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Epidemiology of Bovine Respiratory Disease in Australian Feedlot Cattle

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Abstract

Bovine respiratory disease (BRD) is the most important cause of morbidity and mortality in feedlot cattle, both in Australia and worldwide. BRD broadly describes a complex of diseases involving the respiratory system in cattle. It has a multifactorial aetiology involving a combination of susceptible animals, infectious agents and stressors.

Most published BRD research is from studies conducted in North America, but there are important differences between Australian and North American beef cattle production and feedlot management practices. The relative importance of particular risk factors at the population level would also be expected to differ between Australia and North America because animal entry characteristics, management practices and environmental exposures differ. In addition, there are conflicting results from the literature relating to associations between some risk factors and BRD.

To address these issues a nationwide cohort study was conducted in Australian feedlot cattle to quantify strengths of associations between numerous putative risk factors and BRD, and to determine the population-level impact of relevant risk factors. The main cohort study population comprised 35,131 animals nested within 1,077 groups nested within 170 cohorts (feedlot pens) nested within 14 feedlots. In addition, a subset of 7,450 animals was selected for inclusion in a nested case-control study. The objectives of this study were to: (i) describe the seroprevalences of antibodies to four viruses at induction (i.e. processing at entry) (ii) describe changes in serostatuses six weeks after induction, and (iii) investigate associations between serological risk factors and BRD occurrence.

Data relating to numerous putative risk factors were collected from several sources during the course of the study. Novel use of lifetime animal movement data obtained from a nationwide database allowed detailed analysis of putative risk factors describing each animal's prior mixing history, group dynamics, lifetime saleyard exposure and timing of the animal's move to the feedlot. Laboratory analysis of serum samples and nasal swabs allowed the differentiation of animals persistently or transiently infected with bovine viral diarrhoea virus (BVDV). Hence the effects of exposure to BVDV on BRD incidence in the main cohort study population could be assessed.

Causal diagrams were used to inform model building by considering a priori biologically plausible pathways. Multilevel Bayesian logistic models were utilised to estimate the effects of putative risk factors. In addition, a parsimonious model was built and used to determine the partitioning of outcome (i.e. BRD) variance at different hierarchical levels; this was used for identifying the most appropriate level for interventions and further

research. Population-level effects of important risk factors were calculated and used to rank risk factors and identify management strategies with the largest potential overall effects in reducing BRD risk in Australian feedlot cattle.

Several management-related risk factors were identified as having a marked effect on BRD risk at both the animal level and the population level. Factors related to the animal's lifetime mixing history, feedlot move timing and the numbers of animals in groups established at least two weeks before feedlot entry were all very important. The practice of sharing water troughs between feedlot pens had a very large effect; this previously unreported risk factor is readily amenable to intervention. Exposure to BVDV had a moderate population-level effect, providing a measure of the expected impact in feedlot populations if effective programs to prevent BVDV entry into feedlots were implemented. Animal factors (breed, sex and weight) and broad non-specific factors (feedlot region and season of induction) had modest to large population-level effects.

Animals that were seropositive to any of four viruses at induction were generally at reduced risk of BRD compared to those that were seronegative, although those with low antibody levels to BVDV appeared to be at increased risk. Animals that were seropositive to increasing numbers of viruses at induction were at reduced risk of BRD compared to those seropositive to fewer viruses. Seroconversion or seroincrease to any of four viruses during the first six weeks on feed was associated with increased risk of BRD. Animals that seroincreased to one virus were at increased risk, and animals that seroincreased to two or more viruses were at markedly increased risk of BRD compared to animals whose serological status did not change.

The studies described in this thesis have identified several important management-related risk factors that are amenable to interventions with the potential to markedly reduce BRD incidence in Australian feedlot populations.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer-reviewed papers

HAY, K. E., BARNES, T. S., MORTON, J. M., CLEMENTS, A. C. A. & MAHONY, T. J. 2014. Risk factors for bovine respiratory disease in Australian feedlot cattle: Use of a causal diagram-informed approach to estimate effects of animal mixing and movements before feedlot entry. *Preventive Veterinary Medicine*, 117, 160-169 (Hay et al., 2014)

Conference presentations, abstracts and posters

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Contributions by others to the thesis

The National Bovine Respiratory Disease Initiative (NBRDI) was already underway at the commencement of my candidature. The NBRDI was jointly funded by Meat and Livestock Australia (MLA) and the Queensland Government. The NBRDI was co-ordinated by The University of Queensland and the Department of Agriculture, Forestry and Fisheries. My studies comprised the major epidemiological analyses of data derived from the NBRDI.

Dr Tamsin Barnes (The University of Queensland School of Veterinary Science, Gatton, Queensland) was my principal advisor as well as the NBRDI operations manager. She provided ongoing input about veterinary epidemiological methods. She critically reviewed all chapters of the thesis.

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Dr Paul Horwood, Dr Rebecca Ambrose and Dr Beth Fowler performed laboratory testing for viral sequencing and virus and bacterial detection

Ms Jenny Gravel, Ms Margaret Commins and other members of the Queensland Department of Agriculture, Forestry and Fisheries virology research laboratory group were responsible for sample handling, processing and storing along with serological testing

Statement of parts of the thesis submitted to qualify for the award of another degree

None

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Keywords

bovine respiratory disease, cattle, feedlot, risk factor, serology, seroprevalence, multilevel modelling, bayesian statistics

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Fields of Research (FoR) Classification

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List of abbreviations

ACF	Auto-correlation factor
ALFA	Australian Lot Feeders' Association
BCoV	Bovine corona virus
BoHV-1	Bovine herpes virus type 1
BOM	Bureau of meteorology
BPI3	Bovine parainfluenza virus type 3
BRD	Bovine respiratory disease
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine viral diarrhoea virus
CI	Confidence interval
Cred Int	Credible interval
Ct	Cycling threshold
DIC	Deviance information criterion
DOF	Days on feed
ELISA	Enzyme-linked immunosorbent assay
H. somni	*Histophilus somni*
IBR	Infectious bovine rhinotracheitis
ICC	Intra-class correlation coefficient
M. bovis	*Mycoplasma bovis*
M. haemolytica	*Mannheimia haemolytica*
MCMC	Markov chain Monte Carlo
MCSE	Monte Carlo standard error
MLA	Meat and Livestock Australia
NBRDI	National Bovine Respiratory Disease Initiative
NLIS	National livestock identification system
NLIS ID	National livestock identification system identification string
NSW	New South Wales
NT	Northern Territory
OR	Odds ratio
P. multocida	*Pasteurella multocida*
PACF	partial autocorrelation factor
PAF	Population attributable fraction
PAR	Population attributable risk
PCR	Polymerase chain reaction

PI	Persistently infected with BVDV
PIC	Property Identification Code
PQL2	Second order penalised quasi-likelihood
Prob	Probability
QAAFI	Queensland Alliance for Agriculture and Food Innovation
Qld	Queensland
qPCR	Real-time quantitative RT-PCR
Ref	Reference category
RFID	Radio frequency identification device.
ROC	receiver operating characteristic
RT-PCR	Reverse transcription polymerase chain reaction

List of abbreviations of variables used in analyses

Age	Estimated age range at induction in months
Arrival to day0	Time between arrival at the feedlot PIC and day 0
BoHV-1 ind	Bovine herpes virus type 1 induction serostatus category
BoHV-1 change	Change in BoHV-1 serology between induction and follow-up, where 'change' may be measured by a) composite variable, b) seroincrease or c) seroconversion
BoHV-1 comp	Bovine herpes virus type-1 composite change variable: (up, no change, initially high)
BoHV-1serocon	Seroconversion to BoHV-1 between induction and follow-up
BoHV-1seroinc	Seroincrease to BoHV-1 between induction and follow-up
BPI3 ind	Bovine parainfluenza virus type 3 induction serostatus category
BPI3 change	Change in BPI3 serology between induction and follow-up, where 'change' may be measured by a) composite variable, b) seroincrease or c) seroconversion (i.e. seroincrease in animals initially seronegative)
BPI3 comp	BPI3 composite change variable: (up, no change, initially high)
BPI3serocon	Seroconversion to BPI3 between induction and follow-up
BPI3seroinc	Seroincrease to BPI3 between induction and follow-up
BRD50	Bovine respiratory disease occurring within the first 50 days at risk
Breed	Breed category
BRSV ind	Bovine respiratory syncytial virus induction serostatus category
BRSV change	Change in BRSV serostatus between induction and follow-up, where 'change' may be measured by a) composite variable, b) seroincrease or c) seroconversion
BRSV comp	BRSV composite change variable: (up, no change, initially high)
BRSVserocon	Seroconversion to BRSV between induction and follow-up
BRSVseroinc	Seroincrease to BRSV between induction and follow-up
Bunk space	Linear meters of space at the pen feed bunk per head in the home pen
BV_vacc	Prior vaccination with Bovilis MH™ occurring at least 14 days before induction and reported in the vendor questionnaire
BVDV ind	Bovine viral diarrhoea virus induction serostatus category

BVDV change	Change in BVDV serostatus between induction and follow-up, where ‘change’ may be measured by a) composite variable, b) seroincrease or c) seroconversion
BVDV comp	BVDV composite change variable: (up, no change, initially high)
BVDVserocon	Seroconversion to BVDV between induction and follow-up
BVDVseroinc	Seroincrease to BVDV between induction and follow-up
BVDV_cht	BVDV active in the cohort: BVDV detected in any animal-level or pooled test
BVDV_group-28_PI	BVDV-PI animal in group-28
BVDV_grp_cht	BVDV-PI in group-28 and BVDV active in cohort (no no; yes no; yes yes)
BVDV_PI_animal	The animal is persistently infected with BVDV
Cohort fill	Cohort fill duration
CohortN	Number of animals inducted into the cohort
Day0 to close	Interval between day 0 and cohort close date
Dentition	Number of permanent incisors present on day 0
DOF1 to day0	Interval between the first day on feed and day 0
Feedlot region	Region where feedlot is located
FeedlotN	Average total number of cattle on feed in the feedlot during the animal’s induction month
FeedlotN40	Average total number of cattle less than 40 days on feed in the feedlot during the animal’s induction month
Grain 60%	Time from the first day on feed until the ration contains 60% grain
Grain pre	Cattle have ever previously been fed grain as reported in the vendor questionnaire
Grain type	Type of grain in the ration
Grain1	Percentage of grain in the ration on day 0
Grain21	Percentage of grain in the ration on day 20
Group-13	Group animal was part of 13 days before day 0
Group-13N	Number of animals in the animal’s group-13
Group-28N	Number of animals in the animal’s group-28
Group-91N	Number of animals in the animal’s group-91

Induction year	Year of induction
Intended DOF	Intended number of days on feed at induction
Mix first	Time interval prior to day 0 during which the animal was first comingled (mixed)
Mix first summary	Composite variable derived from Mix first and Mix summary
Mix history	Composite mixing history variable: Mix pre-27, Mix -27 to -13 and Mix -12 to close
Mix pre-27	Animal was mixed with animals from a different PIC prior to day -27
Mix pre-90	Animal was mixed with animals from a different PIC prior to day -90
Mix summary	Mixing history summary variable; (Mix pre-27 and Mix -27 to close)
Mix VQ	On-property mixing as reported in the vendor questionnaire
Mix-12 to close	Number of group-13s that were mixed between day-12 and cohort close date
Mix-27 to -13	Animal was mixed with animals from a different PIC between days -27 and -13
Mix-27 to close	Number of group-28s that were mixed between day-27 and cohort close date
Mix-90 to -28	Animal was mixed with animals from a different PIC between days -90 and -28
Move_FL	Timing and duration of animal's move to the feedlot
Move_time	Total estimated transport time for the move from the source PIC to the feedlot
Pen density	Number of standard cattle units per square meter in the home pen
Pen join	Number of pens adjoining home pen
Pen shade	Pen was/was not shaded
Pen water	Shared pen water
PI	Persistently infected with BVDV
PIC	Property Identification Code
PV_vacc	Prior vaccination with Pestigard™ occurring at least 14 days before induction and reported in the vendor questionnaire
Rain	Total estimated rainfall in the first 7 days beginning on day 0
Rhinogard	Rhinogard™ vaccine was administered at induction

Season	Season of induction
Selection batch	Batch in which animals were in when selected for the case-control study (1 or 2)
Sex	Animal's sex
Sex cht	Sex of the cohort: (male, female or mixed)
Source region	Region defined by the animal's PIC's geographic location 28 days before induction
Supp pre	Cattle have ever previously been supplementary fed (e.g. conserved forage) as reported in the vendor questionnaire
SY -12 to 0	Animal had a saleyard transfer between days -12 and 0
SY -27 to -13	Animal had a saleyard transfer between days -27 and -13
SY pre-27	Animal had a saleyard transfer prior to day -27
Temp max	Mean daily maximum temperature for the first 7 days beginning on day 0
Temp min	Mean daily minimum temperature for the first 7 days beginning on day 0
Temp range	Mean daily range in temperature for the first 7 days beginning on day 0
Test batch	ELISA test kit batch used for serological testing of case-control samples
Time_move1	Interval during which the earliest transfer between PICs occurred
VirusN_ind	Number of viruses the animal is seropositive to at induction
VirusN_seroinc	Number of viruses the animal had a seroincrease to between induction and follow-up
VitADE	Vitamins A, D and E administered at induction
Weight	Induction weight
Weight cht	Mean induction weight for animals in the cohort
Weight diff	Difference between the animal's induction weight and the mean cohort weight
Wind	Mean daily maximum wind speed for the first 7 days beginning on day 0
Yard wean	Animal was yard weaned and if so, interval of time kept in yards after weaning as reported in the vendor questionnaire

1 Literature Review

1.1 Introduction

Broadly, bovine respiratory disease (BRD) describes a complex of diseases involving the respiratory system in cattle and is particularly problematic where cattle are kept in intensive or confined conditions, such as in feedlot operations. While it is generally agreed that the disease occurs when there is a combination of susceptible animals, infectious agents and stressors, some results from studies investigating the risk factors for BRD are conflicting. This is probably due to the multifactorial nature of BRD, the complexity of the interaction of numerous factors, and the difficulty in measuring the effects of exposures at several levels and in controlling for potential confounders.

Most large scale studies of BRD in feedlot cattle have been conducted in North America. However, there are important differences between Australian and North American beef cattle production and feedlot management practices as well as in prevailing weather conditions and infectious agents. Thus, results from North American studies may not be generalisable to the Australian industry. In North America, cattle typically enter the feedlot at a younger age and lighter weight, often at or soon after weaning (Horwood et al., 2014). In Australia, most cattle are weaned onto pasture and typically enter the feedlot for finishing at an average age of about 18 months (Dunn et al., 1993). Therefore, the amount and timing of commingling prior to feedlot entry would be expected to differ considerably. In addition, it is likely that the particular strains or subtypes of pathogens involved in the BRD complex in Australia differ from those seen in other countries, so improved epidemiologic knowledge about the local pathogens is important.

The Australian feedlot industry's production is valued at 'approximately $2.7 billion annually and is estimated to employ 2,000 people directly and 7,000 indirectly' (ALFA, 2011). Nationally there are 397 accredited feedlots, with the majority of these located in Queensland and New South Wales. Of approximately 900,000 cattle on feed in September, 2014, 31% were in NSW and 60% were in Queensland, reflecting the distribution of feedlot operations in Australia (ALFA, 2014). Of the cattle on feed, 57% were in feedlots with a capacity greater than 10,000 and 37% were in feedlots with a capacity between 1,000 and 10,000.

BRD is the most important cause of morbidity and mortality in Australian feedlot cattle with an estimated annual cost to the feedlot industry of around $40million (Sacket D, 2006). Therefore, a more comprehensive understanding of the risk factors of BRD in Australian feedlots is needed to inform management decisions about how to reduce the impact of this disease.

BRD has been the subject of much research over the last 40 years. The early epidemiological studies were useful in identifying factors for further research. There have been huge advances over this period in our knowledge and understanding of complex mechanisms involved in host-pathogen interactions. Our knowledge of genetic associations, pathogen structure, biochemistry, immunology and molecular and cellular biology has expanded greatly. However, BRD remains an important disease in feedlot populations worldwide. Progress in understanding and controlling this disease is likely to depend on a contextual and broad understanding of population-level factors as well as a detailed understanding of the disease pathogenesis. As a corollary of this, reducing the incidence of BRD in feedlot cattle requires 'a holistic approach addressing genetic, environmental, pathogenic and immunological factors' (Snowder, 2009).

This literature review gives an overview of the main pathogens implicated in the BRD complex, and the biological pathways thought to be involved in the pathogenesis of BRD. Cohort and case-control studies which have investigated risk factors for BRD in feedlot populations will be reviewed in detail to assess the quality of evidence from individual studies, considering both internal and external validity. Comparisons and contrasts between the North American and Australian feedlot industries will be made where indicated. Then, evidence of associations between individual risk factors and BRD will be evaluated along with the evidence derived from other relevant fields of research to relate epidemiological associations to biologically plausible causal pathways. The rationale of using causal diagrams to inform analyses will be reviewed.

1.2 Clinical signs and diagnosis of BRD in feedlot cattle

A range of clinical signs have been associated with respiratory disease in cattle. In international studies, the reported clinical presentation of BRD has included signs such as nasal discharge, ocular discharge, increased respiratory rate, laboured breathing, soft cough, depression, lethargy, lack of rumen fill, slow moving and elevated rectal temperature (Sanderson et al., 2008, Gardner et al., 1999, Duff and Galyean, 2007, Thompson et al., 2006). In an Australian study, BRD diagnosis was based on a

combination of two of a panel of signs which included ‘dyspnoea, nasal and/or oral discharge, lethargy and inappetence’ without being referrable to any other system (Cusack et al., 2007). Even with a consistent panel of clinical signs, considerable variability between feedlot personnel in the level of observation and assessment of animals would be expected due to factors including feedlot management protocol, current workload, training and experience (Thomson, 2005).

In addition, epidemiological studies investigating the risk factors of BRD have used different case definitions, adding to difficulty in comparing results across studies. North American studies have often used ‘treatment for BRD’ as an outcome measure (Sanderson et al., 2008, Babcock et al., 2010), but when measured as treatment cost this fails to distinguish between incidence and duration (Martin et al., 1982). Some North American studies refer to ‘undifferentiated fever’ as another manifestation of BRD (Wildman et al., 2008, Van Donkersgoed et al., 2008, Booker et al., 1999) . Several studies have reported BRD mortality (usually confirmed by pathology) as the outcome of interest (Loneragan et al., 2001, Martin et al., 1982, Ribble et al., 1995d).

A recent literature review and Bayesian analysis of two studies which met the inclusion criteria of allowing cross classification of BRD using both clinical signs and lung lesion scoring in the post weaning phase, reported that diagnosis of BRD based on clinical signs had relatively low sensitivity (61.8%) and specificity (62.8%) when compared with lung lesion scoring after slaughter (White and Renter, 2009a). Of cattle with clinical signs during the feeding period 26% had no lung lesions (White and Renter, 2009a). The first study reviewed involved a population of 202 North American Charolais calves monitored from feedlot entry post weaning to slaughter after 150-151 days (Gardner et al., 1999). The second study population was 2,036 calves from two South African feedlots which were monitored from feedlot entry to slaughter at a mean of 137 days (Thompson et al., 2006). Although limited generalisability was acknowledged, it does illustrate likely limitations in sensitivity and specificity based on clinical signs alone (White and Renter, 2009b). While it is reasonable to expect that a proportion of cattle displaying clinical signs of BRD would recover without developing persistent lung lesions, the relatively low sensitivity suggests that using clinical signs alone could result in an underestimation of the incidence. Meanwhile, low specificity may result in the incidence being overestimated.

1.3 BRD incidence and temporal patterns in feedlot populations

Despite difficulties in comparing studies, it is apparent that the cumulative incidence of BRD in feedlot populations during the time on feed varies markedly among populations, feedlots and pens within a feedlot and over time in the same location. In a large North American study (US central and southern plains study), the cumulative incidence within cohorts varied from 0 to 36% over the 45 day observation period (Cernicchiaro et al., 2012). In another large North American feedlot study, the cumulative incidence of BRD over 12 weeks on feed was estimated at 5.5% (Sanderson et al., 2008), while in a Canadian calf study, 21% of calves were treated for BRD in the first 28 days following feedlot entry (Macartney et al., 2003a). In an Australian study 4.5% of animals across 6 feedlots developed BRD or had fever at entry (Dunn et al., 1993). In studies involving individual Australian feedlots the cumulative incidence of BRD during the time on feed was estimated at 5.8% (Appleby, 1995) in one study while in another study investigating prophylactic antibiotic administration at feedlot entry, 19% of the 209 control animals (un-medicated) developed BRD during the 73 day period on feed (Cusack, 2004).

Studies agree that the peak occurrence of BRD is during the first 3-4 weeks on feed (Wilson et al., 1985, Healy et al., 1993, Dunn et al., 1993), but cases may occur sporadically when overall incidence is low. However, it must be recognised that BRD is a heterogeneous complex and that the epidemiological profile of BRD incidence in feedlots can be expected to vary reflecting the immunological status of the animals, level of environmental stress and the pathogens involved. A recent review compared the temporal patterns of BRD occurrence in ten United States commercial feedlots during the first 100 days on feed over the period 2000-2008 (Babcock et al., 2010). The study population comprised 1,226,806 cattle from 7,553 cohorts. Cohorts exhibiting similar temporal patterns defined by the daily percentage of cases relative to the total number of cases within the cohort were grouped together, producing a measure of the temporal pattern of new cases (cumulative incidence summed to 100%) rather than the cumulative incidence of BRD per cohort. Seven temporal patterns were described, differentiated by the timing of onset and rapidity of increase of the cumulative incidence. While most patterns fell within the early time on feed, three of the patterns had a cumulative incidence of less than 60% by day 45 (Babcock et al., 2010).

1.4 Pathogenesis of BRD

1.4.1 Stress

Stress is believed to play a role in increasing the severity and duration of respiratory tract infections in both humans and animals. This is thought to be mediated through a complex interplay between the neuroendocrine and immune systems with both the sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axes playing key roles. Acute stress results in the release of catecholamines and endogenous glucocorticoids which have anti-inflammatory actions. However with chronic stress, persistent activation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system with resultant elevations of cortisol, adrenaline and noradrenaline results in a compensatory down-regulation of receptor expression or function contributing to corticosteroid resistance (Miller et al., 2009). There is some evidence that stress alone can modulate immune function via elevated levels of chemokines and cytokines in dendritic cells and macrophages leading to activation of type-2 helper cells (Haczku and Panettieri, 2010).

Many different chemicals and receptors are involved in the neuroendocrine response and activation of acute phase protein reactions is a normal immunological reaction to stressors (Arthington et al., 2008). Studies have demonstrated differential serological, haematological and biomarker profiles in response to stressors commonly associated with increased risk of BRD such as transportation or weaning. Oxidative stress biomarkers were elevated in transport stressed calves in one study (Chirase et al., 2004), while increases in serum cortisol release, neutrophilia (Buckham Sporer et al., 2007) and changes in acute phase proteins have also been reported (Arthington et al., 2008, Buckham Sporer et al., 2008). Early-weaned calves have been demonstrated to have a lessened response compared to control calves weaned on the day of transport to the feedlot (Arthington et al., 2005). In one experiment, calves subjected to weaning and maternal separation followed by challenge with bovine herpesvirus type 1 (BoHV-1) and bacteria had significantly higher mortality and stress biomarkers than control calves that had been previously weaned (Hodgson et al., 2012). In a further study involving 665 randomly selected heifers blood sampled at entry into a North American feedlot, subsequent clinical signs of BRD were associated with decreased plasma glucose and lactate concentrations (Montgomery et al., 2009).

While differential changes in stress biomarkers suggest that the neuroendocrine and immune systems are modulated by stress, this process is clearly dynamic and complex

and a more complete understanding of molecular mechanisms is required (Aich et al., 2009, Aich et al., 2007). Research in recent years has investigated the possible use of biomarkers for early identification and prediction of BRD (Nikunen et al., 2007, Orro et al., 2011).

1.4.2 Pathogens and immune status

The clinical presentation of the BRD complex does not require the presence of specific pathogens, but can develop with different combinations of viruses and bacteria. Hence, the relative importance of particular pathogens is likely to reflect the dynamic relationship between the presence and virulence of pathogens and both the animal and herd-level immunity. Bovine viral diarrhoea virus (BVDV), BoHV-1, Bovine respiratory syncytial virus (BRSV) and Bovine parainfluenza virus type 3 (BPI3) are four important viruses which have been associated with BRD. BRSV and BPI3 are primary respiratory pathogens while BVDV affects multiple systems (Panciera and Confer, 2010) and BoHV-1 also causes reproductive disease (Fulton et al., 2013). Bovine corona virus (BCoV) may also have an important role in the BRD complex (Hick et al., 2012). Respiratory viruses are generally spread between animals by aerosol over short distances or by contact with respiratory tract secretions. In most settings, the majority of viral infections are thought to be subclinical; for example, an estimated 70-90% of BVDV infections are subclinical (Ridpath, 2010).

Viruses have several mechanisms whereby their survival and dissemination in cattle populations are enhanced. Latent infection occurs when the virus lays dormant within the host for extended periods of time but is able to reactivate under suitable conditions (e.g. stress of transport) so that previously infected animals may excrete virus and act as a source of infection for in-contact animals. BoHV-1 displays latency and may reactivate, while there is some evidence for long term BRSV survival in lymphoid tissue (Ellis, 2009). Animals infected in-utero with BVDV may become persistently infected and excrete large quantities of virus after birth (Ridpath, 2010). When animals are crowded together during transport, saleyard transfer or in feedlot pens, there is ample opportunity for exposure to, and spread of viruses.

Under conditions of stress, viral damage to respiratory mucous membranes, biological synergism and immunosuppression predispose animals to secondary infection by ubiquitous bacterial organisms and can result in the clinical presentation of BRD (Ellis, 2009). Bacterial adhesion is enhanced in virus-infected respiratory mucosal cells and

colonization occurs more readily in surfaces damaged by viral infection (Panciera and Confer, 2010). Changes in the innate and adaptive immune systems include altered alveolar macrophage function, suppression of lymphocyte proliferation, programmed cell death and modification in the release of inflammatory mediators (Panciera and Confer, 2010). Biological synergy refers to specific situations where the presence of one organism promotes or enhances infection with another organism. For example, proinflammatory cytokines released by cells infected with BoHV-1 increase expression of the β-2 integrin molecule (the receptor for the leukotoxin produced by *Mannheimia haemolytica*) on the surface of alveolar macrophages and neutrophils leading to further cell death and inflammation (Ellis, 2009). In cases of fatal BRD, there is often more than one pathogenic species isolated from lungs at necropsy, with patterns of isolation supporting the concept of pathogen synergy (Booker et al., 2008b).

Clues about the potential role of specific viruses in the BRD complex comes from the isolation or detection of the organisms from post mortem samples such as lung lesions from animals dying from pneumonia. In one study, BVDV types 1 and 2, often in combination with BPI3 or BRSV were detected in lung samples (Fulton et al., 2000). A recently published Australian study investigating mortality in live-export cattle found that respiratory disease was the most commonly diagnosed cause of mortality, being responsible for 59% (107/180) of deaths. Of animals with lung necropsy samples, evidence of infection (histology or nucleic acid detection) was demonstrated in 66% (130/195) of animals. Of these, 72% had evidence of bacterial infection, 22% had mixed viral and bacterial infections, 3% had viral infections and no agents were detected in 3% (Moore et al., 2014). BCoV and BVDV were the most commonly detected viruses in about 10% of animals, BoHV-1 and BRSV were detected in less than 5% and BPI3 was not detected (Moore et al., 2014). However, viruses that play an important role in initiating disease may not be present in lesions several weeks after the onset of clinical signs.

1.4.3 Bovine viral diarrhoea virus

Bovine viral diarrhoea virus (BVDV) which belongs to the pestivirus genus within the Flavivirus family is one of the most extensively researched agents involved in the BRD complex. There are two different genotypes (BVDV-1 and BVDV-2) and the single stranded RNA genome is subject to point mutation resulting in heterogeneity between subtypes in genotype, biotype and virulence (Ridpath, 2010). There are two biotypes defined by their behaviour in cell culture (cytopathic and non-cytopathic), both of which can cause acute infection. The non-cytopathic biotype is more common in nature and infection

of bovine foetuses with this biotype between 28 and 125 days' gestation may result in persistently infected (PI) animals (Ridpath, 2010). How infection manifests in PI animals ranges from congenital abnormality to calves of clinically normal appearance, but which are more prone to developing BRD or other chronic illness or fatal mucosal disease. PI animals persistently shed the virus, particularly at times of stress and may be a major source of infection and maintenance of BVDV in cattle populations (Ridpath, 2010). As such, the identification and removal of PI animals from cattle populations has been advocated as an important BRD control strategy.

Numerous BVDV-1 strains are often present in the population (Bachofen et al., 2008). While seroprevalence studies have shown that BVDV is distributed worldwide, subtypes may vary in different regions. Only BVDV-1 has been identified in Australia, with the majority of isolates classified as BVDV-1c; this remains genetically distinct from subtypes identified in North America and Europe (Mahony et al., 2005, Ridpath et al., 2010).

Acute respiratory tract infection with BVDV in normal cattle (non PI animals) is an important factor contributing to BRD morbidity and mortality in feedlot populations. BVDV spread is via aerosol over short distances or direct contact with infected animals. Infection of nasal mucosa is followed by lymphatic spread. The incubation period is usually 5-7 days with viraemia usually lasting less than 15 days, but this varies with the virulence of the strain, stress factors and the presence of secondary pathogens. Although damage to respiratory mucosa and lymphoid tissue may contribute to clinical signs, the major ways that BVDV is thought to contribute to BRD is viral immunosuppression, mediated via lymphoid cell death and reduced function, and biological synergism with other agents (Ridpath, 2010). BVDV infection leads to reduced innate immunity by suppression of interferon production, phagocytosis, chemotaxis and microbicidal activity, and reduced acquired immunity by impairment of T-lymphocyte function. Studies have consistently isolated BVDV from lungs of cattle dying of pneumonia (Ridpath, 2010, Fulton et al., 2002)

The detection of serum antibodies to BVDV indicates prior exposure to BVDV in an unvaccinated animal (Lanyon et al., 2014). Rising antibody levels suggest active infection within the previous 10-12 weeks (Lanyon et al., 2014). Acutely or transiently infected (TI) animals should become seropositive within two to three weeks of infection. At the herd level, a high level of seropositivity to BVDV indicates past or current infection, and hence that it is likely that the herd has been exposed to a PI animal, while low seropositivity in a

herd suggests animals have not been exposed, but are susceptible to infection (Lanyon et al., 2014).

Animals persistently infected with BVDV are thought to be a major source of infection in feedlots (Ridpath, 2010). Despite a low prevalence of PI animals, in a feedlot setting large numbers of animals may be exposed to BVDV because a single PI animal excretes large quantities of virus. North American studies have estimated the prevalence of PI animals entering feedlots at between less than 0.1% (Taylor et al., 1995) to 0.55% (comprising 25 of 4,530 calves tested from 5 of 30 herds), (Fulton et al., 2009b). In one study six of 2,000 (0.3%) market-sourced light-weight yearling steers arriving at seven North American feedlots were identified as PI animals (Loneragan et al., 2005). In another study comprising young light weight auction-sourced cattle in 240 pens in a single feedlot 86 of 21,743 animals (0.4%) were PI animals and 74 pens (30.8%) contained a PI animal (Fulton et al., 2006). In a further study, an estimated 62% of pens were exposed to PI animals either in the same pen or in adjacent pens, including 43% of pens that had PI animals in the pen. Mortality in PI animals was about 26% compared to 2.4% in non-PI cattle (Hessman et al., 2009). In earlier studies, PI animals comprised 2.6% of those developing chronic disease and 2.5% of mortalities (Loneragan et al., 2005). Although PI animals tend to be clustered by arrival group (Loneragan et al., 2005) a single PI animal was identified in three of five herds in one study (Hessman et al., 2009).

1.4.3.1 Diagnosis of PIs

Differentiating persistently from transiently infected animals is important for eradication and control programs and in determining the level of challenge faced by in-contact animals in a feedlot environment. Reverse transcription polymerase chain reaction (RT-PCR) techniques enable the detection of viral nucleic acid. Real-time quantitative RT-PCR (qPCR) techniques allow the amount of nucleic acid detected to by quantified; qPCR has excellent sensitivity and specificity for BVDV with Ct values related to the amount of viral RNA present in the processed sample (Bhudevi and Weinstock, 2001). Relatively low levels of virus shed during transient infection may be detected with qPCR (Bhudevi and Weinstock, 2003) so both transient and persistent infection may be detected. The absence of infection in a subsequent sample collected at least 19 days after the first has been suggested to differentiate acute from persistent infection (Meyling et al, 1990). In one study the duration of positive qPCR tests for transiently infected animals was less than two weeks (Nickell et al., 2011) but repeat testing after a minimum of four weeks has been generally recommended (Lanyon et al., 2014). However, a recent study reported that

BVDV infected recovered and immune animals continued to carry the virus in peripheral blood mononuclear cells for 98 days or more and demonstrated experimental transmission by injection of blood into uninfected calves (Collins et al., 2009). qPCR may be used to detect any PI animals contributing to pools of up to 50 animals (Lanyon et al., 2014).

Immunohistochemistry to detect BVDV antigen in ear notch tissue samples has been used in the diagnosis of PI animals in several North American studies (Fulton et al., 2009b, Hessman et al., 2009, Loneragan et al., 2005). Although sensitivity has been reported at 100%, specificity for detecting PI animals may be lower; one study reported positive immunohistochemical staining and antigen ELISA tests on ear-notch samples for up to 90 days from transiently infected animals that tested negative by virus isolation and RT-PCR (Cornish, 2005).

1.4.4 Bovine herpesvirus 1

Bovine herpesvirus 1 (BoHV-1) belongs to the alphaherpesvirus group, which primarily affect the upper respiratory and reproductive tracts. Respiratory spread between animals is by nose-to-nose contact and aerosol over short distances. Infectious virus is shed in nasal secretions for 10–14 days during acute respiratory infection (Gibbs & Rweyemamu 1977). This is followed by invasion of respiratory epithelial cells and entry to the cell nucleus where viral DNA replicates. Cell lysis and cell to cell transfer occurs and the virus also invades nerve cells in the respiratory tract. Latency is a characteristic feature of this virus which can then lead to reactivation and replication in respiratory epithelial cells, resulting in BoHV-1 outbreaks in closed herds (Ellis, 2009). BoHV-1 latency occurs at immuno-privileged sites in the peripheral nervous system, especially in the trigeminal ganglion, following productive viral infection (Rock et al. 1987; Rock et al. 1992; OIE 2000). Latency may also occur in tonsillar lymphoid cells and peripheral blood lymphocytes (Mweene et al. 1996). Cattle that are seronegative for BoHV-1 antibodies may be latently infected with BoHV-1 (Hage et al. 1998). There is no evidence for persistent productive infection with BoHV-1.

Typical respiratory lesions include erosion and ulceration of respiratory mucous membranes, especially the trachea, resulting in the clinical syndrome of infectious bovine rhinotracheitis (IBR). Uncomplicated IBR usually resolves in 7-10 days. However, secondary bacterial infection results in severe lower respiratory tract infection manifesting as clinical BRD. Damage to tracheal epithelium interferes with the normal mucocilliary escalator function allowing bacteria to enter alveoli. In addition, BoHV-1 infection results in

immunosuppression by enhancing apoptosis of CD4+ T lymphocytes, down-regulating interferon production, reducing antigen presentation and clearance of virus infected cells (Ellis, 2009).

Three subtypes of BoHV-1 have been described, BoHV-1a, BoHV-1.2a and BoHV-1.2b based on associated clinical signs and genetic content. Only BoHV-1.2b strains have been isolated in Australia (Snowdon 1964; Studdert 1989; Smith et al. 1993; Young et al. 1994; Smith et al. 1995). These are considered less virulent than the BoHV-1 strains identified in other countries and cannot be transmitted to the foetus of infected pregnant cows (Young et al. 1994).

1.4.5 Bovine respiratory syncytial virus

Bovine respiratory syncytial virus (BRSV) is a paramyxovirus which replicates in cell cytoplasm. Replication is associated with genetic and antigenic variation. Although latency is not a feature of this virus, there is evidence that some animals become long-term carriers, possibly from ongoing virus survival in lymphoid tissue (Ellis, 2009).

Infection is by contact with nasal secretions or short distance aerosol spread. Respiratory epithelial cells, including those in airways and pulmonary parenchyma cells become infected. Cell death is thought to occur by apoptotic mechanisms. A viral protein, virokinin, has a role in smooth muscle constriction and may contribute to bronchoconstriction. Synergism with commensal bacteria is thought to occur by non-specific interruption of the surface epithelium. Speculated roles in interferon inhibition and the excretion of proinflammatory cytokines, especially in younger animals have been proposed (Ellis, 2009).

1.4.6 Parainfluenza virus type 3

Bovine parainfluenza virus type 3 (BPI3) is a single stranded RNA virus of the paramyxoviridae family (Ellis, 2010). The viral envelope contains haemagglutinin neuraminidase glycoprotein, which binds sialic acid in mucous and allows attachment to and penetration of many cell types in the respiratory tract, and homotrimeric fusion glycoprotein which allows fusion of the viral envelope with the host cell membrane. Intracytoplasmic viral replication occurs and results in intracytoplasmic eosinophilc inclusion bodies (Ellis, 2010).

Infection is by contact with nasal mucous or aerosol droplets, so is enhanced in crowded conditions such as in markets, on transport trucks or in feedlots. Most uncomplicated

infections cause mild signs of fever, nasal discharge and cough which resolve within 10 days. Histopathological changes include bronchiolitis, bronchitis and alveolitis with cell death and inflammatory infiltration (Ellis, 2010).

1.4.7 Bovine coronavirus

In addition to the viruses described above, other viruses have been implicated in BRD in feedlot cattle. Bovine coronavirus (BCoV) is an enveloped pleomorphic pneumoenteric RNA virus (Saif, 2010). Seroprevalence studies suggest it is ubiquitous in cattle populations worldwide. Respiratory infection is usually associated with mild clinical signs but may cause pneumonia in calves and has been implicated in some BRD outbreaks in feedlot cattle (Saif, 2010, Fulton et al., 2011). Recent Australian studies have implicated BCoV as an important virus involved in the BRD complex in feedlot (Hick et al., 2012) and live-export (Moore et al., 2014) cattle.

1.4.8 Bacteria involved in the BRD complex

Bacteria commonly isolated from bacterial pneumonia lesions are ubiquitous normal nasopharyngeal commensal organisms (Panciera and Confer, 2010). However, upon infection of the lower respiratory tract they produce virulence factors which promote colonization, cause tissue damage and severe inflammatory responses while evading the immune response. Many of the isolates exhibit resistance to antimicrobial treatment (Panciera and Confer, 2010), but because most cases would have been treated with antibiotics, there is considerable selection pressure for resistant organisms in these animals.

In one study, the relative proportions of the three major bacterial isolates from BRD necropsy samples from beef cattle received at a North American laboratory over an eight year period were examined*. Mannheimia haemolytica* comprised 46% of bacterial isolates, *Pasteurella multocida* comprised 35% of isolates and the remaining 19% were *Histophilus somni* isolates (Welsh 2004).

Mannheimia haemolytica, formerly known as *Pasteurella haemolytica*, was the first BRD-related bacterium described and remains one of the leading pathogens isolated from cases of severe bacterial pneumonia. The organism is commensal, residing in the tonsils and the nasopharynx of clinically normal cattle. It produces many virulence factors such as protein adhesins, enzymes and a ruminant specific leukotoxin, which result in pulmonary inflammation and alveolar and vascular damage leading to acute fibrinous or fibribopurulent pleuropneumonia in the cranioventral lung lobes (Confer, 2009).

In one study, *M. haemolytica* was demonstrated in more than 80% of lungs from cases with peracute to subacute pneumonia, and 40% of cases of chronic pneumonia (Booker et al., 2008b). In another study, fatal pneumonia where *M. haemolytica* was isolated occurred a mean of 19 days after diagnosis, but where it was not isolated, the mean was 33 days (Fulton et al., 2009a).

Pasteurella multocida is a common gram negative commensal bacterium of the nasopharynx. While it typically causes a cranioventral bronchopneumonia, the severity of lesions vary, probably because of frequent concomitant infections with other bacteria and differences in animal factors (e.g. age) and the chronicity of disease (Confer, 2009).

Histophilus somni (formerly *Haemophilus somnus*) is recognised as an important bacterial pathogen in BRD. It is a gram negative pleomorphic rod. Many of the virulence factors are related to membrane proteins. In immune system interactions, IgG2 is most important in protection, whereas IgE binding to the bacterial major outer membrane protein is related to immunopathogenesis. Histamine production by the organism as well as histamine release by mast cells is thought to result in oedema, vasoconstriction and bronchoconstriction in alveoli. Endotoxin or lipooligosaccharide mediates apoptosis of bovine endothelial cells, by activating the complement cascade and platelets, resulting in chemotaxis of inflammatory cells and death of endothelial cells (Nunnery et al., 2007). Both the major outer membrane protein and endotoxin exhibit antigenic variation, which complicates effective vaccine production (Corbeil, 2007). Fibrinous pneumonia is also typical of *H. somni* involvement.

Mycoplasma bovis belongs to the Mollicutes class of bacteria. These are characterised by the lack of a cell wall, which is thought to explain certain properties such as their resistance to β-lactamase antimicrobials and their limited environmental survival because of dependence on host animals to provide adequate nutrients (Caswell et al., 2010). The organism displays antigenic variation in adhesin and variable surface lipoproteins which are important in adhering to host cells and evading antibody binding (Caswell et al., 2010).

Mycoplasma bovis is a commensal organism on mucosal surfaces of the respiratory, intestinal and genital tracts and mammary glands. It is thought that stress, transport or handling increases shedding of *M. bovis* in nasal secretions which in feedlot conditions would result in spread to in-contact animals (Caswell et al., 2010). Although the role of *M. bovis* as a pathogenic organism in BRD is controversial, (Caswell et al., 2010), the organism is more prevalent in the lungs of animals with pneumonia than in those without, especially in chronic pneumonia and in caseo-necrotic bronchopneumonia where it has

been found in 98% of cases (Haines et al., 2001). In a Canadian study investigating BRD mortality, *M. bovis* was present in 50-60% of peracute to subacute pneumonia and in 90% of chronic pneumonia (Booker et al., 2008b). In another study, fatal pneumonia occurred an average of 70 days after diagnosis when *M. bovis* was isolated and 33 days when it was not (Fulton et al., 2009a). *M. bovis* was recently reported to be the most frequently detected and widely distributed pathogen in nasal swabs collected from BRD cases and tissue samples collected at necropsy in Australian feedlot cattle, most commonly in combination with BoHV-1 (Horwood et al., 2014).

1.5 Seroepidemiology of BRD

1.5.1 Seroprevalence and case-control studies

Seroprevalence studies give an indication of the level of exposure in the population and have shown that many of the viruses implicated in BRD are ubiquitous in cattle populations. In unvaccinated populations, seroprevalence generally increases with the age of the animals but the effects of other factors such as herd size are inconsistent (Solís-Calderón et al., 2007, Taylor et al., 2006). A series of serological surveys used two-stage cluster sampling to determine seroprevalences in unvaccinated animals in Mexican beef herds. In one survey of 564 animals from 35 herds, 54% of animals were seropositive to BoHV-1 and 97% of herds had at least one seropositive animal (Solis-Calderon et al., 2003). In another serological survey of 560 animals from 40 Mexican herds, 14% of animals were seropositive to BVDV and 60% of herds had at least one seropositive animal (Solis-Calderon et al., 2005). In a further survey, of 756 animals tested in 54 beef herds, 91% were seropositive to BRSV, and of 728 animals tested from 52 herds 86% were seropositive to BPI3; 100% of herds had at least one animal seropositive to these viruses (Solís-Calderón et al., 2007).

Reported levels of seropositivity to BoHV-1 in Australian cattle populations ranged from 15% to 96% (Smith et al. 1995). In an Australian serological survey of 617 mature aged cattle from 10 cattle farms in northern South Australia, all farms had a moderate to high percentage of cattle seropositive to BoHV-1 (30-78%), BPI3 (26–72%) and BVDV (9-97%) (Durham and Paine, 1997). A serological survey of breeding females from 250 beef and dairy herds in Queensland conducted during 1994-95 found that 11% of herds were seronegative to BVDV and in a further 38% of herds antibodies were detected in cows but not heifers. The seroprevalence of BVDV increased with age (Taylor et al., 2006). In an Australian study seroprevalences at feedlot entry for 500 sentinel cattle in 24 feedlot pens

in 6 feedlots over 18 months were 68% for BVDV, 13% for BoHV-1, 57% for BPI3 and 27% for BRSV (Dunn et al., 1993).

Many studies have used case-control designs to investigate the seroepidemiology of specific viruses in feedlot cattle. Although case-control study designs may not allow accurate estimates of population-level seroprevalences, the estimated seroprevalences in animals entering feedlots have been reported between 20% and 40%, for BVDV and less than 15% for BoHV-1, (Blackall et al., 2001, Dunn et al., 1993, Fulton et al., 2000, Fulton et al., 2002, Martin et al., 1989, Martin et al., 1990, Martin and Bohac, 1986, Martin et al., 1999). Seroprevalences to BRSV ranged from 4-62% and seroprevalences to BPI3 ranged from 11-87% at feedlot entry in North American studies (Martin et al., 1989, Martin et al., 1990, Martin and Bohac, 1986, Martin et al., 1999, Martin et al., 1998b, O'Connor et al., 2001, Fulton et al., 2002). In recent years, widespread vaccination against respiratory pathogens has been promoted in several countries and eradication programs against BVDV and BoHV-1 have been implemented in some European countries. Hence, seropositivity at feedlot entry in these populations may be due to prior vaccination or natural exposure.

1.5.2 Associations between serostatus and change in serostatus and risk of BRD

Serostatus at feedlot entry has been associated with risk of BRD. Although seropositivity to a particular virus indicates prior exposure to that virus and is often used as a proxy measure for immunity, being seropositive to a particular virus does not necessarily equate to immunity against all strains of that virus. This may partly explain inconsistent results in reported associations between seropositiivty at feedlot entry and risk of BRD. In addition, in stressed immunological suppressed cattle, the serological response may not translate to an effective defence against disease.

Case-control studies utilising paired serology (two or more samples over time from the same animal) have had an important role in making inferences about the role of viruses. Several research groups have investigated serological risk factors for BRD. They have reported results from a varying number of animals, often at a single or small number of feedlots and often with repeated studies of similar design over consecutive years. Most of these groups have simultaneously investigated associations between serological risk factors (for a number of viruses and bacteria) and BRD occurrence. Several studies have demonstrated that seroconversion during time on feed is associated with BRD (Healy et

al., 1993, Dunn et al., 1993, Martin and Bohac, 1986, Martin et al., 1990, Martin et al., 1998a). However, in some studies, odds of seroconversion to particular agents did not vary significantly between BRD cases and controls. Some of these studies have lacked sufficient power to detect effects. For example, a study of 59 cases and 60 controls from the same group of animals in a Canadian feedlot found little variation in odds of seroconversion to BVDV and BRSV between BRD cases and controls (Allen et al., 1992). Studies in single populations have limited ability to assess the effects of viruses that may not be circulating in the population at the time of the study or in populations with high herd-level immunity to particular viruses.

Increasing serological measures (seroincrease) between sampling times is used as a proxy for exposure. ‘Seroconversion’ has been used to describe a substantial increase in titre in some studies, but has been restricted to seroincrease in animals initially seronegative in others. Increasing serological titres during time on feed, and seroconversion in particular have been associated with BRD incidence. A discussion of serostatus and its relationship with BRD occurrence for specific agents follows.

1.5.2.1 BVDV

In feedlot populations, seroincrease to BVDV during time on feed is common, although most animals seroconvert without showing clinical signs of disease (Booker et al., 1999). Based on seroincrease, the cumulative incidence of infection with BVDV in North American studies varied between 22 and 68% (Martin et al., 1989, Martin et al., 1990, Martin and Bohac, 1986, Martin et al., 1999, Martin et al., 1998b, O'Connor et al., 2001). In an Australian study the cumulative incidence of infection with BVDV during the first six weeks on feed was 68% (Dunn et al., 1993).

In North American studies, higher arrival BVDV antibodies have been associated with decreased risk of BRD in several studies (Booker et al., 1999, Martin et al., 1989). Seroconversion to BVDV has been associated with increased risk of BRD at both the animal-level and the group-level (Martin et al., 1990, Martin and Bohac, 1986, O'Connor et al., 2001). In an Australian study, seroconversion during the first six weeks on feed was associated with BRD risk (Dunn et al., 1993).

Further North American studies have consistently reported isolating BVDV from BRD case samples and demonstrated associations between both BVDV serology and virus detection and incidence of BRD (Fulton et al., 2000, Fulton et al., 2002).

1.5.2.2 BoHV-1

The incidence risk for infection with BoHV-1, based on seroincrease between sampling times varied from 2 to 6% in North American studies. Low entry titres to BoHV-1 were associated with increased risk of BRD at the animal level in one study (Martin et al., 1989, Martin and Bohac, 1986) but this was not evident at the group level (Martin et al., 1990), nor where the risk of active infection was low (Martin et al., 1999). In an Australian feedlot population, seroprevalence at entry was 13%, 30% of susceptible animals seroconverted by 6 weeks and seroconversion was associated with increased risk of BRD (RR 1.97; 95% CI 1.23- 3.18) (Dunn et al., 1993). Interestingly, in this study further sampling prior to slaughter at approximately 70 days revealed that 59% of those that were seronegative after 6 weeks on feed had subsequently seroconverted.

1.5.2.3 BRSV

The cumulative incidence of infection with BRSV (seroincrease) varied between 8 and 86% in North American studies (Martin et al., 1989, Martin et al., 1990, Martin and Bohac, 1986, Martin et al., 1999, Martin et al., 1998b, O'Connor et al., 2001). Associations between low initial BRSV titres and BRD risk or between seroincrease to BRSV and BRD risk have been inconsistent (Allen et al., 1992, Martin et al., 1989, Martin et al., 1990, Martin and Bohac, 1986). In an Australian study, 27% of animals were seropositive at entry and 57% of susceptible animals seroconverted by 6 weeks on feed but seroconversion was not associated with BRD risk (Dunn et al., 1993).

1.5.2.4 BPI3

The cumulative incidence of infection with BPI3 (defined as a seroincrease between sampling times) varied between 24 and 72% in North American studies (Martin et al., 1989, Martin et al., 1990, Martin and Bohac, 1986, Martin et al., 1999, Martin et al., 1998b, O'Connor et al., 2001). Although some studies reported low seroprevalence at entry and seroconversion to BPI3 were associated with increased risk of BRD (Martin et al., 1989, Martin and Bohac, 1986), this has been inconsistent (Allen et al., 1992, Martin et al., 1999). In an Australian study, 57% of animals were seropositive at induction and 48% of susceptible animals seroconverted by 6 weeks on feed, but seroconversion was not associated with increased risk of BRD (Dunn et al., 1993). BPI3 has been isolated from BRD cases in combination with other agents (Fulton et al., 2000).

1.5.3 Limitations of serological studies

In interpreting results from case-control it is important to keep in mind some limitations. BRD is a heterogeneous disease. Findings from observational studies are likely to depend on numerous factors and are subject to selection bias and complicated because of the effects of unmeasured or unknown confounders and the dynamic relationships between immune status and viral challenge at several hierarchical levels (e.g. animal, herd, cohort, feedlot). Small studies often lack sufficient power to detect effects and studies constrained to a limited population (e.g. single feedlot, single time period) are more likely to lack external validity. In settings where vaccination against respiratory disease is practiced at arrival and vaccination causes seroincrease, it is impossible to differentiate seroincrease due to vaccination from seroincrease due to natural exposure and hence, establish if vaccination is effective in producing immunity. In unvaccinated animals, studies usually assume that increasing serological titres over several weeks for animals on feed under feedlot conditions provide evidence of recent exposure and active infection with the virus. If seroincrease or seroconversion has occurred significantly more frequently in cases than controls, then the effect of that virus in that population can be estimated (e.g. using odds ratios). However because paired serum samples provide a snapshot at only two points in time, they give a limited view of changes in antibody levels over time. Depending on the timing of exposure relative to sampling, a high initial titre may reflect current infection rather than immunity, and these animals may be more likely to succumb to secondary bacterial infection than animals that have protective antibodies from exposure many weeks or months previously. Animals in which serological titres are unchanged between the two sampling points could include animals with very different exposure histories; those that had protective antibodies at arrival and were not challenged as well as those that were exhibiting increasing titres due to recent exposure at first sampling and falling titres at second sampling. Some studies have collected and analysed samples collected at more frequent intervals, but these are generally from small populations and may lack power.

1.6 BRD risk factors

An overview of studies investigating multiple risk factors is presented below, followed by an evaluation of the evidence linking putative risk factors to BRD. Studies which focused on investigating particular risk factors or that employed design features (e.g. matching) that limit the interpretation of other relationships will be reported in the detailed risk factor section of the review.

Some of the terminology used in earler studies has been modified to make it consistent across studies or comparable with that used in this thesis. Thus, ‘pen’, ‘cohort’ or ‘lot’ were designated ‘cohort’ (a collection of animals in a feedlot pen); this was often the unit a analysis. North American studies often referred to ‘auction markets’; these ‘markets’ are the equivlaent of ‘saleyards’ described in the Australian context throughout the thesis. ‘Yard weaning’ is a practice whereby calves are held in pens (yards) for several days or weeks after weaning and provided with supplementary feed (e.g. conserved forage or grain). This contrasts with ‘paddock’ weaning whereby calves are immediately placed on pasture after separation from their dams.

1.6.1 Cohort studies investigating the roles of multiple risk factors for BRD in North American feedlot populations

Most studies investigating the epidemiology of BRD in feedlot populations have been conducted in North America. The Bruce County study was conducted over a period of three years from 1978-1980 in Ontario, Canada and investigated risk factors for all-cause treatment costs and percentage all-cause mortality diagnosed by post mortem examination in the first 6-8 weeks after arrival at the feedlot in 52,889 feeder calves (mostly sourced from western Canada and transported to Ontario by truck or train) comprising 116 groups, arriving at 63 feedlots (Martin et al., 1982). The final group-level analyses included groups of more than 35 calves and feedlots that contributed data to at least 2 years of the study. The explanatory variables examined were divided into management-objective, demographic (e.g. mixing within 3 weeks of arrival, number in group, average weight, source of origin, breed and sex), processing (e.g. vaccination, castration, dehorning) and ration (e.g. ration components and composition) variables (Martin et al., 1982). Details were also collected relating to housing and pen characteristics. The main findings were that larger or commingled groups from different sources, groups that received respiratory vaccines at processing or groups started on a ration with a high percentage of corn silage were at increased risk of all-cause mortality and had higher treatment costs. This early study was important in identifying priority areas for further research. However, BRD morbidity was not differentiated from all-cause morbidity and it was not possible to disentangle the effects of multiple risk factors (e.g. commingling and rations fed) occurring simultaneously.

A retrospective record review of 229 animals in a western Canadian feedlot reported associations between market origin and starting on a high grain ration and BRD (Wilson et al., 1985). However, this small study had limited power and low external validity and was

unable to adequately adjust for confounding (e.g. the number of groups mixed) and separate the effects of these factors.

Alexander et al (1989) studied 17,696 cattle in 95 lots entering a western USA feedlot over an 8 month period and investigated the number of days taken for a cohort to fill, sex, number of groups in a cohort, average environmental temperature change during the first 14 days and pregnancy checking of heifers as risk factors. Cohorts that filled over a number of days with cattle from different sources were at increased risk. Male cohorts were at increased risk and pregnancy checking heifers resulted in increased risk of BRD.

The Canadian fatal fibrinous pneumonia studies were a series of retrospective cohort studies investigating risk factors for fatal fibrinous pneumonia in spring-born calves purchased from 42 Canadian auction markets between September and December, over four years from 1985 to 1988 and transported to a single feedlot (Ribble et al., 1995d, Ribble et al., 1994). Analytic subsets comprised from 32,645 to 58,885 animals. Animal-level risk for fatal fibrinous pneumonia varied between years but a consistent pattern was observed within seasons. In years when there was a higher incidence of fatal fibrinous pneumonia, risk was clustered within truckloads, and increased with a high level of mixing. No associations were observed between distance travelled, weather conditions or shrink (body weight loss during transport) and fatal fibrinous pneumonia (Ribble et al., 1995d).

The Meat Animal Research Centre studies in USA used a retrospective cohort design and reported investigations into BRD incidence in a closed population in research facility feedlots over 15 years from 1987 to 2001 (Mugglicockett et al., 1992, Snowder et al., 2006). Animal-level analyses revealed that BRD risk was associated with birth year, sex, age of the dam and Hereford breed.

An Iowa study included animals entering 18 feedlots between 2002 and 2006 (Reinhardt et al., 2009). Respiratory morbidity and lung lesion score were two of several animal-level outcomes examined. There was no consistent association between disposition score (a measure of temperament) and respiratory morbidity or lung lesions. Low entry weight and continental breeds (compared to > 50% Angus breed) were associated with increased risk of respiratory disease treatment.

Sanderson (2008) reported a prospective cohort study in which 122 pens of cattle (20,136 animals) from 102 feedlots (selected from a stratified random sample of United States

feedlots) were followed for 12 weeks post feedlot arrival. Mixed sex groups, multiple source groups and increased transport distances were associated with increased risk of BRD at the pen level. Other putative risk factors examined, but which did not remain significant in the final multivariable model in this study included the year and quarter of arrival, number of animals in the pen, pen density, metaphylaxis, preconditioning, parasite treatment, and a variety of respiratory vaccines (administered at arrival with or without follow up vaccination). The lack of a standardised case definition was a limitation of this study, as it is in many observational studies. In addition, the study did not have sufficient data to adequately evaluate prior management practices that may have been a source of confounding.

A series of recent retrospective cohort studies have used convenience samples of existing data to investigate associations between cohort-level risk factors and BRD incidence in feedlots located in the central and southern high plains regions of the United States (Cernicchiaro et al., 2012, Cernicchiaro et al., 2012a, Cernicchiaro et al., 2012b). The BRD case definition used in these studies was based on an initial BRD diagnosis together with the administration of an antimicrobial agent. For studies investigating the effects of distance transported and weight loss during transport the outcome was the cumulative incidence of BRD over the entire feeding period. One study used data from 14,601 cohorts entering 21 US commercial feedlots between 1997 and 2009. Only single sex cohorts of more than 20 animals with a mean body weight of more than 227 kg and with data on the source of origin were included. Distance travelled, region of origin, mean body weight, cohort sex, season, cohort size and arrival year were found to be significantly associated with BRD cumulative incidence. Significant interactions were detected between distance travelled and each of the covariates describing source region, mean arrival body weight, cohort sex and season (Cernicchiaro et al., 2012a). Another study using data from 16,590 cohorts entering 13 feedlots between 2000 and 2008 found that weight loss during transport was associated with increased BRD morbidity and the effect was modified by cohort sex, season and mean cohort body weight (Cernicchiaro et al., 2012b). In a further study, 1,904 cohorts (restricted to single sex cohorts with a mean weight range from 227 to 363kg) entering nine US commercial feedlots with daily BRD incidence records between September and November in 2005-2007 were included. The outcome of interest was daily BRD incidence over the first 45 days on feed and weather variables comprised the main exposures of interest (Cernicchiaro et al., 2012). Cohort-level covariates considered included cohort size, number of days on feed, mean arrival body weight, month and year

of arrival and a respiratory risk score (as assessed by feedlot staff at entry and based on factors such as mean body weight, source and transport time). In the final multivariable model, cohort sex, mean arrival body weight category, year and month of arrival, BRD risk score and cohort size were all significantly associated with daily BRD incidence (Cernicchiaro et al., 2012).

A further retrospective cohort study conducted by this group of researchers included cohort-level data for 54,416 cohorts from 16 US feedlots in four states (Babcock et al., 2013a). In this study the outcome of interest was combined all-cause mortality and culling risk. These were associated with mean cohort weight, cohort sex, and arrival month. In utilising large databases, these studies have sufficient power to detect effects of higher-level risk factors. However, because of the very high power of these studies, they may have detected effects that were statistically 'significant' but of minor importance; this is a particular concern when multiple interactions are assessed. In addition, they were not able to assess animal-level data.

1.6.2 Australian studies

One Australian study investigated risk factors for BRD in sentinel cohorts of cattle across six feedlots in three eastern Australian states (Dunn et al., 1993). The study enrolled 5,306 animals comprising one cohort per feedlot per season over 18 months. The age at entry ranged from 12 to 27 months and the weight ranged from 146 to 469kg. Animals were mostly steers and mostly British breeds and cohort size ranged from 100 to 389 animals. A subset of this population was used to investigate the seroepidemiology of BRD as described above.

In an Australian experimental study, the effects of weaning management and prior vaccination were investigated (Fell et al., 2002). Three weaning treatments were compared in groups of steer calves entering a large commercial New South Wales feedlot during autumn in three consecutive years from 1993 to 1995. Animals matched on weight, breed and source were weaned at 7-9 months of age according to their designated method; yard weaning for 10 days with hay or silage, yard weaning for 10 days with hay or silage plus handling and bunk training or paddock weaning without supplementation or handling. They were then grazed on pasture for a period of 6 to 9 months before feedlot entry. Experimental vaccines against the major BRD pathogens were given to half of each group 1-2 months before feedlot entry. Disease status, weight gain and behaviour were monitored prior to feedlot entry and during the time on feed (about 90 days). Both yard

weaned groups experienced consistently lower morbidity compared to the paddock-weaned controls (Fell et al., 2002).

Two studies investigated risk factors in single feedlots. A retrospective study was conducted on a population of 33,321 cattle entering a single New South Wales (NSW) feedlot over an 11 month period in 1994 (Appleby 1995). Animal-level analyses revealed that male cattle, and lighter weight animals were at increased risk and Herefords were at markedly increased risk of BRD. Backgrounding (defined as resting on pasture) prior to induction was protective. Risk also varied with season of induction (increased risk in autumn) and region of origin in this study.

In a prospective cohort study including 2,468 saleyard-sourced cattle (weighing approximately 340kg) entering 13 pens in a New South Wales feedlot during a single season (winter, 2004), the occurrence of BRD during the time on feed (over approximately 70 days) was significantly associated with breed, and a moderate correlation between minimum daily temperature and daily BRD incidence was reported. BRD mortality was more common in male cattle (Cusack et al., 2007).

1.7 Putative risk factors

1.7.1 Animal entry characteristics

1.7.1.1 Genetics and breed

The heritability of resistance to BRD, investigated in the Meat Animal Research Centre population has been estimated in the range 0.04 to 0.08 (Snowder et al., 2006), with higher estimates in populations with higher BRD incidence. When converted to a continuous scale, the estimated heritability was a modest 0.18 where overall BRD incidence was 17%. Studies in this population have shown that Herefords and Pinzgauer were at increased risk of BRD in the feedlot and there was no apparent advantage in heterozygosity. In a bull testing facility population, Herefords and Angus breeds were at increased risk of BRD compared to European breeds (Hägglund et al., 2007).

In an Australian study, survival analysis indicated that Herefords (hazard ratio 10.3), Murray Greys (hazard ratio 6.4) and Angus (hazard ratio 4.9) were at significantly increased risk of BRD compared to the reference category which comprised mainly European or European cross breeds (Cusack et al., 2007). In another Australian study, Herefords and British breeds were at increased risk compared to *Bos indicus* cattle (Appleby, 1995)

Plausible biological pathways linking breed to increased BRD risk may involve genetic variation resulting in differential immune responses, both to natural infection and to vaccine challenge. Recent studies have found serum immunoglobulin responses to vaccination with a modified live BRSV vaccine had a high heritability (Glass et al., 2011).

1.7.1.2 Sex

Male calves have been reported to be at increased risk of BRD compared to female calves in several North American studies, but in some studies this may have been confounded by additional stress associated with castration of bull calves soon after arrival. Although intact bull calves were at increased risk of BRD compared to steer calves in a randomised block trial, castration status at arrival could have been confounded by many other factors in market-sourced cattle (Richeson et al., 2013). Steers have been shown to be at increased risk of BRD compared to heifers in several studies (Alexander et al., 1989, Appleby, 1995, Snowder et al., 2006).In cohort-level analyses, mixed sex cohorts were at increased risk of BRD in one study (Sanderson et al., 2008) while other studies indicated male cohorts were at higher risk than female cohorts, but no mixed sex cohorts were included (Cernicchiaro et al., 2012a, Cernicchiaro et al., 2012, Cernicchiaro et al., 2012b).

Steers were at increased risk of all-cause mortality in an Australian study (Cusack et al., 2007) in contrast to a large American study in which heifers were at increased risk of mortality (Loneragan et al., 2001).

1.7.1.3 Age and weight at feedlot entry

Because induction weight may be associated with several potential confounders, in determining the effect of weight, it is important to consider whether risk estimates appropriately control for confounding. Within breed, age and weight are correlated. Also, heavier older animals are more likely to have been commingled many months prior to feedlot entry than calves sent to a feedlot soon after weaning, so confounding due to prior mixing history should be considered. Weight may also be confounded by the past nutritional plane and health status. Animals in poor condition at feedlot entry may be more at risk of developing BRD due to factors contributing to the low induction weight, rather than lower weight itself. Thus, ideally weight should be considered alongside age, condition score, breed and sex. If potential confounders are unknown or unmeasured, then effect estimates may be biased.

In North America, cattle typically enter the feedlot at a younger age and lighter weight, often at or soon after weaning. For example, in the Meat Animal Research Centre study,

the average age at entry was 5.8 months at an average weight of around 200kg (Snowder et al., 2006). In Australia, most cattle are weaned onto pasture and typically enter the feedlot for finishing at an average age of about 18 months (Dunn et al., 1993). Dentition may be regarded as a proxy for age in older animals. However, permanent incisors erupt around 2 years of age, so this measure lacks the ability to more finely differentiate between cattle without permanent incisors in the 6-18 month age group. For young cattle, weight at feedlot entry would be expected to be correlated with age.

In a bull testing facility, younger age at arrival was associated with fever (used as a proxy for BRD) (Townsend et al., 1989). Dentition was not associated with BRD in an Australian study (Dunn et al., 1993).

Lower animal-level induction weight has been linked to increased BRD risk in several studies (Appleby, 1995, Reinhardt et al., 2009, Sanderson et al., 2008), particularly those that include a relatively broad weight range. Sanderson et al. (2008) reported that higher entry weight was significantly associated with reduced BRD incidence in one study comparing cattle < 250kg to cattle > 318 kg (IRR= 0.18, $p<0.0001$); 72% of cattle were yearling (12-18mths old) while 28% were calves (6-12 months old) at entry.

In cohort-level analyses, the mean cohort weight was significantly associated with BRD in some populations (Cernicchiaro et al., 2012a, Cernicchiaro et al., 2012, Cernicchiaro et al., 2012b), but these estimates were not adjusted for animal-level weight.

1.7.1.4 Temperament

Several studies have investigated the effects of temperament or disposition on BRD risk. Average daily gain and economic advantage were highest in docile cattle, but morbidity due to BRD was also highest in cattle with docile temperaments while mortality was highest in aggressive cattle in one study (Busby, 2006). It has been noted that nervous or flighty animals may be more likely to mask signs when being observed. Thus, aggressive cattle with less severe signs may be less likely to be diagnosed with BRD than calm cattle. However, in a further study, there was no consistent association between disposition score and respiratory morbidity or lung lesions (Reinhardt et al., 2009).

An Australian experiment of factorial design investigated temperament score and weaning method. Twelve animals with the worst temperament score (based on flight time and crush behaviour assessment) from a group of 50 that were paddock weaned with minimal handling and no supplementary feeding were compared with matched controls which

consisted of 12 animals with the best temperament scores from a group of 100 that were yard weaned and hand fed for 10 days. Cattle entered a feedlot six months after weaning. Blood samples were collected at the start and end of weaning, and at days 1, 5 and 85 after feedlot entry. The nervous group had significantly higher cortisol levels at all stages, lower average daily gain, and higher morbidity compared to the calm group, but the relationship is confounded by different weaning management exposures (Fell et al., 1999)

1.7.2 Management of cattle prior to feedlot entry

1.7.2.1 Weaning management, preconditioning and backgrounding

Prior management history of cattle entering feedlots has long been considered important in determining susceptibility to BRD and hence BRD incidence at the feedlot (Schipper et al., 1989, Wieringa et al., 1976). To reduce the level of concurrent stress associated with the transition from cow-calf herds to the feedlot, 'preconditioning' programs were introduced. This term, coined by the North American industry, refers to presale management practices but the components may vary. Recommended procedures include weaning, castration and dehorning (if required) at least one month before feedlot entry with the introduction of roughage and concentrates, bunk training, parasite control and vaccination (Schipper et al., 1989, Woods et al., 1973). Although early studies reported inconsistent findings, a review of preconditioning programs concluded that it was associated with reduced morbidity and mortality after feedlot entry (Schipper et al., 1989). However, it was usually not possible to separate the effects of the different components, and early studies were subject to selection bias and confounding (Taylor et al., 2010b). For example, market-sourced cattle with unknown histories are more likely to have undergone additional stress due to the auction process itself, increased transportation and yarding time and disruption to their social hierarchy as well as exposure to additional pathogens due to mixing of cattle from multiple sources compared to the preconditioned or vaccinated cattle that were from a single source.

More recent studies generally conclude that preconditioning is beneficial, but difficulties remain in comparing studies because of differing definitions of 'preconditioning'. Although study designs have improved compared to earlier studies, the possibility of selection bias and bias due to non-blinding of owners remains in some studies (Taylor et al., 2010b). In one North American study, comparison of a conventional market-sourced group with a group of preconditioned animals and a group of pre-vaccinated animals revealed preconditioned and pre-vaccinated animals had significantly fewer hospital treatments and

lower mortality rates than market-sourced cattle during their time on feed (Roeber et al., 2001).

In a further study, calves sourced from a single auction market during autumn (fall) in 1999 and 2000 were followed during their time on feed using a prospective cohort design. This study investigated 211 groups (minimum of 20 animals per group) comprising 12,313 animals purchased by 112 buyers over 2 years (Macartney et al., 2003b). Groups of calves from special auctions (i.e. certified as previously castrated, dehorned and fully vaccinated against respiratory disease) were differentiated from groups of calves that had been preconditioned (i.e. previously castrated, dehorned and vaccinated but also weaned with the introduction of concentrate rations for at least 30 days prior to transport) and from 'control' groups of calves sold through conventional auctions where the prior management history was unknown. The outcome was initial treatment for BRD during the first 28 days on feed. Control groups were matched with the special auction groups in that the matched groups were assembled within two weeks of each other. Covariates included group size, mean body weight, number of calves treated, number that died, number of source farms, number of days to assemble group, number of days until hay removed, metaphylaxis, sex, and sale type; year, owner and group within owner were fitted as random effects. Vaccinated groups were at reduced risk and preconditioned groups were at markedly reduced risk (OR 0.22) of BRD compared to the control groups in this study (Macartney et al., 2003a).

Another North American study used a randomised 2 x 3 + 1 factorial design to investigate the effects of market origin, prior vaccination, commingling and weaning management on BRD incidence in feedlot cattle. Single-source cattle that were retained on the source property for 45 days after weaning were at reduced risk of BRD compared to market-sourced cattle or cattle from the same single source that were commingled with auction market sourced cattle at feedlot entry (Step et al., 2008). In this study, prior vaccination did not confer additional benefit over and above weaning, but these cattle were not subjected to additional stress associated with the auction market process. In one study, 'preconditioning' was not associated with reduction in BRD morbidity, but the definition used did not specify any required time interval, and relied on retrospective feedlot reports (Sanderson et al., 2008).

In Australia, the majority of cattle enter feedlots at around 6 -18 months after weaning (Walker et al., 2007), so do not have to contend with the stress of weaning at a time

proximal to the stress of feedlot entry. Yard weaning is a practice that involves holding the calves in stock yards or small paddocks, usually for up to two weeks after weaning with the introduction of conserved forage or grain. They become accustomed to these feed types as well as water troughs and possibly feed bunks. This process is also thought to be important in the formation of a social hierarchy under conditions of higher population density and also includes exposure to human contact and handling (Colditz et al., 2006). An Australian study involving two experimental groups of about 200 animals, each with a 3 x 2 factorial design, compared three weaning methods: traditional abrupt separation followed by paddock weaning, yard weaning with hay or silage and yard weaning with hay or silage plus bunk training with the introduction of grain. Half of each group also received experimental BRD vaccines (Walker et al., 2007). Yard weaned animals had significantly better weight gain at the feedlot than paddock weaned animals with the best performance in yard weaned vaccinated animals. Yard weaned animals also had reduced BRD morbidity, but this was not significant at the 95% level in this study (Walker et al., 2007).

'Backgrounding' has been used to describe the assembly and preparation of animals at intermediate farms prior to entering feedlots. In North America, these are referred to as 'stocker' farms and may be used as a step in the supply chain between cow-calf producers and feedlot enterprises (Thomson and White, 2006). In Australia, the term 'backgrounding' has generally been applied to the period of time immediately prior to feedlot entry and refers to the assembly of cattle on pasture at a location close to the feedlot for varying times prior to them being placed on a full ration in a feedlot. In this context, backgrounding is controlled by the feedlot owners rather than intermediate producers. However, it is common practice for animals to be weaned and held on the farm of origin before being on-sold to one or more other farmers before entering Australian feedlots. This process may be more comparable with that practised in North American stocker farms, except that weaning is usually temporally removed from the sale process.

1.7.2.2 Vaccination

In several North American studies, calves previously vaccinated (i.e. before feedlot entry) against BRD pathogens have been shown to be at reduced risk of developing BRD compared to non-vaccinated calves. However, prior vaccinations are often administered as part of preconditioning programs so it can be difficult to differentiate the vaccine effects from other factors such as pre-weaning and bunk training. As discussed above, vaccinated calves purchased through special auctions were at reduced risk compared to non-vaccinated calves purchased through conventional auctions in one study (Macartney

et al., 2003a). However, in another study prior vaccination did not confer additional protection over and above pre-weaning and holding calves on pasture for 45 days before feedlot entry (Step et al., 2008). Similarly, vaccination regimes were not significantly associated with reduced BRD incidence in a large national survey (Sanderson et al., 2008).

Despite widespread use of respiratory disease vaccines in feedlot cattle, studies reporting on viral vaccine efficacy have reported equivocal findings (Larson, 2005, Taylor et al., 2010b). Although antibody levels to some viruses at feedlot entry have been associated with reduced risk of BRD and antibody production occurs in response to vaccines administered under experimental conditions, vaccination at feedlot entry may not necessarily translate into effective immunity for immunocompromised animals (Larson, 2005, Taylor et al., 2010b)

A recent review of the evidence relating to the effectiveness of vaccination against bacterial pathogens in mitigating the effects of BRD concluded that there was potential benefit from vaccination with *M. haemolytica* and *P. multocida* vaccines but there was no evidence of an effect of vaccination with *H. somni* vaccine (Larson and Step, 2012). Studies investigating both prior vaccination and initial vaccination at feedlot entry were included in this review.

While viral vaccines have been available for many years in North America, Australian vaccines against some respiratory pathogens have been developed more recently (Colditz et al., 2006). Bovilis MH™ is an inactivated vaccine against *M. haemolytica* which became commercially available in Australia in 2004. Pestigard™ is an inactivated vaccine registered to reduce reproductive losses due to BVDV (pestivirus). It is also claimed to reduce losses associated with BRD. Vaccination with Bovilis MH™ and Pestigard™ is recommended prior to feedlot entry.

Preconditioning, backgrounding and vaccination programs have been advocated to reduce the incidence of BRD at feedlots. However, many studies have not been able to adequately disentangle the effects of several risk factors that are occurring concurrently in the period of time prior to arrival at the feedlot. Hence, evaluation of interventions is problematic because they involve varying combinations of factors such as the amount of commingling, different market sources, prior vaccination history, introduction of grain-based rations, handling and bunk training. Although variable timing of vaccination

and variation in immune competence at feedlot entry may explain some of the inconsistent findings, there is a lack of randomised controlled studies in commercial feedlot populations to evaluate vaccine efficacy under field conditions, both in North America and in Australia.

1.7.3 Management factors related to processing and the formation of a cohort

1.7.3.1 Vaccination at feedlot entry

The efficacy of vaccination depends on the type of vaccine, the timing of vaccination relative to challenge and the immune competence of the animal. Equivocal results from studies evaluating vaccination are likely to be largely affected by differences in these factors among different populations. As discussed above, some reviews have not distinguished prior vaccination from vaccination at feedlot entry. For most vaccines, prior vaccination is recommended (Taylor et al., 2010b). The effects of using modified live vaccines at processing are equivocal (Richeson et al., 2008).

Rhinogard™ is a modified live BoHV-1 vaccine (developed in Australia) that is delivered intra-nasally. It is registered as a product to improve feed conversion and help protect against IBR but does not claim to be effective in reducing BRD morbidity in feedlot cattle. Rhinogard™ is a thymidine kinase negative mutant of a mildly pathogenic Australian BoHV-1.2b strain (V155) first isolated in 1964 from a case of infectious bovine pustular vaginitis (Snowdon 1964; Brake & Studdert 1985; Smith et al. 1993). Early trials with the V155 strain demonstrated mild transitory signs of clinical disease and an antibody response which persisted at maximal levels from 7 to 21 days after infection (Bagust 1972). Rhinogard™ has been claimed to rapidly induce local immunity and is marketed as an aid to improving weight gain by reducing the impact of BoHV-1 infection. Recombination with or conversion to wild type viruses has not been demonstrated. Other commercial vaccines including combination vaccines against these agents have become available more recently. Rhinogard™ is frequently administered at feedlot entry. Despite widespread use in feedlots, no randomised controlled trials have been conducted under feedlot conditions to evaluate efficacy at reducing BRD morbidity or mortality.

1.7.3.2 Market origin, commingling and group size

Although several North American studies have identified market origin as a significant risk factor for the development of BRD (Gummow and Mapham, 2000, Step et al., 2008, Wilson et al., 1985) the effects of market-sourced cattle could be confounded by several factors such as the number of sources the cattle came from, transport and yarding time

and the number of animals in the group. For example, a study of four groups within a single pen identified that cattle sourced from auction markets and started on a high grain ration were at increased risk compared to the group of mainly farm-assembled cattle that were started on 10% grain with adaptation time (Wilson et al., 1985). Similarly, in identifying the clustering of fatal fibrinous pneumonia by truckload or pen, the researchers did not have sufficient information to determine if this may have been clustered by original source. Nonetheless, an early recommendation of purchasing cattle from the property of origin rather than through auction markets (Woods et al., 1973), is widely promoted. No studies have reported the effects of timing of exposure to auction markets relative to feedlot entry on risk of BRD at the feedlot.

Commingling of cattle from multiple sources has been consistently associated with increased risk of BRD. However, in many studies, the effects of commingling were not able to be disentangled from the effects of other factors such as the number of animals in the group or market origin. In early studies, commingling was often identified as one of a combination of factors associated with BRD risk (Alexander et al., 1989, Martin et al., 1982, Ribble et al., 1994). For example, in the Bruce County study, heterogeneous groups defined by source or timing of arrival, number of cattle per group and mixing of cattle groups after feedlot arrival were significantly associated with BRD mortality (Martin et al., 1982). The definition of ‘source’ was also problematic in this study because cattle sourced from auction markets may have come from many different farms. Hence, larger groups may have been larger due to recent commingling of animals from several source farms. Incidence of BRD was highest in groups of calves from multiple sources compared to fewer sources independent of group size in one study (Ribble et al., 1995c)

Commingling may occur if animals are added to a cohort over a number of days or if multiple groups are combined at entry. A recent study reported that cohorts of cattle comprised of multiple source groups were at increased risk of BRD (IRR: 2.0) after adjusting for several confounders in a multivariable model (Sanderson et al., 2008). Although there was a univariable association between cohort size and BRD, this did not persist in the adjusted model. Although an increased number of animals in the cohort (cohort size) was associated with increased incidence risk of BRD in several recent studies (Cernicchiaro et al., 2012, Cernicchiaro et al., 2013, Cernicchiaro et al., 2012b), this association could have been confounded by increased commingling in larger cohorts, because the number of groups forming the cohort and the length of time those groups had been formed were unknown. In a study investigating market origin, commingling and

weaning management, the group of steers commingled with ranch sourced cattle were at intermediate risk of BRD compared to ranch sourced cattle (lower risk) and auction-market sourced cattle (higher risk) (Step et al., 2008), although it was not clear whether this was due to disease in the ranch sourced or market sourced animals in the commingled group.

Overall, commingling and market origin appear to be important risk factors for BRD. However, studies have not been able to adequately disentangle the effects of these factors and the role of group or cohort size remains unclear. Studies have not investigated the dynamics of group formation, group stability and the amount and timing of mixing prior to feedlot arrival. It is biologically plausible that risk of BRD may vary depending of the timing of these events prior to feedlot entry, so this is an area of research which warrants further investigation.

1.7.3.3 Distance travelled and transport stress

There are some conflicting conclusions in the literature regarding the effect of transportation on BRD incidence in feedlots. Early North American studies returned equivocal results regarding the effect of transport on BRD incidence (Cole et al., 1988). No effect of distance travelled to the feedlot on fatal fibrinous pneumonia was reported in Canadian feedlot calves (Ribble et al., 1995b).

As discussed above, early studies were often unable to separate the effects of transport from other factors such as commingling or weaning. In a four way comparison of preconditioned/ non-preconditioned and long haul transport (15 hours) versus short haul transport (2.7 hours) in cattle sourced from the same property, there was no significant difference in BRD morbidity between groups although a significant interaction was noted between transport time/ preconditioned status and shrinkage (Schwartzkopf-Genswein et al., 2007). However, larger studies suggest a positive association between distance or time transported and BRD morbidity. In a stratified random sample of US feedlots, a positive association between distance transported and BRD morbidity was reported such that there was a 10% increase in pen-level morbidity for each additional 160 km transported (Sanderson et al., 2008). The US central and southern high plains study also reported an association between distance travelled to the feedlot and BRD morbidity and all-cause mortality (Cernicchiaro et al., 2012a). The effect of distance transported was modified by several other factors, including the source region of the cattle, cohort sex, cohort mean arrival body weight and season (Cernicchiaro et al., 2012a). A further study by this group of researchers revealed an association between loss of body weight during

transport and BRD morbidity (Cernicchiaro et al., 2012b). Risk factors for body weight loss during transport included higher ambient temperatures and time on the truck (Gonzalez et al., 2012).

1.7.3.4 Vitamins and mineral supplements

A review of nutritional supplementation with trace minerals and vitamins concluded that there was no evidence that the majority of additional supplementation reduces BRD morbidity, although vitamin E may have a role in reducing BRD morbidity (Duff and Galyean, 2007). However, a more recent review and meta-analysis of studies investigating the administration of injectable vitamins A, D and E at processing or supplementing at levels higher than those recommended by the United States National Research Council guidelines concluded that these practices did not result in reduced morbidity in feedlot cattle (Cusack et al., 2009).

1.7.3.5 Metaphylaxis with antibiotics

In North America use of metaphylactic (i.e. prophylactic) antibiotics on arrival at feedlots has been advocated as an effective protocol to reduced BRD morbidity, mortality and economic loss, especially in high risk populations (Nickell and White, 2010). Similarly, use of tilmicosin at arrival has been shown to significantly reduce BRD morbidity in saleyard sourced high risk Australian feedlot cattle (Cusack, 2004). While metaphylaxis with antibiotics at arrival has been demonstrated to reduce the incidence of BRD in feedlot cattle, there are concerns about the problems of antibiotic resistance and the introduction of antibiotics into the food chain (Rérat et al., 2012, Watts and Sweeney, 2010, Checkley et al., 2010)

1.7.4 Management factors related to the exposures during the time on feed

1.7.4.1 Exposure to PI animals

Studies investigating the association between exposure to PI animals on BRD in populations of feedlot cattle have returned inconsistent results. In one study, in-contact animals (including those in adjacent pens) had a higher incidence of BRD than animals not exposed to PI animals such that 15.9% of BRD treatments could be attributed to PI contact. Cattle in adjacent pens were included as cross-pen social interactions were observed and water troughs were shared between pens. However, when the definition of ‘exposure’ was restricted to animals in the same pen as a PI animal, the increased risk was not significant (Loneragan et al., 2005). O’Connor et al (2005) found that the presence

of a PI animal was not associated with increased disease incidence in commingled groups in a study of 5,041 calves across 50 pens; the lowest incidence was observed in single-sourced groups with a PI animal. This is supported by another study involving 7,123 calves from 25 pens that reported BRD incidence did not differ significantly between pens with and without PI animals (Booker et al., 2008a). Hessman et al (2009) compared five classifications of PI exposure (PI in pen or adjacent pen with or without removal of PI animals within 72 hours, and no PI exposure in pen or adjacent pen) in a feedlot population of 15,348 animals (0.5% PIs). Although animals exposed to PIs for the duration of the study had reduced average daily gains and higher mortality rates compared to unexposed animals, the effects of exposure on BRD incidence were inconsistent. (Hessman et al., 2009).

A study involving animals previously vaccinated against BVDV reported no differences in BRD morbidity between controls (unexposed) and different PI exposure times and proximity groups (Elam et al., 2008). Virulence of BVDV varies between subtypes. In one study, animals in pens with BVDV-1 PIs had higher morbidity but the presence of BVDV-2 PIs in a pen appeared to be protective (Ridpath, 2010). Thus, it appears that the effect of a PI animal within a pen is variable among populations and will likely depend on many factors such as the level of exposure, virulence of the BVDV strain and the herd and animals-level immune statuses of in-contact animals.

Inconsistent results may occur because studies using pens or cohorts as the unit of analysis may lack sufficient power to detect important effects. In addition, it has usually not been possible to assess the effects of natural exposure to PI animals prior to exposure in the feedlot pen. The extent to which prior exposure to animals PI with particular strains of BVDV confers protection in in-contact animals in feedlot settings is unknown.

1.7.4.2 Ration characteristics

Early BRD studies identified a collection of factors that were associated with high BRD morbidity and mortality. Thus, groups exposed to a high level of commingling were also fed a high percentage of corn silage in the starting diet and were found to have increased morbidity and mortality in the Bruce County study (Martin et al., 1982). A further study found that auction-market sourced cattle started on a high percentage of grain in the ration were at markedly increased risk compared to farm-assembled animals started on 10% grain with adjustment time (Wilson et al., 1985). However, these studies were not able to separate the effects of rations from a high level of commingling.

Despite considerable research, consistent associations between ration characteristics and BRD incidence have not been demonstrated (Duff and Galyean, 2007). On balance, a review concluded that higher concentrate diets with increased energy density may increase average daily gain in stressed newly received cattle without substantially impacting BRD incidence (Duff and Galyean, 2007). However, studies have identified marked variation in individual animal feeding behaviour, rumenal pH and microbial ecology responses to the introduction of highly fermentable diets (Schwartzkopf-Genswein, 2005). Because most studies investigating relationships between rations and BRD have used pen-level data, they have been unable to adequately assess the effects of nutritional factors at the animal level. Technology is now available to allow animal-level monitoring of feeding and watering behaviour so future research has the potential to better define the role of diet at the animal level (Schwartzkopf-Genswein, 2005).

1.7.4.3 Housing and pen characteristics

It is widely recognised that although respiratory disease occurs in cattle under grazing conditions, the incidence and severity of BRD is far greater where animals are kept under intensive or housed conditions. In a national survey of a stratified sample of French farms, dairy herds were at significantly increased risk compared to beef herds, which the authors noted may be due to more frequent open range management systems in beef herds (Gay and Barnouin, 2009). In addition to feedlot populations, BRD is often the major cause of morbidity and mortality in pre-weaned dairy calves and weaned heifer calves (Callan and Garry, 2002). Risk factors relating to pens and housing have been more widely researched within these populations, so some recommendations aimed at reducing BRD in feedlot populations have been extrapolated from these populations. Risk factors for BRD in intensive production systems include factors which increase exposure to and density of respiratory pathogens, so interventions aimed at modifying the environment to reduce such exposure have been recommended (Callan and Garry, 2002). Respiratory viruses spread through oral or nasal contact and over short distances by aerosol and have limited survival in the environment. Thus, reducing overcrowding, ensuring optimal ventilation in barns and using physical barriers and separate feed bunks and water troughs have been suggested. Separate individual pens have been advocated for pre-weaned calves and limiting the number of feeder calves in a pen to seven has been associated with reduced BRD incidence (Callan and Garry, 2002). While this may be feasible for housed calves or in small populations of feedlot animals housed in barns, economic feasibility would be expected to preclude the widespread use of such measures in large feedlot operations.

Clearly the type of housing or design of feedlot pens needs to be appropriate for the prevailing weather conditions. Although it is biologically plausible that sharing pen water or feed troughs between pens may contribute to increased incidence of BRD, and shared water troughs were suggested as a possible mechanism whereby exposure of PI animals may affect animals in adjoining pens (Loneragan et al., 2005), I am not aware of studies which have investigated this in feedlot populations.

Heat stress has been identified as a problem for feedlot cattle in some areas where combinations of animals factors (e.g. *Bos taurus* breeds, dark coat colour) and environmental factors (high ambient temperature, high humidity, solar load and low air movement) result in production loss and sometimes increased mortality (Brown-Brandl et al., 2006, Brown-Brandl and Jones, 2011, Mader et al., 2006). Ways to ameliorate these effects in feedlot populations have been the focus of considerable research (Brown-Brandl et al., 2006, Brown-Brandl and Jones, 2011, Davis et al., 2003, Gaughan et al., 2010, Gaughan et al., 2013, Gaughan et al., 2008, Mader et al., 1999, Mader and Davis, 2004, Mader et al., 2006, Sullivan et al., 2011). Recommendations have included providing pen shade, using a water sprinkling system in pens and altering the timing of ration delivery (Davis et al., 2003, Gaughan et al., 2010, Mader and Davis, 2004). Under hot conditions, cattle seek shade, so a limited area of pen shade coverage would be expected to lead to higher animal density in shaded areas. In addition, the provision of pen shade or water sprinklers in feedlot pens might be expected to interact with ambient weather factors to influence the environmental survival and transmission of pathogens involved in the BRD complex. To my knowledge, these relationships have not been investigated.

Although BRD management protocols routinely list environmental features including pen density, bunk space and shade as being important considerations in the management of BRD, few studies have reported on the effects of these factors on BRD incidence in feedlot populations. While higher pen density may contribute to stress, the effects would be expected to vary with other factors such as the stability of the social hierarchy, which in turn depends on how many groups of cattle are commingled in a pen and how long the group has been established. Although it has been suggested that research should be directed at investigating segregation of animals by arrival group and exploring group size independent of pen density (Larson, 2005) little work has been done to evaluate such measures. Pen-level investigations do not adequately capture animal-level differences, so further work needs to be done to establish the effects of pen characteristics at the animal level.

1.7.4.4 Feedlot staffing and number of cattle on feed

The importance of feedlot staffing in health outcomes for feedlot cattle has been noted, with anecdotal evidence from unpublished United States data indicating that the number of feedlot staff (pen riders, processors and veterinarians) per 10,000 cattle explained a large proportion of the variability in death loss across feedlots (Thomson, 2005). In addition, the difficulty in hiring and retaining suitably skilled and motivated staff may be a problem. Feedlot surveys have identified that BRD incidence is higher in larger capacity feedlots (MLA, 2001, Sanderson et al., 2008) but many of the factors driving this association remain unclear.

1.8 Broad environmental risk factors

1.8.1 Year, season and month of arrival

Variation in BRD incidence with the year of entry has been described in several studies (Martin et al., 1988, Snowder et al., 2006, Cernicchiaro et al., 2012, Cernicchiaro et al., 2012a). This association is likely to be complicated by factors such as cycles in herd level immunity, pathogen load, and environmental conditions influencing the plane of nutrition prior to feedlot entry, numbers of cattle on feed in feedlots and extremes in weather conditions. In the closed Meat Animal Research Centre population, the authors suggest the lower incidence in later years may have been due to the increased availability and use of respiratory disease vaccinations (Snowder et al., 2006).

In several North American studies, increased incidence has been observed in the autumn (fall). A Canadian study investigated the timing of feedlot entry and patterns of auction market sales over four years in relation to the outcome of fatal fibrinous pneumonia (Ribble et al., 1995a). A consistent pattern was observed in that calves entering the feedlot in November had a greater risk of mortality than those entering in September or December, with the peak risk occurring 2- 4 weeks after the peak time for the volume of calf sales (Ribble et al., 1995a). However, it has been suggested that this association could be confounded by increased numbers of young/low weight high risk animals entering feedlots at this time of the year. In one study univariable associations between arrival quarter and cohort-level BRD incidence did not persist in multivariable models (Sanderson et al., 2008)

Recent large North American studies have reported increased risk of BRD in the autumn and summer (Cernicchiaro et al., 2012b, Cernicchiaro et al., 2012a). In a large study restricted to animals arriving in the fall, month of arrival was significantly associated with daily BRD incidence at the cohort level (Cernicchiaro et al., 2012). A nationwide stratified

random sample of French cattle herds found that herd-level BRD incidence was highest during winter (Gay and Barnouin, 2009).

There was some evidence of a seasonal association with BRD incidence in an Australian feedlot survey (MLA, 2001). BRD incidence was highest in autumn in one study (Appleby, 1995), but did not vary with season in another study (Dunn et al., 1993). 'Season' is likely to be a proxy for several undefined risk factors and these may vary in different locations; this may contribute to different findings between studies.

1.8.2 Weather

In the US central and southern high plains study, weather variables that were significantly associated with BRD risk included maximum wind speed (3-4 day and 5-7 day lag), mean wind chill temperature (3-4 day, and 5-7 day lag), and temperature change (5-7 day lag) (Cernicchiaro et al., 2012). Several interaction terms between weather variables and between weather variables and demographic variables were also significant. High BRD risk score cattle were at increased risk across categories of weather variables compared with low risk cattle (Cernicchiaro et al., 2012). In an Australian study, a moderate correlation between minimum daily temperature and occurrence of BRD was noted after accounting for serial correlation of temperature between days (Cusack et al., 2007).

Studies have identified differential behaviour amongst different cattle breeds in response to environmental heat stress. In one study, black Angus cattle displayed increased heat stress induced behaviour compared to light skinned cattle (Brown-Brandl et al., 2006). In this study population, signs of heat stress as measured by respiratory rates and panting scores manifested in temperatures about 25^0C. Risk factors for heat stress included breed, higher condition score (increased in fatter animals), excitable temperament and prior treatment for BRD (Brown-Brandl et al., 2006). As discussed above, the role of pen features such as shade area and environmental conditions on animal-level BRD risk warrants further research. A comprehensive environmental stress index has been developed (Mader et al., 2010) and this may be useful in further research into the effects of prevailing weather conditions and pen features on BRD incidence in feedlot cattle.

1.8.3 Source location and feedlot location

Source region has been linked to BRD incidence in several studies (Appleby, 1995, Cernicchiaro et al., 2012a) and was found to be an effect modifier of the association between transport distance and BRD incidence rates in the latter study. However, source

region may be subject to confounding by important covariates such as prior mixing of groups, market origin, transport duration, breed and environmental adaptation.

1.9 Use of causal diagrams to inform modelling

BRD is recognised as a multifactorial disease complex, meaning that there are multiple factors influencing the development of BRD. Models of causation provide useful conceptual frameworks when seeking to better understand complex interrelationships between exposures and outcomes (Dohoo et al., 2009). In the component-cause model, elements can be viewed as necessary, sufficient or component causes (Dohoo et al., 2003, Rothman, 2002). For BRD, no one individual factor is necessary (i.e. BRD can't occur without it) or sufficient (i.e. BRD will occur if the factor is present). Causal complements are the component causes that make up a sufficient cause; for BRD, these comprise a combination of pathogenic organisms, environmental stress and a susceptible immune system. Figure 1-1 presents some putative risk factors for BRD within a general component-cause model. Since many different organisms and other factors are components of BRD causation, it is virtually impossible to design the perfect experimental study to establish causation for specific factors. Indeed, while various pathogens have been shown to induce respiratory tract infection experimentally, clinical BRD has not been reproduced in this way.

Observational epidemiological studies are the most commonly used approaches to understand BRD causation; however a major drawback is that it is not possible to measure all possible component causes. Analyses incorporating random effects take into account unmeasured and unknown confounders at the group level. However, when investigating an outcome as complex as BRD, *a priori* consideration of potential confounders and the attempt to adequately measure these form an important part of study design and analysis. Causal diagrams facilitate an informed approach to model building with postulated and potential relationships defined based on *a priori* knowledge and plausible biological pathways.

Causal diagrams aid in classifying causes as direct or indirect depending on whether there are intervening variables between the exposure and the outcome. In a causal diagram, the direct effect of an exposure is indicated by a single arrow directly linking the exposure and outcome variables. An indirect effect of an exposure is indicated by a pathway through a sequence of arrows passing through one or more intervening variables to the outcome variable. The total effect of an exposure variable is the sum of the direct and all indirect

effects of that exposure on the outcome. (Dohoo et al., 2009). Hence, causal diagrams allow the separate estimation of total and direct effects, both of which may be of interest to researchers and industry stakeholders.

