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Epidemiology and management of bovine respiratory disease in feedlot cattle

Part A: Epidemiology study

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Abstract

While many of the risk factors that contribute to feedlot cattle developing bovine respiratory disease (BRD) are widely recognised, the disease continues to be a major source of economic loss to the industry. A prospective observational study of 35,160 cattle from 170 cohorts (cattle assembled together in a feedlot pen following induction) from 14 feedlots across Australia was conducted to identify and quantify risk factors for development of BRD. Within the study population, 18.2% of cattle developed BRD and 98% of BRD cases occurred with the first 50 days on feed. Results supported prior beliefs regarding many risk factors associated with risk of BRD development including breed, induction weight (which may be due in part to age and/or body condition), size of pre-arrival groups, season of induction, yard weaning, prior vaccination with Pestigard™ and prior vaccination with Bovilis MH™. In addition, the study made novel use of data from the National Livestock Identification System to investigate time-specific effects of mixing and transfers through saleyards and the timing and duration of the move to the feedlot. The findings of this study provide the basis for the future development of a management tool to allow feedlot operators to minimise the impact of BRD on their enterprises.

Executive summary

Bovine respiratory disease (BRD) continues to cause substantial costs to the Australian lot feeding industry and has an adverse effect on animal welfare. Due to the complex nature of BRD, industry has found it very challenging to manage effectively. This is despite the general consensus that many of the risk factors ("things" that either reduce or increase the levels of disease) are known. In response to this ongoing issue, the National Bovine Respiratory Disease Initiative (NBRDI) was established to quantify the risk factors associated with this disease. A broad range of possible risk factors was investigated, many of which have been historically considered by industry to be important in the development or prevention of BRD. The estimated effects of risk factors were based on the population of cattle in this study. As these cattle were representative of cattle across medium and large Australian feedlots at the time of the study, the conclusions are generalisable to this larger population but may not apply to cattle on smaller feedlots or future populations at larger feedlots.

The NBRDI was a prospective observational study whereby cohorts (sometimes referred to as pens or lots) of cattle were enrolled in participating feedlots during the period from December 2009 to May 2011. To assess pathogen exposure, samples were collected for laboratory assays from study cattle at induction, after approximately six weeks on feed and when diagnosed with BRD. Animal-specific and cohort-specific data were obtained for all cattle. Vendors of some cattle were also surveyed, to gather data on pre-feedlot management practices such as weaning methods and on-farm use of vaccines. At the completion of the observational phase of the project 35,160 cattle had been enrolled in 170 cohorts at 14 predominantly large feedlots throughout Australia.

There was considerable variation in the incidence of BRD between cohorts and feedlots. Overall 18.2% of cattle were treated for BRD with the peak incidence of disease being between 15 to 30 days after induction. Approximately 97% of all BRD cases occurred within 50 days of induction. A late BRD "spike" which has been the subject of industry discussion in recent years was generally not seen. The BRD case fatality risk (i.e. the proportion of BRD cases that died within 50 days of onset of BRD) was 3.4% with nearly half these deaths occurring within the first ten days after initial treatment. Overall 0.7% of cattle died from BRD and of these 18.6% had not received any treatment.

The NBRDI findings clearly demonstrated that animal factors play a role in determining the animal's risk of BRD. The study confirmed a long-held industry belief that breed contributes to risk. The study was dominated by Angus cattle (56%) and as a result, this breed was used as the reference group against which other breeds were compared. Herefords were at twice the risk of BRD compared to Angus, whereas tropical breeds and cross breeds were at only half the risk. An unexpected finding was that Murray Grey cattle were at less risk than Angus cattle and further investigations into this are warranted. The weight of cattle at induction also contributes to the risk of BRD development, with heavier cattle (≥ 400 kg) less likely to develop disease than lighter cattle (< 400kg) and with risk progressively decreasing with increasing weight. Surprisingly, as there was no evidence of a large effect of dentition, cattle aged 22 months or older were at increased risk of BRD compared to younger cattle. There was no obvious explanation for this but the finding was restricted to the subset of the study cattle for which vendor information was available; it is possible the results were confounded as the older cattle in this subset

were of similar weight to younger cattle. There was some evidence that risk was lower in heifers compared to steers.

One of the most novel aspects of this project was the utilisation of the data from the National Livestock Identification System (NLIS) database to assess these details for each animal. This information was used to trace not only movements of cattle between properties (including to feedlots) but also mixing of cattle from different properties. This study demonstrated that it is not simply whether or not cattle from different properties are mixed but when they are mixed that has a big impact on the risk of BRD. Importantly, mixing of cattle in the time period leading up to induction is critical to their risk of BRD; cattle mixed in the period from 12 days prior to induction until the cohort is formed with four or more other groups are at increased risk compared to those mixed with only two or three groups during this time period. Cattle mixed at least one month prior to induction are at reduced risk of BRD compared to those that had not been mixed before one month prior to induction. No conclusion could be reached about mixing in the time period from two to four weeks before induction as very few of the study cattle were mixed during this time period.

The study found that saleyard transfers (an animal had a pair of records on the same date in the NLIS data with the transfer types described as “saleyard in” and “saleyard out”) in the 12 days before induction increased the risk of BRD over-and-above the increased risk attributed to mixing. Cattle transferred through saleyards at least 27 days before induction were at reduced risk of BRD, while those with a saleyard transfer from 27 to 12 days before induction were at increased risk. However, further statistical analyses showed that both these effects were mediated primarily through the effects of mixing i.e. the risk of BRD would have been similar if cattle had just been mixed during these periods without having a saleyard transfer.

Cattle in groups of at least 100 that have been established at least 13 days prior to induction are at reduced risk. In the study population, groups were commonly stable for several months prior to induction so it is likely that this benefit requires the group of cattle to be together for at least a month prior to induction.

The protective effects of early mixing of cattle may be due to a number of factors. Mixing of cattle well prior to induction probably allows for the establishment of a stable social group and this removal of one source of stress at the feedlot reduces the risk of BRD. Similarly, early infection with viruses facilitated by mixing well prior to induction may ensure that animals have acquired immunity to that particular agent by induction, and thus reduced the likelihood that re-exposure to that agent at the feedlot will result in BRD.

Undoubtedly, the use of the NLIS data in this study has allowed the identification of previously unexplored risk factors for BRD. The challenge that remains is how to distil this into a useful resource that feedlot operators can and will apply. Currently access to the database is restricted and the analyses conducted in the current study are far from trivial. Some consideration should be given to the development of a tool that uses NLIS data for the rapid assessment of the risk factors linked to these data without compromising the confidentiality of the system. This might be achieved by linking the number of lifetime property changes, the timing of these changes, which other cattle were on the property concurrently, and whether the cattle had a saleyard transfer to the animal identifier but still maintaining the privacy of cattle vendors. From a feedlot operator perspective, this could be valuable information if it was available at the time of purchase in assessing the potential BRD risk of the group.

The study analyses also looked at pen characteristics as possible risk factors for BRD. Somewhat surprisingly these analyses identified a previously unreported risk factor; cattle in pens with water troughs that were shared with an adjacent pen were at three and a half to five times greater risk of developing BRD compared to cattle in pens where water troughs could not be accessed by other cattle. On the basis of this finding, lot operators replacing water troughs should consider locating them where they cannot be accessed by cattle from adjacent pens.

Results supported the widely recognised belief that cattle are at greater risk of BRD in autumn compared to spring. Interestingly though, risk was similarly high in the summer and was also increased in winter compared to spring, but to a lesser extent. Weather variables, averaged over the first week of induction, were considered to try and explain these seasonal effects but no consistent patterns were found. Further investigation using higher resolution data and more complex, computationally intensive methods should be considered to investigate this further.

There is a great deal of industry interest in the importance of bovine viral diarrhoea virus (BVDV, also known as pestivirus). This study identified cohorts where BVDV was present, due to transient infections and/or the presence of persistently infected animal(s). Cattle in cohorts where BVDV was present were at higher risk of BRD than those in cohorts with no evidence of BVDV. This effect was consistent regardless of whether or not the cattle had been in a group with a persistently infected animal for at least 28 days before induction.

As part of this study, one of the most comprehensive analyses ever undertaken was conducted on a subset of BRD cases (N = 3,725) and an equal number of control animals to determine to which of the viruses commonly associated with BRD the study cattle were exposed. The sampling strategy permitted identification of viruses cattle had been exposed to prior to arrival at the feedlots and viruses cattle were exposed to during the first few weeks on feed. Similar to previous studies, the results suggested that exposure to bovine herpesvirus 1 (BoHV-1 or IBR) before arrival at the feedlots is much less common compared to the other three viruses investigated. Despite this, there was serological evidence of infections early in the animals' time on feed for all four viruses, with substantial numbers of cattle becoming seropositive during the first six weeks after induction. Exposure to any of the four viruses investigated in this study increased the risk of cattle developing BRD. Of interest was that none of the viruses appeared substantially more important than any of the others in increasing the animals' risk of BRD. The more of the four viruses cattle were exposed to during the first few weeks on feed, the greater risk of that animal developing BRD. The serological data supported the notion that prior exposure to these viruses and the presence of high levels of antibody to the viral agents reduced the risk of BRD. These findings suggest that effective vaccines should be useful in reducing BRD in feedlots. Unfortunately, no determinations could be made on the protective effects of BoHV-1 vaccination in this study due to the way the current vaccine was used in the study population (either all cattle at a feedlot were vaccinated or no cattle at a feedlot were vaccinated, and feedlots with higher risk cattle were more likely to use BoHV-1 vaccine). To assess the impact of this vaccine on BRD risk a dedicated randomised controlled trial is required. Reduced risks of BRD was observed in subsets of study cattle that were vaccinated on farm with either Pestigard™ or Bovilis MH™ vaccines.

Many other factors were assessed but no definitive conclusion about the increase or decrease in risk of BRD could be reached. For some factors, including presence of pen shade, amount of bunk space per animal and prior grain feeding, results were

suggestive of an effect but the estimates were imprecise. For other factors, including numbers of cattle on feed and many of the weather, ration and pen variables, no conclusion could be reached. This was usually because there was little variation in these factors within participating feedlots. The current study design provides the framework and methodology for a future retrospective study using routinely recorded data to address this deficiency in a highly cost effective manner.

Collectively the findings of this study provide the basis for the future development of an extension process including a management tool to allow feedlot operators to minimise the impact of BRD on their enterprises. To achieve this, it is recommended that a panel of key stakeholders work together with the research team to drive out and own practical and evidence-based messages that can be widely adopted, and to define extension processes that will facilitate adoption.

Glossary

The purpose of this glossary is to provide a working definition for terms used in this report. It is not intended to represent a single "correct" definition for the various terms.

Animal ID	Radio frequency identification device (RFID) number; consisted of a 3 digit manufacturer's code, a space and a unique 12 digit serial number
Animal-level risk factor	Each animal has an individual value for the risk factor; values can vary between animals within a group e.g. induction weight
Bovilis MH™	Inactivated vaccine registered as an aid to the control of respiratory disease caused by *Mannheimia haemolytica*
BRD mortality incidence risk	The percentage of cattle in the population that died from BRD
Case fatality risk	The percentage of cattle that met the BRD case definition when first pulled and the pull occurred within the first 50 days of the start of time at risk, which subsequently died for any reason within 50 days of the pull.
Case-control study	A type of study where a group of cases and a group of controls are selected. The frequency of each exposure of interest in the cases is then compared to that in the controls
Closed population	No animals enter or leave the population during the time period of interest
Cohort	Animals assembled together in a feedlot pen following induction
Cohort close date	The latest day 0 for an animal in that cohort
Cohort fill duration	The number of days in the induction period
Cohort formation	The process by which animals are selected to form a cohort
Cohort-level risk factor	A risk factor for which all animals in the same cohort must have the same category of exposure e.g. mean cohort weight
Cohort open date	The earliest day 0 for an animal in that cohort
Cohort study	A type of study where exposure statuses of animals at the start of the study are known (although this may be determined retrospectively). Animals without the disease of interest are enrolled and observed for the duration of the study period, and the incidences of disease compared between groups of animals with differing exposure statuses.
Consulting veterinarians	Veterinarians who regularly provide animal health and/or nutritional services to the feedlot industry who were consulted throughout the study period
Day 0	For feedlots that did not practice pre-assembly, day 0 was the animal's induction date. For feedlots that practiced pre-assembly, day 0 was the day the animal was moved from pre-assembly facility to the feedlot pen.

