A SEROEPIDEMILOGICAL INVESTIGATION OF UNDIFFERENTIATED BOVINE RESPIRATORY DISEASE

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by

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ABSTRACT

A SEROEPIDEMILOGICAL INVESTIGATION OF UNDIFFERENTIATED BOVINE RESPIRATORY DISEASE

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This thesis investigated the statistical association of titres to *Pasteurella hemolytica, Hemophilus somnus,* bovine viral diarrhoea virus and bovine corona virus, with undifferentiated bovine respiratory disease (UBRD) at three feedlots in Ontario, Canada. The prevalence of exposure to the agents prior to arrival at the feedlot and the incidence of infection during the study period were estimated using the "proxy" variables, arrival titre and change in titre. Titres to *Pasteurella hemolytica* and bovine viral diarrhoea virus were examined to elucidate their behaviour. However as more is known about the sero-epidemiology of these two organisms they also represented a point of reference for the behaviour of the *Hemophilus somnus* and bovine corona virus titres.

A factorial design was used to randomise vaccination against both *Hemophilus somnus* and *Pasteurella hemolytica.* The nonvaccinated (for each antigen) animals served as monitors of natural infection.

Higher arrival titre to all agents were sparing for subsequent disease risk. It was suggested that *Pasteurella hemolytica* and bovine viral diarrhoea virus were causally

related to UBRD because change in titre was associated with increased UBRD risk in this or other studies. For *Hemophilus somnus* and bovine corona virus, no evidence existed that infection was associated with increased risk of UBRD treatment.

Animals treated for UBRD late in the study period tended to show little or no evidence of exposure to *Hemophilus somnus*. As this was not observed for *Pasteurella hemolytica* titres, this suggested that exposure to *Hemophilus somnus* was inhibited in animals receiving additional antimicrobials for UBRD treatment.

The conclusion was drawn that *Hemophilus somnus* and bovine corona virus were not causally related to UBRD occurrence. Higher arrival titres *Hemophilus somnus* and bovine corona virus may indicate a functioning immune system in these calves, rather than indicating that titres are protective against the specific organism causing subsequent disease.

Dedication

For Mum and Dad

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None of us ever anticipated that I would continue to formally study for quite this long but it has been worthwhile. Thank you.

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Chapter 1 Introduction

Undifferentiated bovine respiratory disease (UBRD) continues to represent a significant disease in the North American feedlot industry. Estimates of the annual cost to the United States feedlot industry of deaths due to UBRD approach $1 billion (US) and the cost of preventative and therapeutic measures to deal with UBRD are estimated at $ 3 billion (US) (1). In the United States, as of the 30th January 2000, approximately 14 million cattle where on feed, while in Canada, 1.5 million cattle were on feed in western Canada at this time (2,3). If the cost of UBRD in western Canada feedlot industry is proportion to the USA estimates, then death costs amount to approximately $10 million US and treatment and preventative measures $30 million US. Although the cost of UBRD seems large, health related costs still only represent approximately 8% of the total cost of production, while feed costs make up around 80-90% of the cost of production. It would seem that huge changes in profitability are not likely to occur with improved health. However, since the feedlot industry is characterised by low per head profit margins and large numbers of cattle to maintain economies of scale, fluctuations, either increases or decreases, from the anticipated cost of UBRD per head on a feedlot can significantly affect profit margins (4). UBRD also represents a welfare issue in beef cattle that needs to be continually addressed.

Treatment for clinical respiratory disease in feedlots is often successful and has traditionally been used to the control the impact of UBRD (5). However, it has become increasingly apparent that a large number of animals with UBRD go undetected

throughout the feeding period (1,6). This does not diminish the importance of antibiotic treatment for identified clinical cases as a control measure for UBRD. However, it does highlight the importance of the prevention of UBRD, because unidentified cases do not benefit from these treatments and probably constitute an additional cost to production of beef (6).

The key to the prevention of any disease is the identification of factors that are associated with its occurrence. In UBRD, many factors are known to be risk factors, most importantly the placement of calves that are in transition from nursing to weaning into feedlots. If this class of animals was eliminated from the market then UBRD would be a much lesser concern. Yearling animals and countries that do not feed young calves have considerably lower UBRD treatment rates (7,8). However, because of the nature of the North American beef cattle production cycle, this option is not readily available to many feedlots. Therefore, research has focused on identifying the other component causes of UBRD in this class of cattle, in particular the identification of the causal agents of UBRD.

At present a "shotgun" approach to the prevention of UBRD is taken by many feedlots. This includes prophylactic treatment of all animals at arrival with antimicrobials and the administration of multiple vaccines. Vaccines presently targeted at the feedlot industry for the prevention of UBRD are aimed at providing protection against *Pasteurella haemolytica*, *Haemophilus somnus,* infectious bovine rhinotracheitis, bovine viral diarrhoea virus, bovine parainfluenza-3 and bovine respiratory syncytial virus infection. For some of these vaccines there is evidence of efficacy (9,10) . The

diversity of vaccines on the market and the continued importance of UBRD suggest that the epidemiology of these agents in UBRD is not clear.

Two agents recently incriminated as possible causal agents of UBRD are *H. somnus* and bovine corona virus. With regards to *H. somnus,* recently published studies had reported an association between smaller or no titre change to *H. somnus* with increased risk of undifferentiated bovine respiratory disease (UBRD) (11,12). This finding, though consistently reported in the literature, is incongruous with the present interpretation of titre changes used in sero-epidemiology and our understanding of the role of *H. somnus* in UBRD (13,14). The role of bovine corona virus was investigated because this virus has been causally associated with UBRD occurrence. For example, in a recent publication on respiratory disease in cattle, it was stated that "Bovine respiratory coronavirus is an emerging pathogen causing upper and lower respiratory disease in feedlot cattle' (15) . This statement implied that the causal association between UBRD and BCV has been clearly established, although critical evaluation of the literature would suggest that this is not the case (15,16,17).

Therefore, the aim of this project was to examine in detail the behaviour of titres to *H. somnus* and bovine corona virus and, hopefully, to come to more solid conclusions about their role in UBRD. These agents have been implicated as agents of UBRD, using a variety of study techniques, including experimental studies, case studies and observation studies; however, definitive evidence for their role in UBRD is still lacking. Titres for other agents, *P. haemolytica* and bovine viral diarrhoea virus, were also examined because more is known about their sero-epidemiology in relation to UBRD and thus they provide a reference point for examination of the other titre's behaviour. The

hope being, in the long run, that if the agents that cause UBRD can be more definitively determined, then preventative UBRD programs could be targeted at particular agents with increased efficacy, rather than the present "shotgun" approach to UBRD prevention.

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Chapter 2 Background and Literature Review

2.1 An Introduction to Undifferentiated Bovine Respiratory Disease

Undifferentiated Bovine Respiratory Disease (UBRD) is a disease complex affecting all classes of cattle but the term is most commonly reserved to describe respiratory disease in feedlot cattle. In feedlot cattle, UBRD is a diagnosis achieved by elimination i.e., a dull, usually anorectic, animal with an abnormal respiratory pattern without clinical signs attributable to other body systems is usually assigned a diagnosis of UBRD. Thus, UBRD represents a complex of diseases that may include pneumonia, pleuritis, myocarditis, laryngitis and tracheitis (1). These diseases may be caused by multiple agents, working alone or together, including, but not limited to, *Pasteurella haemolytica*, *Haemophilus somnus*, parainfluenza-3 virus (PI-3), bovine viral diarrhoea virus (BVDV) and infectious bovine rhino-tracheitis virus (IBR). In addition, a variety of stressors can act as component causes of the syndrome. The non-specific clinical and pathologic nature of the disease complex coupled with the possible presence of multiple infectious agents make the study of UBRD extremely difficult. Due to its broad definition UBRD has also been referred to as undifferentiated fever (UF) or just bovine respiratory disease (BRD).

Although UBRD occurs in all classes of feedlot cattle its major impact is in recently weaned, lightweight (200- 400 KGs) calves where overall treatment rates vary from 10 to 50%, with 65% to 80% of these treatments attributed to UBRD (2,3,4).

Overall mortality rates vary from 0 to 10%, with more extreme numbers occasionally reported, and estimates of the percentage of fatalities attributable to UBRD ranging from 30 to 70% (2,3,4). The proportion of mortalities that are attributable to UBRD varies widely because of differences in disease occurrence in particular years and the definition of what represents a UBRD death (1,3,5). In comparison, yearlings rarely have greater than 10% UBRD treatment rates and mortality from UBRD is rare (6).

As alluded to earlier, calves are susceptible to UBRD due to the interplay between host, infectious agent and environmental factors during the transition period from nursing to weaning to independent feeding. During this transition calves show an increased susceptibility to disease because they are stressed and may have decreased nutrient intake due to unfamiliar food sources and feed delivery systems. These stresses are coupled with increased exposure to pathogens due to mixing and transportation. Even well managed pre-weaned calves are susceptible to UBRD because other risk factors, such as large fluctuations in temperature, are likely to occur at the time when most calves are marketed (5).

The epidemic curve of treatment for UBRD has been reported. The majority of cases are treated in the first twenty days post-arrival, and treatment rates peak at around 6- 10 days post-arrival (6,7). In addition to morbidity curves, veterinarians and feedlot managers often plot the first day of treatment for animals that subsequently die; these plots are referred to as the day of fatal disease onset (FDO) curves (2,8). These cause-specific mortality epidemic curves show that, for most common causes of death, the median day of FDO occurs in the first 10 to 20 days post-arrival (Table 2.1).

2.1.1 The Role of Study Design and Diagnostic Tests in the Investigation of Causes of UBRD.

Undifferentiated bovine respiratory disease has been studied extensively. The majority of published papers have focused on the pathogenesis and aetiology of the disease complex, and within these fields the majority of studies have been experimental (9). Other large-scale observational studies and clinical field trials have had as the general aim to establish what "causes" UBRD.

When making casual inferences, all study designs have limitations, however, some are able to provide more information than others about a particular disease. Also within study designs, diagnostic tests or tools (surveys) used to differentiate diseased from non-diseased animals or the presence or absence of exposure are inherently not 100 % sensitive or specific (10). For UBRD studies the diagnostic tests used to establish exposure status to pathogens have traditionally been pathogen culture from the respiratory tract or putative agent titres. Studies based on culture results have been problematic because of difficulties associated with correctly distinguishing case from control animals and therefore this approach has not yielded useful results for making causal inferences. Culture is also labour intensive and apart from *P. haemolytica* has been used for few studies of interest in this thesis.

The use of antibody titres in sero-epidemiological studies does not overcome the problem of disease misclassification, however serology has other advantages over culture. Serum collection is significantly easier than naso-pharyngeal swabs (NPS) and bronchoalveloar lavage (BAL) and it avoids difficulties trying to culture pathogens that are hard to isolate such as *H. somnus* (this is made more difficult by the frequent use of

antibiotics in feedlots). Also the presence of antibodies to a large number of agents may be detected using a single sample and quantitative estimates of the level of antibodies can be derived by titration i.e., high or low titre. However, this approach has not overcome all of the difficulties associated with studying this complex disease.

As discussed, the definition of a "UBRD case" is subjective and depends on the personnel selecting diseased animals. The extent to which disease misclassification occurs in feedlot studies using this system has been investigated. A recent longitudinal study examined treated and untreated feedlot steers at the abattoir and found that 78% and 68% of treated and untreated animals respectively had lung lesions at slaughter (11). This study also found that the presence of lung lesions at slaughter was associated with a 0.076-kg reduction in daily weight gain during the study period, suggesting that the lesions were biologically significant and affected production (11). A review paper suggests that several other studies have reported similar findings (12). This suggests that the sensitivity of disease classification is at best moderate, i.e. the probability of an animal with UBRD being detected and treated is only fair. Unfortunately, abattoir studies are not useful in determining the specificity of disease classification. The same study reported that many previously treated animals (22%) showed no lung lesions at slaughter. The authors correctly concluded that this does not reveal information about the specificity of disease classification. The authors point out that the disease classification may have been correct and treatment resulted in the prevention of permanent tissue damage (11). Although no published information exists, it would seem likely that the specificity of disease classification in feedlots is probably high, i.e. the probability of a well animal being identified as well is high (13). The effects of this misclassification on

statistical analysis of the data are discussed in detail in Section 2.3.1. However generally, nondifferential misclassification of disease status results in a bias of study results towards the null, so associations found are actually likely to be stronger than reported. Nevertheless, associations of a small magnitude will be difficult to detect in low power studies.

Researchers have attempted to improve the differentiation of "cases" from "controls" in feedlot studies by refining the criteria that represent a case. To remove the subjectivity associated with the selection of animals pulled from the pen scoring systems based on attitude, respiratory rate, gut fill, the presence of coughing or nasal discharge are used. Some researchers leave the "pulling" to the subjective assessment of the pen riders and try to discriminate cases from controls at the chute-side; using elevated rectal temperature as a means of identifying cases (14). Although the presence of rectal temperature above 105.0^0F at arrival is associated with increased subsequent risk of UBRD, it is not clear how useful the commonly used cut-off of 104.5^0F at case selection is at distinguishing diseased animals from non-diseased animals (15). Finally, some researchers distinguish cases from controls retrospectively using the measurement of other serum factors such as fibrinogen (16). Most use a combination of several systems (17,18). These "systems" aim to improve the sensitivity and specificity of disease classification, but the effectiveness of the " systems" has not been investigated (19).

2.2 What is Sero-epidemiology, and How Is It Used, With Reference to General Infectious Disease Investigation and The Study of Undifferentiated Bovine Respiratory Disease ?

Sero-epidemiology refers to the study of diseases in populations using serological data. Sero-epidemiology has historically been used with the greatest success to describe the epidemiology of diseases associated with a single causal agent that produces obvious clinical signs ensuring that the timing of disease occurrence is known. Examples of diseases with these characteristics include small pox and chicken pox (20). This information enables the establishment of a clear association between the humoral response and infection. The sensitivity and specificity of the serological test can then be established and the credible serological test made available for use in large population studies. In recent years, serological epidemiological studies have been extended to study more complex diseases and the interpretation of the data is more problematic.

In most feedlot studies the use of serological data has been aimed at establishing associations between putative agents and the disease complex, using the titre as a proxy for evidence of exposure to the agent. Antibody responses usually are expressed as titres or the proportion of the study population that are deemed to be sero-positive or sero-negative. The cut-off point (titre level or signal to positive ratio) representing sero-positivity or sero-negativity is made subjectively and often by the individual researcher. Ideally, the information reported from a prevalence serological study (data collected at one point in time) should include the frequency distribution of titres, percentage sero-positive and sero-negative, geometric or arithmetic means of titres and standard deviations (depending on coding used) (21). In longitudinal studies, this information

should be available for each point in time measured, as well as the frequency distribution of titre changes and the percentage of animals that sero-converted during the full study period. Since the definition of sero-conversion is inconsistent in the literature, being described as a change from sero-negative to sero-positive, a four-fold increase in titre, or a 2-dilution change in titre, there is a need for authors to specify the definition used (21,22,23,24) . A longitudinal study, looking at titre changes over a defined period of time, provides information about the incidence of infection.

Sero-epidemiology is also used to provide data in an attempt to associate a humoral response (antibody titre) to an agent with disease occurrence, susceptibility or resistance, i.e. risk factor analysis. This type of study may aim to establish that a particular agent is associated with disease or that a particular test is useful in diagnosis of a disease. The former use may occur early in disease investigation when disease agents are unknown, the latter is more likely when the disease agent is known but diagnosis is difficult. In either case, it should be remembered that statistical associations between a titre and disease do not imply a causal relationship between the agent and the disease (discussed later in Section 2.2.1).

Prevalence studies frequently associate susceptibility with the absence of, or low titres to, the putative disease agent, and resistance with the presence of, or high titres to, a putative disease agent. In making causal inferences, this simplistic interpretation ignores the possibility that presence of a titre does not equate to resistance. Furthermore, even in diseases where an association between the presence of antibodies and resistance to a disease has been established, single titre measurements are difficult to interpret due to the varying effects of primary and anamnestic responses to antigen exposure on titre levels,

the temporal association of primary or anamnestic response with disease occurrence and the effect that various subclasses of antibodies may have on test results. Data from longitudinal sero-epidemiology surveys can identify associations between an increase in titre and disease occurrence. In the simplest terms, the increase in titre is seen as evidence of recent or current exposure or infection. For commensal organisms the difference between exposure and infection can be confusing, as these animals are already infected and the change in titre must be due to renewed contact between the organism and the animal that triggers an immune response i.e., re-exposure. To avoid this linguistics problem for the purposes of this thesis, exposure and infection are used interchangeably and defined to mean that the animal comes into contact with the organism in a manner that is sufficient to trigger an antibody response. It is understood that this contact could result from initial infection, re-infection or re-exposure, as would occur with commensal organisms such as *P. haemolytica*

2.2.1 The Appropriate Interpretation of Titres in Sero-epidemiological Studies

When interpreting serological data it is important to bear in mind that immunity and the presence of antibodies are not the same thing, although they are frequently related (20). Furthermore, titre change does not indicate clinical disease. The results of exposure may be clinical or sub-clinical disease or non-consequential changes in health status. Inconsequential exposure with one agent may occur concurrently with a significant infection with another organism. In addition, it is not possible without detailed serial sampling and antibody typing to determine if a change in titre represents a primary or anamnestic response to antigen exposure.

With reference to the agents being studied for this thesis very little information is available on the timing and duration of serum titres especially in feedlot calves. For *P. haemolytica,* studies of indirect agglutination titres in 8-week-old calves suggest that animals with low titres respond to exposure by the production of serum antibodies reliably within 14 days of exposure , but not reliably within 6 days of exposure. Animals with high titres prior to exposure showed no increase or a decrease in titres within 3 weeks of exposure (25). Confer *et al* (26) examined the response to vaccination with 5 different *P. haemolytica* bacterins and live *P. haemolytica*. Animals were vaccinated at day 0 and day 7 and all animals (n = 68) had low (<10) *P. haemolytica* somatic antigen and leucotoxin neutralisation titres at the start of the experiment. All titres remained low or unchanged after exposure, but repeat exposure to all bacterins and the live bacteria at 7 days caused large increases in *P. haemolytica* somatic antigen titres. Leucotoxin neutralisation titres increased only after repeat exposure to live *P. haemolytica* (26). Unfortunately, Confer *et al* (26) did not examine the effect of exposure on animals with pre-existing titres. With reference to the sero-epidemiology of UBRD, this would suggest that titres at arrival to *P. haemolytica*, could be a function of exposure that occurs during transit from the farm to the feedlot due to initial infection or re-exposure to a commensal organism; however, this is not known. For purposes of the current study on UBRD we will infer that antibody levels on arrival probably reflect past infection with the organism, although the possibility exists in animals that have been transported for a long time e.g. from western to eastern Canada , that arrival titres are due to current infection. We also infer that increasing titres after arrival indicate current exposure or recent exposure during transit, although it is possible that animals with very long transit

times may have sero-converted by the time of initial sample collection and will show titre decreases or no change.

2.2.2 The Role of Study Design in Providing Information About The Sero-epidemiology of Undifferentiated Bovine Respiratory Disease

The most difficult aspect of sero-epidemiology is establishing a causal association between the titre (arrival or change in) and the disease. As mentioned previously, three main types of studies have been used to determine associations between serological data and UBRD. Their contribution to our knowledge of UBRD will be reviewed in turn.

2.2.2.1. The Use of Serological Data From Experimental Studies Investigating Undifferentiated Bovine Respiratory Disease

Experimental studies involve the use of UBRD models in highly controlled settings. Cattle are exposed to known agents, the serological response is measured and then a determination of any association between the occurrence of disease and the measured titre is pursued. In these studies researchers are able to control the number of agents to which the animals are exposed and they have knowledge about the previous exposure history of the animals (usually sero-negative animals are used), avoiding some of the discussed previously difficulties associated with interpreting titres (Section 2.2.1). However, these studies frequently lack the statistical power to detect differences that may be important when applied on a larger scale. This is because experimental studies are often small and, as such, have large variances in their measure of association estimates. Also, the cattle are frequently from one herd and thus respond very similarly to the challenge (and as such, have high intraclass correlation coefficients). Also, as many

variations exist in the challenge models (dose, route, age of animal and species), extrapolation of the results of experimental studies to the "real" disease process and "real" population is questionable (27). Furthermore, the variety of assays, antigens and protocols used make comparisons across studies invalid (28). Even though experimental studies provide useful information in the study in UBRD direct extrapolation of experimental findings to the field disease should be done with due consideration of these limitations.

2.2.2.2. The Use of Serological Data From Observational Studies Investigating Undifferentiated Bovine Respiratory Disease

Observational feedlot studies are another means of determining associations between the humoral immune response to an organism and UBRD. The humoral response may be interpreted to indicate the prevalence of prior exposure at arrival (arrival titre) or the incidence of exposure during the study period (change in titre).

This style of study may have greater external validity because the disease is the field UBRD, but the complex nature of the disease and the various feedlot contexts in which investigations take place mitigate against internal validity. Thus, the results of a study may be questionable if confounding variables, factors associated with the titre (arrival or change) and the study outcome, are not controlled through design or analysis. Fortunately confounders may be effectively controlled by several methods. At the design stage excluding known confounders from the data removes their effect on estimates of association. For example, if breed or region of origin were known to be factors affecting UBRD occurrence then the study design may restrict the study to one breed or region,

thereby excluding that confounder. Matching on a confounder will also control for confounding i.e. selecting a case and a control matched by breed or region.

However because most confounders in feedlot studies are not easily removed at the design stage, observational studies tend to control for confounders by including them in the statistical analysis and therefore "controlling" for their effect on the outcome of interest and the explanatory variable of interest. When controlling confounders at the analysis stage, the aim is to examine the effect of the "confounding variable" on the variables of interest. This is done by adding or removing the "confounding variables" and looking for changes in the measure of association (the coefficient) between the variable of interest and the outcome. If the coefficients of the variables of interest are not changed by the addition or removal of the "confounder variable" then that variable is not a confounder i.e. its presence is not biasing the association of the variable of interest with the outcome. The disadvantage of controlling for confounding variables at the analysis stage of the study is that many variables, those of direct interest to the study and potential confounders, need to be measured on many cattle, making this type of study prohibitively expensive. Also, unknown confounding variables can not be measured and therefore controlled at either the design or analytical stage.

Finally, because the feedlot environment is not controlled, exposure to multiple organisms usually occurs during the study period resulting in changes in titres to those organisms. It will not be clear which organism is responsible for disease in any one animal, but fortunately many of these problems can be controlled by multiple variable regression analysis.

2.2.2.3. The Use of Serological Data From Vaccine Trials Investigating Undifferentiated Bovine Respiratory Disease

Vaccine trials, ranging from small experimental trials to large field trials, have been used to study UBRD. A properly-designed field trial is able to demonstrate protection from a vaccine, if it is effective, provided it has sufficient power to detect the effect, but these studies are less able to show that a titre to a particular antigen is protective against disease (29).

Vaccine studies aim to demonstrate reduced disease occurrence in the vaccinated group. Correlations between titres from vaccine studies are not evidence that the titre measured is protective, merely that the antibody titre is correlated with protection in vaccinated animals. Since most cattle vaccines contain multiple antigens and titres to all of these may not be measured, there is no guarantee that the correlation between measured titre and disease occurrence will exist when the animals are infected naturally. The correlation may be the result of pattern of antigen exposure that is a function of vaccine preparations and not natural agent exposure.

Therefore vaccine studies will rarely provide conclusive proof of an association between a humoral response, and by extrapolation, an agent. Information from all study types is useful to establish associations between humoral responses, putative agents and UBRD and therefore validate conclusions drawn from sero-epidemiological surveys.

2.3 The Effects of Measurement and Misclassification Errors on Sero-epidemiological Studies Examining Undifferentiated Bovine Respiratory Disease

The uses of serological data are not only complicated by the vagaries of interpretation of titres but also because mis-measurement (continuous variables) and mis-classification (discrete variables) error is common in feedlot studies. This error in measurement or classification results in biased measures of association, such as odds ratios and relative risk.

2.3.1 The Outcome Variable: Undifferentiated Bovine Respiratory Disease

As previously discussed in Section 2.1.1, feedlot studies define treated animals as cases and untreated animals as non-cases and despite attempts to improve the sensitivity and specificity of this classification, the accuracy of this disease classification is questionable. Therefore, in feedlot studies the outcome is frequently misclassified and results in bias of the study findings. If it is possible to assume that misclassification is independent and non-differential, the direction of this bias is predictable and towards the null hypothesis, so authors and readers can adjust for this bias.

Independence of misclassification means that the probability of misclassification for disease status and exposure status is equal to the product of the corresponding misclassification probabilities (30).

i.e. pr (D'E'| DE) = pr(D'| DE) × pr (E'|DE) where

- pr (D'E'| DE) is the probability of being classified as not diseased and not exposed given that the true state of being is that the animal is diseased and exposed

- pr(D'| DE) is the probability of being classified as not diseased given that the true state of being is that the animal is diseased and exposed
- pr (E'| DE) is the probability of being classified as not exposed given that the true state of being is that the animal is diseased and exposed.

Misclassification is non-differential if the sensitivity and specificity of disease classification are independent of exposure status, or visa versa (30). In feedlot studies it seems reasonable to assume that misclassification of disease is non-differential, i.e. sick sero-positive animals will be detected with the same sensitivity and specificity as sick sero-negative animals.

Therefore, if study results for any study, not just sero-epidemiological studies, report a statistical association between an agent and disease occurrence, we would expect that the "true" strength of the association is even greater than estimated by the study. This should always be remembered when interpreting the magnitude of an effect estimated by the study, as knowledge that the magnitude of the association is actually underestimated, often adds weight to a study's ability to make causal inferences about the disease. However the disadvantage of non-differential misclassification is that it decreases the power of studies, so weak associations often go undetected.

2.3.2 The Independent (Exposure) Variable : Titres

Sero-epidemiological feedlot studies may also suffer from misclassification of the exposure status, i.e. titre. The extent of misclassification is a function of the serological test sensitivity and specificity. The effects of test sensitivity and specificity and the prevalence of the disease agent in the population on the study results should be

considered during sero-epidemiological studies. Despite international guidelines that provide detailed instructions for establishing the sensitivity and specificity of serological tests, many serological tests are not validated due to the difficulties associated with this process (31,32,33). Problems include finding sufficient animals of known negative and positive disease status to establish sensitivity and specificity, and the limited application of many tests for purposes other than in-house research, which limits the resources available for extensive test validation.

False positive and false negative serological results occur for a variety of reasons and the common reasons are listed in Table 2.2. The probability of false positives and false negatives should be considered when interpreting serological data.

For statistical analysis, serological data can be classified as class variables (sero-positive or sero-negative) or on a continuous scale (titre). For class and continuous independent variables of interest, misclassification of the true state, i.e. classifying an animal as sero-positive when it is truly sero-negative, or low interclass correlation in repeated measurement of a continuous variable, will again bias the measurement of association towards the null hypothesis. This leads to an increased likelihood of a type II error, again assuming misclassification is non-differential and independent (30,34).

With regard to these assumptions, it is feasible that misclassification of exposure is actually differential, i.e. the sensitivity and specificity of the laboratory test is not the same for sick and well animals. Some serum components may be differentially distributed between sick and well animals and these components may interfere with the serological test, resulting in different sensitivity and specificity's depending on disease status. However, there are no published studies specifically addressing this issue in the

diagnosis of UBRD. Therefore for the present, the assumption that misclassification of exposure status is non-differential is accepted with caution.

The overall result of misclassification of the independent variable is the same effect as misclassification of the outcome, a reduction in the power of the study to detect associations and a reduction in the magnitude of associations detected as statically significant. Therefore, this adds extra weight to the finding of studies that do find associations.

2.3.3 Misclassification of Covariates

The authors of many feedlot studies control for covariates to improve the estimates of association gained from the study and therefore to improve the understanding of the disease. Again these covariates may be class or continuous variables. Misclassification of these covariates can have varied effects on the measure of association depending on the type of covariate.

Misclassification of a class covariate will bias the association estimate towards the null and as discussed previously this will result in a conservative estimate of associations. However misclassification of a class covariate will affect the degree of heterogeneity in strata odds ratios and unfortunately this effect is unpredictable and will either exaggerate or mask the true heterogeneity of association measure across the strata. (35).

Misclassification of continuous covariates results in bias of the association estimate in either direction, toward or away from the null, but usually the magnitude of coefficient is decreased (36). The seriousness of this misclassification increases with

the degree of correlation between the independent variables and the magnitude of the errors of measurement. If the degree of correlation between the exposure of interest and the co-variate is small and the degree of imprecision in the measurement of the covariate is small then the bias will be toward the null (34). Imprecision in the measurement of the exposure of interest will not fully offset the bias in parameter estimates (34). More importantly in extreme cases, when both the exposure of interest and the covariate are very imprecisely measured and the correlation between the two is high, the bias in parameter estimates may be large enough for the coefficient estimates to be in the wrong direction, fortunately this situation is uncommon (36).

In summary, it is expected that when no relationship exists between the exposure of interest and the outcome, and the covariate and exposure of interest are negatively (positively) correlated, then the covariate coefficients will be biased towards the null and the exposure of interest will appear to have a protective (negative) effect (34). If an association does exist between the exposure of interest and the outcome, then misclassification will decrease the magnitude of the coefficients of the covariate and the exposure of interest and in rare situations this may cause a reversal in the sign of the coefficients (34,36).

Fortunately, the result of most misclassification is that parameter estimates are biased towards the null, giving more weight to associations identified as significant and less to those that are rejected. However if unexpected or non-sensical statistical associations occur during model building, the researcher should examine the degree of correlation and misclassification of the variables. This information should be used to determine if the coefficients are likely to be biased and possibly in the wrong direction,

rather than searching endlessly for obscure biological explanations for these statistical associations found during model building.

2.4 A Review of the Published Literature Relating Agents to Undifferentiated Bovine Respiratory Disease, With Emphasis on Sero-epidemiology.

As mentioned, all published studies have some limitation in their ability to provide information about the disease and agent of investigation and all diagnostic tests return some false positive and false negative results. These limitations would initially appear to prevent researchers from making causal inferences about agents and disease occurrence from a single study. However when a body of research is available for review, a general framework is available to help establish a causal association between agents and disease, with each study adding to the body of evidence to suggest or refute a causal association.

These criteria include the presence of a time sequence suggestive of causality, the strength of association, the presence of a dose-response relationship, biologically sensible findings that are coherent with the present knowledge of the disease and consistency of finding across studies and particularly study types (37,38).