There is much literature discussing the use of causal diagrams to inform data analysis (Chesterton et al., 1989, Dohoo et al., 2009, Greenland et al., 1999, Hewson et al., 2006, Martin and Meek, 1986). In constructing a causal diagram, the researcher needs to put considerable thought into biologically plausible pathways and whether each arrow is placed appropriately (Dohoo et al., 2009). In estimating total effects, one must first identify variables that need to be included, such as potential confounders of the relationship between the exposure of interest and the outcome. For each exposure of interest, any variable postulated as both a) causing the exposure and b) on a separate pathway leading to the outcome is a possible confounder. Care needs to be taken to adjust appropriately for confounders, whilst not introducing bias through uncontrolled conditional associations. When controlling for a factor, variables which cause that factor become conditionally associated, and at least one of those variables also needs to be included to appropriately adjust for this (Dohoo et al., 2009). No intervening variables between exposure and outcome are included when estimating total effects, but these are included when estimating direct effects. Causal diagrams explicitly indicate which variables are 'intervening' for a specific exposure. The set of covariates identified in the causal diagram that are appropriate to include in models to estimate the total or direct effects of an exposure on an outcome are referred to as an 'adjustment set' (Textor et al., 2011). However, to appropriately adjust for confounding it may not be necessary to include all of these covariates to block any potentially confounding pathways. The 'minimal sufficient adjustment set' identifies a set comprising the minimum covariates required for a particular model. For a particular relationship, there may be one or more such sets (Textor et al., 2011). Although several methods have been proposed for selecting variables to include in multivariable models to estimate total or direct effects, all have similar features (Dohoo et al., 2009, Greenland et al., 1999, Shrier and Platt, 2008, Textor and Liskiewicz, 2011). Traditional methodology for identifying confounders and intervening variables involving systematic assessment of paths for each exposure individually becomes unwieldy and subject to error with a large complex dataset. Automation of this process should therefore reduce error. The DAGitty software (Textor et al., 2011) provides a tool that will identify minimal sufficient adjustment sets to assess total and direct effects of the exposure variable of interest on the outcome.

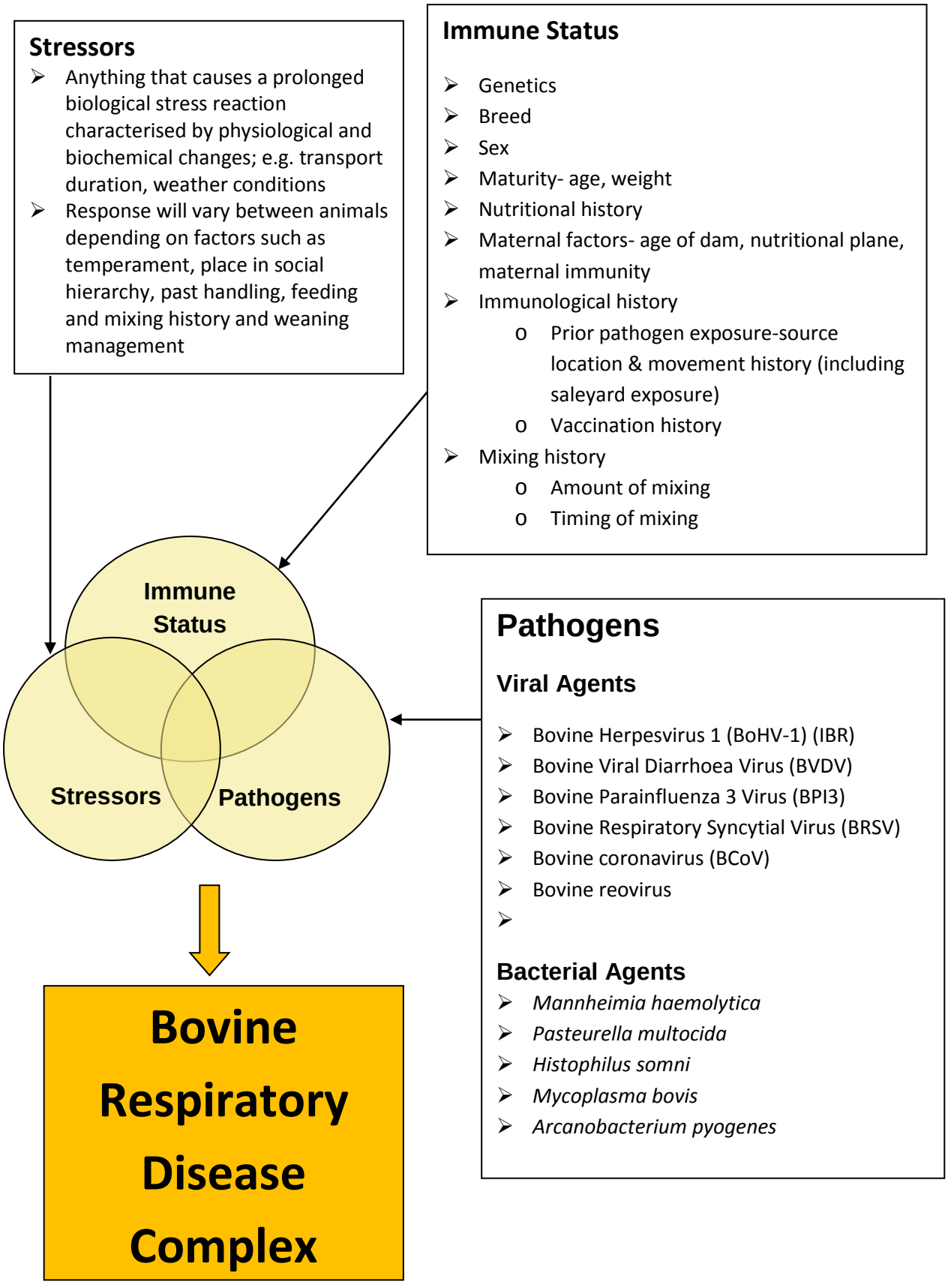

Figure 1-1: Conceptual Framework illustrating interplay of risk factors for BRD

2 Thesis Overview

2.1 Thesis overview

As discussed in Chapter 1, the term ‘bovine respiratory disease’ (BRD) describes a complex of diseases involving the respiratory system in cattle. BRD is the most important clinical disease complex and the major cause of mortality in feedlot cattle, both in Australia and worldwide. BRD has a multifactorial aetiology involving a combination of factors that increase susceptibility of animals, including stressors, and infectious agents. As discussed in Chapter 1, the majority of BRD research has been conducted in North America. However beef cattle management and production systems in Australia differ markedly from those in North America, and the relative importance of different pathogens and risk factors in the Australian feedlot industry might be expected to differ from those in North America. The National Bovine Respiratory Disease Initiative (NBRDI) was a nationwide project which aimed to describe the incidence of BRD in Australian feedlot cattle, quantify associations between BRD occurrence and numerous putative risk factors, determine the population-level impact of relevant risk factors, and make industry recommendations about how to reduce the incidence of BRD in Australian feedlots.

My research consisted of two studies, a prospective longitudinal study and a prospective nested case-control study, conducted within the NBRDI project. In this chapter, I present the conceptual framework, research questions and aims of my thesis within the context of the NBRDI.

In Chapter 3, I provide an overview of the study design and study population before outlining study procedures, data collection, data validation and data management. In Chapter 4, I describe the case definition, importance of clustering in the study population and derivation of exposure variables, and then present assessment of variable quality. I then detail the rationale behind construction of a causal diagram which was used to inform model selection for use in statistical analyses.

In Chapter 5, I provide a description of the occurrence of BRD in the study population. Analyses relating to the cohort study population are detailed in Chapters 6, 7 and 8. The estimation and interpretation of total and direct effects of putative risk factors are described in Chapter 6, while the population-level estimates of these effects are calculated and used to rank risk factors in Chapter 7. The construction and assessment of a parsimonious model used to determine the partitioning of variance in the occurrence of BRD is detailed

in Chapter 8. A comparison of effect estimates across modelling methods and between software packages is discussed in Chapter 9. Chapter 10 details the descriptive epidemiology relating to pathogens detected from biological samples collected during the cohort study. Serological risk factors were assessed in the case-control study; this is described in Chapter 11. The key findings, conclusions and recommendations are presented in Chapter 12 along with a discussion of the strengths and limitations of the studies.

2.2 Hypothesis, conceptual framework and aims

2.2.1 Overall alternative hypothesis

Occurrence of bovine respiratory disease in Australian feedlot cattle is not random. There are proximal causes of BRD, consisting of host immune status and exposure to specific pathogens and stressors. These proximal causes are in turn influenced directly and indirectly by other factors ('risk factors'), which can be measured and quantified.

2.2.2 Overall null hypothesis

Occurrence of bovine respiratory disease in Australian feedlot cattle is random. Any observed associations between risk factors and BRD are due to random (i.e. sampling) variability.

2.2.3 Conceptual framework

Proximal causes of BRD broadly include combinations of stressors, pathogens and immunologically susceptible animals as discussed in the literature review (Chapter 1). It is not possible to measure all of these causes in large numbers of individual animals; nor can feedlot managers directly influence these proximal causes to reduce the incidence of BRD. Risk factors increase the risk of animals being affected by BRD through their effects on these proximal causes. Risk factors can act sequentially, where risk factor 'A' affects (alters frequency of exposure to, or function of) risk factor 'B' which, in turn, affects risk of BRD occurrence through effects on proximal causes. Any particular risk factor can affect multiple other risk factors as well as risk of BRD through its effects on one or more proximal causes.

Causal pathways were postulated based on *a priori* knowledge linking groups of putative risk factors to BRD occurrence; only risk factors for which data collection was planned were included and a concept map was developed as shown in Figure 2-1. Upon receipt and exploration of the study data, specific analysis variables to describe putative risk

factors were derived and a postulated causal diagram developed based on this concept map (Section 4.7). The causal diagram was used to identify covariates to include in models to estimate the total and direct effects of variables of interest; this is described in Chapter 6.

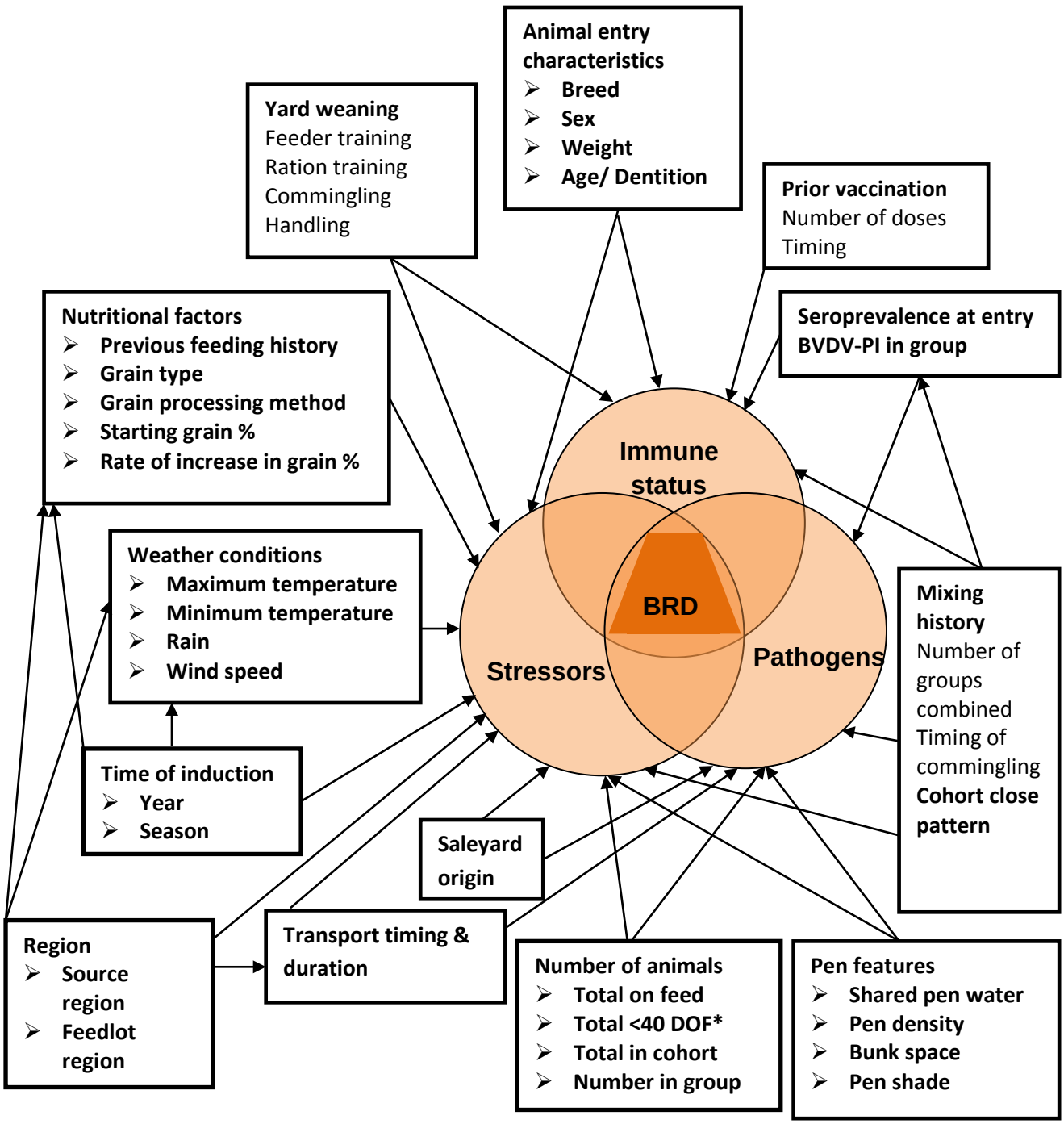

Figure 2-1: Concept map showing proposed links between putative risk factors and proximal BRD causes. Risk factors in bold with bullet points were proposed distinct variables, for which data collection was planned, related to the classification indicated; unbolded risk factors were components of the putative risk factors indicated.
*days on feed

2.2.4 Research questions and aims

My PhD project was conducted within the framework of the NBRDI. The overall objectives of this broader project were to:

1. Identify and quantify the impact of the critical risk factors associated with BRD
2. Determine the role of Bovine viral diarrhoea virus in the occurrence of BRD
3. Determine the role of infectious agents in predisposing animals to developing BRD
4. Assess the impact of current and improved detection methods for BRD on ultimate performance of animals diagnosed with BRD
5. Develop a support tool for feedlot managers and advisors that determines the economic benefits of management practices that reduce BRD incidence
6. Deliver to industry a best-practice manual to minimise the impact of BRD on the feedlot sector

The specific aims of the project addressed in my PhD thesis were as follows:

1. Describe BRD incidence for cohorts of cattle over time and by feedlot.
2. Define typical performance for BRD incidence based on the distribution of observed performance in cohorts in a selected population of Australian feedlots
3. Estimate the proportion of variation in BRD occurrence at animal, group, cohort and feedlot levels
4. Assess the strength of association between ‘known’ and potential risk factors and BRD occurrence
5. Identify priority preventive strategies and areas for further research and extension by estimating population attributable risks and fractions for BRD risk factors (and for groups of risk factors)
6. Estimate the proportion of variation in BRD incidence that is explained by identified risk factors
7. Describe the prevalence of persistently infected carriers (PI animals) of Bovine viral diarrhoea virus in cattle arriving at a selected group of Australian feedlots
8. Assess associations between exposure to PI animals and subsequent occurrence of BRD
9. Describe the seroprevalence of infection with BRD pathogens at induction
10. Assess associations between animal serostatus at induction and seroconversion to four viruses and subsequent BRD incidence

Aims 1 and 2 are addressed in Chapter 5. Chapter 6 addresses aim 4, Chapter 8 addresses aims 3 and 6 and Chapter 7 deals with the 5th aim. The prevalence (aim 7) and

role (aim 8) of PI animals are dealt with in Chapters 6 and 10. Throughout the thesis ‘BVDV’ implies BVDV-1because this is the only genotype that has been identified in Australia. Chapter 11 details the nested case-control study which addresses aims 9 and 10.

3 Project Overview, Data Management and Validation

3.1 Introduction

In this chapter I provide an overview of the NBRDI, including the study design and study population. I then outline study procedures, data collection, data validation and data management. The information detailed in this chapter is relevant to all subsequent chapters.

3.2 Project overview

3.2.1 Background

The NBRDI was a long-term project involving detailed planning, coordination, data validation, advanced statistical analyses and interpretation. Decisions about putative risk factors of interest and data collection methods were informed by literature review and industry consultation. I was not involved in the planning or initial stages of the NBRDI because this had been completed before my work commenced in 2011. The majority of my initial work included data validation and management and the derivation of variables; an overview of this process is provided below and a detailed description is given in Appendix 1 (Chapter 14). This also details procedures for collecting biological samples (blood samples, nasal swabs and necropsy samples)

3.2.2 Animal ethics

Approval for research conducted in Queensland, South Australia and Western Australia was approved by the University of Queensland Animal Ethics Committee (Approval Certificate numbers: SVS/383/07/MLA, SVS/495/08/MLA and SVS/125/10/MLA (NF)). Research in New South Wales was approved by the University of New England Animal Ethics Committee (Approval Certificate number AEC09/027).

3.2.3 Key study terminology

It was necessary to define a number of specific concepts during the project. Typically, groups of animals purchased from pasture-based beef farmers (‘vendors’) were trucked from the beef farms, or from a saleyard, to the feedlot vicinity. An ‘arrival group’ was defined as a group of animals from a single ‘source’ (which may have been a single vendor or multiple vendors if animals were purchased through saleyards). The number of days between the arrival date (i.e. date of arrival at the feedlot location) and the day when

animals were placed ‘on feed‘ (i.e. being fed a full feedlot ration in a feedlot pen) varied. ‘Preassembly’ referred to the practice of assembling cattle on pasture close to the vicinity of the feedlot for a period of time prior to them going on feed in the feedlot.

‘Induction’ referred to routine ‘processing’ procedures and treatments (e.g. identification tagging, weighing and vaccination) and the associated collection of animal-level electronic records before or shortly after animals were placed on feed in a feedlot pen. The induction date was defined as ‘day 0’, the baseline time point from which animals were monitored for onset of BRD in the cohort study. For the majority of study animals, the arrival date, first day on feed and day 0 occurred within one day, but some animals were placed on feed prior to day 0. For preassembled cattle, the interval between arrival and induction was longer. Time at risk was an animal-level measure of time relative to the induction date; positive time at risk indicated days following day 0 and negative time at risk indicated days preceding day 0. In the cohort study, animals were monitored for onset of BRD from their first day at risk (‘day 1’) which was the day following the induction day. ‘Group-#’ (i.e. ‘group minus #’) described the group that each animal was part of based on its location on day - # (i.e. on the day # days prior to the animal’s induction day). For example, animals that were at the same location on day -13 that were later part of the same cohort comprised a unique group-13; animals from the same location on day -13 that were later part of a different cohort comprised a different group-13.

A ‘cohort’ was defined as a collection of animals placed together ‘on feed’ in a feedlot pen after induction; all animals placed together in a pen constituted a cohort. Thus, the cohort study population consisted of multiple cohorts. For some cohorts, not all animals were inducted on the same day. A ‘closed cohort’ was defined as one in which all animals were inducted on the same day, while in an ‘open cohort’, animals were inducted over more than one day. The cohort close date (‘cohort close’) was the last date on which animals were inducted into the cohort. At induction, cohorts of cattle were assigned a cattle class, one component of which was the ‘intended days on feed’ (i.e. the anticipated number of days that cohort would remain on feed at the feedlot). Cohorts of cattle remained together during the first 50 days at risk unless individual animals were removed due to disease signs, or they died in the pen. Once the cohort was established, no additional animals were added within the first 50 days at risk. Once cohort animals were shipped to the abattoir (always after day 50), remaining animals in the cohort may then have been mixed with animals from other pens. ‘Follow-up’ referred to animals being moved from their feedlot pen to a race for further ‘processing’ (sample collection, weighing) then returned to

their pen on the same day; this occurred at approximately 42 days after induction. The home pen described the feedlot pen in which the animals comprising a cohort spent the majority of time during their first 50 days at risk.

3.2.4 Pilot study

A pilot study was conducted where 1,576 animals from nine cohorts at three feedlots were enrolled and followed up between August 2007 and February 2008 to test the proposed methods for sample and data collection.

3.2.5 Sample size estimates

Sample size calculations were informed by retrospective data from three feedlots from an extended period of time and considered the multilevel structure of the data and consequent clustering. The variance inflation factor or design effect for animals within a cohort was estimated based on assumed intra-class correlation coefficients (ρ, ICC) of 0.1 and 0.15, and an assumed mean cluster size (m) of 235 (i.e. mean cohort size). Using the formula depicted in Equation 1, the calculated design effect was 24.4 where ρ=0.1 and 36.1 where ρ=0.15.

Equation 1: $$\boldsymbol{Design\ effect = 1 + (m - 1) * \rho}$$

Winpepi® (Version 10.7, July 2010) software was used to estimate sample sizes. The sample size defined by the number of enrolled cohorts was calculated and tabulated for differing risk factor prevalences and odds ratios. Although initially aiming for a sample size of 200 cohorts, this was reviewed on several occasions after the initial proposal; by August 2010 it was apparent that due to financial and logistical issues with enrolling cattle this could not be achieved. At this time, the sample size was revised down based on the calculations detailed in Table 3-1 and Table 3-2; it was agreed that a revised target of 170 to 175 cohorts would give sufficient power to detect risk factors that would be important at the population level.

The following data were used to estimate the required number of cohorts:

- Incidence risk in the non-exposed – interim estimate 0.2 (August 2010)
- Mean cohort size – interim estimate 235 (August 2010)
- ICC estimate from retrospective data from three feedlots – 0.1-0.15
- Prevalence (%) of the risk factor among cohorts – 1, 5, 10, 20, 50
- Odds ratio – 1.2, 1.5, 2, 3, 4, 5
- Significance – 0.05

- Power – 80%

Table 3-1: Total number of cohorts required to detect the effect of a cohort-level exposure with 80% power and significance level 0.05, assuming an incidence risk for BRD of 0.2 in non-exposed animals, an ICC of 0.1 and a mean cohort size of 235.

		Odds Ratio					
		1.2	1.5	2	3	4	5
Prevalence of risk factor (cohort-level)	0.01	14,372	2,670	832	292	178	126
	0.05	3,002	560	176	64	38	28
	0.10	1,588	298	94	34	20	16
	0.20	898	170	54	20	12	10
	0.50	582	112	36	14	10	6

Table 3-2: Total number of cohorts required to detect the effect of a cohort-level exposure with 80% power and significance level 0.05, assuming an incidence risk for BRD of 0.2 in non-exposed animals , an ICC of 0.15 and a mean cohort size of 235

		Odds Ratio					
		1.2	1.5	2	3	4	5
Prevalence of risk factor (cohort-level)	0.01	21,262	3,948	1,230	432	262	186
	0.05	4,440	828	260	94	56	40
	0.10	2,350	440	140	50	30	22
	0.20	1,328	250	80	30	18	14
	0.50	862	166	54	20	12	10

3.2.6 Outcome

Animals that were suspected by feedlot staff to be unwell during their time on feed were removed by staff from the home pen and taken to the hospital crush for examination and treatment as required before being either placed in the hospital pen or returned to their home pen. The main outcome variable for the cohort study analyses (BRD50) was the onset of BRD (i.e. meeting the case definition described in Section 4.1) between the animal's 1st and 50th day at risk.

3.3 Study population selection

The target population was Australian feedlot cattle in medium to large commercial feedlots. Study feedlots were selected by purposive sampling. The inclusion criteria for the source population were as follows:

1. The feedlot was licensed under the National Feedlot Accreditation Scheme administered by the Australian Lot Feeders Association (ALFA)
2. The feedlot used computerised record keeping for at least all animal-level and within-animal-level data

3. At time of enrolment, feedlot management and staff were considered able and willing to collect required samples and provide requested data for cattle inducted into the study over a two year period

Initially, most of the larger feedlots (i.e. with a capacity of 5,000 head or more) in Australia that met these criteria were approached and invited to participate. Because the target of 16 participating feedlots was not reached, the source population was expanded and medium capacity feedlots (more than 1,000 head) were also invited to participate. Although criterion (3) was met by all enrolled feedlots, some feedlots that participated early in the project were unable to continue to enrol cattle for the duration of the study period and other feedlots became involved at a later stage.

Fourteen feedlots participated in the study. Nine feedlots had a physically constructed capacity of at least 10,000 head, three feedlots had a capacity from 5,000 to <10,000 head and two had a capacity from 2,000 to <5,000 head.

Within study feedlots, feedlot managers were originally requested to select one cohort for the study every 8 weeks from the cohorts being assembled in the usual management of their feedlot. To minimise bias in the selection of cohorts, feedlot staff were asked to describe the cohorts being inducted during the agreed enrolment period so that the project team could randomly select the cohort to be enrolled as a study cohort. However, this process was impractical for feedlot staff, and both the timing and selection of cohorts did not proceed as planned. Managers generally selected cohorts in a pattern that was convenient to them (i.e. when they had labour available for sampling at induction).

A prospective longitudinal study was conducted whereby a subset of cattle inducted into study feedlots was selected, exposure statuses of animals assessed, and occurrences of BRD identified. The full study population comprised all animals inducted into study cohorts enrolled in the study. The longitudinal study involved monitoring study animals for BRD from induction to removal from the feedlot for slaughter, with the prospective collection of data and biological samples. The study population had a multilevel hierarchical structure such that animals were clustered within group-13s which were clustered within cohorts which were clustered within feedlots.

3.4 Data collection, validation and management

3.4.1 Feedlot data

Numerous data types from several sources were collected for study animals as illustrated in Figure 3-1 and detailed in Appendix 1 (Section 14.2). The majority of data were sourced from the participating feedlots. Management practices and typical profiles for incoming cattle were established during face-to-face interviews with feedlot managers at the beginning of the study.

Animal-level induction files provided data about identifiers (animal identification numbers, induction sequence, tail tag, pen identification), relevant dates (arrival date, induction date and first day on feed), entry characteristics (weight, sex, dentition, breed, cattle class) and source (vendor details, saleyard). For most feedlots, treatments administered at induction were detailed in animal-treatment-level files. Feedlots that practiced preassembly provided an additional file describing treatments administered at arrival. Animal identification, date and weight were recorded at follow-up and at exit (i.e. before being trucked from the feedlot to the abattoir), and carcass details were also provided post slaughter.

Animals that were suspected to be unwell during their time on feed were removed from the home pen and taken to the hospital crush for examination and treatment, when required, before being placed in the hospital pen or returned to the home pen. Feedlots were requested to supply complete records for any animals that were taken to the hospital crush, or died in their home pen, for any reason. At a minimum, these data consisted of the date, diagnosis or predominant signs (‘ailment’, and, for animals from some feedlots, ‘pull reason’), and treatment records detailing the medication used and dosage. In addition, dates and reason that animals left the cohort for any other reason were requested (for example, if animals moved to a different pen after the majority of the cohort animals were sent to slaughter)

For each cohort, induction and post-slaughter questionnaires provided group and cohort-level data. Contact details for vendors that sold groups of cattle directly to the feedlot were provided. Some feedlots provided cohort-treatment-level files detailing treatments or procedures administered at induction. Pen characteristics, ration details and information about the numbers on feed at the feedlot around the induction time were also provided. The quality of ration data supplied varied considerably among participating feedlots; much of the data that was originally requested in relation to rations (e.g. detailed ration analyses for each cohort) was not supplied. Hence, after consulting with feedlot

veterinarians to determine the ration variables of major interest, efforts were focused on obtaining a more limited range of complete ration data. Although on-site weather station data were requested; these data were not available for the majority of feedlots. Weather data were instead obtained from national weather databases.

3.4.2 Vendor questionnaire data

Vendors (i.e. cattle producers) who sold 20 or more animals directly to the feedlot and for whom contact details were provided by feedlot personnel were mailed a copy of the vendor questionnaire (Section 14.2) with explanatory details. Vendors were given the option of completing the hard copy of the questionnaire and either mailing or faxing to a member of the project team, completing the questionnaire online or arranging a telephone interview. Follow-up phone calls and/or emails were made when additional contact details were available and the questionnaire was not returned. Vendor questionnaire data differentiated animals bred on the vendor's farm from those purchased from another source and provided group-level information about presale management, including weaning management, nutritional history (feeding of conserved forage, grain or supplements), vaccination history (against respiratory pathogens), on-farm mixing history and the timing of management procedures or purchase.

Of a total of 1,257 arrival groups enrolled into the longitudinal study, questionnaires were sent to 579 (46% of groups). Of the 579 groups for which questionnaires were sent, responses were received for 238, giving a group-level response rate of 41%. Of the responders, 17 vendors supplied two or more arrival groups; usually to the same feedlot. Of the main cohort study population, 19% (238/1,257) of arrival groups comprising 31% (10,721/35,131) of animals had returned vendor questionnaire responses.

3.4.3 National Livestock Identification System data

Australia's National Livestock Identification System (NLIS) requires that all cattle are individually identified with a unique identification string, with the animal's string allocated by application of an ear tag or a rumen bolus ('NLIS device'), before they leave their farm of origin. Each farm is identified by a unique 'Property Identification Code' (PIC). In Australia, cattle producing farms are referred to as 'properties'. Under the NLIS, saleyards and feedlots are also allocated unique PICs. The system relies on registered users electronically scanning animals every time they are moved from one PIC location to another ('transfers') and uploading these data to an online national electronic database. The database records the PIC of issue (the first recorded PIC for each NLIS device,

usually the animal's property of birth) and source and destination PIC, transfer date, transfer type and waybill (i.e. document which accompanies cattle being transported) number for all transfers. Transfer type distinguishes between transfers to or from saleyards and 'point to point' transfers (between two PICs, neither of which are a saleyard). The PIC of issue, NLIS device replacement details and lifetime transfer data were provided from the NLIS database for study animals. Geographical coordinates for each PIC were also obtained, from the individual state NLIS coordinators, to estimate transport times and to facilitate mapping of PIC locations.

3.4.4 Sample collection and laboratory data

Blood samples, nasal swabs and necropsy samples were sent to the Queensland Alliance for Agriculture and Food Innovation (QAAFI) laboratory at the University of Queensland where serum samples were identified, processed and stored. Sample validation and data management involved linking samples and test results to individual animals as described in Appendix 2 (Section 15.2).

3.4.5 Database development

A database was developed to store data and to ensure all study data were readily accessible, allowing data to be linked and extracted at the appropriate level. Of the participating feedlots, most used commercial software (StockaID® or Possum Gully®) while the others used custom software. Most data were provided in Excel® spreadsheets or comma separated value text files. Animal-level data were compiled, cross-checked and validated for each cohort as the study progressed. Data validation was performed using a combination of the Microsoft Excel®, Stata® and Microsoft Access® programs as described in Appendix 2.

The derivation of variables for use in analyses was performed using the Stata® statistical software package. After the majority of the data were collected, cleaned (checked and corrected) and verified, and the basic categorical variables were derived, a Microsoft Access® relational database was assembled containing all of the raw and cleaned data along with important intermediate (e.g. group allocation at time points of interest) and analysis variables. The relational database was modified at the end of the study to ensure that all of the final biological test results (e.g. animal-level BVDV test results), final sample locations and final analysis variables were included.

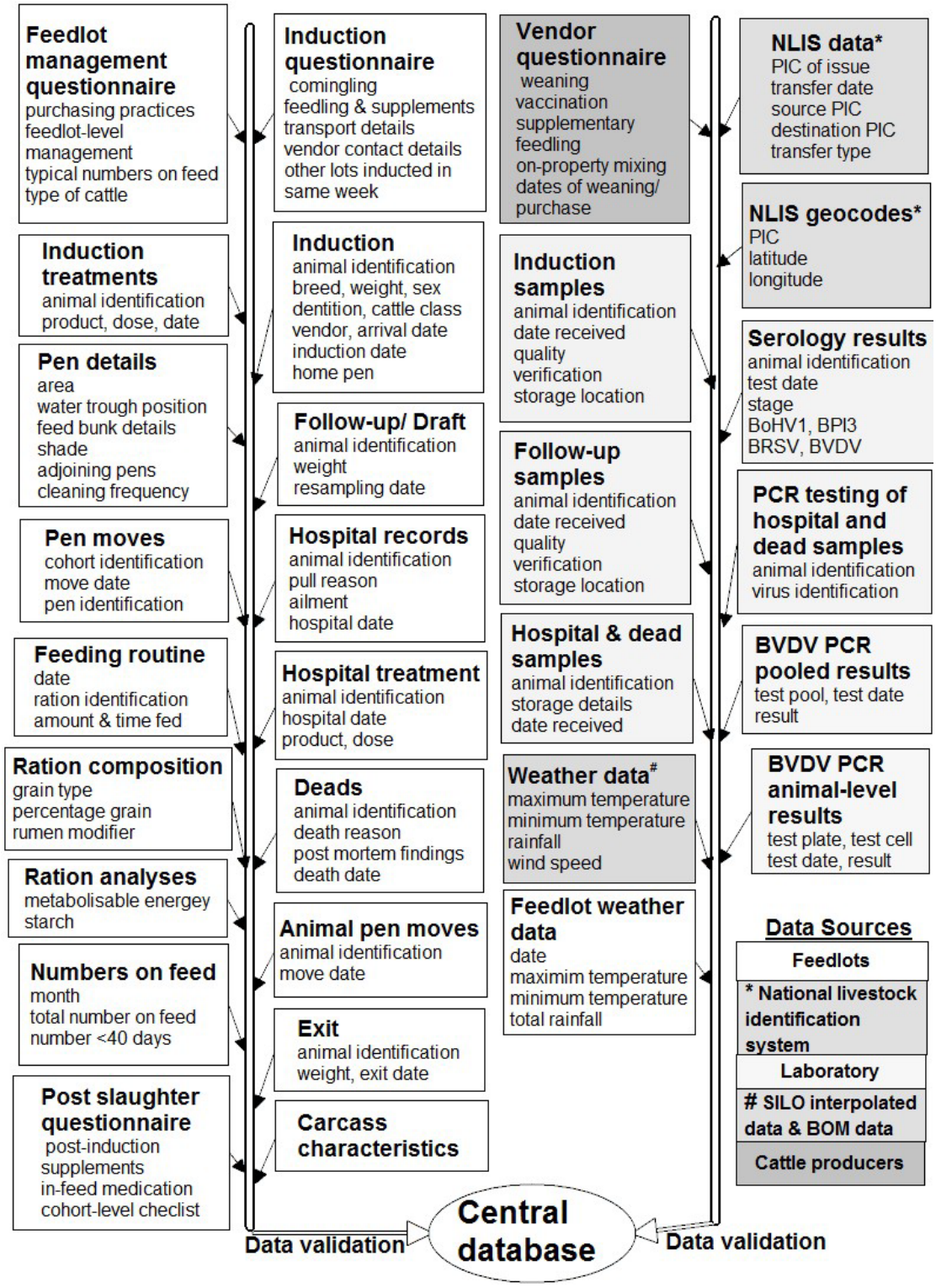

Figure 3-1: Sources of data for the NBRDI
#SILO: Scientific information for land owners, BOM: Bureau of meteorology

4 Variable Derivation and Descriptive Overview of the Study Population

In this chapter I describe the case definition and discuss the importance of clustering in the study population. I outline the derivation of exposure variables and detail the assessment of variable quality. I then describe the distributions of exposure variables in the study population, including any underlying continuous variables or intermediate variables from which the final analysis variables were derived. Thus, I describe the spatial distribution of the study population along with mixing and moving patterns of study animals prior to arrival at the feedlot. I then detail the rationale behind construction of a causal diagram which was used to inform model selection for use in statistical analyses.

4.1 BRD case definition

The outcome variable for the main cohort study was the development of BRD on or between the first and the 50th day at risk (day 1 to day 50). Only the first instance when an animal was removed to the hospital pen was considered for each animal. Those whose first diagnosis was other than BRD were assumed to not have contracted BRD by day 50. This was preferable to using subsequent diagnoses to identify possible BRD cases in these animals because removal of animals to the hospital pen was considered to markedly increase risk of subsequently contracting BRD due to close contact with BRD cases in the hospital pen in association with stressors due to handling etc. The case definition of BRD was based on the information supplied by the feedlots from their hospital records. With the StockaID® software, ‘Pull Reason’ referred to the reason the pen rider removed the animal from the cohort and the ‘Ailment’ field was completed based on a more complete examination in the hospital crush. By contrast, other software only provided a ‘Pull Reason’ which was based on the assessment of the animal after being removed and examined.

A case was defined as an animal removed to the hospital pen in which the ‘Pull Reason’ and ‘Ailment’ (if applicable) were both consistent with the diagnosis of BRD. Pull reasons that were identified as BRD included ‘Respiratory’, ‘Pneumonia’, ‘IBR’, and ‘BRD’. Ailments were considered consistent with the ‘Pull Reason’ provided signs were referable to the respiratory system. A cross tabulation of the ‘Pull Reason’ and ‘Ailment’ fields considered in the classification of BRD is presented in Table 4-1. The shaded area represents animals included in the final BRD case definition and the unshaded area provides the numbers of

animals with non-specific signs or inconsistent pull reasons and ailments that were considered as potential BRD cases but were subsequently excluded from the case definition (N=129). Feedlots used a range of terms for the diagnosis of respiratory-related disorders, some of which were specific to individual feedlots (e.g. 'honker', Table 4-1)

Table 4-1: Cross tabulation of 'Pull Reason' against 'Ailment' for all potential BRD cases; shaded cells indicate the cases, and hence the case definition, used (N=6,406)

Ailment \ Pull Reason	BRD	IBR	Pneumonia	Respiratory	Honker	Necrotic laryngitis	Non eater	Non-eater/waster	Observe	Restart		Total
			10	5,045			2					5,057
BRD	217										3	220
IBR		267									2	269
Respiratory				764							7	771
Pneumonia	1		98									99
Breather	2											2
Honker			1		1							2
Noisy breathing	1											1
Necrotic laryngitis	1					1						2
Non eater				3			16	2			2	23
Observe				2					10			12
Pain relief			4									4
Depressed	43											43
Buller				1								1
Hollow/slow moving	1											1
Slight depression	1											1
Slow moving	3											3
Stiff/ slow moving	3											3
Restart										21		21
Total	273	267	113	5,815	1	1	18	2	10	21	14	6,535

4.2 Cohort study population subsets

The study population comprised all animals inducted into study cohorts. Animals with a time at risk greater than zero and not lost to follow up (i.e. they were either known to be with their study cohort on their fiftieth day at risk, or to have been removed from the study cohort or died by their fiftieth day at risk as determined from the animal-level electronic records provided by feedlots) were eligible for inclusion in the main cohort study. The availability of data and the application of different inclusion criteria resulted in several datasets. The relationships between these cohort study datasets are illustrated in Figure 4-1. Of a total of 35,160 animals inducted into study cohorts, five had zero time at risk and

24 were lost to follow up, so 35,131 were included in the main cohort dataset. The 29 animals ineligible for inclusion in the main cohort dataset were blood sampled at induction and hence were included in the determination of cohort-level PI status detailed in Chapter 10; they were known not to have BRD on day 0. 'Analysis subsets' comprised subsets of the population in which additional variables were available for analysis or different causal pathways were postulated. The preassembly dataset comprised all animals (N=5,614) from the three feedlots that assembled cattle on pasture close to the vicinity of the feedlot prior to induction (regardless of whether the individual animals were preassembled). The vendor questionnaire subset comprised animals for which vendor questionnaire responses were received. The vendor-bred subset comprised animals born on the vendor's property and the prior vaccination subset comprised animals purchased before 10 months of age in addition to the vendor-bred animals. The nested case-control subsets are described in Chapter 11. Stata® datasets were assembled for each of the analyses subsets described above.

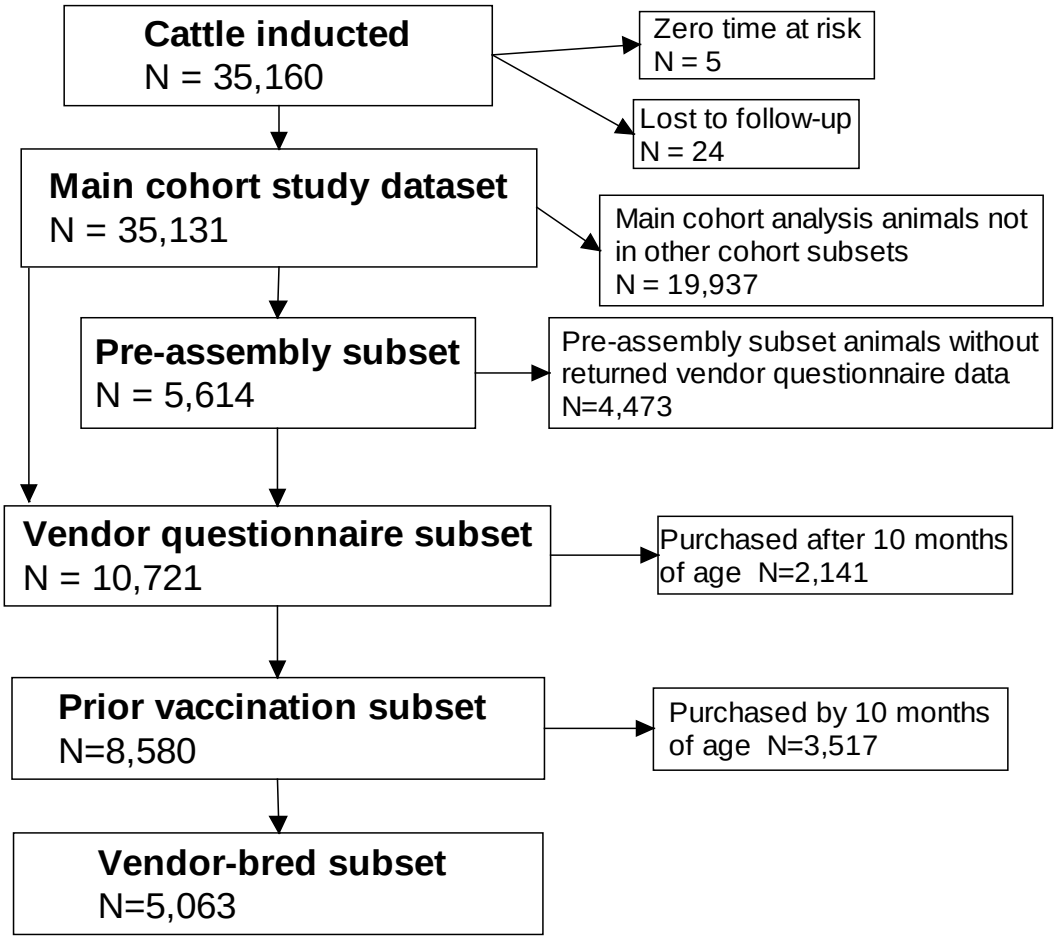

Figure 4-1: Flowchart detailing relationships between cohort study populations

4.3 Unit of analysis

The individual animal was chosen as the unit of analysis for all main analyses because this approach offers the most informative use of data collected at the animal level. Thus, analyses of putative risk factors assess the effects of these factors on the risk that an individual animal would contract BRD by day 50, should that animal be exposed to this risk factor. Alternative units of analysis were at group-13, cohort or feedlot. If a higher level unit of analysis had been used, some animal level and group-13 level information would have been lost by aggregating and summarising animal-level data. The ecological fallacy could also have occurred if a higher level unit of analysis had been used, if relationships derived from aggregated data were assumed to also apply at lower levels when this was not the case. However, it is also important to be aware of the reverse situation (i.e. the atomistic fallacy) whereby relationships at the animal level are assumed also to apply at the herd level or cohort level when this is not the case (e.g. herd-level immunity versus animal-level immunity). Some descriptive statistics were at estimated at higher levels. For particular research questions these 'cluster-level' analyses are appropriate to assess the effects of risk factors on the cluster-level incidence of BRD. This is discussed further in Chapter 12 as part of the recommendations for further research.

4.4 Clustering of data

4.4.1 Variables to account for clustering

It is important to consider clustering in study design and analyses to ensure that study power is adequate, and that estimates of effect and precision are valid. Many statistical methods assume that, for the outcome (i.e. dependent) variable (occurrence of BRD by day 50 in this project), observations are independent (i.e. after accounting for any explanatory variables in a model, the outcome status of one individual is independent of that in another individual). Animals within naturally-occurring 'clusters' may not be independent of each other; for example, animals in the same cohort would be expected to be more similar to each other than animals in a different cohort in a different feedlot and some degree of similarity may remain even after fitting explanatory variables in a model.

The study population had a nested hierarchical structure with four levels such that animals (level 1) were clustered within arrival groups (level 2) which were clustered within cohorts (level 3) which were clustered within feedlots (level 4). While feedlot and cohort adequately described the expected clustering at the two higher levels, numerous alternatives for accounting for clustering at level 2 were available. Defining level 2 as the animal's arrival

group may not have been optimal as animals in an arrival group may have originated from several farms with different management histories. I postulated that the risks of animals contracting BRD by day 50 would be expected to be more similar for animals in the same group for longer periods of time before induction than for animals that were together for only a short period of time before induction.

To inform the choice of variable used to account for clustering at level 2, the NLIS data were used to identify groups of animals at specified time points in relation to day 0. Time points chosen were day -1, day -7, day -13, day -30, day -90, day -180 and day -365. The groups at these time points were termed group-1, group-7, group-13, group-30, group-90, group-180 and group-365, respectively. A further group (group_origin) was identified using each animal's PIC of origin, where the PIC of origin was defined as the earliest recorded PIC of issue (a few animals had records indicating that the NLIS device had been replaced) provided this was not the feedlot PIC. For each group definition, exploratory analyses were conducted using logistic regression part way through the study using records for 20,253 animals (all cohorts with complete animal-level records supplied by feedlots at that time were included). Null mixed-effects models were fitted using the *xtmelogit* function in Stata® with feedlot, cohort and group as hierarchical random effects. The estimated variances at each level (feedlot, cohort, group) were obtained from the models and the latent variable threshold approach was used so the variance at the animal level was assumed to be $\pi^2/3$, or 3.29 (Snijders and Bosker, 2012). The within-group intra-class correlation coefficients were calculated as the sums of the percentages of total variances accounted for collectively by feedlot, cohort, and group. The design effects, giving the magnitude of the effects of clustering on study sample size for group-level variables, were then estimated using the formula described in Equation 1 (Dohoo et al., 2009):

The mean group size for each group-level variable was the mean cluster size. The group-level variables used, proportions of variance at each level, and results of design effect calculations are listed in Table 4-2. The intra-class correlation coefficients for clustering of animals in the same group were similar across the various definitions of group. The observed increases in design effect with group definitions closer to day 0 were therefore mostly a function of increasing cluster size. Based on these results, group-13 was selected as the identifier for the lowest level of clustering (level 2) as it was thought to provide the best balance between a larger design effect, potential misclassification into groups where transfer dates were unknown (imputed based on common group transfer

dates) and the biological implications of the timing with respect to exposure to pathogens and formation of a stable social hierarchy. The hierarchical structure of the main cohort study population and definitions of the cluster variables are depicted in Figure 4-2

Table 4-2: Group definitions, distributions of the variance among levels of the hierarchy when different groups were used in a null model, and the design effect for each group definition.
Intra-class correlation coefficient (ICC) relates to clustering of animals in the same group.

Group	No. of groups	Mean group size	Variance				Percentage of variance				ICC	Design effect
			Feedlot	Cohort	Group	Total	Feedlot	Cohort	Group	Animal		
Group_origin	3,926	5.3	3.99	1.02	0.68	8.97	44.4%	11.3%	7.6%	36.7%	0.634	3.73
Group-365	1,756	11.8	3.89	0.99	0.67	8.83	44.0%	11.3%	7.5%	37.3%	0.629	7.79
Group-180	1,224	16.9	3.94	1.02	0.64	8.88	44.3%	11.4%	7.2%	37.1%	0.631	11.03
Group-90	890	23.2	3.89	1.00	0.62	8.79	44.2%	11.3%	7.1%	37.4%	0.627	14.92
Group-30	757	27.3	3.87	0.94	0.60	8.71	44.5%	10.8%	6.9%	37.8%	0.621	17.34
Group-13	695	29.7	3.87	0.90	0.58	8.65	44.8%	10.4%	6.7%	38.0%	0.618	18.75
Group-7	622	33.2	3.86	0.88	0.57	8.61	44.9%	10.2%	6.7%	38.2%	0.617	20.86
Group-1	519	39.8	3.87	0.83	0.56	8.55	45.3%	9.7%	6.5%	38.5%	0.615	24.87

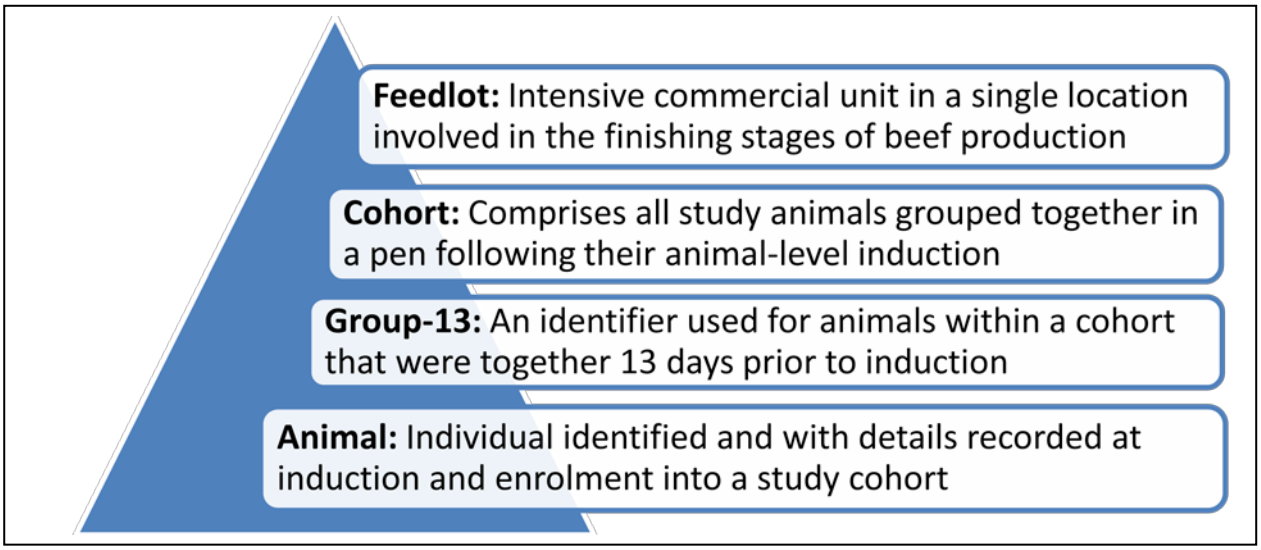

Figure 4-2: Hierarchical study structure: animals were nested within group-13s, which were nested within cohorts which were nested within feedlots

4.4.2 Clustering of exposure variables

Exposure variables could also cluster at different levels in the study population hierarchy. For example, a feedlot-level variable was defined as a variable where all study animals at the same feedlot had the same exposure status. Similarly, if within feedlots, all animals within each cohort were exposed to the same category of a risk factor that risk factor would be considered a cohort-level risk factor. The amount of clustering of exposure variables was important because the study had limited power to detect the effects of

exposures that clustered at higher levels. This was considered in the assessment of variable quality and distribution as described below.

4.5 Exposure variable derivation and assessment

4.5.1 Categorisation and derivation of exposure variables

Large amounts of data were collected during the course of the project. In developing predictor variables for use in analyses, I aimed to fully utilise the rich data set, while considering associations that were biologically plausible. In categorising variables, prior knowledge or industry interest in particular categories were considered and I aimed to create categories with adequate numbers of animals, both in total and in the distributions of animals by category across feedlots. Often the form of the original data influenced the initial selection of categories, which were then modified based on the quality of the data and its distribution. Where categories were sparse, or the distribution was severely unbalanced (i.e. not enough variability within cohorts or within feedlots), statistical power to identify relationships would be expected to be reduced. Sometimes the categorisation of the variables was modified and refined based on initial analyses in combination with prior hypotheses.

All continuous predictors were categorised to avoid incorrectly assuming linearity of associations with the logit of BRD by day 50. Detailed summaries of the distributions of continuous variables were examined and cut points were chosen to ensure a reasonable distribution of animals across categories. The distributions of these categories were then assessed across feedlots; sometimes variables were re-categorised with fewer categories to achieve a better distribution across feedlots.

The derivation of variables is detailed in Appendix 1 (Chapter 14). In the following sections I provide an overview of this process. The distributions of continuous variables used to derive the categorical analysis variables are shown in Table 4-3. Details of the distributions of the final analysis variables used are included in the results tables in subsequent chapters. A summary of variables, including the variable names, abbreviations and definitions are given in Table 4-4 through to Table 4-8, along with an assessment of variable quality and distribution.

4.5.2 Feedlot entry characteristics

Feedlot entry characteristics described animal-level attributes recorded at induction (e.g. breed, weight, sex) and variables derived from them. Induction weights were normally

distributed and ranged from 196 to 756 kg with a mean of 434 kg (Table 4-3). In the final analyses the induction weight variable (Weight; Table 4-4) contained four categories and had a balanced distribution of observations across categories and the majority of feedlots. The mean cohort weight, calculated by summing the individual animal-level induction weights for all animals in the cohort and dividing by the number of animals inducted into the cohort (with non-missing induction weight values), ranged from 315 to 491 kg with mean and median values of 440 kg (Table 4-3). The cohort-level mean induction weight variable (Weight cht; Table 4-4) was a derived categorical variable based on approximate tertiles of the distribution. The difference between mean cohort weight and animal-level induction weight (Weight diff; Table 4-4) described the individual weight difference from the mean cohort weight and ranged between 219 kg below to 326 kg above the mean; 50% of the distribution was within 26 kg of the mean cohort weight (Table 4-3).

The original breed descriptions provided by the feedlots varied from detailed (e.g. Braford X Charbray) to more general (e.g. Angus cross). The frequency distribution of breeds across the population was considered along with prior literature in deriving the final breed classification. Any animal with a tropical breed component was classified as 'Tropical/Tropical cross'. Any animal with a European breed component without a tropical breed component was classified as 'European/European cross'. If there were sufficient numbers of animals across feedlots identified as a particular breed, that breed was included as a separate category (e.g. Murray grey, Hereford). British crosses comprised animals identified as having components of British breeds without tropical or European breed components. The final breed variable (Breed; Table 4-4) contained seven categories (Angus, British cross, Hereford, Shorthorn, Murray Grey, European/European cross and Tropical/Tropical cross).

Sex differentiated steers and heifers, but was clustered by feedlot and cohort. Only 8% of all animals were heifers and these were from only six of the 14 feedlots. Cohort sex (Sex cht; Table 4-4) identified whether the cohort was single sex (steers or heifers) or mixed. Only four feedlots had mixed sex cohorts and only five had heifer-only cohorts. Dentition (Table 4-4) referred to the number of permanent incisors (0, 2, ≥4; 4 and 6 were combined due to sparse numbers). The dentition data were completely missing for one feedlot and were not recorded at the animal level in another. In this latter feedlot, the manager indicated that more than 99% of animals entering the feedlot had only deciduous incisor teeth, so dentition was imputed as zero for all animals in this feedlot. This was consistent with the observed low induction weight range and the reported common

practice of this feedlot purchasing cattle and moving them to the feedlot soon after weaning. The date of birth was not recorded in the NLIS. Dentition was mainly of interest as a proxy measure for age, but does not discriminate between cattle aged less than approximately two years of age (deciduous teeth only); these comprised the majority of the population. The variable describing the estimated age at induction (Age; Table 4-4) was derived for animals in the vendor questionnaire subset based on responses to a series of questions about the animal's age, and timing of management procedures and purchase. Although there was a moderate amount of missing data for this variable (so it could not be estimated for 9.2% of those animals with vendor questionnaire data), it provided an estimate of age which better discriminated between categories of younger cattle than the dentition variable.

Feedlots provided cohort-level descriptions of 'cattle class'; these were usually based on a composite of characteristics such as weight, breed, age, body condition, sex and intended days on feed, but descriptions were not consistent between feedlots. 'Intended days on feed' (Intended DOF; Table 4-4) described the planned time (at induction) that each cohort would be fed in the feedlot before slaughter; this was derived from the cattle class data for use in analyses because it provided a comparable variable across feedlots that may have captured additional information other than that provided by weight, breed and sex.

4.5.3 Management-related exposure variables derived from the vendor questionnaire

Variables describing weaning method, on-property mixing and prior feeding history were derived only for animals in the vendor-bred subset (i.e. animals with vendor questionnaire data that were born on the vendor's property); vendors could obviously not supply full lifetime data for animals they had purchased. Variables for prior vaccination with Bovilis MH™ and Pestigard™ (BV_vacc and PV_vacc; Table 4-4) were derived for animals in the prior vaccination subset; this subset consisted of vendor-bred animals and animals purchased prior to 10 months of age. It was unlikely that animals would have received these vaccinations before ten months of age so absence of vaccination data before this age was expected to cause minimal classification errors. For both vaccines, few animals received multiple doses. So, for each vaccine, a binary variable was derived, indicating administration of one or more doses of the vaccine prior to day -14.

4.5.4 Exposure variables relating to induction treatments

Administration of Rhinogard™ vaccine (a product registered for use as an aid in the control of BoHV-1 infection in feedlot cattle) and vitamins A, D and E at induction were described by dichotomous variables (Rhinogard and VitADE; Table 4-4). These treatments were typically administered to all animals entering some feedlots and to no animals in remaining feedlots, so these were essentially feedlot-level variables. The decision to use Rhinogard™ at induction may have been related to the past incidence of BRD at the feedlot, with feedlots with past high incidences more likely to use Rhinogard™ in study cohorts. Assuming BRD incidence in study cohorts was correlated with past incidence at the same feedlots use of Rhinogard™ would not be independent of feedlot-level random effects and so estimates of effects of Rhinogard™ may be biased by covariates not fitted in the models.

4.5.5 Exposure variables relating to mixing, moving, group size, saleyard exposure and transport prior to induction

The NLIS provided a rich source of data for the study population, and were used to derive numerous exposure variables. In deriving variables for use in final analyses, intermediate continuous variables were usually derived. Summary statistics for those relevant to the derivation of analysis variables are presented in Table 4-3

The term ‘group dynamics’ was used to describe the characteristics of groups, and changes in these groups or their characteristics over time, prior to induction into a feedlot pen. For a group defined at a particular time point (#), this encompassed the definition of the group, geographical location of the group and numbers of animals in the group. Group dynamics also encompassed changes in groups over time as described by mixing (combining groups), moving and saleyard exposure (changing location or being at a particular location).

4.5.5.1 Prior hypotheses and time points of interest

I hypothesised that the effects of mixing with animals from other groups, group size and transfer through saleyards at various times before day 0 would differ depending on both a) the timing of these events relative to the animal’s induction date and b) the animal’s history of mixing at other times prior to day 0. I postulated that mixing prior to day -27 would be protective and that mixing close to induction would be harmful, and that the effect of mixing close to induction would depend on when the animals were first mixed. These postulates were based on the assumption that mixing would increase the chance that animals were

exposed to pathogens and that disruption of the social hierarchy would result in stress. However, after immunity had developed and the hierarchy was re-established, the animal would likely have enhanced immunity and be at reduced risk of BRD at the feedlot with subsequent exposure to pathogens. I also hypothesised that a saleyard transfer close to day 0 might increase risk above and beyond being mixed and moved because of greater exposure to pathogens and additional stress associated with extra mixing and handling at the saleyard. Conversely, I hypothesised that transfer through a saleyard a long time before day 0 may result in reduced risk because of enhanced immunity due to exposure to a wider range of pathogens. I hypothesised that transport would be stressful at any time, but longer duration transport before entry would increase risk. Any increased risk of BRD at the feedlot would depend on the length of time required for transport stress to dissipate.

While recognising that mixing and moving would be correlated, and that saleyard transfers would almost always involve both mixing and moving, I aimed to separate the effects of these exposures. Use of the NLIS data enabled me to define variables describing the group the animal was part of, geographical location of the group and number of animals in that group at time points of interest, and hence mixing, movement and saleyard transfer history between time intervals of interest. In using these data, I assumed that animals at the same PIC at the same time were mixed (which may not have been the case). In addition, data were only available for animals that were part of the study, so the amount of mixing was likely to be underestimated. Days -91,-28 and -13 were chosen based on postulated times for animals to develop immunity following exposure to pathogens or for the effects of stressors to dissipate. Different epidemic curve patterns have been described in feedlot populations, extending for periods of time up to 90 days (Babcock et al., 2010). Days -28, -13, -7 and -2 were chosen to evaluate times for animals to recover from transport stress, with the latter times also used to investigate the duration of transport.

4.5.5.2 Exposure variables describing group dynamics

After determining the grouping of animals at the time points of interest, the numbers of animals in those groups were determined and this distribution was categorised to form variables describing the animal's group size at each of the selected time points (Group-#N described group size at time point #). Because group-13 was the chosen cluster variable, Group-13N was the main variable used to assess the effects of group size. The median number of animals in the animal's group-13 was 64 (interquartile range: 35 to 133, Table 4-3)

Mixing with other animals from other groups was defined as occurring within a time period when animals from two or more groups from the earlier time point were together in the same group at the later time point. Thus, mixing referred specifically to between-PIC mixing among animals enrolled in the study. Using this definition, animals that changed PIC but remained in the same group with no further study animals added were not considered to have mixed. This situation was observed for a small number of animals that had a saleyard transfer as part of the move from one PIC to another.

Mixing was explored using the intermediate variables described in Appendix 1. A variable was derived to investigate the effect of the timing of the earliest mixing event (Mix first; Table 4-5).The median time of first mixing was 166 days prior to day 0 (interquartile range: 0 to 364, Table 4-3). Prior hypotheses suggested that the effect of mixing in particular time periods may not be independent of effects of mixing in other time periods. Possible methods for assessing this included analyses with multiple two and three-way interaction terms, and analyses using a composite mixing variable. Both options were explored. The composite variable was preferred over the interaction term method as output from this type of model was easier to interpret and sparse categories showing similar patterns could be combined. In contrast, in models with interaction terms, combinations with very sparse data resulted in imprecise and potentially misleading effect estimates.

Based on the prior hypotheses and consistent patterns observed in the data, variables describing mixing prior to day -27, from days -27 to -13 and from day -12 to cohort close were used to derive the final composite mixing variable (Mix history; Table 4-5) used in most analyses involving the main cohort dataset. This animal-level variable, had twelve categories determined by various combinations of mixing pre day -27 (yes/no), during days -27 to -13 (yes/no) and days -12 to cohort close (number of group-13s combined: 1, 2 or 3, 4 to 9 or ≥10). A collapsed version of the mixing history variable was derived for use in subset analyses. This four-category variable (Mix summary; Table 4-5) classified animals based on a combination of the binary variable describing mixing prior to day -27 and a variable describing the number of group-28s forming the cohort (<4, ≥4). The median number of group-13s or group-28s forming an animal's cohort was seven (Table 4-3). Because effect estimates relating to mixing from days -27 to -13 derived from the twelve-category mixing history variable were imprecise, a variation of the mixing summary variable was derived (Mix summary composite; Table 4-5) with animals first mixed from days -27 to -13 categorised separately (i.e. first mixed days -27 to -13 and ≥4 group-28s forming the cohort). This allowed a more detailed analysis of this mixing pattern; these

animals were from preassembly feedlots where many small groups would often be combined over a time period of three months or more before induction. The mixing variables were nested (e.g. the mixing summary variable was nested within the mixing history variable) or closely correlated (e.g. Mix first and Mix summary), so these were not fitted in the same model.

An animal was classified as having a saleyard transfer if it moved through a saleyard. A yes/no binary variable was derived for each time period indicating whether or not an animal had been through a saleyard at least once during the time period. Final variables (Table 4-5) included saleyard transfers prior to day -27 (SY pre-27) from days -27 to -13 (SY -27 to -13) and from days -12 to 0 (SY -12 to 0). The last two variables had sparse categories, with less than 3% of animals having a saleyard transfer within each of those time periods.

Each animal's location at the end of the day of interest was determined by its PIC at those times, and moving was defined by a change in PIC location; moving could occur independently of mixing or saleyard transfer if the same group of animals had a new PIC location but no study animals were added. Exploratory analyses supported the hypothesis that there was no large effect of earlier moves between properties prior to the move to the feedlot over and above any effects of mixing with cattle from other PICs. So, to simplify the final analyses, only the timing and duration of the move to the feedlot (i.e. moves where the destination PIC was the feedlot PIC) were considered as these were of greatest interest to industry. For these, the NLIS data were used to determine the source PIC and geographic location and date of the move to the feedlot. If NLIS records were missing or illogical, the feedlot-provided data detailing the animal's arrival date, induction date, tail tag number and arrival group were used to determine the details of the move to the feedlot. The number of days from arrival to day 0 was determined and a categorical version of this variable (Arrival to day0; Table 4-5) was used in the preassembly subset analyses. Within this subset, the median number of days between arrival and induction was 15 (interquartile range: 3 to 32), (Table 4-3).

Transport durations (including estimated travel time, loading and unloading time and driver rest time) were estimated for moves to the feedlot between days -12 and 0; these were then categorised (<6 hours/ ≥6 hours). The median durations of transport were 5 hours and 7 hours for transport from day-12 to day -2 and day -1 to day 0 respectively (Table 4-3). The median number of days between arrival and induction for the main cohort study

population was 0 (interquartile range: 0 to 1, Table 4-3). A composite categorical variable describing the timing and duration of the move to the feedlot (Move_FL; Table 4-5) was derived as the final variable.

Table 4-3: Distribution of continuous variables used in the derivation of analysis variables

Variable	Mean	Median	Range	Interquartile range
Induction weight (kg)	434	438	196 to 756	408 to 466
Mean cohort weight (kg)	440	440	315 to 491	425 to 456
Weight difference (kg)	0	0	-219 to 326	-26 to 26
Number of animals in group-13	88	64	1 to 342	35 to 133
Number of animals in cohort	252	241	17 to 395	175 to 350
Number of group-13s in animal's cohort	8	7	1 to 25	2 to 13
Number of group-28s in animal's cohort	9	7	1 to 29	3 to 14
Days from first mixing event to day 0	205	166	0 to 2,140	0 to 364
Days from arrival to day 0	5	0	0 to 228	0 to 1
Days from arrival to day 0 in preassembly subset	25	15	0 to 228	3 to 32
Transport duration for animals moved to feedlot PIC from day -6 to 0 (hours)	6	5	1.5 to 41	3.5 to 6.5
Transport duration for animals moved to feedlot PIC from day -12 to -7 (hours)	10	7	1.5 to 23.5	4.5 to 23.5
Days from first DOF to day 0	0	0	0 to 13	0 to 0
Days from day 0 to cohort close	2	0	0 to 15	0 to 3
% grain on day 0	41	40	17 to 60	37 to 45
% grain at day 20	66	63	39 to 86	60 to 75
Days to 60% grain	16	15	1 to 158	10 to 20
Number of animals on feed	17,886	19,926	950 to 42,230	8,184 to 26,127
Number of animals <40d on feed	4,624	5,008	269 to 15,930	2,182 to 6,370
Mean maximum temperature (ºC)	24	23	12 to 37	19 to 29
Mean minimum temperature (ºC)	10	9	-2 to 22	5 to 15
Total rainfall in first week (mm)	11	4	0 to 162	0 to 16

4.5.6 Exposure variables relating to the formation of the cohort

The number of animals inducted into each cohort ranged from 17 to 395 (median: interquartile range Table 4-3) and this clustered by feedlot. Hence, only two categories (<200, ≥200) were used in the final variable (CohortN; Table 4-6). The number of days between the first and last animal-level induction date for a cohort ranged from zero to 15 (Table 4-3). Cohort fill duration (Cohort fill; Table 4-6) was defined at the cohort level as the number of days (1 / >1) over which animals were inducted into the cohort. Animals from several feedlots were put on feed in a feedlot pen prior to induction and therefore prior to the study definition of the start of time at risk for BRD (day 1). Accordingly, these animals had additional time to adapt to ration changes and other feedlot management practices before study monitoring for BRD occurrences commenced. For the final analyses the number of days between the first day on feed and induction (median: 0 range: 0 to 13 Table 4-3) was described using a three-category, animal-level variable (DOF1 to day0; Table 4-6) which took the value zero when the first day on feed was day 0. The number of

days from day 0 to cohort close (median: 0 range: 0 to 15, Table 4-3) was captured in another three-category animal-level variable (Day0 to close; Table 4-6) which took the value one for all animals in cohorts filled in one day.

4.5.7 Exposure variables relating to BVDV

4.5.7.1 Prior hypotheses and research questions

As discussed in Chapter 1, BVDV has been consistently associated with BRD, and the most important source of virus is thought to be from PI animals. Hence, in exploring the role of BVDV as a risk factor for BRD, my interest was in determining whether the presence of one or more PI animals in the cohort increased the risk of BRD for other animals in the cohort. I hypothesised that the presence of a PI animal within the cohort would increase risk and I aimed to compare risk for such animals with that for animals in cohorts where no PI animal was identified in the pen but infection with BVDV was present (i.e. virus was detected in any sample from at least one animal from the cohort) and with animals in cohorts with no evidence of BVDV transmission after induction. I further hypothesised that animal-level acquired immunity following natural exposure to a PI animal might result in a protective effect if that exposure occurred at least 28 days prior to induction, but conversely, an animal that was immunologically naïve to BVDV at induction may experience increased risk of BRD following exposure to a PI animal in the cohort. I therefore needed to determine which animals were PI animals and which group-28s and cohorts contained PI animals, and derive appropriate variables to classify animals accordingly. This complex process involved several steps, with sequential laboratory testing and assimilation of results; it is detailed in Chapter 10.

4.5.7.2 BVDV variables examined in the main cohort dataset

A binary cohort-level variable was derived describing whether or not BVDV was detected in PCR analyses of samples from any cohort animal. Pooled induction and follow-up serum samples and individual hospital samples (serum or nasal swab) and necropsy samples (lung or tracheal tissue) were used for this. The presence of BVDV in the cohort (BVDV_cht; Table 4-6) did not distinguish whether or not a PI animal was present in the cohort, only whether any cohort animal, or pool, tested positive to BVDV on a single qPCR analysis; this could have been due to a PI animal or a TI animal.

An animal-level variable (BVDV_PI_animal; Table 4-6) described whether or not the animal was a PI animal. This variable was used in combination with the group-28 variable defined from the NLIS data to determine whether or not a PI animal was present in the

animal's group-28. A composite variable was then derived which classified animals according to whether a PI animal was present in the group-28 and whether BVDV was present in the cohort (BVDV_grp_cht; Table 4-6). This variable took values of 'no, no' if there was no PI animal in the group-28 and no evidence of BVDV being present in the cohort, 'yes, yes' if there was a PI animal in the group-28 (and hence in the cohort) and 'no, yes' If there was no PI animal in the group-28 but BVDV was present in the cohort.

4.5.8 Exposure variables relating to pen characteristics

All pen characteristic exposure variables described each animal's 'home pen' (i.e. where the animal spent the majority of time during its first 50 days at risk of BRD). Shared pen water (Pen water; Table 4-6) indicated whether the water trough(s) could be accessed by animals outside of the pen (yes/no). Stocking density (Pen density; Table 4-6) was estimated as pen area per standard cattle unit. The pen bunk space (Pen bunk; Table 4-6) was calculated as the number of linear meters of feed bunk space per head. Methods of describing the extent and type of shade varied markedly between feedlots, so a dichotomous pen shade variable (Pen shade; Table 4-6) was used in the final analyses indicating the presence or absence of any shade in the pen. The number of pens adjoining the home pen (Pens joining; Table 4-6) was either 1 or 2 as indicated in the original data. Data on pen slope, pen cleaning frequency, pen riding frequency and the distance to the hospital pen (Table 4-6) were not used in the analyses because of missing data, limited variation between feedlots and potential for confounding by feedlot size as indicated in the quality assessment criteria columns. For example, pen cleaning data provided by most feedlots usually were reported as the number of times per year and did not provide specific information about the dates of cleaning of the home pens used by study cohorts.

4.5.9 Exposure variables relating to ration characteristics

After discussion with some of the consulting veterinarians, I hypothesised that a higher percentage of the diet that was grain (percentage grain) at the start of time on feed and on day 20 might be associated with an increase in risk of BRD, as might a rapid increase in the percentage grain in the diet early in the animal's time on feed. Thus animal-level variables describing the percentage grain in the diet on day 0 (Grain1) and day 20 (Grain21) and the time taken for the percentage grain in the diet to reach 60% (Grain60%) were derived (Table 4-7). For most animals, the time points for Grain1 and Grain21 corresponded to the first and 21st days on feed. The choice of 60% as the definition of 'high' grain percentage ensured that this variable could be defined for all study animals; not all animals reached higher cut-points (e.g. 70% grain in the ration). All of the grain

variables were clustered by feedlot. The starting percentage of grain in the ration on an 'as fed' basis ranged from 17% to 60%, with a median of 40% and an interquartile range from 37% to 45% (Table 4-3). On day 20, the percentage of grain in the ration ranged from 39% to 85% with an interquartile range from 60% to 75% (Table 4-3). While the number of days on feed to reach 60% grain in the ration varied greatly (from 1 to 158), the interquartile range was between 10 and 20 days (Table 4-3), and 95% of animals were being fed a ration with at least 60% grain by the 25th day on feed.

Wheat, corn, barley and sorghum were classified as grains; the Grain type variable (Table 4-7) described the type of grain used, but this was highly clustered by feedlot. Data on grain processing method, presence of a rumen modifier, metabolisable energy, and roughage content were not analysed due to correlations with grain type, lack of variability between feedlots, missing data and correlations with percentage grain as indicated in Table 4-7.

4.5.10 Exposure variables relating to numbers of animals on feed in the feedlot

Monthly data describing the total number of cattle (not just study animals) on feed in the feedlot (FeedlotN) and the total number that were less than 40 DOF (FeedlotN40) were derived (Table 4-7). The average number of cattle on feed in the feedlot during each study animal's induction month ranged from 950 to 42,230, with a median of 19,926 and an interquartile range from 8,184 to 26,127 (Table 4-3). The numbers less than 40 days on feed during each study animal's induction month ranged from 269 to 15,927 with a median of 5,008 (Table 4-3). Other variables examined included the proportion of cattle on feed that were less than 40 days on feed, the percentage of cattle on feed in the study animal's induction month compared to the average number on feed for the two previous months, and the percentage of cattle less than 40 days on feed in the study animal's induction month compared to the average number less than 40 days on feed for the two previous months. The comparison to the preceding two months was of interest because increases in staffing levels were considered likely to lag behind increases in the number of animals in feedlots. Thus, if there were many more cattle on feed compared to the preceding two months, the number of cattle per staff member was thought likely to increase, potentially impairing feedlot management quality and so increasing risk of BRD. These latter variables were used in exploratory analyses but not in final analyses due to concerns about the quality of the original data received from most feedlots that were used to derive these variables. The 'physically constructed' capacity (i.e. number of cattle that can be

kept on the feedlot given its existing infrastructure) of each feedlot at the time of feedlot enrolment was also collected but was only used in exploratory analyses as it was closely correlated with the total number of cattle on feed and provided less information because it was a single measure for the duration of the study.

4.5.11 Variable quality assessment

The 'quality' of each analysis variable was assessed using five criteria (denoted A to E in Table 4-4 through to Table 4-8) to determine their suitability for inclusion in further analyses.

A. Missingness for a variable was considered a problem when data for all animals in particular feedlots or cohorts were missing or if a large percentage of animals had missing values.
B. Measurement or misclassification errors: potential for these errors was assessed based on whether it was thought that the derived variable was truly representative of each animal's status for the putative risk factor.
C. Distribution by feedlot: the distribution by feedlot related to how evenly the exposure categories were distributed across feedlots (i.e. the 'balance' in the exposure variable with respect to feedlot). Feedlot-level variables, by definition, had the most extreme imbalance, as all animals in the feedlot had the same value for these variables. Because there were only 14 feedlots in the study, there was very limited power to estimate the effect of any of these variables and any estimates may be biased due to confounding by other unmeasured exposure variables, for both feedlot-level variables and lower level variables that were clustered by feedlot. Less severe issues with distributions by feedlot occurred where most feedlots had animals in each exposure category but some feedlots had no animals in particular exposure categories.
D. Sparse categories were considered a potential problem for variables where one or more categories contained less than 3% of animals.
E. Nesting of variables within other exposure variables and correlation between potentially related exposure variables were assessed. As most variables were ordinal, Spearman's correlation coefficients were used to assess correlations. When variables were nested or closely correlated, one variable was selected for inclusion in any one model based on the dataset being analysed and the quality of the available variables. For example, the mixing summary variable (used in subset analyses) was a collapsed version of the more detailed mixing history variable (used in the main cohort analyses).

Variables measured at the animal level with variability within cohorts and feedlots generally had the highest quality for analysis. Some group-level variables displayed a large amount of variability in values within cohorts (e.g. number of animals in group-13), while others were clustered at the cohort or feedlot level (e.g. time taken to reach 60% grain in the ration). Cohort-level variables had reduced power to detect effects in analyses because the number of units (N=170) was much smaller. Many of these variables were also highly clustered by feedlot, meaning that most cohorts within the same feedlot were likely to have the same value. For example, pen shade status only varied between cohorts within three feedlots. Precision of effect estimates for variables clustered by feedlot was very poor. In addition, effect estimates for these variables may be confounded by unmeasured feedlot-level variables or by lower level variables that were markedly clustered by feedlot, and by such covariates that were measured but not included in multivariable models. Rabe-Hesketh and Skrondal (2012) note that effect estimates may suffer from omitted variable bias and state in relation to multilevel mixed effects logistic regression models: "Although the odds ratios are interpreted as effects keeping the subject-specific random intercepts constant, these random intercepts are assumed to be independent of the covariates in the model and hence do not represent the effects of unobserved confounders, which by definition are correlated with the covariates. Unlike fixed effects approaches, we are therefore not controlling for unobserved confounders" (page 530). These authors also discuss the assumptions of random-effects logistic regression modelling. Of these, failure to meet the assumption of independence of the random effect and the covariates is the major one of concern; lack of independence may result in biased effect estimates and so may limit causal inference about such covariates (Rabe-Hesketh and Skrondal, 2012). Although it is unclear whether these points would be restricted to feedlot-level variables, or would also apply to lower level variables, where particular categories of variables have highly unbalanced distributions across feedlots, I have surmised there may be residual confounding even though a random effect of feedlot was fitted. Hence such estimates should be interpreted more cautiously than if observations were distributed across all feedlots. Accordingly, my ability to assess effects of these variables on BRD risk was limited.

Table 4-4: Definition and quality assessment of exposure variables describing animal-entry characteristics, induction treatments and variables derived from vendor questionnaire data

Variable (abbreviation)	Description	Criteria^ A	B	C	D	E	Notes
Animal entry characteristics							
Breed category (Breed)	Animal-level breed category						
Sex (Sex)	Animal-level sex (steer or heifer)			C			In 6 feedlots, all animals were male
Cohort-level sex (Sex cht)	Sex composition of cohort (steers, heifers or mixed)			C			Only 2 feedlots had mixed sex cohorts and only 5 had female cohorts
Intended days on feed (Intended DOF)	Cohort-level anticipated days on feed (<85, 85-<120, ≥120)			C			8 feedlots have no animals <85 DOF
Number of permanent incisors (Dentition)	Number of permanent incisors at induction (0, 2, ≥4)	A					1 feedlot had no data;1 had inferred data
Age at induction (Age)	Estimated average age of arrival group at induction	A					Vendor questionnaire subset; 9% missing data
Induction weight category (Weight)	Animal-level induction weight category (kg)						
Mean cohort weight (Weight cht)	Cohort-level mean induction weight category (kg)						
Difference between induction weight and mean cohort weight (Weight diff)	Animal –level weight difference category (kg)						
Management-related variables from vendor questionnaire data							Restricted to vendor questionnaire subsets
On-farm mixing (Mix VQ)	Group was mixed with other groups on the farm (yes/no)				D		Vendor-bred subset only; 94% of animals were mixed
Prior grain feeding (Grain pre)	Group ever fed grain on the farm (yes/no)	A					Vendor-bred subset only
Prior supplement feeding (Supp pre)	Group ever fed conserved forage or supplements on the farm (yes/no)	A					Vendor-bred subset only
Yard weaning and duration (Yard wean)	Yard weaning involves keeping cattle in small yards after weaning for variable time periods (no, <7 days, ≥7 days)						Vendor-bred subset only
Prior vaccination with Pestigard™ (PV_vacc)	At least one dose of Pestigard™ vaccine (against BVDV) administered prior to day -14						Prior vaccination subset (vendor bred or purchased by 10 months)
Prior vaccination with Bovilis MH™ (BV_vacc)	At least one dose of Bovilis MH™ vaccine (against *M. haemolytica*) administered prior to day -14						Prior vaccination subset (vendor bred or purchased by 10 months)
Induction treatments							
Rhinogard™				C			Completely clustered by feedlot
Vit ADE				C			Completely clustered by feedlot

^Criteria: A: Missingness, B: Accuracy, C: Distribution by feedlot, D: Sparse categories, E: Correlations between exposure variables

* Not used in any analyses

Table 4-5: Definition and quality assessment of exposure variables derived from the NLIS data describing group dynamics

Variable (abbreviation)	Description	Criteria^ A	B	C	D	E	Notes
Group size							
Number of animals in group-# (Group-#N)	Number of cattle in the group defined # (i.e.13, 28 or 91) days before day 0 (<50, 50 to 99, ≥100)					E	Numbers of animals in groups at different time points were highly correlated; only one variable fitted at a time
Mixing							
Time of earliest mixing (Mix first)	Time interval during which animal was first mixed (pre day -90, day -90 to -28, day -27 to -13, day -12 to 0, never mixed)					E	Correlated with Mix history
Lifetime mixing history (Mix history)	Composite 12-category variable describing mixing pre day -27 (yes/no), from day -27 to day -13 (yes/ no) and the number of group-13s forming the cohort (1, 2 or 3, 4 to 9, ≥10)					E	Correlated with Mix first & Mix summary
Mixing history summary (Mix summary)	Collapsed version of Mix history; composite of mixing pre day -27 (yes/no) and number of group-28s forming cohort (<4, ≥4)					E	Nested within Mix history; for use in subset analyses
First mixing composite variable (Mix first summary)	Composite of Mix first and Mix summary; similar to Mix summary but animals first mixed between days -27 and -13 are in a separate category					E	Correlated with Mix first & Mix summary; use to investigate category first mixed from day -27 to -13
Saleyard exposure							
Saleyard transfer prior to day -27 (SY pre-27)	Animal had been through saleyards at least once prior to day -27 (yes/no)						
Saleyard transfer in interval from day -27 to day -13 (SY -27 to -13)	Animal had been through saleyards at least once between days -27 and -13 (yes/no)				D		Only 2.8% of animals were coded yes
Saleyard transfer in interval from day -12 to day 0 (SY -12 to 0)	Animal had been through saleyards at least once between days -12 and 0 (yes/no)				D		Only 2.7% of animals were coded yes
Feedlot move timing							
Days between arrival and day 0 (Arrival to day0)	Time category during which animal moved to the feedlot PIC (pre day -27, day -27 to day -13, day -12 to day 0) between arrival and day 0 (>28, Arrival to day0)					E	Nested in feedlot move timing; used in preassembly subset
Timing and duration of move to the feedlot (Move_FL)	Composite variable describing the timing and duration of animal's move to the feedlot (pre day -27, day -27 to day -13, <6 hours from day -12 to -2, ≥6 hours from day -12 to -2, <6 hours from day -1 to 0, ≥6 hours from day -1 to 0)			C			Only 4 feedlots had observations in first 2 categories

^Criteria: A: Missingness, B: Accuracy, C: Distribution by feedlot, D: Sparse categories, E: Correlations between exposure variables

Table 4-6: Definition and quality assessment of exposure variables relating to cohort formation, BVDV and home pen characteristics

Variable & abbreviation	Description & categories	Criteria^ A	B	C	D	E	Notes
Cohort formation							
Number of animals in cohort (CohortN)	Cohort-level total number of animals inducted into the cohort (<200, ≥200)						Only 2 categories used because it was clustered by feedlot
Duration of cohort fill time (Cohort fill)	All animals inducted into the cohort on a single day (1) or animals inducted over more than one day (>1)						
Days between first day on feed and day 0 (DOF1 to day0)	First day on feed is: same as day 0 (0), 1 or 2 days before day 0 (1 to 2) or three of more days before day 0 (≥3)			C			8 feedlots had only zero values.
Days from day 0 to cohort close (Day0 to close)	Number of days from day 0 to cohort close date (0, 1 to 6, ≥7)						
BVDV							
PI animal (BVDV_PI_animal)	Animal is persistently infected with BVDV (yes/no)						
BVDV active in cohort (BVDV_cht)	BVDV detected in any sample from an animal in the same cohort (yes/no)					E	Correlated with BVDV_grp_cht
BVDV status of animal's group-28 and cohort (BVDV_grp_cht)	Composite variable describing whether a PI animal was identified in the animal's group-28 and whether BVDV was active in the cohort (no no, yes yes, or no yes)					E	Correlated with BVDV_cht
Home pen characteristics							
Shared pen water (Pen water)	Pen water could be accessed by animals from an adjoining pen (yes/no)			C			8 feedlots have no 'nos'
Number of SCUs per square metre (Pen density)	Calculated from pen area and total standard cattle units (SCUs) derived from the animal level induction weight and table of SCU values (m^2/SCU)				D		5-7 feedlots had no observations in some categories, but good distribution by feedlot overall
Presence of shade in pen (Pen shade)	Part of pen was shaded (yes/no)			C			Highly clustered by feedlot; only 3 feedlots had disparate cohorts
Number of adjoining pens (Pens joining)	Number of pens adjoining home pen and separated by only a fence (1 or 2)						
Bunk space per head (Bunk space)	Calculated from dimensions of the feed bunk and number of animals inducted into the cohort (m/head)	A			D		9 feedlots no observations in lowest category
Pen distance to hospital*	Distance between home pen and hospital pen			C		E	Correlated with feedlot size/capacity
Pen slope*	Slope of home pen	A					
Pen cleaning frequency*	Number of times pen cleaned per year		B	C			Frequency per year does not measure intended cohort level timing
Pen riding frequency*	Frequency of pen riding (i.e. inspection of cattle in the feedlot pen)			C			Little variation between feedlots

^Criteria: A: Missingness, B: Accuracy, C: Distribution by feedlot, D: Sparse categories, E: Correlations between exposure variables; * Not used in any analyses

Table 4-7: Definition and quality assessment of exposure variables relating to rations and numbers of cattle on feed at the feedlot

Variable & abbreviation	Description & categories	Criteria^ A	B	C	D	E	Notes
Ration variables							
Grain type	Type of grain in ration (barley, sorghum, wheat mix or other mix)			C			Highly clustered by feedlot; only 3 feedlots varied grain type between cohorts
Day until 60% grain (Grain 60%)	Number of days from first day on feed until 60% grain in ration on an 'as fed' basis			C			No observations for 7 feedlots in each of two categories
Percentage grain on day 0 (Grain1)	Percentage of grain in ration on day 0 (usually the first day on feed)			C			Highly clustered by feedlot
Percentage grain on day 20 (Grain21)	Percentage of grain in ration on day 20 (usually the 21st day on feed)			C			Highly clustered by feedlot
Rumen Modifier*	Indicated if rumen modifier was added to the ration and the type used			C			Feedlot level; little variation amongst feedlots
Metabolisable Energy (ME)*	ME of ration measured by ration analyses	A		C			Missing values for many animals
Roughage percentage*	Percentage of ration that was roughage	A		C		E	Inconsistent definitions used in original data; correlated with grain%
Grain: roughage ratio*	Ratio of grain to roughage in the diet	A		C		E	Correlated with grain%
Grain processing method*	Method used to process grain			C		E	Completely clustered by feedlot, correlated with grain type
Numbers on feed							
Total on feed (FeedlotN)	Average total number of cattle on feed in the feedlot in the animal's induction month	A		C		E	Highly clustered by feedlot, correlated with capacity
Total <40 days on feed (FeedlotN40)	Average total number of cattle less than 40 days on feed in the feedlot in the animal's induction month	A		C		E	Highly clustered by feedlot, correlated with FeedlotN
Proportion <40 days on feed*	Proportion of cattle less than 40 days on feed in the feedlot in the animal's induction month			C			7 feedlots with no observations in first category
Proportion on feed compared to total on feed during the previous 2 months*	Total on feed compared to the average total number of cattle on feed in the feedlot in the 2 months preceding the animal's induction month	A	B	C		E	Monthly data do not give required detail to estimate short-term changes, correlated with FeedlotN
Proportion <40 days on feed compared to previous 2 months*	Average total number of cattle less than 40 days on feed in the feedlot compared to the average total less than 40 days on feed in the 2 months preceding the animal's induction month	A	B	C		E	Monthly data do not give required resolution to estimate change
Feedlot capacity*	Physically constructed capacity of the feedlot (SCU)		B	C		E	Correlated with number on feed

^Criteria: A: Missingness, B: Accuracy, C: Distribution by feedlot, D: Sparse categories, E: Correlations between exposure variables.
* Not used in any analyses

Table 4-8: Definition and quality assessment of exposure variables relating to environmental exposures (region, induction timing and weather)

Variable & abbreviation	Description & categories	Criteria^ A	B	C	D	E	Notes
Location							
Location of cattle on day -28 (Source region)	Broad regions grouped by similar geography and climate describing location at day -28			C			Most categories have feedlots with no observations
Location of feedlot (Feedlot region)	Qld (north); NSW, WA or SA (south)			C			Completely clustered by feedlot
Time of induction							
Induction season (Season)	Season during which animal inducted (spring, summer, autumn, winter)						
Month of induction (Calendar month)*	Month in which animals were inducted					E	Nested within season
Year and month of induction (Year month)*	Year and month in which animals were inducted				D		
Induction year	Year in which animals were inducted (2008, 2009, 2010)						
Weather variables							
Mean daily maximum temperature in first week from day 0 (Temp max)	Mean maximum temperature derived from averaging daily interpolated data. (°C)					E	Correlation between maximum & minimum temperature
Mean daily minimum temperature in first week from day 0 (Temp min)	Mean minimum temperature derived from averaging daily interpolated data. (°C)					E	
Mean daily range in temperature in first week from day 0 (Temp range)	Mean temperature range derived from averaging daily data (°C)						
Total rainfall in the first week from day 0 (Rain)	Derived from totalling daily rain (mm) from interpolated data						
Mean maximum wind speed in first week from day 0 (Wind)	Mean maximum wind speed (km/h) derived from nearest weather station recording wind speed data (km/h)		B			E	Wind data may not be representative as often measured a long way from feedlots
Wind run below 3 metres*	Total wind run (km) obtained from nearest weather station recording wind run data	A	B			E	Wind data thought not representative as measured a long way from feedlots

^Criteria: A: Missingness, B: Accuracy, C: Distribution by feedlot, D: Sparse categories, E: Correlations between exposure variables.
* Not used in any analyses. Abbreviations: Queensland (Qld). New South Wales (NSW), Western Australia (WA), South Australia (SA)

4.5.12 Exposure variables describing date of induction, source and feedlot regions and weather

4.5.12.1 Timing of the induction date

The calendar timing of the induction date was categorised by calendar month, year-month, year and season (Table 4-8). Because calendar month and year-month had many categories and there were no clear associations between either of these and risk of BRD in exploratory analyses, these were not included in the final analyses. Calendar month was also nested in season; season was chosen as the better quality variable. Final induction timing variables included induction season and year (Season and Induction year; Table 4-8)

4.5.12.2 Weather in the first week after day 0

Because weather can change markedly over short time periods and may affect risk of BRD after a relatively short lag period, the most appropriate methods to examine the effects of these variables would be within a time-varying modelling framework such as survival analysis, or by using a case-crossover design. These analyses were beyond the scope of this project. It was however possible to include crude weather variables within the modelling framework used. Because any effects of weather were hypothesised to have a lagged effect on the risk of BRD and the peak incidence of BRD observed in the study was between two and four weeks on feed, weather variables were derived (Table 4-8) based on observations during the first week after induction for each animal (i.e. observations from days 0 to 6). Original temperature data were approximately normally distributed. The mean maximum daily temperature averaged over days 0 to 6 ranged from 12°C to 37°C, with a median of 23°C, while the mean daily minimum temperature ranged from -2°C to 22°C with a median of 9°C (Table 4-3). Categorical variables were derived, each with four categories, to describe mean maximum (Temp_max) and minimum (Temp_min) temperatures and temperature range (Temp_range) with each categorised into four categories based on their distributions. Although maximum and minimum temperatures were correlated, both were of *a priori* interest so both were retained in analyses. Rainfall had a positively skewed distribution, so the most frequent category (no rain) was the base category for this variable (Rain), with the three remaining categories determined from the distribution. Weather variables were clustered by cohort. The quality of wind data was questionable because the nearest weather station recording

these data was often a long way from the feedlot location; few of the source sites for maximum wind speeds (six feedlots) and wind run data (three feedlots) were within 30 km of the feedlot and wind data from weather stations many kilometres from the feedlot were unlikely to be representative of wind conditions at the feedlot. However, because the effect of wind was of *a priori* interest, a categorical variable was derived (Wind; Table 4-8) describing the average maximum wind gust speed from days 0 to 6. A high percentage of wind run data was missing so this variable was not examined in further analyses.

4.5.12.3 Source and feedlot regions

The source region (Table 4-8) for each animal was determined by the geographical coordinates of its PIC location on day -28. Six source region categories (based on the distribution of observations in the study populations and across feedlots, proximity and similarity in geography and weather patterns) were used in final analyses. The source regions are illustrated in Figure 4-3. A comparison of this map with those illustrated in Figure 4-4 and Figure 4-5 reveals that the majority of the cattle came from the more densely populated cattle-producing regions; hence the relative areas of the regions varied markedly. Participating feedlots were predominantly located in major grain-producing regions. These were grouped into two broad categories to derive the feedlot region variable (Table 4-8); north described feedlots located in the Darling Downs region of Queensland (Qld) and south described feedlots located at latitudes south of the New South Wales/Queensland border (below latitude 29°S). Southern feedlots were mainly located in the Riverina region of New South Wales with one in the southeast of South Australia (SA) and one in the southwest of Western Australia (WA). The locations of the participating feedlots are shown in Figure 4-4

4.6 Description of the study population

Descriptive statistics of the study population which directly informed the derivation of exposure variables were detailed above. Further characteristics of the study population are described in the following section.

4.6.1 Spatial distribution of the study population

Animals were sourced from a range of Australian geographical regions as described above. The spatial distributions of PICs of origin and PIC locations of group-13s are displayed in Figure 4-4.The majority of study cattle originated (i.e. based on the PIC of origin) from New South Wales (51% or 17,641/34,730), Queensland (18% or 6,392/34,730) and Victoria (12% or 4,107/34,730). About 8% originated from the Northern Territory (NT), 6% from Western Australia, 4% from South Australia and 1% from Tasmania. It is clear that animals generally were located closer to the feedlots at day -13 compared to the PICs of origin, but some animals were transported very long distances to the destination feedlots. On day -28, 48% (16,790/35,131) were in New South Wales and 29% (10,035/35,131) were in Queensland. On day -13, 46% (16,250/35,131) were in New South Wales and 30% (10,580/35,131) were in Queensland. The majority of animals (62% of 21,789/35,131) were in southern feedlots. Five of the fourteen participating feedlots sourced cattle from a single source region (as defined at day -28).