Day #	Time points for each animal were described relative to day 0 and were positive when referring to time points after day 0 (e.g. day 50 referred to the 50th day at risk) and negative when referring to time points prior to day 0 (e.g. day -13 referred to the 13th day before day 0).
Days # to #	Time period. #s refer the start and end days of the time period. The numbers of days are relative to day 0 and can be positive or negative
Days on feed	Number of days on which an animal was offered a total mixed ration in a feedlot pen
Deviance information criterion	A numeric measure used to compare the complexity and fit of multiple Bayesian models that use the same data. A lower number indicates a better model for the data
Exposure variable	A variable that describes for each subject whether that subject was “exposed” to a particular event or characteristic. Exposure is used in the broadest sense; for example, an animal's age at induction can be considered to be an exposure. Exposure variables are also called independent variables.
Feedlot-level risk factor	A risk factor where all animals in the same feedlot must have the same category of exposure; e.g. feedlot region
Follow-up sample	Blood sample collected from an animal after about 42 days on feed
Full cohort dataset	Comprises all animals enrolled into study cohort with a time at risk of at least one day that were not lost to follow-up
Group	Collection of study animals that were at the same PIC at the end of a given day and subsequently went into the same cohort
Group-#	Collection of study animals that were at the same PIC at the end of a given day and subsequently went into the same cohort where # refers to the number of days prior to day 0.
Group-issue	Collection of study animals that had the same PIC of issue and subsequently went into the same cohort, provided that the PIC of issue was not the feedlot PIC
Group-level risk factor	A risk factor for which all animals in the same group must have the same category of exposure; e.g. transport time
Home pen	For analysis of putative risk factors relating to pen characteristics; the pen in which the study cohort was housed for the majority of its first 50 days on feed
Hospital crush	A crush used to examine animals removed from their cohort because they are suspected of being unwell by pen riders
Hospital pen	A pen into which animals may have been placed after being pulled from the cohort; separate from the pen where the animals from the cohort were located
Hospital record	A record pertaining to an animal pulled from the cohort for examination and/or treatment. An animal with a hospital record may have then returned to the pen where it was located when pulled or spent time in the hospital pen
Incidence rate of BRD	The number of cattle in the population that were pulled and whose first pull was for BRD per 1000 animal-days at risk
Incidence risk of BRD	The percentage of cattle in the population that were pulled and whose first pull was for BRD

Induction	The process of tagging, weighing, treating and entering animal identifiers into the feedlot system
Induction date	Date animal was first entered in the feedlot computer system
Induction period	Period from when the first animal was inducted into the cohort until the last animal was inducted into that cohort
Induction samples	Blood sample and a nasal swab from all cattle in each study cohort at the time of induction
Intra-class correlation coefficient for BRD	A coefficient that describes the extent of "clustering" of BRD within groups, cohorts and feedlots. Can take values from 0 to 1. For example, if, in some cohorts, all animals acquired BRD and in remaining cohorts, no animals acquired BRD, the intra-class correlation coefficient for clustering by cohort would be 1 i.e. BRD is completely clustered by cohort and there is perfect correlation between animals in the same cohort. In contrast, if the incidence risk of BRD was the same in all cohorts, the intra-class correlation coefficient for clustering by cohort would be 0 i.e. there is no clustering of BRD by cohort
Minimal sufficient adjustment set	Set of variables included in a model to obtain correct estimates for total or direct effects of the variable of interest that would no longer be sufficient if any variable were removed
National Livestock Identification System	The national system operating throughout Australia for the identification and tracing of livestock
NLIS ID	National livestock identification system number comprised of an 8 character Property Identification Code (PIC), a 1 character manufacturer code, a 1 character device type code, a 1 character code for the year of manufacture and a unique 5 character alphanumeric serial number
Odds ratio	The odds of the disease in the exposed group relative to the odds of disease in the reference group
On feed	When animal was in a feedlot pen and being fed a total mixed ration
Outcome variable	Dependent variable; e.g. the development of BRD by day 50
Pen death	Death without a hospital record
Pen rider	Person who observes cattle within a feedlot pen for signs of illness or injury
Pestigard™	Vaccine registered for the active immunisation of cattle against BVDV which will assist in the reduction of losses associated with the BRD complex and other clinical diseases where BVDV is implicated
Physically constructed capacity	The number of cattle that can be kept on the feedlot given its existing infrastructure. This may be equal to or less than the licensed capacity (the number of cattle that the State or other legislative body is willing to support on the feedlot at the current time and in the future).
PIC of issue	Property identification code (PIC) for place where NLIS device is first implanted
Pilot study	Small scale preliminary study used to assess the proposed methodology and logistics before the main study commences

Population attributable fraction for BRD	For a particular risk factor, this describes the proportion (i.e. the fraction) of the incidence risk of BRD in a population that is due to some animals in the population being exposed to the risk factor, assuming that exposure is causal
Population attributable risk for BRD	For a particular risk factor, this describes the absolute amount of incidence risk (i.e. percentage points) of BRD in a population that is due to some animals in the population being exposed to the risk factor, assuming that exposure is causal
Pre-assembly	Process where cattle are kept at pasture in a paddock in the vicinity of the feedlot for a period of time between arrival and "going on feed" i.e. commencement of feeding in a feedlot pen. This process is called backgrounding by some members of the feedlot industry. We have avoided using the term backgrounding (except in the names of files exported from StockaID®) as it has multiple meanings to different people, and can include various combinations of management practices before the animals enter the feedlot.
Prevalence	Proportion of a population with a particular disease (e.g. BRD) or risk factor at a particular point in time
Prospective study	A type of study where the outcome of interest has not occurred at the time the study starts.
Pull	Removal of an animal from the cohort for examination in the hospital crush and treatment, as required
Purchase group	Identifier used for animals entering a particular feedlot on a particular date with the same non-saleyard vendor
Putative risk factor for BRD	An exposure variable that is potentially a risk factor for BRD
Radio frequency identification device	RFID; a sealed transponder that emits a unique number when energised by an external device such as that in a wand or panel reader.
Rhinogard™	Modified live intranasal vaccine registered for the control of bovine herpes virus 1
Risk factor for BRD	An exposure variable that measures a particular event or characteristic that is considered to be a cause or predictor of BRD; animals exposed to that event or characteristic will be at higher risk of subsequently acquiring BRD than non-exposed but otherwise identical animals; if the risk factor is considered to be causal, the exposure directly or indirectly causes BRD; if the risk factor is considered to be predictive but not causal, the increased risk of BRD is because the exposure is correlated with a causal exposure.
Saleyard transfer	An animal was classified as having a saleyard transfer if it had a pair of records on the same date in the NLIS data with the transfer types described as "saleyard in" and "saleyard out".
Sampling frame	List of eligible units from which sample is taken; e.g. for the case-control study it comprised animals that met the eligibility criteria for selection
Selection batch	Set of animals selected at one time for inclusion in the case-control study. In total there were two selection batches.

Selection number	Unique animal identifier used in the case-control study
Seroconversion	An increase in the ELISA result (described in Section 3.5.3) between the induction and follow-up samples for a virus by two or more categories in animals that were seronegative at induction
Seroincrease	An increase in the ELISA result (described in Section 3.5.3) between the induction and follow-up samples for a virus by two or more categories regardless of the induction sample result
Serological variables	Variables derived from the results of ELISAs performed on case-control serum samples
Significance level	Probability of wrongly rejecting the null hypothesis if it is in fact true
Statistical power	Probability that the study will detect a significant difference for a specified association.
Target population	Entire population to which study results are to be extrapolated i.e. cattle in medium/large Australian feedlots
Test batch	Set of serum samples that were tested using plates of the BIOX K 284 ELISA® with the same batch number. In total there were four test batches.
Transfer	Movement of an animal from one location to another as recorded in the NLIS database
Trial ear tags	Ear tags that were sequentially numbered within a cohort. Used by some feedlots to identify study cattle
Vendor questionnaire study	Analysis that includes all animals with vendor questionnaire data that were eligible for inclusion in the cohort study
Vendor questionnaire subset 1	Analysis that includes all animals eligible for inclusion in the vendor questionnaire study that were bred by the vendor
Vendor questionnaire subset 2	Analysis that includes all animals eligible for inclusion in the vendor questionnaire study that were either bred by the vendor or purchased by the vendor aged 10 months or less
Visual identification number	Identification number recorded on an ear tag inserted at induction by most feedlots, an additional tag to a trial ear tag
Yard weaning	Practice whereby weaners are held in small yards or pens for an extended period of time following separation from their dams

List of Abbreviations

BoHV-1	Bovine herpesvirus 1
BPI3	Bovine parainfluenza virus 3
BRD	Bovine respiratory disease
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine viral diarrhoea virus
csv	Comma separated value
C_T	Cycling threshold
DIC	Deviance information criterion
DOF	Days on feed
ELISA	Enzyme-linked immunosorbent assay
IBR	Infectious bovine rhinotracheitis
MCMC	Markov chain Monte Carlo
MLA	Meat and Livestock Australia
NBRDI	National Bovine Respiratory Disease Initiative
NLIS	National livestock identification system
NLIS ID	National livestock identification system identification string
PAF	Population attributable fraction
PAR	Population attributable risk
PCR	Polymerase chain reaction
PI	Persistently infected with BVDV
PIC	Property Identification Code
Ref. cat.	Reference category
RFID	Radio frequency identification device.
SCU	Standard cattle unit

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 herpesvirus 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	BoHV-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 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
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 induciton 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^{TM} 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 induciton 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 present 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 present 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 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 salyeyard transfer between days -12 and 0
SY -27 to -13	Animal had a salyeyard transfer between days -27 and -13
SYpre-27	Animal had a salyeyard 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

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1 Background

Animal health surveys have consistently identified the bovine respiratory disease complex (BRD) as the most important infectious disease of feedlot cattle in eastern Australia.[1, 2] Annual losses to the feedlot sector have been estimated at $20 per head across all animals in the feedlot, placing total industry losses at a minimum of $40 million per year.[3] BRD causes economic loss due to medication costs, mortality, excessive feed inputs associated with increased time on feed, reduced sale prices and associated labour costs. However, evidence for practices used to reduce the incidence of BRD in Australian feedlots is limited. (Refer to "Evaluation of practices used to reduce the incidence of bovine respiratory disease in Australian feedlots", Appendix 1).

A range of microorganisms is involved in BRD with at least four viral and three bacterial species involved. The four viruses most commonly associated with BRD in Australia are bovine herpesvirus 1 (BoHV-1 or IBR), bovine viral diarrhoea virus (BVDV or bovine pestivirus), bovine parainfluenza 3 virus (BPI3) and bovine respiratory syncytial virus (BRSV). Serological surveys have shown that all of these viruses infect feedlot cattle in Australia.[1] A number of bacterial species have also been recognised as important to the BRD complex; these include *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*.

Although the clinical signs of BRD are indicative of infection with one or more of the pathogens listed above, infection alone rarely causes serious illness and other factors are crucial for the development of BRD under field conditions. BRD is a complex multifactorial condition with a number of animal, environmental and management risk factors predisposing cattle to illness. These factors are likely to be mediated through a number of pathways including reductions in systemic and possibly local immunity. Stressors such as weaning, handling at saleyards, transport, dehydration, weather conditions, dietary changes, co-mingling and pen competition may reduce the effectiveness of the immune system, allowing infection with pathogens to lead to the development of more serious clinical syndromes such as BRD. In overseas research, environmental and management risk factors have been identified as contributing to the development of BRD.

In order to improve the management of BRD in the feedlot sector, it is essential to first identify the critical risk factors leading to disease development and then develop management strategies that circumvent the disease development pathway. This project will provide industry with improved management strategies to minimise the

[1] Dunn, S., Godwin, J., Hoare, R., Kirkland, P.D., 1993. Diseases of Feedlot Cattle. Meat Research Corporation.

[2] Sergeant, E., 2001. Survey of Feedlot Diseases in Australia. Meat and Livestock Australia Limited, North Sydney, NSW.

[3] Sackett, P., Holmes, P., Abbott, K., Jephcott, S., Barber, M., 2006. Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers. Meat and Livestock Australia Limited, North Sydney, NSW.

economic impact of bovine respiratory disease on feedlot cattle performance through critical evaluation and identification of key risk factors for disease development.

2 Project objectives

2.1 Overall objectives

1. Conduct an epidemiological study to identify, and quantify the impact of, the critical risk factors associated with BRD development.
2. Determine the role of bovine viral diarrhoea virus and *Mycoplasma bovis* in the occurrence of BRD.
3. Determine the role of infectious agents in predisposing animals to developing BRD.
4. Assess the effectiveness of disease biomarkers for improved detection of BRD through assessment of disease biomarkers.
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.

Each of these objectives is comprised of multiple sub-objectives.

2.2 Objectives addressed in this report

This report describes all research conducted to address the first of the overall project objectives and part of the research conducted to address objectives 2 and 3. The sub-objectives within these three objectives that are addressed in this report are detailed in Table 2-1.

Table 2-1: Specific objectives within overall objectives that are addressed in this report.

Overall objective/sub-objective	Section number
Conduct an epidemiological study to identify, and quantify the impact of, the critical risk factors associated with BRD development.	
Describe BRD incidence for cohorts of cattle over time and by feedlot	Sections 4.1.3 and 5.1
Define typical and achievable performance for BRD incidence based on the distribution of observed performance in cohorts in a selected population of Australian feedlots	Sections 4.1.3 and 5.1
Estimate the proportion of variation in BRD occurrence at animal, cohort, pen and feedlot levels	Sections 4.3 and 5.5
Assess the strength of association between “known” and potential risk factors and BRD occurrences	Sections 4.2, 5.2 and 5.4
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)	Sections 4.2 and 5.6
Estimate the proportion of variation in BRD occurrence/incidence that is explained by identified risk factors	Sections 4.3 and 5.5
Determine the role of bovine viral diarrhoea virus and *Mycoplasma bovis* in the occurrence of BRD.	
Describe the prevalence of persistently infected carriers (PIs) of bovine viral diarrhoea virus (BVDV) in cattle arriving at a selected group of Australian feedlots	Sections 4.2.11 and 5.2.9
Assess associations between exposure to PIs in feedlot cattle in Australia and subsequent occurrence of BRD	Sections 4.2.11 and 5.2.9
Determine the role of infectious agents in predisposing animals to developing BRD.	
Describe the immune status to and prevalence of infection with BRD pathogens at induction at both animal- and cohort- levels in a selected group of Australian feedlots	Sections 4.2.12 and 5.3
Assess associations between animal and cohort status at induction, seroconversion and subsequent BRD incidence using a case-controlled study and mortality	Sections 4.2.12 and 5.4

3 Methodology

3.1 Overview

Two prospective observational studies were used to identify and quantify the impact of the critical risk factors associated with BRD development using a selected population of Australian feedlots. A cohort study was used for analyses of putative non-serological risk factors and a nested case-control study was used for analyses of putative serological risk factors. Both studies were prospective; all cases of BRD occurred after the studies had commenced.[4]

3.2 Rationale for study design

Prospective designs were chosen for both studies because much of the necessary data required could not be obtained retrospectively.

1. Obtaining accurate data from vendors specific to a particular group of animals required contacting them soon after the cattle were sold; data about cattle sold several years previously could not be expected to be reliable.
2. Data obtained from the National Livestock Identification Scheme enabled the derivation of very important variables relating to mixing, moving and saleyard transfers. The NLIS was only fully implemented across all Australian states in 2004. Thus, had the study been conducted retrospectively in 2008 (the planned start date), full lifetime histories would not have been available for all cattle enrolled in feedlots prior to 2008 cattle enter feedlots up to four years of age.
3. Blood samples for all study cattle were required to assess the impact of the presence of animal(s) persistently infected with bovine viral diarrhoea virus and this was of great interest to industry.
4. Blood samples from a subset of study cattle were required to assess the impacts of the four viruses of interest
5. Some data were recorded by feedlot personnel specifically for the purposes of the study.
6. Although much of the data used in the analyses was routinely recorded by personnel at many feedlots, these data were not all retained for an extended period of time; where data had been archived, it may have still been accessible, but some or all old records may have been deleted.