With particular reference to UBRD and the difficulties associated with its study (Sections 2.1.1, 2.2.1, 2.2.2.1, 2.2.2.2 and 2.2.2.3), these criteria enable causal inferences between the agent and the disease. For example, *P. haemolytica* has been consistently associated with UBRD occurrence in many study types (39,40). The association between *P. haemolytica* and UBRD has been shown despite the presence of misclassification bias resulting in decreased reported strengths of association, suggesting that the association is

actually stronger. Furthermore, a dose response has even been shown for *P. haemolytica* antibody titre and mean colony count from nasal swabs (23,41). Given the available information, there is little doubt that *P. haemolytica* is causally associated with UBRD.

Definitive statements referring to causal associations between particular agents and UBRD should be made, in light of information provided by the body of research available, and with consideration of five discussed criteria for making causal inferences. However, it is also important to take into account that some statistically and biologically significant associations may only occur sporadically or in particular regions due to temporal or regional differences in disease patterns. For this reason, attention to the type of study design and its limitations and due consideration for possible regional or temporal factors are important when assessing findings reported in the literature.

The remainder of the review will concentrate on summarising the published findings about four agents and their role in UBRD: *P. haemolytica*, *H. somnus*, bovine coronavirus (BCV) and bovine viral diarrhoea virus (BVDV). For each of these agents a general summary outlining the role of the agent in UBRD is presented, followed by a more detailed discussion of the sero-epidemiology of the agent and the disease. In keeping with the two main purposes of sero-epidemiological studies (Section 2.2) this information is looked at in two sections: (1) information relating to the prevalence of previous exposure, incidence of exposure in feedlots, distribution of antibody titres, and (2) information relating to associations between titres and the disease.

2.5 The Role of *Pasteurella haemolytica* in Undifferentiated Bovine Respiratory Disease

Undifferentiated bovine respiratory disease has been recognised as a disease in cattle since the late 19th century. It is likely that the early syndrome was primarily *P. haemolytica* pneumonia and the occurrence of myocarditis, laryngitis etc was much lower than at present (1). For many years it was debated if *P. haemolytica* was the sole cause of UBRD, hence the older literature sometimes refers to the disease complex as Pasteurellosis and this term may include *Pasteurella multocida* (39). The role of *P. haemolytica* in UBRD has mainly been established by the culture of the organism from necropsy samples (39). Attempts to induce the disease experimentally using *P. haemolytica* have not always been successful, resulting in doubt that *P. haemolytica* was the sole etiologic agent, and leading many authors to suggest that "stress" and other disease agents were needed to create the clinical disease (39). The importance of *P. haemolytica* in UBRD may be diminishing, as the role of other agents, in particular *H. somnus*, gain greater recognition; however, fibrinous pneumonia, characteristic of *P. haemolytica* infection, still remains a significant cause of feedlot mortality (1,2,3,18).

2.5.1 The Sero-epidemiology of *Pasteurella haemolytica*

2.5.2 Descriptive Sero-epidemiology of *Pasteurella haemolytica* in Undifferentiated Bovine Respiratory Disease

For cattle entering feedlots and dairy calves, agglutinating titres to *P. haemolytica* surface antigens are common while neutralising titres to *P. haemolytica* leucotoxin are less common (23,42). This finding is a function of the colonisation pattern of *P.*

haemolytica. Many cattle have commensal colonisation of their nasopharynx with *P. haemolytica* (42). This colonisation results in the production of local secretory immunoglobulin, mainly IgA, and a systemic response to bacterial surface antigens associated with binding to the epithelium which is detected by agglutination (42). As *P. haemolytica* leucotoxin is an exotoxin and is less likely to cross the nasal epithelium fewer animals have titres to the leucotoxin. Anti-leucotoxin titres are thought to be more likely to develop when the animal is stressed and the bacteria invade the lower respiratory tract (42). Nasal colonisation occurs early in life, while events that predispose to lower respiratory tract exposure and the induction of anti-leucotoxin antibody occur less commonly and later in life. Therefore calves arriving at feedlots are very likely to have agglutinating titres but may have low or no antibodies to leucotoxin.

Although several studies have reported agglutination titres to *P. haemolytica* surface antigen, *P. haemolytica* leucotoxin neutralisation titres, and *P. haemolytica* anti-leucotoxin titres determined by ELISA, only Martin *et al.* (23) provided descriptive data (14,23,43). Martin *et al* (23) reported distributions for treated and untreated animals during the study period separately and defined sero-positive as animals with *P. haemolytica* transformed titre values greater than 6 (the mean value) and sero-conversion was defined as a four-fold increase in titre. Approximately 65% of animals had bacterial agglutination titres to *P. haemolytica* at arrival (i.e. sero-positive), and 45% had *P. haemolytica* leucotoxin neutralisation at arrival (i.e. sero-positive). The prevalence of animals sero-positive for *P. haemolytica* surface antigens (agglutinating titres) on arrival was higher in animals that subsequently became cases. The distribution of the titres of both groups appeared to be bell shaped, and little difference existed in the pattern of

distribution of titres between treated and untreated animals. Booker *et al.* (14) also reported quartiles and the range for *P. haemolytica* ELISA anti-leucotoxin titres at arrival, at case and control selection and at approximately 33 days post arrival. Titres in this study followed a bell shaped right skewed distribution, but the number of sero-negative animals was not clear , nor was the definition of sero-positive or sero-negative. These point estimates of sero-prevalence and patterns of distribution from calves on arrival at feedlots are difficult to interpret because of the increased likelihood of the occurrence of recent exposure to *P. haemolytica*. It is not clear if the prevalence of sero-positive animals has risen dramatically since leaving the farm. This is also complicated by differences in transportation times between the two studies and therefore quite different likelihood's of exposure between farm and feedlot; Martin's study predominately used calves transported from western to eastern Canada with an anticipated transit time of 10 days from farm to feedlot, while Booker's study used western calves in western feedlots, with presumably shorter travel times.

Many other studies have reported serological data for various titres but due to study size or design these are of little use in determining the population distribution of *P. haemolytica* titres (18,44,45,46,47).

Exposure to *P. haemolytica* during the feedlot period is common, if changes in titre during the study period are an appropriate indicator of exposure (14,48,49,50) . Martin *et a.* (23) reported on analyses at the individual level that approximately 41% and 46% of treated and untreated animals respectively sero-converted to *P. haemolytica* agglutinating titres, and 71% and 55% respectively of treated and untreated animals sero-converted during the study period to *P. haemolytica* neutralising leucotoxin titres. The

magnitude of titre change ranged from five dilution decrease to a seven dilution increase (dilution factor: twofold). As expected the magnitude of change was negatively correlated with arrival titre (23). At the group level, average sero-positivity (defined as above (23)) was 51% ± 28% for agglutinating titres and 42% ± 26% for *P. haemolytica* leucotoxin neutralising titres(51). The frequency of sero-conversion (defined as above (23)) to agglutinating *P. haemolytica* titres was 41% ± 23% and *P. haemolytica* neutralising titres to leucotoxin were 59% ± 18%. Two other studies have reported geometric means for ELISA titres to *P. haemolytica* anti-leucotoxin at various times during the early feedlot period of calves; however, standard deviations, frequency distributions or results of AVOVA tests for these point estimates or changes in titres were not reported (43,49). Therefore titres to *P. haemolytica* are common in calves prior to arrival at the feedlot and thereafter changes in titres are also common , suggesting that exposure is occurring during the study period of feedlot studies.

2.5.3 The Association Between Humoral Responses to *Pasteurella haemolytica* and Undifferentiated Bovine Respiratory Disease

For cattle arriving at feedlots high bacterial agglutinating titres to *P. haemolytica* at the time of arrival are associated with increased risk of treatment for UBRD during the study period (23). This finding initially seems unusual, since many animals have agglutinating titres to *P. haemolytica* prior to arrival and *P. haemolytica* is thought to be an agent of UBRD. However, the presence of high titres to an agent in a calf arriving at a feedlot, should not be misinterpreted as an indication of resistance, but only an indication of previous exposure (Section 2.2.1). It has been suggested that the presence of

agglutinating titres in the absence of leucotoxin titres is detrimental because agglutinating antibodies in the absence of leucotoxin antibodies enhance contact with bacterial macrophages (52). This hypothesis is supported by the results of challenge studies conducted by Shewen and Wilkie (53).

Titres to the *P. haemolytica* leucotoxin, using ELISA and neutralisation techniques, are also frequently reported in the literature. Titres to this leucotoxin are of interest because it is thought that the leucotoxin plays an important role in the pathogenesis of the *P. haemolytica* pneumonia. Experimental studies suggest that leucotoxin antibody levels are correlated with protection against experimental disease (54) and observational studies suggest that sero-conversion, and increased titre changes, to the leucotoxin during the study period are associated with increased risk of treatment (14,23). Surprisingly, observational studies have failed to show an association between arrival titres to leucotoxin and subsequent disease occurrence (14,23).

Although the serological findings from observational and some experimental studies have supported the concept that *P. haemolytica* is an important agent in UBRD, generally vaccine trials have failed to effectively demonstrate a relationship between humoral responses to vaccines and disease occurrence. A review of field vaccine trials considered only 10 to be well controlled studies, of which only one reported serological data(55). Of these studies four reported a reduction in disease rates, the other six being neutral (55). Other field vaccine trials, aimed at evaluating a commercially available vaccine containing genetically attenuated *P. haemolytica* leucotoxin combined with bacterial extracts from *P. haemolytica* and *H. somnus* have been associated vaccination with a reduction in disease incidence and showed that elevated ELISA titres to the *P.*

haemolytica leucotoxin were associated with this disease reduction (43,49,50,56). Unfortunately, two of these studies used a combined vaccine, protection could not be attributed to a titre for either agent.

Evidence exists therefore, from experimental and observational studies, to suggest that *P. haemolytica* is an agent in UBRD. However, it appears that protective immunity to *P. haemolytica* is complex and therefore present vaccines may not be adequate to prevent disease (42,54,57,58). An as yet undetermined combination of antibodies to the leucotoxin and various membrane proteins may provide the greatest protection against *P. haemolytica* pneumonia (27,40) .

2.6 The Role of *Haemophilus somnus* in Undifferentiated Bovine Respiratory Disease

Haemophilus somnus has been recognised as an agent of disease in cattle since the 1950's. Originally the bacterium was associated with thrombotic meningoencephalomyelitis (TEM) in feedlot cattle. In recent years it has been linked with various other diseases of feedlot cattle, including myocarditis, pericarditis, pleuritis, polyarthritis, *H. somnus* septicaemia and *H. somnus* pneumonia (59). Together these diseases have been called Hemophilosis (1). *Haemophilus somnus* has usually been linked with these diseases through culture of necropsy specimens, resulting in pure or mixed colonies (59,60).

Haemophilus somnus could be an important agent in UBRD by two mechanisms. Firstly, *H. somnus* may cause significant pneumonia that is diagnosed as UBRD or secondly, other manifestations of *H. somnus* infection may be included as UBRD cases

because of the vague nature of their clinical signs and the similarity of these clinical with true UBRD. Unfortunately no studies address either of these issues directly and information from mortality studies can not be extrapolated back to UBRD occurrence. Janzen *et al* (1) suggested that animals with non-specific clinical signs due to *H. somnus* pleuritis, myocarditis, pericarditis and septicaemia were likely to be classified as UBRD cases by feedlot staff. If this were the case then, in studies using treatment or morbidity rates as an outcome, *H. somnus* would play a significant role, but it is not possible to document this (1). Perhaps in support of this suggestion however, Van Donkersgoed *et al* (3) reported that 88% and 43% of *H. somnus* myocarditis and pleuritis deaths were treated for UBRD prior to death. However, this does not mean that these animals had *H. somnus* infection at the time of treatment.

No studies have looked directly at the role of *H. somnus* in true pneumonia cases, nor its contribution to treatment rates in feedlots. Given that there is disagreement in the literature about whether respiratory disease deaths should be included as possibly attributable to *H. somnus*, let alone whether treatment for UBRD is due to *H. somnus*, this area needs considerably more research work (2,3,61).

In summary *H. somnus* may be an important component in the UBRD complex and its involvement is implied by some research findings but to date researchers have been unable to establish this association. It is for this reason that serological epidemiology has been used to investigate the association between UBRD and *H. somnus* in this study.

2.6.1 The Sero-epidemiology of *Haemophilus somnus* in Undifferentiated Bovine Respiratory Disease

2.6.2 The Descriptive Sero-epidemiology of *Haemophilus somnus* in Undifferentiated Bovine Respiratory Disease

Culture surveys would suggest that infection with *Haemophilus somnus* is common in the reproductive tract of male cattle, with prevalence estimates ranging from 0% to 77%, and many animals are carriers of *H. somnus* (62,63,64). Theoretically *H. somnus* remains at the mucosal surface of the prepuce or vagina without invading cells in these carriers, but it is unclear if this carrier state is proceeded by invasion and the infection then overcome, in which case circulating antibodies may be present (63,64,65). Therefore, it is not clear if titres are associated with reproductive or respiratory exposure.

Estimates of the prevalence of titres to *H. somnus* are also complicated by the occurrence of false positives. Cross reactions in serological tests for *H. somnus* have been reported for many bacteria, such as *P. haemolytica, Pasteurella multocida, Salmonella dublin, Actinobacillus lignerisi , Corynebacterium pyogenes* and *E coli* (66,67,68).

The prevalence of sero-positive animals is quite variable and depends on the serological test used. The estimates of prevalence of antibodies in beef cattle herds vary from 69% to 6.8% (69,70). Sanfacon *et al* (70) reported results for the microagglutination test using heated antigen (MAT-H), the complement fixation test (CF) and counterimmunoelectrophoresis (CIEP). Titres and frequency distributions were given for the MAT-H and CFT test ; the distributions were non-normal with 38% and

31% respectively being sero-negative and the distribution of sero-positive animals being skewed to the right. In this study sero-positive animals were defined as those animals with a titre greater than zero.

In the 1990's the sero-prevalence of *Haemophilus somnus* titres to the outer membrane protein components detected using an ELISA was reported for calves entering feedlots in western and eastern Canada. Booker *et al* (14) reported 1st, 2nd and 3rd quartile titres and the range at arrival for animals treated for undifferentiated fever and control animals in western calves. The number of sero-negative animals was not reported but the quartiles and the range suggested a bell shaped, right skewed distribution for both cases and controls at arrival. Martin *et al* (71) reported that 212 western calves had measurable titres on arrival at feedlots; the average being 7.8 ± 1.57 ($\log_4$). In the same study, eastern calves had an average titre of 7.30 ± 1.24 (n= 490). The range of titres was not given. Comparison of titre levels should not be made across studies unless it is known with confidence that the initial dilution and sequential dilutions remained the same. Since this information was not clear for these studies this comparison cannot be made.

It is unclear how common exposure to *H. somnus* is in feedlots. A longitudinal case-control study involving 100 cases of UF by Booker *et al* (14) and a longitudinal cross-sectional study by Martin *et al* (71) both examined changes in titre levels during the study period. Booker *et al* (14) found that titres increased significantly from the day of arrival to day 33, but did not report any descriptive information about these changes in titres and the p value was reported simply as <0.05. Reliance on p-values to determine significance should be avoided because p-values are affected not only by the magnitude of the effect measured (in this case the mean titre change), but also by the standard

deviation of that mean. Therefore, it would be informative to see the magnitude of titre change before agreeing with Booker's conclusion that exposure was common (38).

Evidence from Martin's study suggests that exposure to *H. somnus* in feedlots is not common (71). Martin *et al* (71) found that calves in western Canadian feedlots had a negative average titre change (-0.46 ± 1.27) while calves in Ontario feedlots had only slightly positive titre changes (0.11 ± 1.15) during the first 28 days after arrival at the feedlot. It was not clear if the arrival titres were different from the 28-day titres for either the western or eastern calves, or if the change in titre was different between western and eastern calves.

2.6.3 The Association Between Humoral Responses to *Haemophilus somnus* and Undifferentiated Bovine Respiratory Disease occurrence

The evidence from experimental, observational and vaccine trial studies suggests that *H. somnus* does have a role in UBRD. Large field vaccine trials have shown that vaccination with vaccines containing outer membrane protein (OMP) extracts from *H. somnus* were associated with decreased disease occurrence or mortality (2,43). Other studies reported that animals with high titres to the OMP of *H. somnus* at arrival were at decreased risk of treatment for UBRD in the feedlot (14,71) or experimentally induced pneumonia (72). This combined information suggests that prior exposure and development of antibodies to the OMP to *H. somnus* are protective against UBRD.

Martin *et al* (71) included arrival *H. somnus* titre, change in *H. somnus* titre and province of origin as variables of interest to predict the outcome UBRD occurrence. The published information suggests that arrival titre and change in titre confounded each

other, i.e. the addition of one changed the statistical significance of the other coefficients (actual coefficients not reported). However, the other variables examined during the model building process apparently did not affect the coefficient of the *H. somnus* variables, suggesting that they were unlikely to be confounders. These variables included *P. haemolytica* , bovine corona virus , bovine viral diarrhoea virus , *Mycoplasma bovis*, *Mycoplasma alkalescens,* parainfluenza virus – 3, bovine respiratory syncytial virus and infectious bovine rhinotracheitis virus (IBR), arrival weight, province and arrival group (71). Booker's study also used a multivariable (multiple variable) logistic regression model. The variables available for control included arrival titre, change in titre, rectal temperature at arrival, days on feed at time of selection for convalescent serum collection, and pen effects. The presence of confounding variables however is not clear from Booker' s results, nor is it not clear if Booker *et al.* (14) built separate models for each serological variable available (*P. haemolytica* , *H. somnus* , bovine herpes virus (IBR) , bovine viral diarrhoea virus , *Mycoplasma bovis* and *Mycoplasma alkalescens)* or if all were all included in the same model. Whatever the process of model building, the effect of the addition variables on the variables of interest was not reported, so conclusions about the presence of confounding variables can not be drawn.

Fortunately the direction of the association between the *H. somnus* outer membrane protein antibody titres and disease occurrence across the studies is consistent, adding strength to any causal inference between UBRD and *H. somnus*. The estimated magnitude of the effect does vary between studies, but this is to be expected as differences in the degree of measurement error of the *H. somnus* titre and the outcome are very likely across studies (66,67,68) (Section 2.3.1). The fact that the association is

in the same direction for all studies suggests that the bias is not so extreme as to change the direction of the association, though this can not be proven (Section 2.3.3).

Interestingly despite significant associations between the arrival titre and disease occurrence in multivariable models, no significant differences existed in the average arrival titres for animals treated with UBRD versus those not treated in any study using univariate statistical techniques (56). This suggests that the relationship between arrival titre and disease occurrence is confounded. This confounding would appear, from Martin's *et al* (71) results, to be due to the relationship between change in titre, arrival titre and disease occurrence.

Although data from experimental studies suggests that antibodies to the outer membrane protein of *H. somnus* offer the most protection against pneumonia, no studies have been performed to determine if these same titres are protective against the other components of the Hemophilosis complex included in UBRD (73,74). Therefore, what remains unknown is how important *H. somnus* pneumonia is as a component of UBRD, how protective antibodies to the outer membrane proteins are against the other manifestations of Hemophilosis and what percentage of UBRD treatments in any particular study are attributable to either.

With regards to changes in titre during the study period, Booker *et al* (14) found that control animals had larger increases in *H. somnus* titres than cases and Martin *et al.* (71) found that animals with smaller decreases in *H. somnus* titres during the study period (33 days and 28 days respectively) had a decreased risk of treatment for UBRD (14,71). As both of these studies controlled for the arrival titre to *H. somnus*, this finding is unexpected. In Section 2.2.1 , it was mentioned that change in titre is taken to be

evidence of infection. The findings from these studies seems contradictory to that notion, i.e. evidence of infection is associated with decreased disease occurrence. The difficulty in interpreting these findings is trying to appropriately judge the significance of the magnitude of change of titre, especially in the light of findings in the same studies that increasing *P. haemolytica* anti-leucotoxin titre change is associated with disease occurrence, which seems biologically sensible, i.e. once arrival titre is controlled, animals with higher titre changes are those experiencing sickness.

Martin *et al* (71) suggested that smaller decreases or stable titre may indicate that the immunology of *H. somnus* is different to other organisms (71). Exposure to *H. somnus* in a feedlot may cause a "sopping" up (or overall net use) of arrival antibodies and the greater this " sopping up" the more severe the infection and therefore the more likely the animal is to be treated. Although it is possible that transient decreases in antibody levels do occur in the early stages of infection, it would appear unlikely that this process would last for the 28 day study period. It may be that the large decreases or smaller increases are statistically associated with increased risk of treatment due to an entirely different action. *Haemophilus somnus* is particularly sensitive to antibiotics, and therefore animals treated for UBRD or those exposed to metaphylaxis may have a decreased exposure to *H. somnus* because they receive more antibiotics during the study period. If this were the case, the titre change is a consequence of the disease status , rather than the reverse. Unfortunately, good evidence exists for none of these theories.

It seems to be a consistent finding that higher titres to *H. somnus* at arrival, or higher titres through vaccination, offer protection against UBRD, suggesting that the agent does play a role in the disease. What is not clear, is if infection is actually

occurring in the feedlot during the study period or if the titres to *H. somnus* behave in the manner that is expected. The behaviour of titre changes to *H. somnus* requires further detailed examination, to clarify these remaining questions.

2.7 The Role of Bovine Coronavirus in Undifferentiated Bovine Respiratory Disease

Bovine coronavirus (BCV) has traditionally been associated with neonatal diarrhoea in calves. In these animals maldigestion and osmotic diarrhoea are due to villous atrophy caused by, bovine coronavirus induced, intestinal epithelial cell death (75). The virus has also been linked with respiratory infection in various ages of cattle and bovine coronavirus is possibly a causal agent of winter dysentery in adult cattle (75,76,77).

The evidence that bovine coronavirus causes significant respiratory tract disease in young calves is not compelling. Bovine coronavirus has been isolated from the nasal passages of calves, inoculated with bovine coronavirus, or naturally infected; however, the majority of these animals show no clinical signs or very mild signs of rhinitis, while displaying diarrhoea (78,79,80). These studies may have been limited in their power due to small study sizes; nonetheless, even qualitatively, the evidence for bovine coronavirus causing UBRD is weak. In one experimental study, lower respiratory tract disease was reproduced using bovine coronavirus (81). Other studies have reported bovine coronavirus titre changes and bovine coronavirus isolation from nasal passages of young calves, feedlot calves and adults displaying signs of respiratory disease; however, these studies had no control groups (22,82). The absence of control groups means that no comparison of titre change or isolation prevalence could be made with clinically normal

cattle to determine if an association actually existed with UBRD. The need for control animals is especially important in determining the role of bovine coronavirus in respiratory disease because of the reported high prevalence of titres to bovine coronavirus, between 60 to 100% across multiple beef herds (83). Clark noted that " It has yet to be proven whether this virus is a causative agent of winter dysentery, an opportunistic invader or merely part of normal microflora of the bovine gut." (75). The same can be said for the role of bovine coronavirus in respiratory disease in cattle.

2.7.1 The Sero-epidemiology of Bovine Coronavirus in Undifferentiated Bovine Respiratory Disease

2.7.2 The Descriptive Sero-epidemiology of Bovine Coronavirus in Undifferentiated Bovine Respiratory Disease

As noted, titres to bovine coronavirus are common; between, 65% and 100% of cows randomly selected from 26 beef herds, in a Quebec study, were sero-positive to bovine coronavirus (83). The calves from these dams had similar high levels of bovine coronavirus sero-positivity. Calves born to sero-positive dams were 3.15 times more likely to be sero-positive than calves born to sero-negative dams. Storz *et al* (84) reported that bovine coronavirus could be isolated from the majority of calves in two respiratory disease outbreaks (64/105 and 89/120). Martin *et al* (71) reported that 83% of calves arriving at feedlots were sero-positive to bovine coronavirus-VN, but the frequency distribution was not reported. The overall arrival titre was 5.54 ± 3.79 ($\log_4$), although titres were lower for eastern calves. Western treated calves had lower arrival titres that their non-treated penmates (85). Exposure to bovine coronavirus in the feedlot

is thought to be very common. Martin *et al* (85) reported that 370/604 calves sero-converted in the first 28 days of the feedlot study. All animals that were sero-negative at arrival sero-converted during the study period and arrival titre and sero-conversion were strongly and negatively correlated (85).

Faecal shedding of free coronavirus has been reported in 5% and coronavirus immunoglobulin complexes detectable in 70% of 121 healthy cattle from a single herd (86). Unfortunately no studies have examined the prevalence of free coronavirus or immune complex to bovine coronavirus in the nasal passages of normal cattle.

2.7.3 The Associations Between Humoral Response to Bovine Coronavirus and Undifferentiated Bovine Respiratory Disease

Although bovine coronavirus has not been shown to be pneumo-pathogenic, high titres for bovine coronavirus have been statistically associated with protection against UBRD (83,85). In the only published study looking at the role of bovine coronavirus in UBRD of feedlot cattle, higher bovine coronavirus neutralisation titres (BCV –VN) in western and eastern feedlot calves at arrival were associated with decreased risk of treatment for UBRD and higher weight gains in the 28 days after arrival (85). Given the lack of experimental evidence that bovine coronavirus actually causes respiratory disease, other biological explanations for this statistical association should be investigated. Bovine coronavirus titres at arrival may be a proxy for an unknown variable that is associated with disease occurrence. The bovine coronavirus titre at arrival may also be confounded by another unmeasured variable; however Martin's *et al* (85) study did control for a large number of other agents thought to be associated with UBRD

occurrence. The fact that titre change is not associated with disease occurrence or weight gain suggests that current bovine coronavirus infection is not a cause of disease in the feedlot. Why higher titres at arrival are protective is still unexplained.

Studies on beef calves mixed with cows with a very high prevalence of bovine coronavirus titres, where exposure should occur readily, find that low bovine coronavirus titres are an indicator of increased risk of respiratory diseases (83). These authors suggest that bovine coronavirus is probably not an agent of disease, but rather that low bovine coronavirus titres or sero-negativity indicate an immaturity or weakness in the immune system (83). Interference by maternal antibodies is one possible mechanism of this immaturity (83). Experimentally, maternally derived passive immunity has been shown to delay the development of an active immune response to some bovine coronavirus proteins in young calves however, this finding was only reported for young calves (< 2 months) (76,83). Even if maternal antibody interference was the mechanism responsible for the association between bovine coronavirus and UBRD in Ganaba *et al*'s (83) study, it seems unlikely that this interference would persist to the time that calves are sold to feedlots, but this may warrant further investigation.

2.8 The Role of Bovine Viral Diarrhoea Virus in Undifferentiated Bovine Respiratory Disease

Bovine viral diarrhoea virus (BVDV) is capable of causing a large number of diseases, however its role as a primary respiratory pathogen is unclear (87). The virus is occasionally the sole pathogen isolated from the lungs of animals with UBRD and

pneumo-pathogenic strains have been identified during experimental studies (88,89,90). However, experimental challenge has produced only mild respiratory disease and it is far more common for BVDV to be isolated in association with other respiratory pathogens (24,88,91). Experimentally, respiratory disease is reproducible when BVDV infection occurs prior to, or concurrent with, infection of cattle with other putative UBRD organisms, such as IBR, *P. haemolytica* and BRSV. (90,92,93,94).

Therefore, present knowledge about the pathogenicity of BVDV suggests that it is likely an indirect rather than a direct cause of UBRD. There is considerable evidence that infection with BVDV, rather than being pneumo-pathogenic, causes immunosuppression (95) and there are several reports suggesting that infection with BVDV somehow facilitates infection with other organisms, either by enhancing the virulence of the organism or changing the nature of the disease. Organisms for which this effect is reported include *P. haemolytica* and the viral respiratory pathogens (91,93,96,97). The mechanism of this action is unclear, especially *in vivo*, although experimental evidence suggests that BVDV infection depresses lymphocyte numbers and impairs neutrophil and lymphocyte function. The circumstantial and experimental (*in vitro / in vivo*) evidence for immunosuppression caused by BVDV has been reviewed by Potgieter *et al* (87) .

2.8.1 The Sero-epidemiology of Bovine Viral Diarrhoea Virus in Undifferentiated Bovine Respiratory Disease

2.8.2 The Descriptive Sero-epidemiology of Bovine Viral Diarrhoea Virus in Undifferentiated Bovine Respiratory Disease

Understanding the prevalence and distribution of titres to BVDV in the population is confused by the variety of serological tests available to measure titres. Most studies use BVDV viral neutralisation titres (BVDV - VN) produced in response to the immunodominant major structural glycoprotein 53 (gp53). Titres are also measurable to glycoproteins 25 and 48 and the non-structural protein p 80 but there is no evidence that these are neutralising antigens (95). The sensitivity and specificity of BVDV-VN titres varies with the strain of BVDV used because several antigenic epitopes exist on gp53, and these vary with strains (98). This antigenic diversity results in exposure to different antigens *in vivo* that are different than those used in the *in vitro* tests. Common strains used in laboratory tests include the Singer, Oregon C24V, NADH; in the same laboratory each strain will return different titres for the same animal (99). The occurrence of test differences means that paired samples tested at the same laboratory are necessary to evaluate the serological response. Ideally it would more suitable to use a test that detected a common BVDV antigen. An ELISA designed to measure antibody levels to a common gp 53 epitope has been reported but the epitopes involved were not described, so the response of this test may also vary between animals, unless the test is based solely on a highly conserved epitope (100). Agreement between VN and the ELISA antibody

titres varies. It has been reported as poor (Kappa statistic = 0.15 ± 0.039) (100) and excellent (Kappa statistic = 0.8 – calculated from data provided) (24).

In one study, the prevalence of VN titres to BVDV varied from 67% for New York strain, 67% for Oregon and 81% for Singer strain. These data were from a population of Charolais-cross calves, of unspecified age but still at pasture (101). Other studies using VN titres have reported a lower prevalence of sero-positive animals at arrival at the feedlot. Martin *et al* (23) reported the percent sero-positive for VN -BVDV for individual cases and control animals, as being 32% and 42% respectively and these were significantly different. This population represented western and eastern calves arriving at feedlots in Ontario. Among the sero-positive animals, most had titres of 1 and 2 ($\log_2 2$ and $\log_2 4$) (45 % / 44% for case / controls respectively) and the range was 6 or 7 ($\log_2$) for cases or controls respectively. At the group level, the prevalence of sero-positivity was around 37 %. All groups (n=14) had some positive animal (51).