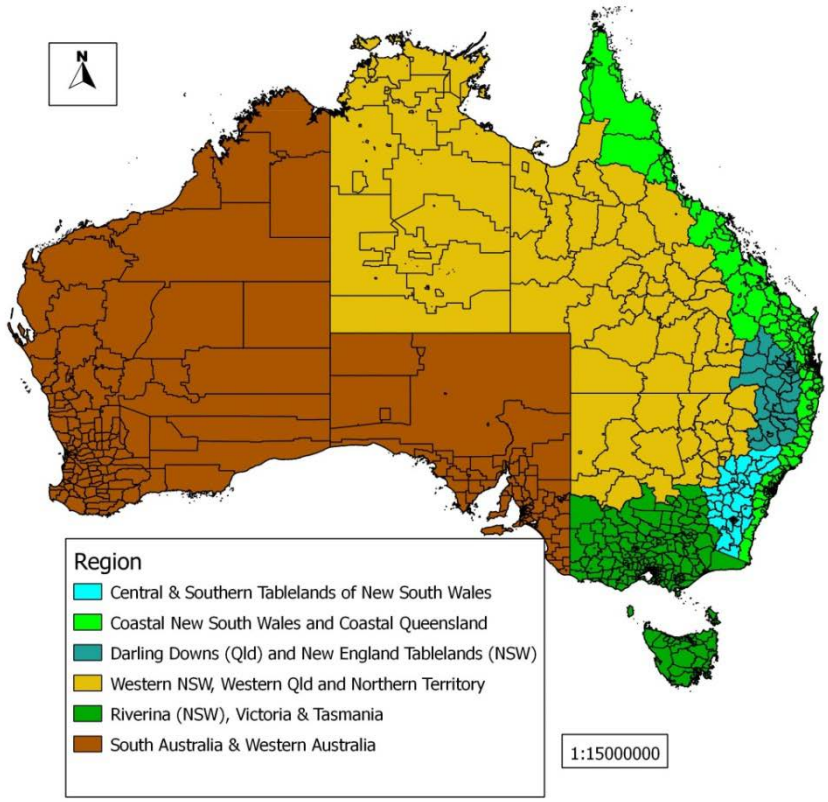

Figure 4-3: Classification of source regions defined 28 days prior to animals' induction into study cohorts.
Regions crossing state borders include state abbreviations for New South Wales (NSW) and Queensland (Qld)

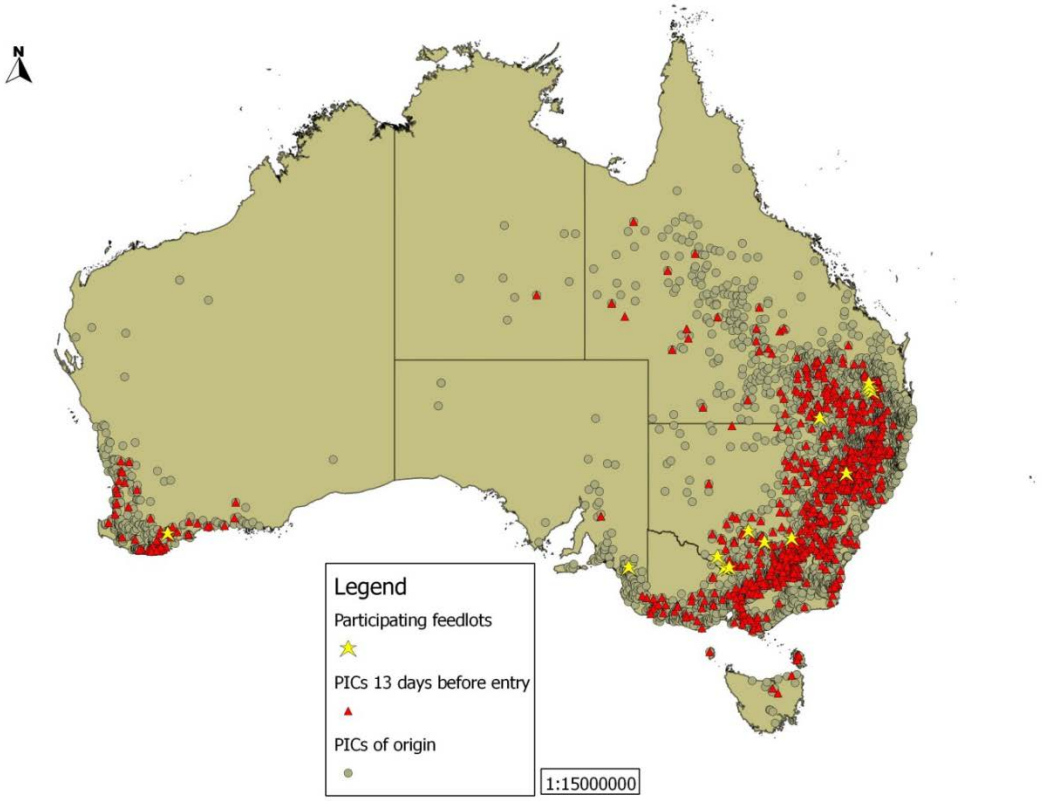

Figure 4-4: Locations of participating feedlots, PICs of origin and PIC-13s (i.e. locations defining original groups and group-13s respectively

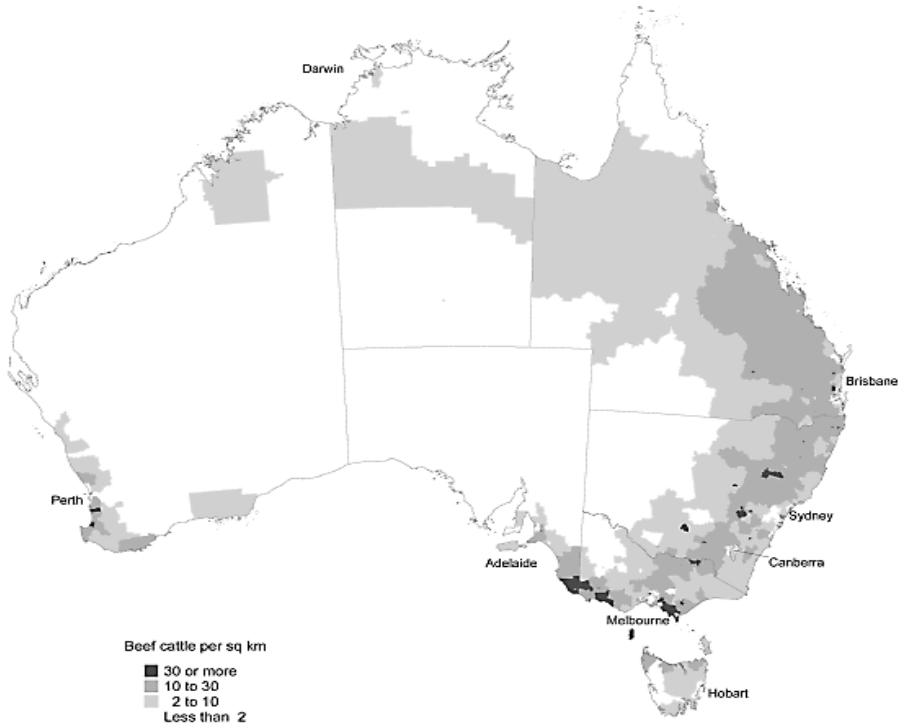

Figure 4-5: Beef cattle density in Australia based on 2000-2001 Agricultural Census data
Source: (ABS, 2005)

4.6.2 Temporal distribution

Study animals were inducted from March 2009 to December 2011. Six of the fourteen feedlots enrolled animals in each of the three years and four feedlots enrolled cattle in only a single year. Animals were inducted in all seasons, with similar proportions inducted in each season (ranging from 20.6% (7,235/35,131) in summer to 28.5% (10,019/35,131) in winter).

4.6.3 Group dynamics

As outlined in Section 4.5.5 and detailed in Appendix 1 (Section 14.4), NLIS data were used to derive analysis variables to describe mixing history, group size, saleyard exposure and the timing of the move to the feedlot. Relevant summary statistics were presented in Section 4.5.5 above. Important additional information was derived from the NLIS data, allowing a more in-depth understanding of group dynamics within the study population. The distributions of continuous variables contributing to this understanding are shown in Table 4-9. The majority of groups comprised a small number of animals and distributions of the numbers of animals per group were positively skewed. Median group size ranged from two (group_origin) to 18 (Group-13) (Table 4-9). Many such groups often formed a cohort. When summarised by group, the median number of original groups forming a cohort was 58, the median number of group-28s was 13 and for group-13s it was 11 (Table 4-9).

However, some groups contained many animals, so when summarised by animal, the median number of animals in the animal's group ranged from 21 (group_origin; Table 4-9) to 64 (Group-13; Table 4-3). Similarly, the median number of group-13s or group-28s forming an animal's cohort was seven, while the median number of group_origins was 21(Table 4-9).

The number of groups defined by the animals' PICs of origin (i.e. their original groups) was 6,234 (Table 4-9). These groups were from 4,848 PICs; some PICs contributed animals to more than one cohort over the course of the study. The number of group-13s was 1,077. The geographical locations of the PICs determining the original groups and group-13s are illustrated in Figure 4-4.

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The median number of lifetime PICs (excluded PICs that the animal transited through for less than 48 hours; most commonly saleyard PICs) was three, and ranged from two to 10 (Table 4-8). Of animals with a single transfer (from the PIC of origin to the feedlot), 10% (1,421/14,091) moved to the vicinity of the feedlot prior to day -27, 6% (880/14,091) moved between days -27 and -13 and 84% (11,790/14,091) moved 12 days or less before induction. For animals transported to the feedlot 6 days or less before day 0, the median estimated distance was 310 km (range: 13 to 2,530 km, interquartile range:190 to 422 km). For animals transported to the feedlot between 12 days and 7 days before day 0, the median estimated distance was 367km (range: 15 to 1,334 km, interquartile range:130 to 679km).

For the 70% (24,656/35,125) of animals within the 52% (565/1,077) of group-13s that had been mixed prior to day -12, the median length of time the animal's group-13 was stable (i.e. no study animals entered or left in the time interval) was 169 days (Table 4-9). For the remaining 30% (10,469/35,125) of animals from 48% (512/1,077) of group-13s, the original group and the group-13 were equivalent; these animals had only two lifetime PICs (i.e. PIC of origin and feedlot PIC) and were assumed to have been in stable groups for life and to have not mixed prior to day -12.

Table 4-9: Summary statistics for additional continuous variables derived from the NLIS data relating to group dynamics. Units may be animals, groups or cohorts depending on variable.

Variable	Total number of units	Median	Range	Interquartile range
Number of animals in animal's original group	34,730	21	1 to 256	6 to 55
Number of animals in animal's group-28	35,131	59	1 to 342	31 to 115
Duration of animal's stable group-13 (days)	24,677	169	2 to 865	64 to 299
Number of lifetime PICs per animal	34,730	3	2 to 10	2 to 3
Days from day 0 to transfer before transfer to the feedlot	20,666	272	1 to 2,129	175 to 372
Distance transported for animals moved to the feedlot vicinity from day -6 to 0 (km)	30,175	310	13 to 2,530	190 to 422
Distance transported for animals moved to the feedlot vicinity day -12 to -7 (km)	1,279	367	15 to 1,334	130 to 679
Size of original group	6,234	2	1 to 256	1 to 5
Size of group-28	1,264	14	1 to 342	4 to 38
Size of group-13	1,077	18	1 to 342	6 to 43
Size of cohort	170	186	17 to 395	140 to 280
Number of original groups in cohort	6,234	58	1 to 138	32 to 81
Number of group-28s in cohort	1,264	13	1 to 29	7 to 17
Number of group-13s in cohort	1,077	11	1 to 25	7 to 16

Figure 4-6 illustrates the animal-level distribution of the duration of a stable group-13 (i.e. before and after day-13) for animals in the main cohort study population that had been mixed prior to day -12. There are separate graphs for animals that were/were not in the vicinity of the feedlot on day-13 (i.e. had been moved to the feedlot PIC before or on day -13). More than 75% (18,630/24,656) of these animals were in group-13s that were stable for at least two months. Although the most frequent category contained animals that were in a stable group-13 for less than one month, this category mainly comprised animals that had been preassembled at the vicinity of the feedlot.

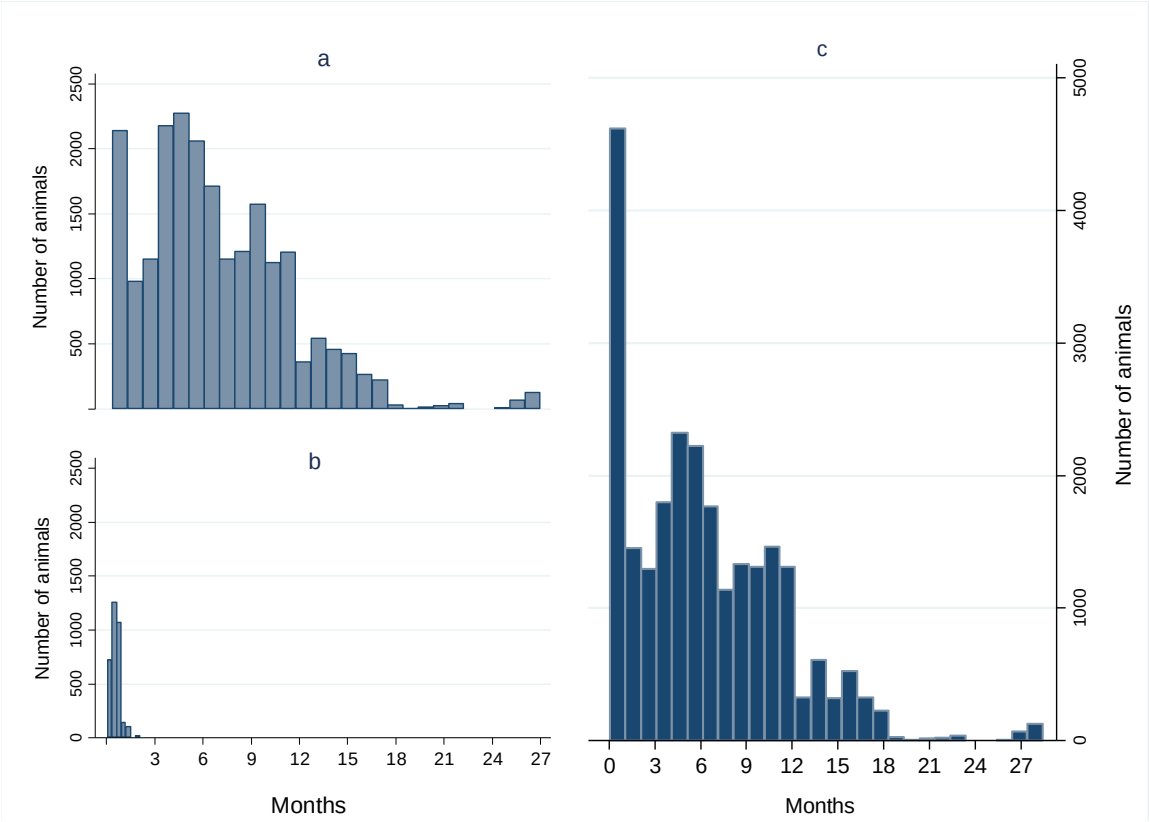

Figure 4-6: Histograms displaying duration of group-13 stability (i.e. before and after day-13) for animals that had mixed prior to day -12.
Animals not at the feedlot PIC (a) are displayed separately from those that were in the vicinity of the feedlot on day -13 (b); these are then combined (graph c)

4.7 Causal Diagram

A causal diagram was developed with postulated interrelationships between proposed direct and indirect causes of BRD (Figure 4-7). This diagram visually depicts all proposed causal pathways between exposure variables of interest, and between these and BRD. Variables were included in the diagram only if they were of

adequate quality. Some of the variables included in Figure 4-7 were used in subset analyses; for example, those used in the vendor questionnaire subsets are within ellipses and those used in the case-control analyses are within boxes.

Each arrow in the causal diagram depicted a hypothesised causal pathway in which one variable (the variable from which the arrow starts) might at least partly determine the status of another (the variable to which the arrow points). This type of diagram is also known as a directed acyclic graph because each pathway is constrained to one direction only (i.e. no double-headed arrows are allowed and any two variables can be directly related by only one arrow). Direct pathways are those where the variables are linked by an arrow that passes directly from one to the other and not via any other variable that was included in the diagram. A variable with a pathway directly to BRD depicts a direct effect of that variable. Direct pathways do not indicate that there is, in reality, no intervening variable; rather, they simply indicate that none of the variables included in the diagram are intervening. These pathways are numbered (in boxes), with the justifications provided in Table 4-10 referencing the relevant sections of the literature review (Chapter 1). Indirect pathways are those where a variable is linked to another via one or more intervening variables; depicted as a sequence of arrows so the pathway can be traced passing through these intervening variables by following the sequence of arrows in the correct direction. There may be multiple indirect pathways from any particular variable to any other particular variable. Effects mediated in this way are known as indirect effects. Table 4-11 gives a summary of the pathways (numbered in ellipses) between variables and the rationale or justification for the presence and direction of arrows is provided. The total effect of a variable on BRD is the sum of the direct and all the indirect effects for that variable on BRD.

The diagram was constructed after examining the evidence from the literature, considering industry opinion and assessing biological plausibility of pathways. In addition, the direction of some arrows was based on the temporal sequences of the hypothesised effect. In some instances, crude associations using the cohort study dataset were assessed before a pathway was drawn in the diagram. In a few instances, there were logical causal arguments for having arrows in either direction so both variations of the diagram were considered in the modelling process. For example, the variable ‘intended days on feed’ is closely linked to weight, breed, sex

and dentition. From a temporal perspective, weight, breed, sex and dentition are determined before the animal arrives at the feedlot and its category of intended days on feed chosen, so arrows should go from weight, breed, sex and dentition to intended days on feed, as shown in Figure 4-7. However, feedlot personnel may decide first to assemble a cohort with animals in a particular ‘intended days on feed’ category, so would then choose to buy animals of specific weight, breed, sex and dentition. In this case, arrows from intended days on feed to weight, breed, sex and dentition would better represent the causal pathway based on the temporal sequence in decision making.

This diagram was used to inform the total and direct effects modelling processes. When causal diagrams are used to inform variable selection for analyses, failure to include a pathway is a stronger claim than including pathways that are potentially true (Shrier and Platt, 2008). Accordingly, some pathways that were biologically plausible but for which there was little additional evidence were included. The justifications provided for these pathways in Table 4-10 and Table 4-11 are shown as ‘?’.

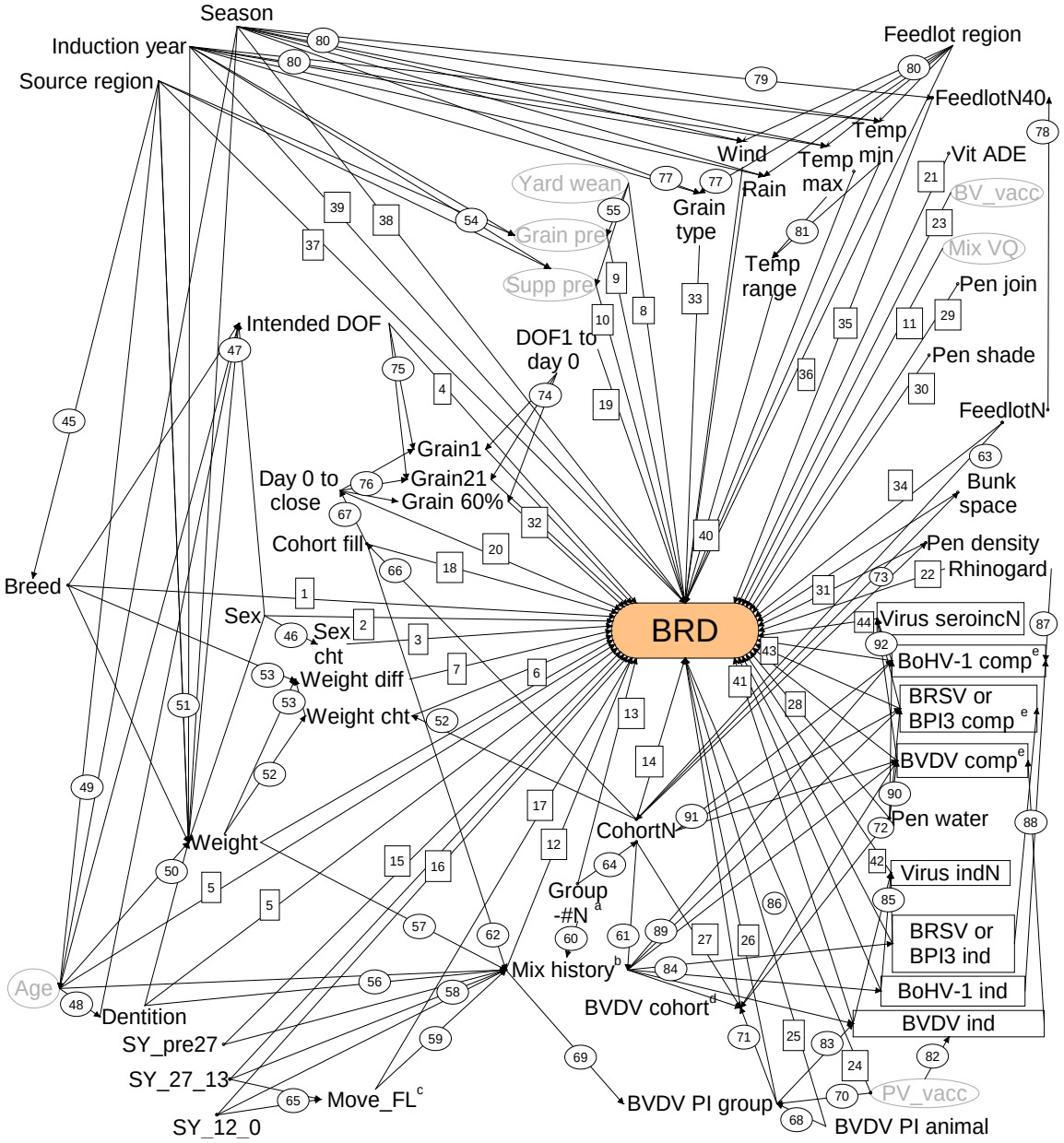

Figure 4-7: Causal diagram depicting proposed pathways linking putative risk factors with BRD.

Variables measured in the vendor-questionnaire subsets are enclosed in ellipses and those from the case-control subset are shown in boxes. Variables with superscripts (a-e) had equivalent substitute variables that were used in some analyses (e.g. collapsed version used in subset analyses):

[a]Group-#N: Group-28N or Group-13N

[b]Mix history, Mix summary, Mix first, Mix first summary

[c]Move_FL, Arrival to day 0

[d]BVDV cohort, BVDV_grp_cht, BVDV_grour-28_PI

[e]BVDV comp, BVDVserocon (equivalent for each virus)

Table 4-10: Brief justification for and description of variables hypothesised as directly causing BRD in the causal diagram and relevant literature review section

Path	Variable description	Summary of prior evidence and/or hypothesised effects on risk of BRD	Reference section
1	Breed: breed category	Herefords and British breeds at increased risk	1.7.1.1
2	Sex: heifer or steer	Males are probably at increased risk	1.7.1.2
3	Sex cht: sex mix at the cohort level	Mixed sex cohorts may be at increased risk	
4	Intended DOF: Estimated days on feed at induction	Risk varies with cattle class, possibly even after adjusting for weight, breed and sex	?
5	Dentition: number of permanent incisors	Dentition is a proxy for age.	1.7.1.3
	Age: estimated age at induction in vendor questionnaire subset	Younger animals at increased risk	
6	Weight: Induction weight	Lighter induction weight associated with increased risk	1.7.1.3
	Weight cht: mean cohort induction weight	Increased risk for cohorts with lower mean weight	
7	Weight diff: Individual difference from cohort mean induction weight	Animals that are lighter than the mean cohort induction weight may be at increased risk	?
8	Yard weaning: Yard weaned as reported in vendor-bred subset	Yard weaning reduces risk of BRD?	1.7.2.1
9	Grain_pre: Prior grain feeding history	Prior feeding of grain, conserved forage or supplements may reduce risk because animals adapt more quickly to feedlot rations	1.7.2.1
10	Supp_pre: Prior feeding of conserved forage or supplement		
11	Mix_VQ	On farm mixing may reduce risk	
12	Mix history: composite variable describing lifetime mixing up until cohort close	Increased risk with commingling immediately prior to induction.	1.7.3.2
	Mix summary: collapsed version of mix history	Reduced risk with backgrounding where one component is commingling several weeks prior to induction	
	Mix first: time of earliest mixing		
13	Group-#N: Number of animals in a group # days before day 0	Having more animals assembled at least 13 days prior to entry is protective?	1.7.3.2
	Separate variables used for #=-13, -28 & -91		
14	CohortN: Number of animals in a cohort	Increased risk with increased cohort size	
15	SY_pre27: saleyard transfer prior to day -27	Reduced risk through commingling at that time	?
16	Saleyard transfers in time intervals:	Cattle sourced from auction sales at increased risk compared to ranch sourced cattle	1.7.3.2
	SY_27_13: between day -27 & day -13		
	SY_12_0: between day -12 & day 0		
17	Move_FL: Composite variable describing timing and duration of move to feedlot	Increased risk with longer transport distances immediately prior to induction	1.7.3.3
	Arrival to day 0: Number of days between arrival and day 0	Moving to the vicinity of the feedlot at least 28 days before induction may be protective	
18	Cohort fill: Cohort fills on a single day or not	Open cohorts are at higher risk but this may be due to the effects of increased commingling in open cohorts	1.7.3
19	DOF1-day 0: Days from animal level first day on feed to day 0	Animals with longer adaption time may be expected to have reduced risk	1.7.3
20	Day 0 to close: Days from induction to cohort close	Longer adaptation time to ration change and pen density may reduce risk	
21	VitADE: vitamin A, D & E injection given at induction	Studies have not demonstrated efficacy against BRD	1.7.3.4
22	Rhinogard: Rhinogard vaccine given at induction	Vaccination against BoHV-1 at induction may reduce risk but prior evidence is equivocal	1.7.3.1
23	BV_vacc: Prior vaccination with BovilisMH vaccine	Prior vaccination with BovilisMH reduces risk of BRD	1.7.2.2
24	PV_vacc: Prior vaccination with Pestigard	Prior vaccination with Pestigard reduces risk of BRD	1.7.2.2

Path	Variable description	Summary of prior evidence and/or hypothesised effects on risk of BRD	Reference section
25	BVDV_PI_animal: animal is a PI	PI animals are at increased risk of BRD	1.7.4.1
26	BVDV_PI_group: PI animal in group-28	Prior exposure to a PI in the group-28 may reduce risk	
27	BVDV cohort- one of the following:		1.7.4.1
	BVDV_cht_YN: BVDV active in cohort	Presence of BVDV in cohort increases risk	
	BVDV_PI_cht: PI in cohort	Presence of a PI animal in the cohort increases risk	
	BVDV_PI_grp_cht: Composite variable describing if a PI was in the group-28 or if BVDV was active in the cohort	Animals previously exposed to a PI in the group-28 may be at reduced risk relative to those not exposed	
28	Pen water: binary variable indicating if pen water can be accessed by animals outside of home pen	Shared pen water may increase risk of BRD through increased exposure to pathogens	1.7.4.3
29	Pen join: number of pens joining home pen	More joining pens may increase risk through increased exposure to pathogens	
30	Pen shade: pen shade & area of shade per standard cattle unit	Pen shade may reduce risk of BRD indirectly through reducing heat load stress	1.7.4.3
31	Pen density: pen area per standard cattle unit at cohort close	Higher pen density and lower bunk space may increase risk trough increased stress and increased exposure to pathogens	
	Bunk Space: linear bunk space per head at cohort close		
32	Grain1: grain percentage in ration on day 0	High grain ration increases risk especially if introduced rapidly	1.7.4.2
	Grain21: grain percentage in ration on day 20		
	Grain60pc: Days from day 0 until ration contains 60% grain		
33	Grain type: Type of grain in ration	Rate of grain fermentation in rumen increases risk?	
34	FeedlotN: Estimated average total cattle on feed in induction month	More animals on feed increase risk?	1.7.4.4
35	FeedlotN40: Estimated average total cattle <40days on feed in induction month	More animals less than 40 days on feed increases risk?	
	Feedlot region: location of feedlot		1.8.3
37	Source region	BRD risk varies with source region?	1.8.3
38	Season: season of induction	Increased risk in autumn	1.8.1
39	Induction year: year of induction	Risk differs with year of birth	
40	Weather variables averaged from day 0 to 6	Increased risk with any conditions causing environmental stress such as lower minimum daily temperature, increased daily temperature range or higher maximum daily temperature	1.8.2
	Temp max: mean daily maximum temperature		
	Temp min: Mean daily minimum temperature		
	Temp range: Mean daily temperature range		
	Rain: total rainfall	Hypothesised interactions between rainfall, wind speed and temperature. E.g. Cold wet windy conditions expected to increase risk	
	Wind: Mean wind speed		
41	BoHV1 ind, BPI3 ind, BRSV ind, BVDV ind: Induction serology status for virus indicated	Low induction titre to BVDV and BoHV1 increases risk Low induction titre to BPI3 and BRSV may increase risk	1.5.2
42	Virus indN: number of viruses seropositive to at induction		1.5.2
43	BoHV1 comp, BPI3 comp BRSV comp, BVDV comp: Change in serostatus between induction and follow-up at approximately 42 days	Increasing BVDV titre associated with increased risk of BRD	1.5.2
44	Virus seroincN: Number of viruses animal seroincreases to between induction and follow-up		

Table 4-11: Brief justification for and description of pathways hypothesised as connecting exposure variables in the causal diagram

Path	Variables in path(s)	Justification
45	**Source region** → Breed*	Particular breeds are more suited to or popular in different regions
46	**Sex** → **Sex cht**	The gender mix in the cohort depends on the individual gender
47	**Breed** → Intended DOF* **Sex** → **Intended DOF*** **Dentition** → **Intended DOF*** **Age** → Intended DOF **Weight** → Intended DOF*	Intended days on feed is related to entry characteristics of cattle which depending on the feedlot may be based on weight, gender, breed and age (or dentition) of the animals in addition to their condition score which has not been measured
48	**Age** → Dentition	Dentition is dependent on age
49	**Source region** → Age Season → **Age**	
50	Dentition → Weight Age → Weight	Weight is correlated with age
51	**Season** → **Weight** Induction year → **Weight*** **Source region** → Weight*	Age and weight at induction may vary depending of the source region, season
52	Weight → Weight cht CohortN → Weight cht	The average cohort weight depends on the individual weight and the number in the cohort.
53	Weight cht → Weight diff Weight → Weight diff Breed → Weight diff	The Individual difference is dependent on the breed, individual weight and the average cohort weight
54	**Induction year** → **Grain_pre** **Induction year** → **Supp_pre** **Source region** → **Grain_pre** **Source region** → **Supp_pre**	Prior supplementary feeding depends on source region and year
55	**Yard weaning** → **Grain_pre** Yard weaning → **Supp_pre**	Feeding conserved forage or grain is part of the yard weaning protocol
56	Dentition → Mix history* Age → Mix history	Older animals (dentition) would have had more opportunity for mixing
57	Weight → Mix history*	
58	SY_pre27 → Mix history* SY_27_13 → Mix history* SY_12_0 → Mix history*	Animals having saleyard transfers are more likely to be mixed with other groups
59	Move_FL → Mix history*	The timing of the feedlot move will likely influence mixing history
60	Group-#N → Mix history	If there are less animals in a group it is likely more groups are mixed
61	CohortN → Mix history*	To have a larger cohort it is likely more groups were mixed
62	Cohort fill → Mix history*	If a cohort is open it is likely that more groups are mixed upon induction
63	FeedlotN → CohortN*	
64	Group-#N → CohortN*	
65	SY_12_0 → Move_FL* SY_27_13 → Move_FL*	Saleyard transfer requires that animals are moved. For transfers within the last month it will influence total transport time to the feedlot
66	**CohortN** → **Cohort fill***	The cohort close pattern can be determined by the required cohort size
67	**Cohort fill** → **Day 0 to close**	
68	**PI animal** → **BVDV PI group**	
69	**Mixing** → **BVDV PI group**	The presence of a BVDV PI animal within a group-28 or cohort will be more likely with increased mixing
70	**PV_vacc** → **BVDV PI group**	
71	**BVDV PI group** → **BVDV cohort** **Mixing history** → **BVDV cohort** **BVDV PI group** → **BVDV cohort**	A BVDV-PI in a cohort means there must have been a PI animal in at least one group forming the cohort
72	**Pen water** → **BVDV cohort**	
73	CohortN → Pen density* CohortN → Bunk space*	Pen density depends on the pen size and the number of animals in the cohort

Path	Variables in path(s)	Justification
74	DOF1-Day 0 → **Grain1** DOF1-Day 0 → **Grain60%** DOF1-Day 0 → **Grain21**	Animals on feed before induction may be receiving a higher percentage of grain on day 0 and this will also influence time taken to reach 60% grain
75	**Intended DOF** → Grain1* **Intended DOF** → Grain21*	Starting and 21 day percentage grain may depend on intended days on feed, with short fed animals reaching a higher percentage sooner
76	Day 0 to close → **Grain1*** Day 0 to close → Grain21* Day 0 to close → **Grain60%***	Animals in an open cohort will have variable rates of grain% on days1, day 21 and therefore time taken to 60% grain
77	**Season** → Grain type **Feedlot region** → Grain type **Induction year** → Grain type	Type of grain available varies with season and feedlot region Supply and cost of different grains may vary in different years
78	FeedlotN → FeedlotN40*	The number of animals less than 40 days on feed is related to the total number on feed
79	Season → N<40d*	The influx of animals and so the number less than 40 days on feed varies by season
80	Feedlot region → Weather Induction year → Weather **Season** → Weather	Weather conditions (i.e. Temp min, Temp max, Temp range, Wind and Rain) during the first week on feed (maximum & minimum temperature, wind speed and rainfall) vary with the feedlot region, season and year of induction
81	Temp min → Temp range Temp max → Temp range	
82	PV_vacc → BVDV ind	
83	BVDV PI group → BVDV ind	
84	Mix history → BRSV ind Mix history → BPI3 ind Mix history → BHV1 ind Mix history → BVDV ind	Entry serology would be expected to be related to past mixing history. Animals with prior mixing history would be exposed to more organisms before induction then animals not previously mixed.
85	**BRSV ind** → Virus indN BPI3 ind → Virus indN **BHV1 ind** → Virus indN **BVDV ind** → Virus indN	The number of viruses the animal is seropositive to at induction is the sum of the four viruses testing positive
86	BVDV_cht → BVDV comp	
87	Rhinogard → BHV1 comp	Rhinogard at induction might be expected to result in seroconversion?
88	Induction serology → Composite serology	The composite serology category depends on the induction serology for each virus
89	Mix history → BRSV comp Mix history → BPI3 comp Mix history → BHV1 comp Mix history → BVDV comp	Change in serology is related to induction serology so is related to mixing history
90	Pen water → BoHV-1 comp Pen water → BRSV comp Pen water → BPI3 comp Pen water → BVDV comp	Change in serology is related to induction serology so is related to mixing history
91	CohortN → BoHV-1 comp CohortN → BRSV comp CohortN → BPI3 comp CohortN → BVDV comp	Change in serology is related to exposure to viruses which may be increased with more animals in a cohort
92	BRSV comp → Virus seroincN BPI3 comp → Virus seroincN BHV1 comp → Virus seroincN BVDV comp → Virus seroincN	Change in serology is related to induction serology so is related to mixing history

*These associations were included based, in part, on evidence of an association based on crude analysis of data from the cohort study dataset including low p-values

5 Descriptive epidemiology of BRD

5.1 Introduction

The primary purpose of this chapter is to report descriptive epidemiology of BRD on medium to large Australian beef feedlots. The incidence of BRD was examined over time, by feedlot region, across feedlots and across cohorts within feedlots. Mortality within the study population is also described.

The relationships between the main cohort study population and cohort population subsets was illustrated and described in Section 4.2. The main cohort study population consisted of 35,131 of the 35,160 animals that were enrolled into study cohorts. These 35,131 animals were nested in 1,077 group-13s within 170 cohorts within 14 feedlots.

The BRD case definition used in the study was detailed in Section 4.1. Animals were classified as having BRD based only on the ′pull reason′ and 'ailment' at their first hospital examination. Cumulative incidences of BRD therefore described the percentages of all animals that met the BRD case definition at their first hospital examination; animals meeting the BRD case definition subsequently were not included. The outcome variable for cohort study analyses was a binary variable describing whether or not animals met the BRD case definition on or between the first and 50th day at risk (BRD50).

5.2 Distribution of study population across feedlots

The distribution of the main cohort study population across feedlots is illustrated in Table 5-1. The number of cohorts contributed per feedlot ranged from three to 21 and the number of group-13s per feedlot ranged from three to 262. Of the 35,131 animals in the main cohort study population, the number of animals contributed per feedlot ranged from 466 (1.3%) to 6,114 (17.4%). The mean number of animals per cohort ranged from 113 to 337 across feedlots.

Table 5-1: Distribution of main cohort study population across feedlots

Feedlot	No. animals	No. group-13s	No. cohorts	No. animals per cohort (mean)	No. animals per cohort (range)
A	633	35	4	160	143 to 179
B	5,364	189	19	316	63 to 350
C	539	24	5	113	75 to 145
D	6,114	262	22	305	105 to 395
E	2,193	77	17	146	56 to 239
F	466	3	3	156	151 to 160
G	2,999	87	21	180	80 to 285
H	2,982	56	20	150	130 to 180
I	2,569	38	14	222	17 to 241
J	5,616	212	18	337	62 to 355
K	1,536	41	9	196	87 to 252
L	500	5	3	173	129 to 208
M	1,927	12	8	250	180 to 280
N	1,693	36	7	242	229 to 250

5.3 BRD incidence in cohort study populations

Of all study animals removed from their cohort for examination in the hospital crush, 77.3% (6,406/8,285) met the BRD case definition at first examination, giving a BRD cumulative incidence of 18.2% (6,406/35,131) of the main cohort study population. The majority of animals that had BRD when first examined were examined during their first 50 days at risk, giving a 50-day BRD cumulative incidence of 17.6% (6,200/35,131) in the main cohort study population.

The preassembly subset comprised all animals (N=5,641) from the three feedlots that assembled cattle on pasture at a location close to their feedlot prior to them being placed on feed in a feedlot pen. Two of these feedlots preassembled all cattle while the third preassembled about half of the study animals. The 50-day cumulative incidence of BRD in this subset was 3.3% (188/5,641). The vendor questionnaire subset comprised 31% (10,721/35,131) of animals from the main cohort study population; the 50-day cumulative incidence of BRD in this subset was 18.7% (2,006/10,721). The prior vaccination subset comprised 24% (8,580/35,131) of animals in the main cohort study population and included vendor-bred animals and animals that were purchased by 10 months of age. The 50-day cumulative incidence of BRD was 18.6% (1,597/8,580) in the prior vaccination subset; 94% of these animals (8,065) had sufficient data to be included in the analyses investigating prior vaccination with Pestigard™ (BVDV vaccine) or BovilisMH™ (*Mannhaemia haemolytica* vaccine). Animals purchased before 10 months of age had a lower

50-day cumulative incidence (14.6% or 513/3,517) compared to vendor-bred animals. The vendor-bred subset (animals born on the vendor's farm) comprised 14% (5,063/35,131) of animals in the main cohort study population and 47% (5,063/10,721) of the vendor questionnaire subset; the 50-day cumulative incidence of BRD was 21.4% (1,084/5,063) in this subset.

5.4 Distributions of BRD

5.4.1 Epidemic curve

As described above, the 50-day BRD cumulative incidence in the full cohort study population was 17.6% (6,200/35,131). The histogram displayed in Figure 5-1 shows the distribution of these 6,200 BRD cases by time at risk when diagnosed. 63% (3,899/6,200) of these animals were diagnosed between days 14 and 28, and 90% (5,554/6,200) were diagnosed between days 7 and 35.

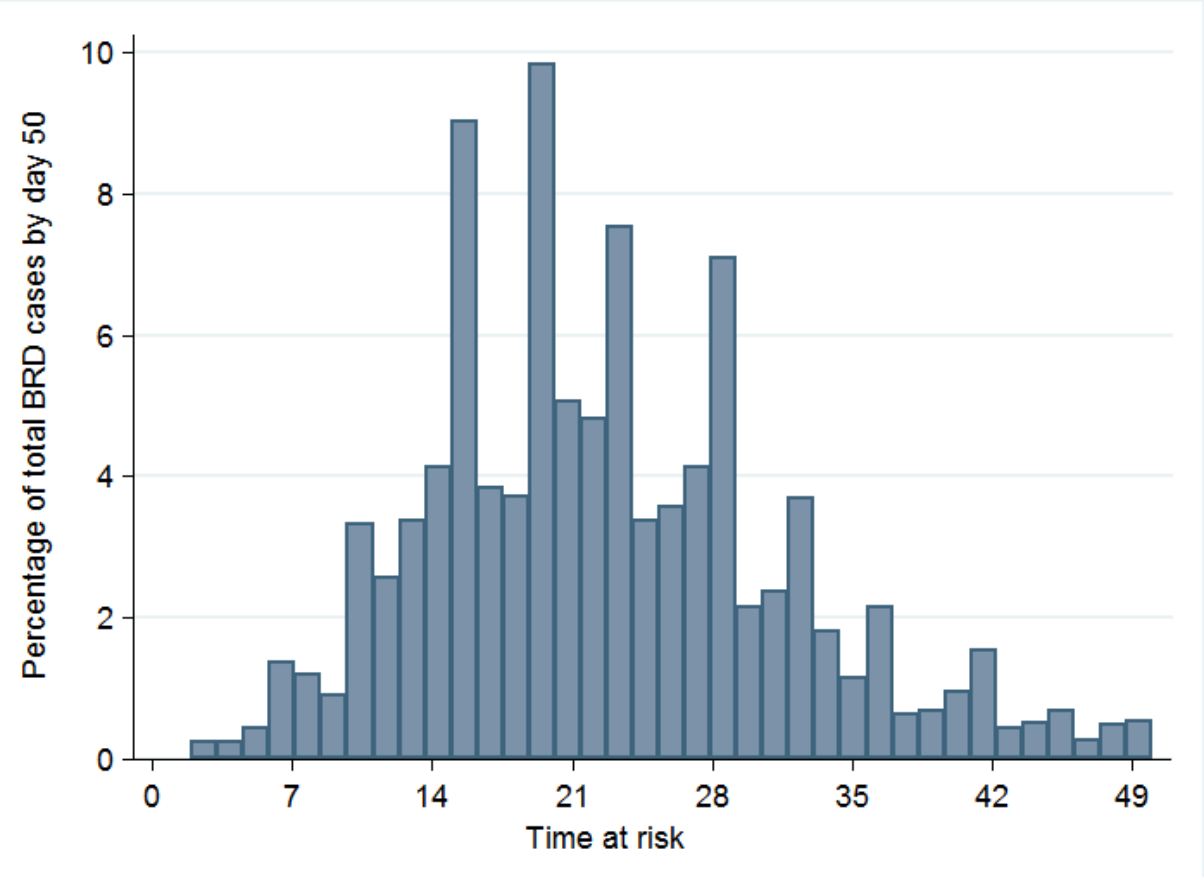

Figure 5-1: Distribution of time at risk for the 6,200 animals that met the BRD case definition by day 50 at risk

5.4.2 Distribution of BRD over time and by feedlot region

The 50-day cumulative incidences of BRD by the quarter in which the animal was inducted (combination of year and season) for the main cohort study population are displayed in Figure 5-2. There are four seasons in a year; spring commences on September 1st, summer on December 1st, autumn on March 1st and winter on June 1st. Only a small number of study animals were inducted during autumn in 2009, so these were included in the winter 2009 category.

BRD 50-day cumulative incidence varied between the quarters, with a tendency for a higher percentage of animals inducted during autumn and summer to develop BRD compared to those inducted during winter and spring, although there was some variation in seasonal patterns between years. The distribution was stratified by feedlot region in Figure 5-3. This illustrates a marked disparity between feedlot regions, with a consistently higher cumulative incidence in southern feedlots compared to those located in Queensland. Peak incidence occurred in southern feedlots in the autumn of 2011, closely followed by the summer of 2009-10. The 50-day cumulative incidence in southern feedlots ranged between 15 and 35%, which contrasts with the observed range of 1% to 19% in Queensland feedlots, with only two quarters having a cumulative incidence above 10%.

5.4.3 Distribution by feedlot and cohort

There was a large amount of variability in the 50-day cumulative incidence of BRD at both feedlot and cohort levels as shown in Figure 5-4 and Figure 5-5. At the feedlot level, cumulative incidences ranged between 0.1 and 45%, while at the cohort level, they ranged from 0% to 72%. There was also variability between cohorts within feedlots, with generally more variability within feedlots that contributed more cohorts.

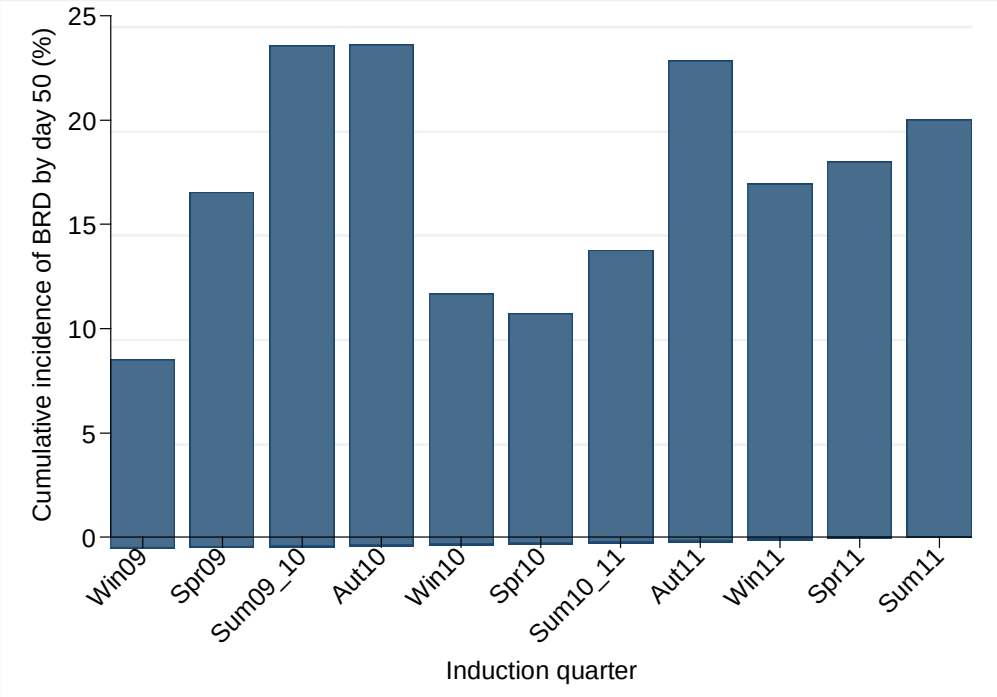

Figure 5-2: BRD 50-day cumulative incidence by induction quarter for the full cohort study population.
A quarter comprises the season and year of induction (e.g. Win09 refers to winter in 2009).

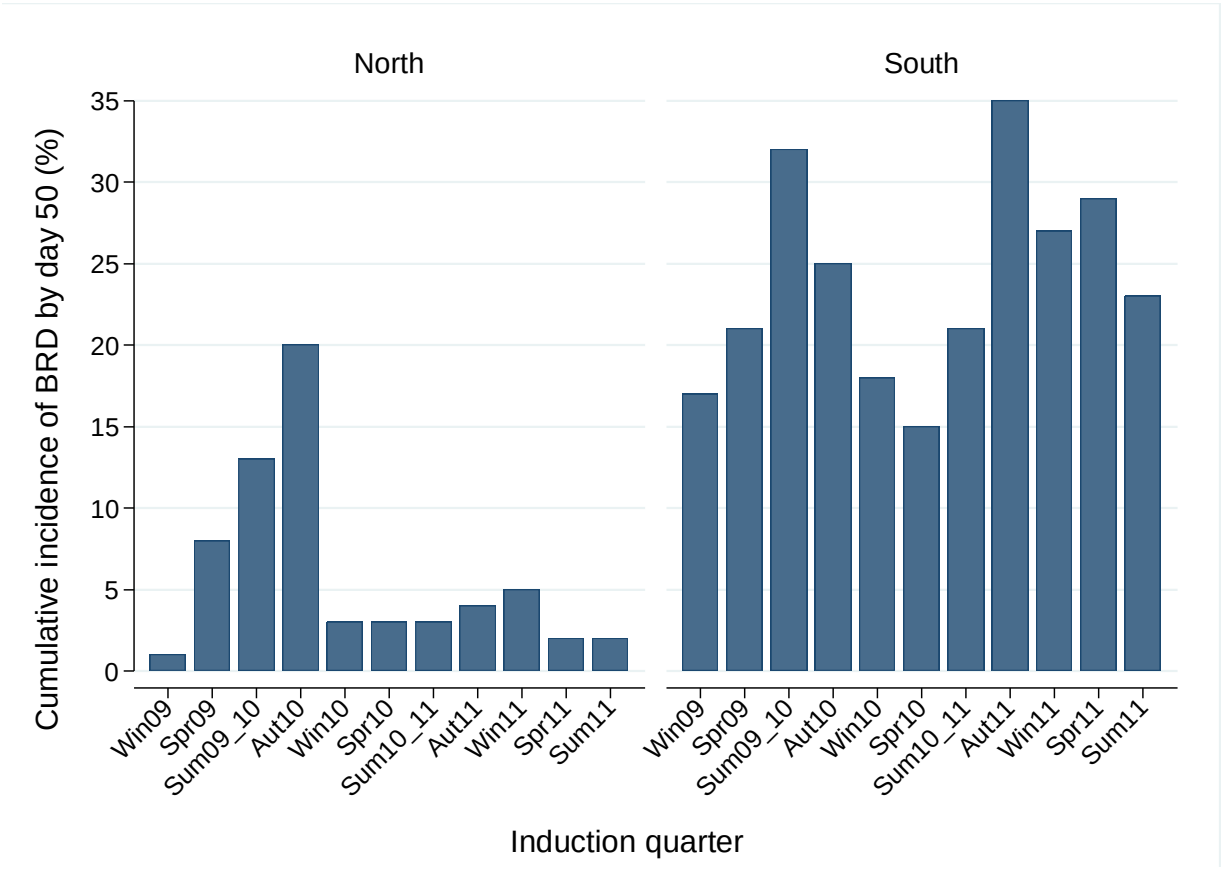

Figure 5-3: BRD 50-day cumulative incidence by induction quarter for the full cohort study population stratified by feedlot region.
A quarter comprised the season and year of induction (e.g. Win09 refers to winter in 2009).

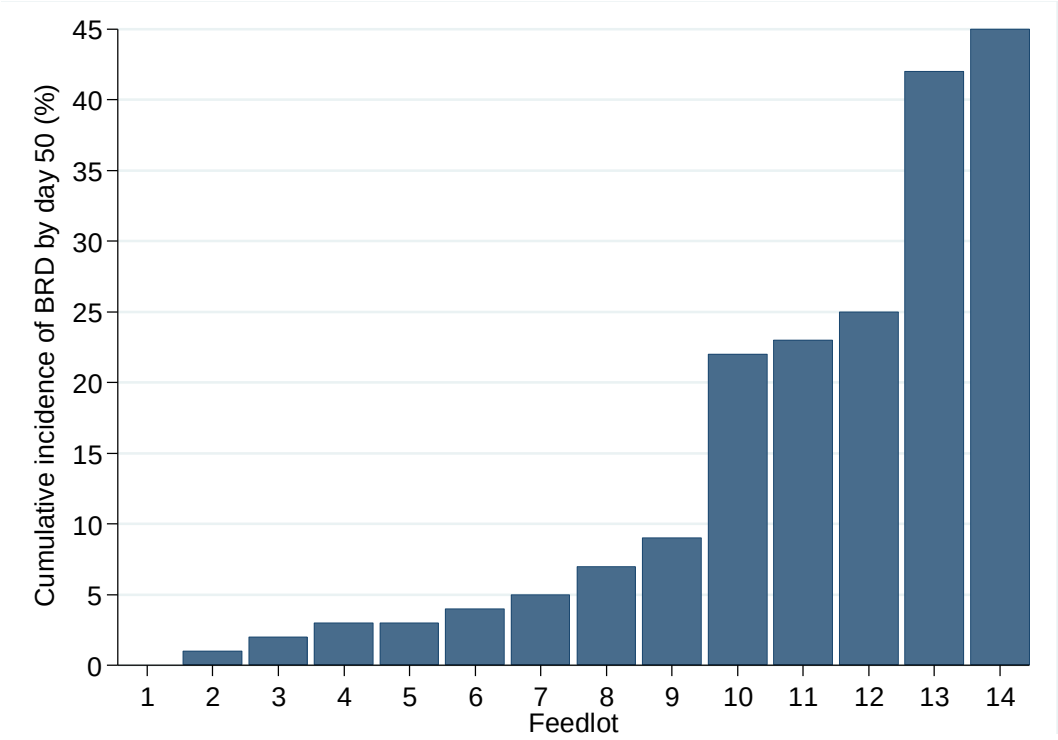

Figure 5-4: Histogram showing pooled 50-day cumulative incidences of BRD by feedlot, arranged in order of incidence

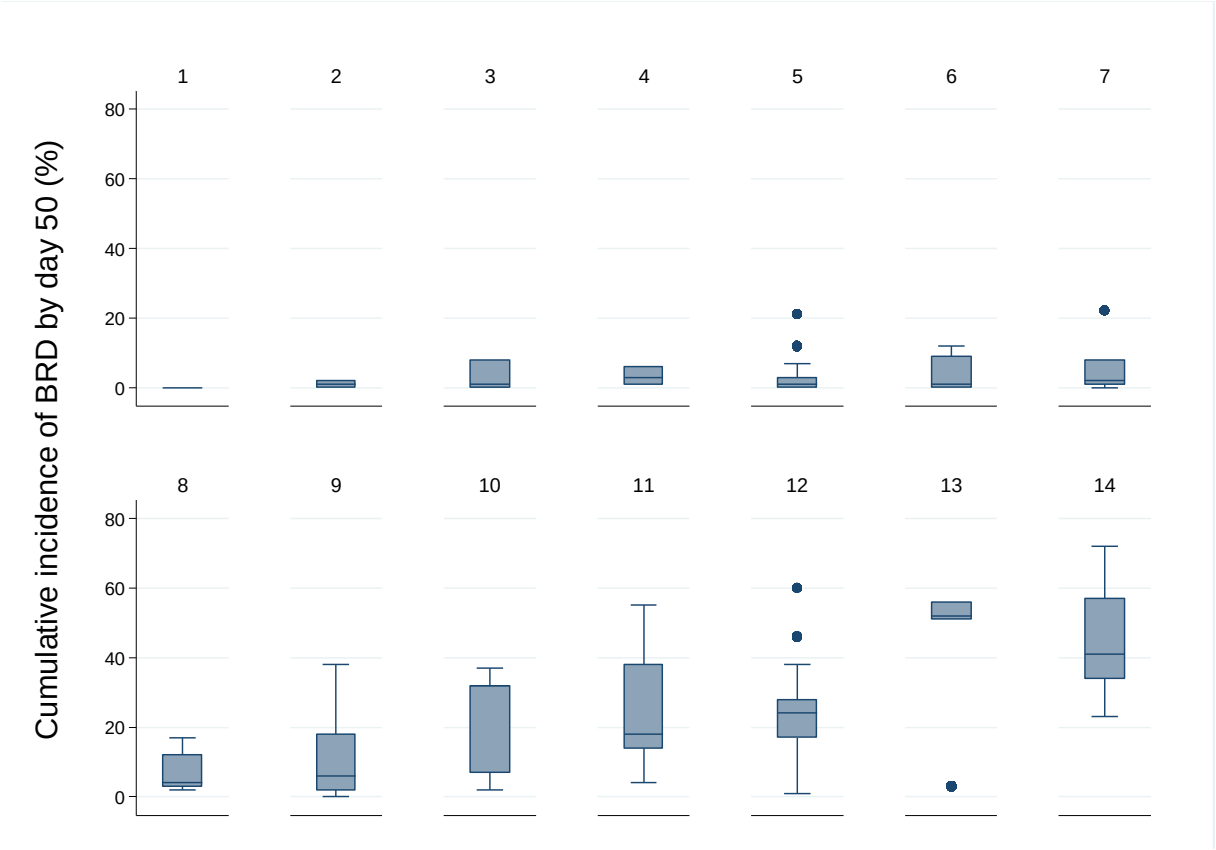

Figure 5-5: Distributions of 50-day cumulative incidence of BRD by cohort within feedlot, with feedlots arranged in ascending order of pooled cumulative incidence

5.5 Overall clinical disease incidence

A total of 8,284 animals comprising 23.6% of the cohort study population had at least one recorded hospital visit during their time on feed. By our case definition, 17.6% of the population was diagnosed with BRD at their first hospital visit within the first 50 days on feed. A further 0.6% (206/35,131) of animals were diagnosed with BRD at first diagnosis after the first 50 days on feed. A further 0.4% of study animals (129/35,131) was first diagnosed with signs that may have indicated respiratory disease but which did not meet the case definition and 5.0% of the population (1,749/35,131) had a non-respiratory diagnosis when first diagnosed. Of animals with a hospital record, 77.3% (6,406/8,284) of all first diagnoses in the study population met our BRD case definition.

5.6 BRD mortality

Mortalities in study animals during their time on feed were identified from feedlot reports of the dates and reasons for death. Deaths were attributed to BRD when the reported reason for death was directly referrable to the respiratory system. Deaths with the following reasons for death were classified as deaths from BRD: ‘BRD’, ‘bronchopneumonia’, ‘fibrinous pneumonia’, ‘lung abscess’, ‘IBR’, ‘pleurisy’, ‘pneumonia’, ‘respiratory’ and ‘tracheitis’.

Of the 35,131 animals in the main cohort study population, a total of 460 animals died during their time on feed, giving a pooled all-cause cumulative mortality of 1.3% (460/35,131). Of these, 52% (239/460) of deaths were attributable to BRD, and the pooled BRD cumulative mortality was 0.7% (239/35,131). BRD cumulative mortalities varied considerably between feedlots, ranging between 0% (3 feedlots had no BRD deaths) and 2% (median 0.5%). A further 20% (93/460) of the deaths met the BRD case definition before dying, but their death was not attributed to BRD (including six with a reason for death recorded as ‘unknown’ or euthanasia). The reason for death was also recorded as ‘unknown’ or euthanasia for a further 4% (20/460) of animals that did not meet the BRD case definition.

Of the BRD deaths, 72% (173/239) met the BRD case definition at first hospital examination, 9% (22/239) did not meet the BRD case definition at first hospital examination, and 18% (44/239) died of BRD without a hospital record (i.e. pen

deaths). Deaths attributed to BRD occurred from 2 to 148 (median: 25; interquartile range: 18 to 34) days after the start of the animal's time at risk (induction day). Pen deaths attributable to BRD occurred most commonly from days 15 to 45 after the start of the animal's time at risk.

Of the 6,200 animals that were diagnosed with BRD at the first hospital visit between the animal's 1st and 50th day at risk, 218 subsequently died (of any cause) within 50 days of being diagnosed, giving a case fatality risk of 3.5% (218/6,200). Among these deaths, 71% (154/218) were attributed to BRD. The interval between first diagnosis and death ranged from 0 to 48 days (median 7; interquartile range: 2 to 15).

5.7 Conclusions

The 50-day cumulative incidences of BRD in the study population varied markedly, by feedlot region, feedlot, cohort within feedlot and season. Animals in southern feedlots had a much higher 50-day cumulative incidence of BRD than animals in northern feedlots. This descriptive epidemiology highlights wide variation in 'typical' BRD incidence in the study population. The sources of this variation will be investigated further in Chapter 8. The descriptive statistics detailed in this chapter indicated that BRD was the major cause of clinical disease in the main cohort study population, being responsible for about 77% of first hospital diagnoses. Of animals meeting the cohort study case definition (with a first hospital diagnosis of BRD between the 1st and 50th days at risk), the case fatality risk was 3.5%. The overall BRD mortality risk was 0.7%, with BRD causing at least half of all feedlot deaths.

6 Cohort Study Analyses: Estimation of Total and Direct Effects

6.1 Introduction

As described in Section 4.1, the outcome measure was the occurrence of BRD, based on clinical signs of respiratory disease, at the earliest hospital examination from days 1 to 50 inclusive (BRD50). As detailed in Section 4.2, the main cohort study population comprised 35,131 animals nested within 1,077 group-13s nested within 170 cohorts nested within 14 feedlots. The preassembly subset comprised animals from three of the 14 feedlots that practiced a management system whereby animals from different farms were assembled on pasture close to the feedlot for various periods of time prior to induction. The vendor questionnaire subsets were the full vendor questionnaire subset (i.e. had a returned vendor questionnaire), the vendor-bred subset (comprising animals bred on the vendor's farm) and the prior vaccination subset (vendor bred or purchased by 10 months of age). In this chapter, I describe the analyses of data from all of these cohort study datasets to determine the total effects, and relevant direct effects, of putative risk factors on the BRD50 outcome. This chapter addresses the research aim of assessing the strength of association between known and potential risk factors and BRD occurrence.

6.2 Materials and methods

6.2.1 Causal diagrams for cohort study subsets

The rationale for using causal diagrams to inform model selection was discussed in Section 1.9. The theoretical causal diagram linking all measured putative risk factors with other risk factors and with the BRD outcome was illustrated and described in Section 4.7. Because data were not available for all putative risk factors for all study animals, subset analyses were used to assess the effects of some risk factors. To facilitate the selection of models to estimate the total and direct effects of exposures of interest, separate causal diagrams were constructed for subsets of the data. These subset causal diagrams included variables that only were measured for animals in the analysis subset, along with any variables required for any models specific to that subset. Thus, variables consisted of all postulated intervening or confounding variables that would be required to be fitted in any model to estimate

the effects of any exposures of interest. Sometimes collapsed versions of variables were included in subset diagrams to facilitate the fitting of models to smaller datasets. For example, a 12-category variable was used to assess the effects of mixing history but a collapsed version (four-category mixing summary) was used in the vendor questionnaire subset analyses.

The causal diagrams used to inform model building to estimate the effects of exposures measured in the full cohort study (Figure 6-1), the preassembly subset (Figure 6-2) and the vendor questionnaire subsets (Figure 6-3) are shown below. These diagrams were used to inform the choice of covariates when estimating the effects of each putative risk factor as reported in this chapter. For example, when deriving a model to estimate the total effect of weight difference (Weight diff, animal-level difference from mean cohort weight) on BRD, induction weight, mean cohort weight and breed comprised the minimal sufficient adjustment set (Table 6-5).

6.2.2 Software and model determination

The DAGitty software (Textor et al., 2011) was used to identify minimal sufficient adjustment sets to assess total and direct effects of the exposure variable of interest on the occurrence of BRD. The causal diagram was reproduced within the DAGitty web interface (Textor et al., 2013). Each variable of interest was sequentially identified as the exposure of interest and the list of variables in the minimal sufficient adjustment sets was copied and pasted into a Microsoft Excel® (version 2010) spreadsheet.

The data had a four-level nested hierarchical structure and four-level models were fitted when possible. Multilevel multivariable models were fitted using the software package MLwiN® (version 2.27). This was run from within the Stata® statistical software package (version 12). The runmlwin program (Leckie and Charlton, 2013) was utilised to facilitate the transfer of data and statistics between the two packages. This enabled use of the more flexible multilevel modelling procedures provided in MLwiN® combined with the functionality provided by Stata®. Results were then compiled and formatted in Microsoft Excel®.

Separate multivariable models were fitted with each adjustment set; where multiple minimal sufficient adjustment sets were possible for the same exposure variable, separate models were fitted and results were compared. The only exceptions to this

were some variables in the vendor questionnaire datasets, where alternative models were disregarded because one or more covariates in the adjustment set were of poor quality or because sparse distributions in the subsets meant that models failed to run or converge. For all biologically plausible interactions of interest, both variables were selected as exposures to determine the minimal sufficient adjustment sets. The exposure variables of interest, their interaction terms and covariates were then fitted to obtain the desired estimates of effect.

Direct effects were of particular interest when an important total effect of a variable may have been due to intervening variables. For example, the total effect of saleyard exposure would be expected to be partially mediated through the effect of mixing and moving, so when drawing conclusions it is informative to consider both direct and total effects. Where direct effects were estimated, the approach described above was implemented, but instead using the minimal sufficient adjustment set for the direct effect. For direct effects, in addition to confounders, these sets included all intervening variables and confounders of the intervening variable(s). Direct and total effects were compared where relevant.

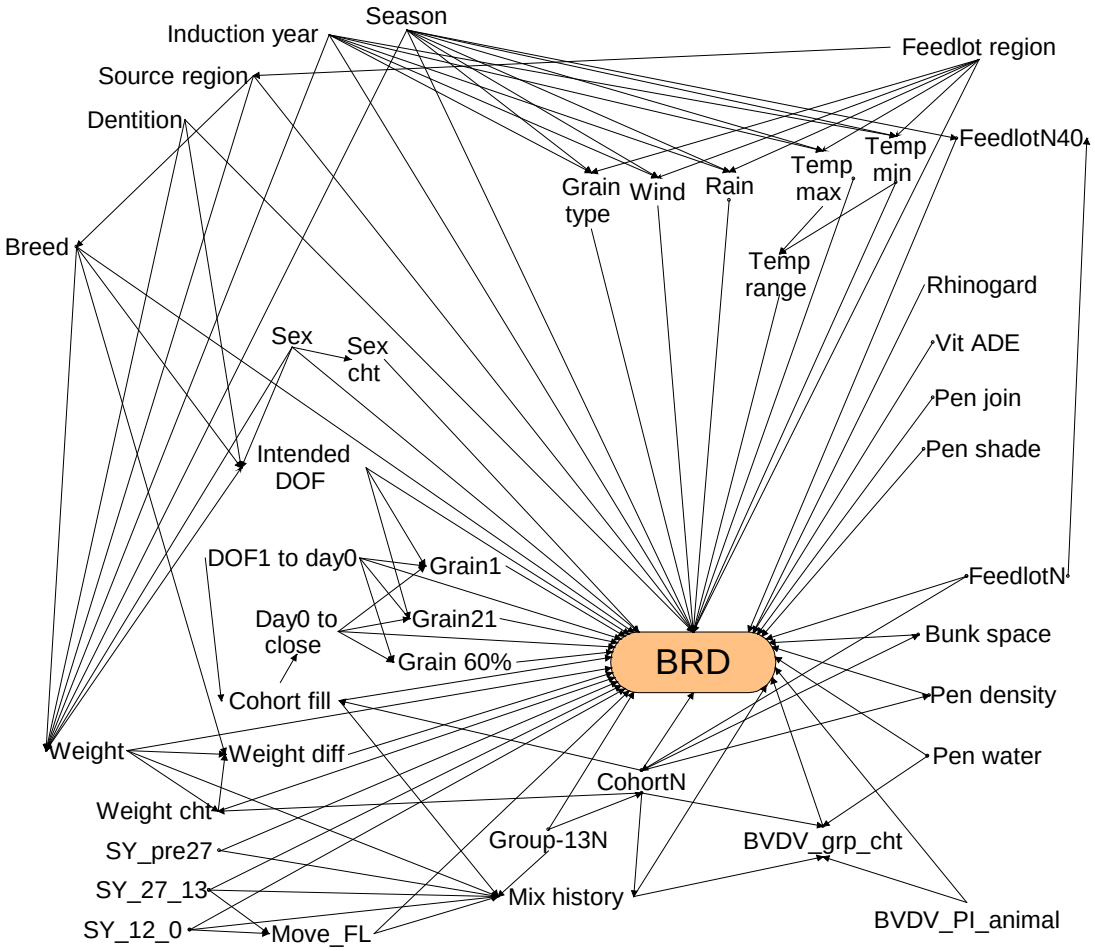

Figure 6-1: Causal diagram depicting pathways relevant for the determination of total and direct effects of putative risk factors investigated in the full cohort dataset.

Group-28N or Group-91N were substituted for Group-13N to determine the models for these variables.
BVDV_chtYN, BVDV_PI_grp28, BVDV_chtPI were substituted for BVDV_grp_cht as required.

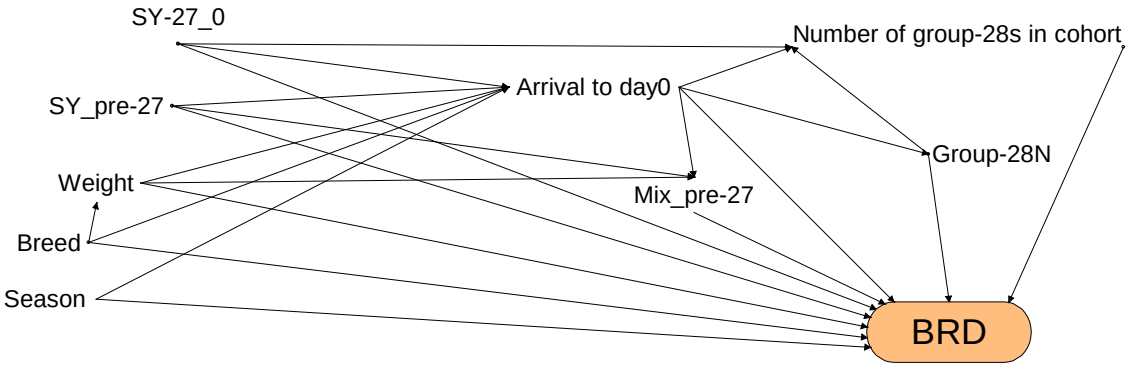

Figure 6-2: Causal diagram used to inform analyses of the preassembly subset

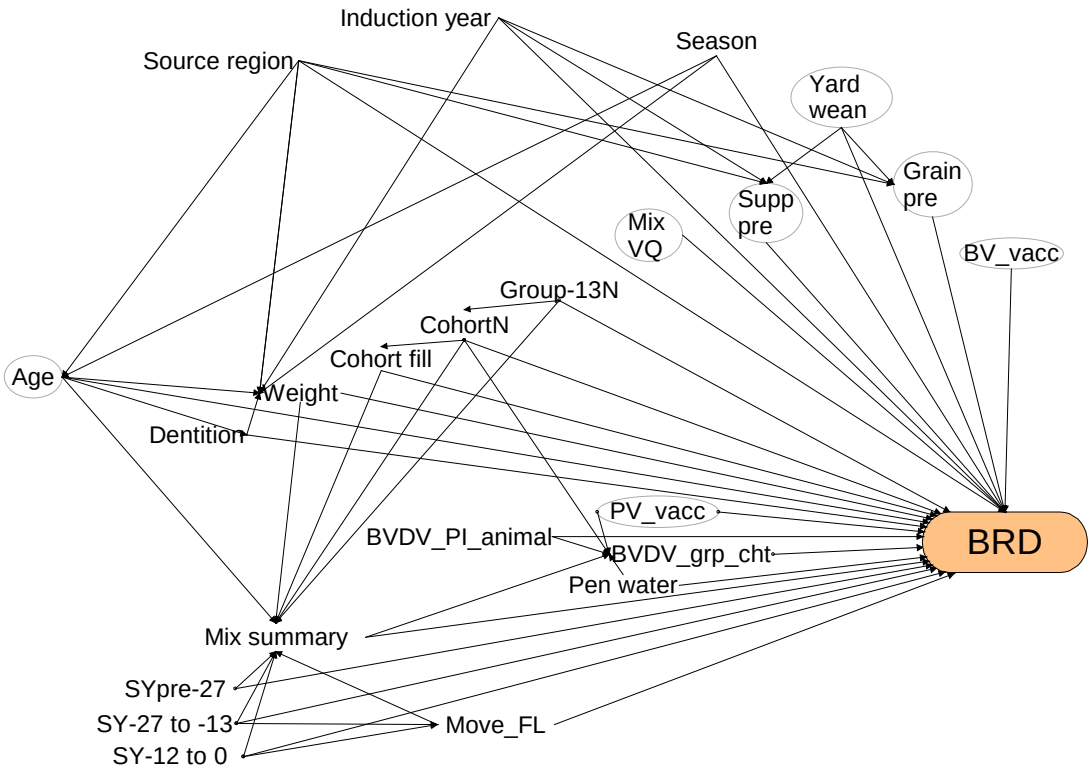

Figure 6-3: Causal diagram depicting pathways relevant for the determination of total and direct effects of putative risk factors investigated in the vendor questionnaire subsets.
BVDV_chtYN was substituted for BVDV_grp_cht as required.

6.2.3 Modelling methodology

6.2.3.1 Model specification

Multilevel mixed effects Bayesian logistic regression models were fitted to assess effects of each exposure variable of interest. The odds are related to the probability of a binary outcome (π), such that odds= π / (1- π). The logit transformation (ln(π/(1- π)) of the probability of BRD was modelled as a linear function of fixed and random effects.

The general form of the model may be described as:

Equation 2:

$$BRD_{ijkl} \sim Bernoulli(\pi_{ijkl})$$

$$\text{logit}(\pi_{ijkl}) = \beta_0 + \boldsymbol{\beta}_1 \boldsymbol{x}_{ijkl}^{(1)} + \boldsymbol{\beta}_2 \boldsymbol{x}_{jkl}^{(2)} + \boldsymbol{\beta}_3 \boldsymbol{x}_{kl}^{(3)} + \boldsymbol{\beta}_4 \boldsymbol{x}_{l}^{(4)} + \upsilon_l + \nu_{kl} + \omega_{jkl}$$

$$\upsilon_l \sim N(0, \sigma_\upsilon^2)$$

$$\nu_{kl} \sim N(0, \sigma_\nu^2)$$

$$\omega_{jkl} \sim N(0, \sigma_\omega^2)$$

where:

π_{ijkl} is the fitted probability of BRD in animal i within group-13 j within cohort k within feedlot l.

β_0 is the intercept

$\boldsymbol{x}_{ijkl}^{(1)}$ is the vector of animal-level covariates with coefficients $\boldsymbol{\beta}_1$

$\boldsymbol{x}_{jkl}^{(2)}$ is the vector of group-13-level covariates with coefficients $\boldsymbol{\beta}_2$

$\boldsymbol{x}_{kl}^{(3)}$ is the vector of cohort-level covariates with coefficients $\boldsymbol{\beta}_3$

$\boldsymbol{x}_{l}^{(4)}$ is the vector of feedlot-level covariates with coefficients $\boldsymbol{\beta}_4$

υ_l is a scalar of feedlot-level random effects with mean 0 and variance σ_υ^2

ν_{kl} is a scalar of cohort-level random effects with mean 0 and variance σ_ν^2

ω_{jkl} is a scalar of group-13-level random effects with mean 0 and variance σ_ω^2

Depending on the minimal sufficient adjustment set and the number of levels that were able to be fitted, not all terms may have been included in the model for a particular variable.

Dummy variables for each level of the categorical predictor variables were created as is required by MLwiN®. The default multilevel modelling methods used in MLwiN® involve first utilising quasi-likelihood approximation methods followed by estimation using iterative generalised least squares (Browne, 2012). Second order penalised quasi-likelihood methods were used to obtain starting values for Markov

Chain Monte Carlo (MCMC) estimation. MCMC methods enable multilevel models to be fitted to datasets where alternative likelihood-based approaches are often not able to be used. Models were fitted using default Gaussian prior distributions with extremely large variances for the fixed effect parameters and weakly informative Wishart priors for the variance matrices(Browne, 2012).

MCMC methods involve repeatedly sampling from the conditional posterior distribution of each parameter as this is equivalent to sampling from the joint posterior distribution, which is the distribution of interest. Metropolis Hastings sampling methods are the default in MLwiN® when multilevel logistic models are fitted. After many iterations, summary measures from the Markov Chain provide posterior predicted estimates for the unknown parameter values (Browne, 2012). For most variables it was possible to fit four-level models thus including all defined hierarchy levels. However, four-level models did not always fit (e.g. where data were missing at the cohort level), so estimation was performed using three hierarchy levels (i.e. without group-13). For a few variables, problems with convergence using iterative generalised least squares were noted and investigated.

6.2.3.2 Model diagnostics and assessment

Diagnostic plots and summary statistics were monitored to assess convergence in all MCMC models. A time-series plot of posterior predicted values of coefficients enables the assessment of stationarity. Non-stationarity is visualised by a time series trajectory plot that wanders widely and indicates poor mixing. Non-stationarity is due to the sampling process being ‘stuck’ in one part of the parameter space rather than sampling from the entire sampling space; this can be caused by high autocorrelation (Hoff, 2009). The kernel density plot provides a smoothed visualisation of the shape of the posterior distribution. The auto-correlation factor (ACF) is a measure of dependence of iterations in a chain, averaged over all samples; a lower ACF indicates a more efficient MCMC chain. The ACF should reduce exponentially with increasing lag (number of iterations between observations) to behave like a first order autoregressive time series (Browne, 2012). The partial autocorrelation factor (PACF) shows the autocorrelation between iterations at different lags having accounted for the iterations in between so that the point where subsequent values are essentially zero indicates that the chain is adequately independent (Browne, 2012).

An example of a model displaying good convergence and model diagnostics is presented in Figure 6-4. This model was run to estimate the total effects of saleyard exposure prior to day -27 (SY pre-27), on the BRD50 outcome. The variable is defined at the animal level, so observations would be expected to be reasonably independent, although they may be correlated within some group-13s. The time series plot of the parameter estimate at each iteration of the MCMC chain is shown in graph 6-4a. It displays good mixing with stationarity and evidence of good coverage of the parameter space, suggesting that convergence has been reached. The posterior distribution displayed in the kernel density plot (graph 6-4b) is unimodal, with a reasonably narrow spread of values around the mode (-0.2). The ACF plot (graph 6-4c) shows low autocorrelation by lag 40 and the PACF (graph 6-4d) reaches zero at around lag 8.

The effective sample size is estimated from the number of iterations divided by the ACF so that where chains display high autocorrelation, the effective sample size will be lower. In the saleyard exposure example, the effective sample size was 973 after 20,000 iterations. Generally, an effective sample size of more than 200 is sufficient to obtain a reasonable estimate provided other diagnostics are adequate (Browne, 2012).

The Monte Carlo standard error (MCSE) is a measure of the accuracy of the estimate and the trajectory plot displays the estimated MCSE of the posterior mean against the number of iterations (Browne, 2012). With high autocorrelation, the number of independent iterations is much lower than the total number of iterations, so the MCSE will be higher, which will be reflected by wide credible intervals. For saleyard exposure prior to day-27 (Figure 6-4e), the MCSE reached a low value (between 0.001 and 0.0015) after running 20,000 iterations.

For clustered data, especially for feedlot or cohort level variables, the effective sample size was lower, chains will be inefficient and a very long chain results in only a modest number of independent observations because all animals within a cluster have the same value for the variable of interest. For example, shared pen water was a cohort-level variable and highly clustered by feedlot.

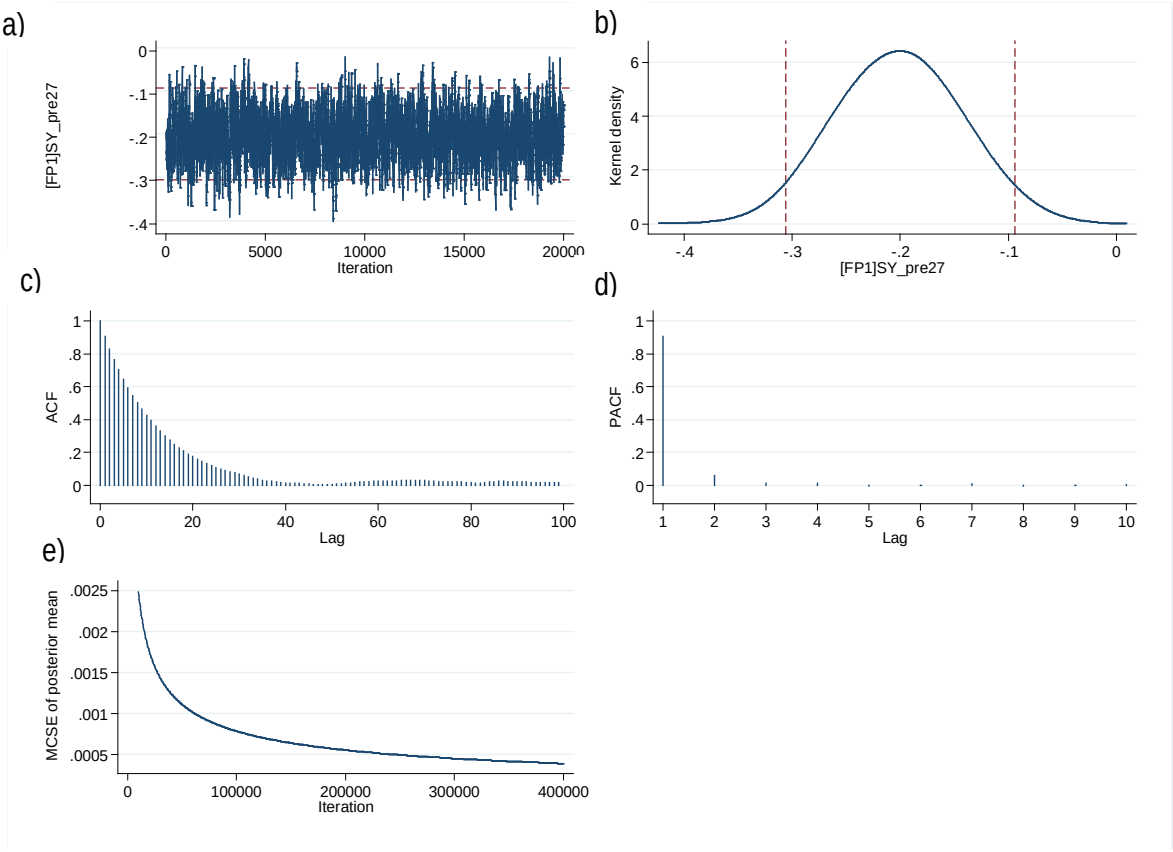

Figure 6-4: Diagnostic trajectory plots for saleyard transfer prior to day -27 from total effects model estimating the effect of SY pre27 on BRD50 (20,000 iterations)

Figure 6-5 shows the diagnostic plots obtained from an MCMC chain run for 100,000 iterations to estimate the total effect of shared pen water on the occurrence of BRD. A lack of convergence is evidenced by non-stationarity, poor mixing, high autocorrelation and a high MCSE. This is reflected in the effective sample size which was only 63 after 100,000 iterations. Sometimes running a longer chain results in a better convergence and a sufficient effective sample size, but for this variable, an MCMC chain run for 500,000 iterations resulted in an effective sample size of 110 with only modest improvement in model diagnostics. Slow mixing and high autocorrelation were commonly observed for variables clustered at higher levels (cohort or feedlot level variables). In more severe instances, problems such as bimodal or flat distributions were noted. Convergence was improved by reparameterising these models as described below.

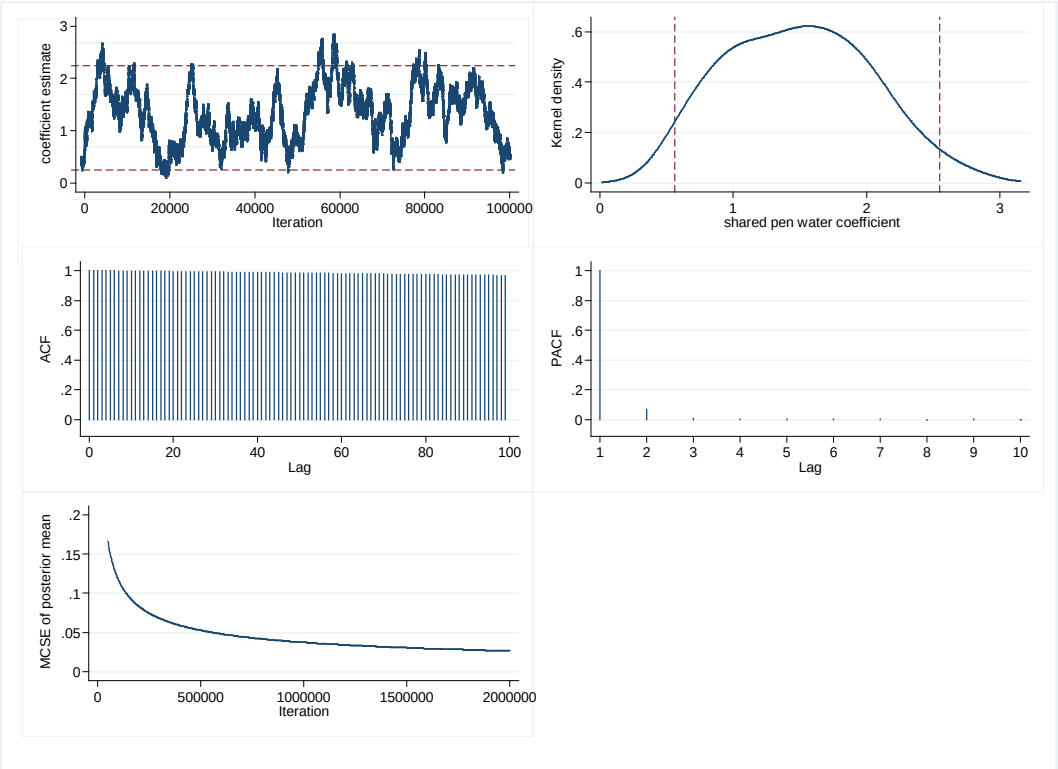

Figure 6-5: Diagnostic trajectory plots for shared pen water on BRD50 from total effects model run for 100,000 iterations

Other diagnostics output by MLwiN® assist in estimating the required chain length for a given model. The Rafferty Lewis diagnostics were checked to determine the length of Markov chain required to produce an estimate of the 95% credible interval accurate to two significant figures. The Brooks-Draper diagnostic gives the number of iterations required to quote point estimates with an accuracy of two significant figures, but for cohort-level variables with high autocorrelation between observations, the number of proposed iterations required to achieve this level of precision was often prohibitive. Thus, models for higher level variables were reparameterised and rerun to achieve more accurate estimates evidenced by markedly improved model diagnostics.

6.2.3.3 Model reparameterisation

If convergence was not achieved, models were specified in a different way (reparameterised) by using orthogonalisation and hierarchical centring. Orthogonalisation is a method whereby orthogonal vectors of predictors are created which span the same parameter space as the variables of interest (Browne, 2012).

The product of a pair of orthogonal predictor vectors will be zero and the effect of each orthogonal parameter should be independent, so where parameters are updated separately, mixing of MCMC algorithms will be improved (Browne, 2012). Hierarchical centring addresses correlation between fixed effects and residuals. The default Gibbs sampling algorithm produces an un-centred estimate for the random effects with respect to the fixed effects. Hierarchical centring centres the random effects estimates on the intercept (i.e. a function of the fixed effects). This results in improved model fit provided the correlation between the intercept and the centred random effects is less than it was prior to centring (Browne, 2012). Hierarchical centring was generally employed at level three (i.e. cohort-level random effects were centred on the intercept).

Reparameterising the model estimating the total effects of shared pen water (described above), by applying orthogonalisation and hierarchical centring at level three, resulted in a much improved effective sample size of 353 after 100,000 iterations, although the trajectory plots still displayed high autocorrelation and non-stationarity. Upon running this model for 500,000 iterations, the final estimate of effect was obtained with the diagnostic trajectory plots displayed in Figure 6-6. While there is still autocorrelation and the MCSE is about 0.003, the trajectory plot and kernel density plots indicate adequate mixing and evidence of convergence. The effective sample size for this model was 1,861 and at 6,934 the Brooks Draper diagnostic suggests that the point estimate is accurate to 2 significant figures. This contrasts with a Brooks Draper diagnostic of three million in the original model. These techniques are now recommended for most logistic regression models run in MLwiN®, but they are not specified as the default (Browne, 2012).

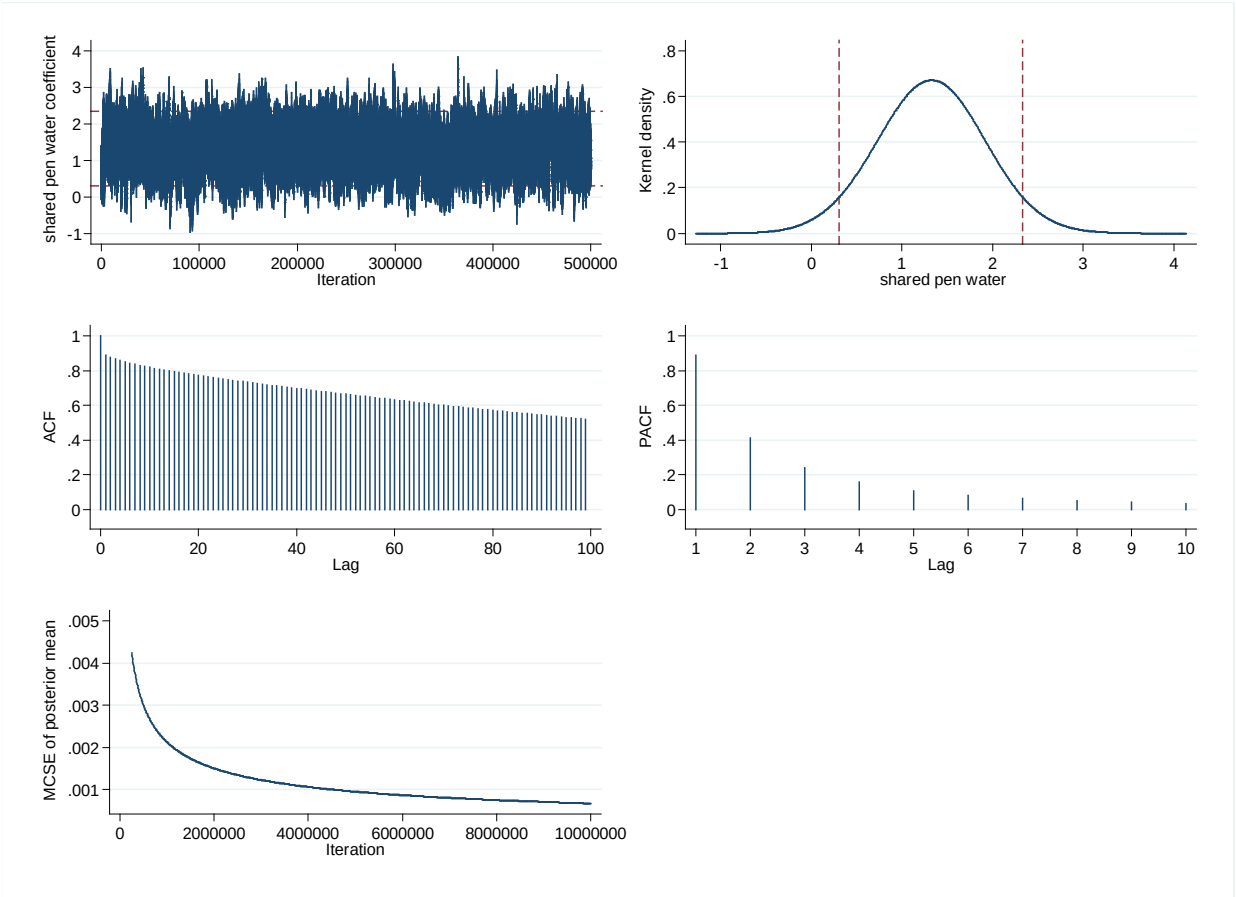

Figure 6-6: Diagnostic trajectory plots for shared pen water on BRD50 from total effects model (with orthogonalisation and hierarchical centring) run for 500,000 iterations

6.2.4 Estimation of main effects

For each minimal sufficient adjustment set for each variable of interest, multilevel MCMC models were estimated using the initial values obtained from second order penalised quasi-likelihood estimation using iterative generalised least squares. The reference categories were chosen to facilitate logical comparisons while considering the distributions across the study population. Sometimes models were rerun with different reference categories, where these were of interest (e.g. mixing history).

Models were initially run for 10,000 iterations after a burn-in of 500 (the default) to assess diagnostic output. The maximum value for the Rafferty Lewis diagnostic was used as a guide in deciding required chain lengths to provide a 95% credible interval accurate to two significant figures. Some animal-level variables (e.g. breed, mixing history) were clustered at higher levels, so longer chains were indicated.

Automated Stata® .do files were written specifying the estimated required chain length. The model diagnostics described above were assessed for all models and

longer chains were specified and models were rerun where necessary. The number of iterations used in final models was commonly 100,000 and usually ranged from 50,000 to 200,000, but very long chains were sometimes run (e.g. 1 million for the feedlot region variable) in an attempt to achieve better convergence. As described above, reparameterisation of models resulted in much improved convergence with shorter chains for higher level variables. More efforts were made to achieve accurate estimates for variables with evidence of an effect based on the 95% credible intervals. Output from the final models was copied into an Excel® spreadsheet and relevant model diagnostics were recorded.

6.2.5 Assessment of interactions

Interaction terms investigated included those that were specified *a priori* based on prior literature and industry interest (based on discussion with feedlot veterinarians and feedlot managers) as well as those considered biologically plausible where the main effects of the variables were significant. For several of these, biological plausibility was based on the threshold effect, such that where an animal is simultaneously exposed to both risk factors, the risk was hypothesised to be higher than that predicted by the model not including the interaction terms. Only those interactions with joint Wald p-values of <0.05 from second order penalised quasi-likelihood models were considered further and these models were then estimated using MCMC methods as described above. Odds ratios and 95% credible intervals were derived using the post estimation *lincom* command in Stata® following model convergence. Although the estimates obtained in this way may differ slightly from the estimates obtained from re-running the MCMC models with different reference categories, the estimates were considered adequate for comparisons across categories and visualising the effects through graphs. Estimates were compiled in an Excel® spreadsheet and imported to Stata® to produce graphical displays which were examined along with the point estimates and 95% credible intervals to decide on meaningful interactions to report. For example, some significant interaction term estimates were highly imprecise, not conducive to meaningful interpretation and did not add additional information to that obtained from the main effects; results from these models were not reported.

6.3 Results

6.3.1 Reporting and interpretation

Results for putative risk factors were grouped into three broad categories and reported within the relevant sections below. These were animal-entry characteristics (e.g. breed, induction weight), management risk factors (either prior to arrival or at the feedlot) and broad environmental risk factors (e.g. season, source region). Tables within each section follow a common format. For each risk factor the crude distributions of animals and BRD 50-day cumulative incidences are presented first. Subsequent tables present the effect estimates and details of models fitted. Footnotes indicate where analyses relate to subsets rather than the full cohort dataset. Within the broad categories, variables may be presented in further subgroups. For example, pen characteristics and ration details form separate subgroups of the management risk factors

The results reported are ‘cluster specific’. This means that the effect estimates compare two animals within the same cluster (i.e. within group-13s which in turn are within cohorts within feedlots). Results reported include point estimates of the mean adjusted odds ratios (OR) with their 95% credible intervals (95% cred int) and the probability that the estimate was less than or greater than one (prob </>1). The 95% credible intervals were based on sampling of the posterior distribution, and record the intervals within which the central 95% of the estimates fall. The probability that the estimate was less than or greater than one was output from MLwiN® as the Bayesian p-value which gives the proportion of samples drawn from the posterior distribution where the odds ratio is less than or greater than one, depending on where the point estimate falls. Hence, if the point estimate of the effect size indicates decreased risk, then the ‘prob </>1’ gives the proportion of samples drawn from the posterior distribution where the odds ratio is greater than one. Bayesian methods do not constrain the posterior distribution to a normal distribution and values are based on observed probabilities rather than hypothetical repeated sampling. Estimates were relative to the reference category (Ref) and were adjusted for the minimal sufficient adjustment set (adjustment set) of covariates as indicated. The number of observations in the model (N) and number of hierarchical levels included (level) are also indicated.

Total effect estimates are reported for all variables and direct effects are also reported where this is of interest. Important differences are indicated in the text below where this generally means that there was evidence of an effect and the 95% credible interval was relatively narrow, but may refer to other estimates which are suggestive of a large effect even though the estimate was imprecise. This may occur, for example, because of reduced power to detect an effect for variables that are clustered at the cohort level.

6.3.2 Animal entry characteristics

6.3.2.1 Breed

The distribution and crude BRD 50-day cumulative incidences by breed are shown in Table 6-1. The most common breed was Angus (56% of animals), tropical breeds and tropical crosses comprised about 16% of the population, European breeds about 4% and the remainder were of other breeds of British origin or derivation.

The risk of BRD varied considerably between different breeds (Table 6-2). Compared to Angus cattle, Herefords were at markedly increased risk (OR: 2.0, 95% credible interval: 1.5 to 2.6) and British breed crosses were at slight to moderately increased risk (OR: 1.2, 95% credible interval: 1.0 to 1.4). Tropical breeds and crosses (OR: 0.5, 95% credible interval: 0.3 to 0.7) and Murray Greys (OR: 0.5, 95% credible interval: 0.3 to 0.8) were at moderate to markedly decreased risk.

6.3.2.2 Sex

Most cattle in the study population were steers (92% of animals, Table 6-1) and most of the cattle in the study were in cohorts comprised of steers only (88%, Table 6-1). Heifer only and mixed-sex cohorts were restricted to a small number of feedlots. The total effect estimates suggest that heifers were at reduced risk compared to steers (OR: 0.7, 95% credible interval: 0.4 to 1.1, Table 6-2). The estimates for the effect of the sex of the cohort on the risk of BRD were very imprecise so no conclusion was possible, probably because the distribution of the categories was clustered by feedlot (Table 6-2).

6.3.2.3 Intended days on feed

About half of the cattle in the study population were intended to be on feed for at least 120 days (53%, Table 6-1). There was no evidence of a moderate or large effect of intended days on feed on the risk of BRD (Table 6-2).

6.3.2.4 Age and dentition

Of animals in the vendor questionnaire subset with sufficient data to estimate age, just over half (55%, Table 6-1) were aged 16 to <22 months at the start of time at risk. Cattle aged at least 22 months were at moderate to markedly increased risk of BRD compared to those aged 16 to <22 months (OR 1.6, 95% credible interval: 1.3 to 2.1, Table 6-2). Direct effect estimates for age (i.e. after adjusting covariates such as weight and mixing history) were similar to total effects (Table 6-3). The induction weights for cattle aged at least 22 months at induction (median: 446 kg, interquartile range: 416 to 474 kg) were similar to the animals in the reference category (median: 446 kg, interquartile range: 422 to 468 kg).

Most cattle in the study population had no permanent incisors (81% of animals, Table 6-1); these cattle were probably less than two years of age. There was no evidence of a moderate or large effect of dentition on the risk of BRD (Table 6-2).

6.3.2.5 Induction weight

Most cattle in the study population were either 400 to <440 kg (31%) or 440 to <480 kg (34%, Table 6-4) at induction. About half of the cattle were in cohorts where the mean weight was 425 to <455 kg (50% of animals, Table 6-4). The study population was evenly distributed among the four categories of weight difference from the mean cohort weight (Table 6-4).

Compared to light cattle (<400 kg), the risk of BRD was reduced with increasing induction weight, with consistent estimates between the two models using different minimal sufficient adjustment sets. Risk was markedly reduced in the heaviest category, ≥480 kg (OR: 0.6, 95% credible interval: 0.5 to 0.7 in both models, Table 6-5). After adjusting for individual animal weight, there was no evidence of a large effect of the mean cohort weight or difference in weight from the mean cohort weight on risk of BRD (Table 6-5).