Thus, the prospective study design enabled reliable data to be collected and readily accessed for most variables of interest.

In addition, with a prospective design, data quality could be assessed on an ongoing basis and any queries could be made within a short time after data collection. A

[4] Dohoo, I., Martin, W., Stryhn, H., 2010. Veterinary Epidemiologic Research. VER Inc Charlottetown, Canada.

cohort study was used for analyses of putative non-serological risk factors. Risk factors could also have been assessed using a case-control study but a cohort design also allowed estimation of prevalences of exposure, and impacts of risk factors on incidence of BRD for the entire population. In addition, rare exposures could be assessed with greater precision with a large cohort study than with a case-control study with the same number of BRD cases as in the alternative cohort study and an equivalent number of controls.

A nested case-control study was chosen for analyses of putative serological risk factors rather than a cohort study because the cost of testing sera from all animals included in the cohort study was prohibitive, and high statistical power could still be achieved with a case-control design.

Both studies were conducted at the animal level, i.e. the unit of analysis was the individual animal. For the cohort study, this method enabled the effects of risk factors at different levels (from animal level to feedlot level, Section 3.3.4) to be considered. It also enabled estimation of the proportions of variance at each of these levels and estimation of the proportion of variance at each of these levels that was explained by various risk factors.

3.3 Cohort study design

3.3.1 Background

The target population was cattle in medium to large Australian feedlots. The initial proposal was to conduct a prospective cohort study involving 16 feedlots. At each feedlot, two pens would be identified and, over an 18 – 24 month period, 13 cohorts (animals assembled together in a feedlot pen following induction i.e. the process of tagging, weighing, treating and entering animal identifiers into the feedlot system) kept in these pens would be enrolled. With an estimated 150 – 200 head per cohort, approximately 32,000 – 40,000 head were expected to be enrolled. The goal was to have sufficient power to identify cohort-level and pen-level risk factors as well as those at the animal level. It was apparent at an early stage that identification of feedlot-level risk factors would be beyond the scope of a study of this size.

After discussions with potential collaborating feedlots, it was apparent that, after induction, cattle were often moved between pens within the feedlot. In addition, logistical constraints might prevent enrolment of each cohort that would be kept in selected pens, for example the additional personnel required for sample collection might not be available when these cattle were inducted. As altering the normal movement patterns of cattle between pens would be contrary to the observational nature of the study, the constraint on the use of specific pens was removed.

In addition, feedlot veterinarians and others in the feedlot industry indicated that factors to which cattle had been exposed prior to arrival could be important. Accordingly, the study design was modified to collect pre-arrival data, by including a vendor questionnaire for a subset of enrolled cattle and obtaining movement data from the National Livestock Identification System (NLIS) for all enrolled cattle. Details on desired sample size of cohorts and total number of cattle are included in Section 3.3.5.

Identification of risk factors relating to prior or on-feedlot exposure to known viral respiratory pathogens required serological data from two points in time. Due to budget constraints, it was not possible to test sera from all cattle in the study, so a nested case-control study was also conducted whereby sera from a subset of cases

and controls were tested and analyses of serological risk factors restricted to this subset (Section 3.4).

The project was known as the National Bovine Respiratory Disease Initiative (NBRDI). The duration of the project extended well beyond the originally proposed timeline due to issues with compensation for participating feedlots which necessitated a major increase in budget, difficulties obtaining animal ethics approval to conduct research in NSW, refusal of many feedlots to participate and a slower than proposed rate of enrolments by participating feedlots.

3.3.2 Consulting veterinarians

Veterinarians regularly providing animal health and/or nutritional services to the feedlot industry were consulted throughout the duration of the project. They provided input into study design, encouraged feedlots to participate, assisted with sample collection on some feedlots and gave valuable feedback on interim results.

3.3.3 Putative risk factors

Putative risk factors were identified in conjunction with consulting veterinarians and other stakeholders in the feedlot industry. Those discussed at a workshop with consulting veterinarians are described in more detail in the MLA publication associated with this project (Evaluation of practices used to reduce the incidence of bovine respiratory disease in Australian feedlots, Appendix 1) and are listed below:

Putative risk factors associated with cattle preparation

- Practices with some supporting evidence
    - Yard weaning
    - Pre-vaccination with bovine herpesvirus 1 (BoHV-1) vaccine (Rhinogard™)
    - Pre-vaccination with *Mannheimia haemolytica* vaccine (Bovilis MH™)
    - “Resting” at property (on grass) in stable social group for 4 weeks before going to feedlot
    - Reducing “time in hand” (transport times)
    - Choosing animals with good weight for age
    - Not using Herefords
- Practices with minimal evidence or untested
    - Distance travelled (as opposed to time in transport)
    - Hydration status on arrival at feedlot
    - Truck design/exhaust fumes

Putative risk factors associated with feedlot management

- Practices with good supporting evidence
    - Homogenous units/minimal purchase groups per pen
    - BoHV-1 vaccine on arrival/induction
    - Bovilis MH™ on arrival/induction
    - Reducing time between arrival and induction (“filling pens quickly”)
    - Antibiotics at induction
        - Tilmicosin (Micotil) at induction (not oxytetracycline)

    - Low pen density/bunk space
- Practices with minimal evidence or untested
    - "Add-ons"/re-mixing pen after induction
    - Large weight range within a pen (i.e. >100kg difference)
    - Introductory diet (low nutrient dense starter ration)
    - Removal of PI animals (persistently infected with BVDV)
    - Liquid supplements (i.e. urea/molasses)
    - Pestigard™ at induction
    - Concurrent disease
    - Staffing levels, i.e. pen riders per 10,000 head
    - Gentle cattle handling
    - Dust levels
    - Climate/season
    - Rainfall/mud
    - Use of growth implants
    - Electrolytes in the water on arrival (increase water intake)
    - Sex
- Practices which we know do not work
    - Vitamin E at induction
    - Vitamin A, D and E on induction
    - Probiotics on induction
    - Vitamin C on induction
    - Vitamin B12 on induction

A selection of these putative risk factors were pursued in the study based on joint consideration of availability and practicality of obtaining the data and variability in exposure status amongst study animal and/or prior evidence of effect including biological plausibility.

3.3.4 Hierarchical structure of feedlot data

Risk factors for BRD operate at various levels. If all animals within each feedlot are exposed to the same category of a risk factor, that risk factor would be considered a feedlot-level risk factor (e.g. all animals in one feedlot are vaccinated with Rhinogard™ at induction, no animals in another feedlot are vaccinated, and there are no feedlots where some animals are vaccinated and others are not). Similarly, if within a feedlot, all animals within each cohort are exposed to the same category of a risk factor (e.g. shared pen water), that risk factor would be considered a cohort-level risk factor. In the same way, if within a cohort, all animals within each purchase group are exposed to the same category of a risk factor (e.g. transport duration), that risk factor would be considered a purchase group-level risk factor. Finally, if within a purchase group, animals have an individual measure of exposure to a risk factor (e.g. induction weight) that risk factor would be considered an animal-level risk factor. This pattern was reflected in the project data, where there was a natural nested hierarchical structure with four levels: feedlot, cohort within feedlot, purchase group within cohort and animal within purchase group.

Risk of BRD occurrence would be expected to "cluster" according to this structure. While each animal can be considered as having a particular risk of being affected by BRD, cattle at any one feedlot would be expected to have risks that are more similar

compared to cattle pooled across all feedlots. For example, across all feedlots, average risk of BRD at the animal level may vary between cohorts from 0% to 80%, whereas in a feedlot that has low BRD incidence, risk of BRD may vary between cohorts from only 0% to 10%, and in a feedlot that has high BRD incidence, risk of BRD may vary between cohorts from 30% to 80%. Thus, within a feedlot, animals are more similar than across the entire population. In the same way, within a particular feedlot, cattle in any one cohort would be expected to have risks that are more similar compared to cattle pooled across all cohorts, and within a particular cohort, cattle raised and/or kept together prior to arrival at the feedlot would be expected to have risks that are more similar compared to all cattle pooled.

This hierarchical structure was useful when considering the nature of various risk factors; the hierarchical level at which a particular risk factor occurs has implications for both studying and preventing exposure to that risk factor. The hierarchical structure also affected study sample size calculations and choice of statistical methods. When such clustering is present, individual observations are not statistically independent. Sample size calculations and statistical methods that accounted for this clustering were used.

3.3.5 Sample size calculations

Sample size can be determined based on statistical power calculations. The statistical power of a study is the probability of that study detecting a significant difference at a specified type 1 error level for a given sample size, the smallest true odds ratio of interest, prevalence of exposure and degree of clustering of the outcome. As described above, the study aimed to identify risk factors at the animal, purchase group and cohort levels. As there was clustering of BRD occurrence by cohort, statistical power was expected to be lower for cohort-level risk factors than for group-level and animal-level risk factors, so sample size calculations were performed for risk factors at this level. To perform these sample size calculations, an estimate of the intra-class correlation coefficient was required. For cohorts, this is a measure of the correlation in BRD occurrence between any two animals in a cohort; it can take any value between 0 and 1. For example, if the incidence of BRD was the same in all cohorts, the intra-class correlation coefficient would be 0, whereas if the incidence of BRD was 0% in some cohorts and 100% in the remaining cohorts, the intra-class correlation coefficient would be 1, indicating perfect correlation between animals in the same cohort.

Estimates of the average cohort size and the incidence risk of BRD (i.e. percentage of cattle that were pulled for BRD and whose first pull was for BRD) in animals not exposed to the risk factor of interest were also required. (Pulling is the removal of an animal from the cohort for examination in the hospital crush and treatment as required). Retrospective data were obtained from three feedlots from which the following estimates were derived:

- intra-class correlation coefficient for clustering by cohort: 0.1
- average cohort size: 235
- incidence risk of BRD in animals not exposed to the risk factor of interest: 20%

The number of cohorts required to detect an association between a binary cohort-level risk factor and BRD occurrence also depended on:

- the proportion of cohorts that are exposed to the risk factor
- the smallest increase of interest in incidence of BRD relative to that in the reference group (i.e. the animals not exposed to the risk factor of interest (quantified as an odds ratio - the odds of the disease in the exposed group relative to the odds of disease in the reference group)
- the desired statistical power
- the required significance level (the probability of wrongly rejecting the null hypothesis [i.e. the hypothesis that there is truly no association] given that the null hypothesis is, in fact, true i.e. no association exists)

Using these retrospective estimates, a desired power of 80% and significance level of 0.05, the required numbers of cohorts to detect cohort-level risk factors with prevalences of exposure ranging from 1 to 50% with odds ratios of 1.2 to 5 relative to the unexposed reference category were calculated using WinPepi Compare2 (Version 10.7, Table 3-1). The compromise between cost and desired power resulted in a target of 200 cohorts. As enrolment of cohorts was more protracted than originally expected, the target sample size was reduced to 170 cohorts during the course of the study. This number of cohorts was expected to ensure the study had statistical power of at least 80% i.e. at least 80% probability of detecting a significant association between a cohort-level risk factor to which 20% of the study population were exposed and BRD provided the odds ratio was at least 1.5. These sample size calculations were based on frequentist statistics as this was the proposed method for analysis at this stage of the study.

Table 3-1: Numbers of cohorts required to ensure the study had 80% probability of detecting a significant association between a given cohort-level risk factor and BRD occurrence for various prevalences of exposure and odds ratios, with the significance level set at 0.05, an average cohort size of 235, incidence risk in the unexposed of 20% and an intra-class correlation coefficient of 0.1. Figures in bold represent combinations of prevalence of exposure and odds ratio compared to the unexposed for which the target of 170 cohorts would have adequate power.

Prevalence of exposure to risk factor[1]	Odds ratio					
	1.2	1.5	2	3	4	5
1%	14,372	2,670	832	292	178	**126**
5%	3,002	560	176	**64**	**38**	**28**
10%	1,588	298	**94**	**34**	**20**	**16**
20%	898	**170**	**54**	**20**	**12**	**10**
50%	582	**112**	**36**	**14**	**10**	**6**

[1] Proportion of cohorts that are exposed to the risk factor

3.3.6 Pilot study

A pilot study involving three feedlots was conducted to develop sampling and data collection methods that could be transferred to the main study. A total of nine cohorts were enrolled between August 2007 and February 2008. During this study it became apparent that it was not feasible to measure hip height, and that body condition and frame size could not be estimated with consistently high accuracy and precision by a large number of personnel across multiple feedlots. Several logistical issues arose including matching blood samples and nasal swabs arriving at the laboratory with the

data recorded by the feedlots at induction and downloading required animal-level data from feedlot software. These issues were addressed and modifications were incorporated into the protocol for the main study.

3.3.7 Feedlot selection

3.3.7.1 Issues with enrolment

Due to an economic downturn in the feedlot sector during late 2007, many of the feedlots that were approached to participate in the study were not willing to do so because of labour shortages. Many were also concerned about the amount of compensation being offered for costs incurred by feedlots due to participation in the project, which they considered to be inadequate at $5/animal enrolled. As result of this, the level of compensation was discussed and calculated by the members of the Australian Lot Feeders' Association (ALFA) Research & Development committee, a project review was held and a contract variation with the compensation raised to $30/animal was approved by the MLA Board in November 2008.

3.3.7.2 Inclusion criteria

Feedlots were selected by purposive sampling. Initially the inclusion criteria were as follows:

1. The feedlot was licensed under the National Feedlot Accreditation Scheme
2. The feedlot was serviced by a veterinarian collaborating with the project
3. The physically constructed capacity of the feedlot (number of cattle that can be kept on the feedlot given its existing infrastructure) was at least 5,000 head, ideally at least 10,000 head
4. The feedlot used computerised record keeping for at least all animal-level and within-animal-level data
5. 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

Most of the larger feedlots in Australia that met these criteria were approached at an early stage by one or more of the project team, consulting veterinarians and an MLA representative both during an initial recruitment phase from late 2007 to early 2008 and after the contract variation at the end of 2008. As the target of 16 feedlots was not met, criteria (2) and (3) were relaxed such that feedlots not serviced by a consulting veterinarian and smaller feedlots were also approached. Although criterion (5) 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.

