%)
	>1	8	4	0	13 (6%)
H. somnus vaccine[c]	0	55	90	22	167 (80%)
	1	14	22	1	27 (13%)
	>1	9	5	1	15 (7%)
M. haemolytica vaccine[d]	0	48	101	23	172 (81%)
	1	18	8	2	28 (13%)
	>1	12	0	1	13 (6%)
Non-vaccinated[e]	0	46	93	23	159 (74%)
	1	21	11	2	34 (16%)
	>1	15	6	0	21 (10%)
All groups	0	207 (65%)	381 (88%)	90 (91%)	678 (80%)
	1	67 (21%)	39 (9%)	7 (7%)	113 (13%)
	>1	44 (14%)	15 (3%)	2 (2%)	61 (7%)

[a] The data indicate the absolute number of animals treated and in brackets, the proportion of animals
[b] Somnu-star PH; Biostar Inc., Saskatoon, Saskatchewan
[c] Somnu-star; Biostar Inc.
[d] Pneumo-star; Biostar Inc.
[e] A control group not receiving any of these bacterial vaccines

unless it had a significant impact on titer change (6). Interaction terms were retained if they were significant at $P < 0.1$. GROUP and FEEDLOT were entered as random effects. The cluster specific coefficients of the random effects are not reported.

Results

Thirteen percent of calves were treated for UBRD once, 7% were treated more than once (relapsed). The epidemic curve is shown in Figure 1. Most treatments occurred at Feedlot A (Table II). All the treated animals had a rectal temperature greater than 40°C when selected for treatment, except for 9 animals with incomplete records, which were missing the rectal temperature at the time of treatment.

The frequency distributions of the arrival titers are presented in Table III. The average titer increase to *H. somnus* was 1.8 ± 1.6 (mean ± SD), to *M. haemolytica* leukotoxin ELISA titer 2.1 ± 1.4, to *M. haemolytica* leukotoxin neutralization titer 4.2 ± 4.7 and to the *M. haemolytica* indirect agglutination titer 1.3 ± 1.8. Arrival titers to both organisms varied across feedlots but not across vaccine groups (results not shown). Titer increases also differed by feedlot ($P < 0.05$ — results not shown). Forty percent of animals seroconverted during the study period to *H. somnus*. Fifty-five and 81 percent of animals seroconverted to *M. haemolytica* leukotoxin ELISA and neutraliztion titers, respectively. Seroconversion to *M. haemolytica* indirect agglutination titer occurred in 51% of animals.

In the logistic model examining factors associated with UBRD, vaccination with *M. haemolytica* antigens either alone [β (regression coefficient) = −0.3 ± 0.07 (standard error of the coefficient)], or in combination with *H. somnus* (β = −0.7 ± 0.2) was associated with reduced UBRD risk compared to the non-vaccinated group. There was no significant difference in the UBRD risk of calves receiving the

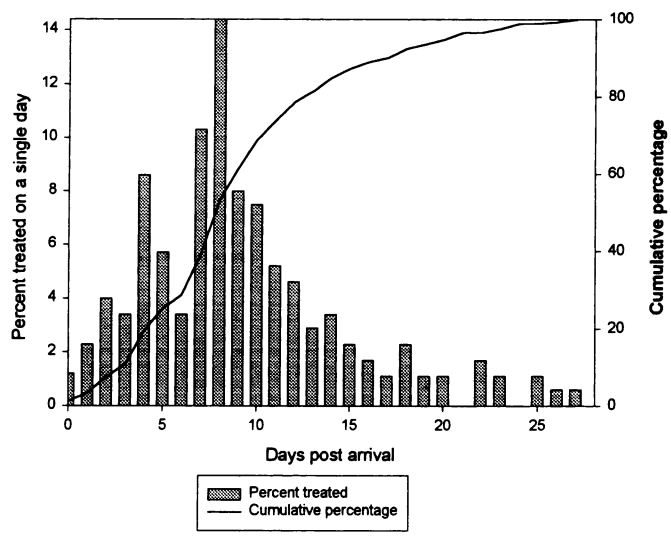

Figure 1. The frequency distribution (%) and cumulative percentage, of days to first treatment for animals treated for undifferentiated bovine respiratory disease at 3 Ontario feedlots over a 28-day study period, 1998.