Table 6-1: Putative risk factors relating to induction characteristics; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing %	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Breed		0.23			
	Angus		19,764	56.4	22.6
	British Cross		4,140	11.8	17.6
	Hereford		1,952	5.6	21.4
	Shorthorn		1,414	4.0	26.0
	Murray Grey		931	2.7	7.1
	European/X		1,318	3.8	3.3
	Tropical/X		5,530	15.8	1.5
Sex		0.00			
	Male		32,260	91.8	18.8
	Female		2,871	8.2	5.3
Cohort sex		0.00			
	Male		30,975	88.2	18.8
	Female^		1,952	5.6	3.0
	Mixed^		2,204	6.3	14.2
Intended days on feed		0.00			
	≥120		18,561	52.8	22.3
	85 to <120		12,615	35.9	15.3
	≤85^		3,955	11.3	3.6
Dentition		1.93			
	0		27,812	80.7	19.3
	2		5,560	16.1	12.9
	≥4		1,082	3.1	10.1
Age* (months)		9.20			
	<16^		1,598	16.4	12.5
	16 to <22		5,326	54.7	23.3
	≥22		2,807	28.9	17.1

^ Categories where 7 or more feedlots had no observations
* Age was analysed using the vendor questionnaire dataset

Table 6-2: Estimated odds ratios for the total effects of putative risk factors relating to induction characteristics on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Breed					(Source region)	N=35,049 4 level
	Angus	Ref				
	British Cross	1.2	(1.0 to 1.4)	0.007		
	Hereford	2.0	(1.5 to 2.6)	<0.001		
	Shorthorn	1.2	(0.9 to 1.6)	0.080		
	Murray Grey	0.5	(0.3 to 0.8)	0.001		
	European/X	0.8	(0.5 to 1.2)	0.169		
	Tropical/X	0.5	(0.3 to 0.7)	<0.001		
Sex					()	N=35,131 4 level
	Male	Ref				
	Female	0.7	(0.4 to 1.1)	0.063		
Cohort sex					(Sex)	N=35,131 4 level
	Male	Ref				
	Female^	1.5	(0.4 to 4.1)	0.349		
	Mixed^	1.4	(0.4 to 3.5)	0.346		
Intended days on feed					(Breed, Weight, Sex, Dentition)	N=34,361 3 level
	≥120	Ref				
	85 to <120	1.2	(0.7 to 1.8)	0.316		
	≤85^	1.1	(0.4 to 2.7)	0.479		
Dentition					()	N=34,454 3 level
	0	Ref				
	2	1.0	(0.9 to 1.1)	0.464		
	≥4	0.9	(0.7 to 1.2)	0.247		
Age* (months)					(Season, Source region)	N=9,731 3 level
	<16^	1.0	(0.7 to 1.3)	0.370		
	16 to <22	Ref				
	≥22	1.6	(1.3 to 2.1)	<0.001		

^ Categories where 7 or more feedlots had no observations
* Age was analysed using the vendor questionnaire dataset

Table 6-3: Estimated odds ratios for the direct effects of age on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Age* (months)					(Cohort fill, CohortN, Weight, SY -12 to 0, SY -27 to -13, SY pre-27, Season, Group-13N, Dentition, Mix summary, Move_FL, Source region)	N=9,522 3 level
	<16^	0.8	(0.6 to 1.2)	0.142		
	16 to <22	Ref				
	≥22	1.6	(1.3 to 2.1)	<0.001		

* Age was analysed using the vendor questionnaire dataset

Table 6-4: Putative risk factors relating to induction weight; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing %	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Induction weight (kg)		0.01			
	<400		7,027	20.0	13.0
	400 to <440		10,767	30.7	21.1
	440 to <480		12,029	34.3	19.2
	≥480		5,303	15.1	13.3
Mean cohort weight (kg)		0.00			
	<425		8,615	24.5	14.0
	425 to <455		17,694	50.4	20.7
	≥455		8,822	25.1	15.2
Weight difference from mean cohort weight (kg)		0.01			
	>20 below		8,425	24.0	20.1
	≤20 below		8,849	25.2	16.4
	≤20 above		9,330	26.6	17.0
	<20 below		8,522	24.2	17.3

Table 6-5: Estimated odds ratios for the total effects of putative risk factors relating to induction weight on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Induction weight (kg) Model A					(Dentition Breed, Grain type, Rain, Wind, Season, Sex, Temp max, Temp min, Source region)	N=34,361 3 level
	<400	Ref				
	400 to <440	0.8	(0.7 to 0.9)	<0.001		
	440 to <480	0.7	(0.6 to 0.8)	<0.001		
	≥480	0.6	(0.5 to 0.7)	<0.001		
Induction weight (kg) Model B					(Dentition, Breed, Induction year, Season, Sex, Source region)	N=34,361 3 level
	<400	Ref				
	400 to <440	0.8	(0.7 to 0.9)	<0.001		
	440 to <480	0.7	(0.6 to 0.8)	<0.001		
	≥480	0.6	(0.5 to 0.7)	<0.001		
Mean cohort weight (kg)					(CohortN, Weight)	N=35,126 4 level
	<425 kg	Ref				
	425 to <455	0.8	(0.4 to 1.3)	0.166		
	≥455	1.0	(0.5 to 1.9)	0.478		
Weight difference from mean cohort weight (kg)					(Breed, Weight cht, Weight)	N=35,044 4 level
	>20 below	1.1	(1.0 to 1.2)	0.114		
	≤20 below	Ref				
	≤20 above	1.0	(0.9 to 1.1)	0.319		
	>20 above	1.0	(0.9 to 1.2)	0.299		

6.3.3 Management risk factors

6.3.3.1 Weaning method

Weaning method was evaluated in the vendor-bred subset comprising animals born on the vendors' farms. The majority of these animals were yard weaned (80%,Table 6-6) and of these 53% were weaned over at least seven days. Yard weaning was associated with a decreased risk of BRD (OR 0.7, 95% credible interval: 0.5 to 1.0,Table 6-7). The effect was similar for those weaned over less than seven and at least seven days. The consistent direct effect estimate (Table 6-8) indicated that the protective effect of yard weaning was not mediated through prior feeding of grain or other supplementary feeding.

6.3.3.2 Prior vaccination

The majority of animals with vendor questionnaire data that were born on the vendor's farm or were purchased prior to 10 months of age had not been vaccinated with Bovilis MH™ (85%) or Pestigard™ (88%) prior to day -14 (Table 6-6). Prior vaccination with Bovilis MH™ was associated with a reduced risk of BRD (OR 0.8, 95% credible interval: 0.6 to 1.0, Table 6-7, and there was some evidence that prior vaccination with Pestigard™ was associated with a reduced risk of BRD (OR 0.8, 95% credible interval: 0.5 to 1.1,Table 6-7).

Table 6-6: Putative risk factors relating to the vendor questionnaire data; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing %	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Yard weaning*		4.6			
	No		983	20.4	31.2
	Yes		3,847	79.7	18.0
Yard weaning detail*		4.6			
	No		983	20.4	31.2
	Yes, <7 days		1,788	37.0	23.8
	Yes, ≥7 days		2,059	42.6	13.0
Prior Bovilis MH™ vaccination (BV_vacc)#		6.2			
	No		6,840	85.0	19.2
	Yes		1,205	15.0	15.4
Prior Pestigard™ vaccination (PV_vacc)#		6.2			
	No		7,063	87.8	19.0
	Yes		982	12.2	16.1

*Analysed in the vendor-bred subset #Analysed in the prior vaccination subset

Table 6-7: Estimated odds ratios for the total effects of yard weaning and prior vaccination as measured in vendor questionnaire subsets on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Yard weaning*					()	N=4,830 3 level
	No	Ref				
	Yes	0.7	(0.5 to 1.0)	0.015		
Yard weaning detail*					()	N=4,830 3 level
	No					
	Yes, <7 days	0.7	(0.4 to 1.0)	0.018		
	Yes, ≥7 days	0.7	(0.5 to 1.0)	0.033		
Prior Bovilis MH™ vaccination (BV_vacc)#					()	N=8,045 3 level
	No	Ref				
	Yes	0.8	(0.6 to 1.0)	0.020		
Prior Pestigard™ vaccination (PV_vacc)#					()	N=8,045 3 level
	No	Ref				
	Yes	0.8	(0.5 to 1.1)	0.054		

*Analysed in the vendor-bred subset #Analysed in the prior vaccination subset

Table 6-8: Estimated odds ratios for the direct effects of yard weaning on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Yard weaning*					(Induction year, Source region, Prior grain, prior supplementary feeding)	N=3,789 3 level
	No	Ref				
	Yes	0.5	(0.2 to 1.0)	0.020		

*Analysed in the vendor-bred subset

6.3.3.3 Mixing

On-farm mixing was evaluated in the vendor-bred subset. The majority of these animals had been mixed on the farm (94%, Table 6-9). There was no evidence of a large effect on the risk of BRD associated with on-farm mixing (Table 6-10).

A number of mixing variables were analysed in the main cohort dataset. The time to first mixing variable described the time interval during which animals were first mixed. About 62% of animals had been first mixed prior to day -90, 5% were first mixed between days -90 and -28, 3% were first mixed between days -27 and -13 and 29% were first mixed between days -12 and cohort close (Table 6-9).

The mix summary variable categorised animals based on mixing prior to day -27 (yes, no) and the number of group-28s forming the cohort (<4, ≥4). The most common mix summary pattern was animals that had been mixed prior to day -27 joining a cohort formed by four or more group-28s (44%; Table 6-9). A further 28% of animals had not been mixed prior to day -27 and joined cohorts formed by four or more group-28s (Table 6-9). The first mix composite variable (Table 6-9) was based on the mix summary variable except that animals first mixed between days -27 and -13 that joined cohorts formed by four or more group-28s (comprising 3% of the total) were in a separate category.

The mixing history variable provided a detailed mixing history based on mixing during three time intervals (pre day -27: yes, no; day -27 to day -13: yes, no; number of group-13s forming cohort). The distribution of mixing history in the population showed that just over one third of animals were mixed prior to day -27 and went into cohorts formed by 10 or more group-13s (‘Yes, no, ≥10’; 22% of animals) or four to nine group-13s (‘Yes, no, 4 to 9’; 16%, Table 6-9). A high level of mixing between day -12 and cohort close was also common in animals not mixed prior to day -27 (‘No, no, ≥10’; 15%; ‘No, no, 4 to 9’; 10%, Table 6-9).

Animals that were first mixed prior to day -90 (OR 0.6, 95% credible interval: 0.5 to 0.7, Table 6-10) or between day -90 and day -28 (OR 0.6, 95% credible interval: 0.4 to 0.8, Table 6-10) were at moderate to markedly reduced risk compared to animals first mixed between days -12 and cohort close. For animals first mixed between days -27 and -13 there was no evidence of a large effect (OR 0.9, 95% credible interval: 0.5 to 1.4, Table 6-10) and for those not mixed ever the effect estimate was highly imprecise so no conclusion was possible from this model. The protective effect of mixing prior to day -27 was evident in the mixing summary variable, which clearly demonstrated that compared to animals mixed prior to day -27 in cohorts formed by less than 4 group-28s, animals not mixed prior to day -27 in cohorts formed by less than 4 group-28s were at markedly increased risk (OR 2.1, 95% credible interval: 1.3 to 3.3, Table 6-10). It also demonstrated that animals subjected to a higher level of mixing close to induction (i.e. mixed pre day -27, 4 or more group-28s in cohort) were at markedly increased risk (OR 2.3, 95% credible interval: 1.3 to 3.6, Table 6-10), with the largest effect in animals not mixed prior to day -27 joining cohorts formed by more than 4 group-28s (OR 3.6, 95% credible interval: 2.1 to 5.7, Table 6-10). The first mix composite variable helped to clarify the risk for animals first mixed between days -27 and -13, which were also at markedly increased risk (OR: 3.0 95% credible interval: 1.4 to 5.7, Table 6-10) compared to animals first mixed prior to day -27 in cohorts formed by less than 4 group-28s.

Risk of BRD varied considerably between the different categories of the more detailed mixing history variable (Table 6-11). Compared to the reference category of animals that had been mixed prior to day -27 and went into cohorts formed by 2 or 3 group-13s ('Yes, no, 2 or 3'), those that had not been mixed prior to day -12 and were mixed with either 4 to 9 ('No, no, 4 to 9': OR 3.6, 95% credible interval: 1.8 to 6.1) or more than 10 group-13s ('No, no, ≥10': OR 3.5, 95% credible interval: 1.8 to 6.2) were at highest risk. Animals not mixed between day -27 and cohort close ('Yes, no, no': OR 1.1, 95% credible interval: 0.5 to 2.4) had a similar level of risk to the reference group. Estimates for animals mixed between days -27 and -13 were very imprecise, probably because the categories used were sparsely populated and did not distinguish the amount of mixing during this time interval. Although animals may have joined cohorts formed by less than 4 group-13s, the cohorts were usually formed by 4 or more group-28s. The direct effects of mixing history were similar to

those observed for the total effects estimates (Table 6-11). Assuming there were no other pathways, this indicates that most of the effect of this risk factor was mediated through the direct pathway, rather than through the presence of a PI animal in the group-28 and BVDV activity in the cohort (the only postulated indirect pathway).

6.3.3.4 Group and cohort size

As discussed previously (Section 4.6.3), the majority of animals had been in stable groups for an extended period of time before being moved to the feedlot. Hence, the numbers of animals in groups defined at different time points close to induction (e.g. day -13 or day -28) were highly correlated. The effects of group size therefore need to be interpreted alongside results for mixing and feedlot move timing. Because group-13 was the cluster variable used to identify 'group' in the study population hierarchy, the number of animals in group-13 (Group-13N) was the main group size variable used in analyses to estimate total and direct effects of group size and as a covariate in adjustment sets for other variables. Numbers of animals in group-13s were fairly evenly distributed across the population with 39% of animals in group-13s with less than 50 animals and 33% in group-13s with 100 or more animals (Table 6-12). The pattern was very similar for group-28s, with 42% of animals in group-28s with less than 50 animals and 30% in groups with 100 or more animals. The change in the percentage of animals in group-91s with less than 50 animals (49%) was largely driven by the preassembly management practice. The majority (65%) of animals were in cohorts comprised of 200 animals or more (Table 6-12).

Compared to animals from group-13s with less than 50 animals, animals from group-13s with 50 to 99 animals were at moderately reduced risk (OR: 0.8, 95% credible interval: 0.7 to 0.9,Table 6-13) and animals from group-13s with 100 or more animals were at markedly reduced risk of developing BRD (OR: 0.5, 95% credible interval: 0.4 to 0.7, Table 6-13). Group sizes defined at other time points (day -28 or day -91) were also investigated and included instead of group-13N in some models where appropriate. The effect estimates for the numbers of animals in group-28s and group-91s were consistent with those observed for the numbers of animals in group-13 (Table 6-13). Compared to animals from group-28s with less than 50 animals, animals from group-28s with 50 to 99 animals were at moderately reduced risk (OR: 0.8, 95% credible interval: 0.6 to 0.9) and animals from group-28s with 100 or more animals were at markedly reduced risk of developing BRD (OR: 0.5, 95%

credible interval: 0.3 to 0.6). For group-91s, compared to animals from group-91s with less than 50 animals, animals from group-91s with 50 to 99 animals were at slight to moderately reduced risk of developing BRD (OR: 0.8, 95% credible interval: 0.7 to 1.0, Table 6-13) as were animals from group-91s with 100 or more animals (OR: 0.7, 95% credible interval: 0.5 to 1.0, Table 6-13). The estimates for the effect of the number of animals in the cohort on the risk of BRD were imprecise (≥200 animals OR: 1.2, 95% credible interval: 0.7 to 1.8, Table 6-13), so no conclusion was possible.

The direct effects of the number of animals in group-13 and group-28 were of a similar magnitude to their respective total effects (Table 6-14), although the direct effects for group sizes of 100 or more animals were attenuated compared to the total effects. Assuming there are no other pathways, this indicates that most of the effect of group size is mediated through the direct pathway, rather than through either the number of animals in the cohort or mixing history (the only postulated indirect pathways).

6.3.3.5 Saleyard transfers

About a third of the cattle in the study (36%) had at least one saleyard transfer prior to day -27. However, only 3% of the cattle in the study were exposed to saleyards between days -27 and -13 and a further 3% of the cattle in the study were exposed to saleyards from days -12 to 0 (Table 6-15).

The total effect estimate indicated that animals that had been exposed to a saleyard prior to day -27 were at reduced risk compared to those that had not (OR 0.8, 95% credible interval: 0.7 to 0.9, Table 6-16). However, there was no evidence of a direct effect (OR: 1.0, 95% credible interval: 0.9 to 1.1, Table 6-17) indicating that the effect of this risk factor was mediated through mixing history (the only postulated indirect pathway) rather than through the direct pathway.

The total effect estimate indicated that animals that had been exposed to a saleyard between days -27 and -13 were at moderate to markedly increased risk compared to those that had not (OR 1.9, 95% credible interval: 1.3 to 2.7, Table 6-16. However, the direct estimate was reduced and suggested only a probable slight to moderate adverse effect (OR: 1.3, 95% credible interval: 0.8 to 2.0, Table 6-17). Although this estimate is highly imprecise, it indicates that most, but probably not all, of the effect

of this risk factor was mediated through mixing history (the only postulated indirect pathway) rather than through the direct pathway.

The total effect estimate indicated that animals that had been exposed to a saleyard between days -12 to 0 were at markedly increased risk compared to those that had not (OR 2.6, 95% credible interval: 1.6 to 4.1,Table 6-16). The direct effect was attenuated (OR: 1.6, 95% credible interval: 0.9 to 2.6,Table 6-17) but still important, indicating that exposure to a saleyard during this time period had a negative effect over and above the effects of mixing history (the only postulated indirect pathway).

6.3.3.6 Move to the feedlot

Most of the cattle in the study were moved to the vicinity of the feedlot within a day before day 0; 49% of all animals were transported less than 6 hours during this time interval, and 27% were transported for 6 hours or more (Table 6-18). Only 5% and 6% of animals arrived at the vicinity of the feedlot prior to day -27 and from days -27 to -13, respectively. As animals in this category were restricted to a small number of feedlots, and I hypothesized that in preassembly feedlots, the decision about how long to keep cattle on pasture prior to them entering a feedlot pen would depend on additional factors (breed, season, weight) not relevant to the full cohort dataset, I conducted a subset analysis restricted to animals in the preassembly subset. Within this subset, 31% moved to the feedlot prior to day -27, 30% moved between days -27 and -13 and 39% moved between day -12 and cohort close (Table 6-18).

Compared to animals transported for less than 6 hours within a day before day 0, animals transported for 6 hours or more during this time interval were at slight to moderately increased risk (OR 1.2, 95% credible interval: 1.0 to 1.5, Table 6-19). Animals moved to the vicinity of the feedlot at least 27 days before day 0 were at markedly reduced risk (OR 0.4, 95% credible interval: 0.2 to 0.8, Table 6-19). Point estimates for the effects of being transported to the vicinity of the feedlot between days -27 and -13 and between days -12 to -2 relative to being transported on days -1 or 0 in less than six hours were suggestive of no important effect but the 95% credible intervals were wide. The direct effects of the timing and duration of the move to the feedlot were generally similar to the total effects but less precise, with greater differences in the estimates for exposure categories with very unbalanced distributions across feedlots (Table 6-20).

Within the preassembly subset, animals moved to the vicinity of the feedlot prior to day -27 were probably at reduced risk of developing BRD compared to animals moved between day -12 and day 0, but estimates were imprecise (OR: 0.6, 95% credible interval: 0.2 to 1.5, Table 6-19). Estimates for those moved between days -27 and -13 were too imprecise to reach a conclusion (OR: 1.2, 95% credible interval: 0.4 to 2.7, Table 6-19).

6.3.3.7 Cohort formation patterns

The majority of the cattle in the study were in cohorts that were filled over more than one day (66% of animals, Table 6-18). However, at the animal level, day 0 was the cohort close date for more than half (57%) of the cattle in the study (Table 6-18). For a small percentage of cattle (8%) the cohort close date was at least seven days after day 0 (Table 6-18). For the majority of the cattle in the study, the first day on feed (DOF1) was the same date as the induction date (81% of animals, Table 6-18). Animals for which the first day on feed occurred earlier than the induction date were restricted to a small number of feedlots.

The total effect estimate for cohort fill duration indicated that risk of BRD was increased for animals in cohorts that were filled over more than one day compared to one day (OR: 1.9, 95% credible interval: 1.2 to 2.8,Table 6-19). There was no evidence of a large direct effect in either of the two direct effect models (OR: 1.2, 95% credible interval: 0.6 to 2.2 and OR: 1.1, 95% credible interval: 0.7 to 2.0, Table 6-20) indicating that most of the effect of this risk factor was mediated through one or more of the indirect pathways (mixing history or days from day 0 to cohort close), rather than through the direct pathway.

Animals with a longer period between day 0 and cohort close were at slight to moderately reduced risk compared to animals whose day 0 was the cohort close date (Table 6-19). The direct effect of the number of days from day 0 to cohort close was slightly lower than the total effects (Table 6-20), indicating that most of the effect of this risk factor was mediated through the direct pathway, rather than through the percentage grain on day 0 or day 20 or the time to 60% grain (the only postulated indirect pathways).

There was no evidence of a large effect of the duration between DOF1 and day 0 being one or two days compared to the same day on the risk of BRD. However, the

estimate for the effect of when the duration was least three days was very imprecise probably because this was a sparse category and was restricted to a few feedlots (Table 6-19).

6.3.3.8 Rhinogard™ at induction

Most of the cattle in the study were vaccinated with Rhinogard™ at induction (79%, Table 6-21) and Rhinogard™ use was completely clustered by feedlot (i.e. within feedlots, either all animals or no animals received Rhinogard™). Vaccination with Rhinogard™ was associated with a markedly increased risk of BRD (OR 6.0, 95% credible interval: 0.4 to 24.4,Table 6-22), but the estimate was very imprecise. Assuming Rhinogard™ does not cause BRD, it is almost certain that feedlots with past high BRD incidences preferentially used Rhinogard™, hence the effects of Rhinogard™ on BRD risk cannot be determined from this study.

6.3.3.9 Vitamin A, D and E at induction

About 30% of the cattle in the study were given vitamins A, D and E by injection at induction and this was completely clustered by feedlot (Table 6-21). There was no evidence of a large effect of using Vitamin A, D and E at induction on the risk of BRD (Table 6-22).

6.3.3.10 Presence of BVDV in the cohort and group-28

Of the 35,160 animals inducted into study cohorts, 85 animals (0.24%) were identified as PI animals (BVDV_PI_animal, Table 6-23). Of a total of 1,274 group-28s, 67 (5%) contained at least one PI animal, and 9% of animals were in the same group-28 as a PI animal. The PI animals were distributed among 54 of the 170 (32%) cohorts, from 12 of the 14 feedlots such that 46% of animals were in cohorts that contained at least one PI animal (BVDV_chtPI, Table 6-23). However, transient infection (TI) with BVDV occurred in cohorts in which no PI animals were identified; BVDV was detected in at least one animal from 101 cohorts (59% of cohorts), so only 34% of animals (BVDV_chtYN , Table 6-23) were in cohorts in which BVDV was not detected (i.e. BVDV not detected in any cohort animals). Of the animals in cohorts with an identified PI animal, 20% (3,198/16,040) were determined to have been in the same group-28 as an identified PI animal (BVDV_PI_grp28, Table 6-23).

PI animals were at increased risk of developing BRD compared to animals that were not PIs (OR 1.9, 95% credible interval: 1.0 to 3.2, Table 6-24). Compared to animals

in cohorts where BVDV was not detected, animals in cohorts where BVDV was detected were at similarly increased risk if a PI animal was (OR 1.6, 95% credible interval: 1.0 to 2.4, Table 6-24) or was not (OR 2.0, 95% credible interval: 1.1 to 3.2, Table 6-24) identified in the cohort. Animals in cohorts where BVDV had been identified in any animal (i.e. either PI or TI) were at moderately increased risk of BRD compared to animals in cohorts where BVDV was not detected (OR 1.7, 95% credible interval: 1.1 to 2.5, Table 6-24). In an analysis restricted to those cohorts where a PI animal had been identified, animals from group-28s where a PI animal was identified were not at reduced risk compared to those from group-28s where no PI animals were identified (BVDV_PI_grp28: OR 1.0, 95% credible interval: 0.8 to 1.1, Table 6-24). This was consistent with the results derived from the composite variable (BVDV_grp_cht) in which animals from group-28s with an identified PI animal were at similarly increased risk of BRD (OR 1.6, 95% credible interval: 0.9 to 2.4) as animals from group-28s where no PI animal was identified but in cohorts where BVDV was detected (OR 1.7, 95% credible interval: 1.1 to 2.6, Table 6-24) compared to animals from cohorts where BVDV was not detected.

Table 6-9: Putative risk factors relating to mixing; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
On-property mixing (Mix_VQ)*		0.59			
	No^		322	6.4	27.3
	Yes		4,711	93.6	20.9
Time of earliest mixing (Mix first)		0.86			
	Pre day -90		21,658	62.2	13.6
	Day -90 to -28		1,741	5.0	4.5
	Day -27 to -13		1,034	3.0	11.4
	Day -12 to 0		9,977	28.6	29.2
	Not mixed^		418	1.2	20.6
Mix summary (mixed pre day -27, group-28s in cohort)		0.02			
	No, <4		1,713	4.9	19.6
	No, ≥4		9,790	27.9	28.5
	Yes, <4		8,120	23.1	4.7
	Yes, ≥4		15,500	44.1	17.4
First mix composite (mix first; group-28s in cohort)		0.04			
	Day-12 to 0, <4		1,713	4.9	19.6
	Day-12 to 0, ≥4		8,794	25.0	30.4
	Day-27 to -13, ≥4		992	2.8	11.7
	Pre day-27, <4		8,117	23.1	4.7
	Pre day-27, ≥4		15,500	44.1	17.4
Mix history (pre day -27, days -27 to -13, day -12 to cohort close)		1.14			
	No, no, no^		418	1.2	20.6
	No, no, 2 or 3		1,489	4.3	19.5
	No, no, 4 to 9		3,332	9.6	30.3
	No, no, ≥10		5,112	14.7	31.4
	No, yes, yes		627	1.8	17.2
	No, yes, no^		407	1.2	2.5
	Yes, no, 2 or 3		3,893	11.2	5.7
	Yes, no, 4 to 9		5,411	15.6	16.4
	Yes, no, ≥10		7,795	22.4	20.7
	Yes, yes, yes^		946	2.7	13.7
	Yes, yes, no^		1,958	5.6	3.3
	Yes, no, no		3,342	9.6	3.4

^ Categories where 7 or more feedlots had no observations.
*Vendor-bred subset

Table 6-10: Estimated odds ratios for the total effects of putative risk factors relating to mixing on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
On-property mixing (Mix_VQ)*					()	N=5,033 3 level
	No^	Ref				
	Yes	1.1	(0.7 to 1.6)	0.463		
Time of earliest mixing (Mix first)					Weight, SY -12 to 0, SY -27 to -13, SY pre-27, Group-91N, Arrival to day0)	N=34,725, 4 level
	Pre day -90	0.6	(0.5 to 0.7)	<0.001		
	Day -90 to -28	0.6	(0.4 to 0.8)	0.002		
	Day -27 to -13	0.9	(0.5 to 1.4)	0.260		
	Day -12 to 0	Ref				
	Not mixed^	1.0	(0.2 to 2.9)	0.350		
Mix summary pre day -27, group-28s in cohort					(Cohort fill, Weight, SY --12 to 0, SY -27 to -13, SY pre-27, CohortN, Move_FL, Group-13N)	N=34,726 4 level
	No, <4	2.1	(1.3 to 3.3)	0.001		
	No, ≥4	3.6	(2.1 to 5.7)	<0.001		
	Yes, <4	Ref				
	Yes, ≥4	2.3	(1.3 to 3.6)	0.001		
First mix composite (mix first; group-28s in cohort)					(Cohort fill, Weight, SY -12 to 0, SY -27 to -13, SY pre-27, CohortN, Move_FL, Group-13N)	N=34,726 4 level
	Day-12 to 0, <4	2.2	(1.2 to 3.6)	0.004		
	Day-12 to 0, ≥4	3.6	(1.9 to 6.0)	<0.001		
	Day-27 to -13, ≥4	3.0	(1.4 to 5.7)	0.002		
	Pre day-27, <4	Ref				
	Pre day-27, ≥4	2.3	(1.2 to 3.8)	0.004		

^ Categories where 7 or more feedlots had no observations
* Vendor-bred subset

Table 6-11: Estimated odds ratios for the total and direct effects of mixing history on the risk of BRD by day 50

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set, N, level
Total effects Mix history (pre day -27, days -27 to -13, day -12 to cohort close)					(Cohort fill, Weight, SY -12 to 0, SY -27 to -13, SY pre-27, CohortN, Move_FL, Group-13N) N=34,726 4 level
	No, no, no^	2.4	(0.4 to 7.8)	0.210	
	No, no, 2 or 3	2.3	(1.3 to 3.7)	0.003	
	No, no, 4 to 9	3.6	(1.8 to 6.1)	<0.001	
	No, no, ≥10	3.5	(1.8 to 6.2)	<0.001	
	No, yes, yes	3.2	(1.4 to 6.2)	0.003	
	No, yes, no^	2.2	(0.5 to 6.7)	0.192	
	Yes, no, 2 or 3	Ref			
	Yes, no, 4 to 9	2.7	(1.3 to 4.6)	0.002	
	Yes, no, ≥10	2.1	(1.1 to 3.7)	0.014	
	Yes, yes, yes^	2.1	(0.9 to 3.9)	0.038	
	Yes, yes, no^	2.5	(0.7 to 6.5)	0.087	
	Yes, no, no	1.1	(0.5 to 2.4)	0.455	
Direct effects Mix history (pre day -27, days -27 to -13, day -12 to cohort close)					(Cohort fill, Weight, SY -12 to 0, SY -27 to -13, SY pre-27, CohortN, Move_FL, Group-13N, Shared pen water, BVDV_grp_cht, BVDV_PI_animal) N=34,726 4 level
	No, no, no^	2.9	(0.5 to 9.6)	0.142	
	No, no, 2 or 3	2.2	(1.3 to 3.7)	0.002	
	No, no, 4 to 9	3.1	(1.5 to 5.6)	0.001	
	No, no, ≥10	3.0	(1.3 to 5.7)	0.004	
	No, yes, yes	2.8	(1.1 to 5.9)	0.013	
	No, yes, no^	2.3	(0.5 to 7.0)	0.180	
	Yes, no, 2 or 3	Ref			
	Yes, no, 4 to 9	2.3	(1.1 to 4.2)	0.009	
	Yes, no, ≥10	1.8	(0.8 to 3.5)	0.081	
	Yes, yes, yes^	1.8	(0.7 to 3.7)	0.111	
	Yes, yes, no^	2.5	(0.7 to 6.4)	0.095	
	Yes, no, no	1.1	(0.4 to 2.3)	0.507	

^ Categories where 7 or more feedlots had no observations

Table 6-12: Putative risk factors relating to numbers of animals in a group and moving to the feedlot; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
No. animals in group-91 (Group-91N)		1.12			
	<50		17,109	49.3	20.5
	50 to 99		9,256	26.6	21.0
	≥100		8,374	24.1	8.2
No. animals in group-28 (Group-28N)		0.00			
	<50		14,717	41.9	22.8
	50 to 99		9,843	28.0	21.2
	≥100		10,571	30.1	7.1
No. animals in group-13 (Group-13N)		0.00			
	<50		13,782	39.2	24.1
	50 to 99		9,783	27.9	21.3
	≥100		11,566	32.9	6.9
No. animals in cohort (CohortN)		0.00			
	<200		12,243	34.8	11.5
	≥200		22,888	65.2	20.9

Table 6-13: Estimated odds ratios for the total effects of putative risk factors relating to the number of animals in a group and the timing of the move to the feedlot.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
No. animals in group-91 (Group-91N)					()	N=35,131 4 level
	<50	Ref				
	50 to 99	0.8	(0.7 to 1.0)	0.023		
	≥100	0.7	(0.5 to 1.0)	0.019		
No. animals in group-28 (Group-28N)					()	N=35,131 4 level
	<50	Ref				
	50 to 99	0.8	(0.6 to 0.9)	0.001		
	≥100	0.5	(0.3 to 0.6)	<0.001		
No. animals in group-13 (Group-13N)					()	N=35,131 4 level
	<50	Ref				
	50 to 99	0.8	(0.7 to 0.9)	0.002		
	≥100	0.5	(0.4 to 0.7)	<0.001		
No. animals in cohort (CohortN)					(Group-13N, FeedlotN)	N=35,131 4 level
	<200	Ref				
	≥200	1.2	(0.7 to 1.8)	0.254		

Table 6-14: Estimated odds ratios for the direct effects of the number of animals in a group on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
No. animals in group-13 (Group-13N)					(CohortN, Cohort fill, Weight, SY -12 to 0, SY -27 to -13, SY pre-27, FeedlotN, Mix history, Move_FL)	N=34,726 4 level
	<50	Ref				
	50 to 99	0.8	(0.7 to 1.0)	0.009		
	≥100	0.6	(0.4 to 0.8)	0.001		
No. animals in group-28 (Group-28N)					(CohortN, Cohort fill, Weight, SY -12 to 0, SY -27 to -13, SY pre-27, FeedlotN, Mix history, Move_FL)	N=34,726 4 level
	<50	Ref				
	50 to 99	0.8	(0.7 to 1.0)	0.005		
	≥100	0.7	(0.5 to 1.0)	0.016		

Table 6-15: Putative risk factors relating to transfers through a saleyard; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Saleyard pre day -27		1.14			
	No		22,223	64.0	18.7
	Yes		12,507	36.0	15.7
Saleyard days -27 to -13		0.00			
	No		34,162	97.2	17.8
	Yes		969	2.8	11.2
Saleyard days -12 to 0		0.00			
	No		34,200	97.4	17.6
	Yes		931	2.7	21.4

Table 6-16: Estimated odds ratios for the total effects of putative risk factors relating to moving through a saleyard on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Saleyard transfer pre day -27					()	N=34,730 4 level
	No	Ref				
	Yes	0.8	(0.7 to 0.9)	<0.001		
Saleyard transfer days -27 to -13					()	N=35,131 4 level
	No	Ref				
	Yes	1.9	(1.3 to 2.7)	0.001		
Saleyard transfer days -12 to 0					()	N=35,131 4 level
	No	Ref				
	Yes	2.6	(1.6 to 4.1)	<0.001		

Table 6-17: Estimated odds ratios for the direct effects of moving through a saleyard on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Saleyard transfer pre day -27					(CohortN, CohortFill, Weight, SY -12 to 0, SY -27 to -13, Group-13N, Mix history, Move_FL)	N=34,726 4 level
	No	Ref				
	Yes	1.0	(0.9 to 1.1)	0.486		
Saleyard transfer days -27 to -13					(CohortN, CohortFill, Weight, SY -12 to 0, SY -27 to -13, SY Pre27, Group-13N, Mix history, Move_FL)	N=34,726 4 level
	No	Ref				
	Yes	1.3	(0.8 to 2.0)	0.156		
Saleyard transfer days -12 to 0					(CohortN, CohortFill, Weight, SY -27 to -13, SY Pre27, Group-13N, Mix history, Move_FL)	N=34,726 4 level
	No	Ref				
	Yes	1.6	(0.9 to 2.6)	0.049		

Table 6-18: Putative risk factors relating moving to the feedlot and cohort formation times; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Move to feedlot: days before day 0 and hours of transport		0.00			
	Pre day -27^		1,880	5.4	1.5
	Days -27 to -13^		2,000	5.7	4.6
	Days -12 to -2; <6 h		2,183	6.2	10.9
	Days -12 to-2; ≥6 h		2,339	6.7	8.0
	Days -1 to 0; <6 h		17,139	48.8	19.9
	Days -1 to 0; ≥6 h		9,590	27.3	23.5
Arrival to day 0* (days)		0.00			
	≥28		1,747	31.0	1.5
	27 to 13		1,723	30.5	5.3
	12 to 0		2,171	38.5	3.3
Cohort fill duration (days)		0.00			
	1		12,051	34.3	7.4
	>1		23,080	65.7	23.0
Days from DOF1 to day 0		0.00			
	0		28,386	80.8	18.8
	1 or 2^		4,940	14.1	14.7
	≥3^		1,805	5.1	7.8
Days from day 0 to cohort close		0.00			
	1		20,001	56.9	13.9
	1 to 6		12,408	35.3	23.4
	≥7		2,722	7.8	19.0

^ Categories where 7 or more feedlots had no observations
*Preassembly subset

Table 6-19: Estimated odds ratios for the total effects of putative risk factors relating to the timing of the move to the feedlot and cohort formation

Variable	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Move to feedlot Days before day 0 and hours of transport					(SY -12 to 0, SY -27 to 13)	N=35,131 4 level
	Pre day -27^	0.4	(0.2 to 0.8)	0.004		
	Days -27 to -13^	1.0	(0.4 to 1.9)	0.394		
	Days -12 to -2; <6 h	0.9	(0.6 to 1.3)	0.217		
	Days -12 to -2; ≥6 h	0.9	(0.5 to 1.4)	0.305		
	Days -1 to 0; <6 h	Ref				
	Days -1 to 0; ≥6 h	1.2	(1.0 to 1.5)	0.016		
Days from arrival to day 0*					(Breed, Weight, Season, SY -27 to 0, SY pre-27)	N=5,551 3 level
	≥28	0.6	(0.2 to 1.5)	0.108		
	27 to 13	1.2	(0.4 to 2.7)	0.480		
	12 to 0	Ref				
Cohort fill duration (days)					(CohortN, DOF1 to day0)	N=35,131 4 level
	1	Ref				
	>1	1.9	(1.2 to 2.8)	0.005		
Days from DOF1 to day 0					()	N=35,131 4 level
	0	Ref				
	1 or 2^	0.9	(0.6 to 1.3)	0.213		
	≥3^	1.1	(0.4 to 2.4)	0.481		
Days from day 0 to cohort close					(Cohort fill)	N=35,131 4 level
	0	Ref				
	1 to 6	0.8	(0.7 to 1.0)	0.008		
	≥7	0.7	(0.5 to 0.9)	0.004		

^ Categories where 7 or more feedlots had no observations
*Preassembly subset

Table 6-20: Estimated odds ratios for the direct effects of putative risk factors relating to the timing of the move to the feedlot and cohort formation on the risk of BRD by day 50

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Move to feedlot Days before day 0 and hours of transport					(CohortN, Cohort fill, Induction Weight, SY -12 to 0, SY -27 to -13, SY Pre27, Group-13N, Mix history, Move_FL)	N=34,726 4 level
	Pre day -27^	0.6	(0.2 to 1.2)	0.065		
	Days -27 to -13^	1.3	(0.5 to 2.8)	0.337		
	Days -12 to -2; <6 h	0.9	(0.6 to 1.3)	0.275		
	Days -12 to-2; ≥6 h	0.9	(0.6 to 1.5)	0.346		
	Days -1 to 0; <6 h	Ref				
	Days -1 to 0; ≥6 h	1.2	(1.0 to 1.5)	0.012		
Cohort fill duration (days) Model A					(CohortN, Day0 to close, DOF1 to day0, Weight, SY -12 to 0, SY -27 to -13, SY pre-27, Group-13N, Mix history, Move_FL)	N=34,726 4 level
	1	Ref				
	>1	1.2	(0.6 to 2.2)	0.288		
Cohort fill duration (days) Model B					(CohortN, DOF1 to day0, Grain1, Grain21, Grain60%, Weight, Intended DOF, SY -27 to -13, SY pre-27, Group-13N, Mix history, Move_FL)	N=34,726 4 level
	1	Ref				
	>1	1.1	(0.7 to 2.0)	0.382		
Days from day 0 to cohort close					(Cohort fill, DOF1 to day0, Grain1, Grain21, Grain60%, Intended DOF)	N=35,131 4 level
	1	Ref				
	1 to 6	0.8	(0.7 to 1.0)	0.026		
	≥7	0.8	(0.5 to 1.1)	0.067		

^ Categories where 7 or more feedlots had no observations

Table 6-21: Putative risk factors relating to induction treatments; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Rhinogard™ at induction		0.00			
	No^		7,365	21.0	2.8
	Yes		27,766	79.0	21.6
Vitamin ADE at induction		0.00			
	No		24,518	69.8	17.1
	Yes^		10,613	30.2	18.9

^ Categories where 7 or more feedlots had no observations

Table 6-22: Estimated odds ratios for the total effects of induction treatments on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Rhinogard™ at induction					()	N=35,131 4 level
	No^	Ref				
	Yes	6.0	(0.6 to 24.4)	0.080		
Vitamin ADE at induction (Vit ADE)					()	N=35,131 4 level
	No	Ref				
	Yes^	1.1	(0.6 to 1.9)	0.364		

^ Categories where 7 or more feedlots had no observations

Table 6-23: Exposure variables relating to the presence of BVDV in a cohort and animals persistently infected with BVDV (BVDV-PI animals); distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
PI animal (BVDV_PI_animal)		0.03			
	No		35,034	99.8	17.6
	Yes		85	0.2	27.1
BVDV active & PI identified in cohort (BVDV_chtPI)		0.00			
	No		11,896	33.9	8.7
	PI identified		16,040	45.6	20.0
	TI		7,195	20.5	27.2
BVDV active in cohort (BVDV_chtYN)		0.00			
	No		11,896	33.9	8.7
	Yes		23,235	66.1	22.2
PI animal in group-28 (BVDV_PI_grp28)*		0.00			
	No		12,842	80.1	20.7
	Yes		3,198	19.9	17.3
PI in group-28 and BVDV active in cohort (BVDV_grp_cht)		0.00			
	No, no		11,896	33.9	8.7
	Yes, yes		3,198	9.1	17.3
	No, yes		20,037	57.0	23.0

* restricted to cohorts with an identified PI animal

Table 6-24: Estimated odds ratios for the total effects of the presence of animals persistently infected with BVDV (PI animals) on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
PI animal (BVDV_PI_animal)						N=35,119 4 level
	No	Ref				
	Yes	1.9	(1.0 to 3.2)	0.030	()	
BVDV active & PI identified in cohort (BVDV_chtPI)					(BVDV_PI_animal, CohortN, Shared pen water, Mix history)	N=34,693 4 level
	No	Ref				
	PI identified	1.6	(1.0 to 2.4)	0.039		
	TI	2.0	(1.1 to 3.2)	0.009		
BVDV active in cohort (BVDV_chtYN)					(BVDV_PI_animal, CohortN, Shared pen water, Mix history)	N=34,693 4 level
	No	Ref				
	Yes	1.7	(1.1 to 2.5)	0.010		
PI animal in group-28 (BVDV_PI_grp28)*					(BVDV_PI_animal, CohortN, Shared pen water, Mix history)	N=16,020 3 level
	No	Ref				
	Yes	1.0	(0.8 to 1.1)	0.285		
PI in group-28 and BVDV active in cohort (BVDV_grp_cht)					(BVDV_PI_animal, CohortN, Shared pen water, Mix history)	N=34,693 4 level
	No, no	Ref				
	Yes, yes	1.6	(0.9 to 2.4)	0.041		
	No, yes	1.7	(1.1 to 2.6)	0.009		

* restricted to cohorts with identified PI animal

6.3.3.11 Shared pen water

Most of the cattle in the study were in pens where the water troughs could be accessed by animals in an adjoining pen (82%, Table 6-25). Pen water access status did not vary between study cohorts in ten of the fourteen feedlots, so a subset analysis was also performed restricted to those with disparate values.

Shared pen water was associated with a markedly increased risk of BRD (OR 4.3, 95% credible interval: 1.4 to 10.3, Table 6-26). Results from the subset analysis using only data from the four feedlots with disparate values for study cohorts were

consistent, (OR 4.2, 95% credible interval: 1.5 to 9.3) indicating that the observed increase in risk was unlikely to be due to confounding by feedlot. The direct effect of shared pen water (estimated in the full cohort dataset) was slightly attenuated (OR 3.3, 95% credible interval: 1.1 to 7.8, Table 6-26), indicating that the effect was partly mediated through the presence of BVDV in the cohort (the only postulated intervening variable), but that a strong significant direct effect was also present. Analysis restricted to the case-control dataset provided a similarly large total effect estimate (OR 5.0, 95% credible interval: 1.4 to 14.6, Table 6-26) with an attenuated direct effect estimate (OR 3.3, 95% credible interval: 1.1 to 8.0, Table 6-26). The direct effect was estimated by adjusting for the animal-level change in serostatus between induction and follow-up (after approximately 42 days on feed: up/no change/initially high) to each of four viruses and the presence of BVDV in the cohort (the postulated indirect pathways). This indicated that the effect of shared access to pen water may be partially mediated through active infection with BoHV1, BRSV, BVDV and/or BPI3, but that an important direct effect remained over and above these effects.

6.3.3.12 Other pen characteristics

About two-thirds of the cattle in the study were in pens with some shade (69%, Table 6-25). Pen shade did not vary between study cohorts in 11 of the 14 feedlots. Of the cattle in the study, 70% were in pens that had two (rather than one) other pens adjoining (Table 6-29). The most frequent stocking density was 11 to <14m^2/SCU (41% of animals) or 14 to <17m^2/SCU (31%, Table 6-25). Forty-five per cent of the cattle in the study were in pens with bunk spaces of 0.18 to <0.24 m/head (Table 6-25).

Estimates for the total effect of pen shade (none compared to some) on the risk of BRD were suggestive of increased risk but were imprecise (OR 1.7, 95% credible interval: 0.8 to 3.4, Table 6-27). There was no evidence for a strong effect of the number of adjoining pens on the risk of BRD (OR: 1.1, 95% credible interval: 0.6 to 1.6). Estimates for the total effect of stocking density on the risk of BRD were imprecise probably because the distribution across categories was clustered by feedlot (Table 6-27). Estimates for the total effect of bunk space on the risk of BRD were imprecise but were suggestive of a possible protective effect when bunk space

was ≥0.24m/head compared to <0.18m/head (OR: 0.6, 95% credible interval: 0.2 to 1.2, Table 6-27).

6.3.3.13 Prior feeding history

The majority of animals with vendor questionnaire data that were born on the vendor's property had not been fed grain (77%) but had been fed conserved forage or supplement before leaving the property (84%, Table 6-28). Estimates were suggestive of a probable decrease in the risk of BRD associated with prior feeding of grain, but the estimates were imprecise (OR 0.6, 95% credible interval: 0.3 to 1.1, Table 6-29). There was no evidence of a large effect on the risk of BRD associated with prior feeding of conserved forage or supplement (Table 6-29).

6.3.3.14 Rations

The most commonly fed grain types were barley (48%) and wheat mix (40%, Table 6-28). Grain type was highly clustered by feedlot. Over half the cattle in the study were fed rations on day 0 containing at least 40% grain on an 'as fed' basis (54%, Table 6-28). On day 20, most of the cattle in the study were fed a ration containing 60 to <70% or ≥70% grain on an 'as fed' basis (39% and 32%, respectively, Table 6-28). For most of the cattle in the study, the ration reached 60% grain on an 'as fed' basis between days 7 and 13 or days 14 and 20 (31% and 40%, respectively, Table 6-28).

Estimates for the total effect of grain type on the risk of BRD were imprecise and inconsistent across the models fitted using the three minimal sufficient adjustment sets (Table 6-29). Such inconsistencies were likely to be due to unmeasured feedlot-level variables. The models were supportive of a protective effect of sorghum but the estimates were very imprecise so no conclusion can be reached.

Estimates for the total effects of the percentage of grain in the ration fed on day 0 and on day 20, as well as the number of days until the ration contained 60% grain on the risk of BRD were imprecise probably because the distribution of the categories was clustered by feedlot (Table 6-30). Estimates were consistent across the models fitted using the two minimal sufficient adjustment sets.

6.3.3.15 Numbers of animals on feed in the feedlot

Nearly 40% of the cattle in the study were in feedlots where there were 10,000 to <20,000 total cattle on feed and nearly 40% of study animals were in feedlots where there were 3,000 to <6,000 cattle less than 40 days on feed at the start of or during the animal's induction month (Table 6-31).

Estimates for the total effect of the number of cattle on feed and the number of cattle less than 40 days on feed in the induction month on the risk of BRD were imprecise probably because the distribution of the categories was clustered by feedlot (Table 6-32).

Table 6-25: Putative risk factors relating to pen characteristics; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Shared pen water		0.00			
	No^		6,453	18.4	3.9
	Yes		28,678	81.6	20.7
Pen shade		0.00			
	None		11,141	31.7	9.6
	Any		23,990	68.3	21.4
Number of adjoining pens		0.00			
	1		10,394	29.9	14.7
	2		24,391	70.1	19.1
Stocking density (m^2/SCU#)		0.00			
	11 to <14^		14,266	40.6	21.6
	14 to <17		10,893	31.0	17.8
	17 to <25		5,436	15.5	11.9
	≥25^		4,536	12.9	11.6
Bunk space (m/head)		3.30			
	<0.18^		9,500	28.0	13.5
	0.18 to <0.24		15,253	44.9	22.2
	≥0.24		9,214	27.1	14.3

^ Categories where 7 or more feedlots had no observations

A Standard Cattle Unit (SCU) is equivalent to an animal with a live-weight of 600 kg.

Table 6-26: Estimated odds ratios for the total and direct effects of shared pen water on the risk of BRD by day 50

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Total effects						
Shared pen water					()	N=35,131 4 level
	No^	Ref				
	Yes	4.3	(1.4 to 10.3)	0.005		
Shared pen water (only feedlots with both categories)					()	N=14,210 4 level
	No	Ref				
	Yes	4.2	(1.5 to 9.3)	0.001		
Shared pen water[#]					()	N=7,314 3 level
	No^	Ref				
	Yes	5.0	(1.4 to 14.6)	0.001		
Direct effects					(BVDV_PI_animal, BVDV_chtYN, CohortN, mix history)	
Shared pen water						N=34,693 4 level
	No^	Ref				
	Yes	3.1	(1.0 to 7.7)	0.020		
Shared pen water[#]					(serochange to BPI3, BRSV, BVDV & BHV1, BVDV_chtYN, CohortN, Rhinogard, Mix summary)	N=6,477 3 level
	No^	Ref				
	Yes	3.3	(1.1 to 8.0)	0.019		

^ Categories where 7 or more feedlots had no observations
[#] restricted to case-control dataset

Table 6-27: Estimated odds ratios for the total effects of risk factors relating to pen characteristics on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level, DIC
Stocking density (m^2/standard cattle unit)					(CohortN)	N=35,131 4 level
	11 to <14^	Ref				
	14 to <17	1.0	(0.5 to 2.0)	0.469		
	17 to <25	0.8	(0.3 to 1.6)	0.197		
	≥25^	1.2	(0.5 to 2.5)	0.417		
Bunk space (m/head)					(CohortN)	N=33,967 3 level
	<0.18^	Ref				
	0.18 to <0.24	0.7	(0.3 to 1.3)	0.116		
	≥0.24	0.6	(0.2 to 1.2)	0.073		
Pen shade					()	N=35,131 4 level
	No	Ref				
	Yes	1.6	(0.7 to 3.3)	0.154		
Number of adjoining pens					()	N=34,785 3 level
	1	Ref				
	2	1.1	(0.6 to 1.6)	0.394		

^ Categories where 7 or more feedlots had no observations

Table 6-28: Putative risk factors relating to prior feeding and ration characteristics; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Prior grain feeding (Grain pre)*		20.56			
	No		3,082	76.6	24.9
	Yes		940	23.4	16.4
Prior conserved forage or supplement (Supp pre)*		20.56			
	No		659	16.4	28.8
	Yes		3,363	83.6	21.7
Grain type		0.00			
	Barley		16,825	47.9	25.0
	Sorghum^		2,709	7.7	2.9
	Wheat mix^		14,168	40.3	12.8
	Other mix^		1,429	4.1	7.3
Grain % on day 0		0.00			
	<35%		7,762	22.1	16.5
	35 to <40%		8,322	23.7	32.0
	40 to <45%		9,007	25.6	9.5
	≥45%		10,040	28.6	14.0
Grain % on day 20		0.00			
	<60%^		9,817	27.9	20.1
	60 to <70%^		13,781	39.2	18.3
	≥70%		11,533	32.8	14.8
Days to 60% grain		0.00			
	0 to 6^		3,358	9.6	3.6
	7 to 13		10,821	30.8	14.8
	14 to 20		13,987	39.8	22.7
	≥21^		6,965	19.8	18.6

^ Categories where 7 or more feedlots had no observations
*Analysed in the vendor bred subset; missing % refers to this dataset

Table 6-29: Estimated odds ratios for the total effects of prior grain feeding, prior conserved forage/supplement and grain type on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Prior grain feeding (Grain pre)*					(Yard weaning, Induction year, Source region)	N=3789 3 level
	No	Ref				
	Yes	0.6	(0.3 to 1.1)	0.038		
Prior conserved forage/supplement (Supp pre)*					(Yard weaning, Induction year, Source region)	N=3789 3 level
	No	Ref				
	Yes	1.5	(0.6 to 3.3)	0.227		
Grain type Model A					(Dentition, Breed, Weight, Rain, Wind, Season, Sex, Temp max, Temp min, Source region)	N=34,361 3 level
	Barley	Ref				
	Sorghum^	0.2	(0.0 to 0.8)	0.014		
	Wheat mix^	0.9	(0.3 to 2.4)	0.287		
	Other mix^	0.5	(0.1 to 1.8)	0.099		
Grain type Model B					(Temp min, Induction year, Rain, Wind, Season, Temp max, Source region)	N=35,125 4 level
	Barley	Ref				
	Sorghum^	0.2	(0.0 to 1.2)	0.033		
	Wheat mix^	1.3	(0.2 to 4.0)	0.481		
	Other mix^	0.5	(0.1 to 2.2)	0.135		
Grain type Model C					(Feedlot region, Induction year, Season)	N=35,131 4 level
	Barley	Ref				
	Sorghum^	0.5	(0.0 to 2.1)	0.145		
	Wheat mix^	3.0	(1.0 to 7.4)	0.029		
	Other mix^	1.2	(0.2 to 5.1)	0.388		

^ Categories where 7 or more feedlots had no observations
*Analysed using the vendor questionnaire subset1 dataset

Table 6-30: Estimated odds ratios for the total effects of the percentage grain at day 50 and the number of days to 60% grain on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Grain % on day 0					(Day 0_close, DOF1_day 0, Intended DOF)	N=35,131 4 level
	<35%^	Ref				
	35 to <40%	1.1	(0.5 to 2.5)	0.499		
	40 to <45%	0.8	(0.2 to 1.9)	0.228		
	≥45%	1.1	(0.2 to 2.9)	0.493		
Grain % on day 20 Model A					(Day 0_close, Grain1, Intended DOF)	N=35,131 4 level
	<60%^	Ref				
	60 to <70%^	1.0	(0.8 to 1.3)	0.438		
	≥70%	1.1	(0.5 to 2.0)	0.473		
Grain % on day 20 Model B					(Cohort fill, DOF1_day 0, Grain1, Grain60%, Intended DOF)	N=35,131 4 level
	<60%^	Ref				
	60 to <70%^	0.9	(0.6 to 1.2)	0.213		
	≥70%	1.0	(0.5 to 1.8)	0.454		
Days to 60% grain Model A					(Day 0_close, DOF1_day 0, Intended DOF)	N=35,131 4 level
	0 to 6^	Ref				
	7 to 13	1.2	(0.5 to 2.1)	0.350		
	14 to 20	1.1	(0.4 to 1.9)	0.523		
	≥21^	0.9	(0.3 to 1.6)	0.322		
Days to 60% grain Model B					(Cohort fill, DOF1_day 0, Grain1, Grain21, Intended DOF)	N=35,131 4 level
	0 to 6^	Ref				
	7 to 13	1.1	(0.5 to 2.5)	0.479		
	14 to 20	0.9	(0.3 to 1.9)	0.281		
	≥21^	0.6	(0.2 to 1.4)	0.088		

^ Categories where 7 or more feedlots had no observations

Table 6-31: Exposure variables relating to monthly summaries of numbers of animals on feed in the feedlot; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Number on feed in animal's induction month (FeedlotN)		0.00			
	<10,000		11,538	32.8	5.8
	10,000 to <20,000^		13,818	39.3	18.0
	≥20,000^		9,775	27.8	31.2
Number <40 DOF in animal's induction month (FeedlotN40)		0.00			
	<3,000^		11,240	32.0	6.5
	3,000 to <6,000^		13,622	38.8	18.0
	≥6,000^		10,269	29.2	29.3

^ Categories where 7 or more feedlots had no observations

Table 6-32: Estimated odds ratios for the total effects of monthly summaries of numbers of animals on feed in the feedlot on the animal's risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Number on feed in animal's induction month (FeedlotN)					()	N=35,131 4 level
	<10,000	Ref				
	10,000 to <20,000^	1.4	(0.4 to 3.3)	0.382		
	≥20,000^	1.2	(0.2 to 3.8)	0.493		
Number <40 DOF in animal's induction month (FeedlotN40)					(Season, FeedlotN)	N=35,131 4 level
	<3,000^	Ref				
	3,000 to <6,000^	1.4	(0.4 to 3.2)	0.375		
	≥6,000^	1.2	(0.4 to 2.8)	0.486		

^ Categories where 7 or more feedlots had no observations

6.3.4 Broad environmental risk factors

6.3.4.1 Source region and feedlot region

The most common regions from which cattle were sourced were Darling Downs/New England (25%) and Western NSW/Qld/NT (24%, Table 6-33). Cattle from five of the six source regions went to seven or less of the participating feedlots. The majority of cattle in the study were inducted into southern feedlots (62%, Table 6-33).

There was no evidence of a large effect of source region and models fitted using the two minimal sufficient adjustment sets gave similar results (Table 6-34). Animals from southern feedlots were at markedly increased risk of BRD compared to those from northern feedlots but the total effect estimate was very imprecise (OR: 22.1, 95% credible interval: 1.6 to 99.3, Table 6-35). The direct effect models fitted using the two minimal sufficient adjustment sets gave differing results, one consistent with a reduced direct effect (OR: 11.8, 95% credible interval: 0.5 to 55.8) and the other indicating an effect similar to the total effect (OR: 23.8, 95% credible interval: 0.8 to 132.6, Table 6-35).

6.3.4.2 Timing of induction

The distribution of the induction season for the cattle in the study was fairly balanced across seasons (21 to 29% of animals in each season, Table 6-36). The majority of the cattle in the study were inducted in 2011 (54%, Table 6-36).

Relative to spring, risk of BRD was increased in winter (OR: 1.6, 95% credible interval: 1.0 to 2.3) and markedly increased in summer (OR: 2.4, 95% credible interval: 1.4 to 3.8) and autumn (OR: 2.1, 95% credible interval: 1.2 to 3.2, Table 6-37). The estimates for the total effect of year on the risk of BRD were imprecise so no conclusion was possible.

6.3.4.3 Weather in the first week after day 0

The most frequent means of the daily maximum temperatures in week one were 17 to <23°C (32% of animals) or 23 to <30°C (36%, Table 6-36), and for the daily minimum temperatures in week one, they were 5 to <11°C (36%) or 11 to <17°C (27%, Table 6-36). The means of the daily temperature ranges in week one were commonly 11 to <16°C (63%, Table 6-36). The most frequent rainfall totals in week one were 0.1 to <4 mm (28%) or 4 to <25 mm (37%, Table 6-36), while the means of

the daily maximum wind speeds in week one were most commonly 35 to <45km/h (56%, Table 6-36).

There was no consistent evidence of a large effect of mean maximum temperature, mean minimum temperature or mean temperature on the risk of BRD across the models fitted using the three minimal sufficient adjustment sets (Table 6-37, Table 6-38). One model indicated a protective effect of warmer minimum temperatures but estimates from the other two models were imprecise with point estimates close to one (Table 6-38). There was no consistent evidence of a large effect of total rainfall on the risk of BRD across the models fitted using the three minimal sufficient adjustment sets (Table 6-39) but in all three models there was a possible adverse effect of 4 to <25mm rain compared to no rain. There was no consistent evidence of a large effect of mean maximum wind speed on the risk of BRD across the models fitted using the three minimal sufficient adjustment sets (Table 6-40).

Table 6-33: Putative risk factors relating to source region and feedlot region; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Source region		0.02			
	NSW Central & Southern Tablelands^		6,251	17.8	28.5
	Coastal NSW or Queensland^		1,224	3.5	18.3
	Darling Downs/New England^		8,900	25.3	13.3
	Western NSW/Qld or NT		8,452	24.1	7.8
	NSW Riverina, Victoria & Tasmania^		6,188	17.6	32.5
	South Australia/Western Australia^		4,110	11.7	8.2
Feedlot region		0.00			
	North^		13,342	38.0	5.4
	South		21,789	62.0	25.1

^ Categories where 7 or more feedlots had no observations

Table 6-34: Estimated odds ratios for the total effects of feedlot and source region on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Source region Model A					(Feedlot region)	N=35,125 4 level
	NSW Central & Southern Tablelands^	Ref				
	Coastal NSW or Queensland^	0.9	(0.6 to 1.3)	0.240		
	Darling Downs/New England^	1.3	(1.0 to 1.6)	0.047		
	Western NSW/Qld or NT	1.1	(0.8 to 1.4)	0.333		
	NSW Riverina, Victoria & Tasmania^	0.9	(0.7 to 1.2)	0.171		
	South Australia/Western Australia^	0.9	(0.6 to 1.4)	0.344		
Source region Model B					(Induction year, Rain, Wind, Season, Temp max, Temp min Grain type)	N=35,131 4 level
	NSW Central & Southern Tablelands^	Ref				
	Coastal NSW or Queensland^	0.9	(0.6 to 1.3)	0.238		
	Darling Downs/New England^	1.2	(0.9 to 1.6)	0.062		
	Western NSW/Qld or NT	1.1	(0.8 to 1.4)	0.344		
	NSW Riverina, Victoria & Tasmania^	0.9	(0.7 to 1.1)	0.144		
	South Australia/Western Australia^	1.0	(0.6 to 1.5)	0.457		
Feedlot region					()	N=35,131 4 level
	North^	Ref				
	South	22.1	(1.6 to 99.3)	0.011		

^ Categories where 7 or more feedlots had no observations

Table 6-35: Estimated odds ratios for the direct effects of feedlot region on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Feedlot region Model A					(Breed, Grain type, Weight, Rain, Season, Sex, Temp max, Temp min, Wind, Dentition, Source region)	N=34,361 3 level
	North^					
	South^	11.8	(0.5 to 55.8)	0.066		
Feedlot region Model B					(Grain type, Induction year, Rain, Season, Temp max, Temp min, Wind, Source region)	N=35125 4 level
	North^					
	South^	23.8	(0.8 to 132.6)	0.041		

^ Categories where 7 or more feedlots had no observations

Table 6-36: Putative risk factors relating to timing of the induction period and weather in the first week after day 0; distribution by category, percentage missing and crude 50-day BRD cumulative incidence.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)
Induction season		0.00			
	Spring		9,763	27.8	16.0
	Summer		7,235	20.6	18.7
	Autumn		8,114	23.1	22.4
	Winter		10,019	28.5	14.6
Induction year		0.00			
	2009		4,729	13.5	15.7
	2010		11,593	33.0	16.7
	2011		18,809	53.5	18.7
Mean of daily maximum temperatures in week 1 (°C)		0.00			
	11 to <17		5,294	15.1	18.2
	17 to <23		11,259	32.0	16.7
	23 to <30		12,526	35.7	17.4
	≥30		6,052	17.2	19.5
Mean of daily minimum temperatures in week 1 (°C)		0.00			
	<5		7,879	22.4	21.7
	5 to <11		12,670	36.1	16.7
	11 to <17		9,595	27.3	17.4
	≥17		4,987	14.2	14.1
Mean of daily temperature ranges in week 1 (°C)		0.00			
	6 to <11		5,961	17.0	13.0
	11 to <16		22,045	62.7	18.8
	≥16		7,125	20.3	18.1
Total rainfall in week 1 (mm)		0.00			
	0		7,225	20.6	14.4
	0.1 to <4		9,958	28.4	23.0
	4 to <25		12,895	36.7	17.2
	≥25		5,053	14.4	12.8
Mean of daily maximum wind speeds in week 1 (km/h)		0.00			
	20 to <35		9,166	26.1	18.9
	35 to <45		19,694	56.1	16.1
	≥45		6,271	17.8	20.5

^ Categories where 7 or more feedlots had no observations

Table 6-37: Estimated odds ratios for the total effects of season, induction year and mean maximum temperature during the first week at risk on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Induction season					()	N=35,131 4 level
	Spring	Ref				
	Summer	2.4	(1.4 to 3.8)	0.001		
	Autumn	2.1	(1.2 to 3.2)	0.004		
	Winter	1.6	(1.0 to 2.3)	0.025		
Induction year					()	N=35,131 4 level
	2009	Ref				
	2010	0.9	(0.5 to 1.7)	0.361		
	2011	1.0	(0.5 to 1.8)	0.436		
Mean of daily maximum temperatures in week 1 (°C) Model A					(Dentition, Breed, Grain type, Weight, Rain, Wind, Season, Sex, Temp min, Source region)	N=34,361 3 level
	11 to <17	Ref				
	17 to <23	0.8	(0.6 to 1.0)	0.036		
	23 to <30	0.9	(0.6 to 1.3)	0.267		
	≥30	0.8	(0.5 to 1.3)	0.204		
Mean of daily maximum temperatures in week 1 (°C) Model B					(Grain type, Induction year, Rain, Wind, Season, Temp min, region28)	N=35,125 4 level
	11 to <17	Ref				
	17 to <23	0.8	(0.5 to 1.1)	0.093		
	23 to <30	0.8	(0.5 to 1.3)	0.194		
	≥30	1.1	(0.5 to 2.2)	0.457		
Mean of daily maximum temperatures in week 1 (°C) Model C					(Feedlot region, Induction year, Season)	N=35,131 4 level
	11 to <17	Ref				
	17 to <23	0.8	(0.5 to 1.1)	0.066		
	23 to <30	0.7	(0.4 to 1.1)	0.068		
	≥30	0.9	(0.5 to 1.6)	0.357		

Table 6-38: Estimated odds ratios for the total effects of mean minimum temperature and temperature range during the first week at risk on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Mean of daily minimum temperatures in week 1 (°C) Model A					(Dentition, Breed, Grain type, Weight, Rain, Wind, Season, Sex, Temp max, Source region)	N=34,361 3 level
	<5	Ref				
	5 to <11	1.0	(0.8 to 1.2)	0.478		
	11 to <17	0.6	(0.4 to 0.9)	0.007		
	≥17	0.6	(0.4 to 1.0)	0.025		
Mean of daily minimum temperatures in week 1 (°C) Model B					(Grain type, Induction year, Rain, Wind, Season, Temp max, Source region)	N=35,125 4 level
	<5	Ref				
	5 to <11	1.2	(0.9 to 1.6)	0.166		
	11 to <17	0.8	(0.5 to 1.4)	0.198		
	≥17	0.9	(0.4 to 2.0)	0.369		
Mean of daily minimum temperatures in week 1 (°C) Model C					(Feedlot region, Induction year, Season)	N=35,131 4 level
	<5	Ref				
	5 to <11	1.2	(0.9 to 1.5)	0.159		
	11 to <17	0.8	(0.5 to 1.2)	0.135		
	≥17	1.0	(0.5 to 1.8)	0.454		
Mean of daily temperature ranges in week 1 (°C)					(Temp max, Temp min)	N=35,131 4 level
	6 to <11	Ref				
	11 to <16	1.1	(0.7 to 1.6)	0.404		
	≥16	1.0	(0.6 to 1.6)	0.448		

Table 6-39: Estimated odds ratios for the total effects of total rainfall during the first week at risk on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Total rainfall in week 1 (mm) Model A					(Dentition, Breed, Grain type, Weight, Wind, Season, Sex, Temp max, Temp min, Source region)	N=34,361 3 level
	0	Ref				
	0.1 to <4	1.2	(0.9 to 1.6)	0.139		
	4 to <25	1.3	(0.9 to 1.8)	0.056		
	≥25	1.2	(0.8 to 1.8)	0.237		
Total rainfall in week 1 (mm) Model B					(Grain type, Induction year, Wind, Season, Temp max, Temp min, Source region)	N=35,125 4 level
	0	Ref				
	0.1 to <4	1.2	(0.9 to 1.5)	0.158		
	4 to <25	1.3	(0.9 to 1.7)	0.070		
	≥25	1.2	(0.7 to 1.8)	0.242		
Total rainfall in week 1 (mm) Model C					(Feedlot region, Induction year, Season)	N=35,131 4 level
	0	Ref				
	0.1 to <4	1.2	(0.9 to 1.6)	0.117		
	4 to <25	1.3	(1.0 to 1.7)	0.047		
	≥25	1.2	(0.8 to 1.8)	0.220		

Table 6-40: Estimated odds ratios for the total effects of mean maximum wind speed during the first week at risk on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
Mean of daily maximum wind speeds in week 1 (km/h) Model A					(Dentition, Breed, Grain type, Weight, Rain, Season, Sex, Temp max, Temp min, Source region)	N=34,361 3 level
	20 to <35	Ref				
	35 to <45	1.0	(0.8 to 1.2)	0.489		
	≥45	0.8	(0.6 to 1.0)	0.033		
Mean of daily maximum wind speeds in week 1 (km/h) Model B					(Grain type, Induction year, Rain, Season, Temp max, Temp min, Source region)	N=35,125 4 level
	20 to <35	Ref				
	35 to <45	0.9	(0.7 to 1.2)	0.205		
	≥45	0.8	(0.5 to 1.2)	0.151		
Mean of daily maximum wind speeds in week 1 (km/h) Model C					(Feedlot region, Induction year, Season)	N=35,131 4 level
	20 to <35	Ref				
	35 to <45	0.9	(0.7 to 1.2)	0.239		
	≥45	0.9	(0.6 to 1.3)	0.278		

6.3.5 Interactions

Results of analyses for important interaction terms are displayed in Table 6-41 and shown graphically in Figure 6-7 and Figure 6-8 . There was a significant interaction between breed and season (i.e. the effect of breed differed with season, Figure 6-7). Most notably, the adverse effect of Hereford breed was compounded in autumn. There was also a significant interaction between induction weight and the number of animals in the group-13 (Figure 6-8). The adverse effect of low induction weight was compounded in small groups. However, estimates of interaction terms were very imprecise, so conclusions focus on the main effects.

Table 6-41: Odds ratios for total effects of season and breed, and induction weight and number of animals in group-13, both when interactions terms were fitted, on the risk of development of bovine respiratory disease by 50 days

Combination of variable categories		OR	95% cred int	p-value[a]	Adjustment set	N, level
Season	Breed			*0.023*		
Spring	Angus	Ref			(Source region)	N=35,043
Spring	British X	1.4	(1.1 to 1.8)	0.017		4 level
Spring	Hereford	2.4	(1.2 to 4.5)	0.009		
Spring	Shorthorn	2.0	(1.3 to 3.1)	0.001		
Spring	Murray Grey	0.5	(0.3 to 1.0)	0.043		
Spring	European/X	1.3	(0.3 to 4.8)	0.698		
Spring	Tropical/X	0.4	(0.2 to 0.8)	0.012		
Summer	Angus	2.4	(1.5 to 4.0)	0.001		
Summer	British X	3.4	(2.0 to 5.8)	<0.001		
Summer	Hereford	3.9	(1.7 to 8.8)	0.001		
Summer	Shorthorn	1.8	(0.8 to 4.0)	0.135		
Summer	Murray Grey	0.8	(0.1 to 5.1)	0.832		
Summer	European/X	2.8	(1.2 to 6.4)	0.019		
Summer	Tropical/X	1.4	(0.6 to 3.1)	0.419		
Autumn	Angus	2.0	(1.2 to 3.3)	0.006		
Autumn	British X	2.1	(1.3 to 3.6)	0.005		
Autumn	Hereford	7.7	(3.8 to 15.5)	<0.001		
Autumn	Shorthorn	2.5	(1.2 to 5.0)	0.010		
Autumn	Murray Grey	2.3	(0.9 to 6.1)	0.098		
Autumn	European/X	1.7	(0.7 to 3.9)	0.224		
Autumn	Tropical/X	0.8	(0.3 to 1.9)	0.619		
Winter	Angus	1.8	(1.2 to 2.8)	0.004		
Winter	British X	1.8	(1.1 to 2.9)	0.018		
Winter	Hereford	2.1	(1.2 to 3.6)	0.010		
Winter	Shorthorn	0.8	(0.3 to 1.8)	0.523		
Winter	Murray Grey	0.5	(0.2 to 1.2)	0.138		
Winter	European/X	0.7	(0.3 to 1.6)	0.362		
Winter	Tropical/X	0.7	(0.4 to 1.5)	0.396		
Group-13N	Weight (kg)			*<0.001*	(Sex, Breed,	N=34,361
<50	<400	2.4	(1.9 to 3.0)	<0.001	Dentition,	3 level
<50	400 to 439	1.5	(1.2 to 1.8)	<0.001	Season, Source region,	
<50	440 to 479	1.3	(1.0 to 1.5)	0.024	Induction year)	
<50	≥480	1.1	(0.9 to 1.4)	0.261		
50 to 99	<400	1.2	(1.0 to 1.6)	0.082		
50 to 99	400 to 439	1.4	(1.1 to 1.7)	0.001		
50 to 99	440 to 479	1.2	(1.0 to 1.5)	0.060		
50 to 99	≥480	1.0	(0.8 to 1.3)	0.974		
≥100	<400	1.1	(0.8 to 1.5)	0.472		
≥100	400 to 439	Ref				
≥100	440 to 479	0.9	(0.7 to 1.1)	0.383		
≥100	≥480	0.8	(0.6 to 1.2)	0.280		

[a]Joint Wald p-values obtained from penalised quasilikelihood models are indicated in italics. P-values against each category indicate the Prob </>1 obtained from Bayesian MCMC models

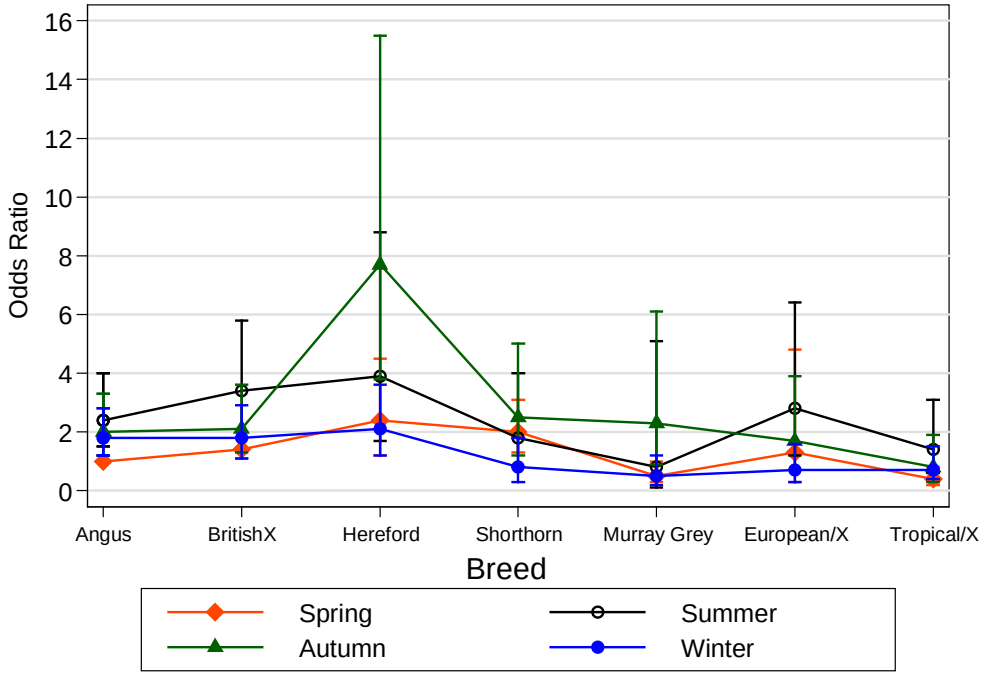

Figure 6-7: Estimates for odds ratios and 95% credible intervals for breed-season combinations derived from a model including an interaction between breed and season.

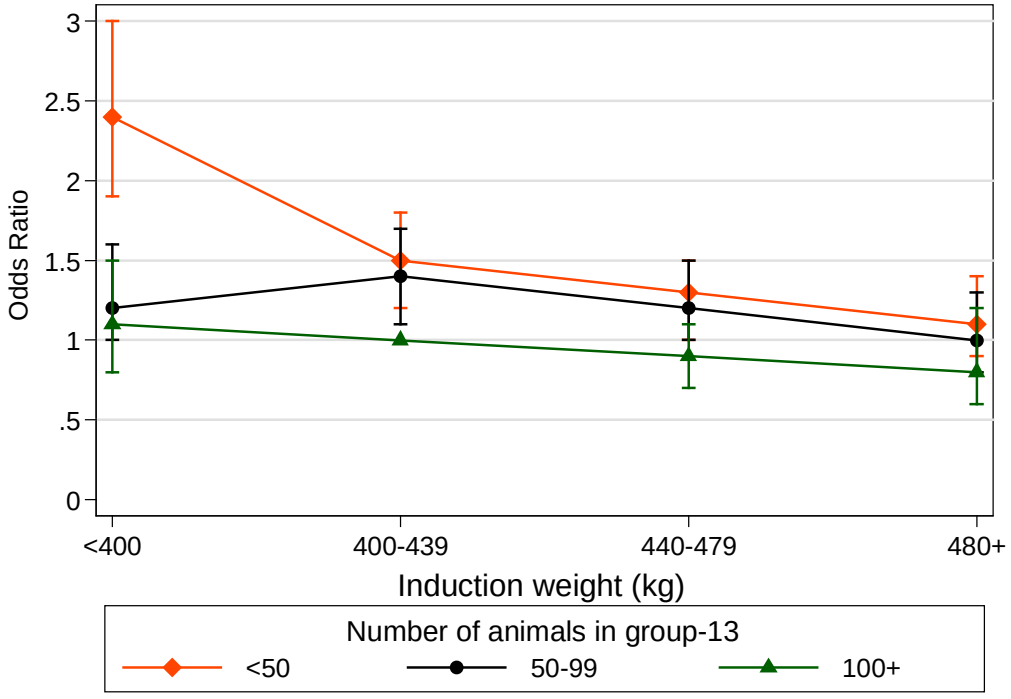

Figure 6-8: Estimates for odds ratios and 95% credible intervals for induction weight-number of animals in group-13 combinations derived from a model including an interaction between induction weight and number on animals in group-13.

6.4 Discussion

The total and direct effects models described in this chapter have been used to identify and quantify the effects of several important risk factors associated with the incidence of BRD in Australian feedlot cattle. Use of a causal diagram to inform model building and the comparison of total and direct effects allows better understanding of causal pathways and hence identification of appropriate interventions.

6.4.1 Animal entry characteristics

The current study has identified and quantified important differences in BRD risk among breeds commonly entering Australian feedlots. Several previous studies have found significant variation in BRD risk between breeds, with increased risk for Hereford cattle, both in feedlots (Cusack et al., 2007, Snowder et al., 2006) and in bull testing facilities (Durham et al., 1991, Hägglund et al., 2007). Consistent with previous Australian reports (Appleby, 1995, Cusack et al., 2007), my results indicated that Herefords were at much higher risk of BRD than other breeds, and that *Bos indicus* cattle were at reduced risk. However, the lower risk observed for Murray Greys in the current study differed from that observed in a prior study involving a single feedlot (Cusack et al., 2007). Further investigation into Murray Greys is warranted.

Differences between breeds may be partly explained by genetic differences; genetic associations with BRD have been described in a genome wide linkage study involving four half-sib families (Neibergs et al., 2011). Despite the difficulty of estimating heritability of a complex disease, low to modest heritability for BRD resistance has been reported (Snowder et al., 2006, Mugglicockett et al., 1992).

Most previous studies have reported that heifers were at reduced risk of developing BRD compared to steers (Alexander et al., 1989, Mugglicockett et al., 1992, Snowder et al., 2006), which is consistent with the results from the current study. One study reported that mixed-sex cohorts were at increased risk of BRD (Sanderson et al., 2008). In my study, heifer only and mixed-sex cohorts were restricted to a small number of feedlots and clustered by feedlot; estimates of effect were imprecise and I cannot draw a conclusion about this. Although dentition may be

regarded as a proxy for age, it is only useful in differentiating animals older than about 18 months to two years (when the first permanent incisors appear). For animals without permanent incisors (the majority of the study population), the youngest animals (<15 months) were generally in the lowest weight category. There was no evidence of a moderate or large effect of dentition on the risk of BRD in the current study. In a subset of the population with vendor questionnaire data, I classified animals into approximate age categories based on ages and timing of reported events (e.g. time and age of weaning or purchase). In this subset it appeared that animals older than 22 months at induction may have been at increased risk of BRD compared to younger animals. This effect persisted in the direct effects model indicating that the effect was over and above the effects of intervening variables (i.e. induction weight, mixing history, saleyard exposure). This association could have been confounded by unmeasured factors that resulted in cattle entering the feedlot at a later age because they had been slower to attain the desired weight for age (e.g., factors causing lower growth rates; nutritional, genetic, immune compromise, parasites) or because of differences in the immune status at induction depending on the timing of when cattle were previously mixed. That these animals' mean induction weight was not significantly different from that of the younger animals in the reference category, provided some evidence that their growth rates may have been lower. Unfortunately, body condition scores were not available for study cattle. Although this was considered in the design phase, it was decided that this was not practical under the high throughput conditions of the majority of participating feedlots.

Previous studies which have included cattle with a wide range of induction weights have reported reduced risk of BRD with increasing induction weight (Appleby, 1995, Gummow and Mapham, 2000, Reinhardt et al., 2009, Sanderson et al., 2008), which is consistent with my results. My study included cattle with a wide weight and age range. Although previous studies have reported increased risk with reduced mean cohort weight (Cernicchiaro et al., 2012, Cernicchiaro et al., 2012b, Cernicchiaro et al., 2012a), these studies used group- or cohort-level analyses and did not adjust for animal-level weight. In my study, there was there was no evidence of a moderate or large effect of the mean cohort weight or of the animal level difference in weight from

the mean cohort weight and risk of BRD. However, weight difference from mean cohort weight was closely correlated with induction weight.

6.4.2 Management risk factors

6.4.2.1 Yard weaning and prior vaccination

Yard weaning, as assessed in the vendor-bred subset, was significantly associated with reduced risk of BRD in my study, with similar effect sizes for the two categories examined after accounting for intervening variables (prior grain, conserved forage or supplement feeding) in the direct effects model. This is consistent with previous work indicating benefits from the practice of yard weaning (Walker et al., 2007). However, the recommended duration of yard weaning could not be assessed with the limited study data. Animals that were yard weaned may have been better accustomed to yards, feed bunks, water troughs, more crowded conditions and handling so that entry to a feedlot pen was associated with less stress compared to animals that were not yard weaned. North American studies agree that weaning at least several weeks prior to sending cattle to the feedlot is beneficial (Macartney et al., 2003a, Step et al., 2008), but this may be associated with several management practices occurring simultaneously such as the administration of vaccine, bunk feeding and commingling of animals.

The effect of prior vaccination against agents causing respiratory disease was evaluated in the subset of animals that were vendor-bred or purchased by 10 months of age as indicated by returned vendor questionnaires. Total effect estimates indicated that prior vaccination with Bovilis MH™, which is registered to protect against *M. haemolytica* was associated with a reduced risk of BRD. My results are consistent with those of a recent meta-analysis which concluded that prior vaccination against *M. haemolytica* was potentially beneficial. However, the 15 studies used in this analysis had variable results with only three demonstrating statistically significant benefits (Larson and Step, 2012).

Pestigard™ is an inactivated BVDV vaccine claimed to reduce reproductive loss and assist in the reduction of losses due to BRD. The total effects estimate for prior vaccination with Pestigard™ vaccine against BVDV provided some evidence that vaccination was associated with a reduced risk of BRD. However, the effectiveness

of prior Pestigard™ vaccination in feedlot conditions with a high level of challenge with BVDV requires further investigation.

6.4.2.2 BVDV in the cohort

BVDV activity in the cohort increased risk of BRD whether PI animal was present or whether there was evidence of transient infection in the cohort. Prior exposure to a PI animal (defined as an identified PI animal in the group-28) did not appear to result in reduced risk of BRD at the feedlot. However, misclassification error could have influenced these results (i.e. animals may have been exposed to PI animals previously but the PI animal was not in their group at the feedlot. BVDV will be discussed further in subsequent chapters.

6.4.2.3 Mixing, saleyard exposure, move timing and group size.

Commingling of animals from multiple sources immediately prior to arrival or at the feedlot has been consistently shown to be associated with increased risk of BRD (Martin et al., 1982, O'Connor et al., 2005, Ribble et al., 1995c, Sanderson et al., 2008, Step et al., 2008). Results from my study demonstrate that the effect of commingling depends on prior mixing history; important differences were observed between categories of cattle with differing mixing histories. By utilising lifetime animal-level data I have been able to examine mixing history in a way that has not, to my knowledge, previously been described. Mixing prior to 27 days before induction was protective. Although first mixing in the interval from day -90 to day -28 occurred in only 5% of the full study population, it was associated with a similar level of reduced risk as prior mixing before day -90. Commingling of cattle from less than four groups within the 12 days preceding induction did not increase risk provided cattle had been mixed prior to 27 days before induction. A high level of mixing (defined by the combination of four or more group-13s forming a cohort) close to induction markedly increased the risk of BRD. The effect was compounded for animals not mixed prior to day -27 and then joining cohorts formed by four or more group-13s. Animals in the study that were first mixed between days -27 and -13 generally joined cohorts formed by four or more group-28s; these were at a similarly markedly increased risk of BRD as those not mixed prior to day-27 and then joining cohorts formed by four or more group-13s. Hence, the overall mixing variable results indicate that the lowest-risk animals are those first mixed prior to day -27 joining cohorts formed by less than four group-28s.

An important finding from this study is that the effects of exposure to saleyards differ depending on the timing of exposure relative to induction. My results show that cattle exposed to saleyards more than 27 days before induction were at lower risk but this protective effect was primarily mediated by factors other than the process of unloading, yarding, holding then reloading at saleyards. This was demonstrated by separately estimating total and direct effects. Similarly, the detrimental total effect of saleyard exposure from day-27 to day -13 should be interpreted in combination with the much attenuated direct effect estimates. Although estimates were imprecise, an increased risk of BRD was demonstrated in animals exposed to saleyards from day -27 to day -13. Although attenuated compared to the total effects, the direct effect was stronger for animals exposed to saleyards from day-12 to day 0 compared to those exposed from day -27 to day -13. This increased risk was over and above the effects of mixing and feedlot move timing. There is a need for the reasons for this increased risk to be defined.

Cattle transported for six hours or more within one day of induction were at slightly increased risk of BRD compared to those undergoing shorter duration transport in this period, which is consistent with findings from recent North American studies (Cernicchiaro et al., 2012a, Sanderson et al., 2008). To my knowledge, prior studies have not investigated the effect of time interval between arrival at the vicinity of the feedlot and induction on BRD risk after induction. My results showed that cattle arriving at the feedlot vicinity more than 27 days before induction were at reduced risk of BRD. I speculated that this may have been overestimated as only three feedlots in the study moved cattle to the vicinity of the feedlot prior to day -12 and there may have been uncontrolled confounding despite having fitted feedlot as a random effect. However, results of analyses restricted to animals from these three feedlots were consistent with a large protective effect, although the odds ratio estimate for cattle moved prior to day -27 was imprecise. My results indicated that the timing of the move to the vicinity of the feedlot was an important contributor to the risk of BRD over and above effects of mixing.

In the current study, animals that were part of a larger group 13 days prior to induction were at reduced risk of BRD. A larger number of animals in a group has been associated with increased risk of BRD in prior studies, but this may be due to the effects of more commingling in larger groups (Martin, 1983, Martin and Meek,

1986, Martin et al., 1982). The interpretation of the effects of ‘group size’ in prior research is problematic because the length of time the group has been assembled was usually unknown. The number of animals in the cohort aligns more closely with group size investigated in other studies, but I do not draw a conclusion about cohort size because it tended to be clustered by feedlot, limiting the power to detect an effect and possibly leading to uncontrolled feedlot-level confounding. I defined group size at a consistent time point for comparison of all study animals, potentially avoiding misclassification bias if effects of group size depend on time before induction when group size is assessed. However, group sizes were often stable for extended periods of time before the move to the feedlot and for the majority of animals the grouping structure did not change dramatically between 3 months and 13 days before induction. Hence, my conclusion is that group size was very important, but the stability of group sizes observed in my study means that the duration of time that the group was formed should be considered alongside the effects of mixing history and feedlot move timing.

As a consequence of being in a larger group, fewer such groups are likely to be mixed to form a cohort, but the similar effect in both total and direct effects models, indicates an important effect over and above that mediated through mixing. Possible additional reasons for the protective effect could relate to a lower level of stress associated with the disruption of their social hierarchy, and if the group is of sufficient size, animals may be exposed to fewer novel pathogens in the feedlot pen.

The total and direct effects of the animal-level variable describing the time between induction and cohort close date suggests that animals that have a longer adaptation time have lower risk of BRD. This may be related to a number of factors including having a longer time in the pen to become accustomed to the pen environment, feed bunks and water troughs, more gradual increase in pen stocking density, and less rapid rates of ration changes early in their period in the feedlot pen, compared to animals entering the cohort on the latest induction date. Effect estimates were similar for total and direct effects; the latter were obtained by adjusting for the cohort fill pattern and variables relating to grain percentages in the ration. Previous studies have found that animals in cohorts filled over more than a day were at increased risk (Alexander et al., 1989, Martin et al., 1982), but concede this is associated with increased levels of commingling. Similarly, in my study the total effect of the cohort-

level variable describing cohort fill duration indicated that animals in cohorts where all animals shared the same induction date were at lower risk of BRD. However, the effect was much attenuated such that there was no evidence of a large direct effect after adjusting for intervening factors including mixing history, the animal-level time between induction and cohort close, the percentage of grain in the ration on days 0 and 20 and time taken to reach 60% grain in the ration, suggesting that the total effect was mediated through some or all of these variables.

There is a need for further research to investigate the reasons for the reduced risk in animals with a longer period between day 0 and cohort close and to establish if these factors could be managed to provide benefit at the cohort level.

6.4.2.4 Vaccination at Induction

The practice of vaccination against BoHV-1with Rhinogard™ at induction was determined at the feedlot level. Although the effect estimates from my results indicates that animals that receive Rhinogard™ at induction are at increased risk of BRD, the estimate is very imprecise and subject to feedlot-level confounding. Feedlots that use Rhinogard™ may have management practices that differ systematically from those that do not give Rhinogard™, or the decision to use it may be due to historical problems with BRD. I am not able to draw a conclusion about the effectiveness of Rhinogard™ using the data from the current study. Other researchers have faced similar issues regarding the interpretation of estimates relating to vaccination at induction, which may partially explain the inconsistent and equivocal results reported in the literature (Taylor et al., 2010b). The association between the use of Rhinogard™ in feedlot settings and BRD incidence should be assessed using a randomised controlled trial.

6.4.2.5 Shared pen water

Pen variables were measured at the cohort level, so there was limited power to detect effects. Despite this, my study has shown a significant and strong association between shared pen water and cumulative BRD incidence. This may be because the spread of pathogens occurs more readily between animals in different pens when animals share a common water source. The direct effect was attenuated after adjusting for active viral infection in the cohort (measured by BVDV being active in the cohort and by serochange to four viruses in the case-control dataset). However,

a strong and significant direct effect remained, suggesting other factors may also be important. I am not aware of any prior reports of this association. Although limited disparity between cohorts within feedlots meant that effect estimates were imprecise, these estimates indicated a consistently strong effect of shared pen water on BRD risk. Given that the installation of water troughs that are not shared between pens could be implemented by industry relatively easily and that the effect estimates were very large, this intervention should be trialled with high priority.

6.4.2.6 Cohort-level risk factors

Cohort-level variables such as other pen features, ration variables and numbers of animals on feed in the induction month were often highly clustered at the feedlot level, so there was even more limited power to detect effects, and no conclusion was possible for most of these risk factors. The lack of 'significant' effects does not mean these factors may not be important.

Other pen characteristics analysed included pen density, bunk space and pen shade. The effect of pen shade may vary depending on feedlot region, season and weather conditions. For example, under hot or cold conditions, animals may congregate under the shaded area, effectively increasing the animal density in part of the pen and enhancing the transmission of viruses between animals. There was possible evidence for increased risk of BRD with some pen shade rather than none but estimates were imprecise so further investigation is required, particularly to adequately investigate different types and amounts of shade and to establish how the effect of pen shade may vary across feedlot region and in different weather conditions. This needs to be considered alongside the important effect of shade in mitigating heat load.

The effects of bunk space and pen density may vary at the animal level over time. Use of a single measure at one time point (i.e. at cohort close) does not take account of lower pen density and increased bunk space during the initial time on feed for animals that joined a cohort more than 7 days before the cohort close date. The finding that animals with a longer period of time between induction and cohort close were at reduced risk compared to animals joining the cohort at cohort close indicates that time-varying changes in pen density, bunk space and rations may be important. Further research is recommended to investigate these cohort-level variables; such

studies may be possible with existing feedlot data (i.e. data recorded routinely at a large number of feedlots).

6.4.3 Broad environmental factors

6.4.3.1 Source and feedlot region

Some previous studies have identified differences in risk in cattle sourced from different regions, but in my study, there was no evidence of a large effect of source region on BRD risk. However, BRD risk varied by feedlot region such that animals inducted into Queensland feedlots (northern) were at much lower risk than animals inducted into feedlots in other states (southern). The feedlots located in Queensland were in the Darling Downs region which has a humid subtropical climate with the majority of rainfall occurring in the warmer months. By contrast, feedlots in southern regions were spread over a very wide area, but the majority were in the Riverina region of NSW. This region has a semi-arid climate with hot summers and cool winters and the majority of rainfall occurs in the cooler months. North American studies have also identified variation in risk depending on feedlot location (Cernicchiaro et al., 2012a). Feedlot region is likely to be a proxy for many risk factors, including unknown or unmeasured factors, so that much of the effect would be expected to be indirect. Adjusting for the highest quality variables (i.e. breed, weight and sex) in the direct effects model did result in marked reduction in the effect estimate, but the effect still remained very large and very imprecise.

6.4.3.2 Timing of induction

BRD risk varied substantially by season of induction in the current study, such that animals inducted during autumn or summer were at increased risk. Increased risk in autumn (fall) has been reported in North American studies, (Loneragan et al., 2001, Ribble et al., 1995a), but this could be confounded by other factors associated with the concentration of young cattle entering feedlots at this time (Taylor et al., 2010a). The effects of season are likely to reflect different factors in different locations, as the components of ‘season’, most obviously defined by weather conditions, would vary markedly by location. In Australia, cattle enter feedlots year round and at a range of ages, so there is less potential for confounding by factors related to a marked seasonal variability in stocking density and induction weight or age. In contrast to my study, a previous Australian study of 5,306 cattle in 25 cohorts showed no

association between season of induction and BRD incidence (Dunn et al., 1993), but this study included fewer animals over a more restricted geographical region and time period.

6.4.3.3 Weather

The strong effects of season and region suggest that weather variables may be implicated as BRD risk factors in Australian feedlot cattle. Although results using crude variables for weather in the first week of induction did not show evidence of effect, further work to explore weather variables as time-varying exposures is warranted. Fluctuations may be important to consider along with a lag time of up to two weeks. Thus, further analytical approaches more suited to time-varying covariates are required to adequately investigate the effects of weather. Previous studies have linked weather conditions to BRD risk in Australia (Cusack et al., 2007), and a recent North American study found an association between BRD incidence and lagged weather variables (wind speed, wind chill and temperature change) (Cernicchiaro et al., 2012).

6.4.4 Interactions

Important interactions were found between breed and season, most notably involving the Hereford breed during autumn. Increased risk was also observed for European breeds, Herefords, Angus and British crosses during summer. This indicates that the risk for Herefords inducted in autumn, for example, is over and above that expected for exposure to each of these factors in isolation. That British and European breeds would be expected to be better adapted to cooler climates may explain the observed interaction between summer and these breed categories.

A significant interaction was observed between induction weight and the number of animals in group-13. Thus, animals in the lowest weight category were at much higher risk if they were also from group-13s with fewer than 50 animals. There was *a priori* industry interest in an interaction between breed and season, but other interactions (including the one between weight and group-13N) were investigated on the basis of the biological plausibility of a threshold effect with compounding of effects. Knowledge of the interactions identified is important for feedlot managers to understand and manage BRD risk in the process of forming cohorts.

6.5 Conclusions

Numerous putative risk factors for BRD have been investigated and many have been found to be probably associated with increased risk of BRD in Australian feedlot cattle. The effects of these risk factors at the population level will be investigated in Chapter 7.

7 Population-level Effects

7.1 Introduction

To identify and quantify the effects of important risk factors in the development of BRD in feedlot cattle, the total effects and selected direct effects were estimated as described in Chapter 6. Effects of particular risk factors on BRD incidence are central, for both industry and individual feedlots. Nonetheless, the effect of a particular risk factor at the population level depends on the prevalence of exposure in the population as well as the strength of association (effect). A strong association is clearly important for those individuals exposed to that risk factor, but if very few individuals in the population are exposed, removing that risk factor (or preventing the effects of that risk factor) will have little impact on the disease frequency across the entire population. So the risk factor is of little importance for the population. Alternatively, a risk factor with only a modest strength of association may be very important for the population if a high proportion of individuals are exposed. Population attributable fractions (PAFs) and population attributable risks (PARs) are population-level measures (i.e. 'population-level effects') that attempt to quantify the effects of risk factors for the population. They can be used to gauge the relative importance of risk factors in the study population. However, for high internal validity of these estimates (i.e. for the estimates to closely reflect the true values in the target population other than random error) estimates of both strength of association and proportions exposed must be unbiased. In this chapter, I present and compare PAFs and PARs derived from total and direct effect estimates using two software packages. These population-level effects are used to rank risk factors and make recommendations aimed at reducing the population-level impact of BRD in Australian feedlots.