3.3.7.3 Feedlot enrolment

Feedlots managers who expressed an interest in participating were visited by a member of the project team. Managers were provided with an information booklet (National BRD Initiative – Information for Feedlot Managers, Appendix 2). Details about the feedlot management protocol were collected at this time via a questionnaire that was completed during a face-to-face interview with the feedlot manager. Where the manager agreed to have their feedlot participate, the manager and staff were provided with a more detailed manual of protocols (National BRD Initiative – Protocols, Appendix 3). Fourteen feedlots participated in the study. Their locations are shown in Figure 4.1 and details of numbers of cattle contributed are

reported in Table 4-1. Nine feedlots had a physically constructed capacity of at least 10,000 head, three feedlots had a physically constructed capacity from 5,000 to < 10,000 head and two had a physically constructed capacity from 2,000 to < 5,000 head.

3.3.8 Cohort selection

Feedlot managers were initially requested to enrol a cohort every eight weeks. The cohort was to be randomly selected by a member of the project team from all cohorts that were expected to be inducted at the feedlot during that week. It became apparent at an early stage that this process was not practical; additional labour was required for sampling cattle as they were inducted into the study and it was not always practical for feedlot personnel to arrange this for randomly selected cohorts. So feedlot personnel proceeded to enrol cohorts into the study when it was logistically feasible for them to do so. Rate of cohort enrolment also varied markedly both within and among feedlots as a result of numbers of cattle being inducted. Cohorts were enrolled between March 2009 and December 2011. The frequency of enrolment of cohorts in 2011 was increased in many feedlots because all study cattle had to be enrolled by the end of 2011 to allow final data analyses to commence in mid-2012.

3.3.9 Full cohort dataset

3.3.9.1 Inclusion criteria

All animals inducted into study cohorts were eligible for inclusion in the main cohort study provided they were not pulled on their induction day or did not die on their induction day as this day preceded the start of time at risk, and provided they were 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). Of a total of 35,160 animals inducted into study cohorts, 35,131 were included in the full cohort dataset (Figure 3.1).

3.3.9.2 Outcome variable – BRD50

The outcome variable for the cohort study was being identified as a BRD case (as defined in 3.7.1) on or between its first and fiftieth day at risk (BRD50). Only the first pull was considered for each animal. For analysis purposes, those whose first pull was for reasons other than BRD were assumed not to have contracted BRD by their fiftieth day at risk. This restriction was imposed as animals could possibly have developed BRD after being pulled for another reason, due to close contact with BRD cases in the hospital pen in association with stressors due to their original reason for being pulled, subsequent handling etc.

3.3.10 Vendor questionnaire datasets

3.3.10.1 Inclusion criteria

The full vendor questionnaire dataset included all animals eligible for inclusion in the cohort study that also had vendor questionnaire data (N = 10,721, Figure 3.1). There were also two vendor questionnaire data subsets. Vendor questionnaire subset 1 included animals eligible for inclusion in the full vendor questionnaire dataset that were bred by the vendor (N = 5,063; Figure 3.1). Vendor questionnaire subset 2 included all animals eligible for inclusion in the full vendor questionnaire dataset that were either bred by the vendor or purchased by the vendor when aged 10 months or less (N = 8,580; Figure 3.1).

3.3.10.2 Outcome variable

The outcome variable for the vendor questionnaire subsets was the same as for the cohort study; being identified as a BRD case (as defined in 3.7.1) on or between its first and fiftieth day at risk.

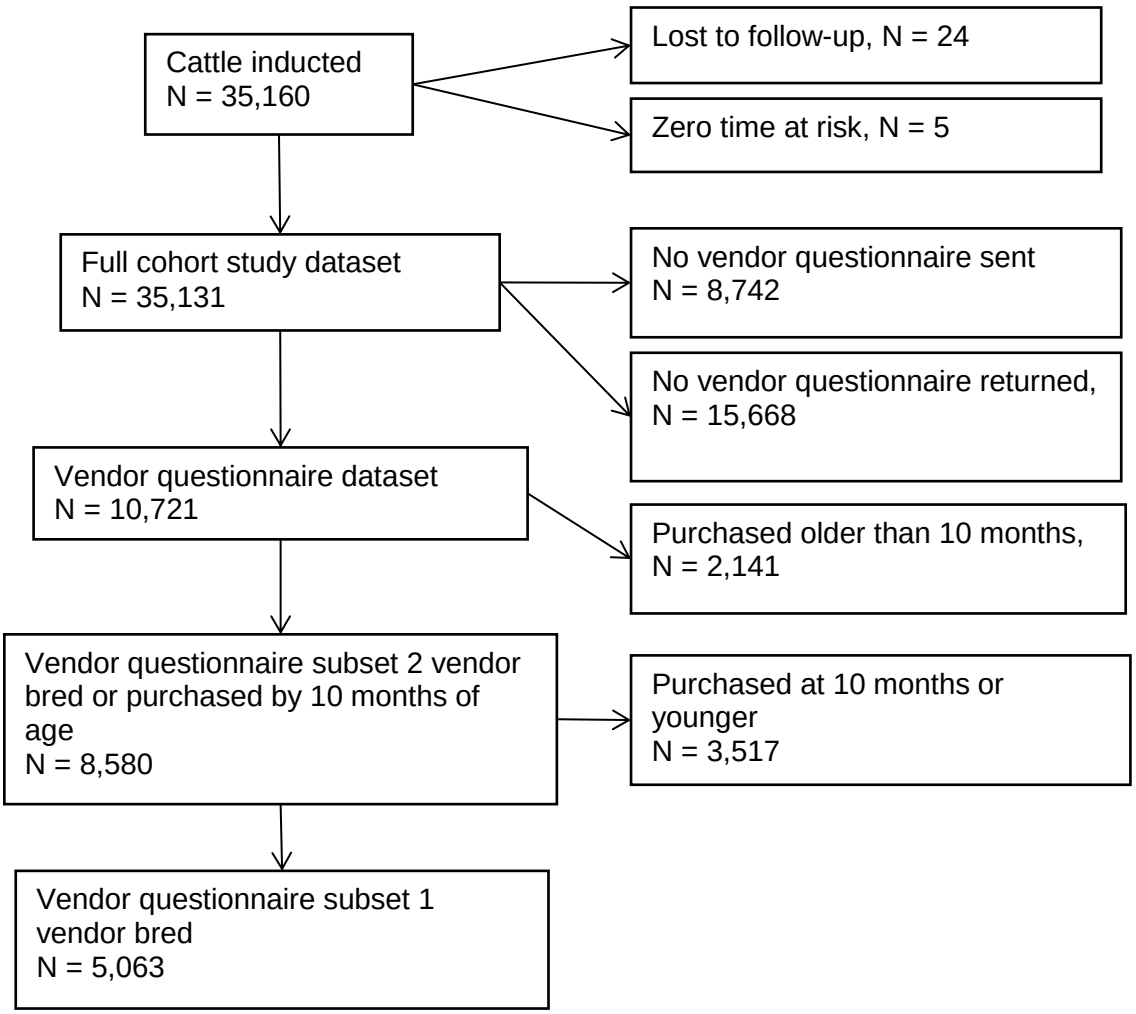

Figure 3.1: Flowchart demonstrating the relationship between the full cohort dataset and the vendor questionnaire datasets (N = number of animals).

3.3.11 Pre-assembly dataset
3.3.11.1 Inclusion criteria

The pre-assembly dataset included all animals from feedlots that practiced pre-assembly. We defined pre-assembly as the process where cattle are kept at pasture in a paddock in the vicinity of the feedlot for a period of time between arrival and "going on feed" i.e. commencement of feeding in a feedlot pen. This process is called backgrounding by some members of the feedlot industry. We have avoided using the term backgrounding (except in the names of files exported from StockaID®) as it has multiple meanings to different people, and can include various combinations of management practices before the animals enter the feedlot.

3.3.11.2 Outcome variable

The outcome variable for the pre-assembly dataset was the same as for the cohort study; being identified as a BRD case (as defined in Section 3.7.1) on or between its first and fiftieth day at risk.

3.3.12 Access to electronic data

Participating feedlots used various software packages for record keeping and data management. Six feedlots used StockaID® (Elynx Pty Ltd), one used Cattle Fattening Records® (Possum Gully), one used an in-house data management system developed by the feedlot manager, and the remaining six feedlots were owned by a single company and used an in-house data management system located at the company's head office. Elynx and Possum Gully were commissioned to develop user-friendly means for extraction of the required electronic data as csv files so that feedlots using these software packages were able to access the animal- and within-animal-level data with minimal effort and then email the files to the project team. In a similar manner, queries were written in the two in-house systems. Thus electronic data received from all feedlots was made as similar as possible. In addition, StockaID® introduced a modification such that a warning appeared on the computer screen at the feedlot hospital crush when an animal identified as a study animal was examined in the hospital crush. This prompted the feedlot personnel to collect the required blood sample and nasal swab.

3.3.13 Animal ethics

Approval for research conducted in Queensland, South Australia and Western Australia was covered by the University of Queensland Animal Ethics Approval Certificates 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, AEC09/027.

3.4 Case-control study design

3.4.1 Introduction and overview of design

To assess associations between serological variables and BRD, initially all animals enrolled in the cohort study were to be tested at induction and follow-up. However, this required very large resources for laboratory inputs so a much more efficient design, a case-control study, was used. Under this design, serum samples from a subset of animals from the cohort study were assayed, markedly reducing laboratory costs without a marked reduction in precision.

As the study population could be considered closed from day 0 to day 35, an unmatched risk-based design was used with the cases and controls selected from animals that, respectively, were pulled for BRD between 7 and 35 days (inclusive) after the animals' cohort close dates or were not pulled for any reason between 0 and 35 days (inclusive) after the cohort close date.

3.4.2 Eligibility criteria

Cases and controls were selected based on the following criteria:

Criteria for both cases and controls

- Cases and controls were defined at the animal level
- Animals were only eligible for selection once
- Exposure statuses were not considered in selecting cases and controls, other than restricting selection to animals from specified cohorts as described in Section 3.8.1 and below
- Induction and follow-up serum samples were adequate (Section 3.6.6) and verified (Section 3.8.4)
- The time interval between induction and follow-up samples was ≤ 60 days

Cases

- Must have remained with their cohort until pulled for BRD
- Must have been first pulled between 7 and 35 days after cohort close date
- The first pull must have met the BRD case definition (Section 3.7.1)

Controls

- Must have been part of their cohort continuously from induction until 35 days from cohort close date (i.e. were not removed for any reason in this time period)

A flowchart showing numbers of animals meeting/not meeting specific inclusion criteria is illustrated in Figure 3.2. BRD cases were restricted to those animals pulled between 7 and 35 days after cohort close date to facilitate interpretation of serological data. If the incubation period (i.e. the period between exposure to relevant infectious agents and onset of BRD signs) is 7 days, some BRD cases occurring before day 7 may have been due, in part, to infectious agents acquired before induction. Similarly, BRD cases occurring after day 35 could be due, in part, to infectious agents acquired after day 28. Assuming seroconversion after first exposure to the viruses studied takes 10 to 14 days, some of these animals may not have seroconverted by follow-up sampling as this was to be conducted around day 42.

3.4.3 Selection method

As described in Section 3.8.1, selection and testing were conducted in two selection batches. At the time of selecting animals for the first selection batch, final numbers of both cohorts and animals that would be enrolled in the study for each feedlot were estimated. For each feedlot, half of the expected final number of cohorts was included in the first batch, except for those feedlots that were enrolled late in the study and so contributed fewer cohorts to the first selection batch.

Following the identification of the sampling frame of eligible animals for the first selection batch, 1979 cases and 1979 controls were randomly selected. This process was subsequently repeated for the second selection batch with equal numbers of cases and controls selected to give a total of 3,725 selected cases and 3,725 selected controls. The total number of cases and controls tested was constrained by the laboratory testing costs. Each selected animal was assigned a unique selection

number that was used to link laboratory results to the individual animal. Selected animals from the first selection batch were assigned to multiple groups, whereby all selected animals whose induction and follow-up samples were stored in the same plate were grouped together, as this facilitated retrieval of samples by laboratory personnel prior to testing. This process was later repeated for the second selection batch.