H. somnus only vaccine (β = −0.3 ± 0.2) compared to the control group. The presence of titers on arrival to all organisms tested reduced the risk of subsequent UBRD; odds ratios of 0.9 and 0.82 for *H. somnus* and *M. haemolytica*, respectively (results not shown).

The treated calves were grouped according to timing of initial diagnosis and treatment, i.e., those that were initially diagnosed and treatment for UBRD within 9 d of arrival (early) and those calves that were initially diagnosed and treatment for UBRD on day 10 or later (late). Calves that were diagnosed and treated for UBRD more than once tended to have smaller *H. somnus* titer increases than

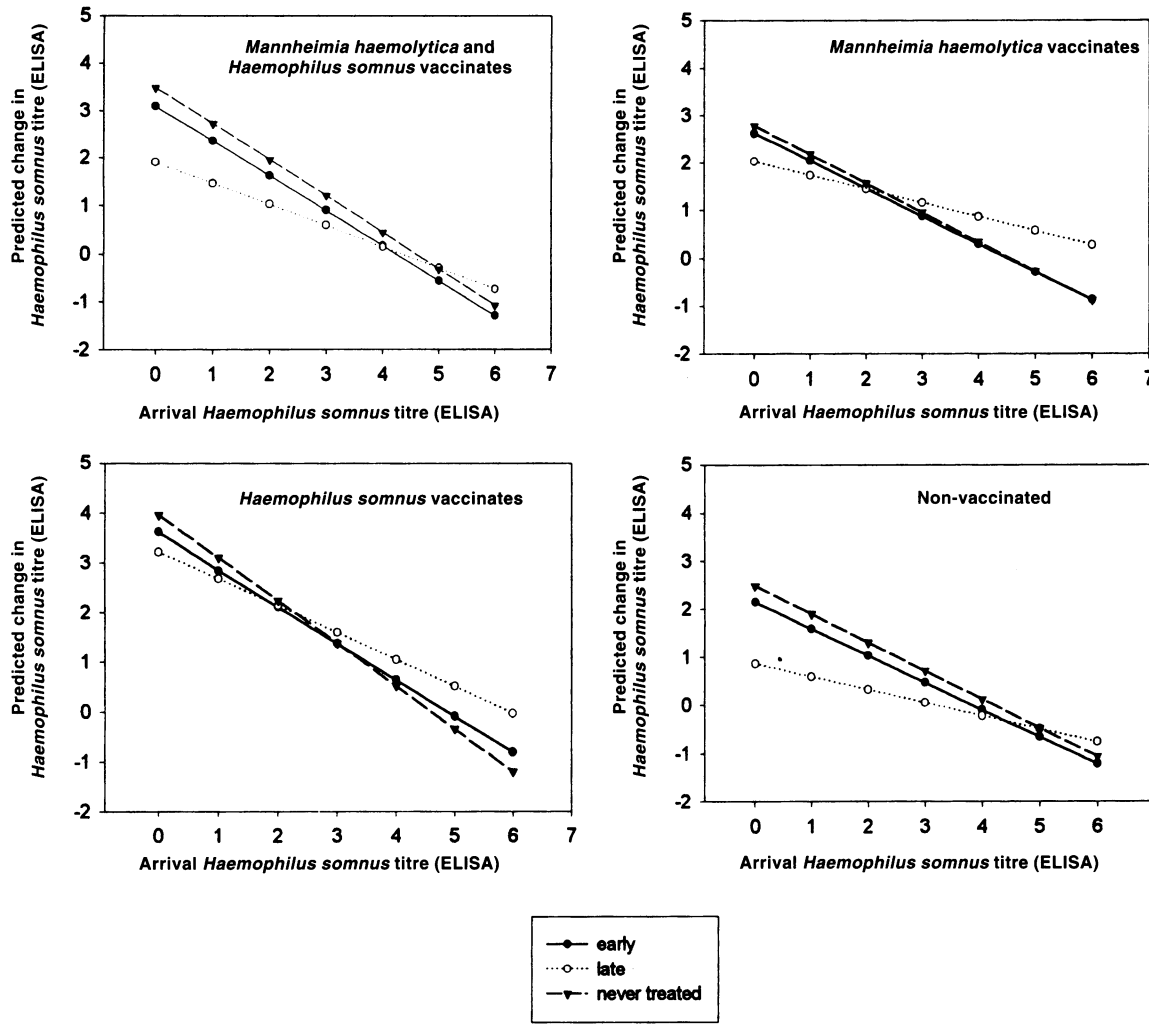

Figure 2. The effect of treatment timing and vaccination group of the predicted behaviour of *Haemophilus somnus* ELISA titer for cattle at 3 Ontario feedlots in 1998.

those that needed to be treated for UBRD only once. Calves that were diagnosed and treated prior to day 9 had larger *H. somnus* titer increases than those initially diagnosed and treated after day 9, but titer changes in relapsed calves did not differ by time of initial treatment (Table IV). The average *H. somnus* titer at arrival for the 3 UBRD timing groups did not differ (results not shown).

In the mixed regression models, UBRD treatment and UBRD TIME were not associated with changes in *M. haemolytica* titers (Table V). However, UBRD treatment and UBRD TIME were significantly associated with *H. somnus* titer change. The associations between timing of treatment and change in *H. somnus* titer are shown in Table VI. Because the interaction effects are not obvious, the predicted titer changes in these models are plotted in Figure 2. Essentially, untreated calves had similar titer increases to calves treated in the first 9 d regardless of vaccine group. Calves with low arrival titers to *H. somnus*, and treated on or after 10 d post arrival, had smaller titer increases than the former 2 groups of calves. *Haemophilus somnus* vaccination increased the *H. somnus* titer change over that in non-vaccinated calves; calves vaccinated with only *M. haemolytica* had intermediate *H. somnus* titer increases. Vaccination with *M. haemolytica* antigens was associated with increased titer change to *M. haemolytica* leukotoxin ELISA ($P < 0.05$), however not to the *M. haemolytica* indirect agglutination or *M. haemolytica* leukotoxin neutralization titer changes (results not shown).