Exposure to BVDV in feedlots appears common. Sero-conversion has been reported in 42% and 33% of cases and controls (23). Arrival titres and change in titres were negatively correlated as expected (23,101). At the group level, the prevalence of sero-conversion was around 31 % (51). Because of the current popularity of BVDV vaccination in feedlot cattle, it is difficult to find studies that report the changes in titres that occur in unvaccinated feedlot animals.

2.8.3 The Association Between Humoral Responses to Bovine Viral Diarrhoea Virus and Undifferentiated Bovine Respiratory Disease

Whether BVDV is a direct or indirect cause of UBRD there is general agreement that it does play a role in UBRD. The difficulty associated with making causal inferences about the association between humoral responses (arrival titres or titre change) to BVDV and UBRD is mainly related to the availability of a large number of serological tests measuring different antigens. This difference was evident in a study by Van Donkersgoed *et al* (101) . In an evaluation of 8 commercial vaccines, sero-conversion to BVDV by VN varied between strains. Among sero-negative animals, vaccination resulted in sero-conversion in 22%, 37% and 56% of animals tested using the New York, Oregon C24V or Singer strains respectively (101). Among sero-positive animals, vaccination resulted in sero-conversion in 20%, 23% and 19% of animals based on tests using the same strains (101). This variation in response to vaccination highlights the difference in titre due to test strain. Failure to find an association between BVDV and UBRD may be due to the strain used to determine the titres, and for the same group of animals, measurement of titres using a different strain may result in an entirely different result.

Because the titres measured are quite different, associations between BVDV and UBRD across sero-epidemiological studies may not be consistent and one criterion for making casual inferences is not met i.e. consistency of findings (37,38). However, as it is know that the behaviour of the titres varies across tests, the criterion of consistency of findings should probably be considered within studies using the same serological test,

rather than across all studies. If findings are consistent across sero-epidemiological studies using the same serological test, then causal inferences can be made.

Observational studies reporting associations between titres to BVDV and BRD are few and most are conducted by the same author, presumably using the same test. Several of these studies report that low titres at arrival and sero-conversion to BVDV during the study period are associated with increased risk of treatment for UBRD (51,102,103). Another study reported a similar relationship, but it is not clear if the antigen tested was the same (14). If it were different, this would add strength to causal inferences made about BVDV serological data and UBRD occurrence.

The authors of an experimental vaccine study reported that vaccination with a commercial modified live virus was protective and induced neutralising titres, while vaccination with inactivated laboratory strains of the Singer, Oregon C24V, and NADL strains also induced neutralising antibodies but failed to protect animals from disease, defined as elevation of temperature and leucopenia (95). This suggested that neutralising titres may be associated with another unmeasured response that is responsible for protection and inactivation of the virus fails to stimulate this protective response. Further research is needed to identify antibodies and titres other than neutralising titres that may be linked with protection, in a similar way that agglutinating and leucotoxin titres both provide information about *P. haemolytica*.

Reviews of the effect of vaccination against BVDV on UBRD in the field, have concluded that no appropriately designed vaccine trial actually exists (55) or that vaccination has no effect or may be detrimental to cattle health (104). The finding that vaccination was detrimental may be attributable to the use of older, more virulent BVDV

vaccines which may have caused immunosuppression and led to an association between vaccination and disease occurrence. However there is no documented evidence of a difference in virulence between older and more recent vaccines. Unfortunately the practice of vaccinating cattle for BVDV is so widespread these days, and the vaccine is so frequently combined with other agents, that it is unlikely that controlled field studies comparing disease rates in BVDV vaccinates versus non vaccinates could be conducted. To determine the role of BVDV in UBRD in the face of mass vaccination, arrival titres and changes in titres can still be measured and associations determined. However, the differences between the strain of virus used in the vaccine and laboratory will continue to confuse comparisons across studies and make consistent findings hard to come by.

2.9 Conclusion

The study of UBRD is perhaps most limited by the difficulties associated with the misclassification of the disease outcome. Misclassification of the serological results constitutes another problem. All diagnostic tests and their studies suffer from the same problem to a greater or lesser degree, regardless of the area of research, but these problems do not necessarily invalidate study findings. The aim for researchers should be to continue to refine all aspects of the study design. In fact as the direction of bias is usually towards the null hypothesis, this adds greater strength to the associations found. Weaker associations are likely to go undetected.

For *H. somnus*, sero-epidemiological studies have provided the best evidence so far that this agent may play a role in UBRD. For *P. haemolytica*, serological data have provided information about the complexity of the immune response and therefore, are

aiding in the development of vaccines. For the viral agents, bovine coronavirus and bovine viral diarrhoea virus, serological data have shown that these agents are statistically associated with the occurrence of UBRD, but the mechanism of this association is not clear. In conclusion, despite previous research efforts, the role of the organisms, *H. somnus* and bovine corona virus, in the UBRD complex have not been clearly established and this thesis aimed to investigate their roles using an observational sero-epidemiolgical study.

Table 2.1: Day of fatal disease onset for cause-specific mortalities as reported in two studies

Cause of death at necropsy	Onset of fatal disease		
	Median	range	n
Fibrinous pneumonia[a](4 year study)	19-22	0-220	N/R
Pneumonia[b]	12	1-30	55
Myocarditis[b]	22	3-36	34
Polyarthritis[b]	18	5-41	15
Pleuritis[b]	22	11 –37	14
Hemophilus septicaemia[b]	17	13-19	4
Thrombotic menigoencephalomyelitis [b]	29	19-29	5

[a] **RIBBLE CS, MEEK AH, JIM GK, GUICHON PT.** The pattern of fatal fibrinous pneumonia (shipping fever) affecting calves in a large feedlot in Alberta (1985-1988). Can Vet J 1995; 36:753-757.

[b] **VAN DONKERSGOED J, JANZEN ED, HARLAND RJ.** Epidemiological features of calf mortality due to hemophilosis in a large feedlot. Can Vet J 1990; 31:821-825.

N/R = not reported

Table 2.2 : Reasons for positive and negative results in serological tests (21)

Positive results	**Status of result**
Actual infection	true +ve
Group cross reactions	false +ve
Non-specific inhibitors	false +ve
Non-specific agglutinins	false +ve
Negative results	
Absence of infection	true -ve
Natural or induced tolerance	false –ve
Improper timing	false –ve
Improper selection of test	false –ve
Non-specific inhibitors e.g. anticomplementary substances tissue culture toxic substances	false –ve
Antibiotic induced immunoglobulin suppression	false –ve
Incomplete or blocking antibody	false –ve
Insenstive test	false –ve

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Chapter 3 The Effect of Arrival Titres to *Haemophilus somnus* and Vaccination with *Haemophilus somnus* on the Probability of Treatment for Undifferentiated Bovine Respiratory Disease and the Change in Weight of Feedlot Cattle

3.1 Introduction

Determining that an organism causes a particular disease under field conditions requires a large body of evidence. In the study of undifferentiated bovine respiratory disease (UBRD) a statistical association between previous exposure and disease occurrence, a change in titre and disease occurrence and reduced disease occurrence after vaccination combine to support inferences about a causal association between UBRD and a specific organism. From an epidemiological perspective, *Pasteurella haemolytica* is thought to be a major agent of UBRD because studies have shown that previous exposure is statistically associated with reduced disease occurrence, concurrent exposure is associated with increased disease occurrence (1,2) and vaccination may be protective(3). However, for *Haemophilus somnus* there is less supporting evidence for a causal role in UBRD. Exposure to *H. somnus* prior to arrival has been associated with decreased risk of treatment (2,4). However, other studies have failed to show that active *H. somnus* infection is associated with increased disease risk (2,4). Furthermore, although *H. somnus* vaccination has been shown to offer some protection against UBRD, the possibility of cross protection offered by *P. haemolytica* vaccines against *H. somnus* casts doubt on the evidence for a causal role for *H. somnus* in UBRD (5,6,7).

Therefore, the aim of the present study was to examine the role of arrival titres to *H. somnus* in predicting the risk of treatment. The null hypothesis was that arrival titres to *H. somnus* are not associated with UBRD occurrence or weight gain. The protection offered by vaccination with *H. somnus* vaccines was also studied using a factorial design with *P. haemolytica* and *H. somnus* vaccines.

3.2 Methods and Materials.

Animal management

A cross sectional observational study was conducted at three Ontario feedlots in fall, 1998. Eight hundred and fifty two cattle were enrolled in the study. The cattle were from a variety of sources; western Canadian calves (Feedlot A) and eastern Canadian calves (Feedlots B /C). The cattle at Feedlot C were from the University of Guelph beef cattle research farms and it was known that these cattle received no vaccines prior to arrival. For the calves at Feedlot A and B, the farm of origin and previous vaccine history were unknown. Only one bull was identified among the cattle (Feedlot B), and castrated at processing. None of the feedlots de-horned cattle at processing or within the 28 day study period. Antibiotics were not included in the ration during the 28-day study period. At processing, the cattle were assigned to four treatment groups. The length of time from purchase to arrival at the feedlot was not known, but all animals were processed within 36 hours of arrival. At the feedlot, during routine processing, the cattle were systematically assigned to one of four vaccine groups: 1) *P. haemolytica* Pneumostar™, Biostar Inc., Saskatoon, Saskatchewan) (PHVACC), 2) *H. somnus* Somnustar™, Biostar Inc., Saskatoon, Saskatchewan) (HSVACC) 3), *P. haemolytica* and *H. somnus* (Somnustar PH™, Biostar Inc., Saskatoon, Saskatchewan) (COMBINED),

and 4) an unvaccinated control group (CONTROL). All animals at Feedlot A also received Pyramid ™ 4MLV, (Ayerst Laboratories, 1025 BLVD Laurentien, Saint Laurent, PQ), while all animals at Feedlot B received Bovishield ™ 4, (SmithKline Beecham Animal Health, 3130 Pepper Mill Court, Mississauga, ON). At processing, the rectal temperature and body weight were recorded, animals individually identified and blood samples collected. Approximately 28 days later (± 4 days) the cattle were weighed and blood samples collected again. During the intervening days the owners of the feedlots were asked to record cattle requiring treatment. No attempt was made to standardise the criteria for selection for treatment between the feedlots. Owners were asked to record the rectal temperature, classify the animals level of depression based on the criterion proposed by Perino *et al.* (8) and clinical signs present when animals where treated. The reason for treatment was recorded as UBRD, unless clinical signs existed that where referable to other body systems. During the study period, two animals were diagnosed with problems other than UBRD, and therefore were excluded from the analysis.

Serology

Day 0 and Day 28 serum samples were analysed for *P. haemolytica* leucotoxin ELISA titres (Biowest Laboratory, Biostar, Saskatoon, Saskatchewan), *P. haemolytica* indirect agglutination titres (P. Shewen laboratory at University of Guelph), *H. somnus titres* ELISA titres (Biowest Laboratory, Biostar Inc., Saskatoon, Saskatchewan) and viral neutralisation titres to bovine corona virus and bovine viral diarrhoea virus (E. Nagy laboratory at University of Guelph). Although not used in the analyses described in this chapter, *P. haemolytica* leucotoxin neutralisation titres were also measured (n= 600) (P.

Shewen laboratory at University of Guelph). Blood samples were collected in 10 millilitre vacuum tubes (Vacutainer, Becton Dickinson, Mississauga, Ontario). The blood was centrifuged and pipetted into microwell plates or 1.5-ml aliquots and stored until assayed. The techniques used to analyse the samples are described in previous reports (4,9,10). The ELISA tests used phosphatase labelled goat anti-bovine IgG (H+L) (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland). For statistical analysis the titres were transformed into an index that represented the dilution (well) of the last positive reaction for all titres as defined by the above techniques (11). The initial dilution of the *P. haemolytica* leucotoxin ELISA was 1/400 and the *H. somnus* ELISA was 1/ 200. For the bovine corona virus neutralisation assay the initial dilution was 1/4, while for the remaining tests the starting dilution was
1 /2 .

Statistical analysis

All data analyses, unless otherwise stated, were performed in SAS Release 6:12 ® (SAS Institute Inc. Cary, NC.). Descriptive analysis of the risk of treatment included the prevalence of treatment during the study period and the epidemiological curve of days to first treatment for all animals. Univariate comparisons of the proportions of treated animals by feedlot and vaccine group were made and significant differences determined using chi-square (χ^2) test for multiple proportions. These calculations were performed in a specially designed computer program using the technique described by Edgington (12).

The factors associated with the risk of treatment were examined using logistic regression techniques in PROC GENMOD. The arrival titres to *H. somnus*, bovine corona virus, bovine viral diarrhoea virus and *P. haemolytica*, and bacterial vaccine

group allocated at processing (VACCINE) were the explanatory variables of interest. Other explanatory variables examined were the calf's rectal temperature and weight at arrival. PROC GENMOD allows for the control of correlation at one level and therefore FEEDLOT was included in the REPEATED statement.

The approach to model building involved initial examination of all main effect variables of interest. Potential confounding variables for the *H. somnus*-UBRD association were added to or removed from the model based on their influence on the coefficients already present in the model. If a variable had a non-significant p value (<0.05: Wald test) and its removal did not materially change the *H. somnus* coefficients the variable was omitted. A significant change in the coefficient was arbitrarily set at a 10 % change in magnitude. After establishing a main effect model, biologically feasible interaction terms were added. Because the addition of interaction terms results in changes in the coefficients of the main effects associated with the interaction, the preferred model was chosen based on a significant decrease in the residual deviance of two compared models, i.e. the G statistic (χ^2 test statistic) with a p value was <0.1

The outcome for the logistic model of disease risk had two values (Y=1 represented treatment for UBRD, otherwise Y = 2) and the response probability modelled was p = Pr (Y = 1|x). Therefore, the main effect model of interest was:

$$g(x)_i = \beta_0 + \beta_1 + \beta_2 + \beta_3 + \beta_4 + \beta_5 + \beta_6 + + \beta_7 + \beta_8 + R_1$$

Where

$g(x)_i$ = log (p /1-p) for the *ith* individual

β_0 = mean of the response variable

β_1 the fixed affect of arrival *P. haemolytica* indirect agglutination titre

β_2 the fixed effect of arrival *P. haemolytica* leucotoxin ELISA titre

β_4 the fixed effect of arrival bovine corona virus titre

β_5 the fixed effect of arrival bovine viral diarrhoea virus titre

β_6 the fixed effect of arrival *H. somnus* titre

β_7 the fixed effect of VACCINE

β_8 the fixed effect of arrival weight

R_1 is the random effect of FEEDLOT (13,14) .

Factors affecting change in weight were assessed using regression techniques in PROC MIXED. Because treatment for UBRD (TRT) could be considered an intermediary step between the association of arrival titre and weight change, models were constructed with and without TRT. The fit of the model was examined by dividing the residuals by the standard deviation of the residuals and plotting these values against the variables of interest (15). One outlying data point was identified, an animal with a –84kg weight change during the study period. The variables FEEDLOT and the GROUP (the group a calf was processed in) where included in the model as random variables. PROC GLM (excluding the random variables) was used to evaluate the models and obtain the multiple coefficient of determination (R^2)

3.3 Results

Thirty-two animals had missing titre data, and were excluded from the analysis. Overall, 20% of animals were treated for UBRD, more than half of these were in Feedlot A (Table 3.1). Of the animals treated, nine did not have the rectal temperature reported,

and of these nine, six had a depression score of two (8). The remaining three animals had no information recorded other than clinical signs. Of the remaining treated animals all had a rectal temperature greater than 40 ^{0} C. The day animals arrived at the feedlot (GROUP) affected the likelihood of treatment (Table 3.2). The non-vaccinated calves had a higher risk of UBRD (25%) than vaccinated calves (Table 3.3). The peak risk of treatment occurred at Day 9 (Figure 3.1), perhaps slightly earlier at Feedlot A (Figure 3.2). The epidemic curves had approximately the same appearance across vaccine groups (Figure 3.3, Table 3.4).

The multiple regression (logistic regression) associations between the variables of interest and risk of treatment are shown in Table 3.4. The *P. haemolytica* indirect agglutination arrival titre was consistently insignificant, therefore it was excluded from the final model. The risk of treatment decreased for calves with higher arrival titres to *P. haemolytica* (leucotoxin ELISA), bovine corona virus or bovine viral diarrhoea virus. Higher arrival titres to *H. somnus* tended to be associated with reduced disease risk, but the relationship was only marginally significant (p=0.06). Vaccines containing *P. haemolytica* antigens reduced the risk of UBRD relative to the non-vaccinated group (OR 95% CI: PHVACC –0.5 to –0.2; COMBINED –1.03 to -0.4). We examined the effect of either random effect, FEEDLOT or GROUP, on the model and the predicted results were the same. Therefore the final model used the higher level of aggregation (FEEDLOT) because this statistically incorporated the effect of lower levels of clustering.

The average weight at arrival was 247 ± 32 kg, and the average weight gain was 27 ± 18 kg (Table 3.5). There was no difference in arrival weight among the feedlots but Feedlot B had a higher weight gain (Table 3.6). Vaccine groups did not differ with

respect to arrival weight or weight gain (Table 3.7). Calves treated for UBRD were lighter at arrival and gained less than untreated calves (Table 3.8). The distribution of weight gain across vaccine groups, and feedlot, was similar. All were somewhat left skewed (Figure 3.4, Figure 3.5).

Two multivariable models describing factors affecting the change in weight are shown in Table 3.9. Model 1 omits the effect of TRT, while Model 2 includes TRT as an explanatory variable. VACCINE was not significant, nor was an interaction between the arrival titre and VACCINE. Thus, these variables were removed from the final models. Arrival weight was negatively associated with weight gain. Higher arrival titres to the *P. haemolytica* (leucotoxin ELISA), *H. somnus* and bovine viral diarrhoea virus were associated with greater weight gains. Treated animals gained 15kg less over the study period than untreated animals. The change in the magnitude of the coefficients of the arrival titres from Model 1 to Model 2 indicates that TRT partially confounded the relationship between the arrival titres and weight gain.

The covariance parameter estimates for FEEDLOT, GROUP and the residual were 10.6, 1.7, and 281.9, respectively. The R^2 for a model using the variables in the PROC MIXED models without TRT (Model 1) was 0.09, while for a model including TRT (Model 2) the R^2 was 0.2.

The multivariable model of factors associated with the change in weight indicated that TRT had a major effect on weight gain during the study period, but the effect varied with the *H. somnus* titre, as did the effect of the arrival weight (Table 3.10). Higher titres to *H. somnus* at arrival were associated with increased weight gains, except in the lighter calves where there was either no association (if treated) or a negative association (if

untreated). In general, heavier calves at arrival gained more weight if they had higher *H. somnus* titres, but less weight, if they had low *H. somnus* arrival titres (Figure 3.6). No interaction between *H. somnus* titre and the titre to other organisms was noted.

3.4 Discussion

Our major intent in this study was to assess and understand any associations between *H. somnus* titres and UBRD or weight gain. In order to make valid causal inferences about an organism we believe there are at least three essential pieces of information needed: the association of arrival titre with disease risk and weight gain, the effect of vaccination in reducing the risk of UBRD or increasing weight gains, and finally a significant relationship between UBRD occurrence and titre change. The first two of these are investigated in this portion of our study.

If infection with *P. haemolytica* is associated with UBRD occurrence, as reported by Martin *et al* (1) and Booker *et al* (2) , then it might be expected that higher titres at arrival, which are surrogate evidence of previous exposure, would be associated with protection. However, because of the dynamic nature of infection, immune response and UBRD occurrence, the overlap in timing of these events could cloud this relationship. The previously reported relationship between *P. haemolytica* indirect agglutination titres at arrival and increased risk of treatment was not observed in this study and the reasons for this are unclear (1). On the other hand, the association we observed between the higher arrival titres to *P. haemolytica* leucotoxin and decreased risk of treatment for UBRD has not been reported by other authors(2). This disparity may be a consequence of the statistical approaches to analysis of the data. The authors of the previous studies

used logistic regression modelling with both arrival titre and change in titre as explanatory variables. The inclusion of the change in titre and the arrival titre in the same model may have masked the effect of arrival titre because the arrival titre and titre change were negatively correlated. This may explain why the previous authors found that only increased titre change to *P. haemolytica* leucotoxin was associated with UBRD occurrence (1,2). The findings of the current study, that a higher *P. haemolytica* arrival titre was sparing for subsequent UBRD were consistent with the general findings in the literature that *P. haemolytica* is an important agent of UBRD. The decreased risk of UBRD occurrence with the use of leucotoxin containing *P. haemolytica* vaccines provided further support for *P. haemolytica* as a causal agent of UBRD (3,7,16).

Support for a causal role of *H. somnus* in UBRD was our finding of the sparing association between higher arrival titres to *H. somnus* (2,4). However, given that vaccination was not protective and that titre change has not been associated with disease occurrence we would suggest another reason for the association. It is possible, that the presence of *H. somnus* titres at arrival is a proxy for a "healthy calf". The notion that titres to an agent may represent a proxy for a healthy calf, rather than implicate that agent as a cause of disease, has been previously suggested by Ganaba *et al* (17) with respect to bovine corona virus. They suggested that although bovine corona virus was statistically associated with respiratory disease, " it is possible that this lack of sero- reaction in some calves could be an indicator of their incapacity to respond immunologically as efficaciously as other calves" (17). A similar mechanism may be true for *H. somnus* titres. Further support for this is that higher arrival titres to all four agents investigated by the current authors were sparing for subsequent UBRD (Table 3.4), and this has been

noted previously by other authors. It is possible that specific immunity played a role in this protection, but it seems more likely to reflect the general ability of a healthy calf to respond to a variety of antigens.

Our results indicated that vaccination with *P. haemolytica* antigens decreased the risk of treatment for UBRD, whereas vaccination with *H. somnus* antigens alone did not. Further, the large overlap of the confidence intervals of the COMBINED and PHAVCC groups indicated that the COMBINED vaccine had approximately the same effect on disease risk as the PHAVCC. Thus we infer that the addition of the *H. somnus* component to the COMBINED vaccine did not provide additional protection. As vaccination against *H. somnus* did not alter the risk of subsequent UBRD, this would not support the thesis that *H. somnus* was a major cause of UBRD in this study.

The main effect models of change in weight suggested that treatment for UBRD was the most significant factor affecting weight gain, among the variables examined. This was expected, since treated animals probably had diminished appetites and those with normal appetites were probably less competitive at the feed bunk and therefore ate less during illness. Any compensatory gains that these animals might display were not evident within 28-days post arrival. The results also provided some reassurance that despite the likely misclassification of disease status within the study animals, the classification system used to differentiate sick from well animals (treated versus untreated) was useful because we would expect that sick animals would gain less than well animals. In addition, despite differences in the outcome (disease risk versus weight gain), both models showed a similarity of the effect of arrival titre; evidence of exposure

to *H. somnus* prior to arrival was associated with decreased disease risk and increased weight gain.

The coefficients for the arrival titres of *P. haemolytica*, *H. somnus* and bovine viral diarrhoea virus on weight change indicated that the arrival titres had an impact independent of the their effect on the risk of being treated. These relationships have been noted before, but beyond the theory that *H. somnus* titres at arrival were a proxy for a healthy calf, the mechanism behind these effects is not clear. Heavier animals with low titres to *H. somnus* gained less weight during the study period. If heavier animals were older at arrival, and if, as suggested in the literature, exposure to *H. somnus* is common prior to arrival at the feedlot, then low *H. somnus* titres in these older animals may have been indicative of a "weak " or "ineffective" immune response to *H. somnus* (18,19,20) . This ineffective response to *H. somnus* may suggest that these animals were somewhat immuno-compromised and therefore more likely to be treated for UBRD and gain less weight. Lighter "younger" animals, with decreased opportunity for exposure to *H. somnus* prior to arrival, would not be expected to have similar weight gains to their older more exposed pen mates.

Initially, we had thought that the inclusion of the random variables FEEDLOT and GROUP would explain a large amount of variability of the weight gained because these variables should account for different feeding practices, etc. However, the covariance parameter estimates and the R^2 of the OLS models suggested that a large amount of the variability in the weight gain was not accounted for by any of the explanatory variables we measured. The unexplained variability may be due to factors such as sources of calves, stress during shipment, and individual genetics.

The owners of the feedlots were instructed to select animals for treatment based on their usual criterion and it would appear that a rectal temperature greater than 40 ^{0} C was one of those criterion. This may be because of the advice received from veterinarians or published literature. This would suggest that the majority of animals classified as UBRD cases did require treatment but does not diminish the possibility that UBRD cases were not identified and therefore were included in the untreated group. This misclassification would result in a bias of the study findings towards the null hypothesis (21) . Likewise, because the sensitivity and specificity of the serological tests was not available, the study results could not be adjusted for any bias due to exposure status misclassification. Generally, misclassification bias results in a bias of study findings toward the null hypothesis (21,22,23,24) . The likelihood and impact of any misclassification should be considered when evaluating the study conclusions.

In summary, although previous exposure to *H. somnus*, as evidenced by arrival titre, was associated with protection against UBRD and increased weight gains, this should not be interpreted as evidence that *H. somnus* caused UBRD. Instead, in the absence of evidence of vaccine efficacy, and given that there was no association of titre change with UBRD risk, we would suggest that the ability to produce antibodies to *H. somnus* may only imply a functional healthy immune system that is capable of producing antibodies to a variety of antigens, some of which, from other agents, are protective against UBRD. Thus, collaborative support for *H. somnus* as a cause of UBRD is lacking (4).

The reduced risk of treatment in animals receiving vaccines containing *P. haemolytica* antigens continues to support the notion that *P. haemolytica* is causally

related to UBRD. Our finding that arrival titres and vaccination with *P. haemolytica* antigens are sparing for UBRD, coupled with reports from other authors that increased titre changes are associated with increased UBRD risk is continuing support for *P. haemolytica* as an agent of UBRD.

Table 3.1 The distribution of the number of animals treated for undifferentiated bovine respiratory disease, by feedlot, at three Ontario feedlots over a 28 day study period, Fall 1998.

	Feedlot A	Feedlot B	Feedlot C
	No. (%)	No. (%)	No. (%)
Not Treated	207 (65 %)	381 (88%)	90 (90%)
Treated	111 (35%)[a]	54 (12%)[b]	9 (10%)[c]
	318	435	99

Within rows, columns with the same superscript do not differ in the proportion of treated animals

Table 3.2 : The number of animals treated for undifferentiated bovine respiratory disease, by GROUP and FEEDLOT, at three Ontario feedlots over a 28 day study period, Fall 1998.

Processing day		10/04	10/05	10/22	10/23	10/24	11/03	11/05	11/08	11/09
FEEDLOT A	Yes			31		8	54	18		
	No			40		20	75	72		
FEEDLOT B	Yes	29	12						6	7
	No	92	78						107	104
FEEDLOT C	Yes				9					
	No				99					

Table 3.3: The distribution of the number of animals treated for undifferentiated bovine respiratory disease, by vaccine, at three Ontario feedlots over a 28 day study period, Fall 1998.

	CONTROL	*H. somnus* only vaccine[x]	*P. haemolytica* only vaccine[y]	*H. somnus* and *P. haemolytica* vaccine[z]
	No. (%)	No. (%)	No. (%)	No. (%)
Not treated	162 (75%)	167 (80%)	172 (81%)	177 (83%)
Treated	55 (25%)[a]	41 (20%)[ab]	42 (19%)[ab]	37 (17%)[b]
	217	209	213	213

Within rows, columns with the same superscript do not differ in the proportion of treated animals

[x] Somnustar ™, Biostar Inc., Saskatoon, Saskatchewan

[y] Pneumostar ™, Biostar Inc., Saskatoon, Saskatchewan

[z]Somnustar PH ™, Biostar Inc., Saskatoon, Saskatchewan

Figure 3.1 The frequency distribution (%), and cumulative percentage, of days to first treatment for animals treated for undifferentiated bovine respiratory disease at three Ontario feedlots over a 28 day study period, Fall 1998.

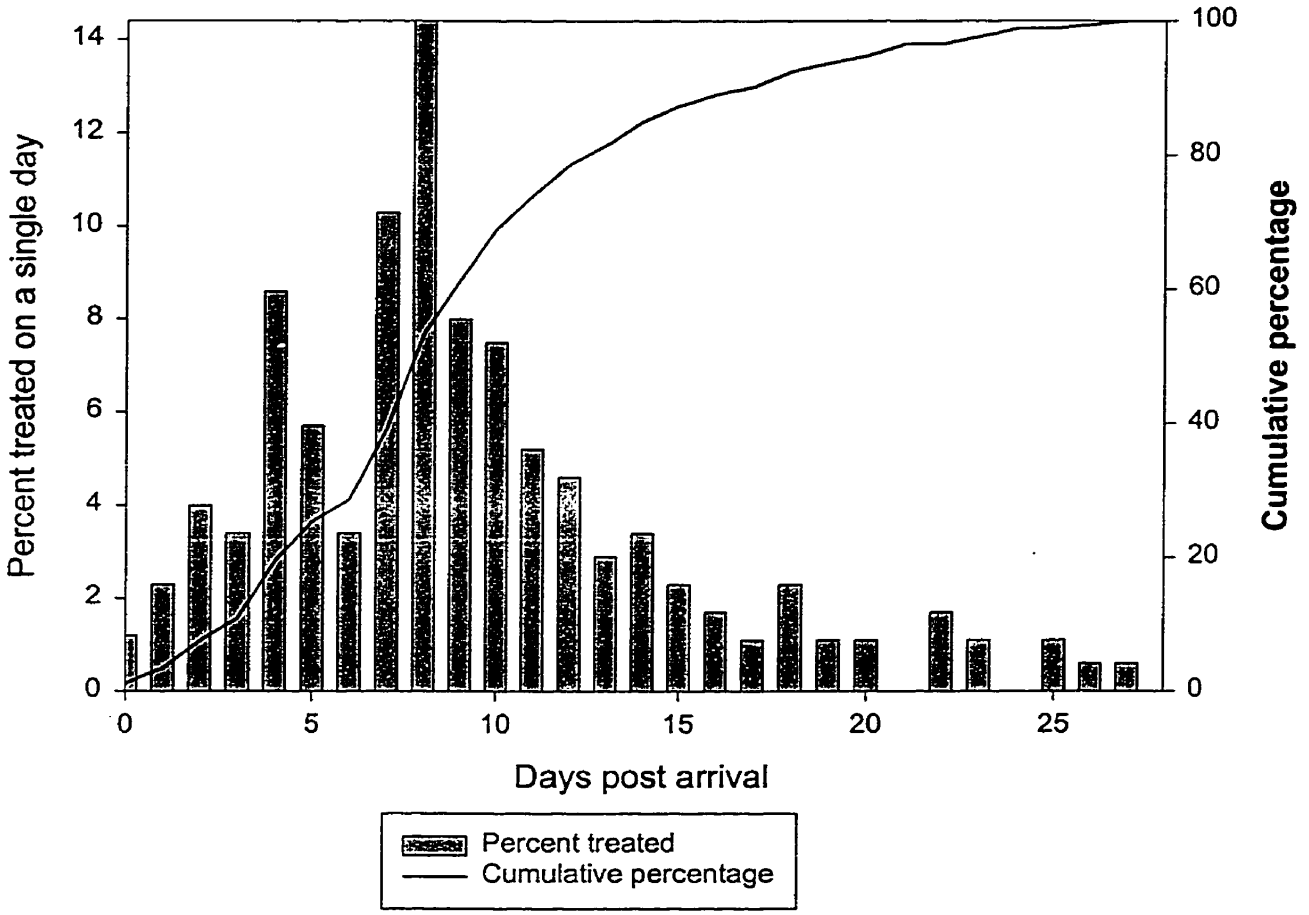


Figure 3.2: The frequency distribution (%), and cumulative percentage, of days to first treatment for animals treated for undifferentiated bovine respiratory disease, by feedlot, at three Ontario feedlots over a 28 day study period, Fall 1998.