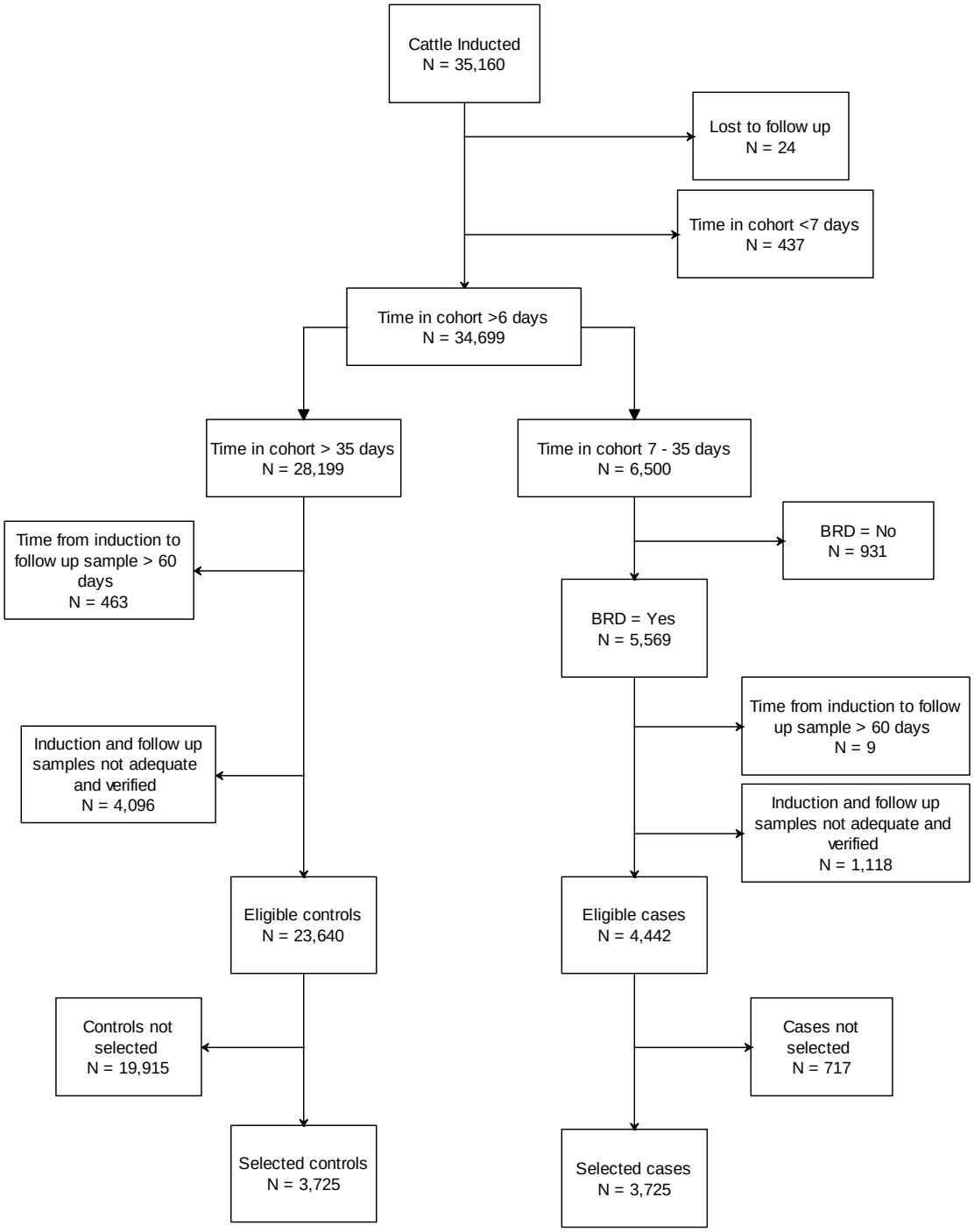

Figure 3.2: Flowchart demonstrating the selection of cases and controls from the cohort study population for the nested case-control study.

3.5 Blood samples, nasal swabs and tissue samples

3.5.1 Sample collection and transport

Feedlot personnel were asked to collect a blood sample and a nasal swab from all cattle in each study cohort at the time of induction (induction samples) and a follow-up blood sample (follow-up sample) as close to 42 days on feed (number of days on which an animal was offered a total mixed ration in a feedlot pen) as possible. They were also requested to collect a blood sample and a nasal swab from all study cattle that were pulled (removed the animal from the cohort for examination in the hospital crush and treatment, as required) for BRD while on feed. In addition, they were asked to collect a lung tissue sample and a tracheal sample at post mortem from any study cattle that died where the death was attributed to BRD.

3.5.1.1 Induction samples

Feedlot personnel notified the project team prior to induction of each study cohort. An induction pack was then sent by courier to the feedlot by a member of the project team. This pack contained the following:

- Pre-labelled 6ml serum tubes (sequentially numbered from one to the number of cattle expected to be in the cohort)
- 50 spare 6ml serum tubes
- Foam racks each to hold 50 serum tubes
- 18 gauge 1” double-ended needles sufficient to blood sample the number of cattle expected to be in the cohort plus spares
- 10 vacuette needle holders
- Nasal swabs sufficient for the number of cattle expected to be in the cohort plus spares
- 1 bin for safely storing used needles
- 2 marker pens
- Multiple freezer blocks
- 1 name and address label for sending the samples to the laboratory
- 1 roll of cling wrap for packaging the serum tubes for return to the laboratory
- 1 form to provide details of number of cattle blood sampled and swabbed and date(s) of sampling
- Plastic box of sufficient size to hold all of the above

Feedlots were requested to follow their standard procedure for inducting cattle into the feedlot. In addition, it was essential that the order in which the cattle were inducted was recorded in some manner so that the sequentially numbered serum tubes could be matched to individual cattle identity numbers. In all cases, this was possible through sequential induction of cattle into the feedlot computer system. In addition, some feedlots chose to use trial ear tags (sequentially numbered ear tags to identify study cattle), whereby tags were numbered from one to the total number of animals inducted into that cohort. These tags also facilitated recognition of study cattle in the hospital.

Each study animal was blood sampled at the time of induction by either a veterinarian or a member of the feedlot personnel who had been trained by a veterinarian. The name(s) of the bleeder(s) for each study cohort were recorded as stipulated in the Animal Ethics Approvals. At least 5ml of whole blood was collected from either the caudal or jugular vein into the tube that matched the sequential number for that animal. The caudal vein was most commonly used. If the vacuum

was lost or the tube was broken during blood sampling, an unlabelled replacement serum tube was used and immediately labelled with the appropriate number. When every tenth animal was blood sampled, at least the last 5 digits of the animal's NLIS number or the entire visual identification number (identification number recorded on an ear tag inserted at induction by most feedlots, an additional tag to a trial ear tag) was recorded on the serum tube to enable the sequential numbering of the serum tubes to be cross-checked against the sequential numbering of the cattle entered into the feedlot computer.

A nasal swab was also collected at the time of induction. Where Rhinogard™ was used the nasal swab was always collected prior to its administration. Each swab was then placed in transport medium and labelled with the same sequential number as the serum tube and every tenth swab was labelled with the last 5 digits of the animal's NLIS number or the entire visual identification number as described above.

If cattle were inducted into the study cohort over more than one day, the sequential numbering was continued from the last number used at the previous session. Blood samples were allowed to clot and the combined serum and clot and nasal samples were kept refrigerated until all cattle had been inducted into the cohort. Samples were then packaged appropriately and transported to the laboratory using the courier preferred by the feedlot.

3.5.1.2 Follow-up samples

Feedlot personnel were requested to collect a second blood sample from cattle as close to 42 days on feed as was feasible. Prior to the follow-up blood sample of each study cohort, a follow-up pack was sent by courier to the feedlot by a member of the project team. This pack contained the following:

- Pre-labelled 6ml serum tubes (numbered from one to the number of cattle in the cohort)
- 50 spare 6ml serum tubes
- Foam racks each to hold 50 serum tubes
- 18 gauge 1" double-ended needles sufficient to blood sample the number of cattle in the cohort plus spares
- 10 vacuette needle holders
- 1 bin for safely storing used needles
- 2 marker pens
- Multiple freezer blocks
- 1 name and address label for sending the samples to the laboratory
- 1 roll of cling wrap for packaging the serum tubes for return to the laboratory
- 1 form to provide details of number of cattle blood sampled and date(s) of sampling

When the induction period (period from when the first animal was inducted into the cohort until the last animal was inducted) was more than one day, the preferred follow-up blood sample date was 42 days after the mid-point of this time period. For logistical reasons some follow-up blood samples were collected up to 75 days after induction (mean: 44.2 days, median: 42 days, standard deviation: 5.4 days, range: 31 - 75 days). Some cohorts where a large number of cattle were pulled for BRD were blood sampled late as feedlot managers were concerned about the potentially negative effects of the additional stress associated with moving and blood sampling.

Blood samples were collected and handled as described in Section 3.5.1.1 and name(s) of bleeder(s) were recorded.

Each follow-up serum tube was matched to the individual animal's data in one of two ways. Feedlots using StockaID® and Cattle Fattening Records® recorded the order in which the cattle went through the crush in the same manner as induction. Thus blood from the first animal was collected in the tube labelled “1”, blood from the second animal was collected in the tube labelled “2” and so on. Other feedlots where cattle had trial ear tags used the tag number to identify the tube into which the blood from that animal was collected. Thus if the first animal going through the crush had trial ear tag 50, the tube labelled “50” was retrieved from the relevant foam rack and the blood sample was collected in this tube, and if the second animal had trial ear tag 24, the tube labelled “24” was retrieved and the blood sample collected in this tube. As at the induction sampling, when every tenth animal was blood sampled the last 5 digits of the animal’s NLIS number or the entire visual identification number was recorded on the serum tube to enable cross-checking. Feedlot personnel were requested to also collect blood samples from cattle that had left study cohorts because they had been pulled for BRD and so had been moved to the hospital pen, if a blood sample had not been collected in the hospital crush within the preceding five days. Blood samples collected in the hospital crush within the five day period were considered suitable replacements for the follow-up blood samples. There was no requirement to collect a follow-up blood sample from cattle that had left the study cohort for other reasons. Samples were kept refrigerated until follow-up blood sampling was complete. They were then packaged appropriately and returned to the laboratory using the courier preferred by the feedlot.

3.5.1.3 Samples from BRD pulls and cattle that died from BRD

Feedlot personnel were requested to collect a blood sample and a nasal swab from all cattle from study cohorts that were pulled for BRD. Prior to enrolment of the first study cohort, a hospital pack was sent by courier to the feedlot by a member of the project team. This pack contained the following:

- 50 6ml serum tubes
- 50 18 gauge 1” double-ended needles
- 50 nasal swabs
- 20 100ml sterile pots for post-mortem samples
- 2 small eskies
- freezer blocks

Subsequent hospital packs were sent to the feedlots when requested. Quantities of the materials in the subsequent packs varied in accordance with requests from the feedlots.

Feedlot personnel were able to identify study cattle when they were examined in the hospital crush via either a warning that was displayed on the crush-side computer if the feedlot was using StockaID®, or the presence of a trial ear tag. A blood sample and a nasal swab were collected and handled as described in Section 3.5.1.1. The serum tube and swab were then labelled with the animal’s NLIS number or the visual identification number, the date of sampling and the name of the feedlot. Samples were kept refrigerated for up to a week and sent to the laboratory appropriately packaged with any other samples that were collected in the time period. Further sampling was not requested if the same animal was subsequently retreated for BRD.

Feedlot personnel were requested to collect tissue samples at post mortem from all study cattle that died from BRD. Tissue samples were collected from the trachea (approximately 5cm) and lungs (approximately $5cm^3$) if the death was attributed to BRD. Samples were placed in sterile pots and labelled with the animal's NLIS number or the visual identification number, the date of sampling and the name of the feedlot. Samples were kept refrigerated for up to a week and sent to the laboratory appropriately packaged with any other samples that were collected in the time period.

3.5.2 Sample management

3.5.2.1 Blood samples

On arrival at the laboratory, blood samples (induction, follow-up and hospital) were stored at 4^oC prior to processing. Samples were visually inspected to make an overall assessment of sample integrity. For high quality samples, where clear separation was observed between the serum and the blood clot, up to 2ml of serum was aspirated and transferred to a 96-well 2ml storage plate. Plates were stored at -80^oC until required. Details of the samples provided by feedlot personnel were recorded as the samples were processed and subsequently transferred to Microsoft® Excel spreadsheets (Induction files, Section 3.6.6.1; Follow-up blood sample files, 3.6.6.2 and Hospital files, Section 3.6.6.3). All details provided on the tube by the feedlot staff were transferred to these spreadsheets. The serum plate number and position of the samples were also recorded.

Some samples received were suboptimal with no visible serum following clotting. In an effort to recover a useable sample for subsequent analysis, these samples were centrifuged or incubated at various temperatures in an effort to extract serum. Where no sample could be recovered, this was recorded on the appropriate spreadsheet. In some cases, particularly where the supply of poor quality samples persisted, the project staff contacted the relevant feedlot to help resolve the situation.

All associated documentation included with a shipment of samples was filed, and also scanned and sent to the project data management team.

3.5.2.2 Nasal swabs

On arrival at the laboratory, nasal swabs (induction and hospital) were stored at 4^oC prior to processing. The nasal swabs were processed in the laboratory to recover any biological material from the swab. The end of the swab was cut off and added to a well in a 96 well storage plate containing 500 µl of serum free media with 2% HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid), 5 × PSF (500 units/mL Penicillin, 500 µg/mL Streptomycin, and 1.25 µg/mL Fungizone x100) and 1% glutamax or phosphate buffered saline. Details provided by feedlot personnel and swab plate number and position were recorded and transferred to spreadsheets (Induction files, Section 3.6.6.1 and Hospital files, Section 3.6.6.3). Plates were stored at -80^oC until required.

3.5.2.3 Tissue samples

On arrival at the laboratory tissue samples were stored at 4^oC. For processing, a qualitative assessment was made regarding the integrity of the sample. In some cases, the samples had been collected many weeks prior to arrival at the laboratory and were considered to be unsuitable for storage or testing due to extensive degradation. These samples were discarded and details recorded in the relevant spreadsheet. Where a sample was deemed suitable for storing and testing, a portion

(approximately 3-4 x 5mm^2) was aseptically removed and stored at -80^{o}C. Plates were stored at -80^{o}C until required. Details provided by feedlot personnel and sample plate numbers and positions were recorded and transferred to spreadsheets (Deads files, Section 3.6.6.4)

Any documentation accompanying the tissue samples was retained by the laboratory staff, scanned and sent to the data management team.

3.5.3 Enzyme-linked immunosorbent assays

Sera were tested using BIOX K 284 ELISA® to evaluate the humoral immune response to bovine herpesvirus 1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus (BPI3) and *Mycoplasma bovis*. The results specific to the *M. bovis* component of the test are not discussed in this report. The *M. bovis* results and conclusions will be reported elsewhere (M. Schibrowski, PhD Thesis, submitted). Tests were conducted according to manufacturer's instructions. Raw optical density results for each test plate were output into 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 and categorise them according to cut-offs provided by the manufacturer. These cut-offs varied among plates with different batch numbers. Each sample was categorised as 0, 1, 2, 3, 4 or 5 for each of the five viruses. Plates with four different batch numbers (test batch) 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.