Discussion

If change in titer represents exposure to the agent during the UBRD risk period, then exposure to *H. somnus* was common, as > 30% of unvaccinated animals seroconverted. This titer change may be due to initial exposure or re-exposure to commensal organisms sufficient to stimulate a circulating immune response. The average change in *H. somnus* titer was 1.8 ± 1.6 (mean ± SD), and vaccination with *H. somnus* antigens increased the magnitude of titer change by 1 to 1.5 dilutions. Thus, calves appear to respond to both natural and artificial challenge with *H. somnus* antigens in a predictable manner.

In earlier studies, current exposure to *H. somnus* did not appear to be as frequent because the average titer change was −0.36 ± 0.97 (1). However, the prevalence of antibody titers to *H. somnus* at arrival, in the current study, was also lower than previously reported. In 1998, Martin et al (1) reported that all animals studied (n = 602) had detectable *H. somnus* titers at arrival and that the

Table III. Frequency distribution of transformed titers to *Mannheimia haemolytica* and *Haemophilus somnus* at arrival for cattle at 3 Ontario feedlots in 1998

Titer	*M. haemolytica* leukotoxin ELISA No. (%)	*M. haemolytica* indirect bacterial agglutination No. (%)	*M. haemolytica* leukotoxin neutralization No. (%)	*H. somnus* ELISA No. (%)
0	134 (16.2%)		1 (0.2%)	282 (34.1%)
1	70 (8.5%)		3 (0.5%)	71 (8.6%)
2	182 (22%)	6 (0.7%)	8 (1.3%)	177 (21.4%)
3	284 (34.3%)	28 (3.3%)	19 (3.2%)	165 (19.9%)
4	136 (16.4%)	119 (14%)	20 (3.3%)	97 (11.7%)
5	21 (2.5%)	221 (26%)	58 (9.7%)	32 (3.9%)
6	1 (0.1%)	249 (29.3%)	67 (11.2%)	4 (0.5%)
7		152 (17.9%)	55 (9.2%)	
8		44 (5.2%)	91 (15.2%)	
9		24 (2.8%)	129 (21.5%)	
10		6 (0.7%)	32 (5.3%)	
11		1 (0.1%)	12 (2.0%)	
12			15 (2.5%)	
13			28 (4.7%)	
14			17 (2.8%)	
15			16 (2.7%)	
16			15 (2.5%)	
17			13 (2.2%)	
18			1 (0.2%)	