7.2 Methods

7.2.1 Risk factors included

PAFs and PARs were estimated for important risk factors that were determined to be probably associated with BRD in this study based on the total and direct effects modelling described in Chapter 6. In addition to risk factors for which a definitive conclusion was reached (e.g. breed, induction weight), results are presented for

population effects of risk factors with qualified conclusions (e.g. sex), novel associations (e.g. shared pen water) or unexpected findings (e.g. age in the vendor questionnaire subset). Population-level effects were estimated for some risk factors related to subset analyses (e.g. vendor questionnaire variables). No population-level effects were estimated for BRD in PI animals; because the animal-level prevalence was extremely low (around 0.24%) so the estimated PAFs and PARs would be negligible. The variable describing whether BVDV was active in the cohort provided more appropriate population-level measures of the effect for BVDV. No interaction terms were considered and estimates were not obtained for the serological exposure variables examined in the nested case-control study reported in Chapter 10, because they cannot be directly manipulated.

Risk factors were grouped into three categories for presentation and discussion. Animal factors referred to animal-entry characteristics (e.g. breed, sex, weight), management risk factors referred to management decisions (both prior on-farm and feedlot management) and 'broad environmental' risk factors (e.g. feedlot region and season of induction) were likely to be proxy measures for the collective effects of multiple undefined factors. Management-related risk factors derived from the vendor questionnaire datasets were presented in a separate graph because these were not derived from the same dataset as the other variables.

7.2.2 Definitions, formulae and estimation

The PAF estimates the proportion of disease incidence in a population attributable to an exposure assuming that the exposure is causal (Dohoo et al., 2009). It is based on the effect estimate and the prevalence of exposure to the risk factor in the population, and may be used to rank risk factors in order of relative importance. The PAF for a particular risk factor may be interpreted as the proportionate reduction in BRD incidence that would occur at the population level if all animals in higher risk categories were replaced with otherwise identical animals but in the lowest-risk category or their risk was reduced to that of the lowest-risk category. Population attributable fractions for multiple risk factors may sum to more than 100% because multiple risk factors may contribute via the same causal pathways.

The PAR describes the amount of disease incidence in a population that can be attributed to a risk factor. So, for an estimated PAF for BRD, the PAR is the

corresponding estimated population level reduction in BRD incidence if all animals were moved to the lowest-risk category for that risk factor.

PAFs for a particular risk factor with *k* categories were calculated using case fractions ($\boldsymbol{CF_i}$); these are the proportions of all animals that developed the outcome (i.e. BRD50) that were in each category *(i)* for the risk factor, and the adjusted relative risks compared to the reference group (RR_i), summed over all categories for categorical variables as shown below (Hanley, 2001):

Equation 3: $\boldsymbol{PAF = \sum_{i=1}^{k} CF_i * {(RR_i - 1)}/{RR_i}}$

This approach can take account of confounding because it is appropriate for use with adjusted relative risk estimates (Dohoo et al., 2009, Hanley, 2001). For a variable with more than two categories, the partial PAF (PAF_i) estimates the amount contributed by each category of the variable.

The PAR is then calculated as the product of the PAF and the overall BRD incidence. Because these effect estimates need to be calculated relative to the lowest-risk group, it was necessary to obtain estimates from models run with the lowest-risk category as the reference category.

As noted above, PAFs and PARs require relative risks (ratios of proportions) but logistic model effect estimates are differences on the logit scale which can be transformed to odds ratios. Where the disease is rare, odds ratios approximate relative risks. However, the crude BRD incidence was not low (17.6%), so PAFs and PARs would have been overestimated if odds ratios had been used in place of relative risks. Accordingly, relative risks were estimated from odds ratios. The observed (i.e. crude) percentage of individuals in the reference category that developed BRD by day 50 ($\mathrm{BRD\%\_ref}$) was used to calculate the odds of individuals in this category developing BRD ($\mathrm{odds\_ref.}$) The adjusted odds ($\mathrm{odds\_adj}$) for all other categories were then estimated by multiplying the adjusted odds ratios (OR) derived from the relevant model by the odds of BRD for the reference category. These odds were then used to calculate the adjusted percentage for individuals in each category that developed BRD by day 50 ($\mathrm{BRD\%\_adj}$). The adjusted relative risk ($\mathrm{RR_i}$) was then obtained by dividing the adjusted percentages of individuals that developed BRD by

day 50 for the category by the percentage that developed BRD by day 50 in the reference category. These steps are described in the formulae below:

Equation 4:

i. $\mathbf{odds\_ref} = (\mathbf{BRD\%\_ref}\ /\mathbf{100})/((\mathbf{1} - \mathbf{BRD\%\_ref})\ /\mathbf{100})$

ii. $\mathbf{odds\_adj} = \mathbf{OR} * \mathbf{odds\_ref}$

iii. $\mathbf{BRD\%\_adj} = \mathbf{100} * \mathbf{odds\_adj}\ /(\mathbf{1} + \mathbf{odds\_adj})$

iv. $\mathbf{RR_i} = \mathbf{BRD\%\_adj/BRD\%\_ref}$

The overall 50-day BRD cumulative incidence was 17.6% for the models fitted using the main cohort study population, 18.7% for the full vendor questionnaire subset (used to estimate age) 18.6% for the prior vaccination subset and 21.0% for the vendor-bred subset used to derive estimates for yard weaning.

7.2.3 Estimation of PAFs and PARs using MLwiN®

Two methods were used to obtain population-level estimates of effect. As described in Chapter 6, multilevel logistic regression modelling was performed using MCMC estimation methods in the MLwiN® (version 2.27) software package run within the Stata® (version 12) program to obtain total and required direct effect estimates for putative risk factors for BRD. Where necessary, models were refitted to obtain effect estimates relative to the reference category with the lowest adjusted risk of BRD. These odds ratio estimates were then copied into a Microsoft Excel® spreadsheet, along with all of the other required data detailed above. The formulae described above were then applied to estimate RR_i and then to produce point estimates for the PAFs and PARs.

7.2.4 Estimation of PAFs and PARs using WinBUGs

The second method utilised an alternative Bayesian modelling software package (WinBUGs®). By programming the WinBUGs® software to fit a mixed effects multilevel level logistic model and also perform the calculations described above, it was possible to obtain effect estimates with associated estimates of uncertainty for the odds ratios, PAFs and PARs.

Equivalent models were constructed using WinBUGs® for both the total effects estimates and selected direct effects estimates. PAFs and PARs were derived by programming nodes to estimate the adjusted percentage of cases, adjusted relative

risk and partial PAF for each category, and hence the total PAF and PAR for each variable, using the formulae described above. The percentage of individuals in the reference category that were BRD cases, odds of BRD for the reference category, and the percentage of all cases that were in the reference category were compiled and imported as fixed data. Non-informative priors were specified. Odds ratios, coefficients, PAFs, PARs and variance were monitored. Diagnostics, including the autocorrelation function, trajectory plot, history trace, quantile plot and kernel density plot, and model output were examined for evidence of non-convergence. If there was evidence of non-convergence, chains were run for longer. Knowledge of the hierarchical level of the variable and the number of iterations used to achieve convergence in the MLwiN® models was used to determine the starting chain length. For example, models for animal-level risk factors were run for 10,000 iterations, but for cohort-level risk factors the starting chain length was 30,000. Chains were thinned where long chains were required. All models were run for a minimum of 10,000 iterations after burn-in of 1,000 iterations. As expected, models for feedlot and cohort-level variables were much slower to converge than models for animal-level variables. Output included the mean values for PAFs and PARs and their 95% credible intervals derived from the posterior distributions for the risk factors of interest.

7.2.5 Ranking of risk factors

Following the estimation and comparison of the population-level effects described above, for each risk factor, biological plausibility of a causal relationship along with evidence from the current study and prior published evidence were assessed. Risk factors investigated were then classified into those determined to have sufficient evidence (as detailed below) to draw conclusions assuming causality, and those for which the evidence was evaluated as being insufficient to draw conclusions. Risk factors with sufficient evidence were then ranked to identify the most important risk factors from the perspective of the Australian feedlot industry. Thus, where factors had similar effect sizes, those amenable to intervention were ranked above those that were not. Because further research may reveal management strategies to address the latter group, they were still considered important and were included in the ranking. Risk factors without sufficient evidence of an effect were not included in this ranking. PAFs were classified as large (>0.4), moderate (>0.2 to 0.4), modest

(>0.05 to 0.2) and small ≤0.05). Risk factors were ranked within these population-level effect size groupings by considering: a) estimated size of effect and the precision of the estimate (direct effect considered first if both estimated) and b) potential for intervention or perceived value of further research

7.3 Results

Within each category of risk factors, results presented include tables and graphs displaying the estimated PAFs and PARs derived from different models and software. In addition, for variables with multiple categories, graphs are presented to illustrate the derivation of the partial PAFs for each category (PAF_i) that contributed to the total PAF. The adjusted relative risks (calculated from the adjusted odds ratios) are represented by colour coded diamonds so that red (RR: >2) indicates markedly increased risk, dark orange (RR: >1.5 to 2.0) indicates increased risk, tan indicates slightly increased risk (RR: 1.1 to 1.5) and grey (RR <1.1) indicates a similar risk compared to the reference category (black diamond). Percentages of all BRD50 cases that occurred within each category are illustrated along with the derived partial PAFs. For dichotomous variables, the partial PAF and PAR are equivalent to those for the non-reference category. PAFs and PARs (i.e. the sums of the partial PAFs and PARs, respectively) for different models and software are illustrated graphically. All presented variables have total effects estimates. Direct effect estimates were only calculated for variables of interest as explained in Chapter 6. Corresponding estimates for the equivalent models run with the WinBUGS® software are presented with associated 95% credible intervals (presented as range plots in the graphs). Because the PAFs and PARs were derived from estimated relative risks based on the odds ratios, the level of uncertainty and variability between estimates mirrors that observed for the odds ratio estimates. If the odds ratios estimates were imprecise, the PAFs and PARs were imprecise, especially when multiple categories had imprecise estimates (e.g. timing of the move to the feedlot.

7.3.1 Animal risk factors

7.3.1.1 Breed

The PAFs and PARs for total effects of breed were 0.56 and 9.8%, respectively, from the MLwiN® model, while the estimates were 0.67 (95% credible interval: 0.54 to

0.77) and 11.8% (95% credible interval: 9.6 to 13.5%), respectively, from the WinBUGS® model (Table 7-1).The lowest-risk reference category for breed consisted of tropical breeds or tropical breed crosses (e.g. Santa Gertrudis, Brahman cross) and comprised 16% of the study population. As shown in Figure 7-1, a large percentage of the study population were British breeds (56% Angus, 6% Hereford, 4% Shorthorn and 12% British breed crosses) that were at markedly increased risk of BRD compared to the reference category. From Figure 7-1 it is evident that of the total PAF for breed, the partial PAF for Angus cattle contributed about 70%.

Thus, overall BRD incidence would be estimated to decline by an absolute amount of 9.8 or 11.8% if all cattle were at the same risk as tropical breed and/or tropical crossbred cattle, equating to proportional reductions in incidence of 0.56 and 0.67, respectively. These results, displayed graphically in Figure 7-2 and Figure 7-3, indicate that breed was a risk factor with large population-level effects.

7.3.1.2 Sex

The majority of the study population (92%) were steers (the higher risk group); they were at increased risk compared to heifers. The total effects estimates for the PAF and PAR of sex were 0.31 and 5.4% in the MLwiN® model and 0.36 (95% credible interval: 0.00 to 0.59) and 6.3% (95% credible interval: -0.1 to 10.5) in the WinBUGS® model (Table 7-1). The estimates of PAF and PAR for sex were very imprecise, as indicated by the wide credible intervals (Figure 7-2, Figure 7-3). Assuming sex is causal, I estimated that it had a moderate population-level effect.

7.3.1.3 Induction weight

The PAFs and PARs for total effects of induction weight were 0.16 and 2.7% from the MLwiN® model and 0.16 (95% credible interval: 0.09 to 0.23) and 2.9% (95% credible interval: 1.6 to 4.1) from the WinBUGS® model (Table 7-1). About 15% of the study population was in the heaviest induction weight category (≥480 kg). The lightest weight animals (<400 kg; 20% of the study population) were at increased risk of BRD and the second lightest weight category (400 to <440 kg; 31% of the study population) was at slightly increased risk compared to the reference category (Figure 7-4).

The fraction of BRD incidence attributed to lower weight categories was estimated at about 0.16 (Figure 7-2), and overall BRD incidence would be estimated to decline by

an absolute amount of 2.7 to 2.9% if all cattle were instead at the same risk as cattle ≥480 kg (Figure 7-2, Figure 7-3). These results indicate that induction weight had a modest population-level effect.

7.3.1.4 Age at induction

The PAFs and PARs for total effects of age were 0.07 and 1.4% from the MLwiN® model and 0.06 (95% credible interval: 0.01 to 0.11) and 1.2% (95% credible interval: 0.2 to 1.9) from the WinBUGS® model (Table 7-1). The estimates were quite imprecise (Figure 7-2 and Figure 7-3). Partial PAFs were not presented graphically because the effect was entirely due to a single category; 29% of animals in the vendor questionnaire dataset (used to estimate these effects) were in the older age category (>22 months) determined to be at increased risk of BRD. If age is causal, it was estimated to have a modest population-level effect.

Table 7-1: Comparison of the population effects (PAFs and PARs) for animal-entry characteristics on the 50-day cumulative incidence of BRD derived from total effects models

Risk factor	MLwiN	WinBUGs	
	PAF	PAF	95% Credible Interval
Breed	0.56	0.67	(0.54 to 0.77)
Sex	0.31	0.36	(0.00 to 0.59)
Weight	0.16	0.16	(0.09 to 0.23)
Age*	0.07	0.06	(0.01 to 0.11)
	PAR	PAR	95% Credible Interval
Breed	9.8	11.8	(9.6 to 13.5)
Sex	5.4	6.3	(-0.1 to 10.5)
Weight	2.7	2.9	(1.6 to 4.1)
Age*	1.4	1.2	(0.2 to 2.1)

* Estimated in the vendor questionnaire subset

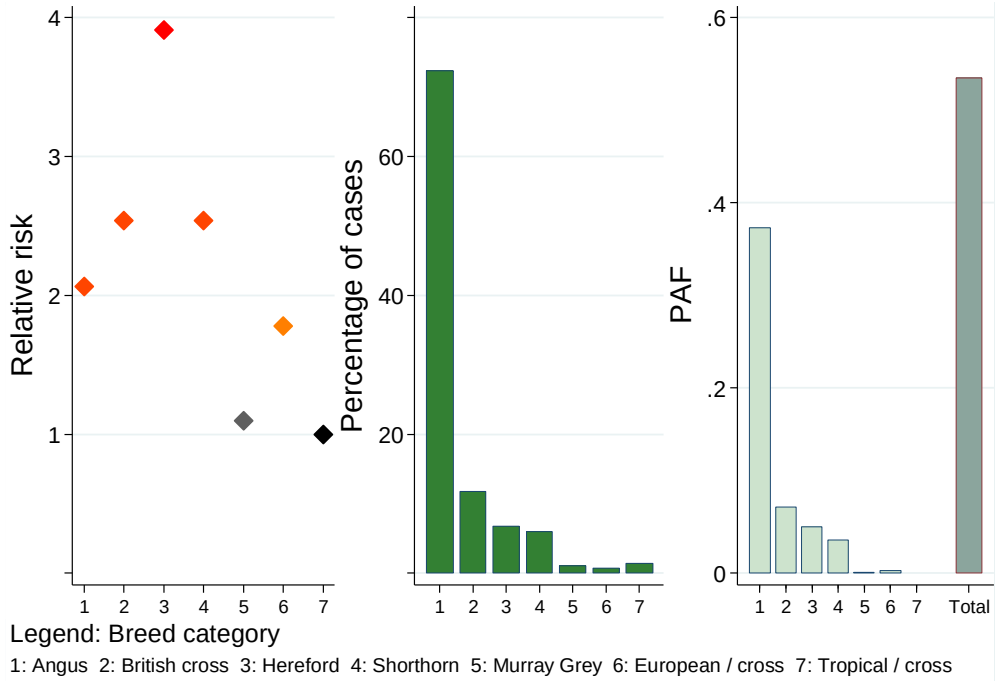

Figure 7-1: Adjusted relative risks, percentages of cases (case fractions), and estimated partial PAFs by breed category, and total PAF for breed derived from the MLwiN® total effects model.

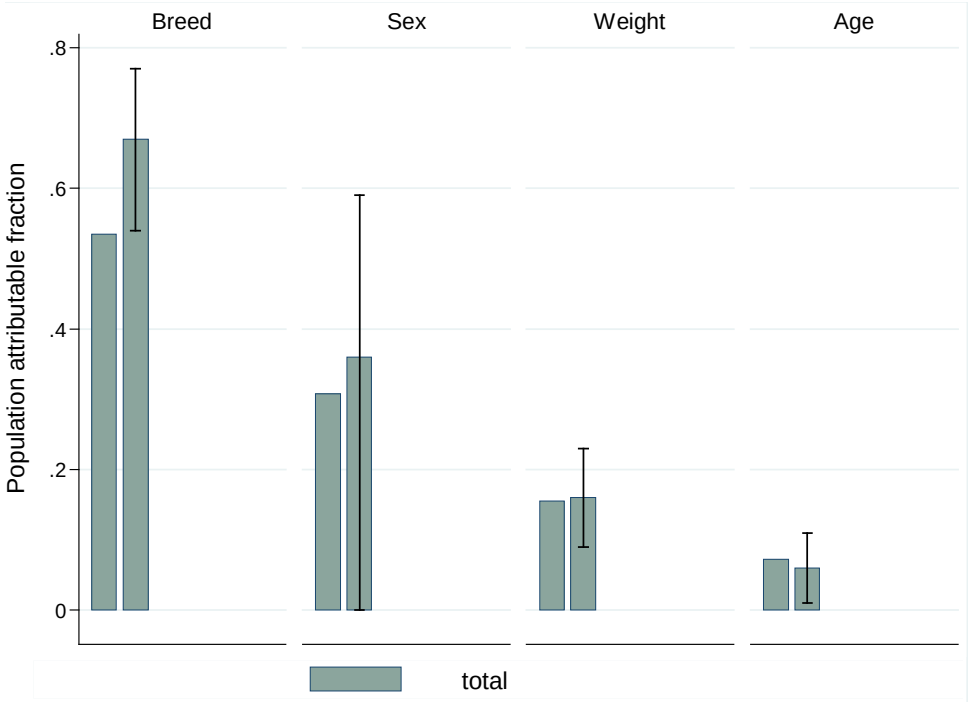

Figure 7-2: Population attributable fractions (PAFs) for animal-entry characteristics risk factors derived from the models fitted using MLwiN® (bars only) and WinBUGS® (with 95% credible intervals) software

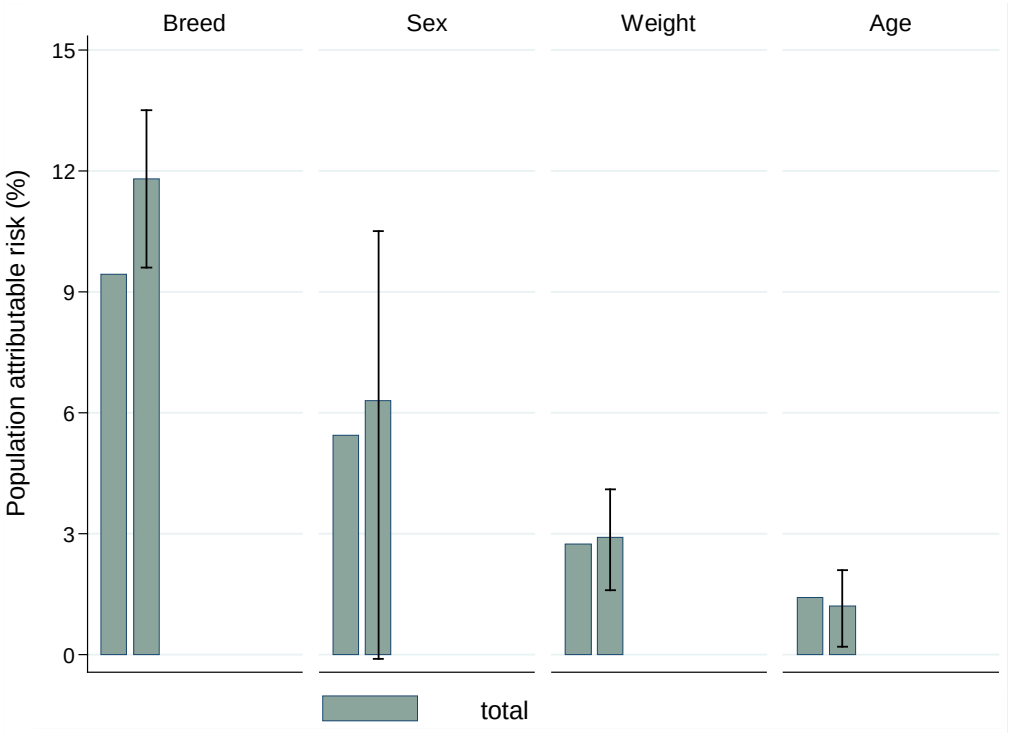

Figure 7-3: Population attributable risks (PARs) for animal-entry characteristic risk factors derived from the models fitted using MLwiN® (bars only) and WinBUGS® (with 95% credible intervals) software

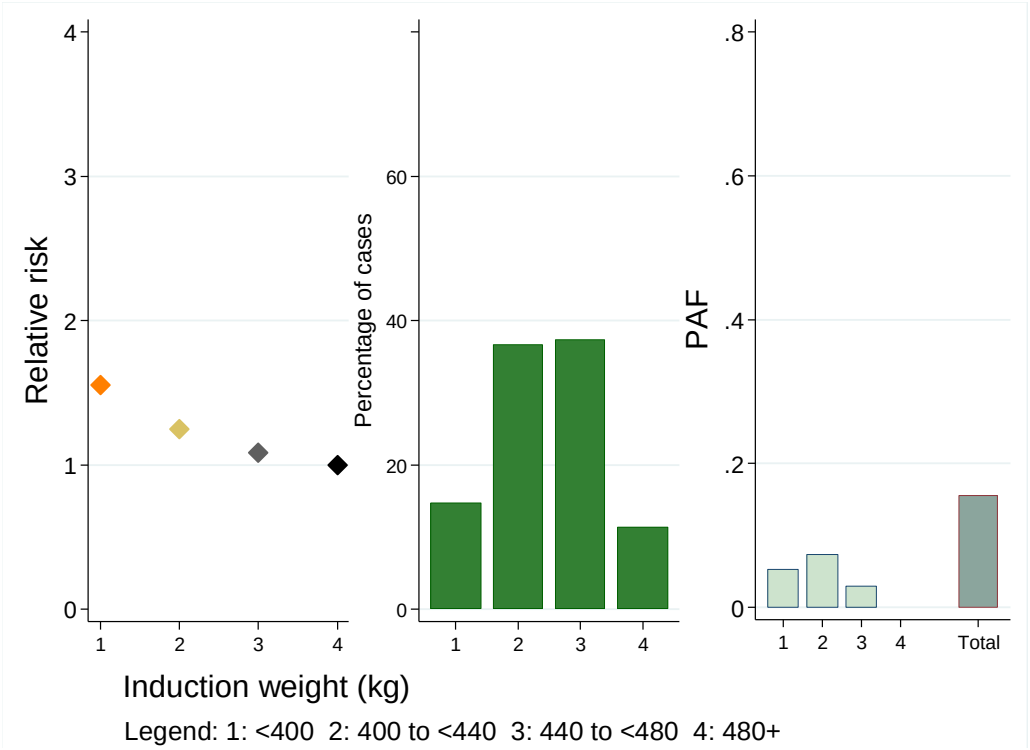

Figure 7-4: Adjusted relative risks, percentage distribution of cases, and estimated partial PAFs across induction weight categories and total PAF for induction weight derived from the MLwiN® total effects model.

7.3.2 Management risk factors

7.3.2.1 Shared pen water

The PAFs and PARs (using the main cohort dataset) for the total effects of shared pen water were 0.71 and 12.5% from the MLwiN® model and 0.70 (95% credible interval: 0.45 to 0.83) and 12.3% (95% credible interval: 7.9 to 14.7%) from the WinBUGS® model (Figure 7-5.). The majority (82%) of the cattle in the population were exposed to shared pen water and exposed cattle were at markedly increased risk of developing BRD. Hence, shared pen water had very large population-level effects. Assuming pen water is causal and the estimates of effect are unbiased, the PAF indicates that if all cattle were offered unshared pen water rather than some being offered shared pen water (or all cattle were at the same risk as those whose pen water was not accessible by cattle in another pen), BRD incidence pooled across all feedlots would be estimated to decline by a factor of 0.7 (i.e. to about 30% of the current incidence). The PAR was 12.4%, indicating that BRD incidence pooled across all feedlots would be estimated to decline by an absolute amount of about 12.4%, from 17.6% to around 5.2%.

The direct effect PAFs and PARs were also very large and overall, the results indicated that shared pen water could be a very important potentially modifiable risk factor at the population level.

7.3.2.2 Feedlot move timing

The estimated population-level effects of the timing of the move to the feedlot were large. The PAFs and PARs for total effects of feedlot move timing were 0.69 and 12.1% from the MLwiN® model and 0.75 (95% credible interval: 0.57 to 0.88) and 13.3% (95% credible interval: 10.1 to 15.5%) from the WinBUGS® model (Table 7-2). The lowest-risk category for the feedlot move timing variable consisted of animals that moved to the feedlot property prior to 27 days before induction (comprising 5% of the total study population). Total effects estimates indicated that all other categories were at markedly increased risk of developing BRD. The highest risk was for animals subjected to longer duration transport within a day of induction. Because the majority of cattle were moved to the feedlot within a day of induction (49% transported less than 6 hours and 27% transported 6 hours or more), very

large partials PAFs were observed for these categories (contributing 91% of the total PAF; Figure 7-6).

The estimates of the PAFs and PARs for the direct effects were slightly lower and much less precise (Table 7-2). Thus, overall BRD incidence would be estimated to decline by an absolute amount of 12.1% or 13.3% if all cattle were instead at the same risk as those moved to the vicinity of the feedlot at least 27 days before day 0. However, estimates of the population level effects of feedlot move timing were imprecise with wide credible intervals (Figure 7-5), and for several categories, including the reference category, all animals were from only a few feedlots.

7.3.2.3 Mixing

The estimated population-level effects of mixing were large. The PAFs and PARs for the total effects of mixing history were, respectively, 0.58 and 10.2% from the MLwiN® model and 0.55 (95% credible interval: 0.32 to 0.72) and 9.7% (95% credible interval: 5.3 to 12.7%) from the WinBUGS® model (Table 7-2). The detailed 12-category mixing history variable was used to estimate population-level effects presented in Table 7-2 and Figure 7-5. The lowest-risk reference category for the mixing history variable consisted of the 11% of animals that were mixed prior to day -27 and joined cohorts formed by two or three group-13s.

The mixing summary variable provided a practical summary version of the detailed mixing history variable; this was used to produce the graphical illustration presented in Figure 7-7. The lowest-risk reference category comprised the 23% of the population that had been mixed prior to day -27 and joined cohorts formed by less than four group-28s. All other categories were at markedly increased risk of BRD. The majority of the population were in mixing summary categories in which four or more group-28s formed the cohort (38% not mixed prior to day -27 and 44% mixed prior to day -27) and when combined with the higher adjusted relative risks for these categories, the resultant high partial PAFs contributed 90% of the total PAF for this risk factor (Figure 7-7).

Thus, overall BRD incidence would be estimated to decline by an absolute amount of approximately 10% (9.7% or 10.1%) if all cattle were instead at the same risk as those mixed prior to day -27 joining cohorts formed by 2 or 3 group-13s. The estimates of the PAFs and PARs for the direct effects were slightly lower and less

precise. Overall, the results indicated that mixing history was a very important risk factor for BRD at the population level, mostly mediated via the direct pathway (i.e. not via the indirect path through BVDV activity in the cohort).

7.3.2.4 Group size

The PAFs and PARs for total effects of the number of animals in the group-13 were consistent between software packages at 0.37 and 6.5% from the MLwiN® model and 0.39 (95% credible interval: 0.23 to 0.51) and 6.9% (95% credible interval: 4.1 to 9.1) from the WinBUGS® model (Table 7-2, Figure 7-5). The lowest-risk reference category (≥100 animals) comprised 33% of the study population. Animals in group-13s with <50 animals (39%) or between 50 and 99 animals (28% of the population) were at increased risk.

Thus, overall BRD incidence would be estimated to decline by an absolute amount of 6.5 to 6.9% if it were possible to ensure that all cattle were at the same risk as cattle from group-13s with 100 or more animals (Figure 7-5). The PAFs and PARs for direct effects were slightly lower, corresponding to the slightly reduced protective effect observed in the direct effects model. These results indicated that the number of animals in group-13 was an important risk factor with a moderate effect at the population level.

7.3.2.5 BVDV present in cohort

The estimated population-level effects of BVDV in the cohort were moderate. The PAFs and PARs were 0.32 and 5.6% from the MLwiN® model and 0.30 (95% credible interval: 0.04 to 0.50) and 5.3% (95% credible interval: 0.73 to 8.89%) from the WinBUGS® model (Table 7-2). The low risk reference category comprised the 34% of the population that were in cohorts where BVDV was not detected. Animals in cohorts where BVDV was detected (66% of the population) were at increased risk of developing BRD. Overall BRD incidence would be estimated to decline by an absolute amount of 5.6% or 5.3% if all cattle were instead at the same risk as those without evidence of BVDV in the cohort (Figure 7-5).

7.3.2.6 Cohort fill duration

The PAFs and PARs for total effects of cohort fill duration were 0.37 and 6.4% from the MLwiN® model and 0.35 (95% credible interval: 0.09 to 0.53) and 6.2% (95% credible interval: 1.7 to 9.4) from the WinBUGS® model (Table 7-2,Figure 7-11). The

low risk reference group comprised animals in cohorts filled on a single day (34% of the population); animals in cohorts filled over more than one day (66% of the population) were at increased risk of BRD

The estimated population-level effects of the cohort fill duration were modest to moderate; overall BRD incidence would be estimated to decline by an absolute amount of 6.2 to 6.4% if all cattle were instead at the same risk as those cohort fill duration was one day (Table 7-2, Figure 7-9).

However, the PAFs and PARs for the direct effects were much lower and the credible intervals included zero, corresponding to the much reduced effect observed in the direct effects model and indicating that the effect was partially mediated by some or all of the intervening variables included in the direct effects model (i.e. mixing history, day 0 to close, grain percentage variables).

7.3.2.7 Days from day 0 to cohort close

The estimated population-level effects of the number of days from day 0 to cohort close were modest. The PAFs and PARs for total effects of the interval between day 0 and cohort close date were 0.16 and 2.8% from the MLwiN® model and 0.16 (95% credible interval: -0.01 to 0.31) and 2.8% (95% credible interval: -0.1 to 5.4) from the WinBUGS® model (Table 7-2, Figure 7-9). The lowest-risk reference category consisted of animals that joined the cohort seven or more days prior to cohort close (8% of the population). For the majority of the population (57%), day 0 and cohort close date were on the same day (Figure 7-10); animals in this category were at slightly increased risk of BRD.

Thus, overall BRD incidence would be estimated to decline by an absolute amount of 2.8% if all cattle were instead at the same risk as those whose cohort close date was day 0 (Table 7-2, Figure 7-9, Figure 7-12). The PAFs and PARs for direct effects were slightly lower, corresponding to the slightly reduced protective effect observed in the direct effects model.

7.3.2.8 Saleyard exposure between day -12 and day 0

The estimated population-level effects of saleyard exposure between day -12 and day 0 were small. The PAFs and PARs for total effects of exposure to saleyards within 12 days of day 0 were only 0.02 and 0.3% from the MLwiN® model and 0.02

(95% credible interval: 0.02 to 0.02) and 0.3% (95% credible interval: 0.3 to 0.3) from the WinBUGS® model (Table 7-2). Although animals exposed to saleyards were at markedly increased risk of developing BRD, only 3% of the study population were exposed. The PAFs and PARs for direct effects were even lower, corresponding to the reduced effect observed in the direct effect model.

The direct effects of saleyard exposure are more relevant because they provide estimates of effect after adjusting for mixing and feedlot move timing. Although the direct effect indicated that saleyard exposure between day -12 and 0 resulted in increased risk of BRD, only 3% of the population were exposed. Therefore, these results indicated that exposure to saleyards from days -12 to 0 was not an important risk factor at the population level in the study population because so few animals were exposed to this risk factor (Figure 7-11).

7.3.2.9 Saleyard exposure between day -27 and day -13

The estimated population-level effects of saleyard exposure between day -27 and day -13 were small. The estimated PAFs and PARs for the total effects of exposure to saleyards between days -27 and -13 were only 0.01 (PAF) and 0.1% (PAR) from the MLwiN® model and 0.02 (95% credible interval: 0.02 to 0.02) and 0.3% (95% credible interval: 0.3 to 0.3) from the WinBUGS® model. The direct effect estimates were even lower (Table 7-2, Figure 7-11), corresponding to the attenuated effect estimates observed in the direct effect model. Although direct effects estimates indicated animals exposed to saleyards during this time were at slightly increased risk of BRD, only 3% were exposed. These results indicated that exposure to saleyards between days -27 to -13 was not an important risk factor at the population level in the study population.

7.3.2.10 Saleyard exposure prior to day-27

The estimated total population-level effects of saleyard exposure prior to day -27 indicated a modestly protective effect (MLwiN®: PAF: 0.09, PAR: 1.7%) and 0.10 (95% credible interval: 0.05 to 0.16) and 1.8% (95% credible interval: 0.9 to 2.7) from the WinBUGS® model. However, there was no direct population-level effect (Table 7-2, Figure 7-11).

7.3.2.11 Prior vaccination with Bovilis MH™

The estimated population-level effects of prior vaccination with Bovilis MH™ were modest. This risk factor was investigated in the subset of the vendor questionnaire data which included animals that were vendor bred or purchased by 10 months of age. The lowest-risk reference category (i.e. vaccinated) comprised 15% of this population and not vaccinating was associated with an increased risk of BRD. The PAFs and PARs for the total effects of prior vaccination with Bovilis MH™ were 0.18 and 3.3% from the MLwiN® model and 0.18 (95% credible interval: 0.01 to 0.32) and 3.3% (95% credible interval: 0.3 to 6.0%) from the WinBUGS® model (Table 7-2, Figure 7-12). Thus, overall BRD incidence would be estimated to decline by an absolute amount of 3.3% if all cattle were instead at the same risk as those that were vaccinated with Bovilis MH™ prior to day -14 (Figure 7-12).

7.3.2.12 Prior vaccination with Pestigard™

The estimated population-level effects of prior vaccination with Pestigard™ vaccine were modest. This risk factor was investigated in the subset of the vendor questionnaire data which included animals that were vendor bred or purchased by 10 months of age. The lowest-risk reference category (i.e. vaccinated) comprised 12% of this population and not vaccinating was associated with an increased risk of BRD. The PAFs and PARs for the total effects of prior vaccination with Pestigard™ were 0.17 and 3.2% from the MLwiN® model and 0.17 (95% credible interval: -0.03 to 0.34) and 3.2% (95% credible interval: -0.6 to 6.3%) from the WinBUGS® model (Table 7-2 and Figure 7-11).

7.3.2.13 Yard weaning

The estimated population-level effects of yard weaning were modest. Yard weaning was investigated only in the vendor-bred subset with returned vendor questionnaire data. The low risk reference category (i.e. yard weaned) comprised 80% of this population and not yard weaning was associated with an increased risk of BRD. The PAFs and PARs were 0.08 and 1.7% from the MLwiN® model and 0.08 (95% credible interval: 0.01 to 0.13) and 1.7% (95% credible interval: 0.2 to 2.8%) from the WinBUGS® model (Table 7-2, and Figure 7-12). Thus, overall BRD incidence was estimated to decline by an absolute amount of 1.7% if all cattle were instead at the same risk as those that were yard weaned.

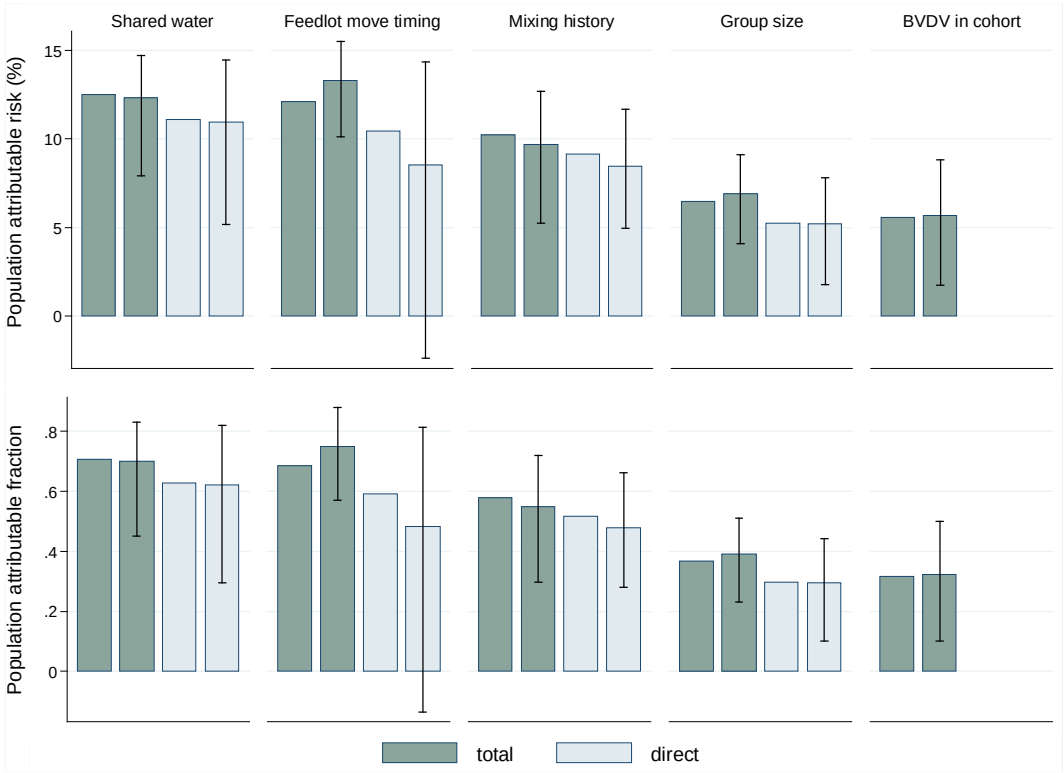

Figure 7-5: PARs and PAFs for management-related risk factors derived from the models fitted using MLwiN® (bars only) and WinBUGS® (with 95% credible intervals) software.

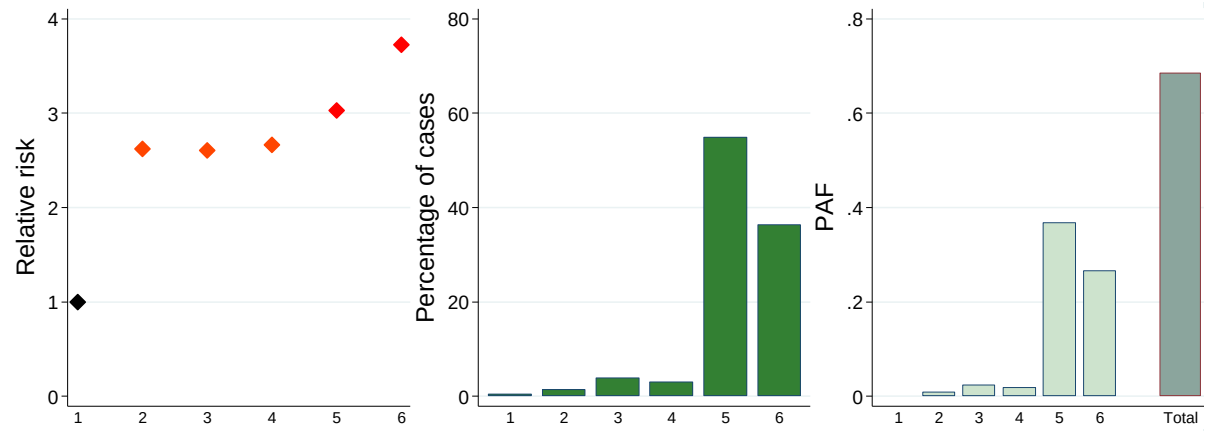

Figure 7-6: Percentage BRD50, adjusted relative risks and partial PAFs from total effects for feedlot move timing on 50-day cumulative incidence of BRD

Table 7-2: PAFs and PARs derived from total effects and direct effects models for feedlot management BRD risk factors

	Total effects			Direct effects		
	MLwiN	WinBUGs		MLwiN	WinBUGs	
Risk factor	PAF	PAF	95% CI	PAF	PAF	95% CI
Shared pen water	0.71	0.70	(0.45 to 0.83)	0.63	0.62	(0.29 to 0.82)
Feedlot move timing	0.69	0.75	(0.57 to 0.88)	0.55	0.48	(-0.14 to 0.81)
Mixing history	0.58	0.54	(0.23 to 0.72)	0.52	0.46	(0.17 to 0.69)
Group size (number of animals in group-13)	0.37	0.39	(0.23 to 0.51)	0.30	0.30	(0.10 to 0.44)
BVDV in cohort	0.32	0.30	(0.04 to 0.50)	0.32	0.30	(0.04 to 0.50)
Prior Bovilis MH	0.18	0.18	(0.01 to 0.32)			
Prior Pestigard	0.17	0.17	(-0.03 to 0.34)			
Days from induction to cohort close	0.16	0.16	(-0.01 to 0.31)	0.14	0.13	(-0.03 to 0.32)
Cohort fill duration	0.37	0.35	(0.09 to 0.53)	0.12	0.26	(-0.15 to 0.53)
Yard weaning	0.08	0.08	(0.01 to 0.13)			
Saleyard day-12 to day 0	0.02	0.02	(0.02 to 0.02)	0.01	0.00	(0.00 to 0.01)
Saleyard day -27 to day -13	0.01	0.02	(0.02 to 0.02)	<0.01	<0.01	(0.00 to 0.01)
Saleyard before day -27	0.09	0.10	(0.05 to 0.16	<0.01	<0.01	(-0.03 to 0.03)
Risk factor	PAR	PAR	95% CI	PAR	PAR	95% CI
Shared pen water	12.5	12.3	(7.9 to 14.7)	11.1*	11.0	(5.2 to 14.5)
Feedlot move timing	12.1	13.3	(10.1 to 15.5)	9.7	8.5	(-2.4 to 14.4)
Mixing history	10.2	9.5	(4.0 to 12.7)	9.1	8.2	(2.9 to 12.1)
Number of animals in group-13	6.5	6.9	(4.1 to 9.1)	5.6	5.2	(1.8 to 7.8)
BVDV in cohort	5.6	5.3	(0.7 to 8.9)	5.6	5.3	(0.7 to 8.9)
Prior Bovilis MH	3.2	3.3	(0.3 to 6.1)			
Prior Pestigard	3.2	3.2	(-0.6 to 6.3)			
Days from induction to cohort close	2.8	2.8	(-0.1 to 5.4)	2.5	2.2	(- 0.5 to 5.6)
Cohort fill duration	6.5	6.2	(1.6 to 9.4)	2.1	4.6	(-2.6 to 9.3)
Yard weaning	1.7	1.7	(0.2 to 2.8)			
Saleyard day-12 to day 0	0.3	0.3	(0.3 to 0.3)	0.1	0.1	(0.0 to 0.2)
Saleyard day -27 to day -13	0.1	0.3	(0.3 to 0.3)	0.1	0.1	(-0.1 to 0.1)
Saleyard before day -27	1.7	1.8	(0.9 to 2.7)	<0.1	<0.1	(-0.6 to 0.6)

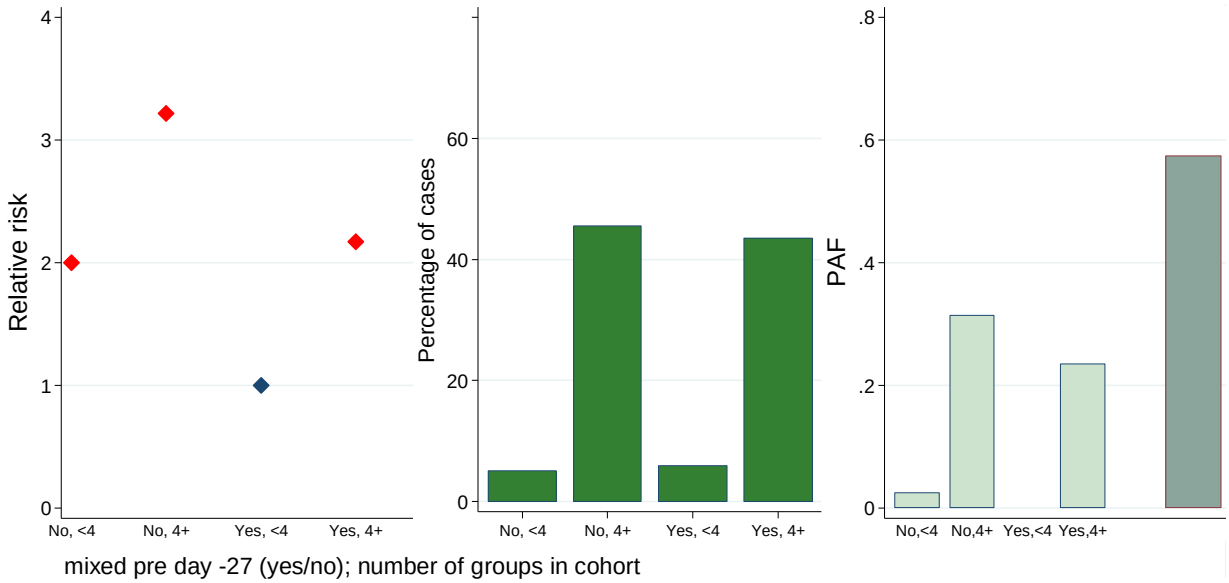

Figure 7-7: Percentage BRD50, adjusted relative risks and partial PAFs from total effects for mixing summary (mixed pre day-27: yes/no; no. group-28s forming cohort) on 50-day cumulative incidence of BRD

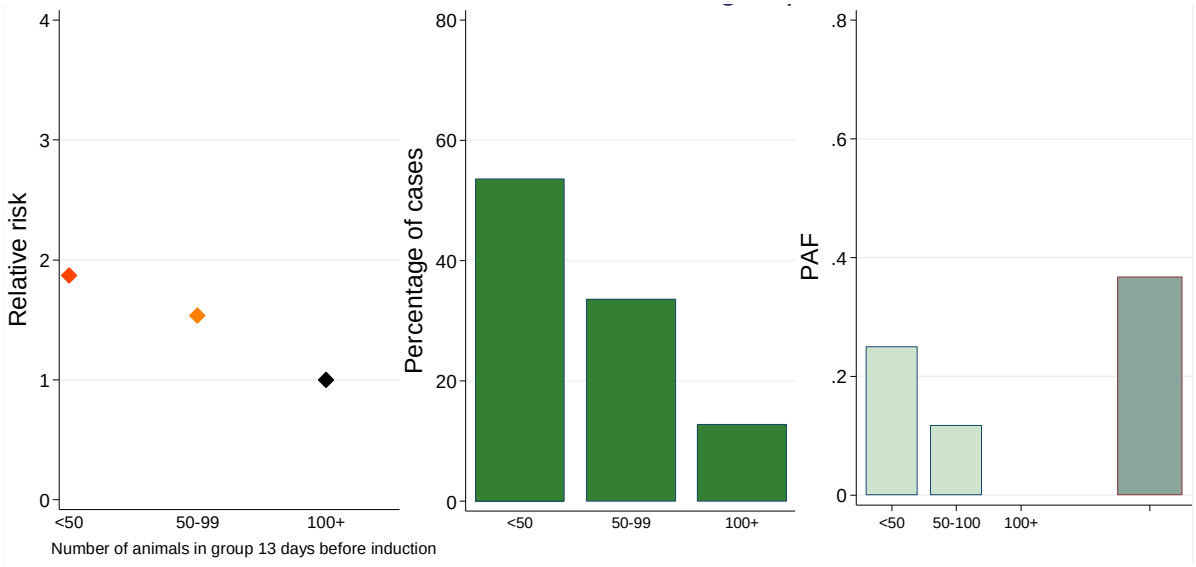

Figure 7-8: Percentage BRD50, adjusted relative risks and partial PAFs from total effects model for effects of number of animals in group-13 on 50-day cumulative incidence of BRD

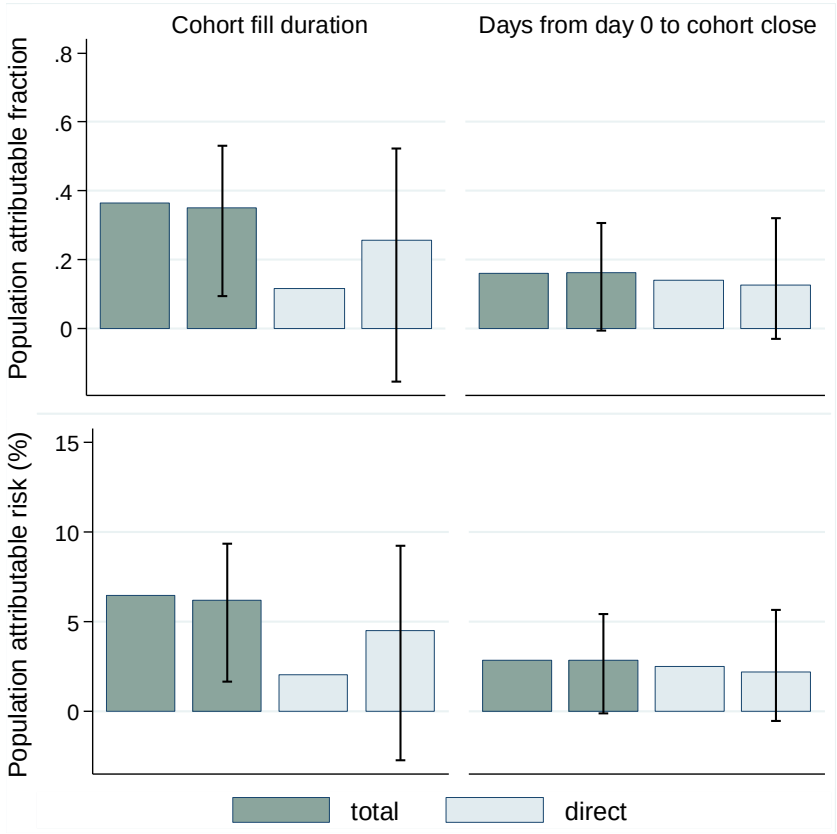

Figure 7-9: PAFs and PARs for management risk factors related to cohort formation derived from the models fitted using MLwiN® (bars only) and WinBUGS® (with 95% credible intervals) software.

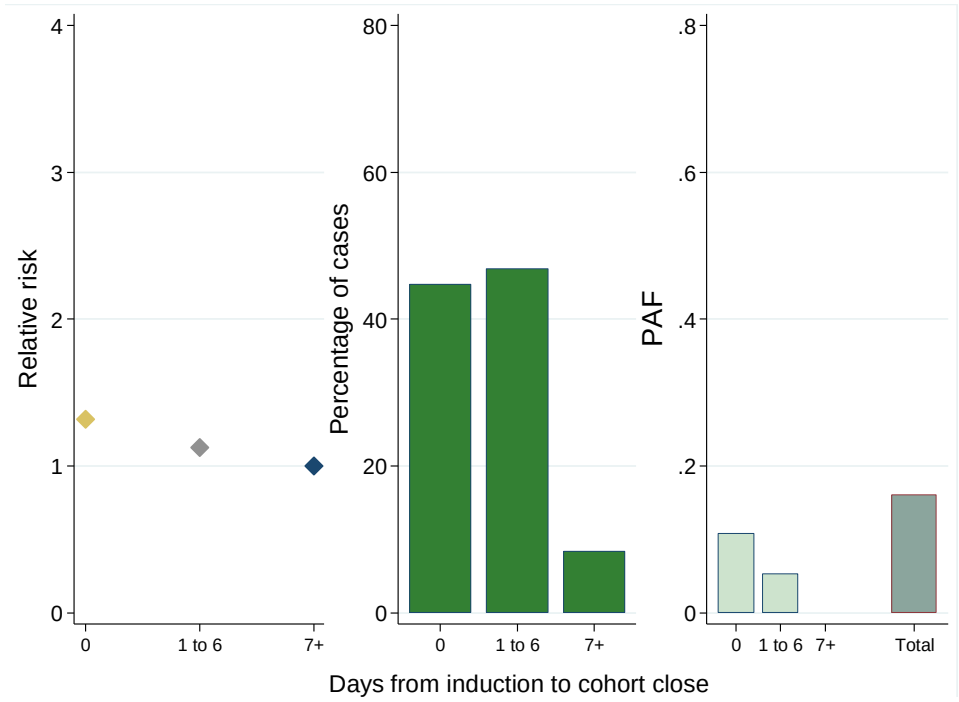

Figure 7-10: Percentage BRD50, adjusted relative risks and partial PAFs from total effects model for number of days from day 0 to cohort close date on 50-day cumulative incidence of BRD

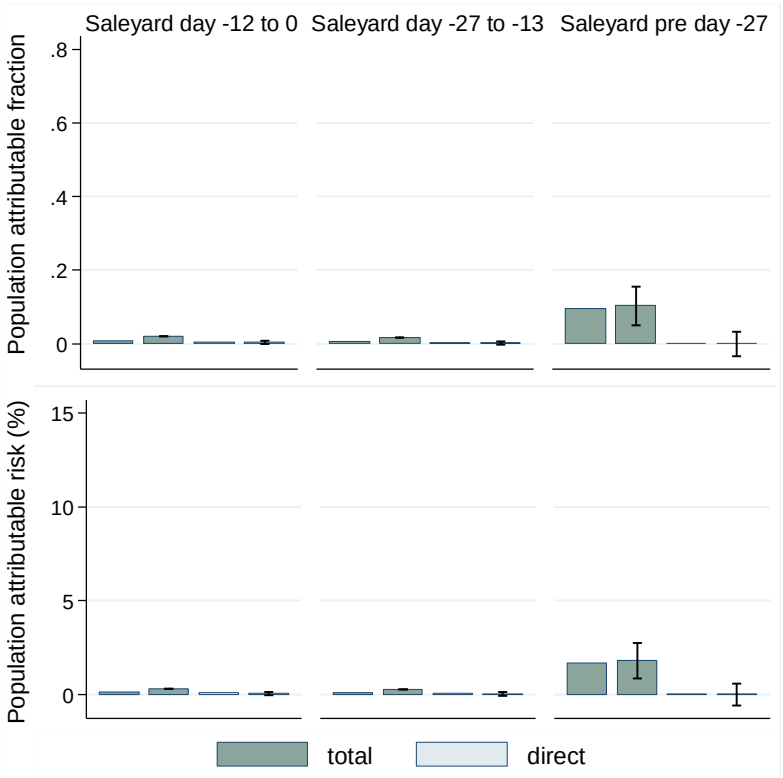

Figure 7-11: PAFs and PARs for management risk factors describing saleyard exposure derived from the models fitted using MLwiN® (bars only) and WinBUGS® (with 95% credible intervals) software.

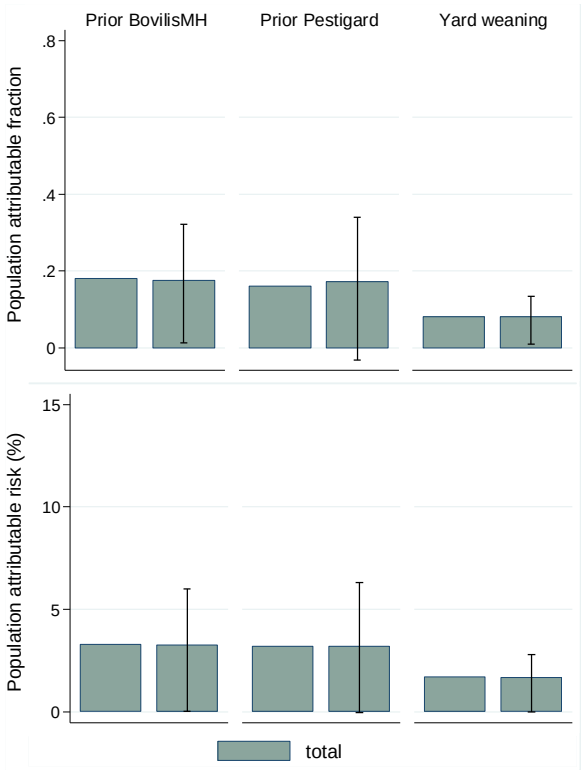

Figure 7-12: PAFs and PARs for management risk factors analysed in the vendor questionnaire datasets; models fitted using MLwiN® (bars only) and WinBUGS® (with 95% credible intervals) software.

7.3.3 Broad environmental risk factors

7.3.3.1 Feedlot region

The majority of the population (62%) were in southern feedlots; these were at markedly increased risk of BRD compared to animals at northern feedlots. The PAFs and PARs for the total effects of feedlot region were very large (Table 7-3, Figure 7-13). Because the direct effect PAF and PAR were also large, there were large direct population-level effects of region over and above covariates included in the model (breed, weight, sex, dentition, source region, grain type and weather variables).

7.3.3.2 Induction season

The estimated population-level effects of induction season were moderate. The PAFs and PARs for the total effects of season were 0.30 and 5.3% from the MLwiN model and 0.28 (95% credible interval: 0.12 to 0.40) and 5.0% (95% credible interval: 2.2 to 7.0%) from the WinBUGs model (Table 7-3, Figure 7-13). About 28% of the population were inducted in spring (the low risk reference group). Animals inducted in summer (21% of the population) or autumn (23%) were at increased risk of BRD and this is reflected in the distributions of partial PAFs (Figure 7-14.). Thus, overall BRD incidence would be estimated to decline by an absolute amount of 5.0% or 5.3% if all cattle were at the same risk as those inducted during spring.

Table 7-3: Comparison of PAFs and PARs derived from total and direct effects models for broad environmental BRD risk factors

	Total effects			Direct effects		
	MLwiN	WinBUGs		MLwiN	BUGs	
	PAF	PAF	95%CI	PAF	PAF	95%CI
Feedlot region	0.80	0.74	(0.62 to 0.81)	0.76	0.72	(0.47 to 0.82)
Induction season	0.30	0.28	(0.12 to 0.40)			
	PAR	PAR	95%CI	PAR	PAR	95%CI
Feedlot region	14.0	13.1	(10.9 to 14.2)	13.4	12.8	(8.8 to 14.4)
Induction season	5.3	5.0	(2.2 to 7.0)			

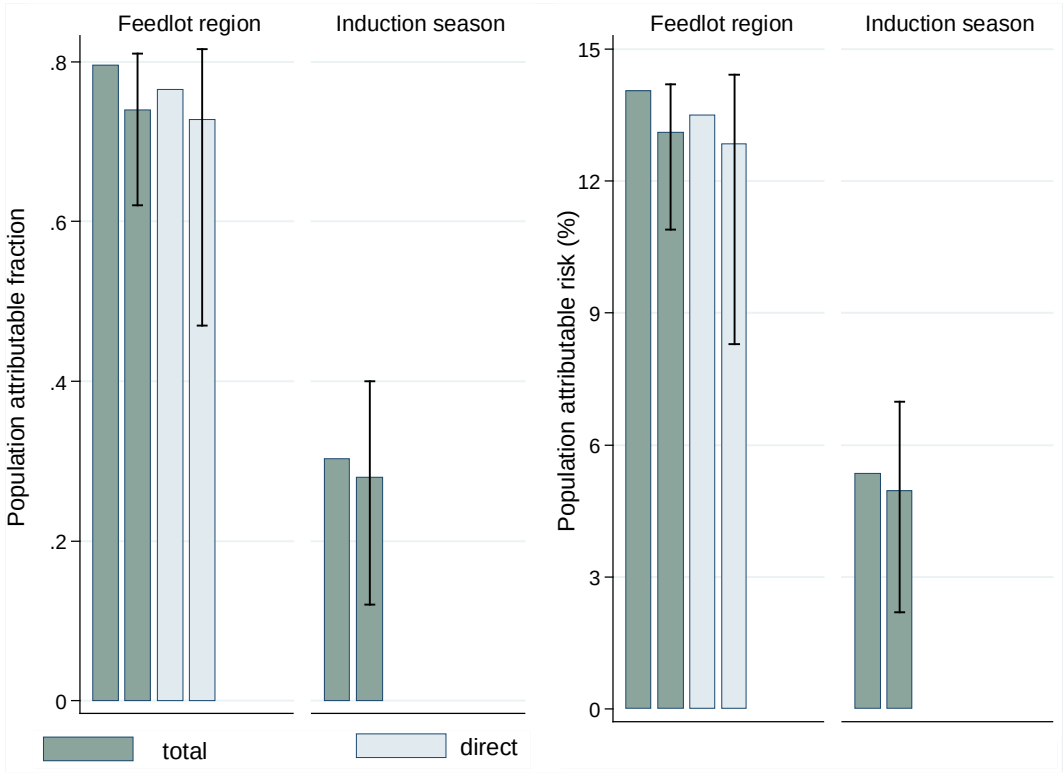

Figure 7-13: PAFs and PARs for broad environmental risk factors derived from the models fitted using MLwiN® (bars only) and WinBUGS® (with 95% credible intervals) software.

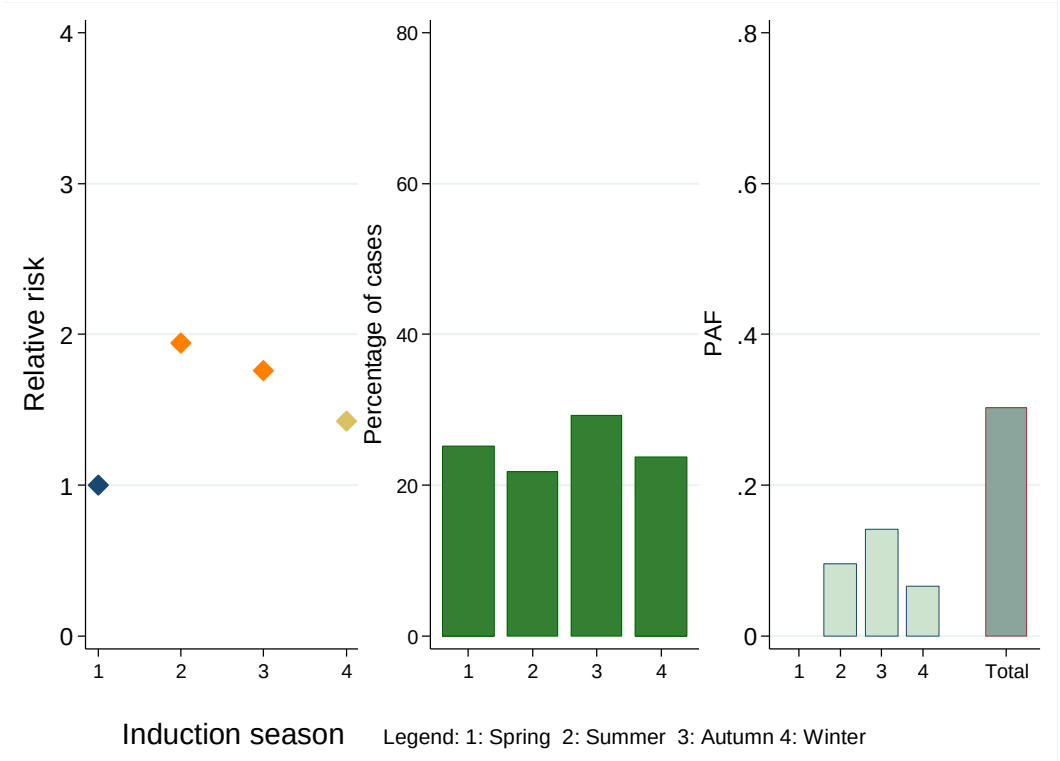

Figure 7-14: Adjusted relative risks, percentage distribution of cases, and estimated partial PAFs across induction season categories and total PAF for induction season derived from the MLwiN® total effects model

7.3.4 Ranking of risk factors

In the preceding section, risk factors were presented within their classification (animal, management, environmental) broadly ordered by their effect estimates. To rank these risk factors in order of importance, the criteria described in Section 7.2.5 were applied. Firstly, risk factors without sufficient evidence of 'causality' were excluded. Age was excluded because the estimates were obtained from a possibly biased subset, the variable was based on crude group averages rather than animal-level measures and the association was not considered to be biologically plausible. As discussed in Section 6.4.1, there was some evidence the association may have been confounded by unmeasured factors such as body condition.

Saleyard exposure between days -27 and -13 and prior to day -27 were not included in the ranking because the direct effect estimates indicated that these risk factors were mediated through mixing and feedlot move timing; hence these intervening variables warrant the attention of industry rather than 'saleyard exposure' a long time before induction.

Remaining risk factors were ranked within the category of population-level effects (i.e. large, moderate, modest, small). Ranking was determined by considering the size and precision of the effect estimates, the relative potential for intervention and/or further research to result in a reduction in BRD incidence at the population level and the quality of the variables. This ranking is presented in Table 7-4. Shared pen water, mixing history, breed, feedlot move timing and feedlot region all had large population-level effects. Shared pen water was ranked first because it had large population-level effects, is amenable to intervention and has a biologically plausible postulated pathway. Mixing history and breed were ranked ahead of feedlot move timing because these were assessed as being better quality variables (i.e. well distributed across feedlots) with plausible biological pathways; there were concerns about the limited distribution of the lowest-risk reference category across feedlots (i.e. only three feedlots had observations in the reference category) and effect estimates for the direct effects were very imprecise. Although feedlot region had the largest PAF, it is lowest ranked out of the variables with a large population-level effect because it was a poor quality variable (feedlot-level and subject to feedlot-level confounding) and was likely to be a proxy for effects of other unmeasured factors.

Nonetheless, the large population-level effects observed are important in prompting further research to better understand these effects.

Of the risk factors with a moderate effect, BVDV in the cohort was ranked above the number of animals in the group-13; the direct effects in the latter variable were attenuated and as discussed in Section 4.6.3, the length of time the group was established varied considerably, so the effect should not be interpreted for the group size of day -13, but rather as the effect of a stable group size over a more extended period of time. The season of induction was ranked next within this group of risk factors with a moderate population-level effect. Further research is recommended to investigate the effects of season in combination with the effects of feedlot region. Sex was ranked lowest within this group because it is unlikely to have much practical application for industry (i.e. the proportion of males entering feedlots is not amenable to intervention and because the majority are male, any tailored interventions would not be practical). Nonetheless, the population-level effect estimates are useful; a management tool based on a predictive model would be expected to have enhanced predictive ability if sex was included in the model.

Risk factors with a modest population-level effect were prior vaccination with BovilisMH^{TM} , or Pestigard^{TM,} induction weight and yard weaning. The first three listed had similar effect sizes but vaccinations were ranked above induction weight because they are more easily amenable to intervention. The two risk factors related to ‘cohort formation’, cohort fill (measured at the cohort level) and days between day 0 and cohort close (measured at the animal level) were combined and included because they warrant further research to better understand the complexities of these relationships.

Exposure to saleyards between days -12 and 0 was the final risk factor included in the ranking and this had only a small effect in the study population. However, in populations where larger proportions were exposed, the population-level effect would be much greater.

Table 7-4: Ranking of identified risk factors for BRD based on population-level effect estimates, biological plausibility, variable quality and potential for intervention or further research

Rank	Risk Factor	Category	Population effect	Conclusion
1	Shared pen water	Management	Large	Shared pen water was probably a major risk factor for BRD at the population level
2	Mixing history	Management	Large	Mixing prior to day-27 was protective; mixing 4 or more grouip-28s to form a cohort increased risk. Cohort formation based on these observations would be expected to markedly reduce BRD incidence at the population level.
3	Breed	Animal	Large	Tropical breeds and crosses were at reduced risk. Management interventions would be expected to be most important for breeds at higher risk
4	Feedlot move timing	Management	Large	Moving to the vicinity of the feedlot at least 27 days before was probably protective but more research is required
5	Feedlot region	Environmental	Large	Variation in BRD risk by feedlot region probably had a major population-level effect but because this is a proxy for other factors, more research is required to investigate the reasons for this
6	BVDV in cohort	Management	Moderate	Eradication of BVDV would be expected to result in a moderately reduced BRD incidence at the population level
7	Group-13N	Management	Moderate	Ensuring that at least 50 animals are assembled in stable groups at least 13 days before induction would be expected to result in a moderately reduced BRD incidence at the population level
8	Season	Environmental	Moderate	Animals inducted during spring were at the lowest-risk, and season of induction had a moderate population-level effect. Season is a proxy for other factors such as weather conditions. More research is required to better understand the association between season and BRD
9	Sex	Animal	Moderate	Steers were probably at increased risk compared to heifers but this has limited application at the population level
10	Prior vaccination Bovilis^{MH}	Management	Modest	Prior vaccination with Bovilis^{MH} probably had a modest population-level effect but this estimate may not be robust because it was analysed in a possibly biased subset of the population
11	Prior vaccination Pestigard^{TM}	Management	Modest	Prior vaccination with Pestigard^{TM} probably had a modest population-level effect but this estimate may not be robust because it was analysed in a possibly biased subset of the population
12	Induction weight	Animal	Modest	Lighter animals were at increased risk resulting in a modest population-level effect
13	Cohort formation	Management	Modest	Further research is required to better understand the relationship between cohort formation variables and BRD
14	Yard weaning	Management	Modest	Yard weaning probably had a modest population-level effect but this estimate may not be robust because it was analysed in a possibly biased subset of the population
15	Saleyard exposure day -12 to day 0	Management	Small	Saleyard exposure within 12 days of induction resulted in only a small population-level effect because few animals were exposed. The effect would be greater in populations where more animals were exposed.

7.4 Discussion

7.4.1 Assumptions and limitations in interpretation of PAFs and PARs

Several assumptions and limitations should be considered when interpreting PAFs and PARs. It is important to keep in mind that the PAFs and PARs are a function of the prevalence of exposure in the population as well as the adjusted relative risks, and as such, their internal and external validity and generalisability are linked to those of these estimates. Risk factors must be causal, estimates must be unbiased and the level of exposure in the population must be representative of the target population before PAFs and PARs can be interpreted as causal population-level effects. Risk factors for which PAFs and PARs were estimated were identified as important because they were probably associated with BRD based on modelling described in Chapter 6.

The methods used in this chapter provide estimated reductions in risk that should be viewed as theoretical maxima rather than likely reductions in practice. The assumptions inherent in these measures have been detailed above. Estimates derived for a particular single risk factor, especially those derived from total effect estimates may be overestimated; the effects of multiple interventions directed at various risk factors will not be additive if the risk factors share common causal pathways. Hence, reported estimates should be interpreted only as a guide to the relative importance of particular risk factors, and their main practical use is in making qualitative statements and comparing risk factors as described in Table 7-4.

Alternative methods have been proposed to estimate 'partial' PAFs from multivariable models (Spiegelman et al., 2007). These methods are useful in estimating the population-level effects of simultaneously changing several 'modifiable' risk factors while considering the distribution and effects of non-modifiable 'background' risk factors (e.g. breed, weight, season) within the population and offer a more realistic expectation of the effects of interventions. However, with the complexity of the dataset, sparse numbers of observations across covariate pattern categories and zero positive outcomes in lowest risk categories, it was not possible to obtain a detailed comparison of population effects of risk factors of interest using these alternative methods.

A further limitation in the methods used to estimate PAFs and PARs related to the multilevel structure of the data. For my calculations, I used the prevalences of exposure at the animal level. However, for exposures that cluster at higher levels such as at the group or cohort level, distributions of exposures assessed at these levels may be more appropriate when estimating population-level effects

7.4.2 Assessing the internal validity of effect estimates

Population-level effect estimates (i.e. PAFs and PARs) are based on estimated relative risks. In this study, I have derived these from odds ratios estimated in multilevel logistic mixed effects models to determine total and direct effects of risk factors of interest. Assessment of the internal validity of PAFs and PARs is therefore inextricably linked to the assessment of the internal validity of the odds ratios. Hence, in this section the term 'effect estimate' applies to both odds ratios and population-level effects and the discussion is relevant to Chapter 6 as well as the current chapter.

Population-level effects were estimated only for those risk factors determined to be probably associated with BRD based on Chapter 6. This was largely determined by the effect size and precision of the estimates. Limited power to detect effects of risk factors clustered at higher levels (i.e. cohort or feedlot level) means that results for many of these factors were inconclusive. Population-level effects were not estimated for these risk factors.

Variables with large, consistent and precise effect estimates, biologically plausible postulated pathways of effect and supporting evidence from prior literature were considered most likely to be causally linked to BRD. These include some animal-entry characteristics and some management risk factors.

7.4.3 Assessing the external validity of population-level effect estimates

Although the study included animals from a broad geographical region, and included medium to large Australian feedlots, only 14 feedlots participated in the study and participating feedlots may have differed in important ways from the target population. Thus, the distribution of exposures observed in the study population may not have

been the same as in the target population. For example, the proportion of cattle sourced from saleyards was very low in the study population, resulting in very low PAFs and PARs for the effect of saleyard exposure between days -12 and 0. If, in fact, a high proportion of cattle in the target population were exposed to saleyards, the population effect would be larger than I have estimated.

7.4.4 Animal entry characteristics

Breed was identified as a very important risk factor with large population-level effects. Because breed was measured at the animal level, was well distributed across feedlots, and because animals were sourced from a wide geographical area, it is reasonable to assume that the distribution is similar to that seen in medium to large Australian feedlots, and that the effect estimates should be relatively unbiased. Previous research provides evidence of an association between breed and BRD and plausible biological pathways have been proposed via genetic susceptibility (Snowder, 2009).

Assuming that breed is causal, then replacing animals whose breeds put them at increased risk of BRD with the reference breed category (tropical or tropical cross breeds) should result in an approximate 50% decline in the pooled BRD incidence (pooled across all feedlots). The distribution of cases is important because the population level effect of a very common breed (e.g. Angus) may be much greater than the effect of a less common breed with a more markedly increased risk (e.g. Hereford). Clearly it is not sensible or practical to suggest that only tropical breeds are inducted into feedlots because market requirements and environmental adaptation need to be considered. The majority of tropically adapted breeds were on feed in northern feedlots and different breeds are better adapted to different environmental conditions. Risk may vary under different environmental conditions; tropically adapted breeds were largely not assessed under conditions prevalent in southern feedlots (e.g. cold wet winters). However, an understanding of the different risk levels of different breeds could be included in an overall assessment of BRD risk for a particular group of cattle.

Lower induction weight was determined to be a moderately important risk factor at the population level. This is consistent with prior research, plausible biological pathways exist (e.g. heavier animals may have a better general health profile or

have a better developed immunity) and because weight was measured at animal level, and was well distributed across feedlots, it would be expected to be representative of the exposure profile in the population. While it is probably not practical to ensure all cattle entering feedlots are in a higher weight category level, knowledge of the expected population-level effects of lower weight may be useful in informing management decisions.

Although the population-level effect estimates for sex were moderately large, estimates were very imprecise, probably because sex was clustered by feedlot. While steers were probably at increased risk compared to heifers, the population level effects were too imprecise to draw a conclusion and there is little practical application for Australian feedlot operators.

The observed population-level effects of age should be interpreted cautiously because these data were restricted to the subset with returned vendor questionnaire data, crude group-level estimates were used and results were contrary to biological plausibility. It is likely that effect estimates are subject to selection bias and uncontrolled confounding.

7.4.5 Management risk factors

Risk factors related to management decisions were of major interest because these are potentially more amenable than animal-entry characteristics to interventions to reduce BRD risk. Some of these interventions may be able to be implemented by feedlot managers while others require the cooperation of farmers or industry on a broader scale. Nonetheless, management risk factors amenable to intervention offer the most promising way of reducing BRD incidence in Australian feedlot cattle.

Ensuring that water troughs are not shared between feedlot pens is a management intervention that is able to be implemented relatively easily. The population-level effects determined from this study indicated that this could have a major impact in reducing BRD incidence. If this factor is truly causal, then changing pen design so that pen water cannot be accessed by outside animals would result in a very large reduction in risk of BRD. However, this association has not previously been reported and it could be confounded by other unmeasured cohort or feedlot level factors. Because this was a cohort-level variable, the effect estimates were imprecise; given

the potentially very large population-level effect of this risk factor, further research is urgently required.

Risk factors related to mixing history, feedlot move timing and group size 13 days prior to induction were of particular interest because they are amenable to feedlot management interventions that have the potential to dramatically reduce BRD incidence in feedlots. These risk factors have substantial PAFs in both total effects and direct effects models indicating they are very important at the population level. That direct effect estimates remained large indicated that there were important direct pathways not explained by intervening variables in the models. Although the total effect estimate of feedlot move timing was quite precise, the direct effects estimates were imprecise. The low risk reference category comprised animals from only three feedlots so estimates could be subject to residual feedlot-level confounding. By contrast, the total and direct effects of mixing history and the numbers of animals in the group-13 were more consistent. Prior literature and plausible biological pathways support a causal role for each of these risk factors. It is likely that the distribution of mixing history and group-13 size are broadly representative of the Australian feedlot population, and that the effect estimates are relatively unbiased, and therefore that these are important factors at the population level. More work is needed to establish the importance of feedlot move timing at the population level because of the limited distribution of the reference category across feedlots and the imprecise direct effect estimates,.

The results indicated that the presence of BVDV in the cohort (i.e. either a PI animal or transient infection) had a moderate population-level effect in the study population. Total and direct effect estimates were consistent such that the incidence of BRD was estimated to drop by around 5.5% if BVDV was not present. Prior evidence from the literature and a biologically plausible pathway support a causal role for BVDV in BRD incidence. Although the measure used was a cohort-level variable, effect estimates were based on laboratory diagnosis at the animal level, and PI animals were detected in in cohorts from 12 of the 14 feedlots (Section 10.2.4.2). This indicated that BVDV presence in the cohort was not clustered by feedlot and the distribution is likely to be representative of that in the population of medium to large Australian feedlots.

The population attributable fraction due to not vaccinating with each of the two vaccines investigated (at least one dose at least 2 weeks before entry) was estimated at 0.18 for Bovilis MH™ and 0.17 for Pestigard™, based on the total effects determined from the vendor bred and purchased by 10 months of age subset of the vendor questionnaire data. Estimates were more precise for Bovilis MH™. Results indicated that vaccinating with Bovilis MH™ or with Pestigard™, may result in a reduction in BRD incidence of around 3% at the population level. However, the validity of the effect estimates should be interpreted with caution because they were derived from a small subset that may not be representative of the total population.

Animals that had a longer period of time between induction and cohort close (7 days or more) were at modestly reduced risk of developing BRD compared to those that joined the cohort on the cohort close date. Because the majority of animals joined within a week before cohort close (rather than longer intervals), there was a moderate population-level total effect. However, this variable is likely to be a proxy measure for other unmeasured factors. Exposures within the pen may be worth assessing in further investigations to explain why animals that had a longer adaptation period were at reduced risk. For example, the study had limited power to determine the pen-level effects of percentage grain in the ration and rates of change in grain in the diet and the effects of pen density and bunk space, because these factors were clustered at the cohort level and sometimes at the feedlot level.

Meanwhile, the total effects modelling for the cohort-level variable describing the number of days taken to fill the cohort (1, >1) indicated that the animals in cohorts that filled on a single day were at reduced risk of BRD, but the direct effects modelling indicated that this was mainly mediated through indirect pathways. In combination with the distribution of exposure across the population, population-level effects derived from total effects were moderate while those derived from direct effect estimates were modest.

These two risk factors were included together in the ranking of important risk factors as ‘cohort formation’. They are both likely to be proxies for other unmeasured factors and results suggested that factors that reduce risk at the animal level (i.e. increased adaptation time-for animals that were the first to be inducted into open cohorts) may not be applicable to the whole cohort (closed cohorts were at reduced risk compared

to open cohorts). Further research is recommended to better understand these relationships.

The population attributable fraction of yard weaning estimated from vendor questionnaire data was modest. As discussed above, the proportion of animals exposed to yard weaning may differ in the broader population. Farmers who chose to respond to the vendor questionnaire may be managers who are more likely to practice yard weaning, so the possibility of selection bias should be considered. It is therefore possible that practicing yard weaning could result in further beneficial effects at the population level if this practice is truly linked to causality, so further investigation is warranted.

The direct effects of saleyard exposure were considered more informative than the total effects. The total effects of saleyard exposure were largely explained by mixing and the timing of the move to the feedlot. While an important direct effect of saleyard exposure in the period between day -12 and day 0 remained, very few animals were exposed and at the population level, the effect was quite small.

7.4.6 Broad environmental factors

Moderate to large population-level effects of broad environmental risk factors were demonstrated; these were included in the ranking to promote further research rather than to identify strategies to reduce risk. The markedly increased risk for cattle in southern feedlots was reflected in very large PAFs and PARs. Because feedlot region is likely to be a proxy for numerous other factors, further investigation is required to understand these effects.

Population-level estimates indicated induction season had a moderate effect. However, season is also likely to be a proxy for other risk factors or exposures that are more common at particular times of the year. Other studies have shown associations between weather variables and BRD, and demonstrated interactions between weather variables and other risk factors. For example, in a North American study, animals with a high ‘BRD risk score’ were at further increased risk when exposed to variations in weather variables than lower risk animals (Cernicchiaro et al., 2012). In the Australian context, the effects of weather variables are the most obvious possible explanatory of contributing factors that could be investigated further to better understand the effects of feedlot region and induction season.

7.5 Conclusions

The population-level effects of important risk factors were estimated. Risk factors with sufficient evidence were ranked on importance (i.e. extension, development and/or research) from the perspective of the Australian feedlot industry. Consideration of whether the risk factors are modifiable and whether they were likely to be ‘causal’ or a proxy for other as yet undetermined causes was incorporated before drawing final conclusions. These conclusions will provide an evidence-based resource to inform industry about strategies most likely to reduce BRD incidence across populations in medium to large Australian feedlots. Conclusions and recommendations are discussed in Chapter 12.

8 Parsimonious Model and Partitioning of Variance

8.1 Introduction

Aims of the analyses reported in this chapter were to estimate the proportions of variation in BRD occurrence at animal, group, cohort and feedlot levels, and to estimate the proportion that is explained by a set of identified risk factors. As described in Section 4.4, the study population had a nested hierarchical structure, with the 35,131 animals included in the main cohort dataset nested within 1,077 group-13s (where group-13 referred to the group an animal was part of 13 days before day 0) nested within 170 cohorts, nested within 14 feedlots. From the descriptive results presented in Chapter 5, it was clear that there were large differences in BRD incidence between feedlots, and between cohorts within feedlots. Sources of these differences can be explored by the estimation of random effects (i.e. the residual variations) at each of these hierarchical levels. Understanding the proportioning of variance in hierarchical data is very useful in determining the level at which interventions or further research would be expected to be of most benefit (Browne, 2012).

In Chapter 6, I estimated total and direct effects of risk factors of interest, but that modelling approach was not appropriate for describing proportioning of variance as the aim was not to identify one set of fixed effects that explained BRD occurrence. In the current chapter, I describe the identification of a parsimonious set of predictors using a semi-automated model building process. Parsimonious models are routinely used to estimate effects, and are sometimes also used as predictive models. Recent North American studies have started reporting investigations into using predictive models for BRD in feedlots (Amrine et al., 2014, Babcock et al., 2013b). Model assessment described later in this chapter included assessing the model fit and discriminatory ability of the final parsimonious model as well as its utility as a predictive model. However, routine model checking procedures have not all been extended to clustered correlated data (Hosmer et al., 2013b).

8.2 Partitioning of Variance

Variance is a measure of the variability of the outcome and in multilevel modelling, variance can be ascribed to different levels of the hierarchy. In a linear mixed model, containing one set of random effects for each level in the hierarchy, the random effects can be interpreted as 'variance components' (Browne, 2012). The null model (one with no explanatory variables added), gives an indication of where the greatest amount of variability in the outcome occurs before the addition of predictor variables. Random effects (or residuals at the hierarchical levels) are random variables (assumed to follow a normal distribution) that represent unexplained variation.

8.3 Methods

8.3.1 Model diagnostics

The statistical software, model specification and model diagnostic assessment detailed in Section 6.2.3 were also applied to the modelling described in this chapter. In addition to the diagnostics described in Chapter 6, the deviance information criterion (DIC) was utilised for model selection in deriving a parsimonious model. The DIC gives an overall measure of model fit by considering the total deviance (a measure of the unexplained variation in the data) and the effective number of parameters; thus explicitly considering the trade-off between model complexity and model fit (Spiegelhalter et al., 2002). The effective number of parameters is a measure of the amount of information needed to fit the data (i.e. model complexity). For the same data, a lower DIC indicates a better model, although small variations can be due to the stochastic nature of the process and a difference of more than three is generally used for model selection (Spiegelhalter et al., 2002).

8.3.2 Model building

All eligible exposure variables were subjected to univariable screening using multilevel logistic regression models with the second order penalised quasi-likelihood (PQL2) method implemented in the MLwiN® software package. Eligible variables included those assessed as being of adequate quality for inclusion in analyses as described in Section 4.5.11, and not nested within or highly correlated with another variable. Where more than one eligible exposure variable measured the same risk

factor, a single variable was selected, based on variable quality and the dataset being analysed (e.g. mixing summary for subsets).

Where possible, models were fitted with a four-level hierarchy (feedlot, cohort, group-13, animal). If a large amount of data was missing (e.g. at the cohort level), this was not possible so three-level models (feedlot, cohort, animal) were fitted instead. All eligible variables with a univariable multiple Wald p-value of less than 0.2 were then fitted in a multivariable multilevel model. A backwards elimination process (using PQL2 methods) was applied to sequentially remove variables with the highest Wald p-value from this model, with variables sequentially dropped if the Wald p-value was greater than 0.1. Dropped variables were then given a chance to re-enter the model (in the reverse order to what they were dropped). This resulted in a 'base' model in which all variables were significant at the 0.1 level.

The base model was then estimated using Bayesian models fitted in MLwiN® (run through Stata®). Initial values were obtained using the PQL2 option within MLwiN®, which were then used with the default non-informative priors. MCMC chains were run with a burn in of 500 and chain length of 50,000 iterations with the application of orthogonalisation and hierarchical centring to improve convergence as described in Section 6.2.3.3. Model diagnostics were assessed and the DIC was recorded to allow comparison of this model with subsequent models containing different groups of variables and/or with the addition of interaction terms.

Where multiple variables measuring essentially the same risk factor were all of suitable quality for inclusion, alternative variables were assessed by substituting them into the final base model. Initial values were obtained and models run as described above and DIC values were used to compare models for the same dataset. For example, the mixing summary and time of first mixing variables were assessed as alternatives to the detailed mixing history variable, but a lower DIC indicated the detailed variable was the most appropriate one to include. The binary cohort-level variable describing whether BVDV was detected in any animal in the cohort was included in the initial modelling process but there were two alternative related variables (BVDV_grp_cht, BVDV_chtPI). The DIC values for equivalent models containing these were essentially the same, so final modelling proceeded with the simpler binary variable.

After the variables in the base model were finalised, all possible two-way interactions involving predictors in that model were tested by fitting each of them separately and using PQL2 model estimation methods (all were considered biologically plausible). Significant interactions (defined when the multiple Wald p-value for all terms in the interaction collectively was <0.05) were investigated by examining and plotting the odds ratios and 95% confidence intervals for different categories of interaction terms. In some instances, despite having a Wald p-value of <0.05, interaction terms were often uninterpretable because of extremely wide credible intervals. Unequal distributions across feedlots and/or sparse or empty categories were also encountered. These interactions were not considered further. The remaining three interactions were sequentially fitted in the model and the model was estimated using MCMC methods as described above. DICs were used to compare models containing each interaction with the base model. For two of these three interactions, the DIC estimates from the interaction models were lower. Both interactions were then entered together into the model together; this resulted in a further reduction in DIC, indicating a better trade-off between model fit and the effective number of parameters than the base model.

Thus, the final parsimonious model used to estimate variance components contained two interaction terms, one between breed and season, and one between induction weight and the number of animals in group-13. Model diagnostics and variance parameters were assessed at different points beginning after 50,000 iterations. Reported estimates from the final parsimonious model were obtained after 300,000 iterations.

8.3.3 Partitioning of variance

In contrast to linear mixed models, multilevel logistic mixed effects models need to be interpreted in the context of ‘conditional’ association (Dohoo et al., 2009). Thus, effect estimates derived from fixed effects in the model are conditional on the random effects (i.e. cluster specific). As with other model parameters, the random effects are estimated on the logit scale. Because the error terms in the logistic model are derived from the binomial distribution, the variance depends on the probability of the outcome, the total variance differs between models. In addition, level 1 variance is on a different scale, so estimating the partitioning of variance presents several challenges (Dohoo et al., 2009). Using a latent variable threshold approach and

assuming the level 1 error term follows a logistic distribution produces a level 1 error term on the same scale as the higher level error terms, which can then be used to estimate the partitioning of variance. This theoretical construct produces a constant value for level 1 variance of $\pi^2/3$ or 3.29 (Dohoo et al., 2009) which was used in the partitioning of variance.

To partition the variance in the final parsimonious model, the estimated variances for the random effects at each level of the hierarchy (i.e. feedlot, cohort, group-13) were obtained from the model fitted using the main cohort dataset The linear predictor was obtained for the fitted model and the variance explained by the fixed effects in the model was obtained as the variance of this variable (Snijders and Bosker, 2012). Hence, the total variance in occurrence of BRD by day 50 was calculated as the sum of the animal-level variance (3.29), the random variances at each other level and the variance explained by the fixed effects. The percentage of variance explained by the model was then derived and the percentages of unexplained variances that were at each of the hierarchy levels were calculated.

8.3.4 Null models

To calculate the partitioning of variance before the addition of any explanatory variables, a four level null model was fitted using the same observations that were included in the final parsimonious model described above. Variance partitioning was performed as described above.

8.3.5 Model diagnostics

Model diagnostics, as described previously (Section 6.2.3) to assess fixed effects, were also examined to assess the random effects from the final parsimonious and null models. Model diagnostics indicated poor mixing and high autocorrelation for the feedlot-level residuals, particularly in the null model. The effective sample size, Brooks Draper, and Rafferty Lewis statistics indicated that convergence was adequate in the final parsimonious model, but the feedlot-level variance estimates from the null model proved to be unstable, changing markedly upon repeated runs. Several repeat runs with chain lengths varying between 50,000 and 500,000 iterations produced variance estimates that ranged between 4.6 and 11.9, while cohort and group-13 level variance remained stable (Table 8-1). In contrast,

repeated estimation of the final parsimonious model also produced stable estimates for all parameters including feedlot-level variance.

The assumption of normality of residuals (i.e. the random effects) was assessed by examining the model diagnostics for the random effects and by inspection of inverse normal quantile plots obtained by using the *qnorm* command in Stata®. For each random effect, this command plotted the quantiles of the observed variable to those from a normal distribution with the same mean and standard deviation as for the observed variable. If the residuals were normally distributed, these quantiles would be identical, and the plotted values would fall along a straight line. The cohort and group-13 level residuals were adequately distributed for this assumption but the feedlot level residuals departed substantially from normality Figure 8-3.

Further investigation of random effects estimates based on the feedlot-level residuals indicated that one feedlot, with an extremely low BRD incidence, was not behaving in an analogous way to the rest of the population. Excluding this feedlot from the analyses resulted in marked improvement; the estimated random effect variance at feedlot level was much more stable and the distribution of feedlot level residuals appeared more normal.

Subsequently, the partitioning of variance procedures described above were repeated using a subset of data that excluded all observations from this feedlot. The final parsimonious model was run with this subset and then the same observations were included in a null model. Model diagnostics were much improved with respect to the feedlot-level variance, although the distribution still departed from normality. Hence, reported results include a comparison of variance partitioning in the study population with and without this feedlot.

8.3.6 Further model fit, discriminatory ability and validation

Cross validation was used to assess the goodness of fit and discriminatory ability of the final parsimonious model. This included the assessment of plots of the observed 50-day cumulative incidence of BRD versus predicted probabilities averaged over group-13s. There has been limited extension of routine methods used to assess model fit and predictive ability in single-level models but some of these may be employed in multilevel models (Hosmer et al., 2013b). Receiver operating characteristic (ROC) curves plot the sensitivity (probability of detecting a case)

against one minus the specificity (where specificity is the probability of correctly classifying a non-diseased animal) over the complete range of possible cut-points and is a measure of the overall discriminatory ability of the model (Hosmer et al., 2013a). If the area under the ROC curve is 0.5, the model has no discriminatory ability, values between 0.7 and 0.8 indicate acceptable discrimination and above 0.8 indicate good to excellent discrimination. A plot of the sensitivity and the specificity against different probability cut-points is also a useful tool. The intersection of these plots determines the cut-point where the sum of the sensitivity and specificity is maximised, which corresponds to the predicted probability cut-point where classification is optimal (Hosmer et al., 2013a).

Following the completion of the modelling process, described above, to derive the final parsimonious model, the discriminatory ability and model fit were assessed by plotting ROC curves. This was repeated with predictions based on a) fixed effects only and b) both fixed effects and random effects. For multilevel mixed effects models, the inclusion of random effects is recommended in the assessment of model fit and predictive ability (Hosmer et al., 2013b). ROC curves were plotted based on each of these predictions for the main cohort study population and the sensitivity and the specificity were plotted against different probability cut-points.

Model cross validation was then performed using separate validation subsets for each feedlot. Each animal was assigned a unique computer-generated random number; these were then sorted by group-13. Half of all animals in each group-13 were then allocated to each ‘validation subset’ and identified by feedlot. Validation models were then fitted (one for each feedlot) by running the final model excluding the relevant validation subset (i.e. the model included all observation except those in the validation subset). The linear predictor was obtained a) based only on the fixed effects and b) based on both the fixed and random effects; these were used to derive predicted probabilities for each animal. Then, using the validation subset only (i.e. including animals with predicted values that were not included in the model used to estimate those values), comparisons were made between the observed and expected values. The predicted probabilities were categorised by decile, and the mean predicted probability was obtained for each decile. These were compared with observed values by fitting and plotting ROC curves. Estimates of areas under the ROC curves were obtained using the rocfit command in Stata®; this fits a binormal

model and the rocplot command then plots the ROC curve. The process was repeated with average predicted probabilities for each group-13 being compared to observed values. The complete process was repeated using a separate validation subset for each feedlot, so that results were obtained for 14 validation subsets. The Hosmer Lemeshow Goodness of fit p-value provides a test statistic for comparing observed versus predicted values. Thus, larger p values mean that the observed and predicted values are not significantly different, indicating a good fit.

In addition, the observed proportion of animals developing the outcome (i.e. BRD50) in each group-13 was compared to the predicted proportion based on the predicted probabilities obtained from the models described above (i.e. excluding the validation subset for each feedlot). These were plotted separately by feedlot. A combined graph was also produced for the main cohort population (excluding group-13s with less than four animals).

8.4 Results

Tables showing the univariable screening results obtained from the parsimonious model-building process are shown in Appendix 3. They are not presented in the main body of the thesis because the main estimates of effects used for inference in the study were derived from the total and direct effects modelling process. Within this framework, univariable results were considered to be of limited interest.

8.4.1 Partitioning of variance

Of the original 35,131 animals eligible for inclusion in the analysis, 34,609 (98.5%) had complete data for all variables that were included in the final parsimonious model. The variables included in the base model were: sex, breed, induction weight, mixing history, timing and duration of the move to the feedlot, number of animals in group-13, number of days from day 0 to cohort close, shared pen water, BVDV active in the cohort and season of induction. Two interactions (breed*season and induction weight*number of animals in group-13) with overall p-values <0.05 had no empty or sparse categories and reduced the DIC by more than three, so were included in the final parsimonious model. Variance estimates from the final parsimonious model and the null model fitted using the same observations are shown in Table 8-1. The fixed effects in the final parsimonious model explained

13.8% (1.11/8.04) of the total variance in BRD. Of the unexplained variance, 36.5% was at the feedlot level, 10.1 % was at the cohort level, 5.9% was at the group-13 level and 47.5% was at the animal level. As described above, the feedlot-level variance estimates for the null model were unstable, with substantially differing estimates each time the model was rerun. This was due to the extremely low BRD incidence in one outlier feedlot. Accordingly reported values are approximate. However, from repeated runs, it was clear that the majority of the variance was at the feedlot level, about 9% was at the cohort level, 5% at the group-13 level and 30% at the animal level.

Comparative results obtained from the dataset in which the outlier feedlot was excluded are displayed in Table 8-2. The feedlot-level variance estimate for the null model was much lower at 2.7, so that the proportion of unexplained variance that was at the feedlot level was estimated at a much reduced 36%. The relative proportion of unexplained variance at other levels was therefore higher, with 13% at the cohort level, 7% at the group level and 44% at the animal level. When the final parsimonious model was run using this subset (i.e.13 feedlots), the fixed effects explained 16.5% (1.18/7.17) of the variance in BRD occurrence.