3.5.4 BVDV testing

Pooled BVDV testing was performed on both induction and follow-up blood samples. Upon arrival at the laboratory, serum was extracted to storage cells and 10µl aliquots of each sample were placed in pooled test wells for quantitative real-time polymerase chain reaction (qPCR) testing. Up to 24 samples from the same cohort were pooled for these tests. Initially the nucleic acids were extracted from the sample pool using VX extraction kit in a 96-well format on the QIAxtractor® according to the manufacturer's instructions (Qiagen). The qPCR analyses were performed as described by Horwood and Mahony.[5] Samples were mixed with the assay components and processed using in Rotor-Gene Q 6000 (Qiagen) thermal cycler to detect the presence of BVDV genomic RNA for 40 cycles A threshold level of 0.05 was specified and the output value in positive tests was a cycling threshold (C_T) which gave the number of cycles completed before the fluorescence exceeded the designated threshold. Thus, a low C_T value indicates that the threshold was exceeded more quickly and is indicative of higher amounts of viral genomic RNA in the sample. Pool extracts were designated positive if the C_T value was ≤35, pool extracts with C_T values >35 but <40 were considered suspect, while pool extracts

[5] Horwood, P.F., Mahony, T.J., 2007. Rapid detection of bovine respiratory disease pathogens. B_FLOT_219 Final Report. Meat and Livestock Australia.

with C_T values ≥40 were considered negative with respect to the presence of BVDV. The plot of the time series displaying fluorescent signal against cycle number typically displayed a sigmoid shape with a steep slope within the logarithmic phase, so assessment of this plot was useful in classifying BVDV-PI animals. Subsequent BVDV testing was conducted on individual samples using the same process.

3.6 Data collection

3.6.1 Details of different data sources

Data were obtained from a range of different sources and at different time points (Figure 3.3).

3.6.2 Animal- and within-animal-level data from feedlots using StockaID®

Animal- and within-animal-level data (the latter referring to data where one animal could have multiple records) for each cohort were exported from feedlot software and sent to the project team after induction, after the follow-up blood sample and after all cattle from that cohort had exited the feedlot. The files and data fields exported from StockaID® are described below.

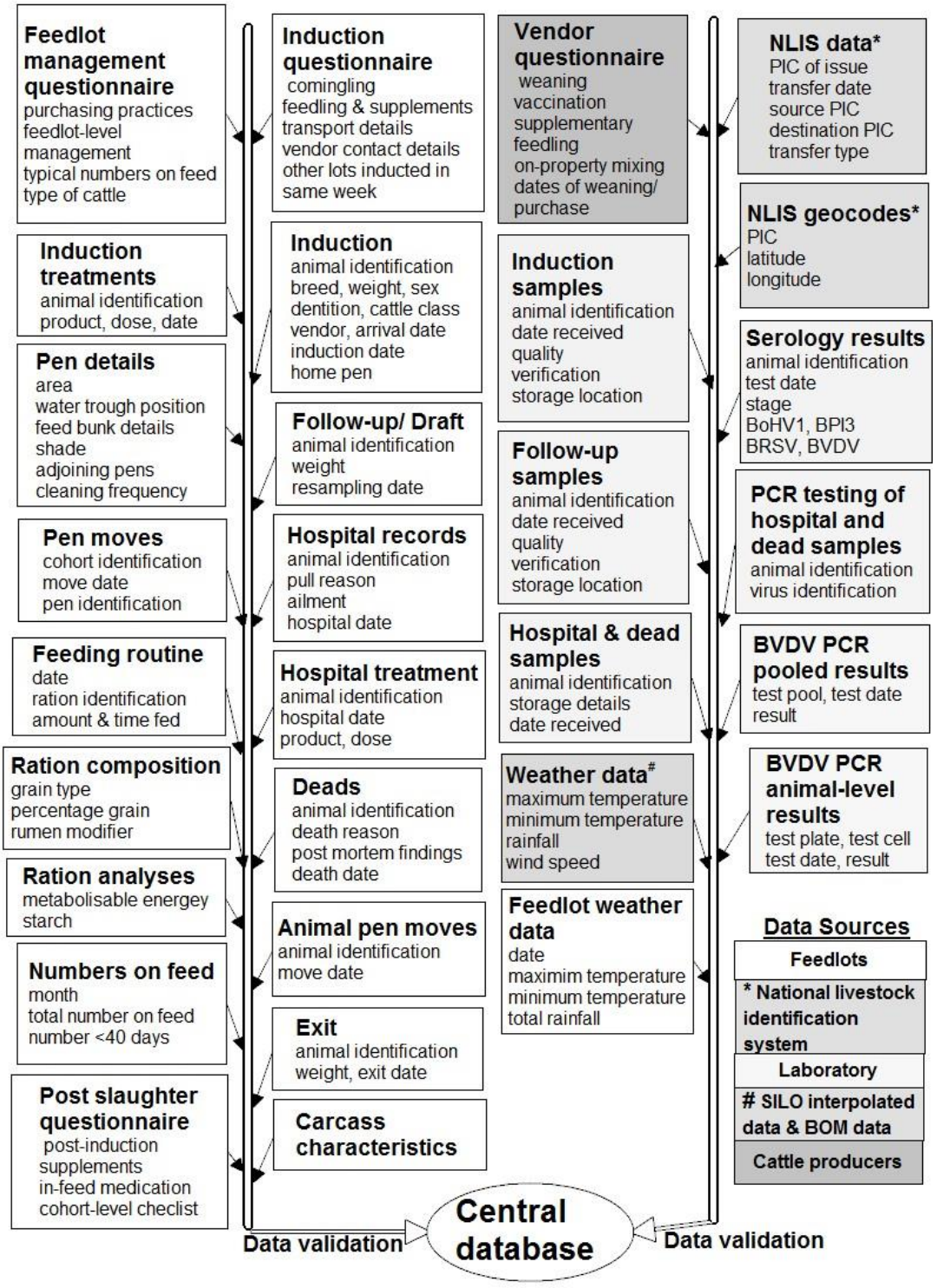

Figure 3.3 Data collected from the sources indicated throughout the study.

3.6.2.1 Induction Sessions files

These files contained one record for each animal in the cohort. They consisted of the following fields, but not all fields were completed by all feedlots:

- Animal ID – Radio frequency identification device (RFID) number comprised of a 3 digit manufacturer's code, a space and a unique 12 digit serial number.
- NLIS ID – National livestock identification system identifier comprised of an 8 character Property Identification Code (PIC), a 1 character manufacturer code, a 1 character device type code, a 1 character code for the year of manufacture and a unique 5 character alphanumeric serial number
- Visual ID – Visual animal identifier usually recorded on an ear tag inserted at induction
- Tail Tag – 8 character PIC identical to that in NLIS ID
- Cattle Class – Feedlot-specific code for target market for all animals in the cohort, usually based on induction weight, breed, sex and intended number of days on feed
- Breed – Feedlot-specific abbreviation for breed, including crosses
- Sex
- Dentition – 0 (or "milk"), 2, 4 or 6 permanent incisors
- Arrival Date – Date of arrival at the feedlot property
- First DOF – Date of the first day on feed in a feedlot pen
- Induction Date – Date animal was first entered in the feedlot computer system. For most feedlots this was the day cattle were blood sampled, received induction treatments, and was either the same date as the first day on feed, or occurred within a few days after the first day on feed. For feedlots that pre-assembled cattle, this was the date the cattle first received any treatment prior to going to the paddock, so it was changed to the "To Backgrounding Date" and the "From Backgrounding Date" (Section 3.6.2.3) was changed to the induction date by the project team to make "induction date" consistent among feedlots.
- Induction Weight – Animal's weight on Induction Date
- Pay Weight – Additional calculated weight estimate. Only supplied by some feedlots. Algorithms for calculation differ between feedlots, so only used for cross-checking extreme weights.
- Off Truck Weight – Additional calculated weight estimate. Only supplied by some feedlots. Algorithms for calculation differ between feedlots, so only used for cross-checking extreme weights.
- Induction Sequence – Sequence number based on the order in which cattle went through the crush for induction, used for matching induction blood samples and nasal swabs to individual cattle. Feedlots using trial ear tags used this number as an additional animal identifier.
- Home Pen – Identifier for pen where animal was when first on feed after induction. Cattle may have stayed in this pen for the duration of their time on feed or may have moved to a different pen depending on feedlot management practices. Note this differed from the home pen definition used for analyses as described in Section 3.6.4.3.
- Lot No – Unique identifier for a group of cattle at the feedlot. In most cases, a lot was a synonym for a cohort but in some cases a lot represented a larger or smaller group of cattle. It was used as a group identifier by feedlot personnel to identify the cattle for which data were to be exported. It was not used for any analyses.

- SAN – Stock advice number. Unique number assigned by StockaID® to each purchase group of cattle.
- Vendor – Name or code for vendor of cattle. Used to define the animal's purchase group.

3.6.2.2 Induction Treatments files

These files contained one record for each animal-treatment at induction (where each product administered to an animal constituted an animal-treatment) for animals in study cohorts. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Induction Date – As above
- Product – Name of product administered on Induction Date
- Dose – Dose of product administered, usually in ml for injectable products.

3.6.2.3 From Backgrounding Sessions files

These files were sent only by those feedlots that pre-assembled cattle. They were identical to Induction Sessions files with the following exceptions:

- From Backgrounding Date in place of Induction Date – This date corresponds to induction date for animals not pre-assembled. It is the date that the animal was blood sampled and received additional treatments, usually within one day of the animal's first day on feed in a feedlot pen.
- From Backgrounding Weight in place of Induction Weight - Individual animal weight on From Backgrounding Date
- From Backgrounding Sequence in place of Induction Sequence - Order in which cattle went through the crush, used for matching induction blood samples and nasal swabs to individual cattle

3.6.2.4 From Backgrounding Treatments files

These files were sent only by those feedlots that pre-assembled cattle. They were identical to Induction Treatments files with the following exceptions:

- From Backgrounding Date in place of Induction Date – This date corresponds to induction date for animals not pre-assembled. It is the date that the animal received the specified treatment.

3.6.2.5 Draft Sessions files

These files contained one record for each animal in the cohort from which a blood sample was collected at the time of the follow-up blood sample. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Draft Date – Date on which follow-up blood sample was collected

- Draft Sequence - Sequence number based on order in which cattle went through the crush for collection of the follow-up blood samples, used for matching follow-up blood samples to individual cattle
- Draft Weight - Individual animal weight on Draft Date

3.6.2.6 Hospital Sessions files

These files contained one record for each animal-examination of an animal from a study cohort, where an animal-examination consisted of examination of an animal in the hospital crush. Thus if an animal was examined on more than one day, it would have multiple records. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Hospital Date – Date the animal was examined in the hospital crush
- Pull Reason – Feedlot-specific code for reason for which pen rider pulled animal from its home pen
- Ailment – Feedlot-specific code for animal's disease, as diagnosed following examination by feedlot personnel in the hospital crush
- Severity – Severity of ailment, only completed at some feedlots
- Course - Feedlot-specific code for course of treatment administered, only completed at some feedlots
- Sequence - Feedlot-specific code for day of the course of treatment administered, only completed at some feedlots
- Treatment – Feedlot specific code for treatment given, only completed at some feedlots
- Temperature – Rectal temperature at time of examination, only completed at some feedlots
- Hospital Weight – Individual animal weight at the time of examination
- New Retreat Repull – Automatically filled field designed to distinguish first pulls, retreatments and repulls. Not consistently defined among feedlots and not used.

3.6.2.7 Hospital Treatments files

These files contained one record for each animal-treatment given to an animal when it was restrained in the hospital crush. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Hospital Date – As above
- Product – Name of product administered on Hospital Date
- Dose – Dose of product administered, usually in ml for injectable products.

3.6.2.8 Deads Sessions files

These files contained one record for each animal in a study cohort that died whilst on feed. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Death Date – Date of death
- Death Weight – Estimated weight at the time of death
- Dead Reason – Reason for which animal was thought to have died
- Autopsy – Yes/no field indicating whether or not a post mortem was conducted
- Autopsy Result – Findings from the post mortem if conducted
- Died In – Identity of pen in which animal died

3.6.2.9 Pen Movements files

These files contained one record for each movement of an animal in a study cohort from one pen to another. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Session Date – Date on which move occurred
- From Pen – Identity of pen from which animal was moved
- To Pen – Identity of pen to which animal was moved

3.6.2.10 Exit Sessions files

These files consisted of one record for each animal in a study cohort which exited the feedlot. Some feedlots did not weigh animals on exit and animal-level exit dates were not available for these feedlots. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Exit Date – Date on which animal left the feedlot
- Exit Weight – Individual animal weight on Exit Date

3.6.2.11 Animal ID Replacements files

These files consisted of one record for each animal in a study cohort for which the RFID was replaced. This file was comprised of the following fields:

- Date Replaced – Date of replacement
- From Animal ID – Original Animal ID already recorded on the computer
- To Animal ID – Replacement Animal ID

3.6.2.12 Carcass Details files

These files consisted of one record for each animal in the cohort from which a blood sample was collected at the time of the follow-up blood sample. They were structured so that data from both left and right sides of the carcass could be included separately, but the right side fields were never used. They consisted of the following fields:

- Animal ID – As above
- NLIS ID – As above
- Visual ID – As above
- Kill Body No – Animal identifier at the abattoir
- Kill Date – Date of slaughter
- Abattoir Live Weight – Individual animal live weight measured at the abattoir
- Carcass Weight – weight of carcass
- P8 Fat – Fat depth measured at the P8 site on the left side
- P8 Fat Right – Fat depth measured at the P8 site on the right side
- Fat Colour – Code for fat colour on the left side
- Fat Colour Right – Code for fat colour on the right side
- Marbling – Code for marbling on the left side
- Marbling Right – Code for marbling on the right side
- Meat Colour – Code for meat colour on the left side
- Meat Colour Right – Code for meat colour on the right side
- Eye Muscle Area Location – site at which eye muscle area was measured
- Eye Muscle Area – Eye muscle area on the left side
- Eye Muscle Area Right – Eye muscle area on the right side
- Firmness – Code for firmness

3.6.3 Animal- and within-animal-files from other data management systems

Comparable file structures were established for transferring data from the other data management systems. The main differences were as follows:

- There was no From Backgrounding equivalent for the feedlot that used the Cattle Fattening Records® software and pre-assembled cattle. However all required dates and the weight at the time of the induction blood sample were recorded in the equivalent to the Induction Session file.
- No animal-level induction treatments were recorded by any of the other data management systems. As treatments were the same for all animals in a cohort, these data were captured at the cohort level.
- Pull reasons were recorded by the other data management systems but ailments were not recorded. Therefore the case definition methodology varied between feedlots using StockaID® and other systems (Section 3.7.1)
- Individual animal pen movements were not available for feedlots using in-house data management systems. For the six feedlots belonging to the same company, movements of individual cattle from the cohort for reasons other than being pulled or death (e.g. transfer to a buller pen) were recorded in an additional file.