Table IV. The change in *Haemophilus somnus* titers for calves, stratified by timing of initial treatment (Early $<$ 10 d after arrival; Late $\geq$ 10 d post arrival) and number of times treated at 3 Ontario Feedlots, 1998

	Once		Relapsed	
Timing of treatment	*n*	mean ± SD	*n*	mean ± SD
Early	64	2.3 ± 0.2^{ac}	35	1.7 ± 0.2^{bc}
Late	46	1.6 ± 0.2^{ad}	17	1.2 ± 0.3^{ac}

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

[c,d] Means in the same column with the same superscript do not differ significantly at $P < 0.05$

average arrival titer for *H. somnus* was 7.5 ± 1.6, considerably higher than the titer of 1.8 ± 1.5 in this study. This discrepancy may be due to true differences between the groups of animals studied, a different starting dilution between the serological tests, or the method of titer transformation used for analysis (1). This highlights the importance of examining titers as relative measures of exposure within a study, rather than as absolute measures of antibody levels that can be compared across studies.

Untreated calves had lower *H. somnus* titers than treated calves. Authors of previous studies have reported a similar relationship between *H. somnus* titer change and UBRD occurrence (1,2). However, the fact that calves that were treated before day 10 had similar titers to untreated calves, and that calves treated after day 9 had lower titers than untreated calves, has not been reported. Calves with a low arrival titer showed evidence of current exposure while those arriving with a high titer had little or no titer changes and these associations did not differ between treated and untreated calves (Figure 2). Regardless of arrival *H. somnus* titer, calves that were not vaccinated and received treatment for UBRD 10 or more days after arrival displayed little or no evidence of titer change to *H. somnus* antigens. The low magnitude of the titer change is consistent with no exposure to *H. somnus*. Within the "*H. somnus* only" vaccinated group, the late-treated calves had similar titer increases to those in the early- and never-treated groups. Therefore, animals that received treatment for UBRD late or those that relapsed, had the smallest increases in *H. somnus* titer, thus the least evidence of current exposure. These observations suggest that *H. somnus* is not a cause of these UBRD cases.

We suggest a possible explanation for the observation that in unvaccinated calves there was evidence of exposure to *H. somnus* in animals treated early, but not in animals treated after day 9, post arrival, based on the duration of antimicrobial exposure. First, unvaccinated calves that were exposed twice to antimicrobials, at least 10 d apart (first prophylactically and then therapeutically), may have had a decreased exposure, or extent of infection, to *H. somnus*, and thus a lower likelihood of an antigen exposure sufficient to trigger an immune response. In contrast, untreated animals were exposed to antimicrobials only once and thus may have had re-infection or a continuing exposure after arrival. Despite receiving 2 doses of antimicrobials (at arrival and about 5 to 7 d later at treatment), animals treated early had the same change in *H. somnus* titer as those animals that were never treated, perhaps because any inhibition of colonization caused by the antimicrobials had "worn off" in sufficient time for them to be re-exposed in the later period post arrival. This pattern was not seen in vaccinated calves because vaccination exposed animals to an antigen challenge that

Table V. Regression coefficients for the association of treatment for undifferentiated bovine respiratory disease (UBRD) with titer changes to *Mannheimia hemolytica* and *Haemophilus somnus*

Outcome		Regression coefficient[a]	SE	*P* value
UBRD treatment				
Change in *M. haemolytica* leukotoxin ELISA titer		0.02	0.07	0.7
Change in *M. haemolytica* leukotoxin neutralization titer		0.03	0.4	0.9
Change in *M. haemolytica* indirect agglutination titer		−0.01	0.1	0.9
Change in *H. somnus* ELISA titer		−0.4	0.09	0.000
UBRD time				
Change in *M. haemolytica* leukotoxin ELISA titer	Early	0.1	0.1	0.3
	Late	0.04	0.1	0.8
Change in *M. haemolytica* leukotoxin neutralization titer	Early	−0.8	0.5	0.9
	Late	−0.2	0.7	0.8
Change in *M. haemolytica* indirect agglutination titer	Early	0.2	0.2	0.3
	Late	0.07	0.3	0.8
Change in *H. somnus* ELISA titer	Early	−0.0	0.1	0.9
	Late	−0.5	0.2	0.008