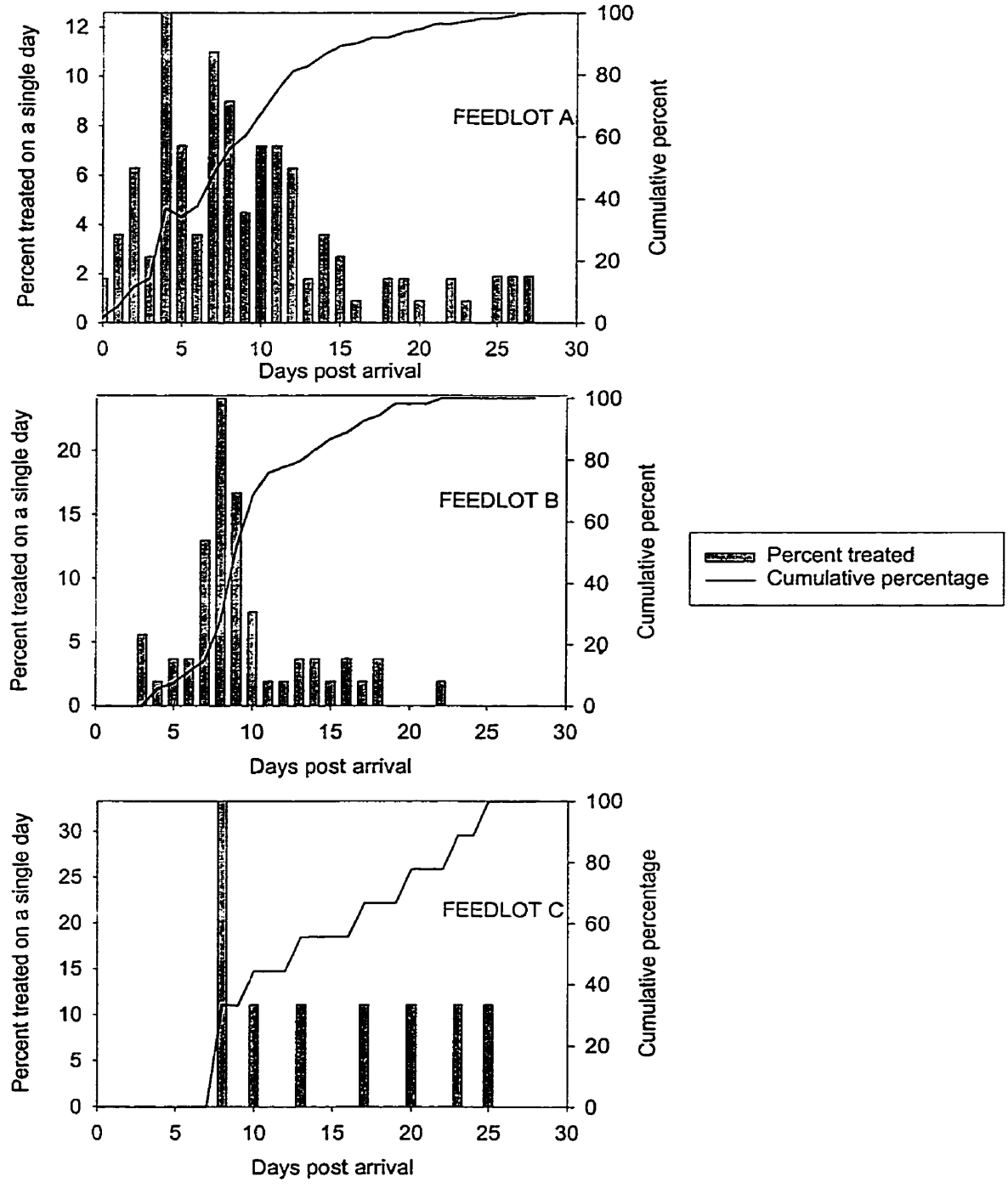


Figure 3.3: The frequency distribution (%), and cumulative percentage, of days to first treatment for animals treated for undifferentiated bovine respiratory disease, by vaccine group, at three Ontario feedlots over a 28 day study period, Fall 1998.

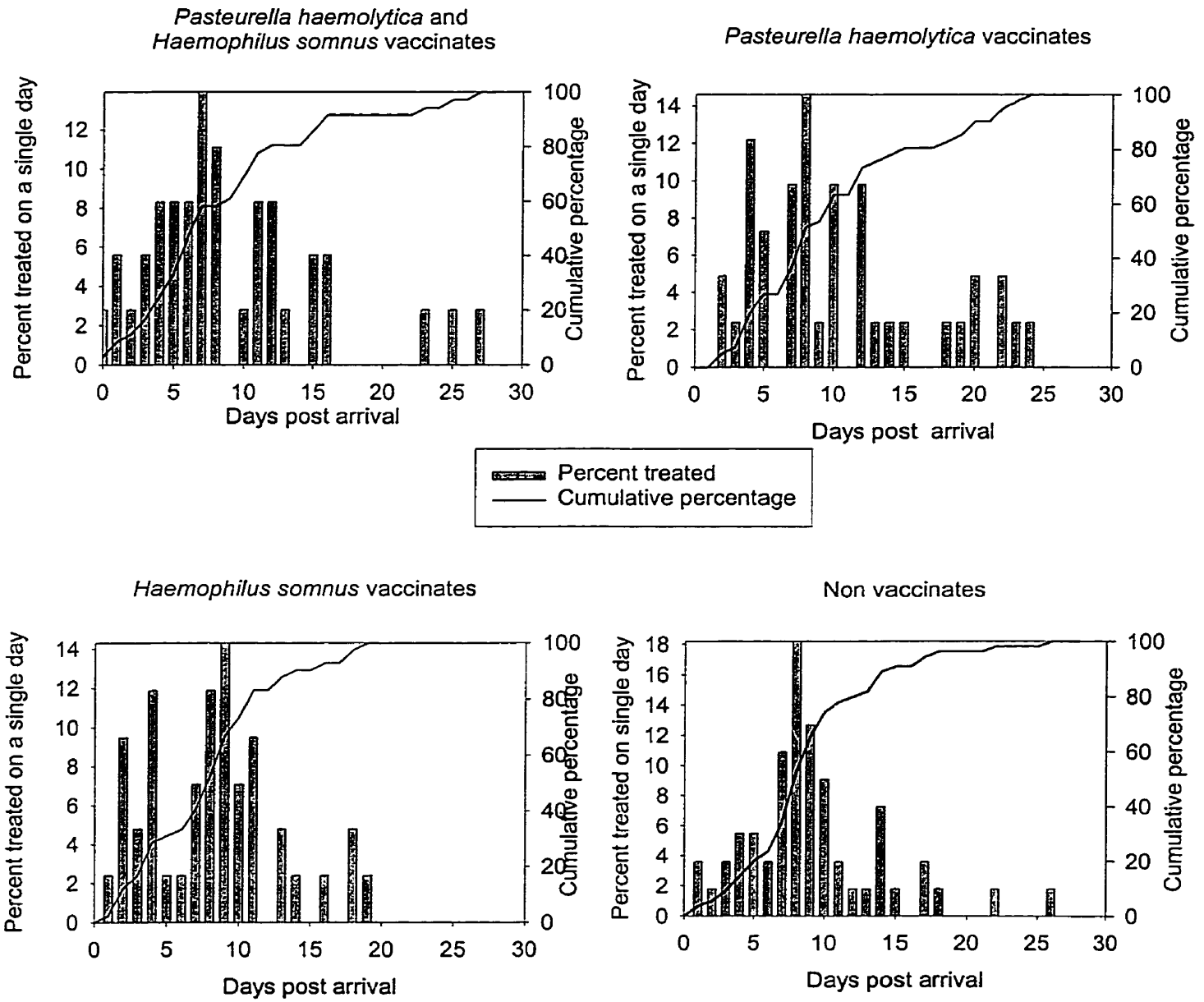


Table 3.4: The predicted effect of vaccine group, arrival titre and arrival weight on the probability of being treated for undifferentiated bovine respiratory disease during a 28 day study on cattle from three Ontario feedlots, Fall, 1998

Variable		Regression Coefficient	SE	p-value
INTERCEPT		1.22	0.9	0.2
VACCINE	COMBINED	-0.7	0.2	0.00
	HSVACC	-0.3	0.2	0.2
	PHVACC	-0.3	0.07	0.00
	CONTROL	0.00	.	.
P. haemolytica leucotoxin ELISA		-0.2	0.04	0.00
H. somnus ELISA		-0.1	0.08	0.06
Bovine corona virus		-0.08	0.04	0.03
Bovine viral diarrhoea virus		-0.1	0.007	0.00
Arrival weight		-0.005	0.004	0.3
Scale		0.96		
DF		805.		
Deviance		732.5		
Log likelihood		-366.2		

Table 3.5: Descriptive statistics for arrival weight and change in weight for cattle at three Ontario feedlots over a 28 day study period, Fall 1998.

Variable	n	mean	S.D.	minimum	maximum
Arrival weight	852	247.2	32.3	160	394
Change in weight	842	27.3	18.9	-84	88.0

Table 3.6: The mean and standard deviation for the change in weight of cattle at three Ontario feedlots over a 28 day study period by feedlot, Fall 1998.

	FEEDLOT A			FEEDLOT B			FEEDLOT C		
	n	mean	S.D.	n	mean	S.D.	n	Mean	S.D.
Arrival weight	318	235.8[a]	34.5	435	247.2[a]	20.8	99	283.4[a]	37.8
Change in weight[∞]	310	23.3[a]	21.4	433	31.8[b]	16.4	99	20.6[a]	16.1

[•] Within rows, columns with the same superscript do not differ

[∞] Controlled for arrival weight

Table 3.7: The mean and standard deviation for the change in weight of cattle at three Ontario feedlots over a 28 day study period, by vaccine group, Fall 1998.

Variable	COMBINED			HSVACC			PHVACC			CONTROL		
	n	mean	S.D.	n	mean	S.D.	n	mean	S.D.	n	mean	S.D.
Arrival weight	213	247[a]	31.2	209	246.0[a]	30.5	213	249.1[a]	31.5	217	245.9[a]	35.6
Change in weight[∞]	207	27.4[a]	18.8	208	29.0[a]	18.6	211	26.5[a]	18.3	216	26.3[a]	19.9

• Within rows, columns with the same superscript do not differ

[∞] Controlled for arrival weight

Table 3.8: The mean and standard deviation for the change in weight at three Ontario feedlots over a 28 day study period for treated and untreated cattle, Fall 1998.

	Untreated		Treated	
	mean	S.D.	mean	S.D.
Arrival weight	249.6[a]	31.5	237.8[b]	33.7
Change in weight[∞]	30.7[a]	16.5	13.5[b]	21.6

• Within rows, columns with the same superscript do not differ

[∞] Controlled for arrival weight

Figure 3.4: The frequency distribution of change in weight for animals for 852 cattle, by vaccine group, at three Ontario feedlots over a 28 day study period, Fall 1998.

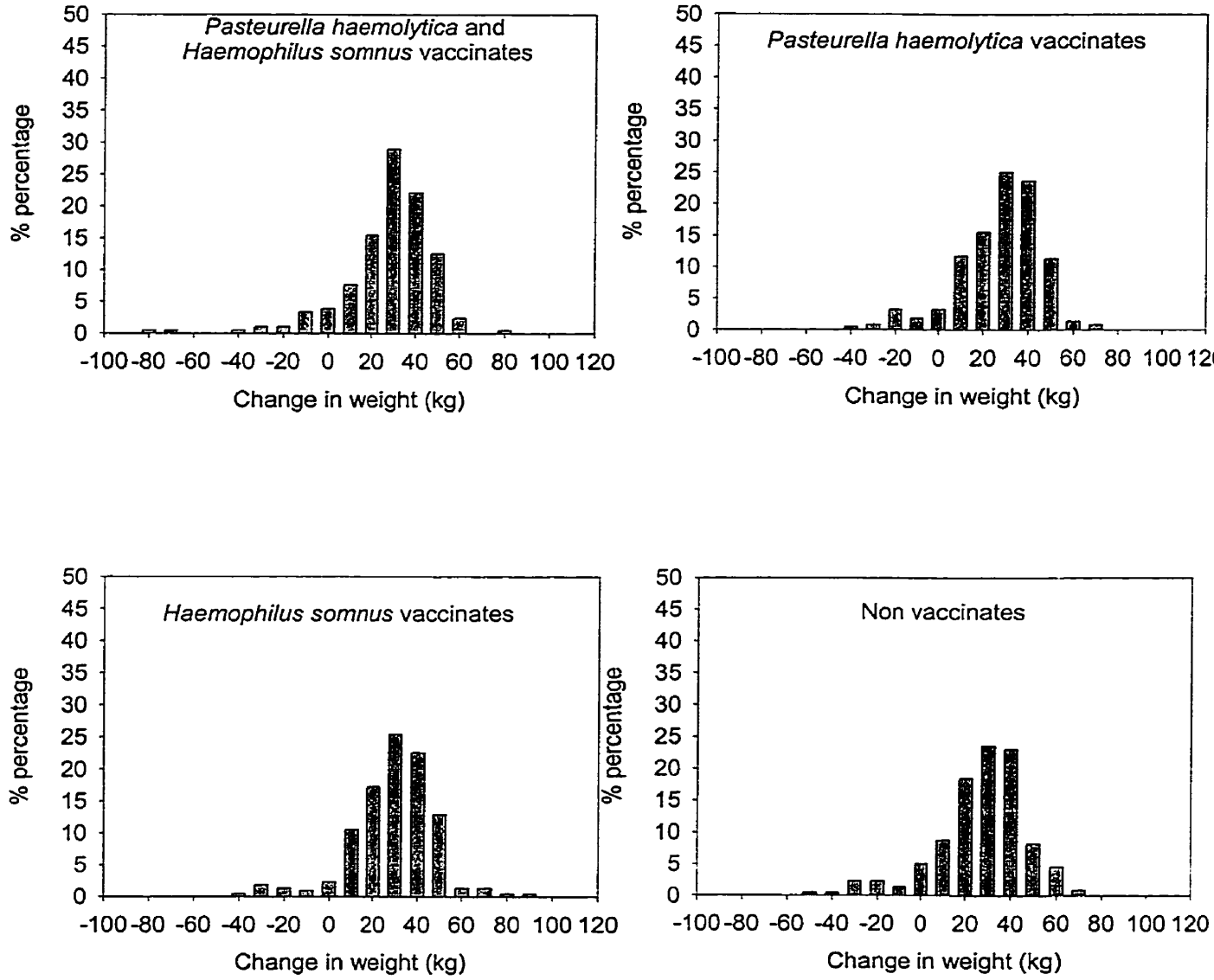


Figure 3.5: The frequency distribution of change in weight for animals for 852 cattle, by feedlot, at three Ontario feedlots over a 28 day study period, Fall 1998.

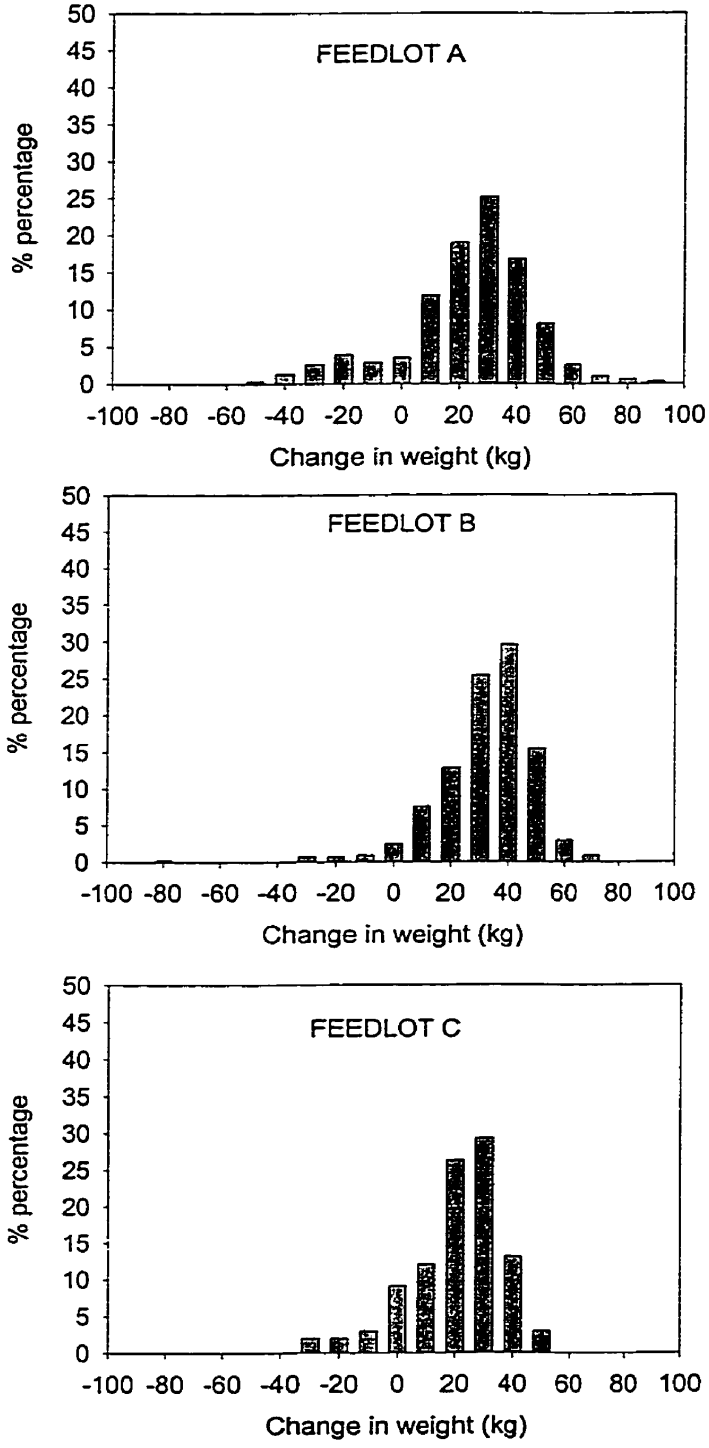


Table 3.9: The main effect models for weight change during a 28 day study period on cattle from three Ontario feedlots, Fall, 1998.

	Model 1			Model 2		
Variable	coefficient	SE	P	coefficient	SE	P
INTERCEPT	26.0	5.9	0.04	35.2	5.7	0.02
P. haemolytica ELISA titre	1.9	0.5	0.000	1.5	0.5	0.004
H. somnus ELISA titre	1.4	0.4	0.001	1.1	0.4	0.006
bovine corona virus titre	0.5	0.4	0.2	0.3	0.3	0.4
bovine viral diarrhoea virus titre	0.8	0.2	0.000	0.6	0.2	0.007
Arrival weight	-0.04	0.02	0.04	-0.05	0.02	0.008
Treatment for UBRD				-15.0	1.6	0.00
DF	805			804		
RLL[1]	-3502.6			-3458.1		
AIC[2]	-3505.6			-3460.1		

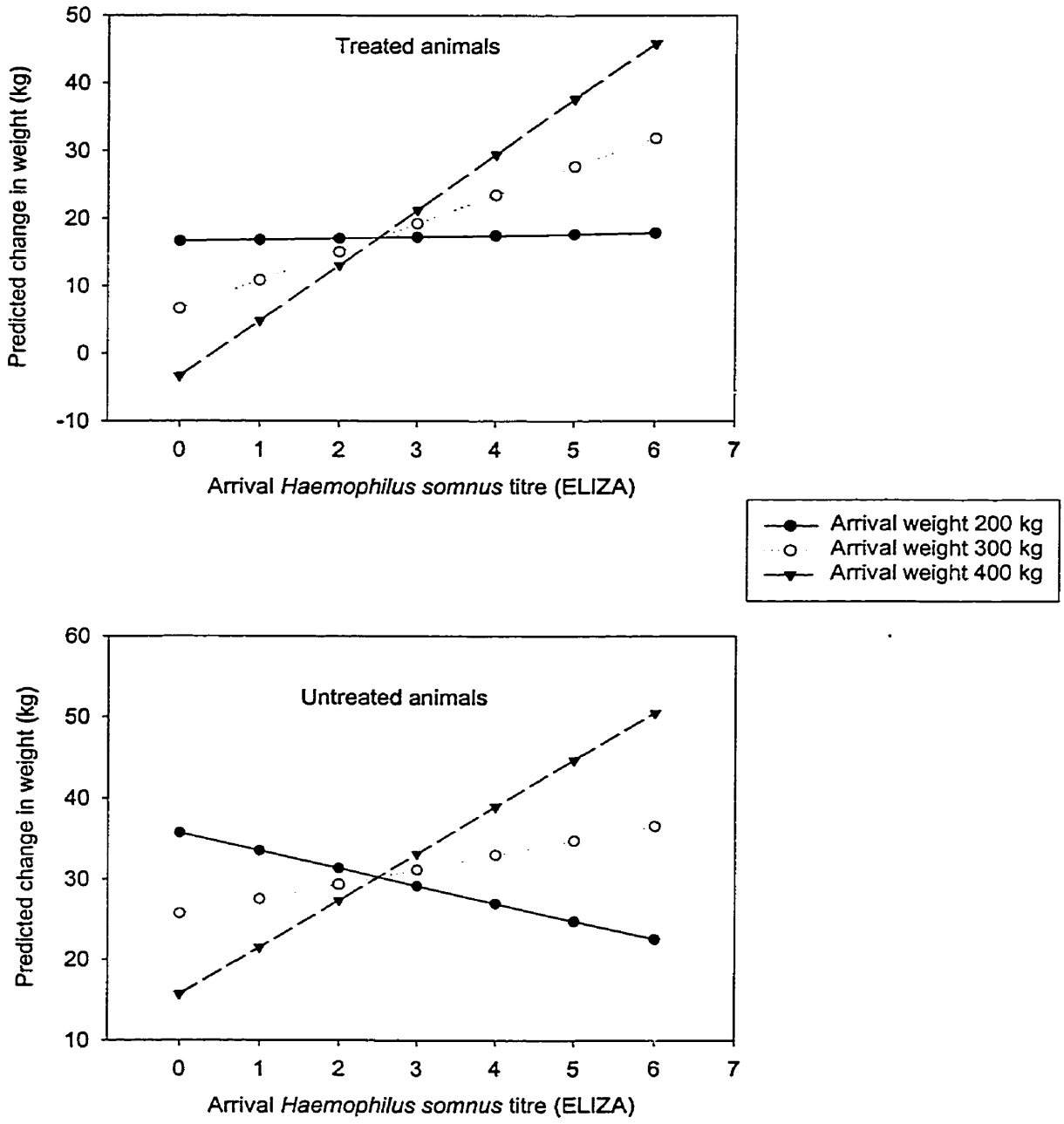
[1]RLL : Residual Log Likelihood [2] AIC : Akaike's Information criterion

Table 3.10: Multivariable model results of change in weight regressed on arrival titres to bacterial and viral agents, and treatment for undifferentiated bovine respiratory disease, during a 28 day study period of cattle in three Ontario feedlots, Fall, 1998.

Variable	Regression Coefficient	SE	p-value
Intercept	55.8	8.0	0.02
Arrival weight	-0.1	0.03	0.00
Treatment for UBRD	-19.3	2.2	0.00
P. haemolytica ELISA titre	1.6	0.5	0.001
H. somnus ELISA titre	-10.2	3.1	0.001
Bovine corona virus	0.2	0.3	0.6
Arrival weight* *H. somnus* ELISA titre	0.04	0.01	0.000
Bovine viral diarrhoea virus * *H. somnus* ELISA titre	0.4	0.1	0.003
H. somnus ELISA titre* treatment for UBRD	2.3	1.04	0.02
DF	813		
RLL	-3451.3		
AIC	-3453.3		

[1]RLL : Residual Log Likelihood [2] AIC : Akaike's Information criterion

Figure 3.6: Predicted behaviour of change in weight affected by arrival *Haemophilus somnus* titre and treatment and weight on the change in weight during a 28 day study of cattle at three Ontario feedlot, Fall, 1998 (Variations of coefficients and statistical effects are shown in Table 3.10)



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Chapter 4 Failure to Find an Association Between Changes in *Pasteurella haemolytica* titres and Undifferentiated Bovine Respiratory Disease Occurrence

4.1 Introduction

In sero-epidemiologic studies, an increase in the serum antibody titre to putative causal agents that is temporally concomitant with disease occurrence has been used as a proxy for exposure to a causal agent of that disease. For example, by regressing the occurrence of undifferentiated bovine respiratory disease (UBRD) on titre change using logistic regression models, increases in titres to *Pasteurella haemolytica* leucotoxin have been associated with an increased risk of UBRD (1,2). However, as most disease in feedlots occurs in the first 7-10 days post arrival it is feasible that titre change would be observed after disease occurrence (3). Technically, the effect of the timing of the proxy variable can lead to invalid causal associations because of the possibility of reverse causation, i.e. being sick may cause the titre change (4). As the second sample, in the previous studies, was not taken until day 28 post arrival it may have been better to model titre change as a function of disease occurrence, as UBRD was probably temporally antecedent to titre change (5). Therefore, in this study we aimed to examine the association between evidence of exposure and disease occurrence by predicting titre change as a function of UBRD occurrence. The null hypothesis was that UBRD occurrence was not associated with the change in titre to *P. haemolytica* leucotoxin or surface antigen and, by extrapolation, that UBRD occurrence was not associated with exposure to the *P. haemolytica*. Two measures of *P. haemolytica* leucotoxin titre, a leucotoxin neutralisation and an ELISA titre, plus an indirect bacterial agglutination titre

to *P. haemolytica* were examined when testing this hypothesis. We further hypothesised that timing of UBRD treatment was not associated with titre change to *P. haemolytica* antigens.

4.2 Materials and Methods

Animal management and serological analysis are described in Section **3.2** and summarised in Table 4.1.

Statistical analysis

All data analyses, unless otherwise stated, were performed using SAS Release 6:12 ® (SAS Institute Inc. Cary NC.). The unit of analysis was the individual animal. The outcome for the analyses was the change in the *P. haemolytica* titre. For statistical analysis, the results of the serological assays were transformed into an index (6). This index represented the dilution, or well, of the last positive reaction as defined by the techniques outlined (7,8,9) and, hereafter, the index will be referred to as the titre. Animals with no reaction were recorded as having a titre of zero.

For regression modelling, transformed arrival titres were retained as continuous numbers but for the titre frequency distributions the numbers were rounded to the nearest integer. Any transformed titre values equal to or lower than the halfway point between dilutions were rounded down, i.e. 1.5 converted to the integer of one not two. The change in titre over the 28-day period was calculated as the difference between transformed titres. Other continuous variables were temperature at arrival and weight at arrival. Treatment for UBRD was classified as a class variable (TRT -2 levels), and other class variables included feedlot (FEEDLOT – 3 levels), calf group defined by day of

processing (GROUP- 9 levels) and vaccine group (VACCINE- 4 levels). For models examining the effect of the timing of treatment, animals were classified as to whether they were treated early (in the first nine days), late (10 days or after) or never treated.

Descriptive statistics for arrival titres and titre change included the geometric mean, standard deviation, range of the transformed titres and frequency distribution histograms. Calves were categorised as sero-positive or sero-negative at arrival, any titre > 0.5 being deemed as sero-positive. Sero-conversion denoted an increase in titre (index) greater than two. For two-fold dilutions test this represents a four-fold increase in titre and for four-fold dilution this represents a 16-fold increase in titre, i.e., for both dilutions a two well increase was required for sero-conversion.

Differences among the vaccine groups and feedlots in arrival titre and titre change were determined using univariate ANOVA techniques (PROC GLM). Odds ratios (OR) were calculated to describe statistical associations between sero-positivity or sero-conversion and risk of treatment. The 95 percent confidence limits for the OR were presented (OR 95% CL). The Pearson correlation coefficients between the arrival titre and the change in titre for *P. haemolytica* were calculated using PROC CORR.

Regression techniques were used to determine factors, including UBRD occurrence, affecting the change in titre to *P. haemolytica* titres. The analyses were performed using PROC MIXED. The model of interest was

$$y_i = \mu + \beta_1 + \beta_2 + \beta_3 + (\beta_1 \times \beta_2) + (\beta_1 \times \beta_3) + (\beta_2 \times \beta_3) + R_1$$

Where

y_i is the change in titre for the individual i

μ = the mean response

β_1 the fixed affect of vaccination group,

β_2 the fixed effect of day zero titre

β_3 the fixed effect of treatment

$\beta_1 \times \beta_2$ is the interaction between arrival titre and vaccination group.

$\beta_1 \times \beta_3$ is the interaction between treatment and vaccination group.

$\beta_2 \times \beta_3$ is the interaction between treatment and arrival titre.

R_1 is the random effect of the GROUP and FEEDLOT

All the explanatory variables of interest were forced into the initial model. For each *P. haemolytica* titre outcome, potential confounding variables included other *P. haemolytica* arrival titres, the arrival titre to *H. somnus*, arrival weight and arrival rectal temperature. To determine if these potential confounding variables should be included in the final model, each variable was added to, or removed from, the model and the resulting effect on the coefficients of the variables involving the effect of treatment for UBRD examined. If the change in the point estimate of the variables involving treatment for UBRD 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. Potential confounders significant at $p < 0.1$ were retained, as were interaction terms. GROUP and FEEDLOT were entered as random effects (10). The cluster specific coefficients of random effects are not reported. The fit of the model was examined by dividing the residuals by the standard deviation of the residuals and plotting these values against the variables of interest. The influence of an animal's data could not be determined because the hat matrix was not provided for mixed models (11). Models examining the effect of timing of UBRD treatment on titre change were built using the same approach.