3.6.4 Other data from feedlots

Other data were collected directly from feedlot personnel. Most data were cohort-specific and were collected at the time of induction and after all cattle from the cohort

had left the feedlot. The number of cattle on feed each month was collected retrospectively. These data were collected using the instruments described below.

3.6.4.1 Cohort induction data

These data were captured in a spreadsheet completed by feedlot personnel after all cattle had been inducted into a study cohort. It consisted of the following cohort- and purchase group-level data:

- Other cohorts inducted during the same week – number of cohorts, number of cattle in each cohort and number of purchase groups in each cohort
- Study cohort – whether any cattle were sick between arrival and induction, how many purchase groups were mixed in the cohort, names of personnel who did the induction blood sample
- Purchase groups contributing to the cohort – number of animals in each group, feeding regimen on arrival, pen in which groups were housed between arrival and induction, whether any mixing and/or splitting of the group occurred between arrival and induction, contact details of the vendor (for vendor-purchased cattle only)

3.6.4.2 Cohort data collected post slaughter

These data were captured in a spreadsheet completed by feedlot personnel after all cattle in the study cohort had exited the feedlot. For many cohorts, these were collected late in the study. They consisted of the following cohort-level data:

- Cohort management – whether any cattle were added to the cohort after the end of the induction period, details of any in-feed medication administered, frequency of pen riding, dates of movement of the whole cohort from one pen to another (only required for those not using StockaID®), pen identifiers for the to and from pens, dates of pen cleaning and whether cattle were mixed with other cohorts after the follow-up blood sample.
- Ration details – number of rations fed, dates for which each ration was fed, number of feeds per day, approximate timing and percentage daily intake provided at each feed, number of days from the end of the induction period until the cohort was fed the final diet and whether any supplements were added to the formulated ration.

3.6.4.3 Pen data

These data were collected on an electronic form completed by feedlot personnel after all cattle in the study cohort had exited the feedlot. Initially feedlots were requested to complete one form for each pen that each study cohort was housed in at any stage while cattle were on feed. It became apparent that at some feedlots cohorts changed pens on multiple occasions in the latter stages of their time on feed, well after the first 50 days on feed (the period when most BRD cases occurred). As the study focussed on risk factors for BRD by day 50, data on these later pens were not required. So feedlots were requested to complete one form per cohort, for the home pen (the pen in which the study cohort was housed for the majority of the first 50 days on feed). The following pen-level data were captured:

- Pen identifier
- Pen length (m)

- Pen width (m)
- Bunk space (m)
- Location of water trough(s) relative to feed trough and fences
- Water accessible to cattle in other pens (Y/N)
- Pen shade – whether any of the pen was shaded, the extent and type of the shading
- Number of adjoining pens (1 or 2)
- Pen slope – direction and angle
- Frequency of pen cleaning (e.g. monthly, every two months)

3.6.4.4 Ration composition and nutritional analyses data

Ration compositions and nutritional analyses were requested for each ration fed to each cohort. These were provided intermittently, after nutritional analyses had been conducted by the feedlot's chosen laboratory or nutritional consultant. For many cohorts, these were collected late in the study. It was originally expected that these data would include a detailed breakdown of ingredients and nutritional analyses that would be comparable between laboratories. However, there was a large amount of variability in the data provided with only the percentage of the ration that was grain and type of grain in each ration provided consistently across feedlots.

3.6.4.5 Post mortem reports

Brief post mortem reports including the date of death, cause of death based on post mortem findings and location of the animal when it died were provided by some feedlots. These reports did not typically contain additional data beyond that recorded in the StockaID® Deads Sessions or equivalent spreadsheet.

3.6.4.6 Numbers of cattle on feed

Near the end of the data collection period, feedlot personnel were requested to provide data on the total number of cattle on feed and the number of cattle less than 40 days on feed during the period from induction of the first study cohort to exit of the last study cohort from the feedlot. For some feedlots, personnel were able to provide these numbers on a daily basis, whereas for other feedlots, personnel were only able to provide averages of daily data for each calendar month either directly or via their consulting veterinarian. In some cases, the number of animals less than 40 days on feed was estimated from records of numbers less than 60 days on feed.

3.6.5 Data from cattle vendors

A questionnaire (National BRD Initiative – Vendor Questionnaire, Appendix 4) was mailed to the vendors of all purchase groups (identified by a common non-saleyard vendor in the Induction Sessions) who sold at least 20 cattle directly to the feedlot and for whom contact details were provided by feedlot personnel. Vendors were provided with a covering letter which gave a brief explanation as to why they had been selected to complete the questionnaire and included a unique group identifier that linked their questionnaire to the specific purchase group. A document providing more detail about the NBRDI and a reply paid envelope were also included (National BRD Initiative – Information for Vendors, Appendix 5). 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.

The questionnaire was divided into four sections. Section A was pre-filled with the unique purchase group identifier and was to be completed by all respondents. It requested full contact details for the vendor, the most common breed of the cattle in the group, whether they were running together as a single group immediately prior to transport to the feedlot and if so, for how long. Several questions followed regarding management between weaning or purchase and yarding prior to sale; details of mixing, handling, vaccination, pre-feedlot preparation programs and feeding. These were followed by questions relating to yarding prior to transport to the feedlot. Section B was to be completed if the cattle had been bred by the vendor. Questions related to management at marking or branding and at weaning with particular reference to mixing, feeding, vaccinations and the type and duration of weaning. Section C was to be completed if the vendor had purchased the cattle. Questions related to the source, approximate age and weight of the cattle at purchase and whether they had been yarded after arrival at the property. Section D was to be completed by all respondents. All questions in this section were open and asked about differences in management if the purchase group was not run as a single group prior to transport and whether the respondent had any further comments about the purchase group of cattle, what they thought was important when preparing cattle for feedlots and whether they had any comments about the questionnaire.

3.6.6 Laboratory samples

3.6.6.1 Induction files

A spreadsheet was received from the laboratory after processing the induction samples for each cohort (Section 3.5.2). It contained one record for each sequential number corresponding to a pair of samples (serum and swab) received from each animal in the cohort. These files consisted of the following fields:

- Feedlot – Feedlot name
- CohortID – Unique four character alphanumeric cohort identifier
- DateIndSampleReceived – Date sample was received at the laboratory
- IndSampleID – Number on serum tube, to be matched with Induction Sequence from Induction Sessions
- OtherRecIndSampleID – Animal identifier recorded on serum tube by feedlot personnel , usually either the Visual ID or the last 5 digits of the NLIS ID
- IndSerumYN – Whether any serum was obtained from the tube. This was no if the tube was returned empty or if it was not possible to separate a sufficient quantity of serum
- IndSerumStoragePlate – Identifier of plate where serum was stored
- IndSerumStorageCell – Identifier of cell where serum was stored
- IndSerumAdequate – Whether serum volume was greater than 200µl.
- IndSerumNotes - Any additional information regarding serum quantity or quality
- IndSwabYN – Whether nasal swab was received
- IndSwabStoragePlate – Identifier of plate where material from nasal swab was stored
- IndSwabStorageCell – Identifier of cell where material from nasal swab was stored
- IndSwabAdequate – Whether material from nasal swab was considered adequate for testing

- IndSwabNotes - Any additional information regarding the nasal swab

3.6.6.2 Follow-up blood sample files

A spreadsheet was received from the laboratory after processing the follow-up samples for each cohort (Section 3.5.2). It contained one record for each sequential number corresponding to a serum sample from a cohort animal. These files consisted of the following fields:

- Feedlot – Feedlot name
- CohortID – Unique four character alphanumeric cohort identifier
- Date42dSampleReceived – Date sample was received at the laboratory
- 42dSampleID – Number on serum tube, to be matched with Draft Sequence from Draft Sessions
- OtherRec42dSampleID – Animal identifier recorded on serum tube by feedlot personnel , usually either the Visual ID or the last 5 digits of the NLIS ID
- 42dSerumYN – Whether any serum was obtained from the tube. This could be no if the tube was returned empty or if it was not possible to separate the serum
- 42d SerumStoragePlate – Identifier of plate where serum was stored
- 42d SerumStorageCell – Identifier of cell where serum was stored
- 42d SerumAdequate – Whether serum volume was greater than 200µl.
- 42d SerumNotes - Any additional information regarding serum quantity or quality.

3.6.6.3 Hospital files

A spreadsheet for each feedlot was regularly updated by the laboratory after processing samples from pulls (Section 3.5.2). It contained one record for each pull sample received. This file consisted of the following fields:

- Feedlot – Feedlot name
- CohortID – Unique four character alphanumeric cohort identifier
- AnimalID – NLIS ID
- VisualID – Animal identifier recorded on ear tag inserted at induction
- InductionID – Animal identifier on trial ear tag based on induction sequence. Usually only one of the three animal identifiers was included on the sample container.
- DatePull – Date of sampling recorded on the serum tube and nasal swab
- DatePullReceived – Date samples were received at the laboratory
- PullSerumYN – Whether any serum was obtained from the tube
- PullSerumStoragePlate – Identifier of plate where serum was stored
- PullSerumStorageCell – Identifier of cell where serum was stored
- PullSerumAdequate – Whether serum volume was greater than 200µl.
- PullSerumNotes - Any additional information regarding serum quantity or quality
- PullSwabYN – Whether nasal swab was received
- PullSwabStoragePlate – Identifier of plate where material from nasal swab was stored
- PullSwabStorageCell – Identifier of cell where material from nasal swab was stored

- PullSwabAdequate – Whether material from nasal swab was considered adequate for testing
- PullSwabNotes - Any additional information regarding the nasal swab

3.6.6.4 Deads files

A spreadsheet for each feedlot was regularly updated by the laboratory after processing samples from cattle that had died whilst on feed and the feedlot personnel had attributed the death to BRD (Section 3.5.2). It contained one record for each animal from which samples were received. This file consisted of the following fields:

- Feedlot – Feedlot name
- CohortID – Unique four character alphanumeric cohort identifier
- AnimalID – NLIS ID
- VisualID – Visual animal identifier usually recorded on an ear tag inserted at induction
- InductionID – Animal identifier on trial ear tag based on induction sequence. Usually only one of the three animal identifiers was included on the sample container.
- DateDeadsSample – Date of sampling recorded on the sample container
- DateDeadsRecieved – Date sample received at the laboratory
- LungYN – Whether lung tissue was included
- LungStoragePlate – Identifier of plate where lung tissue was stored
- LungStorageCell – Identifier of cell where lung tissue was stored
- LungNotes – Any additional information regarding the lung tissue sample
- TracheaYN – Whether a trachea sample was included
- TracheaStoragePlate – Identifier of plate where trachea sample was stored
- TracheaStorageCell – Identifier of cell where trachea sample was stored
- TracheaNotes – Any additional information regarding the trachea sample

3.6.6.5 Serology Results

Spreadsheets for each selection batch consisting of the unique selection number, storage plate and cell references for samples from case and control animals selected for inclusion in the case-control study (Section 3.12.2) were sent to the laboratory so that the appropriate samples were tested (Section 3.5.3). They contained one record for each animal selected. After testing was completed, these spreadsheets were updated the following fields added:

- CP (cherry-picked) plate location; plate#, Ind cell, D42 cell - The new storage location of the induction and follow-up samples from selected animals.
- ELISA: E plate#, Ind cell, D42 cell – The location of aliquots of samples within the test plate.
- Comments - These included the date of testing, an identifier created by the plate reader and notes about individual samples where relevant.

Results from each ELISA plate were exported to a Microsoft® Excel template and files were returned to the data management team. ELISA test plate and cell identifiers were included, along with serological results for antibodies against each of the four viruses that were assessed. Serological results included optical densities and categories for all samples as described in Section 3.5.3.

3.6.6.6 BVDV results

Files containing BVDV test results from polymerase chain reaction (PCR) tests were sent to the project team involved with data management. For the pooled tests, records consisted of the following fields:

- Feedlot – Feedlot name
- CohortID – Unique four character alphanumeric cohort identifier
- Induction or D42 – induction or follow-up samples
- Plate No – Serum storage plate
- Cell No – Serum cell range
- # Samples (x 10µl)- Number of samples in pool
- Pooled Extraction Plate No – test plate
- Pool Cell - test cell
- Date Nucleic Acid Extraction- date of test
- BVDV real time PCR results – negative or positive
- C_T value – cycling threshold value

Animal-level PCR testing proceeded in a number of rounds because the samples identified for testing depended on results from previous testing (described in more detail in Section 3.9.3.11). Spreadsheets for each testing round consisting of the unique animal identification number, storage plate and cell references for the required samples and the type of sample (e.g. induction swab, hospital serum) were sent to the laboratory. They contained one record for each animal. After testing was completed, these spreadsheets were returned to the data management team with fields added which detailed the test plate and cell identification, comments about the sample (e.g. no sample available), C_T value and comments about the test (e.g. possible false positive).

In the final round of testing to identify BVDV-PI animals, ELISA tests to detect BVDV specific antibody on induction samples were requested for animals that were suspected of being BVDV-PIs based on the pooled testing and induction sample testing but for which a second sample was not available. By definition animals testing positive for BVDV antibody were not classified as BVDV-PIs. Samples from these animals along with animals that were in the same group of animals 28 days prior to the start of time at risk were identified for testing to allow a comparison between their results. The final round of results thus also contained the optical density values for the ELISA tests in addition to the PCR results.

3.6.7 Weather data

Initially it was planned to obtain weather data from feedlot weather stations on a regular basis from when the first cohort was inducted until when all cattle from the final study cohort had exited. However, data were inconsistently recorded between feedlots, some data were missing due to malfunctioning weather stations and some feedlots were unable to download the data from their stations. As an alternative, temperature and rainfall data were obtained from the SILO climate database[6] hosted

[6] http://www.longpaddock.qld.gov.au/silo/index.html

by the Science Delivery Division of the Department of Science, Information Technology, Innovation and the Arts. Interpolated data on temperature, rainfall, evaporation, radiation, vapour pressure and relative humidity were obtained for geographic co-ordinates for each feedlot to the nearest 0.05 decimal degree. Only the daily maximum and minimum temperature and rainfall were used to derive variables used for analysis. Data describing wind speed and direction, maximum daily wind gust and daily wind run (below 3 metres above ground level) were obtained from the Weather Station Directory service provided by the Bureau of Meteorology[7] for the nearest weather station to each feedlot that recorded these data.