[a] The never-treated group is the referent. The coefficient describes the change in the outcome for each category change in UBRD or UBRD TIME

was not affected by antimicrobials, hence their antigen challenge continued and led to titer increases. This may explain why the late-treated vaccinated animals had higher titers than the unvaccinated animals, but not as high as those that were presumably naturally re-exposed during the study period, i.e., the early- and never-treated animals. Feedlot C did not use prophylactic antibiotics; therefore, antibiotic exposure could not have been prolonged, nor did any animals that this feedlot require treatment for UBRD twice. Consequently, the association between titer change and both treatment timing and number of times treated was driven by the data from the other 2 feedlots.

If prolonged exposure to antimicrobials resulted in decreased exposure to *H. somnus*, this may also explain why animals that relapsed tended to have less evidence of *H. somnus* exposure than animals treated once. These animals were exposed to a higher dose of antibacterial agents over a longer period of time than others; in effect, the relapsed animals were exposed to antimicrobials throughout the post arrival period. Pharmacologically, however, there is no evidence that the antibacterial agents used to control or treat UBRD were so effective against *H. somnus* that they should inhibit exposure for such a long period of time. An alternative hypothesis would be that animals treated late had their "convalescent" titer collected closer to the time of infection; therefore, these animals were unable to demonstrate large titer changes. However, given that the majority of the late-treated animals were treated at least 10 d prior to sample collection, this seems unlikely (Figure 1).

Exposure to *M. haemolytica* was common prior to arrival and during the study period and we had anticipated a significant relationship between titer change to *M. haemolytica* and UBRD, because such a relationship has been reported previously (2,7). However, none was observed in this study. Initial exposure to *M. haemolytica* occurs with dam/calf contact early in life, at which time the organism colonizes the nasal passages of the animal (8). This contact results in exposure to antigens associated with adherence to the nasopharygeal mucosa and the indirect agglutination titer is a measure of this contact. On the other hand, *M. haemolytica* leukotoxin titers are less likely to develop as a result of this contact, and Shewen (8) suggests that leukotoxin titers develop primarily after deeper colonization, i.e. lower respiratory tract colonization, and, therefore, are a measure of pulmonary exposure to *M. haemolytica*. It appears that the majority of animals had experienced both nasopharyngeal contact and pulmonary exposure to *M. haemolytica* prior to arrival, unlike in previous studies (7). The high prevalence of previous exposure and concurrent exposure may have mitigated against detecting an association between titer change to *M. haemolytica* and UBRD occurrence in this study. The difference in the apparent prevalence of titers to the leukotoxin may have resulted from different techniques and the high initial starting dilution used in the ELISA test. We have no explanation as to why the *M. haemolytica* vaccines did not stimulate significant titer increases in the neutralization of agglutination tests; however, this observation may explain why there were no significant titer increases in calves with UBRD. These observations are not without precedent, however, as only small differences in titer between experimentally challenged, with *M. haemolytica*, and unchallenged calves have been observed (9). There is some evidence (Table VI) that vaccination against *M. haemolytica* may also have induced cross-reacting *H. somnus* antibody production, suggesting either some cross-protection or sharing of some common antigens between these organisms.