The observed instability in variance at the feedlot level obtained from the null model using the full cohort dataset was attributed to one feedlot not behaving in an analogous way to the rest of the population of feedlots. This problem was likely exacerbated because there were only 14 feedlots and because the BRD incidence in that feedlot was extremely low. However, the stable variance values obtained after fitting the final parsimonious model with all 14 feedlots indicated that the fixed effect predictors (e.g. breed, mixing history) largely explained the wide variation between this and other feedlots. Hence, final modelling was completed using the complete dataset; conclusions about the partitioning of variance were drawn from this while comparing results from the restricted dataset where appropriate.

The caterpillar plots displayed in Figure 8-1 (14 feedlots) and Figure 8-2 (13 feedlots) show the estimated feedlot-level random effects with 95% credible intervals ranked in order; a comparison of these plots illustrates graphically how the feedlot-level variance was reduced by the fixed effects in the final parsimonious model. The large amount of variability between feedlots in the null models was reduced in the final

models, with point estimates generally closer to zero with narrower 95% credible intervals.

Figure 8-3 displays the inverse normal quantile plots for the feedlot-level residuals for null and final parsimonious models fitted using either 14 or 13 feedlots (excluding the outlier feedlot). Although some departure from normality was evident, it was clear that the residuals better approximated a normal distribution after the removal of the outlier feedlot. The range in values was reduced between the respective null and final parsimonious models. The cohort and group-13 level residuals met the assumption of normality with values falling close to the straight line on the inverse normal quantile plots.

Table 8-1: Partitioning of variance at each of the four levels in the null and final models and percentages of the variance unexplained by the final model at each of the four levels for the 14 study feedlots.

Partition	Null model	% of unexplained variance	Final model	% of unexplained variance
Feedlot level variance	~ 6*	~ 56*	2.53	36.5
Cohort level variance	~0.99	~ 9	0.70	10.1
Group-13 level variance	~0.56	~ 5	0.41	5.9
Animal level variance	3.29	~ 30	3.29	47.5
Total unexplained variance	~ 10.84		6.93	
Fixed effect variance	n/a		1.11	
Total variance	~ 10.84		8.04	

*Value of feedlot-level variance was unstable in the null model fitted using the full cohort dataset

Table 8-2: Partitioning of variance at each of the four levels in the null and final models and percentages of the variance unexplained by the final model at each of the four levels for a dataset restricted to 13 feedlots after exclusion of one outlier feedlot

Partition	Null model	% of unexplained variance	Final model	% of unexplained variance
Feedlot level variance	2.67	35.5	1.58	26.3
Cohort level variance	1.00	13.3	0.71	11.8
Group-13 level variance	0.56	7.4	0.41	6.9
Animal level variance	3.29	43.8	3.29	54.9
Total unexplained variance	7.52		5.99	
Fixed effect variance	n/a		1.18	
Total variance	7.52		7.17	

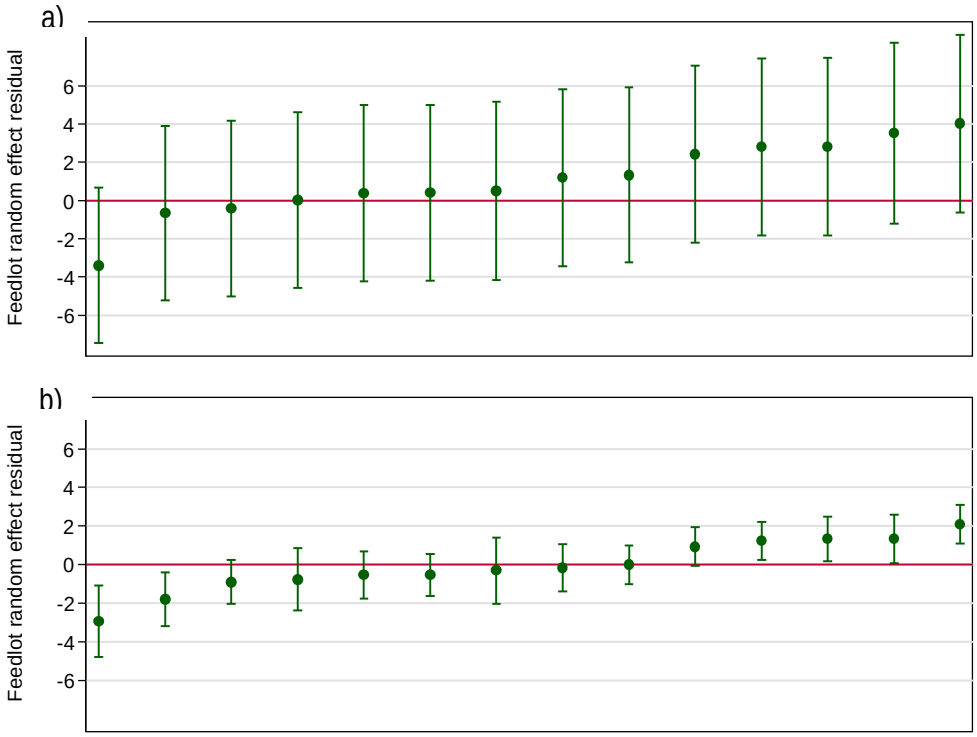

Figure 8-1: Ranked feedlot–level random effects (on logit scale) from the null model (a) and the final parsimonious model (b) fitted using equivalent observations for the 14 study feedlots (point estimates and 95% probability intervals); observations for each feedlot are vertically aligned

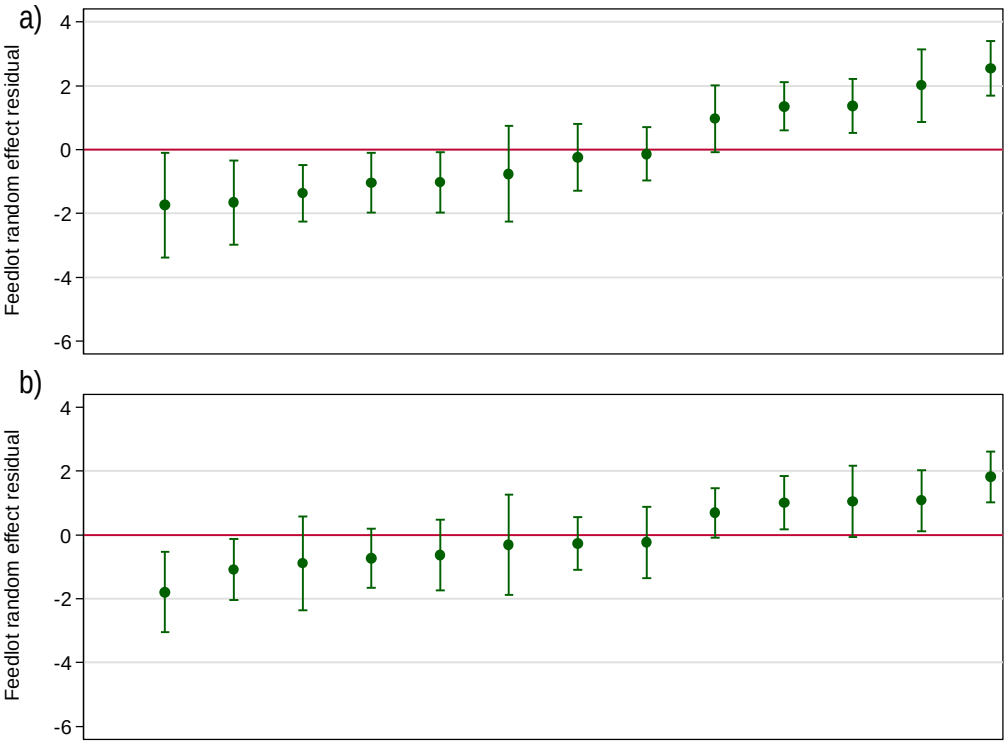

Figure 8-2: Ranked feedlot–level random effects (on logit scale) from the null model (top) and the final parsimonious model (below) fitted using equivalent observations for a dataset restricted to 13 feedlots after exclusion of one outlier feedlot (point estimates and 95% probability intervals); observations for each feedlot are vertically aligned

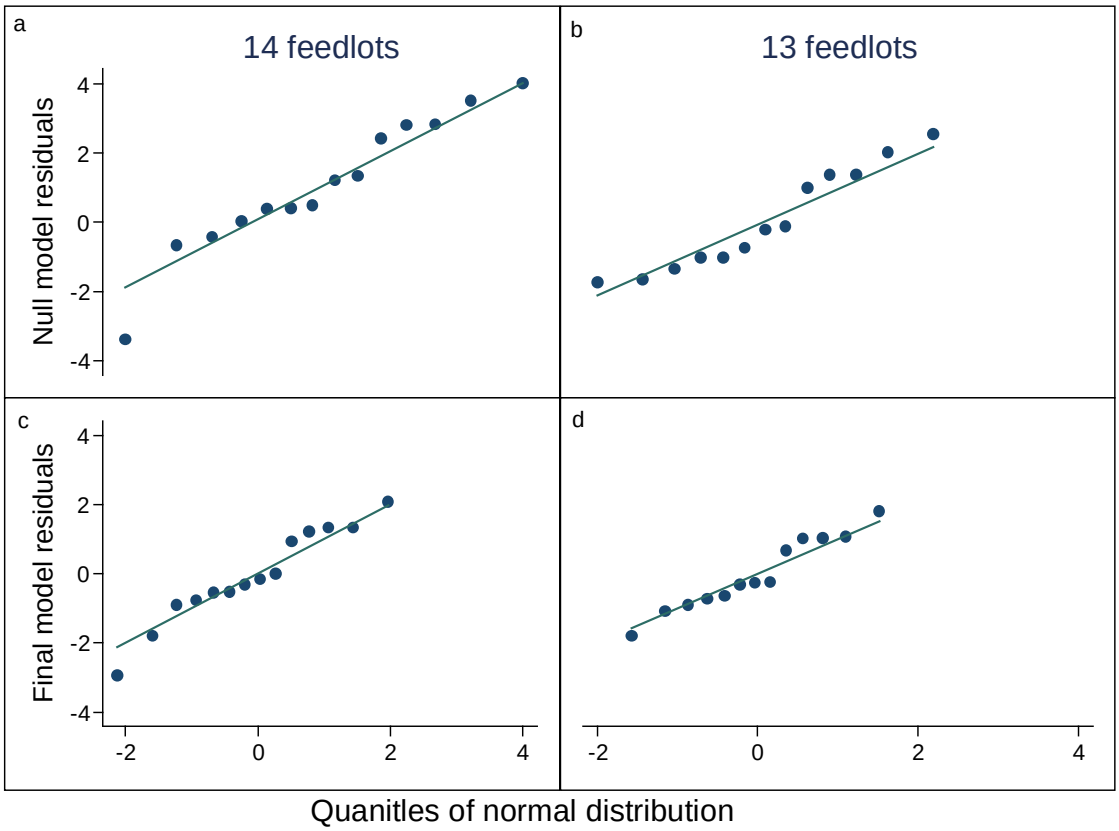

Figure 8-3: Inverse normal quantile plots comparing feedlot-level random effects with normal distribution (logits). Graphs compare the random effects obtained from the null model fitted using 14 feedlots (a) to the final model from the same dataset (c) as well as those obtained from the null model fitted using 13 feedlot (b) with the final model using the same observations (d)

8.4.2 Model assessment and validation

The ROC curve for the final parsimonious model based on predictions from the fixed effects only is shown in Figure 8-4. The area under the ROC curve was 0.74 (95 % CI 0.73 to 0.75), which indicated acceptable overall discriminatory ability. The corresponding ROC curve based on both fixed and random effects is shown in Figure 8-5. The area under this ROC curve was 0.87 (95 % CI 0.86 to 0.87), which indicated good overall discriminatory ability.

From Figure 8-6, it is evident that the intersection of the sensitivity and specificity occurred when these values were 0.69. This occurred when the predicted probability of BRD was about 0.11.

Areas under the ROC curves generated by the model validation process, along with associated 95% confidence intervals, are presented in Table 8-3. Some models failed to run (*) or returned aberrant results ($^{\#}$) (i.e. model predictions in opposite direction to observed results) probably because of very low BRD incidence or limited numbers in the validation subsets. For the two feedlots (including the outlier feedlot described above) with very low 50-day BRD cumulative incidences (<1%), the model was not useful as a predictive model. For the remaining feedlots, the predictive ability of the model was fair to acceptable, ranging from 0.58 to 0.78; it tended to be similar for predicted probability deciles and group-13 averages. As expected, predicted probabilities (averaged over probability deciles) generated with both fixed and random effects produced larger areas under the ROC curve. These predictions were acceptable for five feedlots and good for six feedlots.

The plotted mean observed versus predicted cumulative proportions in Figure 8-7 reveal reasonable agreement in values across feedlots and in the overall cohort study population.

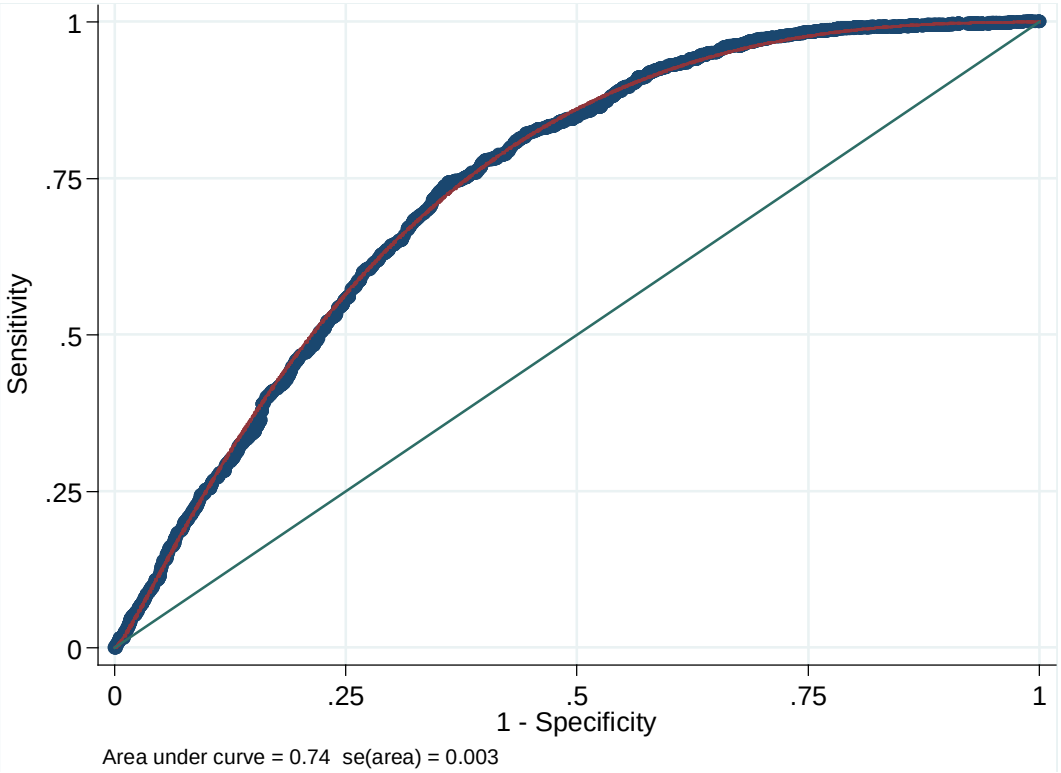

Figure 8-4: ROC plot for predicted probability of BRD50 derived from fixed effects only in the final parsimonious model including interaction terms.

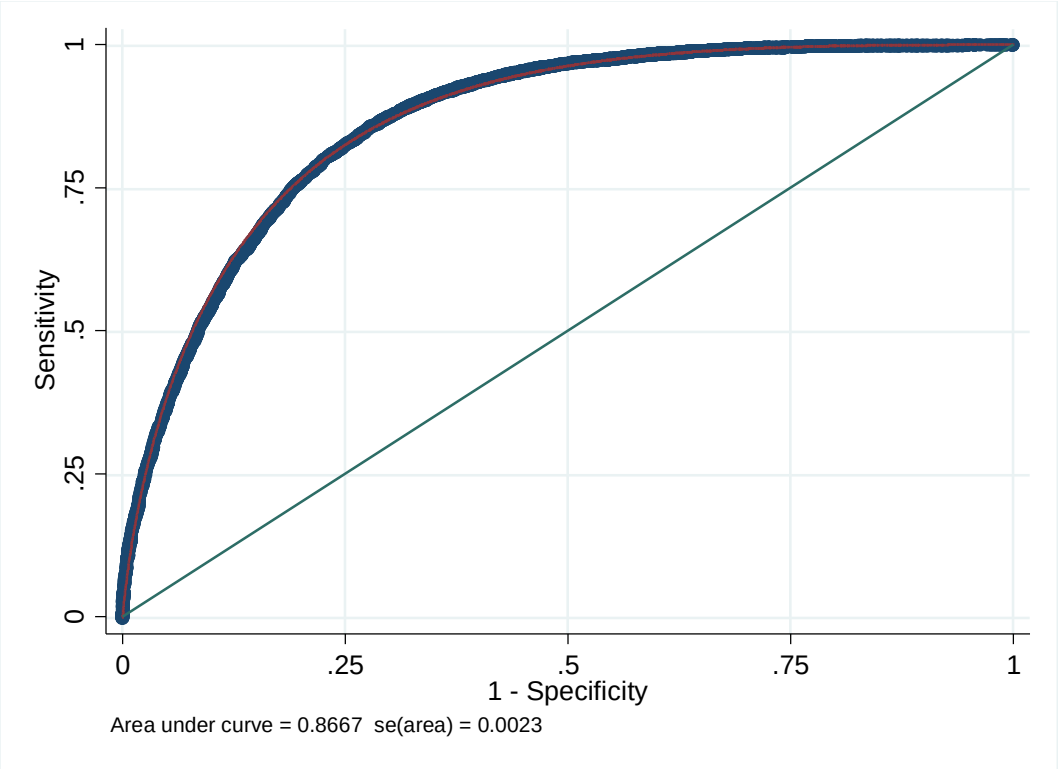

Figure 8-5: ROC plot for predicted probability of BRD50 derived from both fixed and random effects in the final parsimonious model including interaction terms.

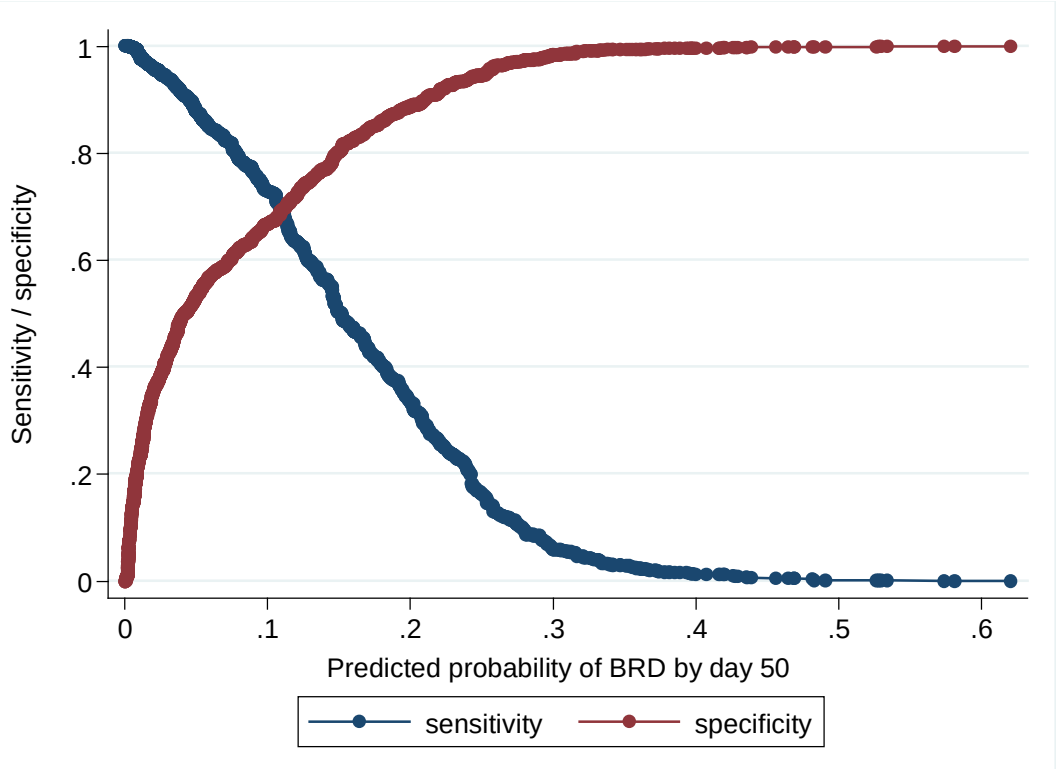

Figure 8-6: Sensitivity and specificity for fixed effects predicted probability from final parsimonious model including interaction terms

Table 8-3: Areas under the ROC curves for validation subsets used to assess the predictive capability for BRD occurrence of the final parsimonious model by feedlot. Separate models were run for each probability decile and group-13 within each validation subset

Feedlot	No. animals in validation subset	Crude 50-day BRD cumulative incidence (%)	Predicted probability decile based on fixed effects only		Group-13 averaged predicted probability based on fixed effects only		Predicted probability decile based on fixed and random effects		Hosmer Lemeshow Goodness of fit p-value (fixed and random effects)
			ROC area	95% CI	ROC area	95% CI	ROC area	95% CI	
A	308	42.0	0.70	(0.63 to 0.77)	0.73	(0.68 to 0.79)	0.81	(0.78 to 0.83)	0.05
B	2,628	9.5	0.75	(0.72 to 0.79)	0.76	(0.72 to 0.79)	0.85	(0.84 to 0.87)	0.76
C	262	2.4	0.70	(0.64 to 0.76)	*		0.89	(0.84 to 1.00)	1.00
D	3,000	22.8	0.61	(0.59 to 0.64)	0.62	(0.59 to 0.64)	0.77	(0.76 to 0.78)	0.67
E	1,072	3.7	0.78	(0.70 to 0.87)	0.75	(0.66 to 0.83)	0.88	(0.86 to 0.91)	0.47
F	232	3.0	0.63	(0.47 to 0.79)	0.66	[#]	0.69	(0.62 to 0.75)	0.85
G	1,478	24.6	0.59	(0.55 to 0.62)	0.58	(0.55 to 0.61)	0.74	(0.73 to 0.76)	0.64
H	1,478	3.2	0.70	(0.64 to 0.76)	0.70	(0.65 to 0.76)	0.87	(0.84 to 0.89)	0.90
I	1,277	4.5	0.69	(0.63 to 0.76)	0.65	(0.58 to 0.72)	0.84	(0.82 to 0.87)	0.18
J	2,749	44.9	0.60	(0.58 to 0.62)	0.59	(0.57 to 0.62)	0.71	(0.70 to 0.72)	<0.001
K	758	22.1	0.59	(0.54 to 0.64)	0.59	(0.53 to 0.64)	0.79	(0.77 to 0.81)	<0.001
L	249	0.8	*		0.33[#]	(0.11 to 0.54)	*		
M	961	0.1	*		*		*		
N	836	6.6	0.64	(0.55 to 0.74)	0.64	(0.55 to 0.74)	0.71	(0.67 to 0.75)	0.65

* ROC fit model did not converge; [#]aberrant result

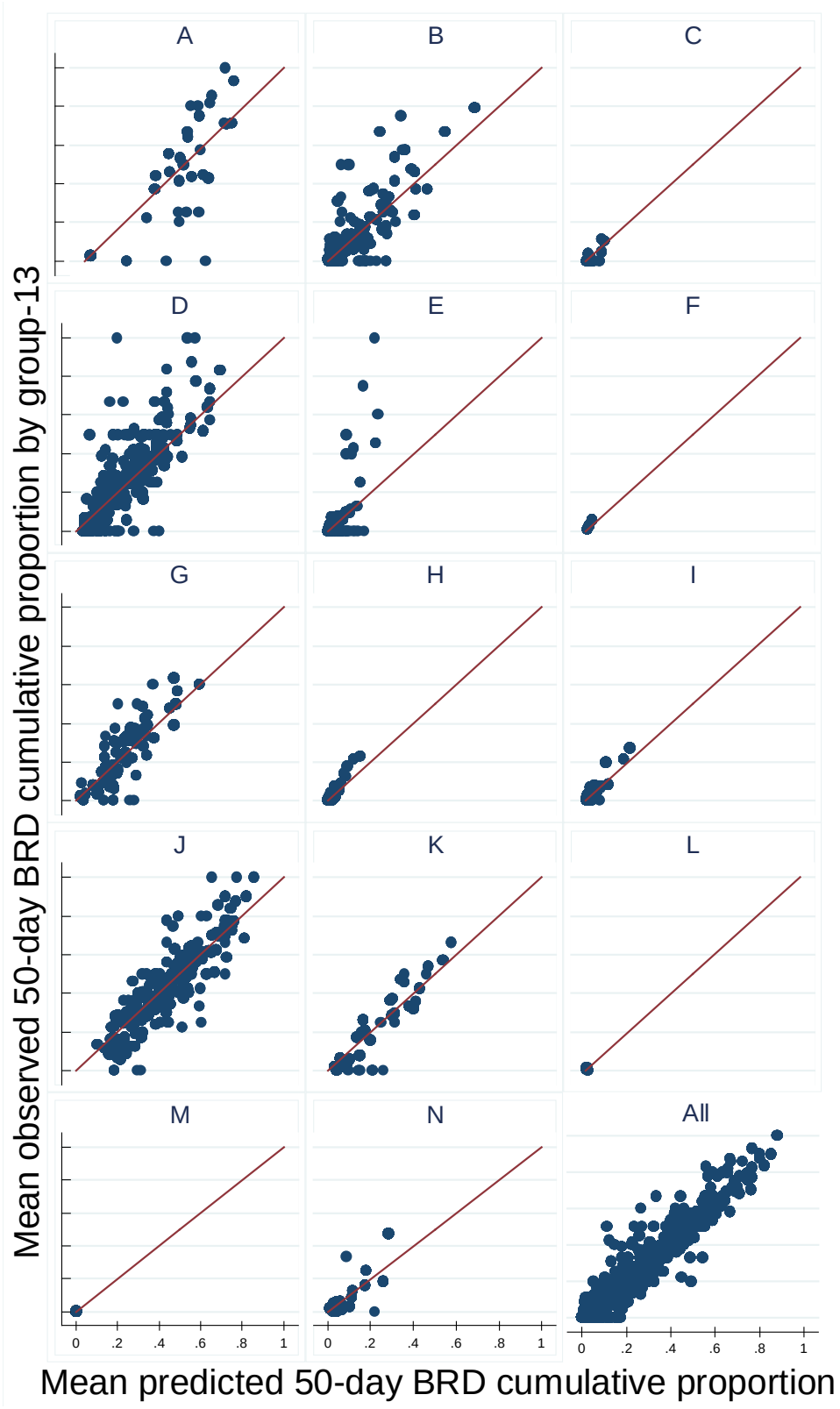

Figure 8-7: Scatter plots of mean observed 50-day cumulative incidences of BRD (y axes) versus mean predicted probabilities (x axes) for each validation subset for each feedlot (labelled A to N) and for the main cohort study population (All) based on predictions from the final parsimonious model

8.5 Discussion

The null models indicated that the majority of the variability in BRD occurrence was at the feedlot and animal levels. For both analyses using all 14 feedlots or the 13 feedlots, the percentages of unexplained variance that was at the animal level increased in the final models over the null models because the total model variance was reduced and animal-level variance was defined as a constant value. Even though no feedlot-level variables were fitted in the models, many explanatory factors at lower levels (animal, group and cohort) tended to cluster at higher levels. So, fixed effects at these levels would have contributed to the substantial reduction in the proportion of unexplained variance that was at the feedlot level. For example, exposures that affect the mixing, movement and grouping of animals around the time of induction are largely determined by feedlot management decisions. To some extent, management decisions also influence the type of cattle (breed, weight and sex) entering the feedlot, although this will also be determined by market factors.

Knowledge of the partitioning of variance is important because it means that research into management strategies at the feedlot level, including strategies influencing lower level factors that cluster by feedlot, would be expected to be most effective at identifying approaches that collectively cause largest reductions in BRD incidence at the population level. Therefore, future research efforts that aim to collect cohort-level and feedlot-level data for a large number of feedlots would be expected to be worthwhile. This research could focus on existing cohort-level database records, requiring minimal input from participating feedlots that already have these records. The challenges would be to ensure sufficient power at the feedlot level, and to measure all important feedlot-level confounders. In this study, I attempted to investigate many of these factors, but limited power and clustering of cohort-level variables have contributed to inconclusive findings.

The variance explained by the parsimonious set of predictors was estimated at around 14% for the complete cohort dataset or 16.5% for the subset excluding the outlier feedlot. Thus, only a modest amount of the variability in BRD incidence was explained by these risk factors.

Analyses of the areas under the ROC curves indicated that overall, the final parsimonious model inclusive of random effects displayed good discriminatory ability

while predictive ability based on fixed effects only was acceptable. When examined by feedlot, discriminatory ability varied. For feedlots a) that contributing sufficient animals to the study such that the validation subset comprised more than about 300 animals and b) that had a BRD cumulative incidence above 1%, ROC analyses revealed that predictive ability was fair to acceptable. The numbers of animals per probability decile was balanced whereas the numbers of animals per group-13 varied and hence were less consistent than those obtained from the probability deciles. Estimates based on very small group-13s or on a limited number of group-13s or in feedlots with a very low overall BRD incidence (<1%), ROC analyses was not useful

The model had reasonable fit when the mean predicted probability by group-13 was assessed against observed values for each validation subset. Because the group-13 most closely approximates the arrival group (i.e. the unit likely to be of most interest to feedlot managers), predictive ability based on this was of interest. My results could be used to develop a tool to predict risk of BRD by arrival group.

8.6 Conclusions

About half of the variability in BRD incidence occurred at the feedlot level. The final parsimonious set of predictors consisted of animal-entry characteristics (breed, induction weight, sex), management factors (shared pen water, BVDV activity in the cohort, mixing history, feedlot move timing, number of animals in group-13, interval between induction and cohort closure) and environmental factors (season of induction). With the exception of feedlots with very low overall BRD incidences, the final parsimonious model had fair to acceptable discriminatory ability in predicting BRD risk for arrival groups, with better predictive capacity for higher risk groups. When random effects were included in the predictive model, predictive ability was acceptable to good across the majority of feedlots. Knowledge of these risk factors could therefore be a useful tool for industry in assessing BRD risk in arrival groups and hence in informing management decisions based on the assessed risk.

The majority of unexplained variance remaining in the final parsimonious model was at the feedlot and animal levels. Therefore further research directed at the feedlot level (including lower level factors clustered at the feedlot level) would be expected to be of most benefit. Existing cohort-level database records could be utilised for this research.

9 Model Comparisons

9.1 Introduction and aims

As described in Section 4.7, a causal diagram was constructed based on *a priori* postulated causal pathways between the exposure variables and the outcome (BRD50) and between exposure variables. This was used to inform model selection to estimate total and direct effects of exposures. For important risk factors identified from these analyses, population-level estimates were then derived. It was sometimes necessary to refit models to obtain estimates relative to the lowest-risk reference category to estimate these effects. Equivalent models were constructed using two software packages (MLwiN® and WinBUGs®). The primary purpose of also estimating these effects with the WinBUGs® software was to obtain associated measures of uncertainty (95% credible intervals).

In Chapter 8, the partitioning of variance at different levels of the study population hierarchy (i.e. feedlot, cohort, group-13 and animal) was described in both a null and a model containing a parsimonious set of exposure variables, including two interaction terms. This model was then evaluated as a predictive model by assessing model fit and discriminatory ability. While the primary purposes of building the parsimonious model were to evaluate the partitioning of variance and produce a predictive model, it is informative to compare estimates of effect obtained using different methodologies and different software packages.

The aims of analyses described in this chapter were to compare total and direct effect estimates of odds ratios, and the PAFs and PARs derived from these with the estimates obtained from the main-effects parsimonious model (i.e. without interaction terms). A second aim was to compare estimates from equivalent models fitted using different software packages (i.e. MLwiN and WinBUGs). Using two separate software packages to estimate odds ratios served two purposes. Firstly, it enabled a comparison of odds ratio estimates between packages; comparison of estimates for complex multilevel analyses with the WinBUGs® estimates are recommended by the MLwiN® software developers (Browne, 2012). Secondly, by programming nodes within the WinBUGs® package it was possible to obtain estimates of uncertainty associated with the estimated population-level effects. .

9.2 Methods

The methods used to derive the total and direct effect estimates were described in Chapter 6 and the methods used to derive the main effects parsimonious model were described in Chapter 8. This modelling was performed using MCMC estimation methods in the MLwiN® (version 2.27) software package run within the Stata® (version 12) program. For variables of interest, population-level estimates of total and relevant direct effects were obtained as described in Chapter 7. Because the population-level estimates require the reference category to be the lowest-risk category, it was sometimes necessary to refit models. Equivalent models were fitted using WinBUGs® software. As part of this process, odds ratios, PAFs and PARs were estimated.

All risk factors that remained in the parsimonious model described in Chapter 8 met the inclusion criteria (based on total effects) for the estimation of PAFs and PARs described in Chapter 7. Only these risk factors are discussed in the current chapter.

To obtain estimates of the odds ratios for the main-effects parsimonious model (i.e. without interaction terms) derived in Chapter 8, this model was refitted in MLwiN® using the lowest-risk reference categories for each variable. To obtain estimates of the PAFs and PARs from this parsimonious model, the formulae described in Section 7.2.2 were applied within a Microsoft Excel® spreadsheet.

To obtain estimates of the odds ratios, PAFs and PARs derived from the parsimonious model using the WinBUGs® software, the equivalent model was fitted. Methods were as described for obtaining estimates of total and direct effects using this package (Section 6.2.3) (i.e. a separate model building process was not used in WinBUGs®). The final main-effects parsimonious model fitted using the lowest-risk reference category for each variable was run for 200,000 iterations after a burn in of 1,000. Orthogonalisation and hierarchical centring at level 3 were applied in the parsimonious model and in all total or direct effects models containing cohort-level variables.

9.3 Results

9.3.1 Animal entry characteristics and environmental factors

Table 9-1 provides odds ratios estimates for the effects of animal-entry characteristics and induction season on BRD from different modelling methods and software packages. Table 9-2 provides the population-level effect estimates for these risk factors. Because the population-level effect estimates are derived from the odds ratio estimates, comparisons of effect estimates are generally consistent across odds ratios, PAFs and PARS. The effect estimates for breed derived for the total effects and parsimonious models were consistent between software packages, and estimates from the parsimonious models were only slightly attenuated compared to the total effects models. The estimated total effects of sex were consistent between software packages. Estimates derived from the parsimonious models were consistent with the total effects, with all indicating that males were at increased risk compared to females, although some 95% credible intervals included unity.

The effect estimates for season of induction derived for the total effects and parsimonious models were also consistent between software packages, and estimates from the parsimonious models were consistent with those obtained from the total effects models.

9.3.2 Management related risk factors

Table 9-3 provides odds ratio estimates for the effects of management-related risk factors on BRD for different modelling methods and software packages. Table 9-4 provides the population-level effect estimates for these risk factors. Estimated total effects derived from MLwiN® and WinBUGS® were consistent and indicated that there was a strong effect of shared pen water on the risk of BRD although the 95% credible intervals were wide. The estimates derived from both software packages for the parsimonious model were consistent and although somewhat attenuated compared to the total effects, a strong effect remained after adjusting for the other variables in the model. Similarly, the PAFs and PARs were consistent between software packages, indicating a very large population-level effect.

Compared to animals moved to the vicinity of the feedlot prior to day -27, total effects estimates indicated all other categories were at markedly increased risk, although

the estimates for some categories varied somewhat between MLwiN® and WinBUGs® models. Parsimonious model estimates were consistent between software packages, but 95% credible intervals were wide and sometimes included unity. Direct effects for categories moved to the feedlot between day -12 and day 0 were attenuated compared to total effects and more consistent with those obtained from the parsimonious models. The PAFs and PARs derived from the parsimonious models were less than those derived from the direct effects models which in turn were less than those derived from the total effects models. The population-level effect estimates obtained from the parsimonious models and from the direct effects models were very imprecise and the effect estimates varied somewhat between software.

The lowest-risk reference category for the mixing history variable consisted of animals that were mixed prior to day -27 and joined cohorts formed by 2 or 3 group-13s. Estimates of effect were consistent across software packages and modelling methods, although estimates from WinBUGs were slightly lower than those obtained from MLwiN. The estimates of the PAFs and PARs for the direct effects were slightly lower and less precise than the total effects estimates; these were consistent with the parsimonious model estimates.

Animals from smaller group-13s were at increased risk compared to animals from group-13s with 100 or more animals with consistent estimates between software. The parsimonious models showed slightly attenuated effects compared to the total effects models; these estimates were consistent with the direct effects.

Effect estimates for the presence of BVDV in the cohort were consistent between software packages indicating increased risk, with slightly attenuated effect estimates from the parsimonious models and for direct effect estimates.

The lowest-risk reference category for the number of days from induction until cohort close consisted of animals that joined the cohort 7 or more days prior to cohort close. Effects estimates were similar across software packages. The PAFs and PARs for direct effects were slightly lower than those derived from the total effects. The estimates derived from the parsimonious model were slightly higher than those obtained from the total effects models.

The effect estimates for saleyard exposure between day -12 and day 0 were very similar between software packages. Estimates from the parsimonious models were attenuated compared to the total effects estimates and consistent with the direct effect estimates.

Table 9-1: Estimated odds ratios (OR) for BRD and 95% credible intervals (Cred Int) derived from total effects and parsimonious models for important animal-entry characteristics associated with 50-day cumulative BRD incidence

	Total effects				Parsimonious model			
Risk factor	MLwiN		WinBUGs		MLwiN		WinBUGs	
& category	OR	95%Cred Int	OR	95%Cred Int	OR	95%Cred Int	OR	95%Cred Int
Animal								
Breed								
Angus	2.2	(1.5 to 3.2)	2.5	(1.7 to 3.5)	2.0	(1.3 to 2.8)	2.0	(1.4 to 2.8)
British cross	2.7	(1.8 to 3.9)	2.9	(2.0 to 4.2)	2.3	(1.5 to 3.2)	2.3	(1.6 to 3.3)
Hereford	4.3	(2.8 to 6.3)	4.7	(3.1 to 7.0)	3.8	(2.5 to 5.5)	3.8	(2.5 to 5.5)
Shorthorn	2.7	(1.7 to 4.1)	3.0	(1.8 to 4.5)	2.2	(1.4 to 3.3)	2.2	(1.4 to 3.3)
Murray Grey	1.2	(0.7 to 1.9)	1.3	(0.7 to 2.1)	1.0	(0.6 to 1.7)	1.0	(0.6 to 1.7)
European/X	1.8	(1.1 to 2.9)	2.0	(1.2 to 3.1)	1.7	(1.0 to 2.7)	1.7	(1.0 to 2.7)
Tropical/X	Ref		Ref		Ref		Ref	
Sex								
Female	Ref		Ref		Ref		Ref	
Male	1.5	(1.0 to 2.6)	1.6	(0.9 to 2.6)	1.5	(0.9 to 2.3)	1.6	(1.0 to 2.5)
Weight (kg)								
<400	1.7	(1.4 to 1.9)	1.6	(1.3 to 1.9)	1.6	(1.3 to 1.8)	1.6	(1.3 to 1.9)
400 to <440	1.3	(1.2 to 1.5)	1.4	(1.2 to 1.5)	1.3	(1.2 to 1.5)	1.3	(1.2 to 1.5)
440 to <480	1.1	(1.0 to 1.3)	1.2	(1.0 to 1.3)	1.2	(1.0 to 1.3)	1.2	(1.0 to 1.3)
≥480	Ref		Ref		Ref		Ref	
Environment								
Season								
Spring	Ref		Ref		Ref		Ref	
Summer	2.4	(1.4 to 3.8)	2.4	(1.3 to 3.9)	2.1	(1.3 to 3.3)	2.1	(1.3 to 3.3)
Autumn	2.1	(1.2 to 3.2)	2.0	(1.2 to 3.3)	2.4	(1.5 to 3.6)	2.4	(1.5 to 3.6)
Winter	1.6	(1.0 to 2.3)	1.6	(1.0 to 2.3)	1.5	(1.0 to 2.2)	1.5	(1.0 to 2.2)

Table 9-2: Estimated population attributable fraction (PAF) and population attributable risk (PAR) of animal entry and environmental risk factors on the 50-day cumulative incidence of BRD derived from total effects and parsimonious models fitted within the MLwiN® and WinBUGs® (with 95% credible intervals) software

	Total effects			Parsimonious model		
Risk factor	MLwiN	WinBUGs		MLwiN	WinBUGs	
	PAF	PAF	95%Cred Int	PAF	PAF	95%Cred Int
Breed	0.56	0.67	(0.54 to 0.77)	0.50	0.48	(0.29 to 0.64)
Sex	0.31	0.36	(0.00 to 0.59)	0.30	0.31	(-0.03 to 0.55)
Weight	0.16	0.16	(0.09 to 0.23)	0.17	0.17	(0.10 to 0.24)
Season	0.30	0.28	(0.12 to 0.40)	0.30	0.30	(0.18 to 0.40)
	PAR	PAR	95%Cred Int	PAR	PAR	95%Cred Int
Breed	9.8	11.8	(9.6 to 13.5)	8.8	8.6	(5.2 to 11.3)
Sex	5.4	6.3	(-0.1 to 10.5)	5.2	5.0	(-1.0 to 9.5)
Weight	2.7	2.9	(1.6 to 4.1)	3.1	3.0	(1.7 to 4.2)
Season	5.3	5.0	(2.2 to 7.0)	5.4	5.3	(3.3 to 7.0)

Table 9-3: Estimated odds ratios (OR) for BRD and 95% credible intervals (CI) derived from total effects, direct effects and parsimonious models from the MLwiN® and WinBUGs software for management-related risk factors

	MLwiN total effects		WinBUGs total effects		MLwiN direct effects		WinBUGs direct effects		MLwiN parsimonious model		WinBUGs parsimonious model	
Variable & category	OR	95%Cred Int	OR	95%Cred Int	OR	95%Cred Int	OR	95%Cred Int	OR	95%Cred Int	OR	95%Cred Int
Shared pen water												
No	Ref		Ref		Ref		Ref		Ref		Ref	
Yes	4.3	(1.4 to 10.3)	5.0	(2.0 to 10.7)	3.1	(1.0 to 7.7)	3.7	(1.5 to 8.9)	2.5	(1.1 to 5.8)	2.9	(1.1 to 6.3)
Feedlot move timing												
Pre day -27	Ref		Ref		Ref		Ref		Ref		Ref	
Day -27 to -13	2.6	(1.4 to 4.5)	1.6	(1.2 to 2.1)	2.6	(1.3 to 4.7)	2.4	(1.1 to 4.4)	2.6	(1.4 to 4.6)	2.7	(1.4 to 4.7)
Day -12 to -2, <6hrs	2.5	(1.1 to 5.2)	2.9	(1.4 to 5.9)	2.1	(0.9 to 5.0)	1.9	(0.7 to 4.5)	1.7	(0.8 to 3.4)	1.9	(0.7 to 3.9)
Day -12 to -2, ≥6hrs	2.6	(1.0 to 5.5)	2.7	(1.2 to 5.7)	2.2	(0.9 to 5.3)	1.9	(0.6 to 4.5)	1.9	(0.8 to 3.9)	2.1	(0.8 to 4.6)
Day -1 to 0, <6hrs	3.0	(1.3 to 6.4)	3.8	(1.8 to 7.8)	2.4	(1.1 to 5.5)	2.4	(0.8 to 5.7)	1.8	(0.9 to 3.8)	2.0	(0.8 to 4.4)
Day -1 to 0, ≥6hrs	3.7	(1.6 to 8.1)	4.6	(2.2 to 9.5)	2.9	(1.3 to 6.8)	2.9	(1.0 to 6.9)	2.2	(1.0 to 4.6)	2.4	(0.9 to 5.3)
Mixing history												
No, no, no	2.4	(0.4 to 7.8)	2.2	(0.4 to 6.8)	2.9	(0.5 to 9.6)	2.8	(0.5 to 8.8)	1.6	(0.3 to 4.6)	1.6	(0.4 to 4.7)
No, no, 2–3	2.3	(1.3 to 3.7)	2.3	(1.3 to 3.7)	2.2	(1.3 to 3.7)	2.2	(1.2 to 3.7)	2.2	(1.3 to 3.6)	2.2	(1.3 to 3.6)
No, no, 4–9	3.6	(1.8 to 6.1)	3.8	(1.8 to 7.0)	3.1	(1.5 to 5.6)	3.0	(1.1 to 5.8)	3.1	(1.7 to 5.3)	3.2	(1.8 to 5.5)
No, no, ≥10	3.5	(1.8 to 6.2)	4.0	(1.9 to 7.7)	3.0	(1.3 to 5.7)	2.9	(1.1 to 6.1)	2.8	(1.5 to 4.9)	2.8	(1.5 to 4.8)
No, yes, yes	3.2	(1.4 to 6.2)	3.4	(1.4 to 7.1)	2.8	(1.1 to 5.9)	2.8	(0.9 to 6.3)	2.8	(1.3 to 5.4)	2.8	(1.3 to 5.4)
No, yes, no	2.2	(0.5 to 6.7)	2.1	(0.4 to 6.1)	2.3	(0.5 to 7.0)	2.1	(0.4 to 6.3)	1.8	(0.4 to 5.1)	1.9	(0.4 to 5.0)
Yes, no, 2–3	Ref		Ref		Ref		Ref		Ref		Ref	
Yes, no, 4–9	2.7	(1.3 to 4.6)	2.8	(1.4 to 5.2)	2.3	(1.1 to 4.2)	2.2	(0.9 to 4.4)	2.3	(1.3 to 3.8)	2.3	(1.3 to 3.9)
Yes, no, ≥10	2.1	(1.1 to 3.7)	2.4	(1.1 to 4.5)	1.8	(0.8 to 3.5)	1.8	(0.6 to 3.6)	1.7	(0.9 to 3.0)	1.7	(0.9 to 2.9)
Yes, yes, yes	2.1	(0.9 to 3.9)	2.2	(0.9 to 4.5)	1.8	(0.7 to 3.7)	1.7	(0.6 to 3.8)	1.6	(0.8 to 3.1)	1.7	(0.8 to 3.1)
Yes, yes, no^	2.5	(0.7 to 6.5)	2.2	(0.6 to 5.9)	2.5	(0.7 to 6.4)	2.2	(0.6 to 5.7)	2.1	(0.7 to 5.1)	2.2	(0.7 to 5.4)
Yes, no, no	1.1	(0.5 to 2.4)	1.1	(0.4 to 2.3)	1.1	(0.4 to 2.3)	1.0	(0.4 to 2.2)	0.9	(0.4 to 1.9)	1.0	(0.4 to 2.0)
Number of animals in group-13												
<50	2.0	(1.5 to 2.8)	2.3	(1.6 to 3.2)	1.7	(1.2 to 2.4)	1.8	(1.2 to 2.4)	1.7	(1.2 to 2.4)	1.7	(1.2 to 2.3)
50-99	1.6	(1.1 to 2.2)	1.8	(1.2 to 2.5)	1.4	(1.0 to 2.0)	1.4	(1.0 to 2.0)	1.4	(1.0 to 2.0)	1.4	(1.0 to 1.9)
≥100	Ref		Ref		Ref		Ref		Ref		Ref	
BVDV in cohort												
No	Ref		Ref						Ref		Ref	
Yes	1.7	(1.1 to 2.5)	1.7	(1.1 to 2.6)					1.6	(1.1 to 2.3)	1.6	(1.0 to 2.3)
Days from induction to cohort close												
0	1.4	(1.1 to 1.9)	1.5	(1.1 to 1.9)	1.4	(0.9 to 2.0)	1.3	(0.9 to 1.9)	1.6	(1.2 to 2.1)	1.6	(1.2 to 2.1)
1 to 6	1.2	(0.9 to 1.5)	1.2	(0.9 to 1.6)	1.1	(0.8 to 1.5)	1.2	(1.0 to 1.4)	1.2	(0.9 to 1.5)	1.2	(0.9 to 1.5)
≥7	Ref		Ref		Ref		Ref		Ref		Ref	
Saleyard days -12 to 0												
No	Ref		Ref		Ref		Ref		Ref		Ref	
Yes	2.6	(1.6 to 4.1)	2.6	(1.5 to 4.1)	1.6	(0.9 to 2.6)	1.6	(0.9 to 2.6)	1.7	(1.0 to 2.8)	1.7	(1.0 to 2.7)

Table 9-4: Estimated population attributable fraction (PAF) and population attributable risk (PAR) of management-related risk factors on the 50-day cumulative incidence of BRD derived from total effects, direct effects and parsimonious models for important feedlot management BRD risk factors

	Total effects			Direct effects			Parsimonious model		
	MLwiN	WinBUGs		MLwiN	WinBUGs		MLwiN	WinBUGs	
	PAF	PAF	95%Cred Int	PAF	PAF	95%Cred Int	PAF	PAF	95%Cred Int
Shared pen water	0.71	0.70	(0.45 to 0.83)	0.63	0.62	(0.29 to 0.82)	0.52	0.52	(0.11 to 0.78)
Feedlot move timing	0.69	0.75	(0.57 to 0.88)	0.55	0.48	(-0.14 to 0.81)	0.47	0.43	(-0.22 to 0.76)
Mixing history	0.58	0.54	(0.23 to 0.72)	0.52	0.46	(0.17 to 0.69)	0.50	0.46	(0.20 to 0.65)
Number of animals in group-13	0.37	0.39	(0.23 to 0.51)	0.30	0.30	(0.10 to 0.44)	0.31	0.29	(0.11 to 0.43)
BVDV in cohort	0.32	0.30	(0.04 to 0.50)	0.32	0.30	(0.04 to 0.50)	0.28	0.26	(0.03 to 0.44)
Days from induction to cohort close	0.16	0.16	(-0.01 to 0.31)	0.14	0.13	(-0.03 to 0.32)	0.20	0.19	(0.04 to 0.32)
Saleyard day-12 to day 0	0.02	0.02	(0.02 to 0.02)	0.01	0.00	(0.00 to 0.01)	0.01	0.01	(0.00 to 0.02)
	PAR	PAR	95%Cred Int	PAR	PAR	95%Cred Int	PAR	PAR	95%Cred Int
Shared pen water	12.5	12.3	(7.9 to 14.7)				9.1	8.7	(1.5 to 14.1)
Feedlot move timing	12.1	13.3	(10.1 to 15.5)	9.7	8.5	(-2.4 to 14.4)	8.3	8.3	(-0.1 to 13.5)
Mixing history	10.2	9.5	(4.0 to 12.7)	9.1	8.2	(2.9 to 12.1)	8.7	9.2	(5.3 to 12.0)
Number of animals in group-13	6.5	6.9	(4.1 to 9.1)	5.6	5.2	(1.8 to 7.8)	5.5	5.2	(1.8 to 7.7)
BVDV in cohort	5.6	5.3	(0.7 to 8.9)	5.6	5.3	(0.7 to 8.9)	4.9	4.6	(0.5 to 7.7)
Days from induction to cohort close	2.8	2.8	(-0.1 to 5.4)	2.5	2.2	(- 0.5 to 5.6)	3.5	3.2	(0.7 to 5.5)
Saleyard day-12 to day 0	0.3	0.3	(0.3 to 0.3)	0.1	0.1	(0.0 to 0.2)	0.2	0.2	(0.0 to 0.3)

9.4 Discussion

In addition to enabling the partitioning of variance at different hierarchical levels, the formulation and estimation of a parsimonious multivariable model provides estimates of the strength of associations between the fixed effects and the outcome after controlling for the other variables in the model. These models may sometimes be useful as predictive models provided the discriminatory ability of the model is adequate. The utility of identifying a group of risk factors in the same model is appealing when trying to model a complex process with its associated uncertainty. When a large number of exposure variables are of interest, an automated model-building approach, or a variation thereof such as that used to construct the main-effects parsimonious model reported here, can allow analyses to be completed in a

much shorter time than that required to construct individual models to estimate total and direct effects for each variable of interest.

Variables included in parsimonious models are often considered to be the 'most important' variables, but with automated model building methods, there is no reason to think this is the case. The question of how to define importance was addressed in Chapter 7 where I evaluated the results obtained from estimating the population-level effects of risk factors of interest derived from total and direct effects models and applied additional criteria to produce a ranked list of those risk factors with sufficient evidence of effect to warrant intervention, further research or selective management strategies. It is useful to compare this list and the associated conclusions with what would have been obtained if only a modified version of an automated model-building process had been applied.

Depending on which other variables are in a parsimonious model, the estimates may include some but not all indirect pathways (and hence reflect neither the total nor the direct effects) and may be biased due to failure to adequately control for confounders. Importantly, confounding may actually be caused by the fitting additional variables because of conditional association bias as described in Chapter 6. Effect estimates will be subject to omitted variable bias if confounders are not included in the model. Nevertheless, effect estimates from models with parsimonious sets of risk factors selected using automated variable selection methods are commonly reported as 'the effects'. From the population-level effect estimates from the parsimonious model presented in this chapter, it was clear that some effects were most consistent with total effects (sex, weight, season), some were more consistent with direct effects (mixing history, number of animals in group-13, saleyard exposure between days -12 and 0), some were lower than both total and direct effect estimates (shared pen water, feedlot move timing), some were lower than the total effect estimates but direct effects were not obtained (breed, BVDV in the cohort) and for one variable, estimates were higher than the total effect estimates (days from induction to cohort close). Although identified for future research, this latter variable was not included in the final ranking of risk factors presented in Table 7-4 for reasons described in Section 7.4.5. The different estimates observed for models fitted to estimate total and direct effects of variables were consistent with expectations given the proposed pathways depicted in the

causal diagrams; comparison of these effects provided information about the causal pathways.

All of the risk factors remaining in the parsimonious model were identified as being associated with BRD in the total and direct effects modelling. The majority of these were also included in Table 7-4 after evaluating other criteria. Variables that were only evaluated in subset analyses were not included in the parsimonious model-building process, so prior vaccination with BovilisMH™ or Pestigard™, and yard weaning were ineligible for inclusion.

The absence of particular risk factors from a parsimonious model does not mean their effect is not 'important'. The only other variable listed in Table 7-4 but not retained in the parsimonious model was feedlot region, which may be because some of the other variables in the parsimonious model explained some of the effect of feedlot region. However, the p–value was very close to the cut-point used of 0.10, and with the limitations of analysing a feedlot-level variable with a dataset containing only 14 feedlots, and because feedlot region is likely to be a proxy for other factors, further investigation is required. Because feedlots were clustered within feedlot regions and the partitioning of variance indicated the importance of the feedlot level of the hierarchy, the population-level effects of feedlot region added little additional information beyond that obtained from the parsimonious model.

Several variables described in Chapter 6 had important total effects but the direct effect estimates were much attenuated, indicating that these were largely mediated through indirect pathways. As expected, these variables did not remain in the parsimonious model. The direct effects modelling had determined that most of the effects of these variables were mediated through indirect pathways. Because these intervening variables (e.g. mixing history) were in the parsimonious model, and there was no direct effect, it would be expected that they would drop out during the parsimonious modelling process. However, the estimation and comparison of total and direct effects and the population-level effects derived from them allowed a more informed analyses which provided insights into causal pathways that would not have been possible from only fitting a parsimonious model. Variables that may have been of particular interest to industry were examined in this way. In particular, the effects of exposure to saleyards at varying times prior to day 0 have been investigated, and

the important role of mixing as a key contributor to the total effects of saleyard exposure has been established. Total and direct effects modelling also provides more insight where apparent inconsistencies occur (e.g. the effects of the animal-level time between day 0 and cohort close and the cohort-level cohort close pattern discussed in Chapter 6). This information is important in directing further research and highlights the importance of considering atomistic and ecological fallacies when determining interventions based on results of modelling complex hierarchical datasets.

Building separate models for numerous risk factors is time consuming and potentially subject to error if the causal diagram is not correct, so a careful and informed consideration of pathways is required. If unmeasured confounders are associated with two or more variables in the diagram, conditional association will be introduced but not controlled because the pathways are unknown. However, in not using a causal-diagram informed approach, the selection of variables is more dependent on chance (Shrier and Platt, 2008).

The estimates of effect for equivalent models were generally consistent between models fitted using MLwiN® and WinBUGs®. However, some differences in effect estimates between packages were noted, especially for higher level variables, or variables with unbalanced distributions across feedlots. MLwiN® estimates generally displayed higher autocorrelation, so for cohort-level variables or variables clustered by feedlot, more accurate effect estimates might be expected from WinBUGS® models (Browne, 2012).

9.5 Conclusions

It was reassuring that the important risk factors identified in the parsimonious model were generally consistent with those identified in Chapter 6. However, a comparison of effect estimates obtained from a parsimonious model with those obtained by total and direct effects modelling served to illustrate how effect estimates derived from parsimonious models may be equivalent to total effects, direct effects or neither for different variables in the same model. While the utility of building a single parsimonious model is appealing, in doing this it is important to recognise limitations.

The approach of using a causal diagram to specifically determine total and direct effects enabled more detailed analysis and comparison of risk factors than would have been possible if only a parsimonious model had been built. As would be expected, not all variables with important total effects remained in the parsimonious model, so where total effects or direct effects are of particular interest, it is necessary to consider causal pathways and build appropriate models based on these. This information provided by causal diagrams about postulated interrelationships between particular risk factors and BRD is useful in promoting a better understanding of complex and seemingly conflicting results, and in adding to the body of evidence informing management interventions likely to reduce BRD incidence or directing future research needs.

The use of a causal-diagram informed approach to model building with a large complex dataset is time consuming. Perhaps a practical compromise would be to construct a causal diagram and identify particular variables (and hence pathways) of *a priori* interest. These variables could then be analysed along with those identified in a parsimonious model to determine and compare total and direct effects. In this way, due consideration is given to causal pathways and research questions of interest along with the need to complete analyses in a timely and cost-effective way.

10 Descriptive Epidemiology of Respiratory Pathogens in Cattle in Australian Feedlots

10.1 Introduction and aims

As detailed in the literature review (Chapter 1), several viral and bacterial respiratory pathogens have been implicated in BRD in feedlot cattle. Some of the initial evidence for the role of particular pathogens (both bacteria and viruses) comes from their detection in necropsy samples from animals that died from BRD (e.g. in lung or tracheal tissue samples). The detection of viruses in nasal swab samples in animals diagnosed with BRD also provides some evidence for a role for particular viruses. Serological studies (usually using a case-control study design) also can provide important evidence; the nested case-control study will be described in Chapter 10. The most consistent evidence from the literature indicates an important role for BVDV and BoHV-1 in the occurrence of BRD. BRSV, BPI3 and BCoV have also been implicated in some populations.

BVDV-1 is the only genotype that has been identified in Australia with the majority of isolates being subtyped as BVDV-1c (Mahony et al., 2005). Hence, 'BVDV' in the context of this thesis refers to BVDV-1. The most important source of BVDV infection in feedlot cattle is thought to be through exposure to persistently infected (PI) animals. As discussed in Section 1.4.3.1, differentiating PI animals from transiently infected (TI) animals presents diagnostic challenges. It has been suggested that because concentrations of virus would be expected to be lower with transient infection than persistent infection, qPCR protocols may be useful in distinguishing PI from TI animals (Lanyon et al., 2014). Hence, a comparison of results obtained from PI and TI animals is presented. Previously isolated Australian strains of BVDV are different from those found internationally. Thus, three of the aims addressed in this chapter were to:

- describe the distribution of PI animals upon arrival at Australian feedlots at the animal, group and cohort levels
- evaluate whether a single qPCR test is useful in differentiating TI animals from PI animals

- isolate and genotype BVDV 1 detected in necropsy and hospital samples

This chapter also presents the descriptive results derived from laboratory testing of biological samples obtained from animals in the cohort study population. This addresses the following research aims:

- to determine which viruses were present in hospital nasal swabs collected at first diagnosis over time and across cohorts
- to determine the presence of selected pathogens in necropsy samples over time and across feedlots
- to isolate and genotype BoHV-1 detected in necropsy and hospital samples

The results obtained from testing hospital nasal swabs collected at first diagnosis from study animals are presented. The results from tests performed on tissue samples collected at necropsy from animals suspecting of having died from BRD (i.e. lung or tracheal samples) are also described.

10.2 Methods

10.2.1 Samples

Blood samples were collected from all study animals at induction and again at follow-up, which was scheduled approximately 42 days after induction. Nasal swabs were collected from all animals at induction. Blood samples and nasal swabs were requested from all animals that were hospitalised due to respiratory signs and necropsy samples (lung and tracheal tissue) were requested from any animals suspected of having died of BRD.

10.2.2 Diagnostic tests for BVDV

To determine the BVDV status of each cohort (i.e. BVDV detected in any cohort animal or the presence of a PI animal in the cohort), testing was performed in a number of stages; animals to be tested at each stage depended on the results of the previous stage of testing.

10.2.2.1 Quantitative real time-PCR

When possible, repeated qPCR assays were used to identify PI animals. This assay has excellent analytical sensitivity and specificity for BVDV with Ct values related to

the amount of viral RNA present (Bhudevi and Weinstock, 2001). Because the expected prevalence of PI animals was low, it was important to minimise misclassification of TI animals as PI animals. BVDV RNA detected in qPCRs may indicate either transient or persistent infection, although the Ct values would generally be expected to be lower for PI animals and repeated testing of TIs would be expected to yield negative follow-up test results. Although the majority of TI animals would be expected to test negative after about two weeks - in one study, the duration of positive qPCR status in TI animals ranged from one to nine days (Nickell et al., 2011) - this period may be extended. Repeated testing with a minimum interval of four weeks is recommended to differentiate PI animals from TI animals (Lanyon et al., 2014).

Assays used to perform qPCRs were developed by the QAAFI laboratory (Horwood and Mahony, 2011). A Ct of 0.05 was used and samples with qPCR values below 35 were categorised as ‘positive’, and samples with values between 35 and 40 were considered borderline. In addition to the Ct value, the plot of the fluorescent signal against cycle number was assessed (for consistency with the expected sigmoid shape of a positive test) when designating statuses for samples with borderline values.

10.2.2.2 Antibody ELISA

In immunologically normal unvaccinated animals, detection of serum IgG antibodies to BVDV indicates prior exposure to BVDV. Antibody ELISA tests have high sensitivity (up to 99%) and specificity (up to 98%) (Lanyon et al., 2014). Acutely infected animals generally become seropositive within two to three weeks of infection and rising antibody levels suggest infection within the previous 10-12 weeks (Lanyon et al., 2014).

At the herd level, a high animal-level prevalence of seropositivity to BVDV indicates previous or current infection, and hence that it is likely that these herds have been exposed to a PI animal. Conversely, a low prevalence of seropositivity in a herd suggests that most animals are susceptible to infection and prior exposure to PI animals is unlikely (Lanyon et al., 2014).

Because PI animals are immunotolerant to BVDV, they do not develop antibodies to BVDV, and would therefore be expected to test negative for BVDV antibodies

(Lanyon et al., 2014). Thus, PI animals would be expected to return a negative ELISA test to BVDV antibodies, while in-contact animals from the same group-28 would be expected to test seropositive at induction (i.e. assuming they were exposed to the PI animal previously when they were together at the same location on or prior to day -28). Animals in a group-28 not previously exposed to BVDV would be expected to be seronegative or show antibody titres following more recent exposure after day -28 (e.g. if exposed during preassembly).

10.2.3 Testing procedure and discriminatory ability of a single qPCR test in differentiating TI animals from PI animals

10.2.3.1 Pooled qPCR testing

To reduce laboratory workload and to keep laboratory costs within budget, BVDV qPCR testing initially was performed using pooled serum samples, with sera from only selected individual animals subsequently tested. Pooled BVDV qPCR testing was performed on both induction and follow-up sera upon receipt at the laboratory. Up to 24 10µl aliquots of samples from animals in the same cohort and from the same time period (i.e. induction or follow-up) were pooled for these tests. Any positive or borderline value was considered a positive pool. All animals with a verified sample in a negative pool were classified as not being PI animals (i.e. negative) (Figure 10-1). Animals without a sample in a negative pool were designated ‘suspect PIs’ and identified for animal-level testing.

10.2.3.2 PI diagnostic criteria

For the reasons described above, to identify PI animals, I aimed to use repeated animal-level qPCR tests with a sampling interval (i.e. between sample collections) of at least 28 days. Induction samples were considered the primary (i.e. first and most informative) sample because a positive qPCR at induction was less likely to be due to transient infection than samples taken after some time on feed (after which animals were more likely to have been exposed to BVDV at the feedlot and have transient infection). Follow-up or hospital samples were used as the second sample. If, at the time qPCR data were collated, an animal already had a result from qPCR testing of a hospital swab (as described in Section10.3) and the interval from induction to the date this sample was taken was at least 28 days, this was used as the second test. If at least two serum samples had been collected (but not already tested), the induction and follow-up sample were used preferentially for diagnosis of

PI animals because these samples typically had a sampling interval of about 42 days.

However, for suspect PI animals where paired samples were not available for testing (i.e. one or both samples were unsuitable for testing), combinations of additional information were considered as detailed below:

- The sampling interval was relaxed to 13 days while considering the Ct values (i.e. repeated test results with low Ct values were more likely to indicate PI animals).

- Antibody levels in suspect PIs and in common group-28 animals were considered (a PI animal would be expected to be seronegative; in-contact group-28 animals would be expected to be seropositive)

- Mixing history and timing of arrival at the feedlot were considered. Induction samples from animals in a stable group (no mixing within 3 months before arrival) that were sampled within a couple of days of arrival at the feedlot provided a more informed snapshot of the group status before arrival. However, positive follow-up samples and induction samples from feedlots which practiced preassembly were considered to be more likely from TI rather than PI animals.

- Statuses of other animals in contact with the qPCR positive animal were assessed. Because the expected prevalence of PI animals was very low and the occurrence of PI animals tends to cluster within herds (Loneragan et al., 2005), and because PI animals shed a large amount of virus, the PI status of animals without sufficient samples for a definitive result was sometimes allocated based on the results of common group-28 animals, provided the group-28 was sufficiently large (>14 animals). For example, consider an animal that was qPCR positive on a single induction sample test, but did not have a suitable sample for any further testing. The animal was part of a group-28 with a total of 30 animals, twelve of which were included in the case-control study. All of the common group-28 animals were seronegative at induction, but the majority seroincreased by follow-up. The pattern suggested these animals had not previously been exposed to a PI animal, so it was

concluded that the suspect PI was probably a TI animal. Because the prevalence of PI animals was probably very low, the probability of this animal being a PI animal was very low. In another example, a suspect PI from a group-28 in which three PI animals were identified was possibly also a PI animal and so was classified with a missing result for PI status.

10.2.3.3 Testing of individual samples

Induction serum samples from all animals with samples in a positive induction pool but without samples in a negative follow-up pool were identified for testing. Animals with a positive induction test or without an adequate induction serum sample for animal-level testing (e.g. original sample of low volume, or sample completely used in case-control testing), remained suspect PIs. The majority of these animals had induction nasal swabs that were then tested as an alternative in the absence of an induction serum sample.

The pooled test results and the animal-level induction test results were compiled and cross checked along with the test results from the hospital and post mortem samples (described in section 10.3 below). Any animal with a negative animal-level qPCR result (induction or hospital) was designated PI negative (Figure 10-1). If the sampling interval was at least 28 days and an animal had positive induction and hospital samples, it was diagnosed as a PI animal. For animals that remained suspect PIs, subsequent rounds of testing involved qPCR testing of follow-up or hospital samples, depending on the availability of samples and the sampling intervals, but paired results with shorter sampling intervals were considered alongside additional information as described above.

For animals unable to be classified using two qPCR tests (i.e. they remained suspect PI animals), serology results obtained from the ELISA tests performed in the case-control study were examined along with any results (ELISA and qPCR) for animals in the same group-28 as the suspect PI animal. If no ELISA test results were available and the suspect PI animal was part of a stable group with at least 15 animals, additional ELISA testing was performed using induction sera samples where possible. Suspect PI animals with a positive qPCR and a negative ELISA antibody test on the same sample (or in samples taken at the same time), with common group-28 animals with high seroprevalence (i.e. the majority of tested

animals were seropositive at induction) were classified as PI animals. Suspect PI animals that were seropositive at induction or follow-up were classified as non-PI animals (Figure 10-1).

PI statuses of animals without sufficient samples for a definitive classification, were determined based on patterns of ELISA and qPCR results in the animal's group-28 and cohort. If BVDV was not identified based on qPCR in any animal in the cohort, animals in that cohort without samples were classified as PI negative. Animals without animal-level test results but in group-28s of 15 or more animals where all other animals were not PI animals were classified as negative (Figure 10-1). Animals with a single positive qPCR, sampled within a few days of arrival at the feedlot and from stable group-28s with 15 or more animals where serology in common group-28 animals indicated transient infections occurred after induction, were classified as PI negative (Figure 10-1). Animals with a single positive qPCR result without an adequate sample for ELISA testing in group-28s where other PI animals had been identified or in group-28s comprising less than 15 animals remained suspect PIs and were classified as missing (Figure 10-1). If a suspect PI animal was the only possible PI animal in induction and follow-up pooled tests (i.e. all other animals in the pool tested negative) it was classified as a PI animal (Figure 10-1).

10.2.3.4 Cross checking

Analysis of pooled samples using qPCR has excellent analytical sensitivity for detecting both PI and TI animals (Lanyon et al., 2014). While the presence of a PI animal in a cohort would be expected to result in positive pools, it is possible that transient infection within a cohort was not detected in samples collected at just two time points (infection could occur and clear within the six-week sampling interval). Thus, most or all cohorts categorised as 'PI positive' based on qPCR testing would be expected to have PI animals present. However, it is possible that some cohorts with at least one TI animal but no PI animals were misclassified as 'negative' instead of 'transient infection'. To test the negative predictive value of this designation, the serology results from the case-control study and results of further induction nasal swab qPCR testing (as described in Section 10.4) were examined and cross-checked against the cohort-level BVDV status classification.

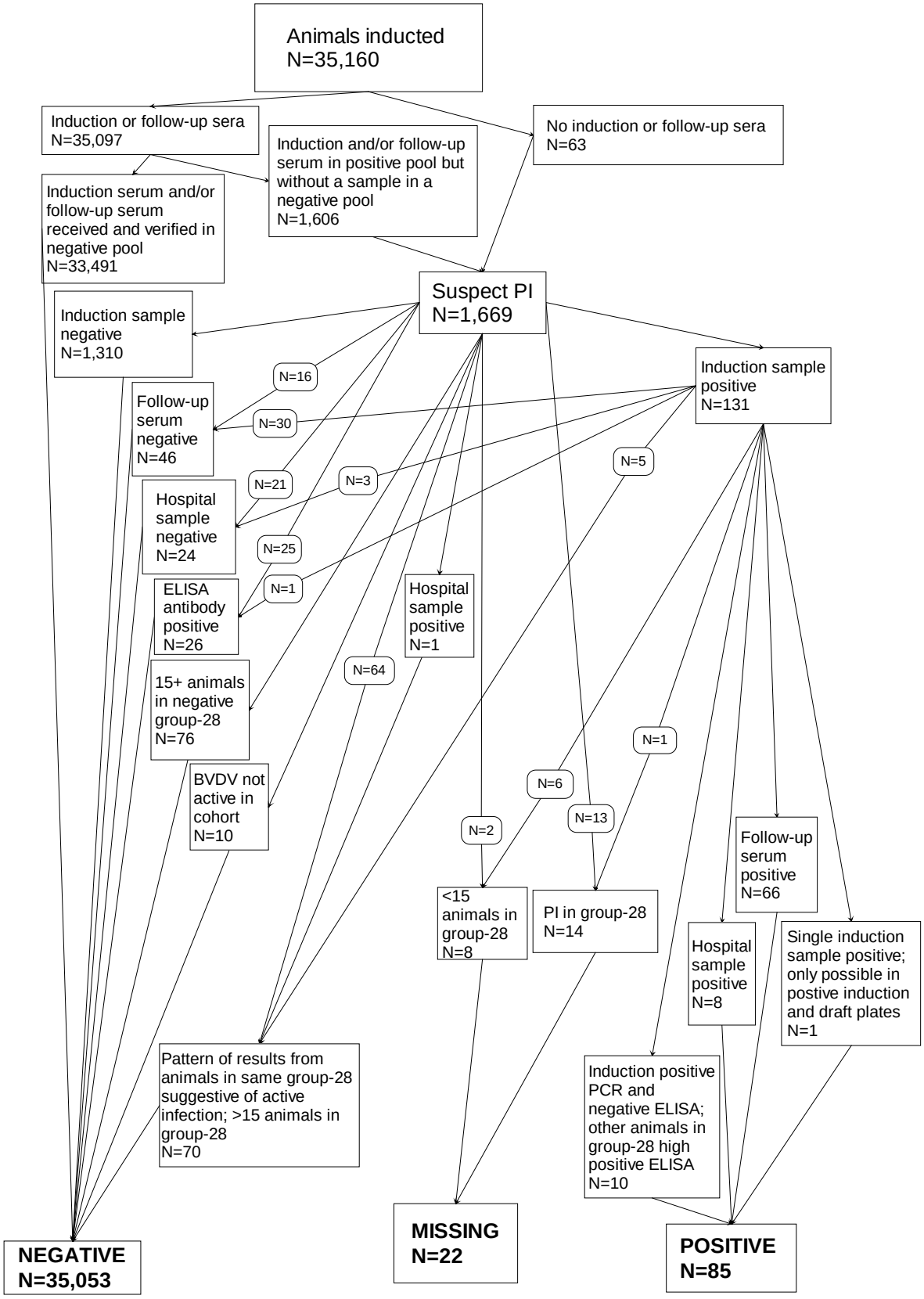

Figure 10-1: Flow chart depicting the determination of animal-level BVDV-PI status in the full cohort dataset

A single positive qPCR test may indicate transient rather than persistent infection and the time taken for this infection to clear may extend beyond the recommended testing interval of 28 days. Hence, the serological ELISA profiles of PI animals that were tested as part of the case-control study were examined and used to cross check the PI status designation. Inconsistencies were noted, but designation of PI status did not change because only a limited number of animals had serology results.

10.2.4 Results

10.2.4.1 PI diagnostic flowchart

A flowchart describing the diagnosis of PI animals is displayed in Figure 10-1. Of 35,160 animals inducted into study cohorts, 35,097 animals had at least one serum sample received and verified at the animal level from the induction or follow-up stage; 32,536 animals had both induction and follow-up samples received and verified.

Any animal with a serum sample received, verified and adequate (N=33,189) or received and verified (N=302) and in a negative pool was deemed negative (N=33,491). This included the 29 animals that were lost to follow-up or had zero time at risk. A total of 1,606 suspect PI animals had samples received and verified in a positive pool but did not have a sample in a negative pool. The 63 animals with neither induction nor follow-up serum samples were also suspect PI animals, and 40 of these had a single other sample (induction swab or hospital sample).

Of the 131 animals with a positive induction qPCR, 74 had a second positive qPCR test (66 follow-up and 8 hospital) while 33 returned negative follow-up (N=30) or hospital (N=3) sample qPCRs. Ten of the 131 animals were seronegative on ELISA testing; these were classified as PI animals. Common group-28 serological profiles (some common group animals highly seropositive) were also consistent with these animals being PI animals. One animal with a positive induction sample was classified as a PI animal because it was the only animal to contribute a sample to positive induction and follow-up plates that did not have a negative test result.

Fourteen suspect PIs from the same group-28 as identified PI animals had their PI status assigned as missing. Of these, two had no samples and 12 had contributed

aliquots to positive induction pools but did not have sufficient diagnostic samples to establish PI status (one had a single positive test). A further eight animals were assigned missing PI status because they were from small group-28s and had no samples (N=2) or had only a single positive test (N=6). Thus, 22 animals had a missing PI status, including seven with a single positive qPCR result.

10.2.4.2 Prevalences of PI animals

From the study population of 35,160 animals, 85 PI animals were identified, giving an animal-level prevalence of 0.24%. The 50-day cumulative incidence of BRD in PI animals was 27% (23/85), which was much higher than in the cohort study population. Of a total of 1,274 group-28s, 67 (5.3%) contained at least one PI animal; a single animal was identified in 55 group-28s, two in seven group-28s, three in four group-28s and four in one group-28. The PI animals were distributed among 54 (32%) of the 170 cohorts, from 12 of the 14 feedlots. Of the 54 cohorts with identified PI animals, a single PI animal was identified in 35 cohorts, two were identified in each of 10 cohorts, three were identified in each of six cohorts and four were identified in each of three cohorts. Fourteen animals classified as missing were in the same group-28 (10 separate group-28s) and cohort (nine cohorts) as identified PI animals. Thus, the prevalence of PI animals was probably underestimated, but the identification of group-28s that contained at least one PI animal was probably accurate. The remaining animals with missing PI status were probably not PI animals. Of these, four with a single positive qPCR result and two without any qPCR results (both in the same small group-28) were in cohorts in which PI animals had been identified, but not in groups where PI animals were identified. The remaining two animals had a single positive test and were in cohorts without an identified PI animal.

BVDV was also present in many cohorts in which no PI animals were identified. BVDV was detected in at least one animal from 47 such cohorts (47% of the 101 cohorts with any positive BVDV qPCR test and 28% of all 170 cohorts) Of study animals, 34% were in group-28s and cohorts that had no PI animals, 9% were in group-28s with an identified PI animal, 37% were in group-28s without a PI animal but in cohorts with an identified PI animal, and 20% of animals were in cohorts in which no PI animals were identified but BVDV was present.

10.2.4.3 Cross checking

Of the 85 PI animals, 31 had a least one ELISA antibody serology result (case-control protocol detailed in Section 11.2.4). The serological profile was as expected in 29 of these animals (i.e. seronegative). One animal was highly seropositive at induction (5) and remained so at follow-up (4). Another animal seroconverted between induction and follow up (i.e. moved from 0 to 5). Both of these animals were diagnosed as PI animals on the basis of paired positive qPCR tests; the intervals between testing were 55 days and 43 days respectively.

Induction nasal swabs from 1,276 animals from 9 cohorts were tested for the presence of BVDV as described in Section 10.4. Positive tests were returned from animals (N=11) from two cohorts. However, both of these cohorts had been designated BVDV negative on the basis of pooled testing of induction sera samples. No PI animals were identified in these cohorts so it is likely that the positive tests were due to transient infection and not PI animals, but these infections were not detected in the pooled testing. All other swab results were in agreement with the pooled qPCR test results.

A cross check of cohort-level seroincrease determined from ELISA testing against the qPCR testing used to designate BVDV status revealed that there was evidence of seroincrease to BVDV in many cohorts in which BVDV had not been identified in any qPCR analyses. Thus, for 51 of the 69 cohorts with negative induction and follow-up pooled qPCR tests in which BVDV was not identified in any study animal, seroincrease to BVDV between induction and follow-up occurred in at least one study animal. In three cohorts with an identified PI animal and one cohort with transient infection, all tested case-control animals were seropositive initially and no seroincrease occurred in case-control animals. In these four cohorts and the remaining 107 cohorts with serological results from case-control animals, serological results were consistent with those obtained from qPCR testing. Thus, in cohorts where BVDV was detected, at least some animals in the cohorts were initially seropositive or seroincreased, whereas in cohorts in which BVDV was not detected serostatus of animals tested in the case-control study did not change. Hence, serological change to BVDV was occurring in many cohorts classified as not infected and having no PI animals, so it was likely the proportion of cohorts that contained ‘transiently infected’ animals was underestimated. If seroincrease between induction

and follow up in any study animal was used to define BVDV activity (i.e. either PI or TI animal present), then BVDV was active in 142 of 161 (88%) cohorts.

10.2.5 Comparison of transiently and persistently infected animals

The distributions of animal-level qPCR results by stage of the test sample and over BVDV PI status are presented in the boxplots displayed in Figure 10-2. Figure 10-3 shows the paired test results for each PI animal (results from the same animal are vertically aligned). While not all transiently infected animals were identified, comparison of results from the 134 animals with a positive animal-level test with those obtained from PI animals is useful in assessing the discriminatory ability of a single qPCR test in differentiating PI animals from TI animals.

All 85 PI animals had a positive induction qPCR test, with a median Ct value of 29 (interquartile range: 28 to 31). With a median Ct value of 37 (interquartile range: 35 to 38), the qPCR values for TI animals were generally higher, but the range of values overlapped (25 to 37 for PI animals and 26 to 40 for TI animals). The induction Ct values for the seven animals with a missing PI status were intermediate.

The distributions of the 66 follow-up Ct values and 8 hospital Ct values for PI animals were similar to the distribution of induction values. Only a single TI animal had a positive qPCR follow-up sample. The Ct values measured on the 58 TI animals with qPCR positive hospital samples were generally higher (median: 34 interquartile range: 30 to 36) than those observed in PI animals, but the range of values extended from 21 to 39.

From these analyses, it was clear that, while the mean and interquartile range of qPCR values for the TI animals was higher than that for PI animals, there was considerable overlap in Ct values between TI and PI animals. Animals with transient infection often returned low Ct values, especially when samples were collected in the hospital crush when first diagnosed with BRD. Hence, the results from this study indicated that a single qPCR test was not useful in discriminating between PI animals and TI animals.

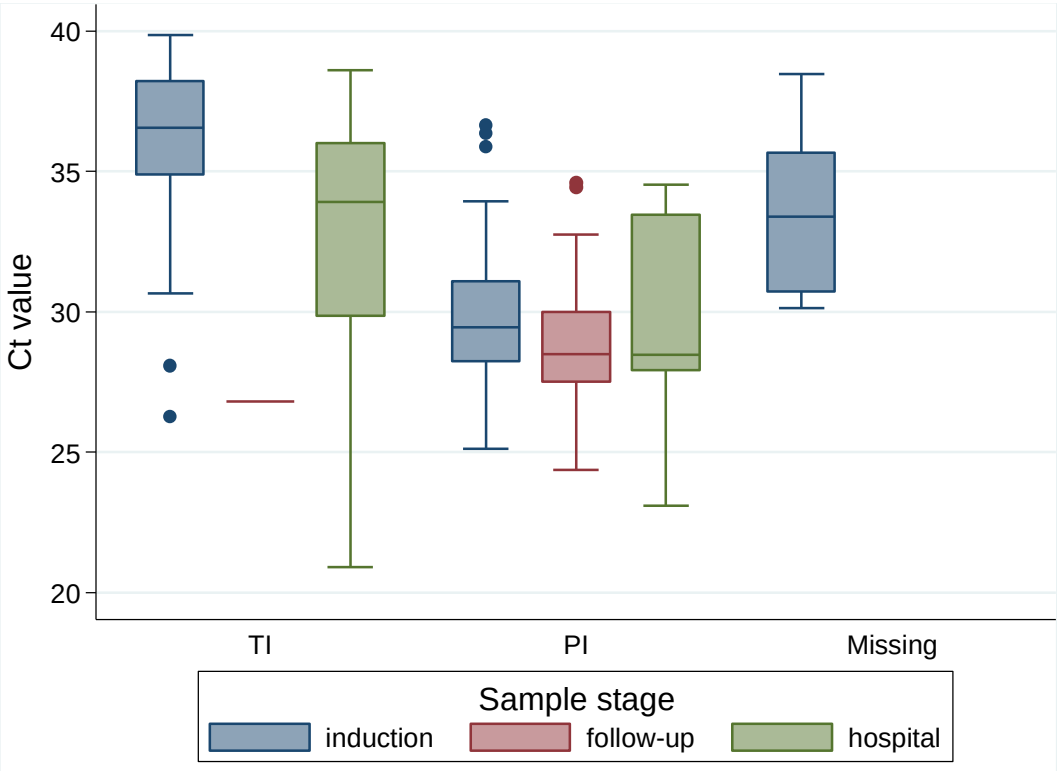

Figure 10-2: Boxplots displaying distributions of individual Ct test results stratified by sample stage and PI status

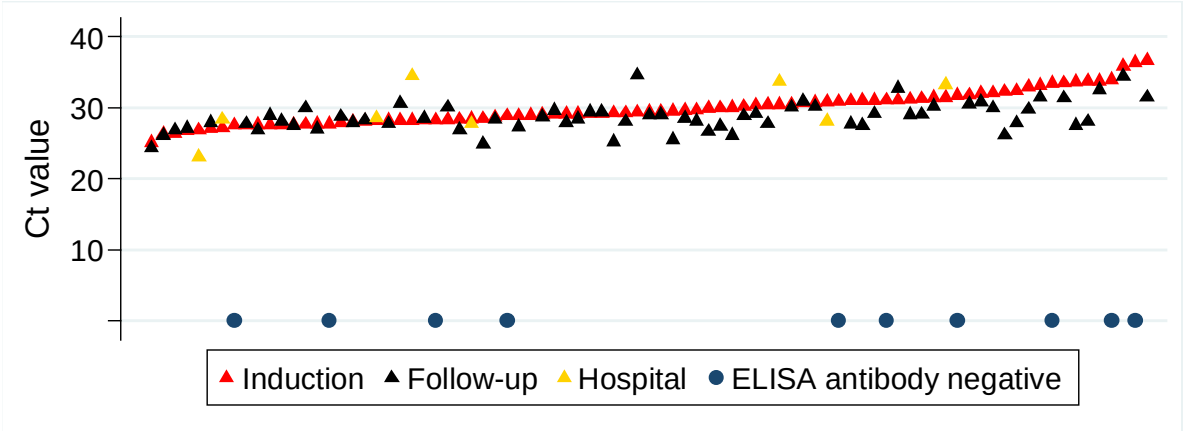

Figure 10-3: Individual test Ct values for 85 PI animals ranked by induction qPCR Ct value. Results from the same animal are vertically aligned.

10.2.6 BVDV genome sequencing

To determine which subgenotypes of BVDV were present in cohorts, partial sequencing of the BVDV 5´ untranslated region (5´-UTR) of the viral genome was performed as described by Mahony et al (2005). In initial testing at the time of pooled BVDV qPCR testing, five positive induction pools and 10 positive draft pools from a total of 13 cohorts were sequenced. In further testing aimed at sequencing samples

from a larger number of cohorts, including all identified PI animals, positive pools were identified for sequencing if a PI animal had contributed to the pool. Other positive pools with Ct values <35 were also identified for sequencing if a PI animal had been identified in the cohort but not in the pool. A total of 74 additional pools were identified for sequencing including 11 pools with Ct values <35 but without an identified PI animal in the pool. Where a PI animal contributed to both induction and follow-up pools, induction pools were used preferentially. Out of the 74 pools identified for sequencing many months after the initial pooled qPCR testing, sequencing was attempted in 62, but only successful in 19. This was probably due the sample being inadequate for further testing or deterioration in the sample over time. Because of the low success rate with these stored samples and because all isolates identified were of the same subgenotype, sequencing was not attempted on the remaining 12 pools. Hence, of the 34 isolates genotyped (15 at the time of initial pooled testing and 19 many months later) all isolates were identified as subtype BVDV-1c (data not shown). Sequenced isolates were from pools derived from 27 cohorts (all of which contained an identified PI animal) from 10 of the 14 participating feedlots. This was consistent with previous Australian BVDV genotyping results (Mahony et al., 2005).

10.3 Viruses detected in BRD cases

10.3.1 Aims

This research aimed to identify viruses in animals hospitalised with BRD, and to describe temporal patterns in frequency of detection of these viruses within cohorts and feedlots.

10.3.2 Nasal swabs from BRD cases

‘Hospital samples’ were collected from study animals as described in Section15.1; one swab was tested per animal. Nasal swabs were received from 4,242 animals, of which 4,086 samples were of adequate quality for testing and verified at the animal level as a first hospital sample collected from an animal meeting the BRD50 case definition. Thus 66% (4,086/6,200) of BRD50 cases had hospital samples suitable for testing. Initially, exploratory qPCR testing was performed whereby all hospital swabs submitted from 14 cohorts (N=568) from five feedlots were tested for the presence of five viruses.

Because a large number of hospital samples were submitted and the number of samples varied across feedlots and cohorts, testing of all submitted samples was not feasible or cost-effective. Hence, a process was subsequently developed to select samples for testing. Time in the cohort was measured from the same starting point (cohort close date) for all animals in the cohort and animals inducted before the cohort close date had negative values if they were first diagnosed with BRD before the cohort close date (but after their individual day 0). Time in the cohort was divided into 10 intervals as shown in Figure 10-4 with fewer days per interval in the peak BRD incidence period (between days 7 and 35). To be eligible for testing, animals needed to meet the BRD case definition when first pulled for BRD, and to have a verified nasal swab sample that was collected at the animal's first hospital examination. I aimed to select a maximum of two animals from each cohort from each time interval. Animals were sequentially selected until two were selected for each cohort-time period combination. If more than two animals were eligible within a step in the process, the animals were sorted by animal identification number and the first two were selected. This was only necessary occasionally. The steps were as follows:

1. Animals whose nasal swabs had already been tested; if more than two animals had already been tested, two were selected using the sequential steps below.

2. Animal had been selected as a case for serological testing in the case-control study

3. Animal was eligible for selection as a case in the case-control study (i.e. time in the cohort between 7 and 35 days and with paired induction and follow-up serum samples collected within a sampling interval of 60 days)

4. Animal was ineligible for the case-control study but had paired induction and follow-up serum samples (e.g. diagnosed outside of the time period of interest in the case-control study)

The rationale for including case-control selected animals first was that they would have additional information to compare with the hospital swab results (i.e. serology),

and similarly, case-control eligible animals were known to have paired sera samples should they be required.

10.3.3 Results

Nasal swabs from a total of 815 animals with BRD were analysed using qPCR analyses to detect the presence of selected viruses. Figure 10-4 shows the percentages of animals that tested positive for each virus within each time interval, Table 10-1 displays the distribution of animals that tested positive by feedlot, and Figure 10-5 shows the numbers of animals that were tested in each time interval. Very few animals were tested in the first time interval (N=3), while the numbers tested in other intervals ranged from 17 (in the interval from days 0 to 6) to 140 (days 21 to 25). BoHV-1 was the most common virus and was detected in 15% (N=124) of samples (Table 10-1). An initial peak (14% of tests positive between 7 to 10 days from cohort close) was followed by a larger subsequent peak between 21 and 50 days; the highest proportion of animals that tested positive (30%) was for those sampled between 31 and 35 days after the cohort close (Figure 10-4).

Bovine coronavirus (BCoV) was the second most commonly detected virus in 7% (N=57) of animals. The apparently high proportion of animals testing positive to BCoV in the first interval represents only one animal out of three tested (Figure 10-4); the proportions of animals that tested positive in other time intervals ranged from 3% to 10%.

BRSV was detected in 4.5% (N=37) of animals, BVDV was detected in 3% (N=28) of animals and only a single sample (0.1% of animals) tested positive for BPI3 (Table 10-1). The proportion of animals that were BRSV positive was greater for animals diagnosed early during the time on feed (peaking at 13% between days 7 to 10 from cohort close; with fewer positive samples in animals diagnosed later (Figure 10-4). The distribution of proportions of animals that were positive to BVDV was flatter across time, ranging from 0% (during the first two intervals) to 5% (between days 16 to 20 from cohort close) of animals tested (Figure 10-4).

From the distribution of animals tested by feedlot, it was clear that a small number of feedlots contributed the majority of samples from hospitalised animals (Table 10-1). These were the feedlots with the highest BRD incidences. Of the 815 animals tested, 27% were from a single feedlot and 75% were from four of the fourteen feedlots. Of

those animals tested, 590 (72%) returned negative results for all five viruses, 25% were positive to a single virus, 2% were positive to two viruses and less than 1% (N=3) were positive to three viruses.

For the five feedlots which contributed more than 50 animals and for three of the five feedlots that contributed between 20 and 50 animals, the distributions of viruses detected across feedlots was generally similar. No viruses were detected in any samples from two feedlots (N=4).

BoHV-1 and BCoV were each detected in samples from 10 of the 14 feedlots, while BVDV was detected in samples from nine feedlots and BRSV was detected in eight feedlots. Of the 124 animals with positive tests to BoHV-1, 10 were also positive to other agents (three each were positive to BCoV and BVDV, two were positive to BRSV and two were positive to BRSV and BCoV). Of the 28 animals testing positive to BVDV, three also tested positive to BoHV-1, two also tested positive to BCoV and one tested positive to BRSV and one was positive to BRSV and BPI3 (Table 10-1)

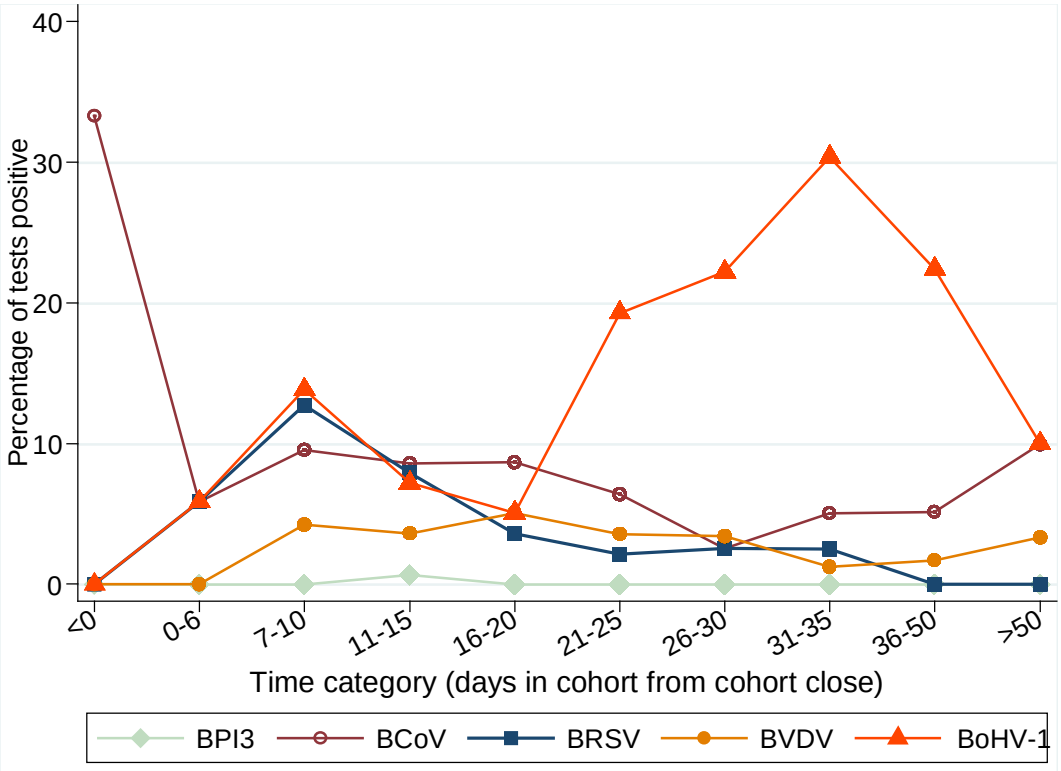

Figure 10-4: Percentages of animals with BRD whose hospital nasal swab tested positive to the viruses indicated, by time from cohort close date to hospital sampling date; total testing is indicated in Figure 10-5

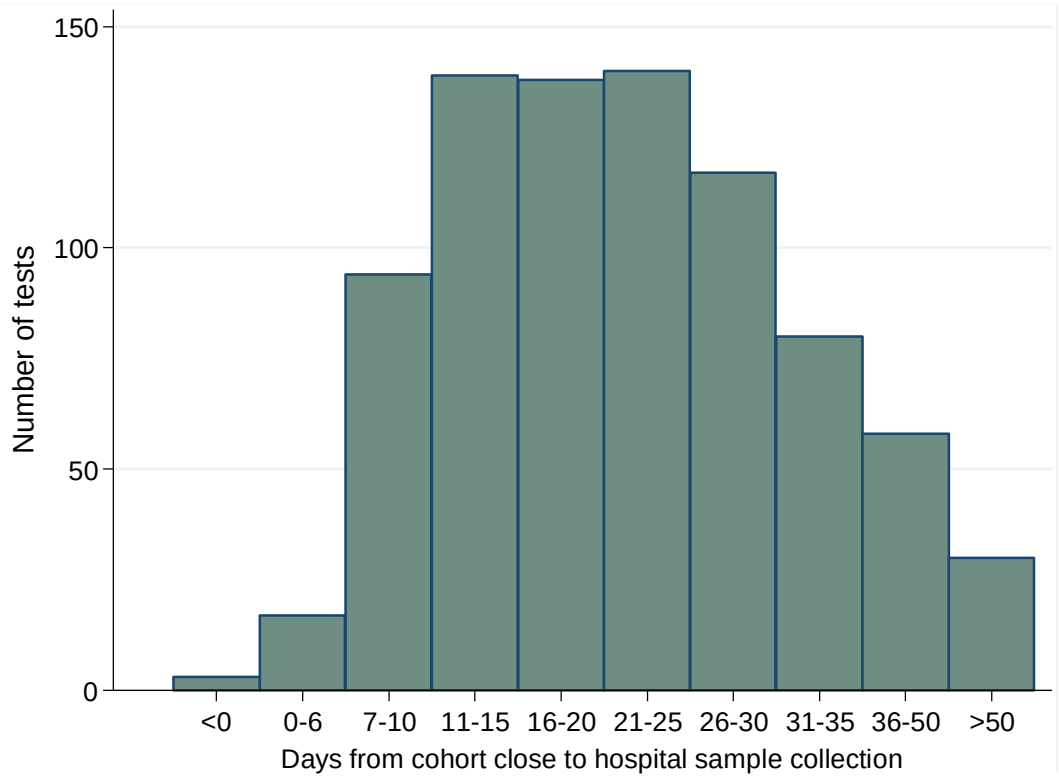

Figure 10-5: Frequency histogram showing number of animal's swabs collected during each time interval that were included in hospital swab qPCR testing for five viruses (BoHV-1, BVDV, BCoV, BRSV and BPI3).