4.3 Results

Due to sample handling errors, 24 animals from the first group of cattle sampled at Feedlot B were not analysed and have no values for day 0 titres. Of these 24 animals, 7 received the combined *P. haemolytica* and *H. somnus* vaccine, 5 received the *H. somnus* only vaccine, 4 were vaccinated with the *P. haemolytica* vaccine, and 8 received no vaccine. At day 28, 6 animals had died and samples could not be collected from 2 other animals. Of the 6 dead animals 5 came from Feedlot A and 1 from Feedlot B, 2 received the combined vaccine, 1 received the *H. somnus* only, 2 received the *P. haemolytica* antigen, and 1 received no vaccine. Another two animals lost their identification and could not be paired when the second samples were taken. In all 32 animals have missing titre data.

Thirteen percent of animals were treated once and seven percent treated more than once, most of these treatments occurred at feedlot A (Table 4.2). Of the animals treated, nine did not have rectal temperature reported, and of these nine, six had a depression score of two (12). Of the remaining treated animals all had a rectal temperature greater than 40^{0} C. Descriptive statistics for the titres are shown in Table 4.3. Table 4.4 shows the frequency distribution of the titres at arrival. Sixteen percent of animals were sero-negative for *P. haemolytica* leucotoxin ELISA, only one animal was sero-negative according to the *P. haemolytica* leucotoxin neutralisation titre and all animals had detectable *P. haemolytica* indirect agglutination titres at arrival. Cattle in feedlot C tended to have lower arrival titres, and titre changes, than cattle at the other two feedlots (Table 4.5). Exposure to *P. haemolytica* leucotoxin appeared to be common, as 55 and 81 percent of animals sero-converted to *P. haemolytica* leucotoxin ELISA and neutralisation

titres respectively. Sero-conversion to *P. haemolytica* indirect agglutination titre occurred in 51% of animals.

Animals that were sero-negative for *P. haemolytica* leucotoxin ELISA titres at arrival were more likely to be treated during the study period than sero-positive animals (OR 95% CL: 1.3 – 3.0). Animals that sero-converted to the *P. haemolytica* leucotoxin ELISA titre were more likely to be treated (OR 95% CL: 1.4 – 2.9) than other calves. The presence of titres at arrival to the *P. haemolytica* leucotoxin neutralisation and *P. haemolytica* agglutination at arrival were associated with reduced UBRD occurrence , if the cut-off for sero-negativity was set at the index eight (8 or 1/512) which approximated the initial starting dilution (1/400) of the *P. haemolytica* leucotoxin ELISA titre. Sero-conversion to the *P. haemolytica* leucotoxin neutralisation titre was not associated with increased disease risk (OR 95% CL: 0.7-2.4). Animals that sero-converted to *P. haemolytica* indirect agglutination were no more likely to be treated for UBRD (OR 95% CL: 1.0 – 2.06) than animals that did not sero-convert.

The Pearson correlation coefficient for the arrival neutralisation and ELISA titre was 0.39 ($p < 0.001$, n= 577) and for the change in neutralisation and ELISA titre it was 0.35 ($p < 0.001$, n = 550). The correlation between the arrival indirect agglutination titre and leucotoxin neutralisation titre was 0.28 ($p < 0.001$, n = 600), and the leucotoxin ELISA titre was 0.28 ($p < 0.001$, n = 826). The correlation between the change in indirect agglutination titre and the change in the leucotoxin neutralisation titre was 0.09 ($p = 0.03$, n= 572). There was greater agreement between the change in the indirect agglutination titre and the change in the leucotoxin ELISA titre as the correlation coefficient was 0.32 ($p <0.001$, n= 815).

For all measures of exposure to *P. haemolytica* i.e. the leucotoxin ELISA, the leucotoxin neutralisation and the indirect agglutination titre, the models predicted that the occurrence of UBRD was unrelated to titre change (Table 4.6, Table 4.7, Table 4.8). In addition, no interaction terms were significant. Higher arrival titres, predicted decreased titre increases subsequently. The vaccines had a significant effect on the change in the ELISA titre, i.e., those animals receiving *P. haemolytica* antigens had higher titre changes. However, for the *P. haemolytica* leucotoxin neutralisation titre and indirect agglutination titre, vaccination with *P. haemolytica* antigens did not affect the change in titre.

The variance component estimates of the random effects are presented in Table 4.9, and suggest that, for *P. haemolytica* leucotoxin ELISA and *P. haemolytica* indirect agglutination titres, FEEDLOT was a major source of variability of the outcome. For *P. haemolytica* leucotoxin neutralisation titres, random error accounted for most of the variation of the outcome.

For all *P. haemolytica* titres the models predicted that the time of treatment for UBRD was not significantly associated with evidence of exposure (results not shown). The only significant term for each titre outcome was the arrival titre. Vaccination with *P. haemolytica* antigens resulted in greater increases in the *P. haemolytica* leucotoxin ELISA titre only.

4.4 Discussion

Based on titres at arrival, a large percentage of calves had been exposed to *P. haemolytica* prior to feedlot arrival. However, a large difference existed in the number of

animals with no detectable titre to the two measures of *P. haemolytica* leucotoxin exposure at arrival. This is probably a function of the difference in the initial starting dilution of the tests and re-enforces the idea that without standardisation, titres are not comparable across tests but are only a relative measure of antibody levels between samples within serological tests. All animals had agglutinating titres at arrival.

The increase in *P. haemolytica* leucotoxin ELISA titre induced by the *P. haemolytica* antigens present in the vaccines used in this study has been reported previously (13). Although no published reports discuss the use of these particular vaccines on the *P. haemolytica* leucotoxin neutralisation and indirect agglutination titres, other vaccines have been shown to increase the antibody levels measured by these tests (14,15). We had anticipated that the leucotoxin titre response to the vaccines would have behaved similarly, in both test methods; however, the results are consistent with results from previous studies that suggest that antibody level changes induced by commercial *P. haemolytica* vaccines vary greatly with the vaccine used (16).

The failure to find a conditional association between evidence of exposure to *P. haemolytica* leucotoxin during the study period and UBRD occurrence was surprising, as previous observational studies have reported that evidence of active infection, determined by an increase in titre, was associated with UBRD occurrence and a large body of literature suggests that *P. haemolytica* is capable of causing UBRD (1,2,17,18). The magnitude of the odds ratio for sero-conversion to *P. haemolytica* leucotoxin in these observational studies was reasonably large and suggested a causal role of *P. haemolytica* in UBRD. Booker *et al* (2) reported that the odds of treatment were between 1.44 – 5.57 (OR 95% CI) higher for animals that sero-converted to the *P. haemolytica* leucotoxin

ELISA titre, while Martin *et al* (1) reported an odds ratio of a similar magnitude for sero-conversion to *P. haemolytica* leucotoxin neutralisation (OR = 2.4, no standard error reported, P <0.05). However, other studies have failed to find a strong association between changes in *P. haemolytica* leucotoxin titres and UBRD occurrence. Martin *et al* (7) reported a significant association between UBRD and changes in *P. haemolytica* leucotoxin neutralisation titres but the magnitude of the difference was small (OR = 1.08, standard error not reported, p <0.1). Hodgins and Shewen (14) also failed to demonstrate a difference in the titre change of vaccinated animals that died from experimental challenge and those that survived, though the number of calves involved was small. For the indirect agglutination titres, evidence of exposure during the study period has rarely been associated with disease occurrence and, when it has, the magnitude of the predicted effect has been small (OR 1.08, p <0.1; no standard error reported) (1,7,14). It should be noted that we did find an unconditional association between sero-conversion to *P. haemolytica* leucotoxin (ELISA) and UBRD, however it appears that the arrival titre confounds this relationship, resulting in its lack of significance in the multiple regression model.

Possible explanations for the failure to identify any association include; that *P. haemolytica* was not an agent of disease, that there was an insufficient gradient in exposure to *P. haemolytica* within the study groups, that the extent of disease misclassification was large enough to reduce the power of the study, or that the results were due to differences in the modelling approach in this versus previous studies.

In the current study we used a different approach to modelling the association between UBRD and titre changes to *P. haemolytica* than has been used previously.

Because the second blood sample for titre change was measured at day 28, we believed that titre change should be regressed on UBRD, not vice versa –as has been done previously. However, this change in approach should not have resulted in a decreased ability to identify the association. Differences in the approach used to control for clustering of the outcome and the levels of clustering identified may also account for the differences in study findings. We controlled for clustering at two levels and the results suggested that FEEDLOT was a major source of variability for the change in titre to *P. haemolytica* indirect agglutination and leucotoxin ELISA titres. Previous studies have accounted for only one level of clustering; a lower level of aggregation than our results would suggest was significant i.e., a fixed pen or group effect (1,2). These factors may account for the difference in conclusions drawn, as the failure to properly account for clustering may bias study results toward the alternative hypothesis (19). Unfortunately because the significance of various levels of clustering are not routinely published, it is not known which levels of clustering should have been included in models predicting titre change.

The nature of UBRD meant that it was not possible to determine if *P. haemolytica* was actually causing disease in this population; however, it was clear from Figures 4.1-4.3, that exposure to *P. haemolytica* occurred in many of the calves. Although there is no evidence that our data on exposure were very different from those reported previously, if there was only a limited range of exposure (most calves being exposed in this study) this mitigates against finding an association of that exposure with the outcome (20). Nonetheless, we examined the relationship between UBRD and *P. haemolytica* in several ways, including looking for possible interactions between arrival titre and vaccine, as

well as examining the effect of time to treatment, and were still unable to find an association between disease occurrence and titre change. Therefore it seems that there was no association between UBRD and titre change to *P. haemolytica* in this study population.

With respect to misclassification of the disease outcome as an explanation for the lack of an association, non differential misclassification of a variable results in a bias of study findings toward the null hypothesis. Further, UBRD misclassification is common in feedlots (10,21). However, although the results were not reported here, misclassification of the disease category may not be important in this study as the weight difference between animals classified as diseased versus non-diseased was quite large and suggested that the classifications were reasonably accurate, though this can not be proven. The owners of the feedlots were instructed to select animals for treatment based on their usual criterion and it would appear that a rectal temperature greater than 40^{0} C was one of those criterion. This may be because of the advice received from veterinarians or published literature. This would suggest that the majority of animals classified as UBRD cases did require treatment but does not diminish the possibility that UBRD cases were not identified and therefore were included in the untreated group. This misclassification would result in a bias of the study findings towards the null hypothesis (10). Likewise because the sensitivity and specificity of the serological tests was not available the study results could not be adjusted for any misclassification bias in the study. Generally, misclassification bias results in a bias of study findings toward the null hypothesis (10,22, 23,24). The likelihood and impact of this misclassification should be considered when evaluating the study conclusions.

In future studies it may be of value to reduce the sampling time to a shorter period such as 10 days. Reducing this time period would decrease the likelihood that the titre had risen and returned to the arrival level during the sampling time and therefore reduce the likelihood of misclassifing the exposure status of the animals. Identifying the isotope of antibody associated with the titre would also address concerns of possible bias in the serological tests and the effect that different isotopes arising from primary or subsequent exposure may have had on the study results.

In conclusion, we were unable to identify any association between *P. haemolytica* exposure and UBRD despite evidence that infection with *P. haemolytica* was common. This finding is contrary to our present understanding of the causal association between UBRD and titre change for *P. haemolytica* leucotoxin titres, but consistent with the previous observational study findings for the *P. haemolytica* indirect agglutination titres.

Table 4.1: Characteristics of the three Ontario feedlots used in the study of *Pasteurella haemolytica* titres, Fall 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's at arrival	Oxytetracycline	Oxytetracycline (<40°C) Tilmicosin (>40°C)	None
Modified live 4-way vaccine at processing	Yes[x]	Yes[y]	No
Sex	Mixed (276 F / 42 M)	Male	Male
Rectal temp at arrival; ^{0}C* (mean ± S.D.)	40.4 ± 0.9[a]	39.9 ± 0.7[b]	39.4± 0.4[c]
Range for temp.	4.2	3.7	2.9
Weight at arrival; kg (mean ± S.D.)	235.0 ± 34.9	247.2 ± 20.2	283.4 ± 37.8
Range of weight (kg)	208.00	129.00	219.00

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

[x] Pyramid ™ 4MLV, Ayerst Laboratories, 1025 BLVD Laurentien, Saint Laurent, PQ.

[y] Bovishield ™ 4, SmithKline Beecham Animal Health, 3130 Pepper Mill Court, Mississauga, ON

Table 4.2 : Distribution of treatments, by feedlot and vaccine group, at three Ontario feedlots used in the study of *Pasteurella haemolytica* titres, Fall 1998.

Vaccine	Times treated	Feedlot A n = 318	Feedlot B n = 435	Feedlot C n = 99	Total
H. somnus and *P. haemolytica* vaccine[v]	0	58	97	22	177 (83%)
	1	14	8	2	24 (11%)
	2	8	4	0	13 (6%)
H. somnus vaccine[x]	0	55	90	22	167 (80%)
	1	14	12	1	27 (13%)
	2	9	5	1	15 (7%)
P. haemolytica vaccine[y]	0	48	101	23	172 (81%)
	1	18	8	2	28 (13%)
	2	12	0	1	13 (6%)
Non vaccinates[z]	0	46	93	23	159 (74%)
	1	21	11	2	34 (16%)
	2	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%)
	2	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.

[v] Somnustar PH ™, Biostar Inc., Saskatoon, Saskatchewan

[x]Somnustar ™, Biostar Inc., Saskatoon, Saskatchewan

[y]Pneumostar ™, Biostar Inc., Saskatoon, Saskatchewan

[z] A control group not receiving any of these bacterial vaccines

Table 4.3 : Descriptive statistics for *Pasteurella haemolytica* titres at three Ontario feedlots, Fall 1998

	n	Geometric mean	Standard deviation	minimum	maximum
Arrival titre					
P. haemolytica leucotoxin neutralisation	600	8.3	3.3	0	17.5
P. haemolytica leucotoxin ELISA	828	2.3	1.3	0	6.3
P. haemolytica indirect agglutination	850	5.8	1.3	2	11
Change in titre					
P. haemolytica leucotoxin neutralisation	573	4.2	4.7	-10.0	23.0
P. haemolytica leucotoxin ELISA	818	2.1	1.4	-1.8	5.9
P. haemolytica indirect agglutination	840	1.3	1.8	-3.5	7.0

Table 4.4 Frequency distribution of *Pasteurella haemolytica* titres at three Ontario Feedlots, Fall 1998

Titre (well no.)	*P. haemolytica* leucotoxin ELISA	*P. haemolytica* indirect agglutination	*P. haemolytica* leuctotoxin neutralization
	No. (%)	No. (%)	No. (%)
0	134 (16.2 %)		1 (0.2%)
1	70 (8.5%)		3 (0.5%)
2	182 (22%)	6 (0.7%)	8 (1.3%)
3	284 (34.3%)	28 (3.3%)	19 (3.2%)
4	136 (16.4%)	119 (14 %)	20 (3.3%)
5	21 (2.5%)	221 (26%)	58 (9.7%)
6	1 (0.1%)	249 (29.3%)	67 (11.2%)
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 4.5 : Descriptive statistics for *Pasteurella haemolytica* titre, by feedlot, at three Ontario feedlots, Fall 1998

Titres	Feedlot A			Feedlot B			Feedlot C		
	n	GMT[+]	SD	n	GMT	SD	N	GMT	SD
Arrival titre									
P. haemolytica leucotoxin neutralisation	126	8.7[a]	3.8	421	8.2[a]	3.2	53	6.9[b]	2.6
P. haemolytica leucotoxin ELISA	318	1.9[a]	1.4	411	2.8[b]	1.1	99	1.9[a]	1.2
P. haemolytica indirect agglutination	318	6.2[a]	1.1	433	5.8[b]	1.4	99	4.7[c]	1.2
Change in titre									
P. haemolytica leucotoxin neutralisation	108	5.8[a]	4.7	421	3.8[b]	4.8	53	3.5[b]	2.9
P. haemolytica leucotoxin ELISA	310	2.7[a]	1.4	409	1.9[b]	1.1	99	1.4[c]	1.6
P. haemolytica indirect agglutination	310	1.8[a]	1.7	431	1.4[b]	1.8	99	-0.1[c]	1.7

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

[+]GMT = geometric mean of titres

Table 4.6 : The effect of vaccination and treatment for undifferentiated bovine respiratory disease on change in *Pasteurella haemolytica* leucotoxin ELISA titre during a 28 day study period on cattle at three Ontario feedlots, Fall 1998.

Variable		Coefficient	SE	P-value
INTERCEPT		2.4	0.4	0.01
Arrival *P. haemolytica* leucotoxin ELISA titre		-0.8	0.02	0.00
VACCINE	*H. somnus* and *P. haemolytica* vaccine[v]	0.7	0.08	0.00
	H. somnus vaccine[x]	-0.08	0.08	0.3
	P. haemolytica vaccine[y]	0.6	0.08	0.00
	Non vaccinates[z]	0.00	.	.
Treatment for undifferentiated bovine respiratory disease		0.02	0.07	0.7
DF		804		
RLL[1]		-1000.4		
AIC [2]		-1003.4		

[1]RLL : Residual Log Likelihood

[2] AIC : Akaike's Information criterion

[v] Somnustar PH [TM], Biostar Inc., Saskatoon, Saskatchewan

[x] Somnustar[TM] , Biostar Inc., Saskatoon, Saskatchewan

[y] Pneumostar [TM], Biostar Inc., Saskatoon, Saskatchewan

[z]A control group not receiving any of these bacterial vaccines

Table 4.7: The effect of vaccination and treatment for undifferentiated bovine respiratory disease on change in *Pasteurella haemolytica* leucotoxin neutralisation titre during a 28 day study period on cattle at three Ontario feedlots, Fall 1998.

Variable		Coefficient	SE	P-value
INTERCEPT		10.4	0.9	0.008
Arrival *P. haemolytica* neutralisation titre		-0.8	0.05	0.00
VACCINE	*H. somnus* and *P. haemolytica* vaccine[v]	0.6	0.4	0.1
	H. somnus vaccine[x]	0.1	0.4	0.7
	P. haemolytica vaccine[y]	0.7	0.4	0.09
	Non vaccinates[z]	0.00	.	.
Treatment for undifferentiated bovine respiratory disease		0.03	0.4	0.9
DF		559		
RLL[1]		-1554.4		
AIC[2]		-1557.4		

[1]RLL : Residual Log Likelihood

[2] AIC : Akaike's Information criterion

[v] Somnustar PH ™, Biostar Inc., Saskatoon, Saskatchewan

[x] Somnustar™ , Biostar Inc., Saskatoon, Saskatchewan

[y] Pneumostar ™, Biostar Inc., Saskatoon, Saskatchewan

[z]A control group not receiving any of these bacterial vaccines

Table 4.8: Comparison of the effect of vaccination and treatment for undifferentiated bovine respiratory disease in *Pasteurella haemolytica* indirect agglutination titre (PHIDA) during a 28 days study period on feedlots cattle from three Ontario feedlots, Fall ,1998.

Variable		Coefficient	SE	P-value
INTERCEPT		5.4	0.9	0.03
Arrival *P. haemolytica* indirect agglutination titre		-0.7	0.04	0.00
VACCINE	*H. somnus* and *P. haemolytica* vaccine[v]	0.01	0.1	0.9
	H. somnus vaccine[x]	-0.2	0.1	0.1
	P. haemolytica vaccine[y]	-0.07	0.1	0.6
	Non vaccinates[z]	0.00	.	.
Treatment for undifferentiated bovine respiratory disease		-0.01	0.1	0.9
DF		826		
RLL[1]		-1485.6		
AIC [2]		-1488.6		

[1]RLL : Residual Log Likelihood

[2] AIC : Akaike's Information criterion

[v] Somnustar PH ™, Biostar Inc., Saskatoon, Saskatchewan

[x] Somnustar™ , Biostar Inc., Saskatoon, Saskatchewan

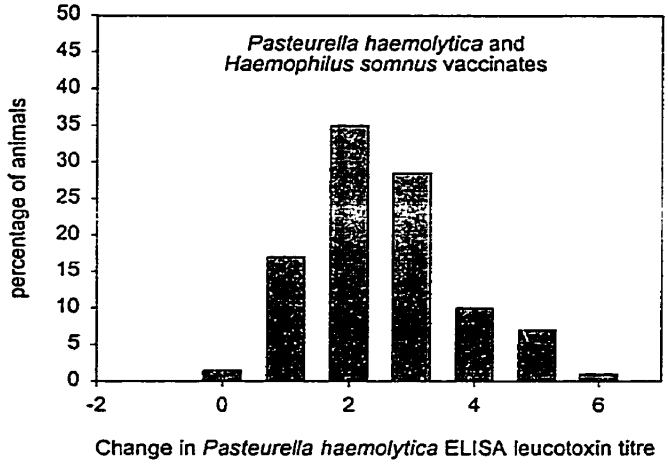
[y] Pneumostar ™, Biostar Inc., Saskatoon, Saskatchewan

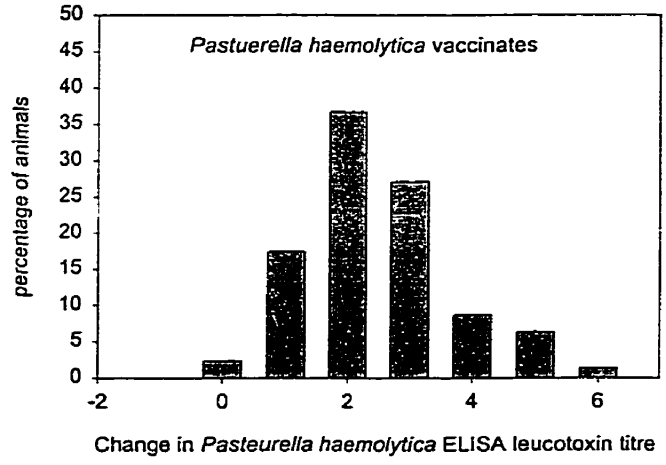
[z]A control group not receiving any of these bacterial vaccines

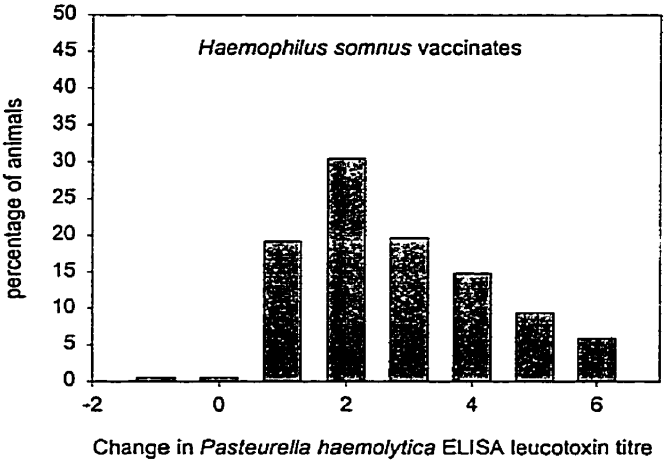
Table 4.9. Covariance parameter estimates for the random effects in PROC MIXED models

Covariance estimates	*P. haemolytica* leucotoxin ELISA titre	*P. haemolytica* leucotoxin neutralisation titre	*P. haemolytica* indirect agglutination titre
FEEDLOT	0.5	0.8	2.5
GROUP(FEEDLOT)	0.01	2.2	0.1
Residual	0.6	1.9	1.9

Figure 4.1 The frequency distribution of the change in *Pasteurella haemolytica* leucotoxin ELISA titre, by vaccine group, at three Ontario feedlots, Fall 1998.







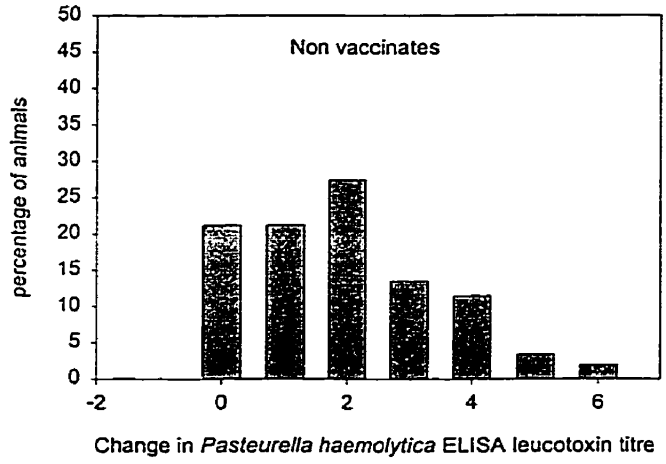


Figure 4.2: The frequency distribution of the change in *Pasteurella haemolytica* leucotoxin neutralisation titre, by vaccine group, at three Ontario feedlots, Fall 1998.

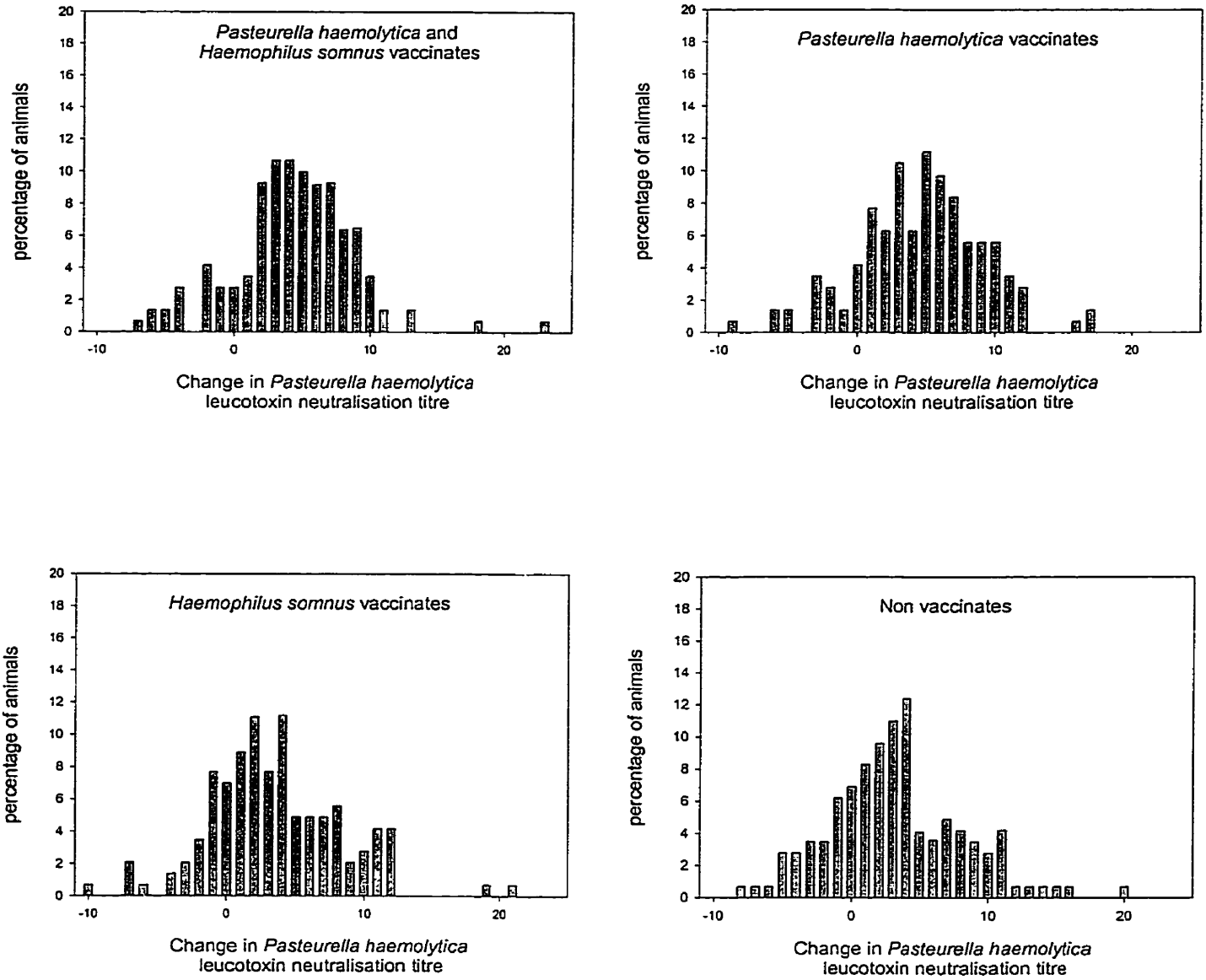
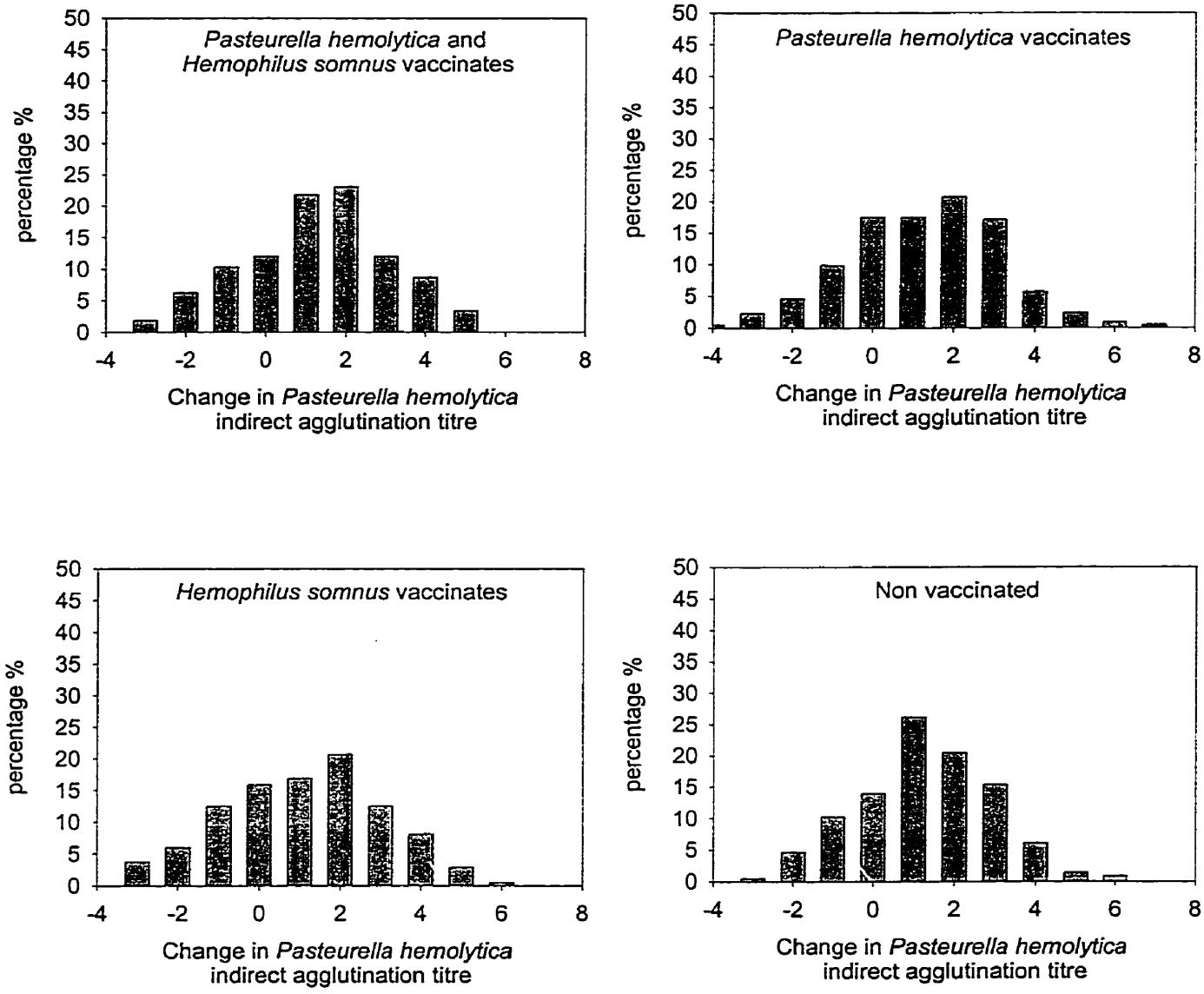


Figure 4.3: The frequency distribution of the change in *Pasteurella haemolytica* indirect agglutination titre for four vaccine groups during a 28 day study period on cattle from three Ontario feedlots, Fall, 1998.