A sparing association between *H. somnus* and *M. haemolytica* titers at arrival was also seen in the model describing subsequent UBRD, although we have not taken this to be evidence that *H. somnus* could be causally related to the UBRD occurrence. An accompanying paper discusses in more detail why evidence of protection from arrival titers alone should not be used as a basis for causal inference in UBRD. Briefly, arrival titers to many organisms, including

Table VI. The association between timing of treatment for undifferentiated bovine respiratory disease (UBRD) and the change in *Haemophilus somnus* ELISA titer in cattle at 3 Ontario feedlots in 1998

Variable Main effects	Levels	Regression coefficient	SE	*P* value
Intercept		2.5	0.2	0.009
H. somnus titer at arrival * Vaccine		−0.6	0.04	0.00
	H. somnus and *M. haemolytica* vaccine	1.0	0.2	0.00
	H. somnus vaccine	1.5	0.2	0.00
	M. haemolytica vaccine	0.3	0.2	0.08
	Non-vaccinated	0.00	—	—
Timing of treatment for UBRD	Early (< 10 d)	−0.3	0.2	0.1
	Late (≥ 10 d)	−1.6	0.3	0.00
	Never	0.00	—	—
H. somnus titer at arrival * Vaccine	*H. somnus* and *M. haemolytica* vaccine	−0.2	0.06	0.004
	H. somnus vaccine	−0.3	0.06	0.00
	M. haemolytica vaccine	−0.02	0.06	0.08
	Non-vaccinated	0.00		
Early (< 10 d) * Vaccine	*H. somnus* and *M. haemolytica* vaccine	−0.06	0.3	0.8
	H. somnus vaccine	0.003	0.3	0.9
	M. haemolytica vaccine	0.2	0.3	0.5
	Non vaccinates	0.00	—	—
Late (≥ 10 d) * Vaccine	*H. somnus* and *M. haemolytica* vaccine	0.05	0.4	0.9
	H. somnus vaccine	0.9	0.4	0.01
	M. haemolytica vaccine	0.9	0.3	0.01
	Non-vaccinated	0.00	—	—
H. somnus titer at arrival *	Early (< 10 d)	0.04	0.07	0.6
TIme of treatment for UBRD	Late (≥ 10 d)	0.3	0.09	0.00
	Never	0.00	—	—

bovine respiratory syncytial virus, bovine coronavirus, *Mycoplasma dispar*, and *Mycoplasma bovis* have been found to be sparing of UBRD without subsequent evidence that actually concurrent infection or exposure is associated with the disease (2,13). Therefore, we believe that this criterion should not be used for establishing causal inferences.

With respect to study design, the sensitivity and specificity of the serological tests, and of the diagnostic process were unknown, but generally, any misclassification would bias the study findings toward the null hypothesis. This is perhaps another reason for the failure to find an association between *M. haemolytica* titer change and UBRD occurrence (6,10–12,). In further studies of this nature, there may be value in reducing the sampling interval to at most 2 wk. The specific sampling regime used by Booker et al (2), in which samples were taken from UBRD cases at the time of diagnosis and from an eligible control animal at that time, is also an excellent format. However, many owners are not willing to subject their "non-sick" calves to the stress of movement and bleeding. If this sampling design is used, it is important to retain the "at time of case" samples from control calves that subsequently become cases of UBRD, consistent with the incidence density sampling design, when making inferences about titer at that time. In addition, identifying the isotope of antibody associated with the titer would address concerns of possible bias in the serological tests and the effect that different isotypes, arising from primary or subsequent exposure, may have had on the study results.

As an overall summary, we found the same relationship between *H. somnus* titer change and UBRD occurrence as was reported previously (i.e. treated animals tend to have lower titers to *H. somnus* relative to untreated animals). However, by examining the effect of timing of treatment and using vaccine induced titers to compare across groups, we were able to determine that this relationship was limited to calves treated after day 9 post arrival. We also found evidence that animals that relapsed had decreased titers to *H. somnus*. We suggest that exposure to antimicrobials over a prolonged period may be the common factor limiting infection with *H. somnus* in these 2 groups of animals. Further, we conclude from this evidence, and the results of previous studies, that *H. somnus* was not associated with UBRD occurrence, and is unlikely, therefore, to be a cause of UBRD in feedlot calves in Ontario. We failed to find an association between titer changes to *M. haemolytica* and UBRD occurrence; however, vaccination with *M. haemolytica* antigens was associated with reduced UBRD. This would suggest, consistent with the literature, that *M. haemolytica* is causally associated with UBRD occurrence, despite the lack of confirmatory associations between UBRD and titer change.

Acknowledgments

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

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