Table 10-1: Distribution of animals with BRD and positive hospital nasal swab test results, and total number of animals with BRD tested by feedlot

Feedlot	BVDV	BoHV-1	BCoV	BRSV	BPI3	Total tested
A	0	5	1	1	0	24
B	3	26	3	4	0	89
C	0	1	0	0	0	2
D	6	23	12	15	0	223
E	1	0	13	1	0	29
F	2	5	0	0	0	11
G	4	28	7	8	0	109
H	0	0	2	0	0	24
I	1	3	1	2	0	25
J	9	20	16	4	1	187
K	1	4	1	2	0	58
L	0	0	0	0	0	3
M	0	0	0	0	0	1
N	1	9	1	0	0	30
Total positive (%)	28 (3.4%)	124 (15.2%)	57 (7.0%)	37 (4.5%)	1 (0.1%)	815

10.4 Virus detection in other samples

To establish whether virus detection would be most appropriate in induction or nasal swabs, exploratory qPCR testing was performed on a number of samples. Initially pools containing material from four swabs (either induction or hospital) from animals in the same cohort were tested using a multiplex assay to detect BoHV-1, BPI3 or BRSV, BCoV and BVDV. However, BVDV results were inconsistent so testing for BVDV was later repeated if sufficient samples were available. Samples in positive pools were tested individually; samples positive for BRSV or BPI3 were tested to distinguish between these viruses.

10.4.1 Other hospital swabs tested

As described above, hospital nasal swabs from 568 animals from 14 cohorts in five feedlots (i.e. all submitted hospital swabs from these cohorts) were tested prior to the application of formal selection criteria. One swab was tested per animal. Of these, 128 were included in the formal hospital swab analyses described above. The distributions of positive tests in the remaining 440 tested animals were compared to those observed in the tested animals included across the whole population; the proportions of positive tests were similar.

10.4.2 Virus detection in induction swabs

To determine which viruses were present in animals at induction into study cohorts, multiplex qPCR analyses were run on pooled nasal swab samples. Material from induction swabs from animals (one swab per animal) from the same cohort was pooled in groups of four. A total of 1,994 induction swabs from animals with suitable samples from 13 cohorts within six feedlots were tested in this way. Table 10-2 displays results of these tests by cohort along with the number of hospital swabs tested from animals in these cohorts with an indication of which viruses were detected in which cohorts (*). For the majority of cohorts, pooled nasal swabs collected at induction tested negative. Samples in positive pools were then tested individually to determine which animals were positive. Of the 50 positive tests, 42 (84%) were from animals from the two cohorts from preassembly feedlots (i.e. animals were assembled on pasture close to the vicinity of the feedlot close prior to induction). Excluding these cohorts, animals positive to BVDV, BoHV1 and BRSV were each identified in only one of the 11 cohorts that were not preassembled, while

BCoV was identified in two. Of the seven cohorts that were not preassembled and with at least five tested hospital nasal swabs, viruses not present at induction were detected in hospital swabs from animals from all seven cohorts.

Table 10-2: Number of animals with positive induction nasal swab qPCR results for the viruses indicated, total induction nasal swabs tested and number of corresponding hospital nasal swabs tested from the cohort indicated.

Cohort	Total animals with hospital nasal swab results	Total induction swabs tested	BVDV	BoHV-1	BCoV	BRSV	BPI3
0218	10	350		*	*	*	
0219	0	79		1	2		
0420	15	224		*	*	2*	
0422	15	112				*	
0421	13	49	*			*	
0719^	1	86			1		
0720	1	90					
0721^	5	10		*		*	
0819^	1	153		1	11	7	
0820^	5	145	8	1	11*	1	1
0913	0	192	3				
1017^	16	355	*	*	*		
1018	15	140		*			
Total	97	1985	11/1276^	3/1985	25/1985	10/1985	1/1985

For each virus, numbers indicate the number of positive induction nasal swab test results,
^ indicates that not all induction samples were tested for BVDV because initial multiplex results were unreliable and there were not sufficient samples for separate BVDV assays,
* indicates that the virus was later detected in at least one hospital nasal swab

10.5 Post mortem samples

10.5.1 Pathogen detection in necropsy samples

Patterns of BRD mortality in the study population were described in Section 5.6. The mortality risk in study animals during the time on feed attributed to BRD was 0.7%. Of animals meeting the BRD case definition, the case fatality risk was 3.5%. For animals with a cause of death reported following a post mortem examination, respiratory disease was implicated in 72% of deaths. Lung and trachea samples were submitted from animals that were necropsied following death from suspected BRD. Testing with qPCR was used to detect the presence of 4 viral and 4 bacterial agents in extracts from these tissues (BoHV-1, BVDV, BRSV, BCoV, *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Mycoplasma bovis)*. These

agents were chosen because they are commonly identified in BRD necropsy samples.

Samples from a total of all 126 animals suspected of having died of BRD (and meeting the definition of a BRD death described in Section 5.6) were tested as shown in Table 10-3. If multiple tissue samples were received from the same animal, pooled testing was used. The most common viruses detected were BoHV-1 and BVDV, in 58 and 39 animals, respectively. The most common bacterium isolated was *M. bovis*, from 121 animals.

Table 10-3: Distribution of viruses and bacteria detected in necropsy samples of animals dying of BRD

No. agents	No. animals	BoHV-1	BVDV	BRSV	BCoV	M. bovis	H. somni	P. mult	M. haem
7	1	1	1	1	0	1	1	1	1
6	2	1	1	1	0	1	1	0	1
6	2	1	0	1	0	1	1	1	1
6	3	0	1	1	0	1	1	1	1
6	8	1	1	0	0	1	1	1	1
5	3	1	1	0	0	1	1	1	0
5	1	1	1	0	0	1	1	0	1
5	1	1	1	0	0	1	0	1	1
4	1	1	0	0	1	1	0	1	0
5	14	1	0	0	0	1	1	1	1
4	12	1	0	0	0	1	1	1	0
4	4	1	0	0	0	1	1	0	1
4	1	1	0	0	0	1	0	1	1
3	1	1	0	0	0	1	0	1	0
3	1	1	0	0	0	1	0	0	1
2	5	1	0	0	0	1	0	0	0
2	1	1	0	0	0	0	1	0	0
5	10	0	1	0	0	1	1	1	1
5	4	0	1	0	0	1	1	0	0
4	3	0	1	0	0	1	0	1	1
4	2	0	1	0	0	1	1	1	0
4	1	0	1	0	0	1	1	0	1
4	1	0	0	0	1	1	1	0	1
5	1	0	0	0	1	1	1	1	1
3	1	0	0	1	0	1	1	0	0
4	10	0	0	0	0	1	1	1	1
3	8	0	0	0	0	1	1	0	1
3	6	0	0	0	0	1	1	1	0
2	7	0	0	0	0	1	1	0	0
3	1	0	0	0	0	1	0	1	1
2	1	0	0	0	0	1	0	0	1
2	1	0	0	0	0	1	0	1	0
1	4	0	0	0	0	1	0	0	0
2	2	0	0	0	0	0	1	0	1
1	1	0	0	0	0	0	0	1	0
0	1	0	0	0	0	0	0	0	0
	126	58	39	9	3	121	104	82	76

Agents: BoHV-1, BVDV, BRSV, BCoV, *Mycoplasma bovis, Histophilus somni, Pasteurella multocida, Mannheimia haemolytica,*

10.6 BoHV-1 genome sequencing

10.6.1 Nasal swabs from hospital animals

Of the 147 animals in which BoHV-1 was detected in hospital nasal swabs (124 from Section 10.3.3 plus 23 from Section 10.4.1), 98% (144/147) had received a modified live BoHV-1 vaccination (Rhinogard®) at induction. It was therefore of interest to determine whether the virus detected in hospital swab samples was the vaccine strain or field strains of BoHV-1. The vaccine strain is a modified live variant of BoHV-1, identifiable by the loss of a cytosine residue within the thymidine kinase gene that results in the loss of kinase activity. To differentiate the vaccine from field strains a selected region of this gene was amplified using conventional PCR. Sequencing of these PCR amplicons can facilitate strain differentiation through either the presence (field) or absence (vaccine) of the cytosine residue. Ninety six of the 147 positive samples from a range of cohorts and feedlots were identified for possible sequencing. If more than one animal from the same cohort had a BoHV-1 positive sample from the same time interval, only one sample was selected for sequencing. Sequencing was attempted on 79 of the samples from vaccinated animals that had tested positive using qPCR analyses several months earlier. Twenty eight samples returned negative PCRs on repeat testing, probably because the sample was inadequate for repeat testing due to degradation. Of those returning repeated positive PCRs and sequence results, none were vaccine strain; 50/51were field virus strains and 1 was a false positive (not BoHV-1). Because none of the tested samples were vaccine strain, sequencing was not attempted on the remaining 17 identified samples.

10.6.2 Necropsy samples

Of the 58 animals in which BoHV-1 was detected in necropsy samples, 56 had received a modified live BoHV-1 vaccination (Rhinogard®) at induction. It was therefore of interest to determine whether the virus in necropsy samples was a vaccine strain or a field strain. Twenty five of the pooled tissue samples from vaccinated animals that initially tested positive to BoHV-1 on qPCR were selected for repeat PCR and sequencing analyses of the thymidine kinase gene. These were selected to cover a range of times to death (from 18 to 67 days from day 0) and from as many feedlots and cohorts as possible. If more than one animal in a cohort had a

necropsy sample, the animal with the shortest interval between BRD diagnosis and death was selected. Of the 25 animals selected, BoHV-1 typing revealed a field strain in 21, the sample from one animal tested negative on repeat PCR and samples from three animals were inadequate for further testing. Thus, the BoHV-1 vaccine strain was not identified in any of the necropsy samples.

10.7 Conclusions

The prevalence of PI animals entering feedlots in the study population was estimated at 0.24%. It was estimated that PI animals were present in about 5% of groups defined at day -28 and about 32% of study cohorts. BVDV was present (i.e. PI and/or TI animals) in 59% of cohorts based on positive qPCR tests. However, seroincrease in at least one study animal occurred in 88% of cohorts with case-control results (161 of 170 cohorts had at least one animal included in the case-control study). Although the PI animals returned lower average Ct results on qPCR testing, there was considerable overlap in Ct values between PI and TI animals; the Ct value was not useful in discriminating between them. Genotyping was performed on 34 isolates; all were identified as subtype BVDV-1c.

BoHV-1 was the most commonly detected virus in hospital swabs (15%), BCoV, BRSV and BVDV were detected in 3 to 7% of cases, while BPI3 was rarely detected. Very few viruses were detected in induction swabs from feedlots where animals were not preassembled. BoHV-1 and BVDV were the most common viruses detected in post mortem samples. Viral genome sequencing determined that BoHV-1 detected in all post mortem samples were field strains. Although BCoV was a commonly identified virus in hospital nasal swab samples, detection in necropsy samples was rare (three positive samples). Bovine coronavirus has only recently been implicated in BRD in Australian feedlot cattle (Hick et al., 2012). Hence, serological testing for BCoV was not included in the NBRDI protocol for the case-control study reported in Chapter 11. Results reported in the current chapter indicate that BCoV was commonly circulating in Australian feedlot cattle. The presence of BCoV in necropsy samples provides evidence that this virus was sometimes implicated in BRD mortalities. Hence, further research is warranted to determine the seroprevalence of BCoV in cattle entering Australian feedlots and to assess associations between infection with BCoV and the occurrence of BRD in Australian feedlot cattle.

The role of viruses in the occurrence of BRD will be discussed further in Chapter 11 after the presentation of results from the nested case-control study.

11 A Case-control Study to Assess the Roles of Four Viruses in the Aetiology of BRD

11.1 Introduction

As discussed in the literature review (Chapter 1), particular viruses have frequently been implicated in the pathogenesis of BRD. A nested case-control study investigating serological risk factors for BRD was performed in a subset of the full cohort study population. Paired serum samples were used to determine serological status to four viruses at induction into the feedlot and at follow-up (approximately day 42). The aims of the case-control study were to describe serostatuses at induction to Bovine herpesvirus 1 (BoHV-1), Bovine viral diarrhoea virus (BVDV), Bovine parainfluenza virus type 3 (BPI3) and Bovine respiratory syncytial virus (BRSV), and changes in serostatuses over time, and to examine associations between serological statuses and occurrence of BRD.

11.2 Methods

11.2.1 Study design and eligibility

A retrospective nested case-control study was conducted with cases and controls selected from animals enrolled in the main cohort study. As described in Section 4.2, the full cohort study population comprised 35,131 animals within 1,077 group-13s within 170 cohorts within 14 feedlots. Blood samples were collected from all study animals at induction and follow-up, which was scheduled at approximately 42 days after induction. Nasal swabs were also collected from all animals at induction. The sampling interval was defined at the animal level as the number of days between induction and follow-up sample collection. The cohort close date (latest animal-level induction date for cohort animals) was the baseline from which time was measured in the case-control study. ‘Time in the cohort’ was defined at the animal level as the number of days spent in the cohort from the cohort close date and was important in determining which animals were eligible for selection in the case-control study.

Each cohort in the study was considered a closed population for 35 days from the cohort close date. Accordingly, an unmatched risk-based design was used, with cases and controls selected from animals that met the case and control criteria described below.

Both cases and controls were defined at the animal level. No exposure statuses were considered in selecting cases and controls other than feedlot when ensuring that some cohorts from each feedlot were included in each selection batch as described below. Cases and controls were selected from those animals that had serum samples from both induction and follow-up which were received, adequate for testing and verified at the animal level, and whose sampling interval was less than or equal to 60 days. Animals were only eligible for selection once, and cases could not also be controls or vice versa.

Eligible cases needed to be part of the cohort at the cohort close date (i.e. not hospitalised for any reason between their individual induction date and the cohort close date). The BRD case definition described in Section 4.1 was applied, such that the reason for removing animals from the home pen and the subsequent diagnosis were consistent and referrable to the respiratory system. Cases were selected from those animals that were first diagnosed with BRD between 7 and 35 days (inclusive) from the cohort close date and this was the first reason for them leaving the cohort. Controls were selected from those animals that remained with the cohort from their induction date until at least 36 days from the cohort close date. Cases and controls were selected in a ratio of 1:1.

11.2.2 Selection method

Following the identification of a sampling frame of eligible animals, the required numbers of controls and cases were selected, respectively, from the populations of eligible controls and cases. Because a previous study had indicated that seroprevalence to a number of viruses of interest was high at feedlot arrival (Dunn et al., 1993), the study aimed to test as many samples as possible within the available budget to ensure sufficient power to detect effects. To facilitate the logistics of laboratory testing, selection of cases and controls was done in two batches. Each ‘selection batch’ consisted of approximately equal numbers of cohorts and approximately half of the cohorts from each feedlot. Projected estimates of the total number of study animals were used to determine the number of animals to select from batch one, which finally comprised 54% of the total. Cases were selected randomly from the eligible case set and controls were selected randomly from the eligible control set from each batch; selected animals were allocated a unique ‘selection number’. Both cases and controls were selected using simple random

sampling from lists of eligible animals without replacement, with computer-generated random numbers. The same sampling procedure was applied to each batch.

11.2.3 Case-control study population

The flow chart displayed in Figure 11-1 illustrates the selection of animals for inclusion in the case-control study using the inclusion criteria described above. Of 35,160 animals inducted, 5 were ineligible because their time at risk was 0 and 24 were lost to follow-up because they were not recorded as being present at the follow-up, there was no exit, hospital, dead or move record, and communication with the feedlot manager did not resolve the status (these animals were also ineligible for the main cohort study). A total of 437 (including the five with zero time at risk) were ineligible because their time in the cohort was less than seven days. The most frequent reason animals were ineligible for selection in the case-control study was the lack of suitable paired serum samples. Of a total of 35,160 animals enrolled into the study, 80% were eligible to be selected either as cases or controls for the case-control study. A total of 4,442 animals were eligible to be selected as cases and 23,640 animals were eligible to be selected as controls. Of these, 3,725 cases and 3,725 controls were randomly selected for serological testing. A very small number of samples from selected animals were subsequently found to be inadequate for testing, but the proportion did not vary between cases and controls; 98% of each returned test results. Of the animals eligible for selection, 16% were eligible as cases and 84% were eligible as controls.

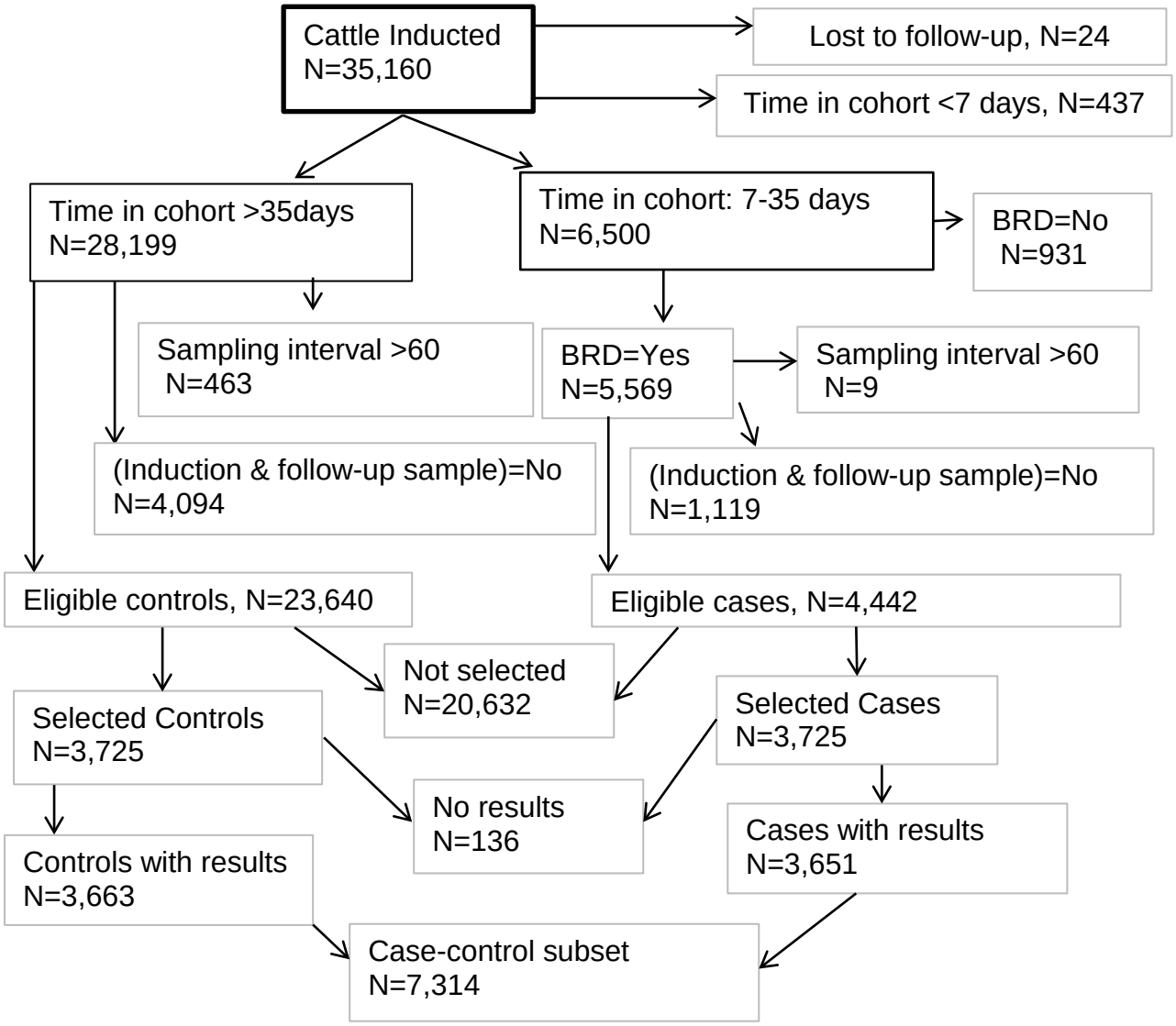

Figure 11-1: Flowchart demonstrating the selection of cases and controls from the cohort study population for the nested case-control study

11.2.4 Serological testing and data linkage

Sera were tested using an indirect multiplex ELISA (BIOX K 284 ELISA®) to evaluate the humoral immune response to BoHV-1, BVDV, BRSV, BPI3 and *M. bovis*. Investigating the role of *M. bovis* was outside the scope of my thesis; such investigations will be reported elsewhere. Tests were conducted according to manufacturer's instructions. Raw optical density results for each test plate were output to a Microsoft® Excel template so that results from each plate occupied one spreadsheet within an Excel workbook. The template applied steps specified in the test kit algorithm to convert the results to optical densities relative to the control sample and categorise them according to cut-offs provided by the manufacturer.

These cut-offs varied slightly between plates with different batch numbers ('test batch'). Plates with four different batch numbers were used during the course of the testing process. Eight induction samples were tested on each ELISA plate along with seven follow-up samples from the same animals. The remaining cells were required for the control serum provided with the test kit. The outstanding follow-up samples were then tested together in catch-up plates as required (i.e. the follow-up samples from each eighth animal from 15 plates were included in a single test plate and tests were run using the same test batch as the induction samples).

The categorised results from each sample were on a 6-point scale, with 0 being considered negative, and 1 through to a maximum of 5 being considered positive. Both the adjusted optical densities and the categorical values were extracted using an automated Excel® add-in. Data were cross checked and any discrepancies were followed up with laboratory staff. In this way, each animal was linked to its unique test results. Results were compiled and linked to a unique test identification code in Excel® before being merged and linked to each animal's selection number and Animal ID in Stata® datasets.

11.2.5 Serological exposure variables

The distributions of the categorical serology results for the induction samples for each virus were examined and some categories were combined to simplify analyses. Induction serology results for each animal were categorised into one of four categories: 0, 1, 2 or 3 and 4 or 5 as shown in Table 11-1. Composite variables to describe the change in serostatus between induction and follow-up for each virus in each animal were derived using the categories (0 to 5) from the induction and follow-up samples (Figure 11-2). 'Seroconversion' was defined as a change from an induction value of 0 to a follow-up value of at least 2. Increases from an induction category of 1, 2 or 3 to a follow-up category of ≥3, ≥4, and 5, respectively, were classified as 're-exposure'. 'Seroincrease' described both seroconversion and re-exposure.

Changes from induction samples with values of 0, 1, 2 or 3 to follow-up samples within one category of the induction sample were classified as 'no change'. 'Initially high' was defined as an induction value of 4 or 5 and a follow-up value within one category of the induction sample. A large drop in serological status between

induction and follow-up was not considered biologically plausible in the feedlot setting within the time frame studied and may have been due to poor sample quality. Thus, serochange variables for animals with a reduction of three or more categories were disregarded and the data value classified as ‘missing’. The raw optical density data was considered in classifying animals with a reduction of two categories. To allow for the loss of information inherent in using categories to describe continuous data, the percentage change in adjusted optical density values between induction and follow-up were considered for these animals. If the change in optical density between induction and follow-up was greater than 40% of the induction values, change in serostatus was coded as ‘missing’ and if it was within 40% of the induction value, it was coded as ‘no change’ or ‘initially high’ depending on the induction value as described above. This classification is illustrated in Figure 11-2.

This classification resulted in virus specific composite serological change variables. The percentages of animals tested with missing values (for reasons described above) for these composite variables ranged from 1 to 5% of the 7,314 animals in the case-control analysis set.

In turn, three simpler analysis variables to describe changes in serostatus were derived. A three-level composite variable for each virus (e.g. BoHV-1 comp, Table 11-1) was derived with the following categories: i) ‘initially high’ – if the change in serostatus was classified as ‘initially high’, ii) seroincrease (‘up’) – if the change in serostatus was classified as ‘seroconversion’ or ‘re-exposure’ and iii) ‘no change’ – if the change in serostatus was classified as ‘no change’

A seroincrease variable (e.g. BoHV-1seroinc, Table 11-1) was derived as a collapsed version of the composite variable for each virus. This variable had two categories: yes for seroincrease, and no for ‘no change’ or ‘initially high’. A seroconversion variable (e.g. BoHV-1serocon,Table 11-1) was also defined for each virus restricted to animals that were seronegative at induction. This variable had two categories: yes for ‘seroconversion’ and no for animals that were 0 or 1 at follow-up.

Table 11-1: Derivation and categories of variables relating to the ELISA serology results

Original data	Range	Category	Variable used in analyses
ELISA optical density category	0 to 5		Virus specific induction serology category* (e.g. **BoHV-1 ind**)
		0	
		1	
		2 or 3	
		4 or 5	
Induction serology and follow-up serology category and optical density values	0 to 5 -2544 to 3638		Virus specific composite serological change variable* (e.g. **BoHV-1 comp**)
		No change	
		Up	
		Initially high	
		Missing	
Virus specific composite serological change variable			Virus specific seroincrease*: increase of at least 2 units between induction and follow-up (e.g. **BoHV-1 seroinc**)
		No	
		Yes	
Virus specific induction serology & composite serological change variables			Virus specific seroconversion*: increase of at least 2 units in animals seronegative at induction (e.g. **BoHV-1 serocon**)
		No	
		Yes	
Induction serology for each virus: (BoHV-1, BVDV, BRSV & BPI3)			Number of viruses animal was seropositive to at induction (**VirusN_ind**)
		0 to 4	
Seroincrease variable for each virus: (BoHV-1, BVDV, BRSV & BPI3)			Number of viruses animal seroincreased to between induction and follow-up (**VirusN_seroinc**)
		0 to 4	

*Equivalent variables were derived for each of the four viruses: BoHV-1, BVDV, BRSV and BPI3
Examples of variables used in final analyses are in bold

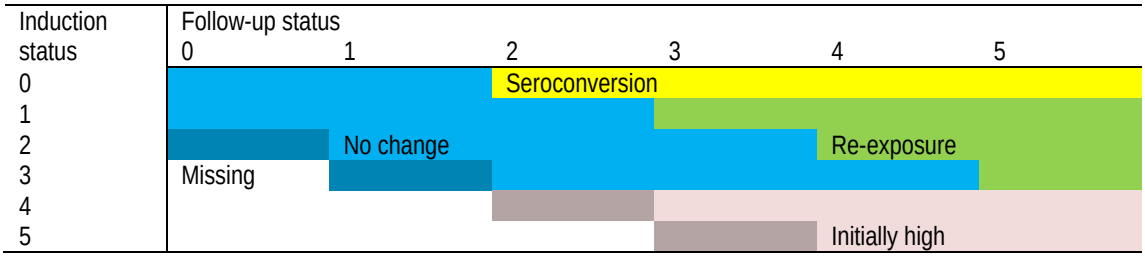

Figure 11-2: Method for classifying change in serostatus based on induction status and follow-up status. Categories were coded as indicated by coloured cells; additional criteria were applied to shaded cells as described above.

Two variables combined data from all four viruses. The number of viruses to which each animal was seropositive at induction (VirusN_ind, Table 11-1) was calculated as the number of the four viruses for which the induction value was at least 1. The number of viruses to which each animal seroincreased (VirusN_seroinc, Table 11-1) was calculated as the number of the four viruses for which the animal was categorised as 'yes' for the seroincrease variable. Animals with a missing value for any one virus variable (i.e. induction serostatus or seroincrease) had a missing value for the respective combined virus variable.

11.2.6 Analyses

11.2.6.1 Descriptive analyses

Cross tabulations for each serological exposure variable against case-control status were produced. As described above, 16% of animals eligible for selection in the case-control study were eligible as cases and 84% were eligible as controls; these percentages were used as weights to estimate seroprevalences in the study population for each of the serological exposure variables. For each category, the weighted average prevalence was calculated as the observed seroprevalence in the cases multiplied by the inverse of 16% plus the observed seroprevalence in the controls multiplied by the inverse of 84%. Correlations between serological variables were assessed using Spearman's correlation coefficients.

11.2.6.2 Effect estimates and causal diagrams

The aims of analyses in the case-control dataset were to determine the total effects of each serological predictor on the risk of BRD. A modified causal diagram was constructed based on the diagram presented in Figure 4-7. The case-control causal diagram illustrated in Figure 11-3 included the serological variables and covariates

relevant to any analyses to estimate the effects of interest (i.e. covariates in the minimal sufficient adjustment set of any serological variable). The diagram in Figure 11-4 shows variables relevant to analyses of the combined virus variables. The rationale for using causal diagrams and the methodology for choosing minimal sufficient adjustment sets to estimate total effects was described in Chapter 6. The DAGitty® software was used to determine which covariates to include in adjustment sets for the serological exposures of interest. Relevant covariates included the number of animals in the cohort (CohortN), whether animals outside the pen could access the water troughs in the animal's pen (Shared pen water), mixing history (Mix summary), vaccination against BoHV-1 at induction (Rhinogard) and whether BVDV was active in the cohort and if so, if a PI animal was identified in the group-28 (BVDV_grp_cht). Nested or correlated variables were not included in the same model (e.g. induction serostatus and the composite serology variables were not fitted together).

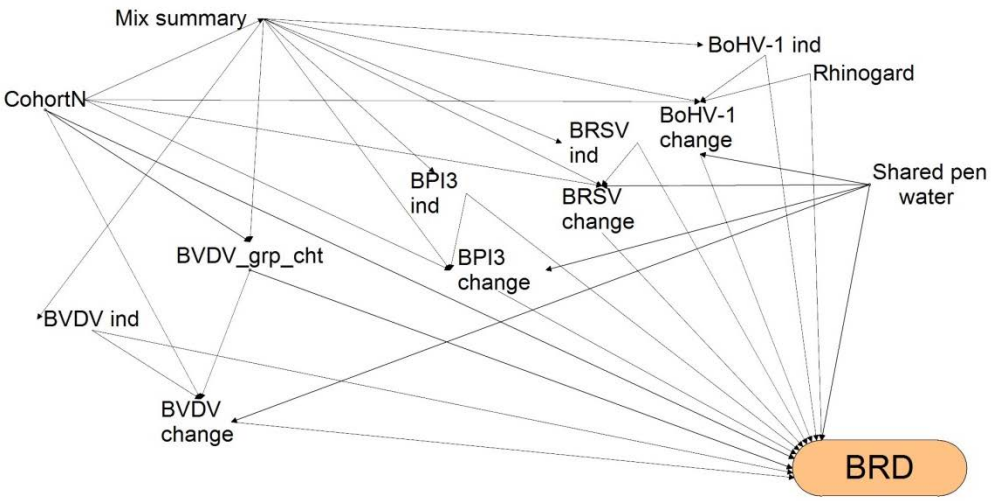

Figure 11-3: Causal diagram showing variables relevant to the case-control study; 'change' variables represent one of the three variables that measured change in serostatus between induction and follow-up, (e.g. BVDV comp, BVDVseroinc or BVDVserocon)

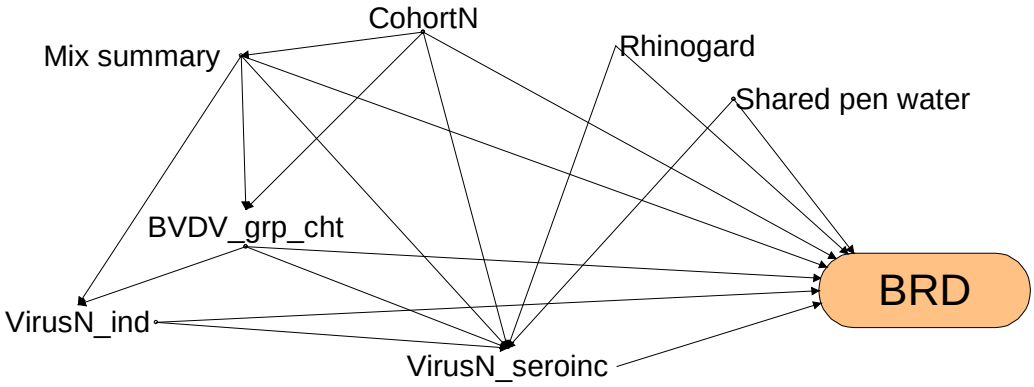

Figure 11-4: Causal diagram showing variables relevant to estimating the effects of 'number of virus' variables in the case-control study.

11.2.7 Modelling

Models were run using Markov chain Monte Carlo (MCMC) methods within the MLwiN® software as described in Chapter 6. PQL2 methods were applied to obtain initial values that were then used in multilevel Bayesian logistic models with MCMC chains run for 50,000 iterations after a burn in of 500. Three hierarchical levels were included in these analyses, with animal fitted within cohort and cohort fitted within feedlot. Test batch and selection batch were included in models as fixed effects. However, for some models with a limited number of observations (e.g. seroconversion models), test batch and selection batch were excluded because models failed to run when these variables were included. Convergence was assessed by examining trajectory plots, autocorrelation factors, MCMC errors, and the Rafferty-Lewis and Brooks-Draper diagnostics as described in Chapter 6. Orthogonalisation and hierarchical centring were applied at the cohort level if any cohort-level variables were included in the model.

To assess interaction terms, all possible two-way interactions were investigated by examining Wald p-values following the fitting of PQL2 models. In addition to interactions between the serological variables, interactions between the BVDV status of the cohort (BVDV_ chtPI) and the BVDV serological variables were assessed. Where the overall p-value for the joint interaction terms was <0.05, model estimation using MCMC methods was planned.

To further assess the effects of a BVDV PI animal in the cohort (BVDV_chtPI), total effects models used to estimate the effects of this variable in the cohort study population (Section 6.3.3.10) were fitted: a) adjusted for initial serostatus to BVDV, b) adjusted for animals that were initially seronegative and c) adjusted for animals that were initially in the seropositive categories of two or higher.

11.3 Results

11.3.1 Correlation between serological variables

Correlations between corresponding serological variables for each of the four viruses were all weak (Table 11-2). When comparing serostatus at induction, all pairwise correlation coefficients were less than 0.2, with the highest being 0.19 for the correlation between BVDV and BRSV. Similarly, there was little correlation between viruses for the composite serochange variables.

Table 11-2: Spearman's correlation coefficients for serological variables ('ind' refers to induction serostatus, 'comp' refers to the composite serochange variable for each virus)

	BoHV-1 ind	BPI3 ind	BRSV ind	BVDV ind	BoHV-1 comp	BPI3 comp	BRSV comp	BVDV comp
BoHV-1 ind	1.00							
BPI3 ind	0.06	1.00						
BRS ind	0.02	0.14	1.00					
BVDV ind	0.08	0.10	0.19	1.00				
BoHV-1 comp	-0.18	0.00	-0.03	0.00	1.00			
BPI3 comp	-0.01	0.55	0.10	0.01	0.07	1.00		
BRSV comp	-0.03	0.02	0.24	0.01	0.08	0.12	1.00	
BVDV comp	0.03	0.12	0.12	0.82	0.05	0.10	0.11	1.00

11.3.2 Seroepidemiology and associations with BRD

11.3.2.1 BoHV-1

The distributions of exposure variables relating to BoHV-1 in the case-control population are presented in Table 11-3. The weighted average seroprevalence (to estimate seroprevalence in the cohort study population) of antibodies against BoHV-1 at induction was 24%. At induction, very few animals were categorised as 4 or 5 (2%) with respect to BoHV-1. The BoHV-1 antibody category increased from induction to follow-up sampling in 48% of animals, and 54% of initially seronegative animals seroconverted. For cattle that did not receive Rhinogard^{TM} at induction, an

estimated 23% exhibited an increase in BoHV-1 antibody category from induction to follow-up sampling, while 27% of initially seronegative animals seroconverted.

Animals that were in induction categories 2 or 3 for BoHV-1 were at reduced risk of BRD relative to induction category 0 (OR 0.7, 95% credible interval: 0.6 to 0.9, Table 11-4). There was no evidence of a large effect of induction categories 4 or 5 but the estimate was imprecise. Seroincrease to BoHV-1 (i.e. 'up' defined as either seroconversion or re-exposure) was associated with a modest increase in risk of BRD (OR 1.4, 95% credible interval: 1.2 to 2.6, Table 11-4) as was seroconversion in initially seronegative animals (OR 1.3, 95% credible interval: 1.1 to 1.5, Table 11-4). However, this effect was not apparent in a subset analysis of animals not given Rhinogard™ at induction; the odds ratio was consistent with a slight protective effect but the estimate was imprecise (OR 0.8, 95% credible interval: 0.3 to 1.6, Table 11-4).

11.3.2.2 BVDV

The distributions of exposure variables relating to BVDV in the case-control population are presented in Table 11-5. The weighted average seroprevalence (to estimate seroprevalence in the cohort study population) for antibodies against BVDV at induction was 69%, with nearly half of the study population categorised as 4 or 5 (49%). BVDV antibody category increased from induction to follow-up sampling in 24% of animals, and 55% of initially seronegative animals seroconverted.

Prior exposure to BVDV (induction categories 2 or 3 and 4 or 5) was associated with a reduced risk of BRD relative to induction category 0 (OR 0.8, 95% credible interval: 0.6 to 1.0 and OR 0.8, 95% credible interval: 0.7 to 0.9, respectively, Table 11-6). The estimated effect of induction category 1 was indicative of a possible increase in risk (OR 1.3, 95% credible interval: 1.0 to 1.7, Table 11-6). Seroincrease to BVDV ('up' category) was associated with a modest increase in risk of BRD (OR 1.3, 95% credible interval: 1.1 to 1.6,Table 11-6) as was seroconversion (OR 1.6, 95% credible interval: 1.2 to 2.1,Table 11-6).

Other results presented in Table 11-6 report associations between the cohort BVDV-PI status and the case-control outcome. The presence of BVDV in the cohort was associated with increased risk of BRD; a similar level of risk was observed whether a PI animal was identified or whether only transient infection (TI) had been

detected in any cohort animal. Analyses adjusted for induction serostatus to BVDV and stratified by induction serostatus to BVDV returned similar findings. Exposure to BVDV in a cohort with an identified PI animal was associated with increased risk of BRD (OR 2.0, 95% credible interval: 1.1 to 3.4, Table 11-6) as was exposure in cohorts in which only TI animals were identified (OR 2.5, 95% credible interval: 1.3 to 4.3,Table 11-6). Animals that were seropositive at induction (serology category 2 or above) were at increased risk of BRD upon exposure to BVDV in the cohort (i.e. either PI or TI animals identified) as were animals that were initially seronegative at induction. A further analysis restricted only to animals that were inducted (and hence first sampled) within a day of arrival revealed similar results with animals exposed to a PI in the cohort or to transient infection in the cohort at similarly increased risk.

11.3.2.3 BPI3

The distributions of exposure variables relating to BPI3 in the case-control population are presented in Table 11-7. The weighted average seroprevalence (to estimate seroprevalence in the cohort study population) of antibodies against BPI3 was 91% (Table 11-7). BPI3 antibody category increased from induction to follow-up sampling in 17% of animals and 54% of initially seronegative animals seroconverted (Table 11-8).

Prior exposure to BPI3 (induction categories 1, 2 or 3 and 4 or 5) was associated with a reduced risk of BRD relative to induction category 0 (OR 0.6, 95% credible interval: 0.5 to 0.7 or 0.8 depending on category, Table 11-8). An increase in BPI3 antibody category (‘up’ category) was associated with a modest increase in risk of BRD (OR 1.4, 95% credible interval: 1.2 to 1.6, Table 11-8). The estimate for seroconversion was also suggestive of increased risk but was imprecise, probably because of the small number of initially seronegative animals (OR 1.4, 95% credible interval: 0.9 to 2.2, Table 11-8).

11.3.2.4 BRSV

The distributions of exposure variables relating to BRSV in the case-control population are presented in Table 11-9. From the case-control study, the weighted average seroprevalence (to estimate seroprevalence in the cohort study population) of antibodies against BRSV was 89% (Table 11-10). BRSV antibody category

increased from induction to follow-up sampling in 29% of animals and 65% of initially seronegative animals seroconverted.

Prior exposure to BRSV (induction categories 1, 2 or 3 and 4 or 5) was associated with a reduced risk of BRD relative to induction category 0 (OR 0.7 or 0.8, 95% credible interval: 0.6 to 0.8 or 1.0 depending on category, Table 11-10). An increase in BRSV antibody category ('up' category) was associated with a modest increase in risk of BRD (OR 1.4, 95% credible interval: 1.2 to 1.7, Table 11-10) as was seroconversion (OR 1.5, 95% credible interval: 1.0 to 2.2, Table 11-10).

Table 11-3: Summary of bovine herpes virus 1 (BoHV-1) induction serology results and change in serostatus between induction and follow-up sampling.

Variable	Category	% Controls	% Cases	Number	%	Weighted %
BoHV-1 induction						
	0	75.5	80.0	5,681	77.8	76.2
	1	12.2	12.6	906	12.4	12.3
	2 or 3	10.4	6.2	606	8.3	9.7
	4 or 5	1.9	1.2	113	1.6	1.8
	Missing			8		
BoHV-1 composite						
	No change	53.9	36.3	3,253	45.1	51.1
	Up	44.9	62.8	3,886	53.9	47.8
	Initially high	1.2	0.9	76	1.0	1.1
	Missing			99		
BoHV-1 seroincrease						
	No	55.1	37.2	3,329	46.1	52.2
	Yes	44.9	62.8	3,886	53.9	47.8
	Missing			99		
BoHV-1 seroconversion						
	No	48.3	32	2,267	39.9	45.7
	Yes	51.7	68	3,414	60.1	54.3
	Missing			0		

Table 11-4: Total effects of bovine herpesvirus 1 (BoHV1) serological variables on the on the risk of being a BRD case.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
BoHV-1 induction category					(Mix summary, Test batch, Selection batch)	N=7,232 3 level
	0	Ref				
	1	0.9	(0.8 to 1.1)	0.252		
	2 or 3	0.7	(0.6 to 0.9)	0.006		
	4 or 5	1.0	(0.5 to 1.6)	0.469		
BoHV-1 composite					(Mix summary, Test batch, Selection batch, CohortN, Rhinogard, Shared pen water)	N=7,211 3 level
	No change	Ref				
	Up	1.4	(1.2 to 1.6)	<0.001		
	Initially high	1.6	(0.8 to 3.0)	0.099		
BoHV-1 seroconversion					(Mix summary, CohortN, Shared pen water, Rhinogard)	N=5,623 3 level
	No	Ref				
	Yes	1.3	(1.1 to 1.5)	0.001		
BoHV-1 seroconversion; No Rhinogard™ at induction					(Mix summary, CohortN, Shared pen water)	N=717 3 level
	No	Ref				
	Yes	0.8	(0.3 to 1.6)	0.205		

Table 11-5: Summary of bovine viral diarrhoea virus (BVDV) induction serology results and change in serostatus between induction and follow-up sampling.

Variable	Category	% Controls	% Cases	Number	%	Weighted %
BVDV induction						
	0	29.3	38.2	2,469	33.8	30.7
	1	4.4	5.9	376	5.1	4.6
	2 or 3	16.6	12.3	1,058	14.5	15.9
	4 or 5	49.7	43.6	3,411	46.6	48.7
	Missing			0		
BVDV composite						
	No change	29.7	22.5	1,845	26.0	28.5
	Up	21.7	34.6	1,999	28.2	23.8
	Initially high	48.6	42.9	3,241	45.7	47.7
	Missing			229		
BVDV seroincrease						
	No	78.3	65.4	5,086	71.8	76.2
	Yes	21.7	34.6	1,999	28.2	23.8
	Missing			229		
BVDV seroconversion						
	No	47.8	29.2	921	37.3	44.8
	Yes	52.2	70.8	1,548	37.3	55.2
	Missing			0		

Table 11-6: Total effects of bovine viral diarrhoea virus (BVDV) serological variables on the on the risk of being a BRD case.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
BVDV induction category					(Mix summary, Test batch, Selection batch, BVDV_grp_cht)	N=7,240 3 level
	0	Ref				
	1	1.3	(1.0 to 1.7)	0.048		
	2 or 3	0.8	(0.6 to 1.0)	0.015		
	4 or 5	0.8	(0.7 to 0.9)	0.002		
BVDV composite					(Mix summary, Test batch, Selection batch, CohortN, BVDV_grp_cht, Shared pen water)	N=7,081 3 level
	No change	Ref				
	Up	1.3	(1.1 to 1.6)	0.001		
	Initially high	1.0	(0.9 to 1.2)	0.478		
BVDV seroconversion					(Mix summary, CohortN, Shared pen water, BVDV_grp_cht)	N=2,446 3 level
	No	Ref				
	Yes	1.7	(1.3 to 2.1)	<0.001		
BVDV_chtPI					(BVDV_an_PI, CohortN, Shared pen water, Mix summary, BVDV_ind)	N=7,241 4 level
	No	Ref				
	PI identified	2.0	(1.1 to 3.4)	0.009		
	TI	2.5	(1.3 to 4.3)	0.003		
BVDV_chtPI^ (seronegative at induction)					(BVDV_an_PI, CohortN, Shared pen water, Mix summary, BVDV_ind)	N=2,446 4 level
	No	Ref				
	PI identified	1.7	(0.8 to 3.0)	0.088		
	TI	1.7	(0.8 to 3.3)	0.076		
BVDV_chtPI* (seropositive at induction)					(BVDV_an_PI, CohortN, Shared pen water, Mix summary, BVDV_ind)	N=4,423 4 level
	No	Ref				
	PI identified	2.5	(1.2 to 4.7)	0.004		
	TI	3.5	(1.6 to 6.8)	0.001		
BVDV_chtPI# (arr_day0 <1)					(BVDV_an_PI, CohortN, Shared pen water, Mix summary, BVDV_ind)	N=6,152 4 level
	No	Ref				
	PI identified	2.2	(1.2 to 3.8)	0.008		
	TI	2.9	(1.4 to 5.3)	0.002		

^Restricted to seronegative at induction

*Restricted to seropositive category 2 or above at induction

Restricted to animals sampled within 1 day of arrival

Table 11-7: Summary of bovine parainfluenza virus (BPI3) induction serology results and change in serostatus between induction and follow-up sampling.

Variable	Category	% Controls	% Cases	Number	%	Weighted %
BPI3 induction						
	0	8.5	11.0	713	9.8	8.9
	1	15.2	15.3	1,114	15.2	15.2
	2 or 3	48.3	48.1	3,525	48.2	48.3
	4 or 5	28.0	25.6	1,962	26.8	27.6
	Missing			0		
BPI3 composite						
	No change	57.4	51.7	3,822	54.6	56.5
	Up	15.8	23.3	1,370	19.6	17.0
	Initially high	26.8	25.0	1,812	25.9	26.5
	Missing			310		
BPI3 seroincrease						
	No	84.2	76.7	5,634	80.4	83.0
	Yes	15.8	23.3	1,370	19.6	17.0
	Missing			310		
BPI3 seroconversion						
	No	48.6	31.6	278	39	45.8
	Yes	51.5	68.4	435	61	54.2
	Missing			0		

Table 11-8: Total effects of bovine parainfluenza virus (BPI3) serological variables on the on the risk of being a BRD case.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
BPI3 induction category					(Mix summary, Test batch, Selection batch)	N=7,240 3 level
	0	Ref				
	1	0.6	(0.5 to 0.8)	<0.001		
	2 or 3	0.6	(0.5 to 0.7)	<0.001		
	4 or 5	0.6	(0.5 to 0.8)	<0.001		
BPI3 composite					(Mix summary, Test batch, Selection batch, CohortN, Shared pen water)	N=7,001 3 level
	No change	Ref				
	Up	1.4	(1.2 to 1.6)	<0.001		
	Initially high	1.1	(0.9 to 1.2)	0.184		
BPI3 seroconversion					(Mix summary, CohortN, Shared pen water)	N=709
	No	Ref				
	Yes	1.4	(0.9 to 2.2)	0.088		

Table 11-9: Summary of bovine respiratory syncytial virus (BRSV) induction serology results and change in serostatus between induction and follow-up sampling.

Variable	Category	% Controls	% Cases	Number	%	Weighted %
BRSV induction						
	0	10.8	14.3	919	12.6	11.4
	1	22.6	24.4	1,719	23.5	22.9
	2 or 3	49.7	45.7	3,487	47.7	49.0
	4 or 5	16.8	15.7	1,189	16.3	16.7
	Missing			0		
BRSV composite						
	No change	55.2	49.4	3,718	52.3	54.3
	Up	28.1	35.1	2,247	31.6	29.2
	Initially high	16.7	15.5	1,145	16.1	16.5
	Missing			204		
BRSV seroincrease						
	No	71.9	64.9	4,863	67.9	70.8
	Yes	28.1	35.1	2,247	32.1	29.2
	Missing			204		
BRSV seroconversion						
	No	36.8	26.1	282	30.7	35.1
	Yes	63.2	74.0	637	69.3	64.9
	Missing			0		

Table 11-10: Total effects of bovine respiratory syncytial virus (BRSV) serological variables on the on the risk of being a BRD case.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
BRSV induction category					(Mix summary, Test batch, Selection batch)	N=7,240 3 level
	0	Ref				
	1	0.8	(0.6 to 1.0)	0.018		
	2 or 3	0.7	(0.6 to 0.8)	<0.001		
	4 or 5	0.8	(0.6 to 1.0)	0.034		
BRSV composite					(Mix summary, Test batch, Selection batch, CohortN, Shared pen water)	N=7,106 3 level
	No change	Ref				
	Up	1.4	(1.2 to 1.7)	<0.001		
	Initially high	1.2	(1.0 to 1.5)	0.014		
BRSV seroconversion					(Mix summary, CohortN, Shared pen water)	N=914
	No	Ref				
	Yes	1.5	(1.0 to 2.3)	0.036		

11.3.3 Exposure to multiple viruses

11.3.4 Induction serology

The induction serological profiles of animals in the case-control study are summarised in Table 11-11. The majority of animals (78%) were seronegative to BoHV-1, while 34%, 13% and 10% were seronegative to BVDV, BRSV and BPI3, respectively. Of animals categorised as seronegative to two or more agents, 74% were seronegative to both BoHV-1 and BVDV and these animals comprised 28% of the population. The remaining 26% of animals seronegative to two or more agents were seronegative to one of these viruses.

The vast majority of animals had antibodies to at least one virus at induction, with only 1.4% of animals being seronegative to all four viruses. About 15% of animals had antibodies to all four viruses and 47% had antibodies to three out of the four viruses. The most common pattern (seen in 41% of animals) was seropositivity to BPI3, BRSV and BVDV, with a further 21% of animals seropositive to BPI3 and BRSV.

Table 11-11: Serological profile of study animals at induction; seronegativity at induction was designated 0 and any positive value was designated 1.

BoHV-1	BPI3	BRSV	BVDV	N	%
0	0	0	0	99	1.4
1	0	0	0	10	0.1
0	1	0	0	268	3.7
0	0	1	0	193	2.6
0	0	0	1	64	0.9
1	1	0	0	54	0.7
0	1	1	0	1506	20.6
1	0	1	0	20	0.3
1	0	0	1	7	0.1
0	0	1	1	253	3.5
0	1	0	1	325	4.4
1	1	1	0	312	4.3
1	0	1	1	65	0.9
1	1	0	1	89	1.2
0	1	1	1	2972	40.7
1	1	1	1	1068	14.6

Combinations of seroincreases are presented in Table 11-12. Of the animals with non-missing values for the seroincrease variables for all four viruses, 21% (1,430/6,720) did not seroincrease to any virus, 38% seroincreased one virus, 27% seroincreased to two viruses, 11% seroincreased to three viruses and 3% seroincreased to all four viruses. Of animals seroincreasing to two or more viruses,

59% seroincreased to either BoHV-1 or BVDV, while the remaining 41% seroincreased to both BoHV-1 and BVDV.

Table 11-12: Distribution of animals by combination of seroincreases between induction and follow-up in animals with non-missing values for all seroincrease variables^

BoHV-1	BPI3	BRSV	BVDV	N	%
0	0	0	0	1,430	21
1	0	0	0	1,469	2
0	1	0	0	217	3
0	0	1	0	474	7
0	0	0	1	417	6
1	1	0	0	298	4
0	1	1	0	104	2
1	0	1	0	607	9
1	0	0	1	491	7
0	0	1	1	177	3
0	1	0	1	109	2
1	1	1	0	210	3
1	0	1	1	327	5
1	1	0	1	126	2
0	1	1	1	86	1
1	1	1	1	178	3

^seroincrease denoted 0 for ‘no’ and 1 for ‘yes’

Based on the weighted average seroprevalences at induction, a high percentage of the cohort study population were estimated to be seropositive to two (28%) or three (49%) viruses at induction (Table 11-13). Based on the weighted average seroincreases between induction and follow-up, seroincrease to one (40%) or two (23%) viruses was common, but an estimated 26% of the cohort study population did not seroincrease to any of the four viruses investigated (Table 11-13).

Compared to animals that were seropositive to all four viruses at induction, those seropositive to less than four viruses were at increased risk of BRD, with risk progressively increasing with seropositivity to fewer viruses. Those seronegative to all of the viruses were at highest risk BRD (OR 2.4, 95% credible interval: 1.3 to 4.3, Table 11-14). Those animals seroincreasing to at least one virus were at increased risk compared to those not seroincreasing to any viruses, with those seroincreasing to at least two viruses at markedly increased risk.

Table 11-13: Summary of number of viruses to which animals were positive at induction and number of viruses to which animals had a positive change in serostatus (increase of at least two categories) by follow-up.

Variable	Category	% Controls	% Cases	N	%	Weighted % in cohort study dataset
Number of viruses animal was seropositive to at induction						
	0	1.0	1.8	99	1.4	1.1
	1	5.5	9.2	535	7.3	6.1
	2	26.6	32.7	2,165	29.6	27.6
	3	50.4	43.7	3,438	47.1	49.3
	4	16.6	12.7	1,068	14.6	15.9
Number of viruses animal seroincreased to by follow-up						
	0	28.5	14.3	1,430	21.3	26.2
	1	41.3	35.5	2,577	38.3	40.3
	2	21.0	32.0	1,786	26.6	22.8
	3	7.5	14.7	749	11.1	8.7
	4	1.8	3.5	178	2.7	2.0

Table 11-14: Estimated odds ratios for the total effects of number of viruses to which animals were positive at induction and number of viruses to which animals had a positive change in serostatus (increase of at least two categories) by follow-up.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N
Number of viruses animal was seropositive to at induction					(Mix summary, BVDV_grp_cht, Test batch, Selection batch)	7,232
	0	2.4	(1.3 to 4.3)	0.002		
	1	1.9	(1.4 to 2.5)	<0.001		
	2	1.3	(1.1 to 1.6)	0.005		
	3	1.1	(0.9 to 1.3)	0.291		
	4	Ref				
Number of viruses animal seroincreased to by follow-up					(Mix summary, CohortN, BVDV_grp_cht, Shared pen water, VirusN_ind, Test batch, Selection batch, Rhinogard)	6,717
	0	Ref				
	1	1.3	(1.1 to 1.6)	0.003		
	2	1.9	(1.5 to 2.3)	<0.001		
	3	1.9	(1.5 to 2.5)	<0.001		
	4	1.5	(0.9 to 2.2)	0.057		

11.3.5 Interactions

All possible two-way interactions were assessed for each group of variables. There were no significant interactions detected (none of the Wald p-values for these terms were below 0.05) between the serological variables at induction, nor were there any

when the composite variables were examined. No significant interaction (P=0.99) was detected between the cohort-level presence of a PI animal and the animal-level BVDV status at induction.

11.4 Discussion

The results of this study supported prior research that suggested exposure to viruses prior to induction was common and that BPI3, BRSV, BVDV and BoHV-1 are ubiquitous in Australian cattle populations. Seroprevalences in our study population were higher than those reported in North American studies. This is not unexpected as cattle entering feedlots in Australia are generally older and have a more extended mixing history than those in North America.

Based on weighted seroprevalences, 69% of the cohort study population were estimated to have antibodies to BVDV in the cohort study population at induction; this is consistent with a prior Australian study that reported a seroprevalence of 68% (Dunn et al., 1993). An estimated 24% of study animals were seropositive to BoHV-1 at induction compared to 13% reported in a previous Australian study (Dunn et al., 1993) and seroprevalences ranging from negligible to 18% in North American studies. In my study, 89% of animals were seropositive to BRSV and 91% were seropositive to BPI3 at induction which is much higher than that reported in North American studies and the previously reported 27% and 57% for BRSV and BPI3 respectively in cattle entering Australian feedlots (Dunn et al., 1993).

My results suggest that animals with BoHV-1 or BVDV serological categories of 2 or more at induction were at reduced risk of BRD, while being seropositive (i.e. category one or higher) to BRSV and BPI3 at induction offered equal degrees of protection. While previous studies generally agree that BVDV and BoHV-1 are consistently associated with increased risk of BRD, studies investigating BRSV and BPI3 have returned inconsistent results. An important strength of my study is that a large number of animals selected from across the cohort study population were included in the case-control study. Hence, the study had sufficient power to detect effects across the population that may not be apparent in smaller studies, or studies confined to single feedlots. Thus, an important role of all four viruses has been defined. Given that both seronegativity at induction and ‘seroincrease’ to any of the viruses was associated with increased risk, it is important to consider the proportions

of animals ‘exposed’. In the case-control study population, 47% of animals were classified as ‘initially high’ to BVDV, 27% were initially high’ to BPI3 and 16% were ‘initially high’ to BPI3 while only 2% were ‘initially high’ to BoHV-1. Hence, because the largest percentage of animals in the population was susceptible to BoHV-1, this virus would be expected to have the greatest impact at the population level.

Animals seroincreasing to any of the four agents were at similarly increased risk of being diagnosed with BRD; odds ratios ranged between 1.3 and 1.4. This indicates that exposure of immunologically naïve animals to any of these viruses at or after induction increased the risk of BRD. This is supported by associations between number of viruses and BRD. The risk of BRD increased as the number of viral agents the animal had antibodies to at induction decreased and as the number of viruses the animal had increasing serological titres for increased. As previously discussed, animals seronegative to two or more agents at induction were invariably seronegative to BoHV1 and/or BVDV and the majority of those seroconverting to multiple agents seroconverted to one or both of these agents. We can therefore infer that these agents were the most important viral contributors to BRD risk in this study population.

11.5 Conclusions

Serological risk factors for each of the four viruses investigated were associated with risk of BRD. Overall, the results support prior literature which suggests that viral infection with BVDV and BoHV-1 are associated with increased risk of BRD. My results provide evidence that exposure to BRSV and BPI3 was also associated with increased risk of BRD. Being seronegative to more than one virus resulted in progressively increased risk of BRD.

For each virus, seroincrease was associated with only a modest increase in risk, indicating that each virus in isolation had only a small effect on BRD risk. Seroincreases to multiple viruses further increased risk, indicating that exposure to multiple viruses was worse than exposure to one virus. However, for animals exposed to all four viruses, the highest odds ratio was only 1.9. Both of these observations indicate the importance of exposure to multiple factors for BRD to occur, highlighting the need to focus on other factors as well as pathogens when planning BRD control.

12 General Discussion and Major Conclusions

12.1 Introduction

The studies described in this thesis were part of an in-depth nationwide epidemiological investigation into BRD in Australian feedlot cattle. In this chapter I link the key findings to the thesis objectives, and summarise the major conclusions and recommendations to industry. I then discuss the strengths and limitations of the studies and comment on the external validity of the findings.

12.2 Key findings

The incidence of BRD in the cohort study population was 18.2% and the 50-day cumulative incidence of BRD was 17.6%. The epidemic curves revealed that peak BRD incidence occurred between day 15 and day 30; 90% of cases occurred between day 5 and 35 and 97% by day 50 (Section 5.3).

BRD incidences varied widely, with some feedlots having marked variation in incidence between cohorts and others having consistently low incidences across cohorts. Thus ‘typical’ performance was difficult to define except in feedlots with consistently low incidences. The distribution of variance at each of the hierarchical levels was examined. In the null model, approximately 56% of the variance was at the feedlot level, 9% was at the cohort level, 5% was at the group-13 level and 30% was at the animal level. The large proportion of variance observed at the feedlot level was consistent with the observations of variability in BRD incidence across feedlots. A parsimonious set of explanatory variables explained 14% of the variability in BRD incidence. Of the remaining (i.e. the unexplained) variance, 36% was at the feedlot level, 10% was at the cohort level, 6% was at the group-13 level and 48% was at the animal level. None of the explanatory variables in this model were feedlot-level variables, the reduction in the proportion of unexplained variance that was at the feedlot level was likely to have been at least partly due to the clustering of lower level explanatory variables by feedlot.

Putative risk factors investigated were classified as animal-entry characteristics, management-related, broad environmental and serological risk factors. Of the animal-entry characteristics investigated, breed, induction weight and sex were

associated with risk of BRD in the cohort study population. Knowledge of these risk factors is of most use in assessing BRD risk for incoming groups of cattle.

Several management-related risk factors were identified as being important. Shared pen water was strongly associated with risk of BRD with cattle in pens with water shared with other pens at markedly increased risk of BRD. Important risk factors related to the assembly of cattle prior to them being placed on feed in a feedlot pen included mixing history, group size and the timing of the move to the vicinity of the feedlot. Animals not mixed prior to day -27 were at markedly increased risk of BRD (compared to those that were mixed). Animals subjected to a high level of mixing (four or more group-13s in the cohort compared to less than four) were also at markedly increased risk. Animals moved to the vicinity of the feedlot prior to day -27 were at markedly reduced risk of BRD compared to animals moved to the vicinity of the feedlot closer to the induction date. Animals in group-13s with less than 50 animals were at increased risk compared to animals in groups with 50 or more animals.

The importance of mixing was also demonstrated through a comparison of total and direct effects for several risk factors that were largely mediated through mixing. This approach has given insight into causal pathways that are important from the feedlot manager's perspective. Saleyard exposure prior to a month before induction was protective; this effect was mediated through mixing. While mixing partly explained the harmful effects of saleyard exposure within a month of induction, there was an increased risk over and above mixing and feedlot move timing. In the cohort study population few animals were exposed to saleyards, so the population-level effects of exposure to saleyards were minimal.

Across the relatively unmixed population of vendor bred cattle and cattle purchased aged less than ten months, prior vaccination with either Pestigard™ or Bovilis MH™ given at least 14 days before day 0 probably resulted in reduced risk of BRD. For vendor-bred cattle, yard weaning probably resulted in reduced risk of BRD. These risk factors were estimated as having modest population-level effects.

Investigations into the roles of pathogens included determining the prevalence of BVDV-PI animals in the study population (Section 10.2), assessing the effect of BVDV in the cohort as a risk factor for BRD in the cohort study population (Section

6.4.2.2), analysing hospital and necropsy samples for specific viruses and bacteria (Chapter 10) and analyses of serological results from the case-control study (Chapter 11).

The animal-level prevalence of BVDV-PI animals across the full cohort study population was estimated at 0.24%. Exposure to BVDV in the cohort was common; qPCR analyses revealed that about two-thirds of study animals were exposed (i.e. either PI or TI animals in the cohort). Serological results from the case-control study (detailed in Chapter 11) revealed that seroincrease to BVDV between induction and follow-up occurred in at least one animal that was tested in 88% of cohorts (animals from 161/170 cohorts were included in the case-control population). Animals exposed to BVDV after induction were at increased risk of developing BRD, and exposure to BVDV in the cohort had a moderate population-level effect. Overall, exposure to BVDV was ranked sixth in importance. The population-level effects give an indication of the expected benefit to feedlots if BVDV were prevented from entering feedlots.

The most common viruses detected in hospital nasal swabs were BoHV-1 and BCoV. Because BCoV has only recently been identified as a potential component of the BRD complex in Australia (Hick et al., 2012), it was not included in the serological testing. The most common viruses detected in necropsy samples were BoHV-1 and BVDV. The patterns of virus detection in the hospital and post mortem samples support the conclusion that BVDV and BoHV-1 play important roles, especially in fatal BRD.

Serological results indicated that infections with the four viruses investigated (measured by seroincrease during the time on feed) were common. Of those animals exposed to any of these viruses at the feedlot, it was usually only one or two of these four viruses; it was rare for animals to be exposed to more than two viruses between induction and follow-up (i.e. approximately day 42).There was evidence that all four viruses were circulating in medium to large Australian feedlots but I found no evidence of all four viruses circulating at any one feedlot at any given time.

Animals that were seropositive at induction to each of the four viruses investigated were at reduced risk of BRD compared to those that were seronegative. For each additional virus individual animals were seropositive to, their risk of BRD decreased

progressively. For each virus, animals that seroincreased were only at modestly increased risk of BRD. Animals that seroincreased to more than one virus were at further increased risk of BRD compared to animals that seroincreased to a single virus. However, the strengths of associations between the serological variables investigated and BRD occurrence were only modest; the separate contributions of each virus to BRD risk were relatively small compared to the main animal-entry characteristics, management-related and broad environmental risk factors.

Animals inducted into feedlots in Queensland (i.e. north) were at reduced risk compared to animals inducted into feedlots in southern areas. Risk of BRD was increased for animals inducted during summer or autumn compared to those inducted during spring. Further research is required to better understand these associations.

Management-related factors identified for future research included those for which results were inconclusive or unexpected, but for which there are plausible biological pathways and/or prior research indicating an association with BRD incidence. Population-level effects were not estimated for these factors (e.g. pen density, pen shade, percentage grain in rations) because inconclusive and very imprecise effect estimates render population-level estimates uninformative. Most of these were cohort-level factors that were clustered at the feedlot level; hence the study lacked sufficient power to allow me to reach conclusions. In addition there may have been residual feedlot-level confounding for some associations. In an extreme case, this may explain the unexpected strong positive association between Rhinogard^{TM} administration at induction and risk of BRD; this warrants further investigation. Assessment of vaccine efficacy is best accomplished through use of randomised controlled trials.

12.3 Conclusions and recommendations

12.3.1 Conclusions about management-related risk factors

- Shared pen water is a major risk factor for BRD at the population level
- Mixing history is a very important risk factor with a major population-level effect
- Moving animals to the vicinity of the feedlot prior to one month before induction markedly reduces risk of BRD at the feedlot
- Animals in a stable group of 50 or more animals established for at least one month are at reduced risk of BRD at the feedlot
- Eradication of BVDV would be expected to result in a moderately reduced BRD incidence at the population level
- Although, assessment of vaccine efficacy is best accomplished through use of randomised controlled trials, my findings indicate that prior vaccination with BovilisMH™ and prior vaccination with Pestigard™ both have modestly protective population-level effects
- Yard weaning probably has a modestly protective population-level effect
- Saleyard exposure within one month of induction increases risk of BRD

12.3.2 Recommendations about management-related risk factors

12.3.2.1 Extension

1. In any newly constructed or renovated feedlot pens, ensure water troughs are not able to be accessed by animals in adjoining pens.
2. Preferentially purchase animals known to have been mixed prior to one month before induction
3. Preferentially purchase larger groups of animals (i.e. 50 or more) that have been together for at least one month.
4. If animals are known to have been in a stable group for at least one month, place the animals together in the pen rather than splitting the group between several pens.
5. If animals have not been mixed prior to a month before purchase, they should be preassembled (i.e. assembled on pasture close to the feedlot in groups of 50 or more for at least four weeks before induction)

6. Cattle sourced from saleyards should be preassembled for at least one month prior to induction.
7. Pens of cattle should be comprised less than four established (i.e. stable for one month or more) groups of 50 or more animals.
8. Administer respiratory vaccines at least two weeks prior to induction
9. Encourage cattle producers to yard wean when possible

12.3.2.2 Further research

1. Investigate the association between shared pen water and BRD incidence further by conducting a retrospective record review of existing cohort-level data across a large number of cohorts within feedlots with disparate values (i.e. with and without pens with shared water troughs)
2. Develop suitable tools that use existing datasets to assess prior mixing, movement and saleyard history of cattle for BRD risk analysis.
3. Perform cost-benefit analyses of preassembly of cattle.
4. Investigate broader industry engagement in preassembly (e.g. by encouraging cattle producers to more actively assume a preassembly role).
5. Perform cost-benefit analyses to assess the benefits of preventing BVDV from entering feedlots.
6. Randomised controlled vaccination trials should be conducted under field conditions so that immunological, serological and clinical responses are appropriately measured. In designing such studies, attention needs to be paid to adequately controlling for confounding due to the many important animal, management-related and environmental risk factors.
    a. conduct a randomised controlled trial to determine the efficacy of Rhinogard™ administration at induction
    b. conduct randomised controlled trials to assess the efficacy of Pestigard™ and BovilisMH™
    c. Investigate any additional benefit of incorporating prior vaccination into a preassembly program
7. Conduct a retrospective cohort study using existing records from a large number of feedlots. Factors of particular interest would include pen characteristics (e.g. pen shade) and possible interactions with weather variables.

8. Conduct trials to investigate factors associated with adaptation to feedlot pens for individual animals (e.g. time-varying changes in stocking density or time to adapt to high grain rations) and whether selectively manipulating these exposures may reduce risk of BRD (e.g. placing ‘high risk’ cattle in pens with lower stocking density with less commingling).

12.3.3 Conclusions about broad environmental risk factors

- BRD risk varies markedly by feedlot region and during different seasons; these factors collectively have a very large population-level effect.

12.3.4 Recommendations about broad environmental risk factors

12.3.4.1 Further research

1. Further research is required to investigate the effects of weather factors on risk on BRD in an attempt to explain some of the variation in risk observed with feedlot different regions and induction seasons

12.3.5 Conclusions about animal-entry characteristics

- Breed is an important risk factor for BRD with a large population-level effect
- Animals with a lower induction weight are at modestly increased risk of BRD
- Steers are probably at moderately increased risk of BRD compared to heifers

12.3.6 Recommendations about animal-entry characteristics

1. Feedlot operators should review selected animal attributes as part of a BRD risk management strategy

12.3.7 Conclusions about serological risk factors

- Active infection with respiratory viruses modestly increases risk of animals developing BRD
- Active infection with more than one virus additional increases risk compared to infection with a single virus
- The effects of infection with viruses are only modest compared to many of the important management-related, animal entry and environmental risk factors

12.3.8 Recommendations about serological risk factors

12.3.8.1 Further research

1. Conduct studies to investigate the role of BCoV in Australian feedlot cattle.

12.4 Strengths of my studies

My studies had numerous strengths. Animals were sourced from a wide geographical area comprising the majority of Australian cattle producing regions and enrolled over a three year period from 2009 to 2011. Even though there were only 14 participating feedlots, they were located in a range of geographical regions (Figure 4-4), and the clustering of feedlots in the major grain-producing areas mirrored that observed in the broader population of Australian feedlots. A large amount of variability in BRD incidences was observed across feedlots and participating feedlots practiced different management practices and fed cattle for a variety of markets.

The collection of data relating to a large number of putative risk factors and confounders facilitated a comprehensive investigation. Many variables were measured and derived at the animal level with minimal missing data, and few animals were lost to follow-up. The distributions of the majority of animal and group level risk factors (e.g. breed, weight, mixing history) were reasonably balanced across feedlots. Thus, selection bias and confounding due to feedlot-associated factors would be expected to be minimal.

The collection of prior management and movement history data was an important strength of the study. In utilising a national animal movement database, I have been able to disentangle the effects of interrelated mixing, saleyard exposure, grouping and feedlot move timing risk factors and quantify their effects at both the animal and population levels. In contrast, most previous studies in large study populations use cohort-level analyses. My study has provided important novel information for several risk factors. Notably, I have shown that the effect of commingling or mixing before induction depends on timing, and on the animal's previous history of mixing. I have also shown that the effects of saleyard exposure depend on the timing relative to induction. I have demonstrated the importance of group dynamics and suggested management strategies to mitigate the effects of BRD through manipulation of mixing and group dynamics.

The use of the causal diagram-informed approach to estimate total effects enabled estimates to be obtained for all risk factors of interest, not just those that would have been included if a single automated parsimonious model had been reported. In addition, the estimated effects at the population level based on total effects represent

the total expected change in BRD incidence if the risk factor is removed from the target population (assuming the relationship is causal and the estimates unbiased). This contrasts with estimates from or based on 'traditional' parsimonious models; these may be total, partial or direct and thus represent the expected change in BRD risk if all other variables in the model are held constant which is not a realistic scenario. The causal-diagram informed approach explicitly considers confounding for each pathway, so conditional association bias is less likely than in a parsimonious model. The ability to estimate direct effects for variables where this effect was of particular interest was an additional benefit. For these risk factors, it was possible to tease out which of the proposed causal pathways were important.

The use of Bayesian modelling facilitated the fitting of four-level models which more realistically account for the complex hierarchical structure of the population than simpler models. These could not always be fitted using maximum likelihood methods; most four-level logistic models attempted in the preliminary analyses stage using the *xtmelogit* function in Stata® did not run. Comparison of results between software packages provided evidence that the models were robust. Understanding of biological and causal pathways was enhanced by comparison of total and direct effects. Use of multilevel random effects models allowed for the determination of effect estimates while accounting for the dependencies at several hierarchical levels. Because the distributions of the outcome and many of the exposures were unbalanced and clustered at the feedlot and cohort-levels, it was important to adequately account for the natural hierarchy in the data during the modelling process. Using this approach, I modelled a complex disease where both exposure variables and BRD occurrence were clustered at higher levels.

The combination of the collection of high quality data about numerous potential confounders, *a priori* consideration of plausible biological pathways through the use of causal diagrams and advanced multilevel modelling techniques would be expected to minimise the effects of confounding in these studies. It has been reported that including random effects for a true cluster-level confounder completely removes the effects of that confounding (Dohoo, 2014), although other authors suggest this is not the case for mixed effects logistic models because, the random effects are assumed to be independent of the covariates, but unobserved

confounders are by definition correlated with the covariates in the model (Rabe-Hesketh and Skrondal, 2012).

The case definition of BRD used for analyses appeared to be robust because the number of non-specific diagnoses was small. Hence, their classification as BRD cases would have had little impact on the results.

The collection of biological samples from a large population of animals over time is another important strength of the study. A large percentage of animals enrolled in the cohort study population (92.5%) had both induction and follow-up blood samples that were received and verified at the animal level; the majority of these comprised the sampling frames from which cases and controls were randomly selected. Hence, selection bias in the case-control study would be minimal. The case-control study included sufficient animals to provide good statistical power to investigate associations between animal-level serological associations and BRD. The collection of biological samples from a large population of animals over time also meant that BVDV-PI animals were able to be identified. Using these results in combination with the known prior group structure enabled determination of prior exposure status to PI animals and novel investigation of the effects of exposure to PI animals prior to induction. Availability of these samples also allowed me to describe other pathogens detected in Australian feedlot cattle.

12.5 Limitations of my studies

There are several limitations inherent in observational studies (Dohoo et al., 2009). The internal validity depends on whether the effect estimates from the study population are representative (apart from sampling error) of those in the source population. Potential sources of bias include confounding, selection bias and information bias (Dohoo, 2014).

While the modelling techniques used would be expected to largely account for confounding, residual confounding due to unknown, unmeasured or poorly measured confounders is possible. Uncontrolled confounding may have contributed to the observed increased risk in older animals in the vendor-questionnaire subset. This could have been confounded by other factors causing animals to be sent to feedlots at an older age; body condition was an unmeasured confounder that may have

influenced these estimates. If the causal diagram was incomplete or incorrect, then estimates may have been biased. Multilevel mixed effects logistic models assume independence of the random effects and the covariates in the model and biased estimates may result if that assumption is violated (Rabe-Hesketh and Skrondal, 2012). For example, the unexpected strong positive association between the administration of Rhinogard™ vaccine against BoHV-1 at induction and risk of BRD was attributed to dependency between the feedlot-level random effects and the administration of Rhinogard™. Feedlots with a traditionally higher incidence of BRD might be expected to be more likely to use Rhinogard™ in subsequent cohorts.

For a study to be free of selection bias, the effect estimates from the study population needs to be representative of those in the source population (Dohoo, 2014). In my study, the source and target populations were both medium to large Australian feedlots. Because the owners or managers of the majority of these feedlots were invited to participate, my source population closely resembled the target population. Although only 14 feedlots participated and selection bias was possible, I believe the participating feedlots were broadly representative of the source and target populations so selection bias at feedlot level should have been minimal. There is the potential that selection bias was introduced in the selection of cohorts because these were not randomly selected as was initially planned. Based on communication with feedlot managers, it was clear that cohort selection was often based on ‘convenience’ (i.e. from a logistical perspective, it was more convenient for them to induct cattle into the study when labour was available and sometimes feedlot veterinarians collected the blood samples so cohorts were selected to coincide with planned visits). Inspection of limited data provided by feedlots about which other cohorts were inducted at the feedlot during the same week or fortnight revealed no systematic differences.

Selection bias may also have impacted results derived from subset analyses (i.e. vendor questionnaire subsets and the preassembly subset). Vendors that completed the questionnaire may have differed in important ways from those that did not return the questionnaire. For example, prevalences of exposure to practices such as yard weaning and prior vaccination in the target population may have been overestimated; this would have resulted in an underestimation of the population-level effects of these risk factors. The observed higher incidence of BRD amongst

vendor-bred animals indicated that these animals were at increased risk compared to the general population; this was not surprising given that this subset was relatively unmixed prior to day -27 and that mixing was strongly associated with BRD risk. If this population was at increased risk of BRD compared to the source population, and the distributions of exposures (i.e. yard weaning and prior vaccination) differed from the source population, it is possible that practices such as yard weaning and prior vaccination resulted in a greater effect in this population than in animals that had acquired immunity through a greater level of commingling prior to day-27. On the other hand, if the prevalence of exposure was higher than that in the source population (i.e. farmers who completed the questionnaire were more likely to yard wean or vaccinate), the population-level effects (PAFs and PARs) may have been underestimated. However, because non-response to the vendor questionnaire was likely to be a surrogate for other unmeasured factors, any resulting bias would be expected to be minimal (Dohoo, 2014).

The effects of timing of the move to the feedlot were estimated for the main cohort population, but the low-risk reference category comprised animals only from preassembly feedlots. Hence, I was concerned that these estimates may have been biased. The BRD incidence in the preassembly subset was much lower than in the main cohort study population. To assess the possible effects of this, an analysis was performed restricted to this subset and additional covariates were included in the model that were postulated to influence the management decision about how long cattle were kept on pasture prior to induction. These results were consistent with those obtained from the main cohort dataset, suggesting that the estimates were valid.

Bias also needs to be considered due to misclassification of both exposure and outcome variables. The study definition of mixing referred specifically to between-PIC mixing among animals enrolled in the study. It is possible that some animals in the study mixed with animals from other PICs that were not in the study. Therefore, there is likely to be some misclassification of animals as not mixed when they were in fact mixed, but not the reverse. Provided such misclassification was non-differential with respect to BRD status, this will have biased effect estimates for mixing towards the null (i.e. the true effects of mixing history are likely to be greater than those reported). Similarly, prior exposure to PI animals in an animal's group-28

was subject to misclassification error. Many more animals may have been previously exposed than I was able to determine from the limited data available. Again, assuming this misclassification was non-differential, the resulting misclassification bias would result in effect estimates biased towards the null. Hence, my findings about no evident effects of prior exposure to PI animals on BRD risk are not definitive.

A further limitation of all observational studies reliant on clinical diagnosis of disease is the potential for misclassification of cases. Some of the variation in BRD incidence between feedlots and cohorts may have been due to differences in factors such as diagnostic criteria, experience and management protocols among feedlot personnel. However, feedlots with high BRD incidences also tended to also have a higher BRD mortality risk, demonstrating that these higher incidences were not largely due to misclassification of healthy animals as BRD cases.

Measurement error may also have impacted the findings. Several variables were measured at higher levels. The quality of the analyses variables derived from these may not have been sufficient to detect an effect. Hence many results related to cohort-level variables for example were inconclusive. For example, the crude summary weather variables used were not of sufficient quality to detect the lagged effects of changes in weather conditions over time on BRD incidence.

A further limitation was that because only 14 feedlots participated, statistical power to detect the effects of higher-level risk factors was limited. This also resulted in limited power to determine the effects of many cohort-level risk factors that were clustered by feedlot. The sample size calculations for the number of cohorts required to estimate the effects of cohort-level risk factors was based on an intra-class correlation coefficient of 0.1 which was derived from retrospective analysis of data from three feedlots. This was markedly lower than the observed cohort intra-class correlation coefficient from the null model of 0.47. It is likely that these three feedlots were more similar to each other than three randomly selected feedlots would have been, so the extent of variability between feedlots was less than across the whole population. This would have had a large effect on the true power of the study compared to the expected power. After accounting for this and the true average cohort size (207 rather than 235 animals), the actual required sample size to have

had the desired precision to estimate cohort-level effects would have been four times greater. Such a study would have been neither logistically feasible, nor financially viable. However, this does in part explain my inability to reach conclusions about some of the putative cohort-level risk factors due to lack of precision of estimated odds ratios.

12.6 Overall conclusion

A large number of important risk factors for BRD have been identified and quantified and their relative importance at the population level has been assessed. The majority of these findings are biologically plausible and support industry belief and prior literature. In addition, several novel associations have been identified or better quantified.

Substantially reducing the incidence of BRD in feedlot cattle requires a holistic approach. Many important risk factors have been identified; to substantially reduce BRD incidence in Australian feedlot cattle would require that the important management-related risk factors are addressed. The relatively modest effect of serological risk factors means that vaccination alone is unlikely to achieve this. Knowledge of important risk factors for purchase groups of cattle could be used to predict BRD risk for these groups. Several of the identified management strategies need to be considered in the framework of other feedlot operational objectives and cost-effectiveness analyses.

13 List of References

ABS. 2005. *Australia's beef cattle industry* [Online]. Australian Bureau of Statistics. Available: http://www.abs.gov.au/AUSSTATS/abs@.nsf/Previousproducts/1301.0Feature%20Article232005?opendocument&tabname=Summary&prodno=1301.0&issue=2005&num=&view= [Accessed December 12 2014].

AICH, P., BABIUK, L. A., POTTER, A. A. & GRIEBEL, P. 2009. Biomarkers for prediction of bovine respiratory disease outcome. *Omics a Journal of Integrative Biology,* 13**,** 199-209.

AICH, P., JALAL, S., CZUBA, C., SCHATTE, G., HERZOG, K., OLSON, D. J. H., ROSS, A. R. S., POTTER, A. A., BABIUK, L. A. & GRIEBEL, P. 2007. Comparative approaches to the investigation of responses to stress and viral infection in cattle. *Omics a Journal of Integrative Biology,* 11**,** 413-34.

ALEXANDER, B. H., MACVEAN, D. W. & SALMAN, M. D. 1989. Risk-factors for lower respiratory-tract disease in a cohort of feedlot cattle. *Journal of the American Veterinary Medical Association,* 195**,** 207-211.

ALFA. Available: www.feedlots.com.au.

ALFA. 2011. *About The Feedlot Industry* [Online]. Available: http://www.feedlots.com.au/index.php?option=com_content&view=article&id=67&Itemid=111.

ALFA 2014. ALFA/MLA feedlot survey.

ALLEN, J. W., VIEL, L., BATEMAN, K. G., NAGY, E., ROSENDAL, S. & SHEWEN, P. E. 1992. Serological titers to bovine herpesvirus-1, bovine viral diarrhea virus, parainfluenza-3 virus, bovine respiratory syncytial virus and pasteurella-haemolytica in feedlot calves with respiratory-disease - associations with bacteriological and pulmonary cytological variables. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 56**,** 281-288.

AMRINE, D. E., WHITE, B. J. & LARSON, R. L. 2014. Comparison of classification algorithms to predict outcomes of feedlot cattle identified and treated for bovine respiratory disease. *Computers and Electronics in Agriculture,* 105**,** 9-19.

APPLEBY 1995. Risk Factors for Bovine Respiratory Disease in a New South Wales Feedlot.

ARTHINGTON, J. D., QIU, X., COOKE, R. F., VENDRAMINI, J. M. B., ARAUJO, D. B., CHASE, C. C. & COLEMAN, S. W. 2008. Effects of preshipping management on measures of stress and performance of beef steers during feedlot receiving. *Journal of Animal Science,* 86**,** 2016-2023.

ARTHINGTON, J. D., SPEARS, J. W. & MILLER, D. C. 2005. The effect of early weaning on feedlot performance and measures of stress in beef calves. *Journal of Animal Science,* 83**,** 933-939.

BABCOCK, A. H., CERNICCHIARO, N., WHITE, B. J., DUBNICKA, S. R., THOMSON, D. U., IVES, S. E., SCOTT, H. M., MILLIKEN, G. A. & RENTER, D. G. 2013a. A multivariable assessment quantifying effects of cohort-level factors associated with combined mortality and culling risk in cohorts of U.S. commercial feedlot cattle. *Preventive Veterinary Medicine,* 108**,** 38-46.

BABCOCK, A. H., RENTER, D. G., WHITE, B. J., DUBNICKA, S. R. & SCOTT, H. M. 2010. Temporal distributions of respiratory disease events within cohorts of feedlot cattle and associations with cattle health and performance indices. *Preventive Veterinary Medicine,* 97**,** 198-219.

BABCOCK, A. H., WHITE, B. J., RENTER, D. G., DUBNICKA, S. R. & SCOTT, H. M. 2013b. Predicting cumulative risk of bovine respiratory disease complex (BRDC) using feedlot arrival data and daily morbidity and mortality counts. *Canadian Journal of Veterinary Research,* 77**,** 33-44.

BACHOFEN, C., STALDER, H., BRAUN, U., HILBE, M., EHRENSPERGER, F. & PETERHANS, E. 2008. Co-existence of genetically and antigenically diverse bovine viral diarrhoea viruses in an endemic situation. *Veterinary Microbiology,* 131**,** 93-102.

BHUDEVI, B. & WEINSTOCK, D. 2001. Fluorogenic RT–PCR assay (TaqMan) for detection and classification of bovine viral diarrhea virus. *Veterinary Microbiology,* 83**,** 1-10.

BHUDEVI, B. & WEINSTOCK, D. 2003. Detection of bovine viral diarrhea virus in formalin fixed paraffin embedded tissue sections by real time RT-PCR (Taqman). *Journal of Virological Methods,* 109**,** 25-30.

BLACKALL, P. J., ANGEN, O., FEGAN, N., BLACKALL, L. L., MUTTERS, R. & BISGAARD, M. 2001. Characterisation of a novel Mannheimia sp from Australian feedlot cattle. *Australian Veterinary Journal,* 79**,** 634-639.

BOOKER, C. W., ABUTARBUSH, S. M., MORLEY, P. S., GUICHON, P. T., WILDMAN, B. K., JIM, G. K., SCHUNICHT, O. C., PITTMAN, T. J., PERRETT, T., ELLIS, J. A., APPLEYARD, G. & HAINES, D. M. 2008a. The effect of bovine viral diarrhea virus infections on health and performance of feedlot cattle. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 49**,** 253-260.

BOOKER, C. W., ABUTARBUSH, S. M., MORLEY, P. S., JIM, G. K., PITTMAN, T. J., SCHUNICHT, O. C., PERRETT, T., WILDMAN, B. K., FENTON, R. K., GUICHON, P. T. & JANZEN, E. D. 2008b. Microbiological and histopathological findings in cases of fatal bovine respiratory disease of feedlot cattle in Western Canada. *Canadian Veterinary Journal,* 49**,** 473-81.

BOOKER, C. W., GUICHON, P. T., JIM, G. K., SCHUNICHT, O. C., HARLAND, R. J. & MORLEY, P. S. 1999. Seroepidemiology of undifferentiated fever in feedlot calves in western Canada. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 40**,** 40-48.

BROWN-BRANDL, T. M., EIGENBERG, R. A. & NIENABER, J. A. 2006. Heat stress risk factors of feedlot heifers. *Livestock Science,* 105**,** 57-68.

BROWN-BRANDL, T. M. & JONES, D. D. 2011. Feedlot cattle susceptibility to heat stress: An animal-specific model. *Transactions of the Asabe,* 54**,** 583-598.

BROWNE, W. J. 2012. MCMC Estimation in MLwiN, Version 2.26. Bristol: Centre for Multilevel Modelling, University of Bristol.

BUCKHAM SPORER, K. R., BURTON, J. L., EARLEY, B. & CROWE, M. A. 2007. Transportation stress in young bulls alters expression of neutrophil genes important for the regulation of apoptosis, tissue remodeling, margination, and anti-bacterial function. *Veterinary Immunology and Immunopathology,* 118**,** 19-29.

BUCKHAM SPORER, K. R., WEBER, P. S. D., BURTON, J. L., EARLEY, B. & CROWE, M. A. 2008. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. *Journal of Animal Science,* 86**,** 1325-1334.

BUSBY, W. D., STROHBEHN, D. R., BEEDLE, P. AND KING, M. 2006. "Effect of Disposition on Feedlot Gain and Quality Grade".

CALLAN, R. J. & GARRY, F. B. 2002. Biosecurity and bovine respiratory disease. *Veterinary Clinics of North America: Food Animal Practice,* 18**,** 57-77.

CASWELL, J. L., BATEMAN, K. G., CAI, H. Y. & CASTILLO-ALCALA, F. 2010. Mycoplasma bovis in Respiratory Disease of Feedlot Cattle. *Veterinary Clinics of North America-Food Animal Practice,* 26**,** 365-+.

CERNICCHIARO, N., RENTER, D. G., WHITE, B. J., BABCOCK, A. H. & FOX, J. T. 2012. Associations between weather conditions during the first 45 days after feedlot arrival and daily respiratory disease risks in autumn-placed feeder cattle in the United States. *Journal of Animal Science,* 90**,** 1328-1337.

CERNICCHIARO, N., WHITE, B. J., RENTER, D. G. & BABCOCK, A. H. 2013. Evaluation of economic and performance outcomes associated with the number of treatments after an initial diagnosis of bovine respiratory disease in commercial feeder cattle. *American Journal of Veterinary Research,* 74**,** 300-9.

CERNICCHIARO, N., WHITE, B. J., RENTER, D. G., BABCOCK, A. H., KELLY, L. & SLATTERY, R. 2012a. Associations between the distance traveled from sale barns to commercial feedlots in the United States and overall performance, risk of respiratory disease, and cumulative mortality in feeder cattle during 1997 to 2009. *Journal of Animal Science,* 90**,** 1929-1939.

CERNICCHIARO, N., WHITE, B. J., RENTER, D. G., BABCOCK, A. H., KELLY, L. & SLATTERY, R. 2012b. Effects of body weight loss during transit from sale barns to commercial feedlots on health

and performance in feeder cattle cohorts arriving to feedlots from 2000 to 2008. *Journal of Animal Science,* 90**,** 1940-1947.

CHECKLEY, S. L., CAMPBELL, J. R., CHIRINO-TREJO, M., JANZEN, E. D. & WALDNER, C. L. 2010. Associations between antimicrobial use and the prevalence of antimicrobial resistance in fecal Escherichia coli from feedlot cattle in western Canada. *The Canadian Veterinary Journal,* 51**,** 853-861.

CHESTERTON, R. N., PFEIFFER, D. U., MORRIS, R. S. & TANNER, C. M. 1989. Environmental and behavioral-factors affecting the prevalence of foot lameness in New Zealand dairy herds - a case-control study. *New Zealand Veterinary Journal,* 37**,** 135-142.

CHIRASE, N. K., GREENE, W., PURDY, C. W., LOAN, R. W., AUVERMANN, B. W., PARKER, D. B., WALBORG, E. F., STEVENSON, D. E., XU, Y. & KLAUNIG, J. E. 2004. Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. *American Journal of Veterinary Research,* 65**,** 860-864.

COLDITZ, I. G., WATSON, D. L., KILGOUR, R., FERGUSON, D. M., PRIDEAUX, C., RUBY, J., KIRKLAND, P. D. & SULLIVAN, K. 2006. Impact of animal health and welfare research within the CRC for Cattle and Beef Quality on Australian beef production. *Australian Journal of Experimental Agriculture,* 46**,** 233-244.

COLE, N. A., CAMP, T. H., ROWE, L. D., JR., STEVENS, D. G. & HUTCHESON, D. P. 1988. Effect of transport on feeder calves. *Am J Vet Res,* 49**,** 178-83.

COLLINS, M. E., HEANEY, J., THOMAS, C. J. & BROWNLIE, J. 2009. Infectivity of pestivirus following persistence of acute infection. *Veterinary Microbiology,* 138**,** 289-296.

CONFER, A. W. 2009. Update on bacterial pathogenesis in BRD. *Animal Health Research Reviews,* 10**,** 145-8.

CORBEIL, L. B. 2007. Histophilus somni host-parasite relationships. *Animal Health Research Reviews,* 8**,** 151-60.

CUSACK, P., MCMENIMAN, N., RABIEE, A. & LEAN, I. 2009. Assessment of the effects of supplementation with vitamin E on health and production of feedlot cattle using meta-analysis. *Preventive Veterinary Medicine,* 88**,** 229-46.

CUSACK, P. M. V. 2004. Effect of mass medication with antibiotics at feedlot entry on the health and growth rate of cattle destined for the Australian domestic market. *Australian Veterinary Journal,* 82**,** 154-156.

CUSACK, P. M. V., MCMENIMAN, N. P. & LEAN, I. J. 2007. Feedlot entry characteristics and climate: their relationship with cattle growth rate, bovine respiratory disease and mortality. *Australian Veterinary Journal,* 85**,** 311-6.

DAVIS, M. S., MADER, T. L., HOLT, S. M. & PARKHURST, A. M. 2003. Strategies to reduce feedlot cattle heat stress: Effects on tympanic temperature1,2,3. *Journal of Animal Science,* 81**,** 649-61.

DOHOO, I., MARTIN, W. & STRYHN, H. 2003. *Veterinary epidemiologic research,* Charlottetown, University of Prince Edward Island.

DOHOO, I., MARTIN, W. & STRYHN, H. 2009. *Veterinary Epidemiologic Research*.

DOHOO, I. R. 2014. Bias--is it a problem, and what should we do? *Preventive Veterinary Medicine,* 113**,** 331-7.

DUFF, G. C. & GALYEAN, M. L. 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. *Journal of Animal Science,* 85**,** 823-40.

DUNN, S., GODWIN, J., HOARE, R. & KIRKLAND, P. D. 1993. Diseases of Feedlot Cattle - DAN.064. Meat Research Corporation.

DURHAM, P. J. K., HASSARD, L. E. & VANDONKERSGOED, J. 1991. Serological studies of infectious bovine-rhinotracheitis, parainfluenza-3, bovine viral diarrhea, and bovine respiratory syncytial viruses in calves following entry to a bull test station. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 32**,** 427-429.

DURHAM, P. J. K. & PAINE, G. D. 1997. Serological survey for antibodies to infectious agents in beef cattle in northern South Australia. *Australian Veterinary Journal,* 75**,** 139-140.

ELAM, N. A., THOMSON, D. U. & GLEGHORN, J. F. 2008. Effects of long- or short-term exposure to a calf identified as persistently infected with bovine viral diarrhea virus on feedlot performance of freshly weaned, transport-stressed beef heifers. *Journal of Animal Science,* 86**,** 1917-24.

ELLIS, J. A. 2009. Update on viral pathogenesis in BRD. *Animal Health Research Reviews,* 10**,** 149-53.

ELLIS, J. A. 2010. Bovine Parainfluenza-3 Virus. *Veterinary Clinics of North America-Food Animal Practice,* 26**,** 575-+.

FELL, L. R., COLDITZ, I. G., WALKER, K. H. & WATSON, D. L. 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. *Australian Journal of Experimental Agriculture,* 39**,** 795-802.

FELL, L. R., WALKER, K. H., REDACLIFF, L. A. & DAVIES, L. 2002. Reducing feedlot costs by preboosting: a tool to improve the health and adaptability of feedlot cattle. Sydney: Meat Research Corporation.

FULTON, R. W., BLOOD, K. S., PANCIERA, R. J., PAYTON, M. E., RIDPATH, J. F., CONFER, A. W., SALIKI, J. T., BURGE, L. T., WELSH, R. D., JOHNSON, B. J. & RECK, A. 2009a. Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. *Journal of Veterinary Diagnostic Investigation,* 21**,** 464-77.

FULTON, R. W., D'OFFAY, J. M. & EBERLE, R. 2013. Bovine herpesvirus-1: Comparison and differentiation of vaccine and field strains based on genomic sequence variation. *Vaccine,* 31**,** 1471-1479.

FULTON, R. W., HESSMAN, B., JOHNSON, B. J., RIDPATH, J. F., SALIKI, J. T., BURGE, L. J., SJEKLOCHA, D., CONFER, A. W. & FUNK, R. A. 2006. Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of subtypes 1a, 1b, and 2a in persistently infected cattle entering a feedlot. *Javma-Journal of the American Veterinary Medical Association,* 228**,** 578-584.

FULTON, R. W., HESSMAN, B. E., RIDPATH, J. F., JOHNSON, B. J., BURGE, L. J., KAPIL, S., BRAZIEL, B., KAUTZ, K. & RECK, A. 2009b. Multiple diagnostic tests to identify cattle with Bovine viral diarrhea virus and duration of positive test results in persistently infected cattle. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 73**,** 117-124.

FULTON, R. W., PURDY, C. W., CONFER, A. W., SALIKI, J. T., LOAN, R. W., BRIGGS, R. E. & BURGE, L. J. 2000. Bovine viral diarrhea viral infections in feeder calves with respiratory disease: Interactions with Pasteurella spp., parainfluenza-3 virus, and bovine respiratory syncytial virus. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 64**,** 151-159.

FULTON, R. W., RIDPATH, J. F., SALIKI, J. T., BRIGGS, R. E., CONFER, A. W., BURGE, L. J., PURDY, C. W., LOAN, R. W., DUFF, G. C. & PAYTON, M. E. 2002. Bovine viral diarrhea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 66**,** 181-190.