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Chapter 5 The Relationship Between *Haemophilus somnus* Titre Changes During the First 28 days in Three Ontario Feedlots and The Occurrence of Undifferentiated Bovine Respiratory Disease

5.1 Introduction

In sero-epidemiological studies, increases in the titre to a putative causal organism are used as a proxy for evidence of exposure and a statistical association between increasing titre changes and temporally related disease occurrence is interpreted as support for a causal role of the organism in the disease (1). For example, titre increases in leucotoxin neutralising titres to *Pasteurella haemolytica* have been associated, consistently, with increased treatment risk (1,2,3). For organisms such as *P. haemolytica* and *Haemophilus somnus,* titre change during the early feedlot period is taken to reflect active infection during that period which may represent initial infection, re-infection or re-exposure (of an already infected animal) to these organisms.

Authors of recent studies examining the association between titre changes to *H. somnus* and undifferentiated bovine respiratory disease (UBRD) occurrence reported that treated animals had smaller titre increases, or larger titre decreases, than untreated animals (3,4). This suggests that either *H. somnus* is not causally related to UBRD or that the immune response reflected in the titre to the agent does not behave in the manner traditionally anticipated for causal agents of UBRD. The objective of the study was to determine if evidence of active exposure to *H. somnus* was associated with UBRD occurrence. The null hypothesis was that change in *H. somnus* titre would not be related to UBRD occurrence, statistically, and by extrapolation that *H. somnus* was not an agent of UBRD. The null hypothesis that timing of treatment for UBRD was not associated

with *H. somnus* titre change was also examined. Vaccination against *H. somnus* was used to identify the response of feedlot calves to an artificial challenge with the agent under the same environmental circumstances.

5.2 Materials and Methods

Animal management and serological analysis are outlined in detail in Section **3.2** and summarised in Table 5.1.

Statistical analysis

All data analyses, unless otherwise stated, were performed using SAS Release 6:12 ® (SAS Institute Inc. Cary NC.). The unit of analysis was the individual animal and the outcome for the analyses was the change in *H. somnus* titre. For the analyses, the results of the serological assays were transformed into an index (5). The index represented the well number of the last positive reaction as defined by the techniques outlined (4,6,7) and corresponds to the negative log of the dilution factor. Hereafter, the index will be referred to as the titre. Animals with no reaction were recorded as having a titre of zero.

For regression, transformed arrival titres were retained as continuous numbers but for the titre frequency distributions, the numbers where rounded down to the nearest integer (values equal to or lower than the halfway point between dilutions were rounded down, i.e. 1.5 converted to the integer of one not two). The change in titre was calculated as the difference between transformed titres at arrival and Day 28. Other continuous variables were temperature at arrival and weight at arrival. Treatment for UBRD was classified as a class variable (TRT - 2 levels), and other class variables

included feedlot (FEEDLOT – 3 levels), calf group defined by day of processing (GROUP- 9 levels) and vaccine group (VACCINE- 4 levels). In models, where time of UBRD occurrence was of interest, calves were classified as either untreated, treated on or after the 10^{th} day post-arrival or treated during the first 9 days post arrival.

Descriptive statistics for *H. somnus* arrival titre and titre change included the geometric mean, standard deviation, range of the transformed titres and frequency distribution histograms. Calves were categorised as sero-positive or sero-negative at arrival, any titre > 0.5 being deemed as sero-positive. Sero-conversion denoted an increase in titre (index) greater than two. For two-fold dilution tests this represented a four-fold increase in titre and for four-fold dilution tests this represented a 16-fold increase in titre, i.e., for all assays a two well increase was required for sero-conversion.

Significant differences among the vaccine groups and feedlots in arrival titre and titre change were determined using univariate ANOVA techniques (PROC GLM). Odds ratios (OR) were calculated to describe statistical associations between sero-positivity on arrival, or sero-conversion, and risk of treatment. The 95 percent confidence limits for the OR were presented (OR 95% CL).

Regression models

Regression techniques were used to determine factors, including UBRD occurrence, affecting the change in titre to *H. somnus*. The analyses were performed using PROC MIXED.

The model of interest was

$$y_i = \mu + \beta_1 + \beta_2 + \beta_3 + (\beta_1 \times \beta_2) + (\beta_1 \times \beta_3) + (\beta_2 \times \beta_3) + R_1$$

Where

y_i is the change in titre for the individual i

μ = the mean response

β_1 the fixed affect of vaccination group,

β_2 the fixed effect of day zero titre

β_3 the fixed effect of treatment

$\beta_1 \times \beta_2$ is the interaction between arrival titre and vaccination group.

$\beta_1 \times \beta_3$ is the interaction between treatment and vaccination group.

$\beta_2 \times \beta_3$ is the interaction between treatment and arrival titre.

R_1 is the random effect of the GROUP and FEEDLOT

All the explanatory variables of interest were forced into the initial model. Potential confounding variables included arrival titres to other agents, *P. haemolytica* leucotoxin neutralisation titre, *P. haemolytica* indirect agglutination titre and *P. haemolytica* leucotoxin ELISA titre, arrival weight and arrival rectal temperature. To determine if confounding variables should be included in the final model, each variable was added or removed from the model and the resulting effect on the coefficients of the variables involving TRT examined. If the change in the point estimate of the variables involving TRT 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 (8)

unless it had a significant impact on titre change. 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 random effects are not reported. The same model building approach was used to examine the effect of timing of treatment for UBRD of the change in *H. somnus* titre. The fit of the model was examined by dividing the residuals by the standard deviation of the residuals and plotting these values against the variables of interest. The influence of particular data points could not be determined because the hat matrix is not provided for mixed models (9).

5.3 Results

Due to sample handling errors, 24 animals from the first group of cattle sampled at Feedlot B were not analysed and have no values for day 0 titres. Of these 24 animals, 7 received the combined *P. haemolytica* and *H. somnus* vaccine, 5 received the *H. somnus* vaccine only, 4 were vaccinated with the *P. haemolytica* vaccine, and 8 received no vaccine. At day 28, 6 animals had died and samples could not be collected from 2 other animals. Of the 6 dead animals 5 came from Feedlot A and 1 from Feedlot B, 2 received the combined vaccine, 1 received the *H. somnus* only, 2 received the *P. haemolytica* antigen, and 1 received no vaccine. Of the animals treated, nine did not have rectal temperature reported, and of these nine, six had a depression score of two (10). Of the remaining treated animals all had a rectal temperature greater than $40\ ^{0}$ C. Another two animals lost their identification and could not be paired when the second samples were taken. In all 32 animals had missing titre data.

Approximately 13% of calves were treated for UBRD once, while 7% were treated greater than once (relapsed). Most treatments occurred at Feedlot A (Table 5.2). The geometric mean titre at arrival was 1.8, as was the geometric mean titre increase (Table 5.3). Arrival titres and titre increases differed by feedlot (Table 5.4). *Haemophilus somnus* vaccination increased the titre change over that in non-vaccinated calves; calves vaccinated with only *P. haemolytica* had intermediate titre increases (Table 5.5). Thirty four percent of calves had no titre to *H. somnus* on arrival (Table 5.6), and were more likely to be treated during the study period than sero-positive animals (OR 95% CL: 1.3 - 2.5). The frequency distribution of change in *H. somnus* titre for each vaccine group is given in Figure 5.1. Forty percent of animals sero-converted during the study period to *H. somnus*, but animals that sero-converted to *H. somnus* were no more likely to be treated than those that did not sero-convert (OR 95% CL: 0.55 – 1.2).

After stratification by timing of treatment, calves that relapsed tended to have lower titre increases than those treated only once (Table 5.7). After stratification for number of times treated, calves that were treated early had larger titre increases than those treated after day 9; titre changes in relapsed calves did not differ by time of initial treatment (Table 5.8).

In the multiple regression model titre change was influenced by both UBRD treatment and arrival titre, as well as by vaccine group. Vaccination against *H. somnus* produced significant titre increases. Overall, calves treated for UBRD had smaller titre increases than untreated calves. The arrival titre by treatment effect interaction indicated that the effect of UBRD on titre change decreased in calves with higher arrival titres

(Table 5.9 and Figure 5.2). The variance component estimates for the random effects were FEEDLOT 0.08, GROUP within FEEDLOT 0.1 and residual 0.9.

The association between timing of treatment and change in *H. somnus* titre is shown in Table 5.10, however because of the interactions the effects are not obvious. Untreated calves had similar titre changes to calves treated in the first 9 days regardless of vaccine group. Calves with low arrival titres to *H. somnus,* that were treated on or after 10 days post arrival, had smaller titre increases than the former two groups of calves. The predicted behaviour of these final models is plotted in Figure 5.3. The variance component estimates for the random effects were FEEDLOT 0.07, GROUP within FEEDLOT 0.1, and residual 0.9.

5.4 Discussion

The prevalence of antibody titres to *H. somnus* at arrival was lower than previously reported. In 1998 Martin *et al.* (4) reported that all animals studied (n= 602) had detectable *H. somnus* titres at arrival and that the average arrival titre for *H. somnus* was 7.5 ± 1.6, considerably higher than the 1.8 ± 1.5 units of titre 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 titre transformation used for analysis (4). This highlights the importance of examining titres as relative measures of prevalence or incidence of exposure within a study, rather than as exact measures of antibody levels that can be compared across studies.

If change in titre is taken to represent the occurrence of exposure during the UBRD risk period, then exposure to *H. somnus* is common. Titre change also was affected by

treatment for UBRD, but this relationship was modified by arrival titre, as indicated by the significant interaction term. The model showed that animals treated for UBRD during the 28-day study period had smaller increases to *H. somnus* than untreated animals. However, as *H. somnus* titre at arrival increased, the effect of treatment for UBRD on titre change diminished. At high arrival titres for *H. somnus*, the change in titre was the same for treated and untreated animals. These findings suggest that we would reject the null hypothesis concerning *H. somnus* and UBRD occurrence. Nonetheless, the relationship was not in the direction expected, that is, we expected to observe increased titre changes with treatment of UBRD, if *H. somnus* was a causal agent of UBRD.

The association of smaller *H. somnus* titre increases in calves with UBRD has been reported previously, that is, animals with undifferentiated fever (UF) had smaller increases in *H. somnus* than control animals over a 33 day period (3). Authors of another study reported that animals treated for UBRD had larger decreases in *H. somnus* titre over a 28-day study period than untreated animals (4). As mentioned previously, exposure to *H. somnus* appeared to be common in the present study. The average change in *H. somnus* titre was 1.8 ± 1.6 (mean ± S.D.), while in the earlier study, exposure to *H. somnus* did not appear to be common as the average titre change was -0.36 ± 0.97 (mean ± S.D.) (4). Overall, in the previous studies, titres to *H. somnus* on arrival were common and high whereas titre increases were small relative to the lower arrival titres and larger titre increases found in this study. Therefore, the situations may have been quite different and the results therefore, not comparable.

As details were available on the timing of UBRD treatment, the association of UBRD and titre change was examined in early and late treated calves. Calves that were treated

before day 10 behaved in a manner very similar to those animals that were not treated in terms of change in *H. somnus* titre. This would mitigate against *H. somnus* being a cause of these UBRD cases. Calves with a low arrival titre showed evidence of exposure while those arriving with a high titre showed little or no titre changes and these did not differ between treated and untreated calves (Figure 5.3). However, regardless of arrival *H. somnus* titre, calves that were not vaccinated and received treatment for UBRD 10 or more days after arrival displayed little or no evidence of titre change to *H. somnus* antigens and the low magnitude of the predicted change in *H. somnus* titre suggested that these animals were not exposed to the *H. somnus* organism. This is also consistent with *H. somnus* not being a cause of these UBRD cases. Within the "*H. somnus* only" vaccinated group, the late treated calves had similar titre increases to those in the early and never treated groups. Therefore animals that received treatment for UBRD late in the study period or those that relapsed during the study period, had the smallest increase in *H. somnus,* thus the least evidence of exposure (Table 5.7, Table 5.8, Table 5.10).

The explanation for the relationship between timing of treatment and evidence of exposure to *H. somnus* remains unclear. Based on the titre changes in unvaccinated calves, there was evidence of exposure to *H. somnus* in animals treated early, but not in animals treated for UBRD after day 9, post arrival. We would suggest two possible explanations for the UBRD-titre change observations. Unvaccinated calves that were exposed twice to antimicrobials, at least 10 days apart (first prophylactically and then therapeutically), may have had decreased exposure to *H. somnus*, and thus no antigen to trigger a response. Untreated animals were exposed to antimicrobials only once and thus may have had re-infection or continuing exposure after arrival. Despite receiving two

does of antimicrobials (at arrival and about 5-7 days later at treatment) animals treated early had the same predicted change in *H. somnus* titre as those animals that were never treated, perhaps because any inhibition of colonisation caused by the antimicrobials "wore off" in sufficient time for them to be exposed in the later period post arrival. Vaccination for *H. somnus* exposed animals to an antigen challenge that was not affected by exposure to antimicrobials, hence their antigen challenge continued and led to titre increases. This may explain why the late-treated vaccinated animals had higher titres 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. Based on the titre responses, vaccination may also have induced *H. somnus* antibody production in the *P. haemolytica* only vaccine group, also, suggesting some cross-protection or common antigens between these organisms.

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 exposure than animals treated once. Animals that relapsed were exposed to antibacterial agents for a longer period of time and hence these calves showed decreased evidence of exposure to *H. somnus* (Table 5.7 and Table 5.8). 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. Further investigation would be required to confirm this hypothesis.

Another hypothesis is that animals treated later may be those animals that had higher arrival titres, and hence it was difficult to detect titre increases in these animals

because of their high initial titre. This would be a function of the method of titre transformation used in the analysis, i.e., a change from dilution 5 to 6 was given equal weight to a change from 1 to 2 despite the former requiring greater net production of antibodies. This theory loses some credibility however, because there was no univariate evidence that the arrival titre of animals treated later, or the arrival titre of calves that relapsed, was different than titres in those treated early or just once.

With regards to the effect that non differential misclassification of the disease status may have had on the study findings, the owners of the feedlots were instructed to select animals for treatment based on their usual criterion and it would appear that a rectal temperature greater than 40 ^{0} C was one of those criterion. This may be because of the advice received from veterinarians or published literature. This would suggest that the majority of animals classified as UBRD cases did require treatment but does not diminish the possibility that UBRD cases were not identified and therefore were included in the untreated group. This misclassification would result in a bias of the study findings towards the null hypothesis (8). Likewise because the sensitivity and specificity of the serological tests was not available, the study results could not be adjusted for any misclassification bias in the study. Generally, misclassification bias results in a bias of study findings toward the null hypothesis (8,11,12,13). The likelihood and impact of this misclassification should be considered when evaluating the study conclusions.

In future studies it may be of value to reduce the sampling time to a shorter period such as 10 days. Reducing this time period would decrease the likelihood that the titre had risen and returned to their arrival level and therefore reduce the misclassification of animals according to exposure status. Identifying the isotope of antibody associated with

the titre would address concerns of possible bias in the serological tests and the effect that different isotopes 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* titre change and UBRD occurrence reported previously (i.e. treated animals tend to have decreased evidence of exposure to *H. somnus* relative to untreated animals). However, by examining the effect of timing of treatment and using vaccine induced titres 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 also had decreased titres to *H. somnus*. We have suggested that exposure to antimicrobials over a prolonged period may be the common factor limiting exposure to *H. somnus* in these two groups of animals. 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.

Table 5.1: Number enrolled, arrival procedures and calf characteristics of calves at three 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[x]	Yes[y]	no
Sex	Mixed (276 F / 42 M)	Male	Male
Rectal temp at arrival; °C (mean ± S.D.) *	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 ± S.D.)	235.0 ± 34.9[a]	247.2 ± 20.2[a]	283.4 ± 37.8[a]
Range of weight (kg)	208.00	129.00	219.00

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

[x] Pyramid ™ 4MLV, Ayerst Laboratories, 1025 BLVD Laurentien, Saint Laurent, PQ.

[y] Bovishield ™ 4, SmithKline Beecham Animal Health, 3130 Pepper Mill Court, Mississauga, ON

Table 5.2 : Distribution [a] of treatments, by feedlot and vaccine group, at three Ontario feedlots used in the study of *Haemophilus somnus* titres, 1998.

Vaccine	Times treated	Feedlot A n = 318	Feedlot B n = 435	Feedlot C n = 99	Total
H.somnus and *P. haemolytica* vaccine[v]	0	58	97	22	177 (83%)
	1	14	8	2	24 (11%)
	2	8	4	0	13 (6%)
H. somnus vaccine[x]	0	55	90	22	167 (80%)
	1	14	12	1	27 (13%)
	2	9	5	1	15 (7%)
P. haemolytica vaccine[y]	0	48	101	23	172 (81%)
	1	18	8	2	28 (13%)
	2	12	0	1	13 (6%)
Non vaccinates[z]	0	46	93	23	159 (74%)
	1	21	11	2	34 (16%)
	2	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%)
	2	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.

[v] Somnustar PH ™, Biostar Inc., Saskatoon, Saskatchewan

[x] Somnustar™ , Biostar Inc., Saskatoon, Saskatchewan

[y] Pneumostar ™, Biostar Inc., Saskatoon, Saskatchewan

[z] A control group not receiving any of these bacterial vaccines

Table 5.3 : Descriptive statistics for *Haemophilus somnus* titres for calves at three Ontario feedlots, 1998

	n	GMT[a]	SD	minimum	maximum
H. somnus titre at arrival	828	1.8	1.5	0	6.3
H. somnus titre at day 28	842	3.6	1.2	0	5.6
Change in *H. somnus* titre	818	1.8	1.6	-3.1	5.7

[a] GMT = geometric mean of titre

Table 5.4 : Descriptive statistics for *Haemophilus somnus* by feedlot, for cattle at three Ontario feedlots, 1998

Titres	Farm A			Farm B			Farm C		
	n	GMT[a]	S.D.	n	GMT	S.D.	n	GMT	S.D.
H. somnus titre at arrival	318	1.6[a]	1.6	411	1.9[b]	1.6	99	2.2[c]	1.2
Change in *H. somnus* titre	310	2.2[a]	1.6	409	1.7[b]	1.5	99	1.1[c]	1.3

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

[a] GMT = geometric mean of titre

Table 5.5: Descriptive statistics for *Haemophilus somnus* titres, by vaccine group, for cattle at three Ontario feedlots, 1998

Titres	Combined PH/HS vaccine[v]			PH vaccine[x]			HS vaccine[y]			Control[z] (nonvaccinated)		
	n	GMT	S.D.	n	GMT	S.D.	n	GMT	S.D.	n	GMT	S.D.
H. somnus titre at arrival	206	1.6[a]	1.6	209	1.8[a]	1.5	204	1.8[a]	1.5	209	1.9[a]	1.6
Change in *H. somnus* titre	200	2.1[b]	1.6	207	1.6[a]	1.4	203	2.4[b]	1.5	208	1.2[c]	1.5

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

[v] Somnustar PH ™, Biostar Inc., Saskatoon, Saskatchewan

[x] Somnustar™ , Biostar Inc., Saskatoon, Saskatchewan

[y] Pneumostar ™, Biostar Inc., Saskatoon, Saskatchewan

[z] A control group not receiving any of these bacterial vaccines

Table 5.6 Frequency distribution of transformed titres at arrival for *Haemophilus somnus* titres for cattle at three Ontario Feedlots, 1998

Titre (well)	*H. somnus* titre at arrival
	No. (%)
0	282 (34.1%)
1	71 (8.6 %)
2	177 (21.4%)
3	165 (19.9%)
4	97 (11.7%)
5	32 (3.9%)
6	4 (0.5%)
7	

Figure 5.1: The frequency distribution of the change in *Haemophilus somnus* titre (ELISA), by vaccine group, for cattle at three Ontario feedlots, 1998.

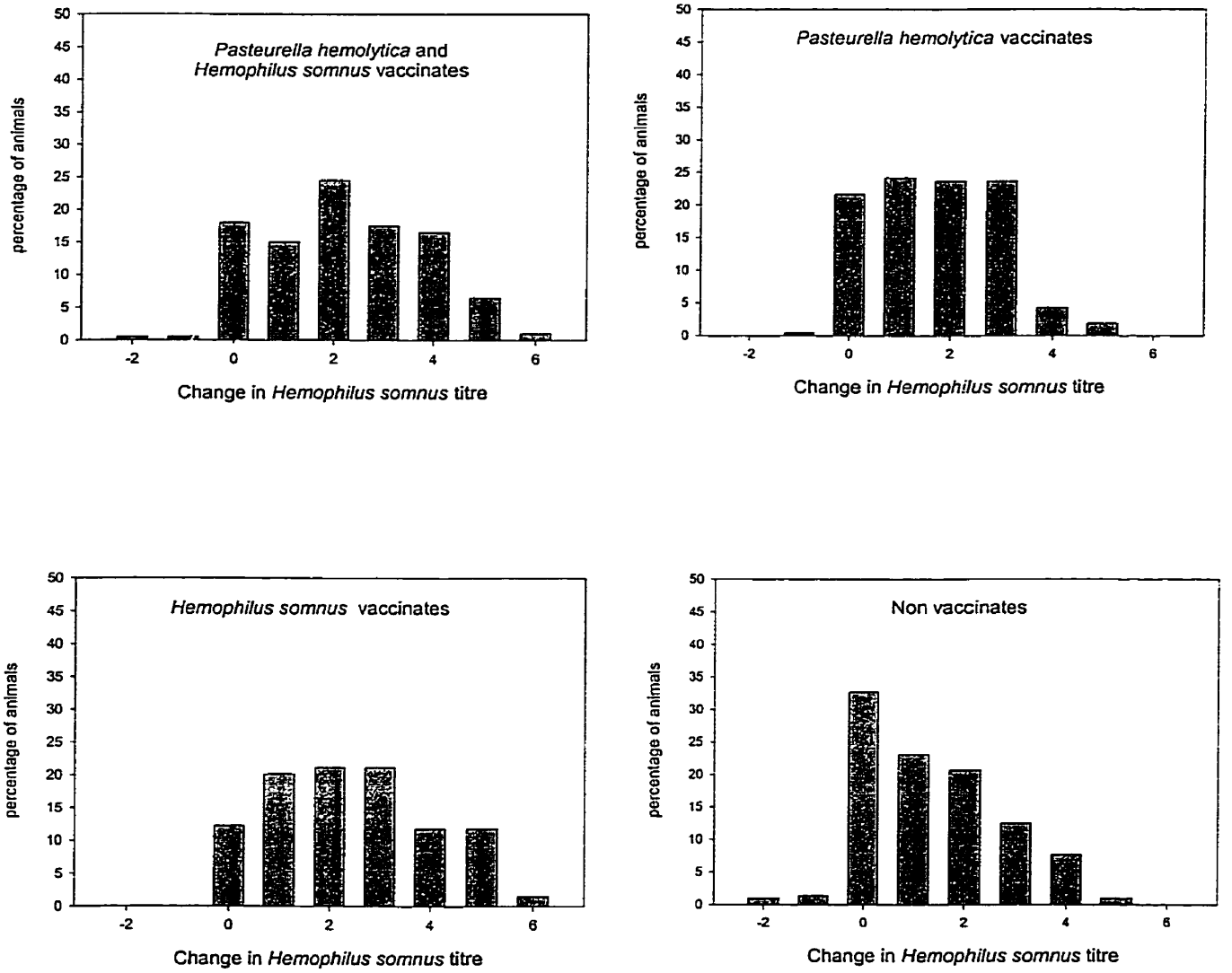


Table 5.7: The association between of number of times treated and change in titre for *Haemophilus somnus* titres for cattle, stratified by timing of initial treatment (Early < 10 days after arrival, Late ≥ 10 days post arrival), at three Ontario Feedlots, 1998,

	Timing of Initial Treatment					
Test	**Early**					
	Once			Relapsed		
	n	mean	S.D.	n	mean	S.D.
Change in *H. somnus* titre	64	2.3[a]	0.2	35	1.7[b]	0.2
	Late					
	Once			Relapsed		
Change in *H. somnus* titre	46	1.6[a]	0.2	17	1.2[a]	0.3

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

Table 5.8: The association between timing of initial treatment and change in titre for *Haemophilus somnus* ELISA titres, stratified by number of times treated for undifferentiated bovine respiratory disease (Early < 10 days after arrival, Late ≥ 10 days post arrival), for cattle at three Ontario Feedlots, 1998

	Number of times treated					
Test	Once					
	Early			Late		
	n	mean	SD	n	mean	SD
Change in *H. somnus* titre	64	2.2[a]	0.2	46	1.6[b]	0.2
	Relapsed					
	Early			Late		
Change in *H. somnus* titre	35	1.7[a]	0.2	17	1.3[a]	0.3

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

Table 5.9: The effect of vaccination and treatment for undifferentiated bovine respiratory disease on change in *Haemophilus somnus* ELISA titre for cattle at three Ontario feedlots, 1998.

Variable		Coefficient	SE	P-value
Main effects				
INTERCEPT		2.4	0.2	0.01
H. somnus titre at arrival		-0.6	0.04	0.000
VACCINE	*H. somnus* and *P. haemolytica* vaccine	1.0	0.1	0.000
	H. somnus vaccine	1.6	0.1	0.000
	P. haemolytica vaccine	0.4	0.1	0.01
	Non vaccinates	0.00	.	.
Treatment for UBRD		-0.6	0.1	0.000
Interaction terms				
H. somnus titre at arrival* VACCINE	*H. somnus* and *P. haemolytica* vaccine	-0.1	0.06	0.01
	H. somnus vaccine	-0.3	0.06	0.000
	P. haemolytica vaccine	-0.02	0.06	0.75
	Non vaccinates	0.00		.
Treatment for UBRD* *H. somnus* titre at arrival		0.1	0.06	0.009
DF		798		
RLL[1]		-1148.8		
AIC[2]		-1151.8		

[1]RLL: Residual Log Likelihood

[2] AIC: Akaike's Information criterion

Figure 5.2: The predicted effect of vaccination, arrival titre and treatment for undifferentiated bovine respiratory disease on changes in *Haemophilus somnus* ELISA titre for cattle at three Ontario feedlots, 1998.

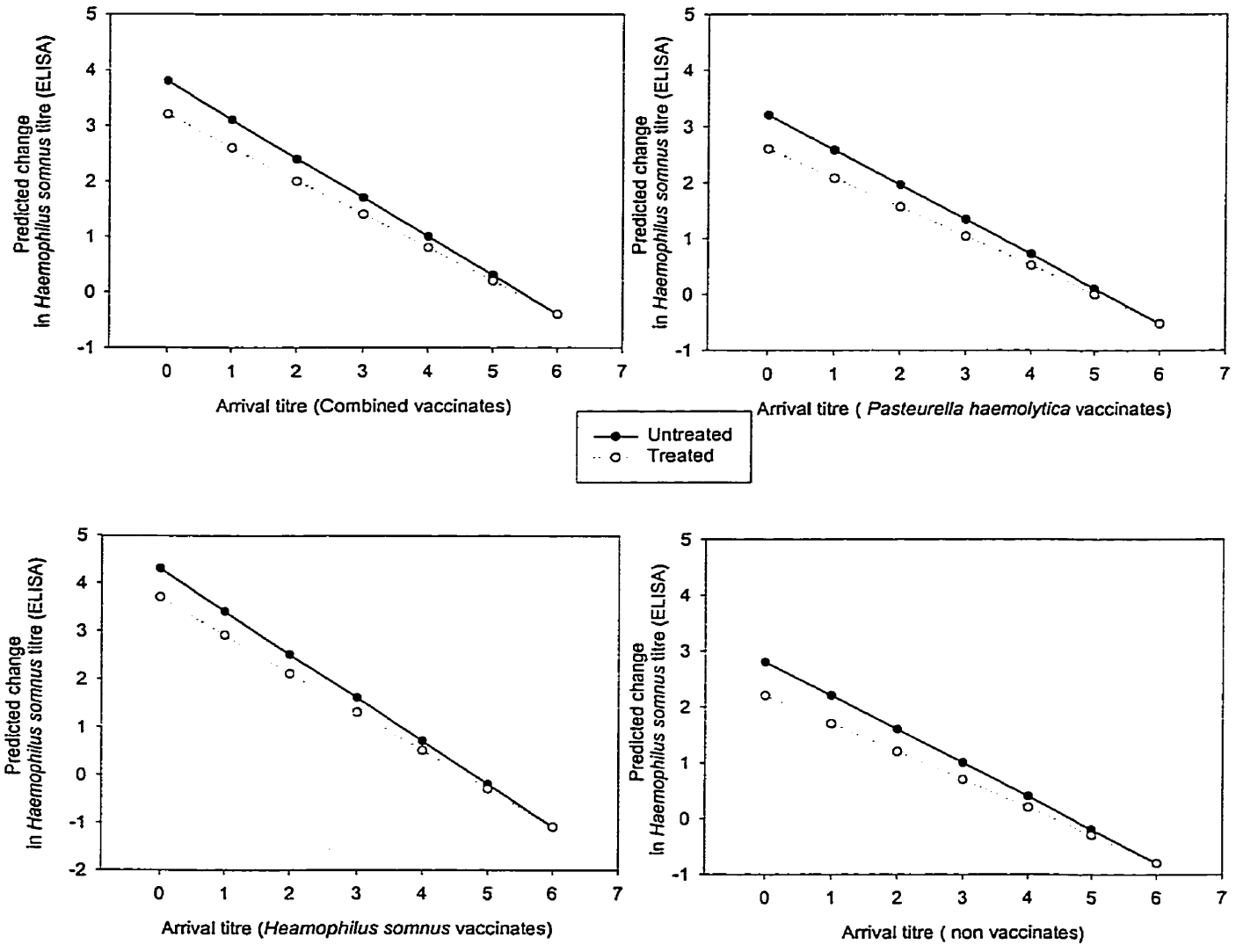
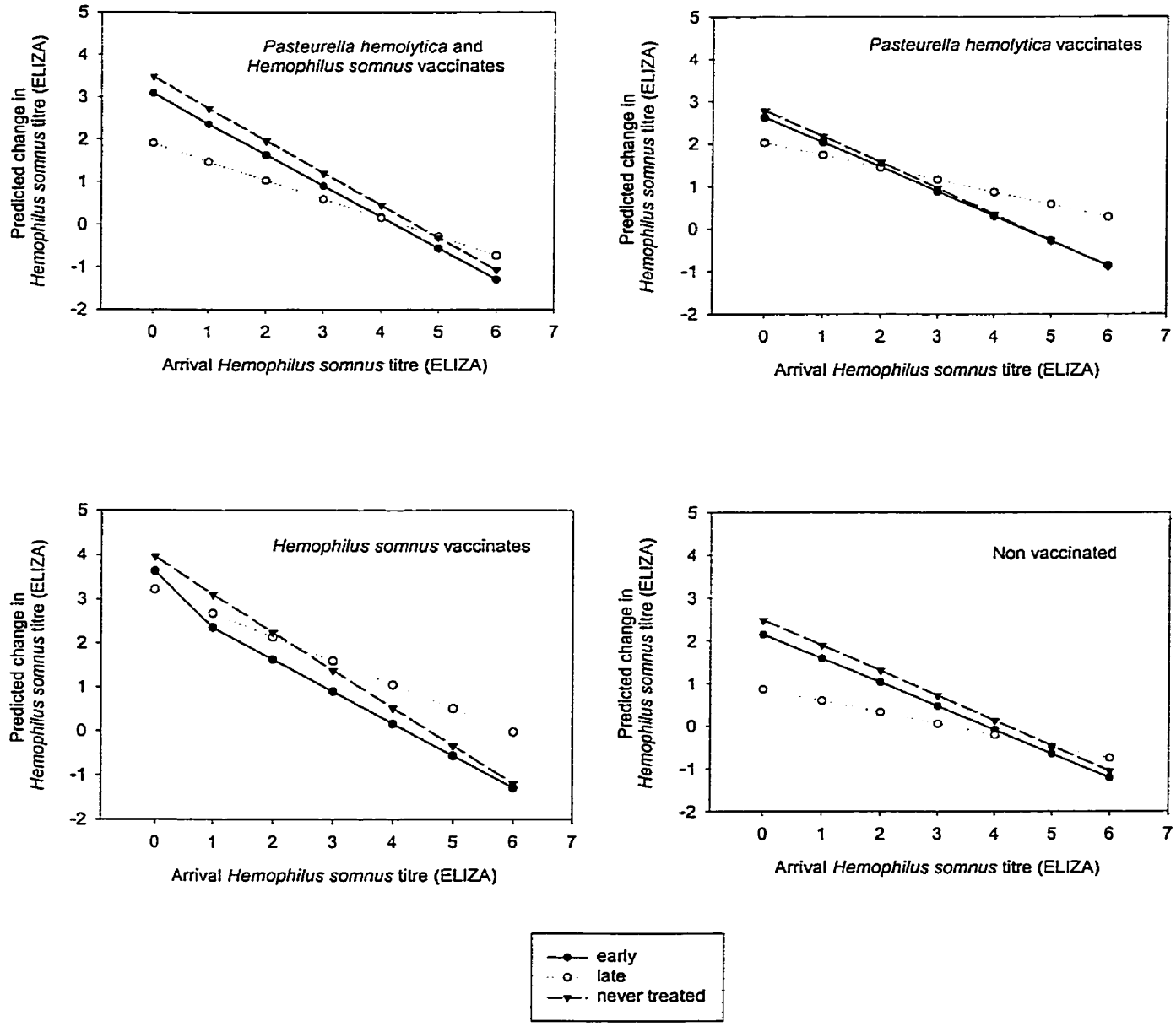


Table 5.10: The predicted effect of vaccination and treatment timing for undifferentiated bovine respiratory disease on the change in *Haemophilus somnus* ELISA titre for cattle at three Ontario feedlots, 1998.

Variable		Coefficient	SE	P-value
Main effects				
INTERCEPT		2.5	0.2	0.009
H. somnus titre at arrival		-0.6	0.04	0.00
VACCINE	*H. somnus* and *P. haemolytica* vaccine	1.0	0.2	0.00
	H. somnus vaccine	1.5	0.2	0.00
	P. haemolytica vaccine	0.3	0.2	0.08
	Non vaccinates	0.00	.	.
Timing of Treatment for UBRD	Early (< 10 days)	-0.3	0.2	0.1
	Late (≥ 10 days)	-1.6	0.3	0.00
	Never	0.00	.	.
Interaction terms				
H. somnus titre at arrival* VACCINE	*H. somnus* and *P. haemolytica* vaccine	-0.2	0.06	0.004
	H. somnus vaccine	-0.3	0.06	0.00
	P. haemolytica vaccine	-0.02	0.06	0.08
	Non vaccinates	0.00	.	.
Early (< 10 days)*VACCINE	*H. somnus* and *P. haemolytica* vaccine	-0.06	0.3	0.8
	H. somnus vaccine	0.003	0.3	0.9
	P. haemolytica vaccine	0.2	0.3	0.5
	Non vaccinates	0.00	.	.
Late (≥ 10 days) * VACCINE	*H. somnus* and *P. haemolytica* vaccine	0.05	0.4	0.9
	H. somnus vaccine	0.9	0.4	0.01
	P. haemolytica vaccine	0.9	0.3	0.01
	Non vaccinates	0.00	.	.
Never*VACCINE	*H. somnus* and *P. haemolytica* vaccine	0.00	.	.
	H. somnus vaccine	0.00	.	.
	P. haemolytica vaccine	0.00	.	.
	Non vaccinates			
H. somnus titre at arrival * time of treatment for UBRD	Early (< 10 days)	0.04	0.07	0.6
	Late (≥ 10 days)	0.3	0.09	0.00
	Never	0.00	.	.
DF		792		
RLL[1]		-1138.9		
AIC[2]		-1141.9		

Figure 5.3: The effect of treatment timing and vaccination group of the predicted behaviour of *Haemophilus somnus* ELISA titre for cattle at three Ontario feedlots, 1998



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Chapter 6 A Descriptive and Analytical Analysis of the Sero-epidemiology of Bovine Corona Virus and Bovine Viral Diarrhoea Virus in Feedlot Steers.

6.1 Introduction

Bovine corona virus (BCV) is a ubiquitous organism in cattle populations, and is frequently isolated from the nasal passages of cattle with clinical respiratory disease (1) (2,3,4,5) A published study on the sero-epidemiology of BCV titres in feedlot cattle found that although higher titres to BCV at arrival were statistically associated with decreased risk of treatment for undifferentiated bovine respiratory disease (UBRD), there was no association between evidence of recent infection (change in titre) and the occurrence of UBRD (6). The failure to associate changing titres, a proxy for exposure within the selected time period, with disease occurrence suggests that BCV may not be an agent of UBRD. The association between previous exposure, as evidenced by titre at arrival, and decreased disease occurrence is not strong evidence that any agent plays a causal role in UBRD occurrence. Since the number of studies examining the roles of BCV in UBRD is very small, the aim of this study was to examine the role of infection with BCV in feedlots and determine if it is associated with increased risk of treatment for UBRD. The null hypothesis was that evidence of previous exposure was not associated with reduced UBRD risk and evidence of current exposure was not associated with increased UBRD risk. The same hypothesis was tested for bovine viral diarrhoea virus (BVDV) to enable comparison with a viral titre about which more was known. Titres to

a number of other agents were controlled during the analysis to improve the validity of any inferences made about the role of BCV in UBRD and to illustrate the role of arrival titre and change in titre in causal inferences made about putative agents of UBRD.

6.2 Methods and Materials

The animal management and serological analysis are described in Section 3.2.

Statistical methods

All data analyses, unless otherwise stated, were performed in SAS Release 6:12 ® (SAS Institute Inc. Cary, NC.). The unit of analysis was the individual animal. Some procedures were applied at higher levels of aggregation. These procedures were the use of viral vaccines and prophylactic administration of antibiotics at arrival.

Descriptive analyses included the calculation of geometric means, standard deviations, minimum and maximum. Differences between the treated and untreated calves, and among feedlots in arrival titre and titre change were evaluated using univariate ANOVA techniques (PROC GLM). Odds ratios (OR) were calculated to describe statistical associations between sero-positivity on arrival, and sero-conversion, and risk of treatment. Calves were categorised as sero-positive or sero-negative at arrival, any titre > 0.5 being deemed as sero-positive. Sero-conversion denoted an increase in titre (index) of greater than two units. For all assays, a two well increase was required for sero-conversion; for a two-fold dilution this represented a four-fold increase in titre and for four-fold dilution this represented a 16-fold increase in titre. Univariate comparisons of the proportion of animals that either arrived sero-positive or sero-converted during the study period at the feedlots, were examined and significant

differences determined using chi-square (χ^2) tests for multiple proportions. These calculations were performed in a specially designed computer program using the technique described by Edgington (7).

Regression analysis

Factors affecting the change in titre to BCV and BVDV were examined using a mixed effects regression model (PROC MIXED). The exposure variable of greatest interest was treatment for UBRD, a class variable with two levels (TRT). Other class variables available for the analysis were the bacterial vaccine group (BVACCINE- 4 levels) and a variable representing vaccination with modified live viral vaccines (VVACCINE – 2 levels). The arrival titres to the putative agents were included as explanatory variables. Other continuous variables available for inclusion in the analysis were temperature at arrival and weight at arrival. The variables, FEEDLOT and GROUP (representing the day animals were processed), were included initially in all models as random effects. The initial main effects model containing the explanatory variables of interest was

$$y_i = \mu + \beta_1 + \beta_2 + \beta_3 + \beta_4 + \beta_5 + \beta_6 + \beta_7 + \beta_8 + R_1$$

Where

y_i is the change in titre for the individual i of BCV or BVDV

μ = the mean response

β_1 the fixed affect of VVACCINE

β_2 the fixed affect of BVACCINE

β_3 the fixed effect of day zero titre (D0BCV or D0BVDV)

β_4 the fixed effect of TRT

β_5 the fixed effect of D0PHALE

β_6 the fixed effect of D0HSOMP

β_7 the fixed effect of D0PHIDA

β_8 the fixed effect of the other viral arrival titre (D0BCV or D0BVDV)

R_1 is the random effect of GROUP nested with (FEEDLOT)

The model building approach was described in Section 3.2.

PROC GLM was used to obtain the multiple coefficient of determination, i.e. the model $R^{2,}$ for the fixed effects in the final PROC MIXED model.

To examine the association between risk of UBRD and previous exposure, logistic regression was used. The method of analysis is described in Section 3.2.

6.3 Results

Due to sample handling errors, 24 animals from the first group of cattle sampled at Feedlot B were not analysed and have no values for day 0 titres. Of these 24 animals, 7 received the combined *P. haemolytica* and *H. somnus* vaccine, 5 received the *H. somnus* vaccine only, 4 were vaccinated with the *P. haemolytica* vaccine , and 8 received no vaccine. At day 28, 6 animals had died and samples could not be collected from 2 other animals. Of the 6 dead animals 5 came from Feedlot A and 1 from Feedlot B, 2 received the combined vaccine, 1 received the *H. somnus* only, 2 received the *P. haemolytica* antigen, and 1 received no vaccine. Another two animals lost their identification and could not be paired when the second samples were taken. In all 32 animals had missing titre data. Of the animals treated, nine did not have rectal temperature reported, and of

these nine, six had a depression score of two (8). Of the remaining treated animals all had a rectal temperature greater than 40 ^{0} C when selected for treatment.

The geometric mean titres for BCV and BVDV at arrival were 3.5 and 1.2 respectively. The average titre change for BCV and BVDV were 2.1 and 3 units respectively (Table 6.1). There were differences in arrival titre and titre change among feedlots for both organisms (Table 6.2). Ninety percent of animals were sero-positive to BCV at arrival (Figure 6.1), and being sero-positive to BCV at arrival was associated with a significant reduction in the risk of being treated (95 % CI: 0.2 – 0.6). Treated animals also tended to have lower average arrival titres (Table 6.3). Fifty percent of animals sero-converted to BCV (Figure 6.2, Figure 6.3) and sero-conversion was not associated with increased risk of UBRD (95 % CI: 0.9 –2.1). However, the change in BCV titres was significantly greater in animals that were treated (Table 6.3).

Sixty-one percent of animals were sero-negative to BVDV at arrival and being sero-positive was associated with decreased odds of treatment (95 % CI: 0.4 – 0.8) (Figure 6.1). The average BVDV arrival titre of animals treated for UBRD was lower than in those animals that remained untreated (Table 6.3). Forty-five percent of animals sero-converted to BVDV during the study period and animals that sero-converted to BVDV were more likely to be treated (95 % CI: 1.3 – 2.8). The change in BVDV titres was significantly higher, a 3.9 titre increase, in animals that were treated compared to those that were untreated during the study period. This association remained when data from Feedlot C, where vaccination for BVDV did not take place, was omitted (Table 6.4).

Regression models

For the models predicting titre change to BCV, the variable, GROUP was not included as a random variable because its inclusion prevented the program, PROC MIXED, from running properly i.e., the G matrix was not positive definitive. Therefore FEEDLOT was used as the random variable to control feedlot effects.

Two regression models used to identify factors associated with the change in BCV titre are shown in Table 6.5. Model 1, is a fixed effects model without clustering (FEEDLOT) controlled, Model 2 is the same fixed effects model but with FEEDLOT included as a random variable. In both models the main variable affecting the change in titre was the arrival titre, the two being negatively correlated. Treatment was not significantly associated with titre change when FEEDLOT was added to the model. The covariance parameter estimate for FEEDLOT was 0.02 and the residual 1.01.

Although the other variables were significantly associated with BCV titre change ($p<0.05$) their coefficients were very small. The R^2 for a model including all the significant fixed effects and with FEEDLOT included as a fixed effect, was 0.71, while a model containing only the arrival BCV titre had an R^2 of 0.70.

When modelling the change in BVDV, the inclusion of VVACCINE and FEEDLOT in the model prevented the model from running, because all of the variability of the FEEDLOT was attributable to FEEDLOT C, and therefore FEEDLOT and VVACCINE represented essentially the same data. Therefore, for the model of factors affecting change in BVDV titre, VVACCINE was included as a fixed effect variable rather than FEEDLOT as a random variable.

The regression model for the change in BVDV titre is shown in Table 6.6. Arrival titre to BVDV was negatively correlated with change in BVDV titre. Treatment for

UBRD was associated with a large increase in titre change to BVDV, even when multiple factors were accounted for in the regression analysis. The model also predicted that those animals with elevated rectal temperatures at arrival and heavier animals were likely to have larger increases in BVDV titre change (Table 6.6). At no point in the model building process, was the interaction between VVACCINE and arrival BVDV titre significant. The R^2 for various models were: the presented model ($R^2 = 0.31$), a model including only the arrival BVDV titre and VVACC ($R^2 = 0.23$)(not shown), a model including only the arrival BVDV titre ($R^2 = 0.08$)(not shown) and a model including only VVACC ($R^2 = 0.1$) (not shown).

When UBRD was regressed on BCV arrival titre, the coefficient for BCV arrival titre was negative and significant ($\beta = -0.08 \pm 0.04$; OR = 0.9 ; $p = 0.05$) (Section **3.3**). When UBRD was regressed on BVDV arrival titre, the coefficient for arrival titre was negative and significant ($\beta = -0.1 \pm 0.09$; OR = 0.9; $p < 0.01$) (Section **3.3**).

6.4 Discussion

Exposure to BCV prior to arrival was extremely common, with 90 % of animals sero-positive at arrival. This, and reports from other authors, would support the notion that BCV is a ubiquitous organism in cattle populations (1,4). Bovine corona virus infection during the early feedlot period, as demonstrated by large changes in titre from day 0 to day 28, was also extremely common, with 50% of animals sero-converting during the study period. However, based on not finding an association of titre change with UBRD in the multiple regression model, we would suggest that BCV was not related to an increased risk of UBRD, a finding reported previously by Martin *et al* (9).

This conclusion is drawn despite the univariate statistic suggesting that average titre changes were larger in treated animals because the multiple regression model controlled for a number of potential confounders of the change in BCV titre and treatment association. Confounding is described as "a distortion of an estimated exposure effect that results in differences in risk between the exposed and the unexposed that are not due to the exposure" (10). It appeared that both FEEDLOT and arrival titre to BCV confounded the relationship between treatment for UBRD and change in BCV titre. FEEDLOT C had relatively few UBRD treatments, smaller titre changes and higher arrival titres (Table 6.2). The confounding effect of FEEDLOT on the impact of TRT on titre change was shown by the large change in the TRT coefficient when FEEDLOT entered the model. Other studies have suggested that an association exists between BCV and respiratory disease occurrence, based on the observation that the organism was frequently isolated from animals with respiratory disease (3,5). However, these studies failed to examine the prevalence of BCV in control animals, and hence are of limited value for such inferences.

The univariate association of positive arrival titres with improved health during the feedlot period, and the reduced risk predicted by higher titres at arrival, was not taken as strong evidence for BCV having a causal role in the UBRD. This is because of the lack of supporting evidence that active infection (shown by titre change) was associated with treatment. An association between arrival titre and reduced disease risk does not necessarily imply that the protection was BCV specific and we prefer to interpret the relationship as evidence of "a healthy animal" effect (4). That is, these calves experience widespread exposure to BCV, probably since birth, and the better the calf can

respond to that exposure, as well as to exposure to other agents, the better its general level of health. In contrast, a failure to respond to that exposure would be taken as evidence of an unhealthy, or at-risk, calf.

The other variables included in the model predicting change in BCV titre have very little practical effect. The coefficients of the variables, *P. haemolytica* and *H. somnus* suggest that their effects are very small compared to the effect of arrival BCV titre (Table 6.5). The minimal change in the R^2 between the full model and a model containing only arrival BCV titre is supportive of this view.

With respect to the use of FEEDLOT rather than GROUP as the random effect for the BCV model, the highest level of clustering was chosen for inclusion as the random variable because the design of the feedlots in the study ensured contact of animals between pens. Therefore, with regards to likelihood of BCV exposure and titre change, animals within the feedlots were more similar than those between feedlots and most of the management decisions were made at the feedlot not the GROUP level.

The findings with respect to BVDV differed from those for BCV. FEEDLOT C represented an unusual situation where almost no natural exposure to BVDV occurred during the study period. Also, the large coefficient for the effect of VVACCINE on BVDV titre change should not be interpreted as a sole effect of vaccination, because some of this effect may have been due to natural exposure as shown in the non-vaccinates. In other studies, the incidence of exposure to BVDV in unvaccinated animals was common during the feedlot period. In fact, the rates of sero-conversion in the vaccinated animals in this study were very similar to the 40 to 50 % sero-conversion rates in unvaccinated animals usually reported (6,11,12).

A number of studies are in agreement with our findings and support the view that high titres to BVDV at arrival are protective against UBRD (6,11,13). As reported elsewhere, change in titre to BVDV was inversely related to the arrival titre (6,11). Because the cattle used in this study were vaccinated at the herd level, rather than at the individual animal level where analysis occurred, it is not possible to make causal inferences about BVDV vaccination. However, we can still test for an association between TRT and titre change, and based on this make inferences about the causal role of BVDV. We do not have a clear explanation as to why, when arrival titre is controlled, higher titre changes to BVDV would be associated with increased risk of disease, in a group of vaccinated animals. However, natural infection with BVDV post vaccination was probably associated with both UBRD and increased titres. Given that the majority of animals were naïve at arrival to BVDV, it may be hypothesised that natural exposure after vaccination resulted in both increased titres due to an anamnestic response and an increased risk of UBRD treatment. Because any titre change as a result of TRT might differ between vaccinated and unvaccinated cattle it would have been preferable to examine interactions between vaccination and TRT on titre change. However, this was not possible in this study.

It also appeared that animals with elevated rectal temperatures at arrival and heavier animals were likely to have larger increases in titre change in BVDV, although the magnitude of the difference between the two extremes of weights and temperature may not be of any practical significance.

With regards to the effect that misclassification of the disease status may have had on the study findings, the owners of the feedlots were instructed to select animals for

treatment based on their usual criterion and it would appear that a rectal temperature greater than 40 ^{0} C was one of those criterion. This may be because of the advice received from veterinarians or published literature. This would suggest that the majority of animals classified as UBRD cases did require treatment but does not diminish the possibility that UBRD cases were not identified and therefore were included in the untreated group. This misclassification would result in a bias of the study findings towards the null hypothesis (14). Likewise because the sensitivity and specificity of the serological tests was not available, the study results could not be adjusted for any bias that may have arisen (14,15,16,17). . The likelihood and impact of misclassification should be considered when evaluating the study conclusions.

In summary, the results of this and other studies support the view that BCV infection is not associated with increased risk of treatment for UBRD, suggesting that BCV does not cause UBRD. In contrast, given that evidence of previous exposure to BVDV predicts lower risk of UBRD treatment, and that such treatment is associated with increased titre changes to BVDV, we infer that BVDV continues to play a causal role as a component of the UBRD complex. These findings are consistent with other literature about BVDV and UBRD (18).

Table 6.1: Descriptive statistics for bovine corona virus and bovine viral diarrhoea virus neutralisation titres for cattle at three Ontario feedlots, Fall 1998.

Variable	n	Geometric mean titre	Standard deviation	minimum	maximum
Day 0 BCV titre	839	3.5	1.9	0	11
Day 28 BCV titre	836	5.6	1.1	0	11
BCV titre change	836	2.1	1.9	-4	9
Day 0 BVDV titre	837	1.2	3.3	0	13
Day 28 BVDV titre	835	4.1	3.5	0	13
BVDV titre change	835	3.0	3.0	-5	13

Table 6.2 : Descriptive statistics for bovine corona virus and bovine viral diarrhoea virus neutralisation titres for cattle, by feedlot, at three Ontario feedlots, Fall 1998.

Titres	FEEDLOT A			FEEDLOT B			FEEDLOT C		
	n	Geometric mean	S.D	n	Geometric mean	S.D	n	Geometric mean	S.D
Day 0 BCV titre	310	3.1^{a}	2.0	430	3.6^{b}	1.7	99	4.7^{c}	0.9
Day 28 BCV titre	309	5.4^{a}	0.8	428	5.7^{b}	1.2	99	5.9^{b}	1.1
BCV titre change	309	2.2^{a}	1.9	428	2.2^{a}	1.9	99	1.1^{b}	1.5
Day 0 BVDV titre	309	1.4^{a}	2.7	429	2.4^{b}	3.1	99	0.1^{c}	1.3
Day 28 BVDV titre	309	4.8^{a}	3.9	427	5.1^{a}	2.8	99	0.1^{b}	1.3
BVDV titre change	309	3.4^{a}	3.7	427	2.6^{b}	2.3	99	0.005^{c}	0.3

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

Table 6.3 : Descriptive statistics for bovine corona virus and bovine viral diarrhoea virus neutralisation titres for cattle at three Ontario feedlots by treatment group, Fall 1998.

	Untreated			Treated		
	n	Geometric mean	S.D.	n	Geometric mean	S.D.
Day 0 BCV titre	672	3.7[a]	1.8	167	3.06[b]	2.02
Day 28 BCV titre	670	5.7[a]	1.0	166	5.4[b]	1.1
BCV titre change	670	2.0[a]	1.9	166	2.3[b]	1.9
Day 0 BVDV titre	672	1.9[a]	3.0	165	1.02[b]	2.0
Day 28 BVDV titre	670	4.2[a]	3.4	165	5.02[b]	3.7
BVDV titre change	670	2.3[a]	2.7	165	3.9[b]	3.7

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

Table 6.4 : Descriptive statistics for bovine viral diarrhoea virus and bovine corona virus neutralisation titres for cattle, by feedlot, at two Ontario feedlots that vaccinated for BVDV at arrival, Fall 1998.

	Untreated			Treated		
	n	Geometric mean	S.D.	n	Geometric mean	S.D.
Day 0 BVDV titre	582	2.2[a]	3.1	156	1.1[b]	2.1
Day 28 BVDV titre	580	4.8[a]	3.2	156	5.3[a]	3.6
BVDV titre change	580	2.6[a]	2.7	156	4.2[b]	3.7

*Means in the same row with the same superscript do not differ significantly at $p < 0.05$

Figure 6.1 : The frequency distribution of change in bovine corona virus and bovine viral diarrhoea neutralisation titres, for all animals, over a 28 day study period, at three Ontario feedlots, Fall, 1998

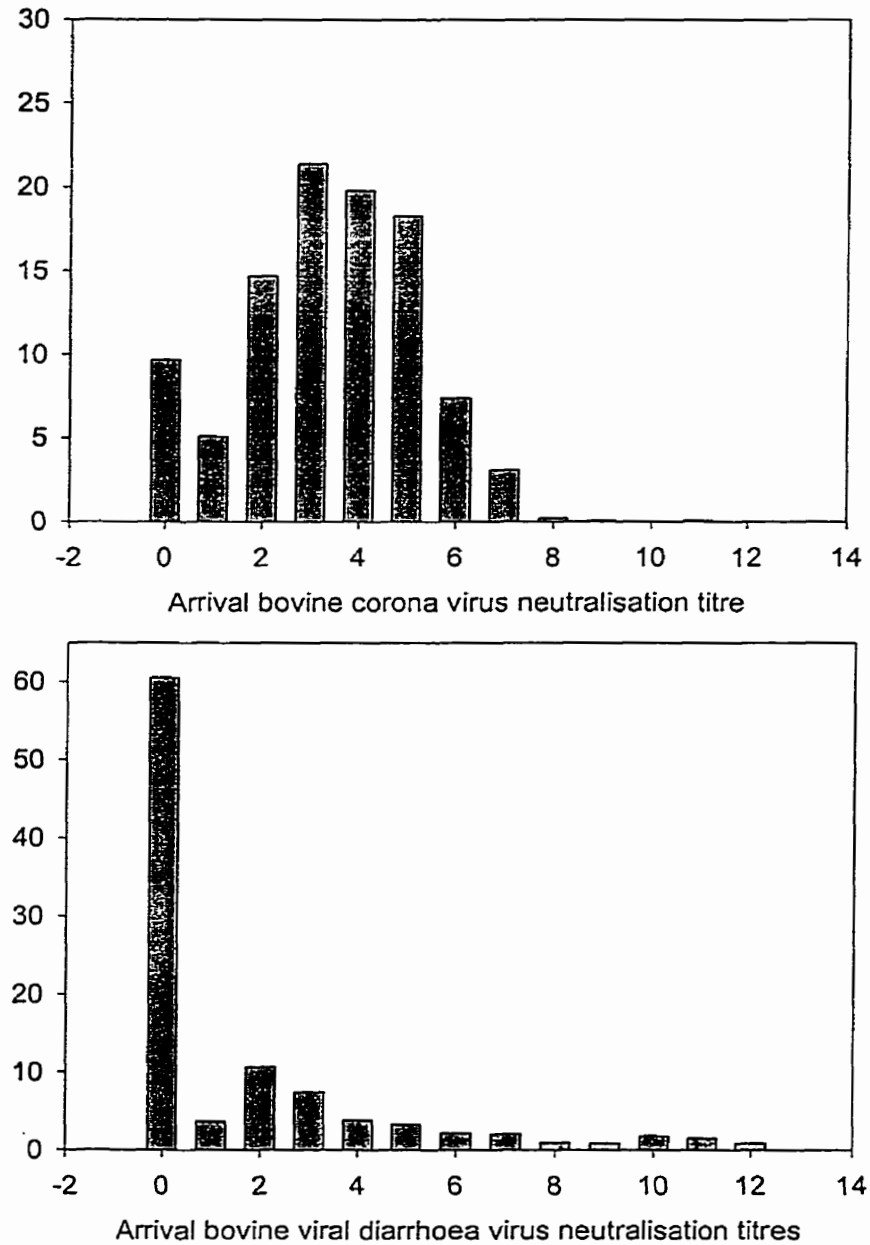

Figure 6.2 : The frequency distribution of change in bovine corona virus and bovine viral diarrhoea neutralisation titres, for treated and untreated animals, over a 28 day study period, at three Ontario feedlots, Fall 1998

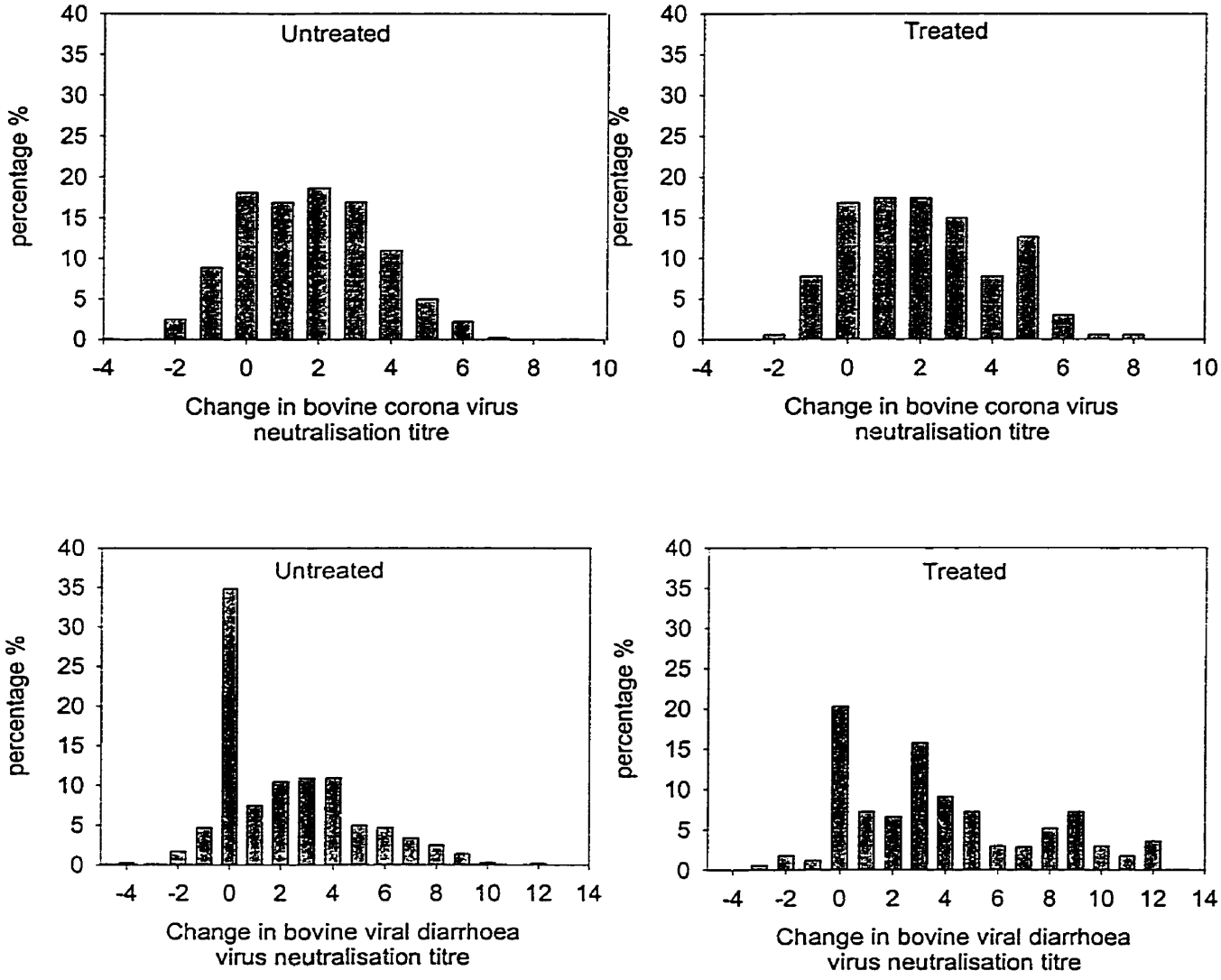

Figure 6.3 : The frequency distribution of change in bovine corona virus neutralisation titres, by feedlot, over a 28 day study period, at three Ontario feedlots Fall 1998.