FULTON, R. W., STEP, D. L., WAHRMUND, J., BURGE, L. J., PAYTON, M. E., COOK, B. J., BURKEN, D., RICHARDS, C. J. & CONFER, A. W. 2011. Bovine coronavirus (BCV) infections in transported commingled beef cattle and sole-source ranch calves. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 75**,** 191-199.

GARDNER, B. A., DOLEZAL, H. G., BRYANT, L. K., OWENS, F. N. & SMITH, R. A. 1999. Health of finishing steers: Effects on performance, carcass traits, and meat tenderness. *Journal of Animal Science,* 77**,** 3168-3175.

GAUGHAN, J. B., BONNER, S., LOXTON, I., MADER, T. L., LISLE, A. & LAWRENCE, R. 2010. Effect of shade on body temperature and performance of feedlot steers1. *Journal of Animal Science,* 88**,** 4056-67.

GAUGHAN, J. B., BONNER, S. L., LOXTON, I. & MADER, T. L. 2013. Effects of chronic heat stress on plasma concentration of secreted heat shock protein 70 in growing feedlot cattle1. *Journal of Animal Science,* 91**,** 120-9.

GAUGHAN, J. B., MADER, T. L., HOLT, S. M. & LISLE, A. 2008. A new heat load index for feedlot cattle1. *Journal of Animal Science,* 86**,** 226-34.

GAY, E. & BARNOUIN, J. 2009. A nation-wide epidemiological study of acute bovine respiratory disease in France. *Preventive Veterinary Medicine,* 89**,** 265-271.

GLASS, E. J., BAXTER, R., LEACH, R. J. & JANN, O. C. 2011. Genes controlling vaccine responses and disease resistance to respiratory viral pathogens in cattle. *Veterinary Immunology and Immunopathology*.

GONZALEZ, L. A., SCHWARTZKOPF-GENSWEIN, K. S., BRYAN, M., SILASI, R. & BROWN, F. 2012. Factors affecting body weight loss during commercial long haul transport of cattle in North America. *Journal of Animal Science,* 90**,** 3630-9.

GREENLAND, S., PEARL, J. & ROBINS, J. M. 1999. Causal diagrams for epidemiologic research. *Epidemiology,* 10**,** 37-48.

GUMMOW, B. & MAPHAM, P. H. 2000. A stochastic partial-budget analysis of an experimental Pasteurella haemolytica feedlot vaccine trial. *Preventive Veterinary Medicine,* 43**,** 29-42.

HACZKU, A. & PANETTIERI, R. A., JR. 2010. Social stress and asthma: the role of corticosteroid insensitivity. *Journal of Allergy & Clinical Immunology,* 125**,** 550-8.

HÄGGLUND, S., HJORT, M., GRAHAM, D. A., ÖHAGEN, P., TÖRNQUIST, M. & ALENIUS, S. 2007. A six-year study on respiratory viral infections in a bull testing facility. *The Veterinary Journal,* 173**,** 585-593.

HAINES, D. M., MARTIN, K. M., CLARK, E. G., JIM, G. K. & JANZEN, E. D. 2001. The immunohistochemical detection of Mycoplasma bovis and bovine viral diarrhea virus in tissues of feedlot cattle with chronic, unresponsive respiratory disease and/or arthritis. *Canadian Veterinary Journal,* 42**,** 857-60.

HANLEY, J. A. 2001. A heuristic approach to the formulas for population attributable fraction. *Journal of Epidemiology & Community Health,* 55**,** 508-14.

HAY, K. E., BARNES, T. S., MORTON, J. M., CLEMENTS, A. C. A. & MAHONY, T. J. 2014. Risk factors for bovine respiratory disease in Australian feedlot cattle: Use of a causal diagram-informed approach to estimate effects of animal mixing and movements before feedlot entry. *Preventive Veterinary Medicine,* 117**,** 160-169.

HEALY, A. M., MONAGHAN, M. L., BASSET, H. F., GUNN, H. M., MARKEY, B. K. & COLLINS, J. D. 1993. Morbidity and mortality in a large irish feedlot; microbiological and serological findings in cattle with acute respiratory disease. *British Veterinary Journal,* 149**,** 549-560.

HESSMAN, B. E., FULTON, R. W., SJEKLOCHA, D. B., MURPHY, T. A., RIDPATH, J. F. & PAYTON, M. E. 2009. Evaluation of economic effects and the health and performance of the general cattle population after exposure to cattle persistently infected with bovine viral diarrhea virus in a starter feedlot. *American Journal of Veterinary Research,* 70**,** 73-85.

HEWSON, C. J., DOHOO, I. R. & LEMKE, K. A. 2006. Factors affecting the use of postincisional analgesics in dogs and cats by Canadian veterinarians in 2001. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 47**,** 453-459.

HICK, P. M., READ, A. J., LUGTON, I., BUSFIELD, F., DAWOOD, K. E., GABOR, L., HORNITZKY, M. & KIRKLAND, P. D. 2012. Coronavirus infection in intensively managed cattle with respiratory disease. *Australian Veterinary Journal,* 90**,** 381-386.

HODGSON, P. D., AICH, P., STOOKEY, J., POPOWYCH, Y., POTTER, A., BABIUK, L. & GRIEBEL, P. J. 2012. Stress significantly increases mortality following a secondary bacterial respiratory infection. *Veterinary Research,* 43**,** 21.

HOFF, P. D. 2009. A First Course in Bayesian Statistical Methods,. *In:* SPRINGER TEXTS IN STATISTICS (ed.).

HORWOOD, P. F. & MAHONY, T. J. 2011. Multiplex real-time RT-PCR detection of three viruses associated with the bovine respiratory disease complex. *Journal of Virological Methods,* 171**,** 360-363.

HORWOOD, P. F., SCHIBROWSKI, M. L., FOWLER, E. V., GIBSON, J. S., BARNES, T. S. & MAHONY, T. J. 2014. Is Mycoplasma bovis a missing component of the bovine respiratory disease complex in Australia? *Australian Veterinary Journal,* 92**,** 185-191.

HOSMER, J. D. W., LEMESHOW, S. & STURDIVANT, R. X. 2013a. Assessing the Fit of the Model. *Applied Logistic Regression.* John Wiley & Sons, Inc.

HOSMER, J. D. W., LEMESHOW, S. & STURDIVANT, R. X. 2013b. Logistic Regression Models for the Analysis of Correlated Data. *Applied Logistic Regression.* John Wiley & Sons, Inc.

LANYON, S. R., HILL, F. I., REICHEL, M. P. & BROWNLIE, J. 2014. Bovine viral diarrhoea: Pathogenesis and diagnosis. *The Veterinary Journal,* 199**,** 201-209.

LARSON, R. E. A new look at reducing infectious disease in feedlot cattle. Plains Nutrition Council Spring Conference, 2005 San Antonio, Texas. Texas A & M University.

LARSON, R. L. & STEP, D. L. 2012. Evidence-based effectiveness of vaccination against Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni in feedlot cattle for mitigating the incidence and effect of bovine respiratory disease complex. *Veterinary Clinics of North America - Food Animal Practice,* 28**,** 97-106.

LECKIE, G. & CHARLTON, C. 2013. runmlwin - A Program to Run the MLwiN Multilevel Modelling Software from within Stata. *Journal of Statistical Software,* 52**,** 40.

LONERAGAN, G. H., DARGATZ, D. A., MORLEY, P. S. & SMITH, M. A. 2001. Trends in mortality ratios among cattle in US feedlots. *Journal of the American Veterinary Medical Association,* 219**,** 1122-1127.

LONERAGAN, G. H., THOMSON, D. U., MONTGOMERY, D. L., MASON, G. L. & LARSON, R. L. 2005. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. *Journal of the American Veterinary Medical Association,* 226**,** 595-601.

MACARTNEY, J. E., BATEMAN, K. G. & RIBBLE, C. S. 2003a. Health performance of feeder calves sold at conventional auctions versus special auctions of vaccinated or conditioned calves in Ontario. *Journal of the American Veterinary Medical Association,* 223**,** 677-683.

MACARTNEY, J. E., BATEMAN, K. G. & RIBBLE, C. S. 2003b. Health performance of feeder calves sold at conventional auctions versus special auctions of vaccinated or conditioned calves in Ontario. *Journal of the American Veterinary Medical Association,* 223**,** 677-83.

MADER, T. L., DAHLQUIST, J. M., HAHN, G. L. & GAUGHAN, J. B. 1999. Shade and wind barrier effects on summertime feedlot cattle performance. *Journal of Animal Science,* 77**,** 2065-72.

MADER, T. L. & DAVIS, M. S. 2004. Effect of management strategies on reducing heat stress of feedlot cattle: feed and water intake. *Journal of Animal Science,* 82**,** 3077-87.

MADER, T. L., DAVIS, M. S. & BROWN-BRANDL, T. 2006. Environmental factors influencing heat stress in feedlot cattle1,2. *Journal of Animal Science,* 84**,** 712-9.

MADER, T. L., JOHNSON, L. J. & GAUGHAN, J. B. 2010. A comprehensive index for assessing environmental stress in animals1. *Journal of Animal Science,* 88**,** 2153-65.

MAHONY, T. J., MCCARTHY, F. M., GRAVEL, J. L., CORNEY, B., YOUNG, P. L. & VILCEK, S. 2005. Genetic analysis of bovine viral diarrhoea viruses from Australia. *Veterinary Microbiology,* 106**,** 1-6.

MARTIN, S. W. 1983. Factors influencing morbidity and mortality in feedlot calves in Ontario. *Veterinary Clinics of North America - Large Animal Practice,* 5**,** 73-86.

MARTIN, S. W., BATEMAN, K. G., SHEWEN, P. E., ROSENDAL, S. & BOHAC, J. E. 1989. The frequency, distribution and effects of antibodies, to seven putative respiratory pathogens, on respiratory disease and weight gain in feedlot calves in Ontario. *Canadian Journal of Veterinary Research,* 53**,** 355-62.

MARTIN, S. W., BATEMAN, K. G., SHEWEN, P. E., ROSENDAL, S., BOHAC, J. G. & THORBURN, M. 1990. A group level analysis of the associations between antibodies to seven putative pathogens

and respiratory disease and weight gain in Ontario feedlot calves. *Canadian Journal of Veterinary Research,* 54**,** 337-42.

MARTIN, S. W. & BOHAC, J. G. 1986. The association between serological titers in infectious bovine-rhinotracheitis virus, bovine virus diarrhea virus, parainfluenza-3 virus, respiratory syncytial virus and treatment for respiratory-disease in Ontario feedlot calves. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 50**,** 351-358.

MARTIN, S. W., DARLINGTON, G., BATEMAN, K. & HOLT, J. 1988. Undifferentiated bovine respiratory disease (shipping fever): Is it communicable? *Preventive Veterinary Medicine,* 6**,** 27-35.

MARTIN, S. W., HARLAND, R. J., BATEMAN, K. G. & NAGY, E. 1998a. The association of titers to Haemophilus somnus, and other putative pathogens, with the occurrence of bovine respiratory disease and weight gain in feedlot calves. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 62**,** 262-267.

MARTIN, S. W. & MEEK, A. H. 1986. A path model of factors influencing morbidity and mortality in Ontario feedlot calves. *Canadian Journal of Veterinary Research,* 50**,** 15-22.

MARTIN, S. W., MEEK, A. H., DAVIS, D. G., JOHNSON, J. A. & ., C. R. A. 1982. Factors associated with mortality and treatment costs in feedlot calves: the Bruce County Beef Project, years 1978, 1979, 1980. *Can J Comp Med. ,* 46**,** 341-349.

MARTIN, S. W., NAGY, E., ARMSTRONG, D. & ROSENDAL, S. 1999. The associations of viral and mycoplasmal antibody titers with respiratory disease and weight gain in feedlot calves. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 40**,** 560-+.

MARTIN, S. W., NAGY, E., SHEWEN, P. E. & HARLAND, R. J. 1998b. The association of titers to bovine coronavirus with treatment for bovine respiratory disease and weight gain in feedlot calves. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 62**,** 257-261.

MILLER, G., CHEN, E. & COLE, S. W. 2009. Health psychology: developing biologically plausible models linking the social world and physical health. *Annual Review of Psychology,* 60**,** 501-24.

MLA 2001. Survey of illnesses in feedlot cattle. Meat & Lifestock Australia.

MONTGOMERY, S. P., SINDT, J. J., GREENQUIST, M. A., MILLER, W. F., PIKE, J. N., LOE, E. R., SULPIZIO, M. J. & DROUILLARD, J. S. 2009. Plasma metabolites of receiving heifers and the relationship between apparent bovine respiratory disease, body weight gain, and carcass characteristics. *Journal of Animal Science,* 87**,** 328-33.

MOORE, S. J., O'DEA, M. A., PERKINS, N., BARNES, A. & O'HARA, A. J. 2014. Mortality of live export cattle on long-haul voyages: pathologic changes and pathogens. *Journal of Veterinary Diagnostic Investigation,* 26**,** 252-265.

MUGGLICOCKETT, N. E., CUNDIFF, L. V. & GREGORY, K. E. 1992. Genetic-analysis of bovine respiratory-disease in beef-calves during the 1st year of life. *Journal of Animal Science,* 70**,** 2013-2019.

NEIBERGS, H., ZANELLA, R., CASAS, E., SNOWDER, G. D., WENZ, J., NEIBERGS, J. S. & MOORE, D. 2011. Loci on Bos taurus chromosome 2 and Bos taurus chromosome 26 are linked with bovine respiratory disease and associated with persistent infection of bovine viral diarrhea virus. *Journal of Animal Science,* 89**,** 907-915.

NICKELL, J. S. & WHITE, B. J. 2010. Metaphylactic antimicrobial therapy for bovine respiratory disease in stocker and feedlot cattle. *Veterinary Clinics of North America - Food Animal Practice,* 26**,** 285-301.

NICKELL, J. S., WHITE, B. J., LARSON, R. L., RENTER, D. G., ROQUE, J., HESSE, R., OBERST, R., PEDDIREDDI, L. & ANDERSON, G. 2011. Onset and Duration of Transient Infections Among Antibody- Diverse Beef Calves Exposed to a Bovine Viral Diarrhea Virus Persistently Infected Calf. *International Journal of Applied Research in Veterinary Medicine,* 9**,** 29-39.

NIKUNEN, S., HÄRTEL, H., ORRO, T., NEUVONEN, E., TANSKANEN, R., KIVELÄ, S. L., SANKARI, S., AHO, P., PYÖRÄLÄ, S., SALONIEMI, H. & SOVERI, T. 2007. Association of bovine respiratory disease

with clinical status and acute phase proteins in calves. *Comparative Immunology, Microbiology and Infectious Diseases,* 30**,** 143-151.

NUNNERY, G. A., VASCONCELOS, J. T., PARSONS, C. H., SALYER, G. B., DEFOOR, P. J., VALDEZ, F. R. & GALYEAN, M. L. 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. *Journal of Animal Science,* 85**,** 2304-2313.

O'CONNOR, A., MARTIN, S. W., NAGY, E., MENZIES, P. & HARLAND, R. 2001. The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to bovine coronavirus and bovine viral diarrhea virus in 3 Ontario feedlots. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 65**,** 137-142.

O'CONNOR, A. M., SORDEN, S. D. & APLEY, M. D. 2005. Association between the existence of calves persistently infected with bovine viral diarrhea, virus and commingling on pen morbidity in feedlot cattle. *American Journal of Veterinary Research,* 66**,** 2130-2134.

ORRO, T., POHJANVIRTA, T., RIKULA, U., HUOVILAINEN, A., ALASUUTARI, S., SIHVONEN, L., PELKONEN, S. & SOVERI, T. 2011. Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus. *Comparative Immunology, Microbiology and Infectious Diseases,* 34**,** 23-29.

PANCIERA, R. J. & CONFER, A. W. 2010. Pathogenesis and Pathology of Bovine Pneumonia. *Veterinary Clinics of North America-Food Animal Practice,* 26**,** 191-+.

RABE-HESKETH, S. & SKRONDAL, A. 2012. *Multilevel and Longitudinal Modeling Using Stata*, Stata Press.

REINHARDT, C. D., BUSBY, W. D. & CORAH, L. R. 2009. Relationship of various incoming cattle traits with feedlot performance and carcass traits. *Journal of Animal Science,* 87**,** 3030-3042.

RÉRAT, M., ALBINI, S., JAQUIER, V. & HÜSSY, D. 2012. Bovine respiratory disease: Efficacy of different prophylactic treatments in veal calves and antimicrobial resistance of isolated Pasteurellaceae. *Preventive Veterinary Medicine,* 103**,** 265-273.

RIBBLE, C. S., MEEK, A. H., JANZEN, E. D., GUICHON, P. T. & JIM, G. K. 1995a. Effect of time of year, weather, and the pattern of auction market sales on fatal fibrinous pneumonia (shipping fever) in calves in a large feedlot in Alberta (1985-1988). *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 59**,** 167-172.

RIBBLE, C. S., MEEK, A. H., JIM, G. K. & GUICHON, P. T. 1995d. The pattern of fatal fibrinous pneumonia (shipping fever) affecting calves in a large feedlot in Alberta (1985-1988). *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 36**,** 753-757.

RIBBLE, C. S., MEEK, A. H., SHEWEN, P. E., GUICHON, P. T. & JIM, G. K. 1995c. Effect of pretransit mixing on fatal fibrinous pneumonia in calves. *Journal of the American Veterinary Medical Association,* 207**,** 616-619.

RIBBLE, C. S., MEEK, A. H., SHEWEN, P. E., JIM, G. K. & GUICHON, P. T. 1995b. Effect of transportation on fatal fibrinous pneumonia and shrinkage in calves arriving at a large feedlot. *Journal of the American Veterinary Medical Association,* 207**,** 612-615.

RIBBLE, C. S., SHOUKRI, M. M., MEEK, A. H. & WAYNE MARTIN, S. 1994. Clustering of fatal fibrinous pneumonia (shipping fever) in feedlot calves within transport truck and feedlot pen groups. *Preventive Veterinary Medicine,* 21**,** 251-261.

RICHESON, J. T., BECK, P. A., GADBERRY, M. S., GUNTER, S. A., HESS, T. W., HUBBELL, D. S. & JONES, C. 2008. Effects of on-arrival versus delayed modified live virus vaccination on health, performance, and serum infectious bovine rhinotracheitis titers of newly received beef calves. *Journal of Animal Science,* 86**,** 999-1005.

RICHESON, J. T., PINEDO, P. J., KEGLEY, E. B., POWELL, J. G., GADBERRY, M. S., BECK, P. A. & FALKENBERG, S. M. 2013. Association of hematologic variables and castration status at the time of arrival at a research facility with the risk of bovine respiratory disease in beef calves. *Journal of the American Veterinary Medical Association,* 243**,** 1035-41.

RIDPATH, J. 2010. The contribution of infections with bovine viral diarrhea viruses to bovine respiratory disease. *Veterinary Clinics of North America - Food Animal Practice,* 26**,** 335-48.

RIDPATH, J. F., FULTON, R. W., KIRKLAND, P. D. & NEILL, J. D. 2010. Prevalence and Antigenic Differences Observed between Bovine Viral Diarrhea Virus Subgenotypes Isolated from Cattle in Australia and Feedlots in the Southwestern United States. *Journal of Veterinary Diagnostic Investigation,* 22**,** 184-191.

ROEBER, D. L., SPEER, N. C., GENTRY, J. G., TATUM, J. D., SMITH, C. D., WHITTIER, J. C., JONES, G. F., BELK, K. E. & SMITH, G. C. 2001. Feeder Cattle Health Management: Effects on Morbidity Rates, Feedlot Performance, Carcass Characteristics, and Beef Palatability. *The Professional Animal Scientist,* 17**,** 39-44.

ROTHMAN, K. J. 2002. *Epidemiology : an introduction,* New York, Oxford University Press.

SACKET D, H. P., ABBOTT K ET AL 2006. Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers. . Sydney.

SAIF, L. J. 2010. Bovine respiratory coronavirus. *Veterinary Clinics of North America - Food Animal Practice,* 26**,** 349-64.

SANDERSON, M. W., DARGATZ, D. A. & WAGNER, B. A. 2008. Risk factors for initial respiratory disease in United States' feedlots based on producer-collected daily morbidity counts. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 49**,** 373-378.

SCHIPPER, C., CHURCH, T. & HARRIS, B. 1989. A review of the Alberta certified preconditioned feeder program (1980-1987). *The Canadian Veterinary Journal,* 30**,** 736-741.

SCHWARTZKOPF-GENSWEIN, K. S., BOOTH-MCLEAN, M. E., SHAH, M. A., ENTZ, T., BACH, S. J., MEARS, G. J., SCHAEFER, A. L., COOK, N., CHURCH, J. & MCALLISTER, T. A. 2007. Effects of pre-haul management and transport duration on beef calf performance and welfare. *Applied Animal Behaviour Science,* 108**,** 12-30.

SCHWARTZKOPF-GENSWEIN, K. S., MCALLISTER T. 2005. Metabolic consequences of feed intake: defining the individual. Plains Nutrition Council Spring Conference, 2005 San Antonio, Texas. Texas A&M Universtity, 47.

SHRIER, I. & PLATT, R. W. 2008. Reducing bias through directed acyclic graphs. *BMC Medical Research Methodology,* 8**,** 70.

SNIJDERS, T. & BOSKER, R. 2012. *Multilevel Analysis: an introduction to basic and advanced multilevel modeling,* London, Sage Publications.

SNOWDER, G. 2009. Genetics, environment and bovine respiratory disease. *Animal Health Research Reviews,* 10**,** 117-9.

SNOWDER, G. D., VAN VLECK, L. D., CUNDIFF, L. V. & BENNETT, G. L. 2006. Bovine respiratory disease in feedlot cattle: Environmental, genetic, and economic factors. *Journal of Animal Science,* 84**,** 1999-2008.

SOLÍS-CALDERÓN, J. J., SEGURA-CORREA, J. C., AGUILAR-ROMERO, F. & SEGURA-CORREA, V. M. 2007. Detection of antibodies and risk factors for infection with bovine respiratory syncytial virus and parainfluenza virus-3 in beef cattle of Yucatan, Mexico. *Preventive Veterinary Medicine,* 82**,** 102-110.

SOLIS-CALDERON, J. J., SEGURA-CORREA, V. M. & SEGURA-CORREA, J. C. 2005. Bovine viral diarrhoea virus in beef cattle herds of Yucatan, Mexico: seroprevalence and risk factors. *Preventive Veterinary Medicine,* 72**,** 253-62.

SOLIS-CALDERON, J. J., SEGURA-CORREA, V. M., SEGURA-CORREA, J. C. & ALVARADO-ISLAS, A. 2003. Seroprevalence of and risk factors for infectious bovine rhinotracheitis in beef cattle herds of Yucatan, Mexico. *Preventive Veterinary Medicine,* 57**,** 199-208.

SPIEGELHALTER, D. J., BEST, N. G., CARLIN, B. R. & VAN DER LINDE, A. 2002. Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society Series B-Statistical Methodology,* 64**,** 583-616.

SPIEGELMAN, D., HERTZMARK, E. & WAND, H. C. 2007. Point and interval estimates of partial population attributable risks in cohort studies: examples and software. *Cancer Causes & Control,* 18**,** 571-579.

STEP, D. L., KREHBIEL, C. R., DEPRA, H. A., CRANSTON, J. J., FULTON, R. W., KIRKPATRICK, J. G., GILL, D. R., PAYTON, M. E., MONTELONGO, M. A. & CONFER, A. W. 2008. Effects of commingling beef calves from different sources and weaning protocols during a forty-two-day receiving period on performance and bovine respiratory disease. *Journal of Animal Science,* 86**,** 3146-58.

SULLIVAN, M. L., CAWDELL-SMITH, A. J., MADER, T. L. & GAUGHAN, J. B. 2011. Effect of shade area on performance and welfare of short-fed feedlot cattle1. *Journal of Animal Science,* 89**,** 2911-25.

TAYLOR, J. D., FULTON, R. W., LEHENBAUER, T. W., STEP, D. L. & CONFER, A. W. 2010a. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? *Canadian Veterinary Journal,* 51**,** 1095-102.

TAYLOR, J. D., FULTON, R. W., LEHENBAUER, T. W., STEP, D. L. & CONFER, A. W. 2010b. The epidemiology of bovine respiratory disease: what is the evidence for preventive measures? *Canadian Veterinary Journal,* 51**,** 1351-9.

TAYLOR, L. F., BLACK, P. F., PITT, D. J., MACKENZIE, A. R., JOHNSON, S. J. & RODWELL, B. J. 2006. A seroepidemiological study of bovine pestivirus in Queensland beef and dairy herds conducted in 1994/95. *Australian Veterinary Journal,* 84**,** 163-168.

TAYLOR, L. F., VANDONKERSGOED, J., DUBOVI, E. J., HARLAND, R. J., VANDENHURK, J. V., RIBBLE, C. S. & JANZEN, E. D. 1995. The prevalence of bovine viral diarrhea virus-infection in a population of feedlot calves in western canada. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire,* 59**,** 87-93.

TEXTOR, J., HARDT, J. & KNUPPEL, S. 2011. DAGitty A Graphical Tool for Analyzing Causal Diagrams. *Epidemiology,* 22**,** 745-745.

TEXTOR, J., HARDT, J. & KNÜPPEL, S. 2013. *DAGitty v.2.0* [Online]. Available: http://www.dagitty.net/dags.html.

TEXTOR, J. & LISKIEWICZ, M. Adjustment criteria in causal diagrams: an algorithmic perspective. Proceedings of the 27th Conference on Uncertainty in Artificial Intelligence, 2011 Corvallis, OR. AUAI Press, 681-688.

THOMPSON, P. N., STONE, A. & SCHULTHEISS, W. A. 2006. Use of treatment records and lung lesion scoring to estimate the effect of respiratory disease on growth during early and late finishing periods in South African feedlot cattle. *Journal of Animal Science,* 84**,** 488-498.

THOMSON, D. U. Mortality in feedyard cattle. Plains Nutrition Council Spring Conference, 2005 San Antonio, Texas. A&M Universtity, 1.

THOMSON, D. U. & WHITE, B. J. 2006. Backgrounding beef cattle. *Veterinary Clinics of North America-Food Animal Practice,* 22**,** 373-+.

TOWNSEND, H. G., MEEK, A. H., LESNICK, T. G. & JANZEN, E. D. 1989. Factors associated with average daily gain, fever and lameness in beef bulls at the Saskatchewan Central Feed Test Station. *Canadian Journal of Veterinary Research,* 53**,** 349-54.

VAN DONKERSGOED, J., BERG, J. & HENDRICK, S. 2008. Comparison of florfenicol and tulathromycin for the treatment of undifferentiated fever in Alberta feedlot calves. *Veterinary Therapeutics,* 9**,** 275-81.

WALKER, K. H., FELL, L. R., REDDACLIFF, L. A., KILGOUR, R. J., HOUSE, J. R., WILSON, S. C. & NICHOLLS, P. J. 2007. Effects of yard weaning and training on the behavioural adaptation of cattle to a feedlot. *Livestock Science,* 106**,** 210-217.

WATTS, J. L. & SWEENEY, M. T. 2010. Antimicrobial Resistance in Bovine Respiratory Disease Pathogens: Measures, Trends, and Impact on Efficacy. *Veterinary Clinics of North America: Food Animal Practice,* 26**,** 79-88.

WHITE, B. J. & RENTER, D. G. 2009a. Bayesian estimation of the performance of using clinical observations and harvest lung lesions for diagnosing bovine respiratory disease in post-weaned beef calves. *Journal of Veterinary Diagnostic Investigation,* 21**,** 446-453.

WHITE, B. J. & RENTER, D. G. 2009b. Bayesian estimation of the performance of using clinical observations and harvest lung lesions for diagnosing bovine respiratory disease in post-weaned beef calves. *Journal of Veterinary Diagnostic Investigation,* 21**,** 446-53.

WIERINGA, F. L., CURTIS, R. A. & WILLOUGHBY, R. A. 1976. The influence of preconditioning on plasma corticosteroid levels, rectal temperatures and the incidence of shipping fever in beef calves. *The Canadian Veterinary Journal,* 17**,** 280-286.

WILDMAN, B. K., PERRETT, T., ABUTARBUSH, S. M., GUICHON, P. T., PITTMAN, T. J., BOOKER, C. W., SCHUNICHT, O. C., FENTON, R. K. & JIM, G. K. 2008. A comparison of 2 vaccination programs in feedlot calves at ultra-high risk of developing undifferentiated fever/bovine respiratory disease. *Canadian Veterinary Journal,* 49**,** 463-72.

WILSON, S. H., CHURCH, T. L. & ACRES, S. D. 1985. The influence of feedlot management on an outbreak of bovine respiratory-disease. *Canadian Veterinary Journal-Revue Veterinaire Canadienne,* 26**,** 335-341.

WOODS, G. T., MANSFIELD, M. E. & WEBB, R. J. 1973. A three year comparison of acute respiratory disease, shrink and weight gain in preconditioned an non-preconditioned Illinois beef calves sold at the same auction and mixed in a feedlot. *Can. J. comp. Med,* 37**,** 6.

14 Appendix 1: Data collection, validation and management and derivation of variables

14.1 Software and data management

The majority of study data were provided as Microsoft Excel® or ASCII text (comma-separated value format) files. Animal-level files were exported from feedlot management software; most feedlots used the StockaID^{TM} program and the remaining feedlots used custom software. For six feedlots, files were compiled in a central head office before being exported.

Original electronic files were stored on a regularly backed up shared drive to which the research team members had access. Any modifications to the data were saved in separate files in the same location, and following the data checking and verification procedure, import files were created for database import.

Data validation was performed using a combination of Microsoft Excel®, Microsoft Access® and the Stata® statistical software package (Version 12, StataCorp). A Microsoft® Access database was developed for storage and linkage of study data.

14.2 Data collection, validation and use

14.2.1 Feedlot management questionnaire

A feedlot management questionnaire was administered once by face-to-face interview at the start of the study. This semi-structured interview was used to determine feedlot-level management practices and typical descriptions and distributions of animal-entry characteristics for animals entering the feedlot. These data were used in combination with other data. For example, the feedlot map often provided pen and bunk dimensions, so this was used to estimate pen density and bunk space for the home pen for each cohort. In addition, these data were occasionally used (after confirming their validity with the feedlot manager) to determine feedlot-level values for variables where no other data were received. For example, some of the information requested in induction questionnaires for each arrival group forming the cohort was not provided because it was standard feedlot protocol (e.g. mixing groups or providing supplements upon arrival and before

induction); feedlot-level variables derived in this way were confirmed with managers during the study. The following list summarises the data collected at this interview:

- Typical profile of cattle entering feedlot
    - Who does the buying; what is the strategy?
    - Description of each cattle class used to classify incoming cattle: class name, average induction and final weight ranges, breed, sex, intended days on feed, what they are fed
    - Percentages from different purchase sources (e.g. paddock/direct, saleyard, custom cattle); is it possible to get history of saleyard-sourced cattle
    - Do you know in advance how many head will arrive when? If so – how far in advance?
    - Are animals bought as a group with feeding class in mind or split later and cohorts built up?
    - When does selection of cattle class occur?
- Typical movement of animals through the feedlot
    - Are groups mixed or split? When does this occur? Is this recorded?
    - Arrival
        - Where are they unloaded to; is this recorded?
        - Are they fed and watered upon arrival
    - Induction (i.e. processing, treatment, recording of animal-level details)
        - Timing in relation to arrival; do you wait until whole cohort is formed and then induct, or induct over multiple days?
        - Over what period is a cohort formed?
        - Are there ever any animals added after induction?
        - What are the typical induction procedures?

    - Do animals go straight to home pen after induction?
    - How does induction weight differ from arrival weight?
    - Which day do you call the first day on feed?
- Rations
    - Is all ration data available?
    - Which rations fed when?
- Pen Riding
    - What do your pen riders look for? How are they trained?
    - Does one person typically ride the same pen each day; is this possible for the study cohorts?
- Home pens
    - What percentages go through smaller and larger pens?
    - Are different pen sizes used for different cattle classes, or are pens based on availability at the time?
    - Are pen movements recorded?
    - Does any mixing or splitting occur when they change pens
    - How can we get this data?
- Treatment/hospitalisation
    - What data is recorded when animals goes through the hospital crush; weight, temperature, treatments?
    - Movement through hospital-where do animals go after examination in the hospital crush?
    - When/ on what basis are animals re-examined; where do they go?
    - What about chronically sick animals

- Draft(s) for selection for slaughter.
    - How many drafts are typical; from what period for each class?
    - Do you always draft out the heaviest or lightest?
    - What are stipulations for supply to abattoirs?
    - Are residual animals typically mixed with equivalent animals from another pen; if so can we obtain records of these movements?
- Are all animals slaughtered?

➢ Logistics

- Who will collect blood samples; is training required? (details required for Animal Ethics)
- Who will be responsible for administration and the provision of data?
- Are you happy for other participating feedlots to know that you are involved?
- Please provide a map of the feedlot (ideally with details of layout, pen dimensions, location of shade, feed bunks and water troughs)

14.2.2 Vendor questionnaire

Table 14-1 summarises the questions asked in the vendor questionnaire and indicates how these data were used in the study. Analysis variables derived from vendor questionnaire data are indicated in bold. Separate sections of the questionnaire collected data for cattle bred on the farm (i.e. ‘vendor bred’) and for cattle purchased by the vendor from another source. Questions relating to the period between weaning or purchase and sale and transport to the feedlot were asked for all cattle in the group sold to the feedlot.

Vendors had the option of completing an online questionnaire, returning a hard copy or participating in a telephone interview. Vendor questionnaires received in hard copy were entered into the online questionnaire so that all responses had equivalent electronic records.

14.2.3 Data collected from feedlots for each cohort

Feedlot staff provided group and cohort-level data in induction and post-slaughter questionnaires relating to each cohort. The main uses for induction questionnaire data (Table 14-2) were in identifying vendors and determining the intended number of days on feed and sometimes the first day on feed (if not supplied at the animal-level). The post-slaughter questionnaire aimed to collect cohort-level information and to provide a check list and prompt that appropriate data had been supplied for each cohort. For some feedlots the format of the cohort-level post-slaughter questionnaire was modified to better fit their reporting process. The aim was to collect the same information for all cohorts. Sometimes all cohort-level data (e.g. pen data, ration data) were provided as responses to the post-slaughter questionnaires, but often data were provided in separate electronic files. Details of group and cohort-level data provided and how they were utilised in the study are provided in Table 14-2, Table 14-3 and Table 14-4.

14.2.3.1 Ration data

Complete details of all ration compositions, nutrient analyses and pen diaries recording changes in rations were requested. I planned to determine the ME, neutral-detergent fibre and percentages of starch and roughage at different time points on feed. However, the quality and detail provided relating to rations varied substantially between feedlots; the supplied data ranged from nothing to complete detailed composition by ingredient with daily feeding routines of mixes of rations. Few rations had laboratory feed analyses results; those that did often did not relate directly to the study cohorts, so these data were not useful for statistical analyses.

Because ME density would be expected to be correlated with the percentage of the ration that was grain (‘grain percentage’) and possibly grain type, it was decided to focus on trying to get complete and consistent data for all cohorts for a more limited number of variables of interest. These were identified as grain percentage in rations over time, grain type, grain processing method, percentage roughage and the use of and type of rumen modifiers as shown in Table 14-3. The quality and detail of these simpler data as supplied varied considerably between feedlots.

14.2.3.2 Numbers of cattle on feed

At a late stage of the study, data were requested to determine the numbers of cattle on feed for the entire feedlot during time periods during which study animals were enrolled. These data were requested to assess whether the total number of cattle on feed, the total number of 'susceptible' cattle on feed and the short-term change in these numbers impacted on BRD risk. The population most at risk in the feedlot (i.e. 'susceptible cattle') was defined as animals less than 40 days on feed because the highest BRD risk is during this period (Babcock et al., 2010). To evaluate the effects of changes in numbers on feed, I aimed to determine monthly averages for each month beginning two months prior to the induction month of study cattle.

There was wide variation between feedlots in the quality of data provided concerning the numbers of cattle on feed, ranging from aggregated data for particular dates (three feedlots) to detailed running inventories of all cattle entering or leaving the feedlot (two feedlots). Where only aggregated monthly totals were provided the proportion of cattle that were less than 40 days on feed was estimated from the management questionnaire data detailing average days on feed for each class. The monthly averages for the total number of animals on feed and number of animals less than 40 days on feed were estimated for each feedlot for the duration of the study (Table 14-4).

14.2.4 Data collected from feedlots for each animal

Data provided by feedlots for each animal and how they were utilised is shown in Table 14-5 and Table 14-6.

14.2.5 Weather data

Although some study feedlots routinely monitored and provided weather data during the study, the high level of missing data limited the use of feedlot-sourced weather data. Therefore, weather data for all feedlots for the time period of interest were instead obtained from SILO (scientific information for land owners) database, which is a weather database hosted by the Queensland Government's Science Delivery Division of the Department of Science, Information Technology, Innovation and the Arts. Daily interpolated data for maximum temperature, minimum temperature and total rainfall were obtained from the SILO database. Wind data obtained for the Bureau of Meteorology were measured at the nearest weather station recording that

information. Maximum temperature, minimum temperature, temperature range, total rainfall and maximum wind gust speed were the final analysis variables used (Table 14-8).

14.2.6 National Livestock Identification System data

Table 14-8 lists data sourced from the National Livestock Identification System (NLIS) database. The geocodes (latitude and longitude) were obtained from the individual state NLIS co-ordinators. These data were used to derive complex analysis variables as described below

14.3 Data validation

Vendor questionnaire data were periodically downloaded, checked and compiled in Microsoft® Excel, before importing, saving and cross checking using Stata®. Induction and post-slaughter questionnaire data were extracted from the files provided and complied in Microsoft® Excel files structured according to the study hierarchy (i.e. feedlot, cohort, group or animal). For example, post-slaughter questionnaire data were extracted to cohort-level files. Supplied ration data were compiled and cross-checked with the aim of retaining as much detail as possible in the database and generating analysis variables that were common across feedlots. For example, where animals shared a common induction date, the calculation of the number of days until the animal was receiving 60% grain in the ration was based on cohort-level feeding regimens and grain percentages in rations, while where animals were added to the cohort over a period (i.e. for open cohorts), this variable was determined at the animal-level.

The questionnaire data compilation and validation process was hampered to some extent by delayed responses from feedlots in providing the data, and the provision of incomplete data. This was followed up on an ongoing basis. Sometimes, management practices were determined at the feedlot level (e.g. provision of electrolytes after arrival or use of rumen modifiers in the ration) so it was possible to fill missing values after confirming with the feedlot manager that management questionnaire responses were applicable to the study cohorts.

For each cohort, the animal-level files were used to track animals through cohorts to identify any discrepancies in a timely way to allow follow up with feedlots as required.

A Microsoft® Access database was created with queries designed to detect inconsistent, implausible or missing data. Microsoft® Excel files were imported into the database and the queries were run and output obtained via a Microsoft® Excel macro that produced summary files that were examined for inconsistencies. The main purpose of performing the check by cohort was to ensure that all animals inducted were accounted for at exit. Hospital, death and cattle movement records provided by the feedlots were checked to establish whether animals missing from the feedlot exit sessions could be accounted for in this way. Several animals had their NLIS identity tags replaced. In the majority of feedlots, animals also had at least one other identification tag, so it was easy to match visual identification numbers in animals where the tag had been lost. The range of dates for arrival, induction, follow-up, hospitalisation, death, exit and slaughter were also checked for consistency at this point.

Weight ranges within each cohort were noted and animal-level average daily gain was estimated from recorded weights at known time points (induction and follow-up). Individual animal weights measured in high-throughput conditions could be expected to have a moderate degree of measurement error. The cross-checking protocol aimed to consider all of the available data and retain the values considered most likely to be accurate. Detailed summaries of all weight variables revealed obvious errors such as weights below 150 kg. Details of all weight measures were inspected for animals with outlying values for induction weight or average daily gain. If a suitable proxy measure (e.g. pay weight measured at time of purchase) was consistent with other values (exit follow-up weight), these were substituted for missing or implausible induction weight values. Table 14-7 details how some examples of raw data for breed and cattle class were categorised. The final breed category analysis variable contained seven categories.

Table 14-1: Vendor questionnaire data collected and main use in study

Data	Use
Identity, address and contact details of vendor's farm; preferred contact method	Administration
Cattle group identifier (provided by research team at contact)	Data linkage
Age in months at marking/branding; month(s) of marking/ branding	**Estimated age at induction** (<16,16 to <22 or ≥22 months)
Month and age (in months) at weaning	
When purchased (month/year); age in months at purchase	
Average weight at purchase	
Most common breed in group	Cross checking
Were cattle castrated at marking/ branding? Were the cattle castrated at weaning?	
Were cattle dehorned at marking/ branding; were they a polled breed?; Were the cattle dehorned at weaning; were they already dehorned?	
Method of weaning (yard/paddock); if yard weaned, number of days kept in yards, type of feed provided and method of feeding	**Yard weaning** (yes <7 days, yes ≥7 days, no)
Number of times yarded/ handled between weaning/purchase and sale; reason for yarding/ handling	
Were the cattle kept in yards after purchase; if so, number of days?	
Were the cattle mixed at marking/ branding? Were the cattle mixed at weaning? Were the cattle mixed between weaning/purchase and sale?	**On-farm mixing** (yes/no)
Number of groups mixed in the period before yarding and transport/sale to the feedlot; number of months the group was together before sale.	
Number of vendors cattle in group purchased from (single/multiple); where were cattle purchased; sale type (weaner sales, saleyards, paddock) and location	
Were the cattle given Pestigard™/BovilisMH™ at weaning; if so approximate date given	**Prior vaccination with Pestigard** (yes/no)
For respiratory vaccines (i.e. Pestigard™, Rhinogard™ and BovilisMH™): date(s) vaccination(s) given between weaning/purchase and sale	
Did the group participate in pre-feedlot vaccination programme? (Feedergard I / Feedergard II / Feedlot Ready / None / Other (please specify)	**Prior vaccination with BovilisMH** (yes/no)
Dates cattle moved on/off native/improved pasture; type of native/improved pasture	
Ever given supplementary feed (i.e. grain, conserved forage, mineral supplement, other)? For each type of supplementary feed: dates fed (from/to), type of feed, method of feeding	**Prior grain feeding** (yes/no)
If yard weaned, type of feed provided and method of feeding	**Prior supplementary feeding** (yes/no)
Number of hours yarded prior to transport to the feedlot (<2, 2-4, 4-6, 6-8, >8)	
Was water available in the yards; if so, were electrolytes added to the water? Was feed available in the yards at the time of sale; if so, type of feed?	

Variables in bold were used in analyses

Table 14-2: Induction questionnaire data collected and main use in study

Data	Use
Other lots inducted over the induction period How many lots? For each lot: Cattle class, Number of head, Type and number of groups in lot (e.g. source)	To assess if inducted lots were similar to other lots placed on feed during induction period
Number of animals in each group Departure time (from property/saleyard) (from documentation that accompanies cattle being transported) Arrival time (if not known give earliest and latest times) Date(s) of any mixing from arrival until induction; Date(s) of any splitting from arrival until induction; Number of groups in study lot Any mixing at the time of induction? Any splitting at the time of induction?	Cross-checked against NLIS data High percentage of missing feedlot-supplied data about arrival and departure times; NLIS data were used to consistently estimate transport times and mixing variables for complete population. Mixing and splitting between arrival and induction tended to be feedlot-level and dates often not reported, so not used in analyses
Participation in any pre-feedlot program (from documentation that accompanies cattle being transported)	Cross checked against vendor questionnaire data
Feeding on arrival; if yes specify amount (kg/head) Water on arrival; if yes, were electrolytes added to the water Liquid supplement after arrival; If yes, specify (e.g. urea, molasses) Were liquid supplements given after induction; if yes specify	Feeding, watering and liquid supplements tended to be feedlot-level; not used in analyses
Were any animals from the study lot sick between arrival and induction?	Almost all ‘no’, not used
Which pen were they in after arrival?	
Date of first day on feed in a feedlot pen	Duration of First day on feed to day 0
Vendor details for each group of paddock sourced cattle	Vendor questionnaire contact
Who collected blood samples at induction?	Administration
Estimated exit date or total days on feed	Used with cattle class to derive Intended days on feed

Variables in bold were used in analyses

Table 14-3: Post-slaughter questionnaire data collected and main usage in study

Data	Use
Were any animals added to the trial lot after the induction period?	Cross-checked against animal-level records
Was there any mixing with other lots after drafts (give dates and details)?	
Did any animals leave the main trial lot or were transferred to another lot for any reason other than the originally intended market; if available, indicate which animals were removed, date they were removed and reason?	
Details of whole lot pen moves (date, pen moved from, pen moved to)	Used to determine home pen
Frequency of pen riding throughout time on feed.	**Pen riding frequency**; little variation (daily in most feedlots); not used in analyses
Dates when pen cleaned; cleaning protocol	Dates usually not provided; not used
Number of rations fed; number of days to final diet	**Time to 60% grain in ration**, **Percentage grain on day 0**, **Percentage grain on day 20**
Feeding routine: number of feeds per day, approximate timing and percentage fed at each feed; (specify if different for different rations)	
For each ration: date fed from, date fed to	
Ration composition: grain type, percentage grain, percentage fibre	Percentage fibre correlated with grain percentage and not consistently defined; not used
Method of grain processing	**Grain type** **Grain processing method**
Was a rumen modifier used? If yes, type?	Feedlot level; little variation; not used
Nutritional analyses: metabolisable energy, crude protein, percentage starch based concentrate,	Much missing data and feed analyses dates often did not correspond to cohort dates; not used
Were any supplements given in addition to the formulated ration (added to feed or water); if yes, please specify both supplement and when given	Feedlot level; not used
Was there any in-feed medication given to the trial lot; if yes, please indicate details & dates?	Generally no; not used

Variables in bold were used in analyses

Table 14-4: Data collected for each cohort or pen and main use in study

Data	Use
Name or number of pen as reported by the feedlot	Pen identifier for data linkage
Pen dimensions (length, width, surface area or plan with dimensions for irregular pens	Calculate surface area (m^2) and **Pen density**
Maximum capacity of pen	Cross-checked against surface area
Length of feed bunk (m)	**Bunk space** per head
Location of feed bunk within pen	
Length of water trough (meters)	
Location of water trough in pen	
Do animals outside the pen have access to the water trough?	**Shared pen water** (yes/ no)
Number of pens joining pen	**Pens joining** (1/2)
Is the pen shaded?	**Pen shade** (yes/ no); amounts and types of shade were generally feedlot level, so only a binary variable was used
If shaded, surface area covered by shade	
Detail of shade type/ material	
How often the pen is cleaned per year	**Pen cleaning frequency**: feedlot level, not used
Distance between the pen and the hospital pen	**Pen hospital distance**: correlated with feedlot capacity; not used
Direction of slope of ground	Inconsistently reported; not used
Amount of slope- %	
Number of cattle on feed at feedlot during the study	Average **Total cattle on feed** in induction month
Number of cattle less than 40 days on feed	Average **Number of cattle less than 40 days on feed** in induction month

Variables in bold were used in analyses

Table 14-5: Data provided by feedlots relating to each animal's identification, source and important dates (as applicable)

Raw data	Description	Use
Lot no.	Identifier used by feedlots to identify pens of cattle; usually a single lot comprised a cohort	Matched to study cohort identifiers
Tail tag	Number on tail tag (applied on farm prior to sale) which can be linked to the source PIC and vendor	Identified most recent source if NLIS ID was missing
SAN	Stock advice number:	Not used
Vendor	Identity of vendor- person/s or company (i.e. paddock, saleyard or preassembly)	Vendor questionnaire contacts
Immediate Source*	Origin of cattle immediately prior to induction	Cross-check against NLIS data
Animal ID	Number on NLIS ear tag; unique identifier	Identifier used to link all animal-level data
NLIS ID	Coded 16 character unique barcode used in the NLIS system, electronically recorded when device is scanned and can be decomposed into state, region, area and locality codes	In addition to Animal ID, other identifiers (NLIS ID, Visual ID, Induction sequence, Draft sequence) were used to verify samples and link animals to laboratory results. The visual ID was used as the linking unique identifier in place of the Animal ID for 2 animals with lost tags.
Visual ID	Identification tag used by feedlots- usually simpler than Animal ID, but may not be unique	
Induction sequence	Order animal put through crush and bled at induction. Some feedlots use numbered tags, so this may be an animal identifier.	
Draft Sequence	Order of sample collection at draft. However, many cohorts that inserted 'Induction number' ear tags at induction used these as identification numbers to collect samples in corresponding tubes.	
Induction Date	Date of Induction at feedlot	Baseline date for main cohort study: **Day 0**
Arrival Date	Date of arrival at feedlot	Dates were cross checked for consistency (against each other and against the NLIS data) and were used to define intervals to define intervals for inclusion criteria (e.g. **Time at risk**, sampling interval) or converted to categorical variables used in analyses (**Arrival to day 0**, **Day 0 to close**, **First day on feed to day 0**)
First Day on Feed	First day on feed at the feedlot	
Draft Date	Date of 42 day sample collection	
Exit Date	Date animal left feedlot	
Move Date	Date animal left cohort (if not recorded in hospital or death records)	
End Date	Assigned based on cohort level information where exit date was missing	
Session Date	Date of pen movement (usually whole cohort)	
Hospital Date	Date of event related to hospital session or hospital treatment	
Death Date	Date of death for animals that died at the feedlot	

Variables in bold were used in analyses

Table 14-6: Data provided by feedlots relating to each animal's physical attributes, diagnosis treatment, death or carcass records (as applicable)

Raw data	Description	Use
Breed	Recorded by feedlot at induction	**Breed category**
Sex	Steer / heifer	**Sex**, **Cohort sex**
Dentition	Number of permanent incisors present at induction	**Dentition**
Cattle class	May describe the anticipated number of days on feed, market and/or breed types	Partly used to define **Intended days on feed** variable
Induction weight	Weight recorded on induction day (day 0)	**Induction weight category** **Mean cohort weight** **Weight difference from mean cohort weight**
Arrival weight	Weight recorded at arrival in preassembly feedlots	Change in weight over time was used in cross checking induction weight. Sometimes pay weight or off-truck weight used as proxies for induction weight (if values were missing or unrealistic).
Pay weight	Weight of beast recorded at sale (used to calculate purchase price)	
Off Truck weight	Weight recorded at arrival.	
Draft weight	Individual weight recorded at draft session	
Exit weight	Weight at exit	
Hospital weight	Body weight recorded for hospitalised animals	
Induction product	Name of product administered or procedure performed at induction	**Rhinogard at induction** (yes/ no)
Induction dose	Amount of product given at induction	**Vitamin ADE** (yes/ no)
Pull reason	Reason animal was pulled from cohort	Cased definition (**BRD50**) was based on 'pull reason' and ailment (if provided)
Ailment	Diagnosis assigned by feedlot staff after examination of animal	
Temperature	Body temperature at hospital examination	
Hospital product	Name of medication used to treat animal	
Hospital dose	Amount of medication given	
Dead Reason	Reason for death as reported by feedlot	Reason for death
Autopsy	Records whether a post mortem was performed (yes/no)	
Autopsy Result	Reason for death as per autopsy result	Cross checked against dead reason
Died In	Which pen the animal died in	Differentiated 'pen deaths' from animals that died in the hospital pen
Carcass data	Kill date, carcass weight, fat, marbling, eye muscle area, firmness	Not used in current studies

Variables in bold were used in analyses

Table 14-7: Derivation and categories of the breed and Intended days on feed variables used in the final analyses.

Data	Examples	Category	Analysis variable
Breed			Breed category (Breed)
	Angus	Angus	
	Red Angus	Angus	
	Hereford	Hereford	
	Polled Hereford	Hereford	
	Shorthorn	Shorthorn	
	Murray Grey	Murray Grey	
	British cross	British cross	
	Angus X	British cross	
	Hereford X	British cross	
	British X European	European/ European cross	
	Limousin	European/ European cross	
	Simmental	European/ European cross	
	Charolais	European/ European cross	
	Gelbvieh	European/ European cross	
	European X	European/ European cross	
	Charbray	Tropical/Tropical cross	
	Santa Gertrudis	Tropical/Tropical cross	
	Droughtmaster	Tropical/Tropical cross	
	Braford	Tropical/Tropical cross	
	Brahman	Tropical/Tropical cross	
	Brangus	Tropical/Tropical cross	
	British/Tropical	Tropical/Tropical cross	
	British/Tropical/European	Tropical/Tropical cross	
	Tropical/European	Tropical/Tropical cross	
	Brahman / Brahman X	Tropical/Tropical cross	
	Wagyu		Excluded
	Unknown		Excluded
Cattle class			Intended days on feed (Intended DOF)
	150D ox	≥120	
	BB	85 to <120	
	60D domestic	≤85	

Table 14-8: NLIS data and weather data collected and main use in study

Data	Use
NLIS data	
Animal ID	Data linkage to animal
National livestock identification system unique identifier (NLIS ID)	Data linkage to animal
Property identification code (PIC): unique identifier used for farms, saleyards and feedlots	Data linkage to location; **source region**, **feedlot region**
PIC of issue (i.e. where NLIS device was first registered); usually this was the original PIC where the animal was born, but if the device had been replaced, it was the PIC where this occurred	PIC of origin
Source PIC- source PIC for transfers recorded in database	PIC locations, transfer dates and transfer types were used to determine saleyard exposure variables (**Saleyard pre-27**, **Saleyard -27 to -13** and **Saleyard -12 to 0**), **Group-#N**, **Mix first**, **Mixing history**, **Mix summary**
Destination PIC- destination PIC for transfers recorded in database	
Transfer date: date of animal transfer between PICs	
Transfer type: type of transfer between PICs (i.e. point to point, saleyard in or saleyard out)	
Name of saleyard	
Waybill number: Identification number of documentation that accompanies animals being transported	Cross checking and correcting data
Change of NLIS ID files (old NLIS ID, new NLIS ID)	Records of a change of NLIS ID; merging data allowed movement history to be determined
Latitude of PIC location	Latitude and longitude used for mapping and determining transport distances and durations and **timing of the move to the feedlot**
Longitude of PIC location	
Location, latitude longitude and altitude of nearest Bureau of Meteorology weather stations recording rainfall, wind or temperature measures	Used to assess how representative weather data may have been
Weather data	
Daily temperature maxima	**Mean daily maximum temperature** (over days 0 to 6)
	Mean temperature range (over days 0 to 6)
Daily temperature minima	**Mean daily minimum temperature** (over days 0 to 6)
Daily total rainfall	**Total rainfall** (over days 0 to 6)
Daily maximum wind speed	**Mean daily maximum wind speed** (over days 0 to 6)

Variables in bold were used in analyses
refers to days of interest relative to day 0

14.3.1 NLIS data validation

NLIS data were obtained for all but four study animals (three missing tags and one recorded Animal ID that did not match any record in the database). Hence, PIC of issue data were obtained for 99.99% (35,156) of the 35,160 animals enrolled into the study. For 340 of these 35,156 animals, the PIC of issue was the feedlot PIC and the prior identification numbers were unknown; thus it was not possible to establish mixing and moving histories from the NLIS data for 344 study animals. Records from the NLIS system were cross checked against the tail tag numbers recorded at induction. Often this allowed me to confirm the most recent source PIC and in some cases the movement history where all animals from that source shared the same PIC of issue which matched a single transfer from that PIC to the feedlot. A total of 34,788 animals (98.8%) had one or more recorded movements between PICs. Each transfer directly between two properties with different PICs was identified by a single 'point-to-point' record. In contrast, a transfer via a saleyard was recorded as two transfers ('saleyard in' and 'saleyard out'). The NLIS required that each animal's transfer history has no missing steps (i.e. that every transfer commences from the animal's most recent PIC before that transfer date). When this requirement is breached NLIS automatically imputes two additional intervening transfers via an unknown PIC, both with the date one day prior to the apparently illogical transfer, a transfer from the animal's most recent PIC before that transfer date to the unknown PIC , and a transfer from that unknown PIC to the known PIC (Table 14-9). The unknown PIC is listed as 'XXXXXXXX', and the unknown waybill numbers may be missing or listed as '1234567'. Of the original 109,987 transfers, in the raw data, 6.1% were imputed by the system, with 3,189 transfers with the source listed as 'XXXXXXXX', and 3,505 transfers with the destination listed as 'XXXXXXX'.

Data validation and correction involved consolidating and simplifying records to create a logical sequence for each animal from its PIC of issue to the feedlot PIC. Where possible, records were combined to form point to point moves, each with a single source and destination. Thus, moves involving saleyard transfers or other PICs where animals were held for less than 2 days were combined to form single point to point moves and editing was noted in a transfer detail variable that was added to the record. Similarly, each transfer with an unknown destination followed by a transfer with an unknown source were replaced by a single transfer between the

two known PICs (i.e. the PIC preceding and the PIC succeeding the intervening, unknown, PIC), with the transfer detail variable recording that the transfer was imputed by the NLIS system.

The number of days between each transfer and the animal's induction date (day 0) was determined and used to sequence transfers. For animals that had only valid transfer dates, the interval between each transfer and da y 0 was used to determine the animal's PIC location at each time point of interest. For transfers imputed by NLIS as described above, the dates were unlikely to be correct. Where animals with an imputed transfer were part of a group of animals that had otherwise identical transfers, the missing PIC locations common to the group were allocated based on known transfer dates for the other animals in the group. If no animals in the relevant group had recorded transfer dates, the imputed transfer date was allocated midway between the two adjoining known transfer dates. Where there was no transfer before the imputed transfer and no common group animals, the imputed transfer date was changed to 180 days prior to the known transfer date as this was more consistent with observed patterns among animals with complete records. Stata® program files were written to automate the NLIS data checking process as much as possible. The vast majority of data were either validated or corrected in this manner. Cohort-level cross tabulations of groups defined at different time points were then examined for inconsistencies. Where this occurred, raw data for groups of animals were examined to establish a 'most likely' scenario.

An example of how the validation and correction process proceeded is illustrated in Table 14-9. Steps 1-8 were executed by the Stata® program file and the last two steps required manual checking and the application of the 'most likely' scenario. The transfers in this example were common to a group of 7 animals. The process for these animals involved the following steps:

1. Delete records for transfers after induction (i.e. source PIC=feedlot PIC)
2. Determine whether the date of the move to the feedlot matched the arrival date
3. Convert saleyard moves to point-to-point moves, while retaining an indicator for saleyard transfer
4. Convert 'XXXXXXXX' to point to point moves, retaining in indicator for 'XXXXXXXX' moves

5. Determine the sequence of transfers for each animal
6. Change imputed transfer dates to missing and determine the intervals between each transfer and the animal's day 0
7. Check if any common group animals had known transfer dates for imputed transfers. Use these to determine group allocation at time points of interest.
8. Allocate remaining missing transfer dates based on assumed transfer dates midway between transfers or 180 days before a single known transfer
9. Examine records where the transfer interval remains at 1 day or where the sequence of moves appears illogical
10. Where records do not make sense, recheck against raw data and decide most logical sequence.

In the example in Table 14-9, the final recorded transfer was deleted because it was later than the arrival date. Transfers occurring on the same day were combined to form point to point transfers and the date of one point-to-point move was changed. On further checking, remaining moves within a one-day interval were identified and examination of the data revealed inconsistencies. In particular, the destination two days after the original move was the same as the original source (which matched the PIC of issue) and this move led to the imputation of another move within the NLIS database to get the animals to a destination PIC which was the same as the destination PIC in the original saleyard transfer. Assuming that saleyard transfers and transfers with a valid source PIC, destination PIC and waybill number are more likely to be correct, it was possible to reconstruct a 'most likely' scenario of transfers. From a total of nine entries in the raw data, the corrected data contained only two transfers, both with an identified source, destination, transfer date and waybill number.

After completion of the validation and correction process, of the 35,160 animals enrolled, 419 animals (1.2%) had no NLIS transfer records, and a further 414 (1.2%) had transfer records but these did not include the transfer to the feedlot. The remaining animals had NLIS records including the transfer to the feedlot PIC, although there were often single day discrepancies between the NLIS transfer date and the feedlot arrival date due to the timing of when records were entered into the respective systems. A total of 51 animals had records from the NLIS change of identification device files which enabled their movement histories to be established.

In the cleaned dataset, 2,387 of the 30,397 transfers (7.9%) that were not moves to the feedlot PIC were imputed by the NLIS system. Most often, these were due to an illogical sequence (i.e. a 'missing' intermediate PIC). Cross checking group-level values often enabled these transfer dates to be assigned based on common group move dates. Transfers close to the time of induction were of particular interest, so all imputed moves within 90 days of day 0 were examined in this way. Use of tail tag numbers (these were used to identify the most recent PIC prior to the feedlot move), NLIS ID (which included the PIC of issue) arrival groups and arrival dates supplied by the feedlots, along with the movement history of animals with common tail tag numbers usually enabled these animals' PIC sequences and dates of transfers to be identified with a high degree of confidence.

Table 14-9: Example of the data validation and correction process applied to a series of transfers common to seven animals.

a) Raw data from NLIS database				
Transfer Date	Source PIC*	Destination PIC*	Transfer Type*	Waybill
12/04/2010	A	SY	SY IN	Unique No. 1
12/04/2010	SY	B	SY OUT	Unique No. 1
13/04/2010	B	XXXXXXXX	P2P	1234567
13/04/2010	XXXXXXXX	C	P2P	1234567
14/04/2010	C	A	P2P	
28/10/2010	A	XXXXXXXX	P2P	1234567
28/10/2010	XXXXXXXX	B	P2P	1234567
29/10/2010	B	FL	P2P	Unique No. 2
18/04/2011	FL	D		
b) Intermediate Step: converting to P2P equivalent				
Transfer Date	Source	Destination	Move type	
12/04/2010	A	B	SY same day	
13/04/2010	B	C	P2P XXX	
14/04/2010	C	A	P2P	
28/10/2010	A	B	P2P XXX	
29/10/2010	B	FL	P2P	
c) Intermediate Step: modifying dates for P2P moves (midway between surrounding moves)				
12/04/2010	A	B	SY same day	
13/04/2010	B	C	P2P XXX	
14/04/2010	C	A	P2P	
22/07/2010	A	B	P2P XXX date change	
29/10/2010	B	FL	P2P	
d) Final corrected data after examining the crude data and deciding logical sequences				
12/04/2010	A	B	SY same day	Unique No. 1
29/10/2010	B	FL	P2P	Unique No. 2

*A, B, C, D: unique PICs, FL: feedlot PIC, SY: saleyard, P2P: point-to-point).

14.4 Derivation of exposure variables from NLIS data

The process for development of exposure variables from the NLIS data is detailed in, Table 14-10 and Table 14-11. Initially, mixing variables were derived for each time

period using the number of groups from the earlier time point that had been combined to form a single group by the later time point. These variables were then categorised into two or three categories based on the distribution of the number of mixing events for each time period. Results of exploratory analyses assessing univariable associations between a large number of time periods and BRD supported the hypothesis that mixing on or before day -28 reduced the risk of BRD after day 0. Exploratory analysis using the time of first mixing variable provided supporting evidence that first mixing prior to day -28 was protective, the level of protection was similar for animals first mixed prior to day -90 and for animals first mixed between days -90 and -28, and that first mixing between days -27 and 0 was harmful. However disaggregating the data into many time periods resulted in sparse data in some categories for some periods. Based on the prior hypotheses and consistent patterns of BRD risk observed in these exploratory analyses, multiple time periods were amalgamated and variables for mixing pre day -27, from days -27 to -13 and from day -12 to cohort close date were jointly used to derive a composite mixing history analysis variable. Categories were selected after tabulating the mixing variables for all the time points to assess their distribution and by examining the effects of various combinations on the risk of BRD. Categories describing the extent of mixing (i.e. the number of groups combined, as distinct from whether or not any mixing occurred) were only retained in the day -12 to cohort close date component of the composite variable as there was only a lot of variability between animals during this time period. When no mixing occurred during days -27 to -13, the day -12 to cohort close date variable was split into no mixing, 2 or 3, 4 to 9 and 10 or more group-13s combined. This distinction was not made when mixing occurred during days -27 to -13 due to sparse data. Thus, although choices of time periods and combinations thereof for defining final exposure variables were partly defined based on results of univariable associations with BRD, the general concept of the effects of mixing, including possible dependencies among time periods prior to day 0, was based on *a priori* hypotheses. The final detailed mixing history variable had 12 categories.

A collapsed four-category version (Mix summary) of the mixing history variable was derived for use in subset analyses. A five-category variable (Mix summary

composite) separated animals first mixed between days -27 and -13 into a separate category to allow a closer examination of first mixing during this time period.

Transport durations were estimated for moves to the feedlot between day -12 and day 0. Estimated road distances and travel times were established by entering the geographical coordinates of the source PIC, intervening PIC where one was present, and the feedlot PIC into Google maps. Depending on the number and type of moves and the estimated time of the journey, additional time was added for driver rest time, transit through an intervening PIC and animal loading time and unloading times, as appropriate. Driver rest time was estimated based on National Transport Commission Basic Fatigue Management requirements, and ranged from zero for journeys under eight hours to eight hours for journeys over 12 hours. Total animal loading and unloading time was assumed as one hour per move (i.e. 30 minutes loading and 30 minutes unloading), so moves with an intervening PIC were allocated an additional hour's transport duration. Estimated times for travel, driver rest time, and animal loading and unloading times were summed to give the transport duration variable in hours for moves to the feedlot between days -12 to 0. The final analysis variable measuring timing of the move to the feedlot was a composite six-category variable (pre day-27, day -27 to -13, <6 hours between days -12 to -2, ≥6 hours between day -12 and -2, <6 hours from days -2 to 0, ≥6 hours from day -2 and 0).

Table 14-10: Derivation and categories of the number in group variables and intermediate variables used to derive mixing variables

Intermediate variable	Range	Category	Analysis variable & notes
Group-#N	1 to 342		**Number of cattle in the group** defined # days before day 0 (Group-#Ncat)
		<50	
		50 to 99	
		≥100	
Number of source groups forming group-91			Mixed prior to day -90 (Mix pre-90): binary variable indicating if mixing has occurred prior to day -90
		No	
		Yes	
Number of group-91 forming group-28			Mixed between days –90 and -28 (Mix-90 to -28)
		No	
		Yes	
Number of source groups forming group-28	1 to 96		Mixed prior to day -27 (Mix pre-27)
		No	
		Yes	
Number of group-28s forming group-13	1 to 29		Mixed between days -27 and -13 (Mix-27 to -13)
		No	
		Yes	
Number of group-28s forming cohort			Amount of mixing between day -27 and the cohort close date (Mix-27 to close)
		<4	Less than 4 group-28s combined to form cohort
		≥4	4 or more group-13s combined to form cohort
Number of group-13s forming cohort	1 to 25		Amount of mixing between day -12 and the cohort close date (Mix-12 to close)
		1	No mixing in interval (1 group-13 forms cohort)
		2 or 3	2 or 3 group-13s combined to form cohort
		4 to 9	4 to 9 group-13s combined to form cohort
		≥10	10 or more group-13s combined to form cohort
Interval between earliest transfer date and day 0			Interval during which the earliest transfer between PICs occurred (Time_move1)
		Pre -90	Prior to day -91
		Day -90 to -28	Between day -90 and day -28
		Day -27 to -13	Between day -27 and day -13
		Day -12 to 0	Between day -12 and day 0

Variables in bold were used in analyses
refers to days of interest relative to day 0 (13, 28 or 91).

Table 14-11: Derivation and categories of the mixing variables used in the final analyses.

Intermediate variable	Category	Analysis variable & notes
Mixing pre day -90, mixing from days -90 to -28, mixing from days -27 to -13, Mixing from day -12 to cohort close, transfer dates, induction date		Time interval during which animal first mixed (**Mix first**): estimated from mixing variables and time of earliest transfer
	Pre day -90	First mixed before day -90
	Day -90 to -28	Between days -90 and -28
	Day -27 to -13	Between days -27 and -13
	Day -12 to 0	Between days -12 and 0
	Not mixed	Not mixed (single group in cohort)
Mixing pre day-27, mixing from days -27 to -13, mixing from days -12 to cohort close date		Mixing history (**Mix history**): composite variable describing lifetime mixing history based on the three interval variables: Mix pre-27, Mix-27 to -13 and Mix-12 to close
	No, no, no	Not mixed ever
	No, no, 2 or 3	Not mixed pre day -12; 2-3 group-13s form cohort
	No, no, 4 to 9	Not mixed pre day -12; 4-9 group-13s form cohort
	No, no, ≥10	Not mixed pre day -12; 10 or more group-13s form cohort
	No, yes, yes	Not mixed pre day -27; mixed days -27 to -13 & day -12 to cohort close
	No, yes, no	Not mixed pre day -27; mixed days -27 to -13; not day -12 to cohort close
	Yes, no, 2 or 3	Mixed pre day -27; not days -27 to -13; 2-3 group-13s form cohort
	Yes, no, 4 to 9	Mixed pre day -27; not days -27 to -13; 4-9 group-13s form cohort
	Yes, no, ≥10	Mixed pre day -27; not days -27 to -13; 10+ group-13s form cohort
	Yes, yes, yes	Mixed pre day -27 & days -27 to -13 & day -12 to cohort close
	Yes, yes, no	Mixed pre day -27 & days -27 to -13; not day -12 to cohort close
	Yes, no, no	Mixed pre day -27; not day -27 to cohort close
Mixing pre day -27, mixing from day -27 to cohort close date*		Mixing history summary (**Mix summary**): collapsed version of mixing history for use in subset analyses
	No, <4	Not mixed pre day -27; less than 4 group-28s form cohort
	No, ≥4	Not mixed pre day -27; 4 or more group-13s form cohort
	Yes, <4	Mixed pre day -27; less than 4 group-28s form cohort
	Yes, ≥4	Mixed pre day -27; 4 or more group-13s form cohort

*A further variable (**Mix summary composite**) separated those first mixed between days -27 and -13 joining cohorts formed by 4 or more group-28s into a separate category
Variables in bold were used in analyses

15 Appendix 2 - Biological samples and laboratory data

15.1 Sample collection and handling

Feedlot managers were issued with a manual detailing the recommended protocol for the collection and handling of blood samples and nasal swabs and detailing the associated data collection required. Blood samples (5 mL of whole blood) from the jugular or middle caudal vein, and nasal swabs were collected from each animal at induction, and blood samples were obtained at follow-up. At induction, samples were collected into numbered tubes which corresponded to the induction sequence number of the animal. In addition, feedlots were requested to write identification numbers (at least the last 5 digits of the animal's NLIS ID number or other visual identification number from additional ear tags) on every tenth tube for use in sample verification. The same process was repeated at follow-up, but some feedlots elected to collect blood in numbered tubes corresponding to the induction sequence (cattle had ear tags corresponding to the induction sequence), so that although 1 in 10 samples were identified with full animal identifiers, these samples were unequally distributed in the sample rack.

Blood samples were allowed to clot and the combined serum and clot and nasal samples (if collected) were kept refrigerated until sampling of all animals in the cohort was completed for that stage (i.e. induction or follow-up). Samples were then packaged appropriately and transported to the laboratory using the courier preferred by the feedlot.

Feedlot personnel were also requested to collect a blood and nasal swab sample from all cattle from study cohorts that were diagnosed with BRD. Necropsy samples (lung and trachea tissue in sterile containers) were requested from animals that died from suspected BRD. Hospital and necropsy samples were typically refrigerated at the feedlot for up to a week before being sent to the laboratory with the other samples. Blood samples, nasal swabs and necropsy samples were sent to the Queensland Alliance for Agriculture and Food Innovation laboratory at the University of Queensland where all samples were identified, processed and stored.

15.2 Laboratory data

15.2.1 Data flow

Laboratory data were supplied in the form of Microsoft® Excel or comma separated value files which were cross-checked in a timely manner. Regular communication with laboratory staff members facilitated efficient and accurate identification and linkage of biological samples and test results to animal records. Any discrepancies or queries arising from inconsistent data were followed up with the laboratory or feedlot as indicated. When corrections were necessary, updated corrected versions were provided by the feedlot or laboratory or documented minor changes were made before saving the original data as a corrected version. For example, if a duplicated Animal ID was recorded but this could be easily corrected based on feedlot information and other recorded identification numbers, the original value was modified, whereas if the induction file was incomplete compared to the samples received, a new version of the induction data was supplied by the feedlot.

Upon receipt of samples, data recorded at the laboratory included date samples received, any identification numbers on the tubes, storage plates and cells, whether samples were received and whether samples were of adequate volume for testing as listed in Table 15-1. Additional notes recorded any discrepancies or issues with the samples.

15.2.2 Sample verification

Verification of biological samples involved ensuring that the induction sequence number matched the sample sequence number, while using the additional animal identification numbers on every tenth tube to ensure accuracy. Sample verification involved deciding if samples could be linked to particular animals (verified ‘A’), were from animals in the study cohort but not able to be linked to a particular animal (verified ‘C’) or not able to be verified (‘N’). Verification of samples obtained at follow-up followed the same procedure whereby the sample records from the laboratory were compared with and matched against the electronic feedlot files.

In a number of cohorts from particular feedlots, no follow-up file was provided, but samples were collected into tubes numbered with induction sequences, so verification was possible. This method meant there were often unused tubes

corresponding to animals that were not present at follow-up. Sometimes samples were received for animals not recorded in the follow-up electronic file. This could occur if animals were in a hospital pen on the day of follow-up and a sample was collected even though details were not recorded electronically at the feedlot. Thus, if a sample was received in a numbered tube that corresponded to an animal that was recorded in hospital then it was verified. Sometimes there was considerable delay in the receipt of final files, so a final verification check against animals that had exited the cohort could not be made until close to the end of the data collection phase.

Variables were derived to describe whether or not animals had samples received, verified and of adequate volume at each stage of testing (Table 15-1). These were used in determining eligibility for inclusion in the case-control study. Separate variables recorded if samples were received and verified but of inadequate volume (Table 15-1). This was used in determining which animals contributed samples to pooled BVDV qPCR tests.

Hospital and necropsy samples were matched with electronic animal-level records. Hospital samples obtained at initial examination and diagnosis were distinguished from those obtained at subsequent examinations.

15.2.3 PCR test results

Pooled test results and animal-level test results from PCR testing were supplied in the form of electronic files which were cross validated by inspecting original laboratory records to confirm that positive pools were correctly allocated to the sample locations indicated. Table 15-2 details qPCR test result data supplied by the laboratory for both pooled and individual tests were appropriate.

15.2.4 ELISA antibody serology results

Serology data output included optical density measurements, and derived manufacturer-defined categories from the ELISA antibody tests. Data were supplied in Microsoft Excel® files based on a common template. An automated Microsoft Excel® add-in was used to extract the data into a single spreadsheet, greatly enhancing the efficiency and accuracy of data assimilation. Unique test IDs were created to link the test output and the animal identification number and stage of sampling. All data were cross checked and discrepancies such as duplicated or

missing results were followed up with laboratory staff. These were mostly due to recording errors and the cross-checking process resulted in complete agreement in linking samples to test results and animals. The final data fields obtained from ELISA tests are summarised in Table 15-3.

Table 15-1: Data relating to samples received at laboratory and pooled BVDV PCR results

Data	Description
Sample storage	
Feedlot	
Lot no.	Provided by feedlot linked to study Cohort ID
Sample ID*	Order of sample in rack, usually animal processing sequence
Other received sample ID*	Visual ID or other identification tag number, used for verification
Date sample received*	Consistency checking against feedlot records
Serum YN*	Serum received (yes/no)
Serum storage plate*	Serum sample storage plate number
Serum storage cell*	Serum sample storage cell number
Serum <100ul*	Indicated if the sample volume was less than that required for pooled testing
Serum adequate*	Indicated if sample volume was adequate for case-control study
Serum notes*	Additional notes relating to sample
Swab YN*	Swab received (yes/no)
Swab storage plate*	Swab storage plate number
Swab storage cell*	Swab storage cell number
Swab adequate*	Indicated if sample was adequate
Swab notes*	Additional notes relating to sample
Pull date	Date sample collected as recorded on documentation
Death date	Death date as recorded on documentation
Lung sample YN	Necropsy lung tissue received
Lung sample adequate	Necropsy lung tissue suitable for testing
Trachea sample YN	Necropsy trachea tissue received
Trachea sample adequate	Necropsy trachea tissue suitable for testing
Trachea storage plate	Trachea sample storage plate
Trachea storage cell	Trachea sample storage cell
Lung storage plate	Lung sample storage plate
Lung storage cell	Lung sample storage cell
After verification	
Animal ID	Updated with feedlot data to ensure data linkage
Serum verified*	Sample linked to unique animal ‘A’ or Cohort ‘C’
Swab verified*	Sample linked to unique animal ‘A’ or Cohort ‘C’
Trachea verified	Sample linked to unique animal ‘A’
Lung verified	Sample linked to unique animal ‘A’
Serum RVA*	Serum received, verified and adequate
Swab RVA*	Swab received, verified and adequate
Serum RV*	Serum received and verified, but not adequate (low volume)

*Separate equivalent variables for induction, follow-up (draft) or hospital (pull) samples

Table 15-2: Data relating to laboratory PCR testing of nasal swabs and serum samples

Data	Description
BVDV PCR tests	
Pooled tests	
Cohort ID	Cohort identification number
Stage	Induction or follow up (draft)
Pool No	Sequence pool number by cohort
Serum storage plate*	Serum sample storage plate number
Serum storage cell*	Range of cells corresponding to serum storage cells
# Samples (x 10ul)	Number of samples in pooled well (up to 24)
Pooled Extraction Plate No	Pooled test plate number
Pool Cell	Pooled test cell number
Date of Nucleic Acid	Date of test
Serum BVDV PCR	Negative or positive
Serum BVDV CT value	Cycling threshold value if result was positive
Individual tests for PI detection	
Animal ID	Animal identification number
Cohort ID	Cohort identification number
Storage plate*	Serum sample or nasal swab storage plate number
Storage cell*	Serum or swab storage cell
Stage	Induction, hospital or follow up (draft)
Test plate	Individual test plate number (also new location of sample)
Test cell	Individual test cell number (also new location of sample)
BVDV PCR	Negative or positive
BVDV CT value	Cycling threshold value if result was positive
*Nasal swab tests^*	
Nasal swab storage plate^	Original induction swab storage location
Nasal swab storage cell^	Single cell or range of 4 cells corresponding to nasal swab storage cells
Cohort ID	Cohort identification number
Stage	Induction or follow up (draft)
NS test plate	Nasal swab pool test plate
NS test cell	Nasal swab test cell
Swab BVDV PCR^	PCR tested for BVDV (negative/positive)
Swab BRSV PCR^	PCR tested for BVDV (negative/positive)
Swab BPI3 PCR^	PCR tested for BVDV (negative/positive)
Swab BoHV-1 PCR^	PCR tested for BVDV (negative/positive)
Swab BCV PCR^	PCR tested for BVDV (negative/positive)
Swab BVDV CT value^	BVDV CT value if BVDV PCR positive
Swab BRSV CT value^	BRSV CT value if BRSV PCR positive
Swab BPI3 CT value^	BPI3 CT value if BPI3 PCR positive
Swab BoHV-1 CT value^	BoHV-1 CT value if BoHV-1 PCR positive
Swab BCV CT value^	BCV CT value if BCV PCR positive

^ Separate equivalent variables for induction or hospital (pull) samples

Table 15-3: Data relating to laboratory ELISA antibody testing of serum samples

Data	Description
Identifiers	
Selection number	Unique identifier for animals selected for case-control study
Storage plate	Serum sample storage plate number
Storage cell	Serum sample storage cell number
CP plate number	Cherry picked (CP) plate where case-control selected animal's samples were relocated prior to testing
CP induction cell	Cell where case-control selected animal's induction serum was relocated prior to testing
CP draft cell	Cell where selected animal's draft serum was relocated prior to testing
ELISA induction plate	Test plate for induction sample ELISA testing
ELISA induction cells	Set of 6 test cells for a single sample (denoted 'induction block')
ELISA draft plate	Test plate for draft sample
ELISA draft cells	Set of 6 test cells for a single sample (denoted 'draft block')
Test date	Date ELISA test performed
Test file	Name of file where test results were stored
Test results	
BHV1^	Serology category for Bovine Herpes Virus Type 1
BVDV^	Serology category for Bovine Viral Diarrhoea Virus
BRSV^	Serology category for Bovine Respiratory Syncytial Virus
BPI3^	Serology category for Parainfluenza Type 3 virus
Mbovis^	Serology category for Mycoplasma bovis
BHV1_OD^	Serology optical density value for Bovine Herpes Virus Type 1
BVDV_OD^	Serology optical density value for Bovine Viral Diarrhoea Virus
BRSV_OD^	Serology optical density value for Bovine Respiratory Syncytial Virus
BPI3_OD^	Serology optical density value for Parainfluenza Type 3 virus
Mbovis_OD^	Serology optical density value for Mycoplasma bovis
Data linkage	
Test ID	Test identification number (based on storage plate and block of cells) allowing the linkage of test results to samples and animals

^ Paired values for induction and follow-up samples

16 Appendix 3: Univariable results

Table 16-1: Distribution and univariable results for induction characteristics

Variable	Category	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
Breed							<0.001
	Angus	19,764	56.4	22.6	Ref		
	British Cross	4,140	11.8	17.6	1.2	(1.0 to 1.3)	0.031
	Hereford	1,952	5.6	21.4	1.7	(1.4 to 2.2)	<0.001
	Shorthorn	1,414	4.0	26.0	1.2	(1.0 to 1.5)	0.100
	Murray Grey	931	2.7	7.1	0.6	(0.4 to 0.8)	0.001
	European/X	1,318	3.8	3.3	0.8	(0.6 to 1.2)	0.239
	Tropical/X	5,530	15.8	1.5	0.5	(0.4 to 0.7)	<0.001
Sex							0.115
	Male	32,260	91.8	18.8	Ref		
	Female	2,871	8.2	5.3	0.7	(0.5 to 1.1)	0.115
							0.380
Cohort sex					Ref		
	Male	30,975	88.2	18.8	0.8	((0.5 to 1.2)	0.250
	Female^	1,952	5.6	3.0	0.5	(0.1 to 2.2)	0.380
	Mixed^	2,204	6.3	14.2			
Intended days on feed							0.987
	≥120	18,561	52.8	22.3	Ref		
	85 to <120	12,615	35.9	15.3	1.0	(0.7 to 1.5)	0.927
	≤85^	3,955	11.3	3.6	1.0	(0.4 to 2.1)	0.904
Dentition							0.794
	0	27,812	80.7	19.3	Ref		
	2	5,560	16.1	12.9	1	(0.9 to 1.1)	0.906
	≥4	1,082	3.1	10.1	0.9	(0.7 to 1.2)	0.517

^ Categories where 7 or more feedlots had no observations

Table 16-2: Distribution and univariable results for variables describing induction weight, number of animals in a group and cohort

Variable	Category	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
Induction weight (kg)							<0.001
	<400	7,027	20.0	13.0	Ref		
	400 to <440	10,767	30.7	21.1	0.9	(0.8 to 1.0)	0.024
	440 to <480	12,029	34.3	19.2	0.8	(0.7 to 0.9)	<0.001
	≥480	5,303	15.1	13.3	0.7	(0.6 to 0.8)	<0.001
Mean cohort weight (kg)							0.413
	<425	8,615	24.5	14.0	Ref		
	425 to <455	17,694	50.4	20.7	0.8	(0.5 to 1.2)	0.21
	≥455	8,822	25.1	15.2	0.9	(0.6 to 1.4)	0.639
Weight difference from mean cohort weight (kg)							0.001
	>20 below	8,425	24.0	20.1	Ref		
	≤20 below	8,849	25.2	16.4	0.9	(0.8 to 1.0)	0.013
	≤20 above	9,330	26.6	17.0	0.9	(0.8 to 0.9)	<0.001
	<20 below	8,522	24.2	17.3	0.8	(0.8 to 0.9)	<0.001
No. animals in group-13 (Group-13N)							<0.001
	<50	13,782	39.2	24.1	Ref		
	50 to 99	9,783	27.9	21.3	0.8	(0.7 to 0.9)	0.004
	≥100	11,566	32.9	6.9	0.6	(0.5 to 0.7)	<0.001
No. animals in cohort (CohortN)							
	<200	12,243	34.8	11.5	Ref		
	≥200	22,888	65.2	20.9	1.1	(0.8 to 1.6)	0.444

^ Categories where 7 or more feedlots had no observations

Table 16-3: Distribution and univariable results for variables describing mixing, saleyard history, feedlot move and cohort close pattern

Variable	Category	No.	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
Mix history (pre day -27, days -27 to -13, day -12 to cohort close)							<0.001
	No, no, no^	418	1.2	20.6	2.2	(0.7 to 7.0)	0.168
	No, no, 2 or 3	1,489	4.3	19.5	2.4	(1.5 to 3.6)	<0.001
	No, no, 4 to 9	3,332	9.6	30.3	3.7	(2.1 to 6.3)	<0.001
	No, no, ≥10	5,112	14.7	31.4	3.9	(2.3 to 6.7)	<0.001
	No, yes, yes	627	1.8	17.2	3.5	(1.9 to 6.5)	<0.001
	No, yes, no^	407	1.2	2.5	1.9	(0.6 to 6.0)	0.288
	Yes, no, 2 or 3	3,893	11.2	5.7	Ref		
	Yes, no, 4 to 9	5,411	15.6	16.4	2.8	(1.6 to 4.8)	<0.001
	Yes, no, ≥10	7,795	22.4	20.7	2.6	(1.5 to 4.5)	<0.001
	Yes, yes, yes^	946	2.7	13.7	1.9	(1.1 to 3.5)	0.030
	Yes, yes, no^	1,958	5.6	3.3	1.3	(0.5 to 3.1)	0.576
	Yes, no, no	3,342	9.6	3.4	0.7	(0.4 to 1.5)	0.377
Saleyard pre day -27							0.001
	No	22,223	64.0	18.7	Ref		
	Yes	12,507	36.0	15.7	0.9	(0.8 to 0.9)	0.001
Saleyard days -27 to -13							0.029
	No	34,162	97.2	17.8	Ref		
	Yes	969	2.8	11.2	1.4	(1.0 to 2.0)	0.029
Saleyard days -12 to 0							<0.001
	No	34,200	97.4	17.6	Ref		
	Yes	931	2.7	21.4	2.1	(1.4 to 3.1)	<0.001
Move to feedlot: days before day 0 and hours of transport							<0.001
	Pre day -27^	1,880	5.4	1.5	0.2	(0.1 to 0.5)	<0.001
	Days -27 to -13^	2,000	5.7	4.6	0.9	(0.5 to 1.5)	0.644
	Days -12 to -2; <6 h	2,183	6.2	10.9	1.0	(0.7 to 1.3)	0.973
	Days -12 to-2; ≥6 h	2,339	6.7	8.0	1.1	(0.8 to 1.6)	0.642
	Days -1 to 0; <6 h	17,139	48.8	19.9	Ref		
	Days -1 to 0; ≥6 h	9,590	27.3	23.5	1.1	(1.0 to 1.3)	0.038

^ Categories where 7 or more feedlots had no observations

Table 16-4: Distribution and univariable results for variables describing cohort formation and induction treatments

Variable	Category	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
Cohort fill duration (days)							0.011
	1	12,051	34.3	7.4	Ref		
	>1	23,080	65.7	23.0	1.7	(1.1 to 2.6)	0.011
Days from DOF1 to day 0							0.677
	0	28,386	80.8	18.8	Ref		
	1 or 2^	4,940	14.1	14.7	0.9	(0.7 to 1.2)	0.425
	≥3^	1,805	5.1	7.8	1.0	(0.5 to 2.0)	0.906
Days from day 0 to cohort close							0.043
	1	20,001	56.9	13.9	Ref		
	1 to 6	12,408	35.3	23.4	0.9	(0.8 to 1.0)	0.041
	≥7	2,722	7.8	19.0	0.8	(0.7 to 1.0)	0.031
Rhinogard™ at induction							0.146
	No^	7,365	21.0	2.8	Ref		
	Yes	27,766	79.0	21.6	3.4	(0.7 to 18.1)	0.146
Vitamin ADE at induction							0.609
	No	24,518	69.8	17.1	Ref		
	Yes^	10,613	30.2	18.9	1.1	(0.8 to 1.6)	0.609

^ Categories where 7 or more feedlots had no observations

Table 16-5: Distribution and univariable results for variables describing BVDV and pen characteristics

Variable	Category	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
PI animal (BVDV_PI_animal)							0.209
	No	35,034	99.8	17.6	Ref		
	Yes	85	0.2	27.1	1.4	(0.8 to 2.4)	0.209
BVDV active in cohort (BVDV_chtYN)							0.009
	No	11,896	33.9	8.7	Ref		
	Yes	23,235	66.1	22.2	1.6	(01.1 to 2.2)	0.009
Shared pen water							0.012
	No^	6,453	18.4	3.9	Ref		
	Yes	28,678	81.6	20.7	2.9	(1.3 to 6.7)	0.012
Pen shade							0.14
	None	11,141	31.7	9.6	Ref		
	Any	23,990	68.3	21.4	1.6	(0.9 to 2.8)	0.14
Number of adjoining pens							0.995
	1	10,394	29.9	14.7	Ref		
	2	24,391	70.1	19.1	1	(0.6 to 1.6)	0.995
Stocking density (m^2/SCU#)							0.633
	11 to <14^	14,266	40.6	21.6	Ref		
	14 to <17	10,893	31.0	17.8	0.9	(0.6 to 1.5)	0.827
	17 to <25	5,436	15.5	11.9	0.8	(0.4 to 1.3)	0.312
	≥25^	4,536	12.9	11.6	1	(0.6 to 1.8)	0.956
Bunk space (m/head)							0.288
	<0.18^	9,500	28.0	13.5	Ref		
	0.18 to <0.24	15,253	44.9	22.2	0.7	(0.3 to 1.4)	0.27
	≥0.24	9,214	27.1	14.3	0.5	(0.2 to 1.2)	0.115

^ Categories where 7 or more feedlots had no observations

Table 16-6: Distribution and univariable results for variables describing rations and induction timing

Variable	Category	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
Grain type							0.268
	Barley	16,825	47.9	25.0	Ref		
	Sorghum^	2,709	7.7	2.9	0.3	(0.1 to 1.3)	0.11
	Wheat mix^	14,168	40.3	12.8	1.1	(0.5 to 2.8)	0.79
	Other mix^	1,429	4.1	7.3	0.7	(0.2 to 2.4)	0.571
Grain % on day 0							0.861
	<35%	7,762	22.1	16.5	Ref		
	35 to <40%	8,322	23.7	32.0	1.1	(0.6 to 1.8)	0.787
	40 to <45%	9,007	25.6	9.5	0.8	(0.5 to 1.5)	0.544
	≥45%	10,040	28.6	14.0	1.2	(0.5 to 2.5)	0.682
Grain % on day 20							0.467
	<60%^	9,817	27.9	20.1	Ref		
	60 to <70%^	13,781	39.2	18.3	1.1	(0.9 to 1.3)	0.219
	≥70%	11,533	32.8	14.8	1.1	(0.8 to 1.6)	0.597
Days to 60% grain							0.059
	0 to 6^	3,358	9.6	3.6	Ref		
	7 to 13	10,821	30.8	14.8	1.2	(0.6 to 2.2)	0.658
	14 to 20	13,987	39.8	22.7	1	(0.5 to 1.9)	0.92
	≥21^	6,965	19.8	18.6	0.8	(0.4 to 1.7)	0.6
Induction season							0.031
	Spring	9,763	27.8	16.0	Ref		
	Summer	7,235	20.6	18.7	1.7	(1.2 to 2.6)	0.006
	Autumn	8,114	23.1	22.4	1.5	(1.0 to 2.2)	0.04
	Winter	10,019	28.5	14.6	1.3	(1.0 to 1.8)	0.094
Induction year							0.842
	2009	4,729	13.5	15.7	Ref		
	2010	11,593	33.0	16.7	0.9	(0.5 to 1.5)	0.649
	2011	18,809	53.5	18.7	1	(0.6 to 1.6)	0.905

^ Categories where 7 or more feedlots had no observations

Table 16-7: Distribution and univariable results for variables describing numbers of cattle on feed and region

Variable	Category	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
Number on feed in animal's induction month (FeedlotN)							0.683
	<10,000	11,538	32.8	5.8	Ref		
	10,000 to <20,000^	13,818	39.3	18.0	1.1	(0.6 to 2.3)	0.716
	≥20,000^	9,775	27.8	31.2	0.9	(0.4 to 2.1)	0.773
Number <40 DOF in animal's induction month (FeedlotN40)							0.974
	<3,000^	11,240	32.0	6.5	Ref		
	3,000 to <6,000^	13,622	38.8	18.0	1	(0.3 to 3.3)	0.94
	≥6,000^	10,269	29.2	29.3	1	(0.3 to 3.2)	0.992
Source region							0.76
	NSW Central & Southern Tablelands^	6,251	17.8	28.5	Ref		
	Coastal NSW or Qld^	1,224	3.5	18.3	0.9	(0.7 to 1.2)	0.551
	Darling Downs/New England^	8,900	25.3	13.3	1.1	(0.9 to 1.3)	0.346
	Western NSW/Qld or NT	8,452	24.1	7.8	1	(0.8 to 1.2)	0.862
	NSW Riverina, Vic & Tas^	6,188	17.6	32.5	0.9	(0.8 to 1.1)	0.45
	SA/WA^	4,110	11.7	8.2	1.0	(0.7 to 1.3)	0.833
Feedlot region						Did not converge	
	North^	13,342	38.0	5.4			
	South^	21,789	62.0	25.1			

^ Categories where 7 or more feedlots had no observations

Table 16-8: Distribution and univariable results for variables describing weather

Variable	Category	Number	Distribution by category (%)	Crude 50-day BRD cumulative incidence (%)	OR	95%CI	p-value
Mean of daily maximum temperatures in week 1 (°C)							0.292
	11 to <17	5,294	15.1	18.2	Ref		
	17 to <23	11,259	32.0	16.7	0.8	(0.6 to 1.0)	0.106
	23 to <30	12,526	35.7	17.4	0.8	(0.6 to 1.1)	0.185
	≥30	6,052	17.2	19.5	1.0	(0.7 to 1.4)	0.87
Mean of daily minimum temperatures in week 1 (°C)							0.475
	<5	7,879	22.4	21.7	Ref		
	5 to <11	12,670	36.1	16.7	1.1	(0.9 to 1.3)	0.566
	11 to <17	9,595	27.3	17.4	0.9	(0.7 to 1.2)	0.599
	≥17	4,987	14.2	14.1	1.1	(0.8 to 1.6)	0.501
Mean of daily temperature ranges in week 1 (°C)							0.792
	6 to <11	5,961	17.0	13.0	Ref		
	11 to <16	22,045	62.7	18.8	1.0	(0.8 to 1.2)	0.679
	≥16	7,125	20.3	18.1	0.9	(0.7 to 1.2)	0.5
Total rainfall in week 1 (mm)							0.472
	0	7,225	20.6	14.4	Ref		
	0.1 to <4	9,958	28.4	23.0	1.1	(0.9 to 1.4)	0.255
	4 to <25	12,895	36.7	17.2	1.2	(1.0 to 1.4)	0.115
	≥25	5,053	14.4	12.8	1.1	(0.8 to 1.5)	0.466
Mean of daily maximum wind speeds in week 1 (km/hr)							0.757
	20 to <35	9,166	26.1	18.9	Ref		
	35 to <45	19,694	56.1	16.1	1.0	(0.8 to 1.2)	0.702
	≥45	6,271	17.8	20.5	0.9	(0.7 to 1.2)	0.466

Table 16-9: Distribution and univariable results for variables derived from the vendor questionnaire data

Variable	Category	% Missing	N	Total %	Total %	OR	95%CI	p-value
Age		9.2						<0.001
(months)	<16^		1,598	16.4	12.5	0.8	(0.6 to 1.2)	0.356
	16 to <22		5,326	54.7	23.3	Ref		
	≥22		2,807	28.9	17.1	1.5	(1.2 to 1.8)	0.001
Prior Bovilis		6.2						0.053
(BV_vacc)	No		6,840	85	19.2	Ref		
	Yes		1,205	15	15.4	0.8	(0.6 to 1.0)	0.053
Prior		6.2						0.056
Pestigard	No		7,063	87.8	19.0	Ref		
(PV_vacc)	Yes		982	12.2	16.1	0.8	(0.5 to 1.1)	0.056
Yard wean		4.6						0.027
	No		983	20.4	31.2	Ref		
	Yes		3,847	79.7	18.0	0.7	(0.5 to 1.0)	0.027
Yard wean		4.6						0.084
detail	No		983	20.4	31.2			
	Yes, <7 days		1,788	37.0	23.8	0.6	(0.4 to 1.0)	0.034
	Yes, ≥7 days		2,059	42.6	13.0	0.7	(0.5 to 1.0)	0.067
Prior grain		20.6						0.306
(Grain pre)	No		3,082	76.6	24.9	Ref		
	Yes		940	23.4	16.4	0.8	(0.5 to 1.3)	0.306
Prior		20.6						0.699
conserved forage or	No		659	16.4	28.8	Ref		
supplement (Supp pre)	Yes		3,363	83.6	21.7	0.9	(0.5 to 1.6)	0.699
On-property		0.6						0.999
mixing	No^		322	6.4	27.3	Ref		
(Mix_VQ)	Yes		4,711	93.6	20.9	1.0	(0.6 to 1.6)	0.999

^ Categories where 7 or more feedlots had no observations