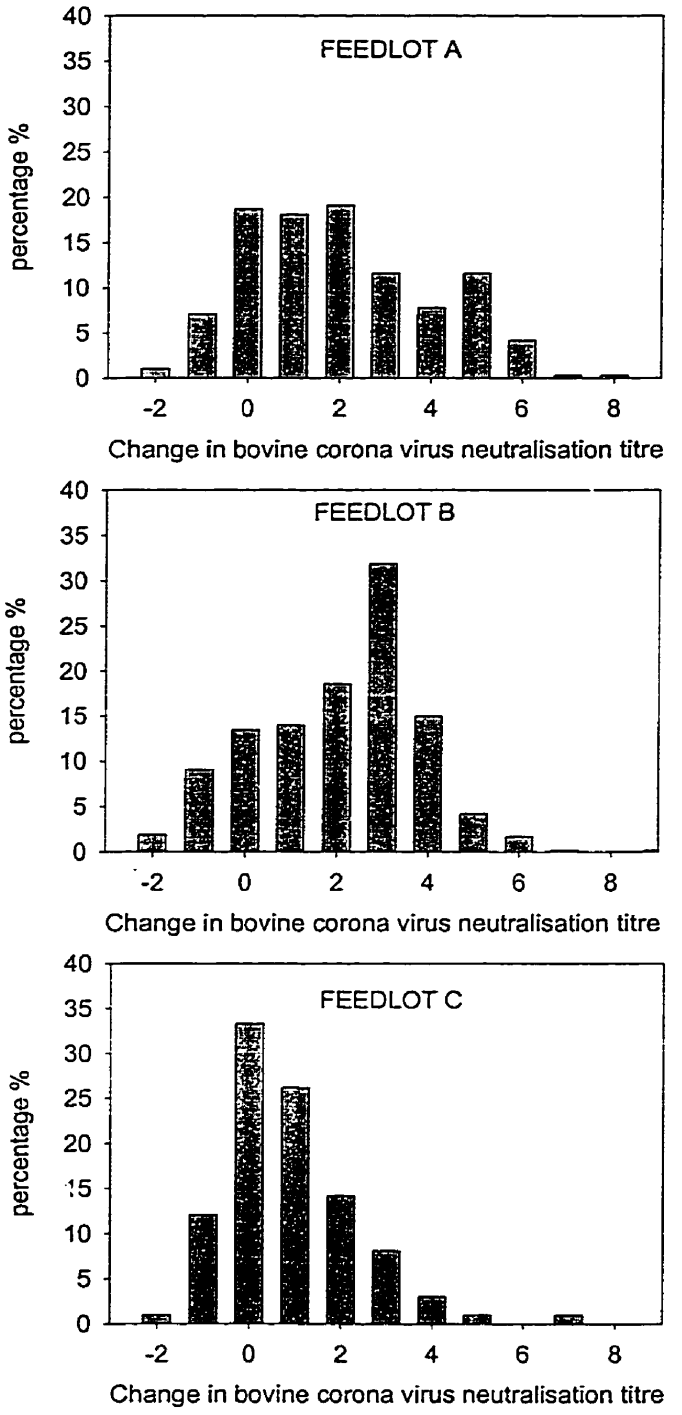

Figure 6.4 : The frequency distribution of change in bovine viral diarrhoea virus neutralisation titres, by feedlot, over a 28 day study period, at three Ontario feedlots Fall 1998.

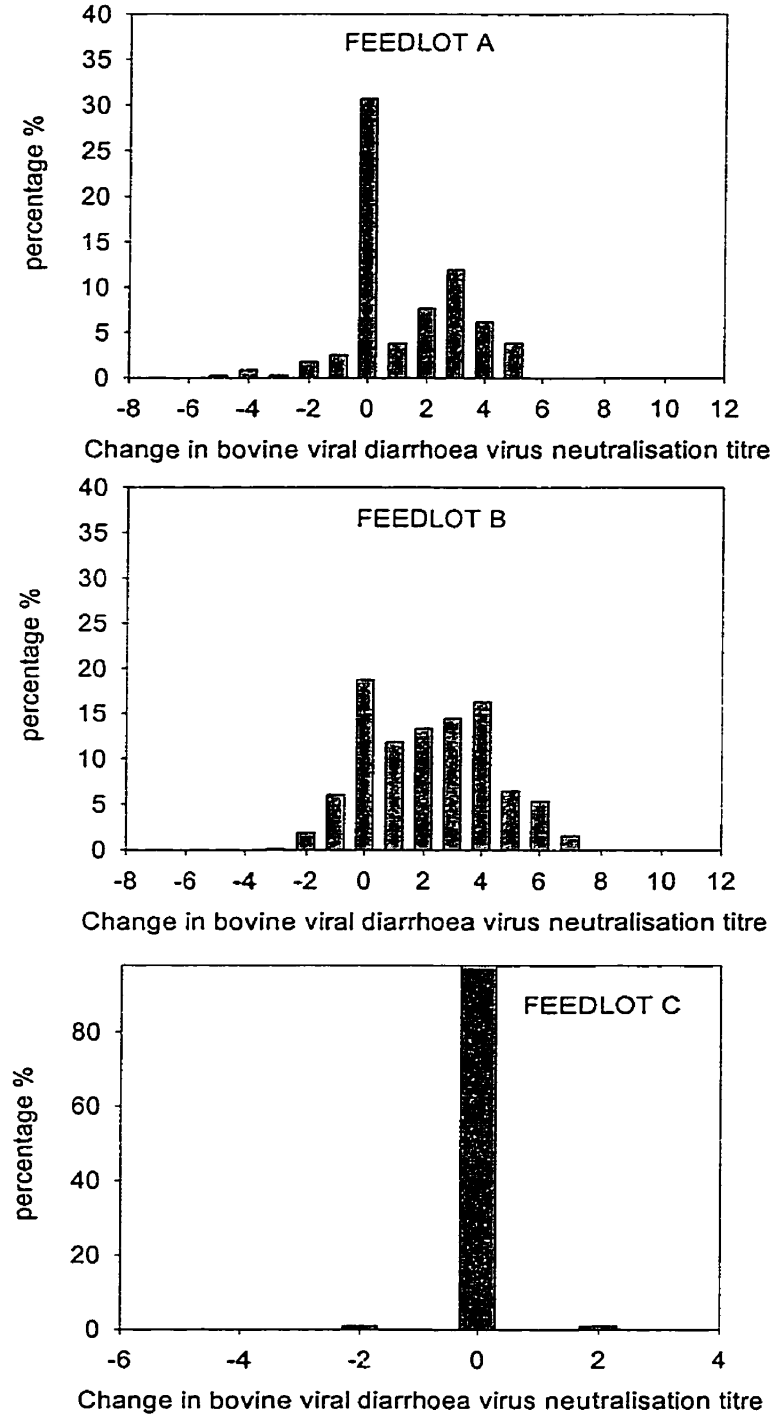

Table 6.5 : Comparison of the association between arrival titres to bacterial and viral agents and temperature at arrival and the change in bovine corona virus neutralisation titres during a 28 day study period on cattle from three Ontario feedlots, Fall, 1998.

	Model 1 : no FEEDLOT effect			Model 2 : FEEDLOT as a random effect		
Variable	Coefficient	SE	p-value	Coefficient	SE	p-value
Intercept	17.5	3.7	0.00	16.9	3.7	0.04
Arrival bovine corona virus titre	-3.3	0.9	0.00	-3.5	0.9	0.00
Arrival *P. haemolytica* titre	0.07	0.03	0.01	0.07	0.03	0.03
Arrival *H. somnus* titre	0.04	0.02	0.06	0.05	0.02	0.04
Temperature at arrival	-0.31	0.09	0.00	-0.3	0.09	0.001
Arrival bovine corona virus titre* Temperature at arrival	-0.19	0.09	0.03	0.06	0.02	0.006
Treatment for UBRD	-0.2	0.09	0.03	-0.12	0.09	0.12
DF	806			805		
RLL[1]	-1177.6			-1174.5		
AIC[2]	-1178.6			-1176.5		

[1]RLL : Residual Log Likelihood

[2] AIC : Akaike's Information criterion

Table 6.6: Comparison of the association between arrival titres to bacterial and viral agents, temperature at arrival and treatment for UBRD and the change in bovine viral diarrhoea virus neutralisation titres during a 28 day study period on cattle from three Ontario feedlots, Fall, 1998.

Variable	Coefficient	SE	p value
Intercept	-22.2	4.6	0.00
Arrival bovine viral diarrhoea virus titre	-0.4	0.03	0.00
Arrival bovine corona virus titre	0.3	0.05	0.00
Arrival weight	0.01	0.003	0.001
Temperature at arrival	0.6	0.1	0.00
Treatment for UBRD	1.2	0.2	0.00
Vaccination with bovine viral diarrhoea virus (herd level variable)	-3.9	0.3	0.00
DF	828		
RLL[1]	-1962.5		
AIC[2]	-1963.5		

[1]RLL : Residual Log Likelihood

[2] AIC : Akaike's Information criterion

6.5 References

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Chapter 7 General Discussion and Conclusion

The preceding chapters of this thesis were aimed at addressing two broad questions: what is the role of infection with *H. somnus* in undifferentiated bovine respiratory disease (UBRD), and what is the role of infection with bovine corona virus in UBRD? The potential role of these agents was investigated because the causal association between these agents and UBRD remains unclear, despite previous research (1,2,3,4,5,6). To answer these questions, two aspects of the association between the immunological response to these agents and the occurrence of UBRD were investigated. The roles of infection with *H. somnus* and bovine corona virus in UBRD occurrence were examined by determining factors that were associated with changes in titre; the latter is interpreted as evidence of current or active infection. If active infection was associated with disease occurrence, this was viewed as strong evidence of a causal association. The second approach was to determine if evidence of previous exposure, based on arrival titre, was associated with the risk of treatment of UBRD. An association between arrival titre and UBRD was interpreted as supportive evidence of a causal association only when evidence of active infection or protection by vaccine was also associated with UBRD occurrence. Otherwise, the reason for an association between arrival titre and UBRD occurrence was unexplained. However, we posited an explanation for these latter observations as a "healthy animal" effect.

An additional aspect of this study was to use vaccines to *H. somnus, P. haemolytica*, or both, in a factorial design to ensure that the some of calves were exposed to these antigens at arrival. Then, the effect of vaccination on both UBRD and titre

change could be investigated. Also, any interactions involving vaccines, for example, the impact of arrival titre, could also be assessed. The data from nonvaccinated animals provided baseline measures for natural exposure and its impacts.

7.1 The Use of Serological Data to Study UBRD

To examine the questions asked in this thesis, serum antibody levels i.e., arrival titre and change in titre, were used as proxies for previous and current exposure respectively. These two measures of antibody levels were then used as variables of interest to ascertain if these agents were associated with disease occurrence.

Change in titre is a "proxy" parameter for current infection, and throughout this thesis it has been used to associate evidence of temporally-related exposure to an organism with disease. Two major criticisms can be levelled at this interpretation of titre change and its use in inferring causal associations. First, it is possible that cross-reactions with other organisms in the serological tests may lead to statistical associations. In addition, in the feedlot, cattle are exposed to numerous organisms and exposure to one organism may offer protection against UBRD from another organisms. We attempted to prevent this bias by controlling for the titres to other key organisms, but the possibility of cross protection is still present. Second, the use of the "proxy" titre change for infection could lead to significant distortions of true causal associations (7). Throughout this thesis when UBRD occurrence and changes in titres were associated, we implied a casual association between that agent and UBRD; however, this inference was open to reverse causation. Reverse causation occurs because of the timing of the measurement of the two events. In the present study, disease occurrence occurred prior to the measurement of

titre change, therefore, exposure and change in titre may occur as a consequence of disease occurrence and therefore exposure may be falsely associated with the antecedent outcome (7).

With respect to inferences about titre on arrival, throughout this thesis we have avoided interpreting an association between evidence of previous exposure (arrival titre) and reduced disease risk as strong evidence of a causal association, unless it was supported by other evidence such as an association between titre change and UBRD occurrence. We have avoided making this inference because, although reduced disease risk associated with previous exposure may be organism specific, we also suspect the association may arise because these animals are generally in better health and have titres to a wide range of organisms. That is, the presence of a particular titre simply suggests that the animal has a well-functioning immune system, and we believe that, on its own, this may merely represent a " healthy calf" indicator. The mechanism of this "healthy calf" indicator is not clear but several possibilities exist. The most readily recognisable linkage is direct antibody cross protection provided by the arrival titre against infection from another organism. Cross reactions in serological tests are frequently seen, and in the context of UBRD, there is evidence that cross reactions occur between *H. somnus* serological tests and *P. haemolytica*, *P. multocida, Salmonella dublin*, and *Esherichia coli,* to name a few (8,9,10). In the present study there was also evidence of cross-reaction between the *H. somnus and P. haemolytica* antigens; either the vaccines stimulate antibody to both organisms or the serological tests could not distinguish between the two very well. Although a cross-reaction between serological tests does not necessarily mean cross protection, for some infectious diseases such cross protection has

been demonstrated. The resistance to small pox infection of people previously exposure to cow pox infection is the classic example of cross protection offered by the antibody to one agent against infection with another organism (11). Also, natural immunity to influenza meningitis caused by *Haemophilus influenza* type b, has been suggested to be due to cross protection from other organisms (12,13,14). Within the context of UBRD, cross protection from antibodies to *H. somnus* may play some protective role against *P. haemolytica* induced UBRD, or visa versa , given the large number of antigens that these two agents share. Having said this about the bacterial antigens, there is no evidence that antibodies produced in response to bovine corona virus infection would offer protection against other viral infections.

Another explanation of the healthy calf effect may be that antibody production is generally higher to a large number of antigens in healthier animals. In human studies, antibody levels to a variety of disease-causing organisms were lower in malnourished but otherwise clinically well children, than in adequately nourished, clinically well children (15). This same study also found that titres to 12 of 14 infectious agents, including tetanus and diphtheria, where lower in children suffering from diarrhoea. The authors did not interpret the higher titres in healthy children as evidence of a casual association between the agent and the disease, but rather as a general indicator of a healthy child. In cattle populations where exposure to *H. somnus* and bovine corona virus is common, it is interesting to speculate that animals that have failed to produce antibodies to these organisms prior to arrival are not actually naïve, but rather they have failed to respond properly to previous exposure. This failure to respond is an indicator of a general weakness in their immune system that will subsequently make them more susceptible to

UBRD. It is for this reason that suggesting a causal association between an agent and UBRD, based solely on protection associated with arrival titres, may not be valid. However, if this association is found, in conjunction with evidence that infection was associated with disease occurrence, we view this as supporting evidence of a causal relationship.

7 .2 Statistical Approach to Analysis

The statistical approach to the analysis of the data in the proceeding papers differed from previously published work in several ways. Although there would have been merit in repeating the analyses with the same methods, we believe the differences reflect an improvement in analytic approach that can serve as a better basis for future research.

7.2.1 The Outcome Variable

Previous sero-epidemiological feedlot studies have used logistic regression to model the risk of disease as a function of arrival titre and changes in titre. This approach was not used for two reasons. To say that change in titre causes disease, which is what is being implied by modelling disease occurrence as a function of changes in titre, is biologically incorrect, although mathematically no real differences are likely to occur. The other reason is that modelling "change in titre" as a function of "treatment" emphasises the proxy nature of the parameter and increases awareness of possible incorrect causal assumptions made by the use of change in titre as a proxy for infection.

We chose to model change in titre as the outcome of interest, as did the previous reports, for ease of interpretation, although Day 28 titre could be an alternative outcome.

We preferred to use "change in titre" because the use of "Day 28 titre" is less intuitive as an indicator of exposure. From a statistical point of view, because they may be measuring the same biological event, modelling "change in titre" may be criticised because it is a derived number, i.e., day 28 titre – day 0 titre (16). This approach to modelling often increases the arrival titre's correlation with change in titre, and leads to some instability (increased standard errors) in estimates of effect. To avoid complications in parameter interpretation, the independent variable Day 0 titre was always included in the model, prior to other independent variable assessment (16). Interactions involving the arrival titre were also investigated.

7.2.2 The Model Building Approach and Detection of Confounding Variables

In this study, models were built based on the magnitude of association of the initial variables of interest with the outcome, and evidence of confounders was tested using the "change in point estimate" of their coefficient (17). A more refined, but infrequently used approach to identify confounding is the change in confidence interval (17,18). Other model building approaches, such as forward or backward stepwise variable selection relying upon the p value of the coefficient to determine the inclusion or omission of a variable, have been strongly criticised in recent years, and some journals will not accept the results of models built in this manner (e.g.. Journal of Epidemiology). For example, reliance on the coefficient p-value to determine the inclusion or omission of a confounding variable can be biased because the test statistic is a function of the magnitude of the variables association with the outcome (the numerator) and the sample size (the denominator), and as such, is considered to be biased (19). The change in the

coefficient of the variable of interest is also impacted by the association between this variable and the confounder. However, confounding should be judged by the change in estimate not by a statistical test. If one relaxes the p-value criteria to approximately 0.15, the two approaches often lead to the same final model (20).

7.2.3 The method used to control for clustering

Another aspect of the statistical approach that differed from other studies was the use of mixed models with random effects to control for clustering of the outcome (21). To obtain valid parameter estimates, regression techniques rely upon the assumption that the units of analysis, in this study the individual, are independent. Violation of the assumption of independence results in biased parameter estimates of variance. The standard errors for fixed effects, at the cluster level, will be underestimated when independence is falsely assumed and as a result the null hypothesis may be falsely rejected (22). The behaviour of coefficients estimated at the individual level is not clear. The magnitude of the standard deviations of the coefficient estimates of individual animal variables when a random effect is included in the model may remain unchanged, increase or decrease, although they are thought to be "better" [personal communication – Dr M Shoukri]. Therefore, it is recommended that clustering be accounted for when independence cannot be assumed (22).

The methods described to account for clustered data include: ignoring the clustering altogether, avoidance of clustering by restricting the study population and assuming no other levels of clustering exist (i.e. one feedlot, ignore the pens), weighted least squares, entering the cluster variables as fixed effects, or inclusion of random effects

(22). Many previous studies have used the "fixed effects" approach to manage the clustering and prevent bias. The advantage of using the random effects method over other methods is that it allows for examination of the variability of the outcome at the levels of clustering. This may be important when implementing health interventions because interventions aimed at levels of clustering with large variation will be more effective than those aimed at levels of clustering with low variability (22). Another advantage is that unless the clusters studied are of particular interest, they are not fixed effects and a single parameter to describe the effect of cluster is more appropriate and statistically efficient than a fixed effect for each parameter (22). The disadvantage of this approach in this study was that the estimate of the variability of the random effect was based on a small number of clustered units. By using FEEDLOT as a random effect in the mixed model, the variance of the FEEDLOT was estimated using only three feedlots, so estimates of the feedlot effect will have a large variance. Therefore the cluster effect of FEEDLOT may bias findings towards the null hypothesis and increase the probability of a type II error. The true variance associated with the outcome due to feedlot may in fact be smaller or larger than that estimated in this study.

In feedlot studies, both the feedlot and the pen might be considered to be important units of clustering; however, no published studies have reported the variance components attributable to these units. Booker *et al* (6) studied one feedlot and controlled for pen but did not explain how this was done or give estimates of the effects. Other authors routinely included the feedlot effect as a fixed variable, which prevents bias, but can not provide information about the importance of the feedlot as a level of clustering (5,23). Knowing the variance components would be useful as it enables closer

critical assessment of studies based on where the majority of variability in the outcome is expected to lie. For example, if the feedlot accounted for a great deal of the variability of the outcome, then parameter estimates for fixed effects in single feedlots studies would have less validity than those based on multiple feedlot studies. However, if the pen were the greatest source of variation of the outcome then studies that control for pen effects should give better estimates of animal level effects.

In this study, GROUP (a surrogate for the group the animal was processed with) and FEEDLOT were considered to be random effects. GROUP was considered a random effect because auction market handling procedures, staffing levels, weather influences, and processing stresses create a similarity between animals, processed on the same day, that is greater than between processing days. The effect of the FEEDLOT was also included as a random effect. Animals within a feedlot are purchased using the same purchasing practices and preferences, and exposed to similar nutrition, management styles and environments that result in similarities between animals on one feedlot.

When possible a nested error structure was used to control for clustering i.e. the effect of GROUP was nested within FEEDLOT (PROC MIXED). If that approach was not possible, because of statistical package limitations, the single random effect FEEDLOT was used to define the level of clustering (PROC GLM). The aim of this was to obtain parameter estimates of cluster level variables that allowed estimation of variance components of FEEDLOT and also to remove the effects of these nuisance variables. As discussed previously, the pen may also be considered a source of clustering; however, because pen allocation was unavailable for one feedlot, this area of clustering had to be ignored. Unfortunately because no published estimates of the

relative importance of the variance components of the levels of clustering in feedlots are available it is not known if the exclusion of this level of clustering resulted in parameter estimate bias.

7.2.4 Examining Time to Treatment as an Explanatory Variable

The other unique approach to analysis in this thesis was the examination of the time to treatment as a factor affecting the outcomes, change in titre and the risk of treatment. Categorisation of the treated animals based on time to treatment provided considerably more information about the behaviour of the change in titres relative to UBRD, especially with regards to the *H. somnus* data then the approach previously used based on UBRD treatment status (yes / no). The choice of 10 days was an arbitrary categorisation that proved to be useful in this study however this could be changed if other authors wished to use this approach.

7 .3 The Study Population.

The study population may limit the external validity of conclusions drawn from this study. Although the study population may not be representative of the target population (i.e. all feedlot cattle) this does not affect the internal validity of the conclusions drawn from the study, which is more important for causal inference than external validity and is a prerequisite for external validity. By using appropriate techniques to examine confounding and control for clustering within the study, the parameter estimates were the best possible and therefore the within-study conclusions should be valid. The external validity, or generalizability, of a study is more a function

of the internal validity of the findings and the other literature available for examination than of how well the study population reflects the target population (20).

7 .4 General Findings

Because so much literature is available on the role of *P. haemolytica* and bovine viral diarrhoea virus in UBRD, the behaviour of titres to these two agents represented a 'yard stick" by which we judged the behaviour of the other two putative agents. The association between larger titre changes to the *P. haemolytica* leucotoxin and disease occurrence has been reported consistently elsewhere the literature, although it was not found in this study (6,24,25). A similar relationship has been reported between disease occurrence and bovine viral diarrhoea virus titre changes (23). However, results of this study suggested that treated animals were likely to have smaller or no titre increases to *H. somnus* compared to other naturally exposed and untreated animals. Thus recent exposure to *H. somnus* was not associated with an increased risk of being treated. This association is not consistent with the "yard stick" behaviour of *P. haemolytica* and bovine viral diarrhoea virus titre changes and UBRD occurrence. If *H. somnus* were an agent causing UBRD we would have expected that, as with *P. haemolytica* and bovine viral diarrhoea virus, animals that became sick would have higher titre changes in response to exposure.

Explanations previously offered either for this lack of association, (or the presence of a reversed association) have included : that *H. somnus* antigens were being consumed at a rate faster than antibody production in sick animals (5) or that the association was "logical" but without further explanation (6). Presumably the latter

author felt that control animals were likely to have larger titre increases because larger titre changes can be associated with a protective anamnestic response. We offered two other possible explanations for this finding based mainly on the additional evidence that the low titre changes occurred only in a subset of animals. The first was that the lack of evidence of exposure was a function of the treatment rather than visa versa. Inhibition of colonisation and infection of calves with *H. somnus* may occur because of the use of antimicrobials to prevent or treat UBRD. Given that the vast majority of the treated calves (165/ 174) received "prophylactic" antibiotics at arrival, animals treated after 9 days post arrival, or treated twice because of a relapse of UBRD, had sub-therapeutic levels of antibiotics in their circulation for longer periods, and perhaps these levels are sufficient to control infection with an organism that is usually highly sensitive to antimicrobials, such as *H. somnus*. Another explanation may lie in the statistical approach used. Animals that were treated later after arrival had higher titres and therefore because of the method used to model titre increase (a constant increase in titre across all initial titres) we were unable to detect titre changes in these animals. This explanation seems attractive; however, the same method of titre analysis was used for *P. haemolytica.* Further, it does not explain why early treated animals that relapsed also showed smaller titre changes. Further investigation is required to provide evidence for either hypothesis.

By using the vaccine field trial design to examine the titres to the bacterial agents we were able to examine the effect of vaccination on UBRD occurrence, and the behaviour of natural and vaccine induced titre changes in well and sick animals. It was expected that vaccinated animals would have larger titre increases than unvaccinated

animals but how UBRD occurrence might affect this response to vaccination was unknown. The data showed that, as anticipated, vaccinated animals had higher titres to *P. haemolytica* than unvaccinated animals. However, unexpectedly, treatment did not affect the response to vaccination. For *H. somnus* it was also possible to determine if the behaviour of the *H. somnus* seen in previous studies was due to the nature of the *H. somnus* antigen or due to the route of exposure. If the decreased antibody production was specifically due to the nature of the *H. somnus* antigen, i.e. regardless of route of exposure vaccination or natural, then both vaccinated and unvaccinated sick animals should have had the same *H. somnus* titre response. In the early and never treated groups, the titres behaved exactly as expected, i.e., those receiving the *H. somnus* vaccine had higher titres than those not receiving the *H. somnus* antigens. Sick *H. somnus* vaccinated animals were able to respond to the *H. somnus* antigens and so the relative lack of antibody production in the sick unvaccinated animals appears to be a function of natural exposure to *H. somnus* rather than due to the nature of the antigen itself.

The presence of the vaccine trial also aided our ability to make causal inferences because protection afforded by vaccines in a randomised clinical trial is viewed as strong evidence of causation, while associations from observational studies are viewed as supportive evidence of causation. Despite this, there remains measure of uncertainty about the causal inferences based on the vaccine trial because of the possibility of cross protection between *P. haemolytica* and *H. somnus*.

In this study, and consistent with other authors, higher *H. somnus* titres at arrival where protective of UBRD occurrence (5,6). However, without supporting evidence that

disease occurrence was capable of inducing titre change, this was not interpreted as supporting evidence for a causal association between UBRD and *H. somnus* infection.

The failure to find an association between evidence of *P. haemolytica* exposure during the study period and UBRD was unexpected, because, as discussed this relationship had been reported previously. This failure may have been due to a lack of gradient of exposure to *P. haemolytica* which would mitigate against finding an association.

A relationship between BCV infection and UBRD occurrence was also not found. Exposure to BCV was extremely common in the feedlots in the study, but there was no evidence that change in titre was associated with treatment. As reported previously, arrival titres to BCV were sparing of UBRD risk (26). However, again, without supporting evidence that current BCV infection is actually responsible for disease, this was not interpreted as evidence of a causal relationship, but rather as a healthy calf effect.

7 .5 Conclusion

Despite previous literature suggesting that *H. somnus* and bovine corona virus are capable of causing UBRD in feedlot cattle, no evidence for the causal association of either organism to UBRD was found in the present study. We found no association between infection and disease occurrence and feel that evidence of an association between previous exposure and protection is not sufficient to make causal inferences. This would suggest that vaccination against UBRD occurrence using vaccines containing these agents would not result in decreased UBDR occurrence. However, given other

research suggesting that *H. somnus* is a common cause of mortality in western Canada, vaccination may still protect against other manifestations of *H. somnus* infection.

Future areas of research arising from this project may include; the examination of other datasets to determine if the frequent use of antibiotics is consistently associated with decreased evidence of *H. somnus* exposure and if this related to the increased observance of other manifestations of *H. somnus* infection e.g., myocarditis, polyarthritis etc. It would also be of interest to further examine the validity of serological evidence of previous exposure when making causal inferences about the role of organisms in UBRD. That is to examine the validity of the proposed "healthy animal" effect.

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