3.6.8 NLIS data

NLIS management approved a request to release data on device details and transfer details for all cattle in the study provided strict privacy and security conditions were met. Data were requested for all study animals. Lists of animal identifiers were supplied to NLIS personnel every 6 to 12 months depending on the frequency with which cattle were enrolled into the study. Data provided for each device were NLIS ID, RFID number, PIC of issue, lifetime status and active (Y/N). For any replaced devices the following data were also provided: NLIS ID, RFID number, old NLIS ID, old RFID number and replacement date. For each transfer (move from one location to another) the following data were provided: NLIS ID, RFID, transfer date, source PIC/saleyard ID, destination PIC/saleyard ID, national vendor declaration or waybill number, saleyard name (for saleyard transfers only) and transfer type (point to point, saleyard in or saleyard out). All data were provided as csv files. Geographical coordinates for most PICs were obtained from individual state NLIS representatives to estimate transport times.

3.6.9 Dust

We originally aimed to collect data on concentrations of dust from two of the participating feedlots. It was proposed that the data would be used in a subset analysis to investigate the effect of dust levels as a risk factor for BRD and to identify whether dust levels were correlated with weather variables. Dust monitors were successfully installed in two feedlots but it was impractical to operate these devices under field conditions. They regularly malfunctioned and a cable on one monitor was eaten by cattle. The monitors were returned to the manufacturers on several occasions. As the cost of repairs and servicing was high and data were at best only collected intermittently the decision was taken to remove the dust monitors from the participating feedlots.

3.7 Case definition

3.7.1 BRD Case

It was impractical for feedlot personnel to record additional data over and above data that they normally recorded for each pull. Therefore BRD cases were defined based on the data normally recorded by the feedlots at the time of examination in the hospital crush. For feedlots using StockaID®, both the Pull Reason and the Ailment

[7] http://www.bom.gov.au/climate/data/stations/

recorded in the Hospital Sessions (see Section 3.6.2.6) were used as both fields were completed for nearly all pulls. Feedlots using other data management systems recorded only the Pull Reason. There was much discussion among the project team and the consulting veterinarians in the planning phase and early part of the study as to whether the BRD case definition should be narrow, including only cattle that were reported to have signs directly related to the respiratory system, or wide, including in addition cattle that were reported to have non-specific signs that were not directly referable to other systems such as “depressed” or “non-eater”. Classification of pulls where there was a discrepancy between the Pull Reason (determined by the pen rider before removing the animal from the pen) and Ailment (determined by the feedlot staff member who examined the animal in the hospital crush) was also discussed extensively.

The case definition chosen required an animal from a feedlot using the StockaID® system to have both a Pull Reason and an Ailment directly referrable to the respiratory system and an animal from a feedlot using any of the other data management system to have a Pull Reason directly referrable to the respiratory system. Earlier concerns that many cattle pulled at an early stage of BRD would be missed using this strict definition were dismissed as 98.0% (6,406/6,535) of all pulls not directly referable to a system other than the respiratory system were classified as BRD cases (Figure 3.4).

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Ailment \ Pull Reason	BRD	IBR	Pneumonia	Respiratory	Honker	Necrotic laryngitis	Non Eater	Non-eater/waster	Observe	Restart	Not recorded	Total
Not recorded			***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

Figure 3.4: Cross tabulation of the Pull Reason and Ailment for all cattle pulled where the Pull Reason and/or Ailment were either directly referrable to the respiratory system, or not directly referable to any other system. The ailment for cattle from feedlots using data management systems where ailment was not recorded is listed as not recorded. Pulls meeting the BRD case definition are within the top left box with numbers for each included combination in bold italic font (N = 6,406).

3.7.2 Death from BRD

For practical reasons, a death from BRD had to be defined based on the data normally recorded by the feedlots at the time of death. The Dead Reason field in the Dead Sessions was completed for nearly all cattle that died, whereas the Autopsy Result field was frequently blank. A death from BRD was defined as a death where the Dead Reason was directly referrable to the respiratory system. Feedlot-specific codes for such Dead Reasons included in this definition were: BRD, bronchopneumonia, fibrinous pneumonia, lung abscess, IBR, pleurisy, pneumonia, respiratory and tracheitis. No distinction was made between natural death due to disease and euthanasia as a management decision for chronically diseased animals.

3.8 Data management and validation

All data received were stored on a shared drive at the University of Queensland. All members of the project team involved with data management had access to this drive. Management of data received from the different sources is detailed below. A

Microsoft® Access database was developed for the storage and linking of all data. This database was assembled after the primary data were compiled, merged and cross-checked in Stata® files and the basic descriptive and analysis variables were derived. Variables derived or results obtained after the assembly of this interim database were stored and managed within Stata® files. The final database will be updated with all of these data.

3.8.1 Animal- and within-animal-level data from feedlots

Animal- and within-animal-level data from feedlots were cross-checked and verified on an ongoing basis as the study progressed. Cohort ID and Group ID fields were added to the Induction Sessions. Cohort ID was a unique identifier for each cohort which could be linked to the Cohort ID field in all the spreadsheets from the laboratory. Group ID was a unique identifier for all cattle from any one purchase group. Cattle arriving on the same day from any one saleyard were given the same Group ID, as were all cattle that arrived on the same day direct from any one vendor. For each cohort, selected animal-level files (Induction Sessions, From Backgrounding Sessions, Draft Sessions, Hospital Sessions, Dead Sessions, Exit Sessions and Animal ID Replacements) were used to track all animals in each cohort to identify any discrepancies in a timely way so that these could be followed up with feedlot personnel as quickly as possible. A Microsoft® Access template was created with queries designed to detect inconsistencies in the data. Microsoft® Excel files were imported into the Microsoft® Access template and the queries were run and output obtained via a Microsoft® Excel macro. This procedure produced summary spreadsheets in a Microsoft® Excel file which were then examined for inconsistencies.

The main purpose of performing this check at the cohort level was to check that all animals inducted were accounted for. Hospital Sessions, Dead Sessions and Pen Movement files were checked to establish whether animals missing from the feedlot Exit Sessions could be accounted for in this way. Several animals had their NLIS ID tags replaced. In most feedlots, animals also had at least one other identification tag, so it was possible for feedlots to replace the NLIS ID and link to the animal's original record. The range of dates for arrival, induction, follow-up sampling, hospitalisation, death, exit and slaughter were also checked for consistency at this point. Spurious weight data were noted, but no follow-up of inconsistent weight data was performed.

It was necessary to compile and validate the animal-level files before animals could be selected for inclusion in the case-control study (described in Section 3.12.2). To spread laboratory workloads, the original plan had been to select cases and controls and test their sera in multiple batches at approximately six monthly intervals. For logistical reasons, selection and testing did not begin as early as originally planned and so was done in only two selection batches. At the time of selecting animals for the first selection batch, anticipated final numbers of both cohorts and animals that would be enrolled in the study for each feedlot were estimated. For each feedlot, half of the expected final number of cohorts was included in the first batch, except for those feedlots that were enrolled late in the study and so contributed fewer cohorts to the first selection batch. The animal-level data for the batches were initially managed separately then merged after completion of data collection. After cross checking and reformatting so that headings and field formats were consistent for data from StockaID® and other data management systems, a Microsoft® Access database comprising Induction Sessions, Draft Sessions, Dead Sessions and Exit Sessions was created for each feedlot for the first selection batch. Data were then exported as Microsoft® Excel files. Files for cohorts in the first selection batch were then imported

into Stata®. The Hospital Sessions data often contained more than one record per animal. A Stata® program file was written to extract the earliest hospital record and this file was then merged with the other animal-level data to produce a working first selection batch dataset. The data were checked and validated in Stata®. At a later stage this was repeated for the cohorts in second selection batch. The datasets were then merged to create a core animal-level dataset and a copy included in the main database in the Animal Level Raw table.

3.8.2 Other data from feedlots

3.8.2.1 Cohort induction data

Cohort-level data were extracted into a cohort-level spreadsheet and imported into the database, into the Cohort Level table. Group-level data from this spreadsheet were extracted into a group level spreadsheet and imported into the database in the Group Level table. For many cohorts, these data were incompletely recorded but management of cohorts was typically consistent within particular feedlots, so in many cases, the missing data could be inferred. Many of the fields in this spreadsheet were not used in the final analyses and some were used for cross-checking purposes only.

3.8.2.2 Cohort data collected post slaughter

Most of the cohort-level data from this spreadsheet were extracted into a cohort-level spreadsheet and imported into the database in the Cohort Level table. For many cohorts, these data were incompletely recorded but management of cohorts was typically consistent within particular feedlots, so in many cases the data could be inferred. Many of the fields in this spreadsheet were not used in the final analyses and some were used for cross-checking purposes only. The dates each ration was fed were extracted into a spreadsheet and imported into a Ration Routine table in the database.

3.8.2.3 Pen data

All data provided for home pens were manually entered into a spreadsheet and imported into the database in the Cohort Pen table.

3.8.2.4 Ration composition and nutritional analyses data

All ration data were manually entered into a spreadsheet and imported into the database in the Ration Detail table. All provided data (composition and nutrient analysis) were retained at this stage. Each ration was given a unique code which was used to link to the Ration Routine table in the database where the date each ration was first fed to each cohort was recorded based on the data provided in the Cohort Questions Post Slaughter.

3.8.2.5 Post mortem reports

The post mortem reports were cross-checked against the records in the Dead Sessions, but they provided no useful additional data.

3.8.2.6 Numbers of cattle on feed

Data on the numbers of cattle on feed were requested late in the study and varied markedly in format and quality. As only the data for the calendar month of induction

for each cohort were of interest, estimates were made in different ways for each feedlot making the best use of the data available. These data were then merged with the relevant datasets in Stata® that had already been developed.

3.8.3 Data from cattle vendors

Vendors had the options of completing the Vendor Questionnaire online, by telephone interview or by returning a hard copy. All hard copy and telephone interview responses were entered into the online questionnaire so all responses were in the same format. The data were downloaded periodically as a Microsoft® Excel spreadsheet and cross-checked using Stata® before importing into the main database in the Group Level table.

3.8.4 Laboratory data

3.8.4.1 Induction files

When the Induction file for each cohort was received from the laboratory, the serum samples and nasal swabs were identified by the number on the tube in which the blood sample had been received and the number on the nasal swab (IndSampleID). Verification was required to ensure that it was valid to assume that the n^{th} blood sample in the sequence of samples was from the n^{th} animal in the Induction Sequence in the Inductions Sessions file from the feedlot. The partial NLIS ID or Visual ID recorded on every tenth tube was used for this purpose. Inconsistencies arose if for example an animal was inadvertently not blood sampled, resulting in the Induction Sequence being out of phase with the IndSampleID by one. Discrepancies were followed up with the feedlots. During this process the following fields were added to the Induction Spreadsheet:

- Animal ID – from Induction Sessions
- NLIS ID – from Induction Sessions
- Visual ID – from Induction Sessions
- Induction Sequence – from Induction Sessions and matched against IndSampleID
- Induction Date – from Induction Sessions
- IndSerumVerified – Recorded as “A” if the sample was verified as linked to an Animal ID, “C” if the sample was verified as coming from an animal in the cohort, but could not be linked to an Animal ID and “N” if the sample could not be verified as coming from an animal in the cohort
- IndSwabVerified– Recorded as “A” if the swab was verified as linked to an Animal ID, “C” if the swab was verified as coming from an animal in the cohort, but could not be linked to an Animal ID and “N” if the swab could not be verified as coming from an animal in the cohort

3.8.4.2 Follow-up blood sample files

When the Follow-up Spreadsheet for each cohort was received from the laboratory, serum samples were identified by the number on the tube in which the blood sample had been received (42dSampleID). For feedlots using StockaID® or Cattle Fattening Records®, verification that it was valid to assume that the n^{th} blood sample in the sequence of samples was from the n^{th} animal in the Draft Sequence in the Draft Sessions file (or Cattle Fattening Records® equivalent) from the feedlot followed the same process as used for the induction samples. Verification differed somewhat for follow-up samples from feedlots that used trial ear tags numbered by induction sequence. Feedlot personnel recorded part of the NLIS ID or Visual ID on every tenth

tube, but because the induction sequence differed from the order in which cattle were blood sampled at follow-up, the doubly numbered tubes were not evenly distributed among sequentially numbered tubes and so provided a less reliable method for cross-checking. However, this method had some inherent cross-checking at the time of blood sampling. If the feedlot personnel had inadvertently used the wrong tube (e.g. 81 instead of 18) when the animal whose tube had been used mistakenly came into the crush the tube was already full. Some of these mistakes could be rectified at the time of blood sampling. Discrepancies identified by the project team were followed up with the feedlots.

For some cohorts no Draft Session file was provided from the feedlot, but in all these cases the feedlots were using trial ear tags so some cross-checking was inherent in the sampling process. In some cases, samples were received from animals that did not appear in the Draft Session provided by the feedlot. This was most likely to occur if an animal was in the hospital pen at the time when most cattle in that cohort were blood sampled. It was then likely to have been blood sampled in a different crush at a different time and its details not recorded electronically. Such samples were usually easily identified following discussion with the laboratory as the samples were usually located in the sample trays after those from the main blood sample.

During the verification process the following fields were added to the Follow-up Spreadsheet:

- Animal ID – from Draft Sessions
- NLIS ID – from Draft Sessions
- Visual ID – from Draft Sessions
- Draft Sequence – from Draft Sessions and matched against 42dSampleID
- Draft Date – from Draft Sessions
- 42dSerumVerified – Recorded as “A” if the sample was verified as linked to an Animal ID, “C” if the sample was verified as coming from an animal in the cohort, but could not be linked to an Animal ID and “N” if the sample could not be verified as coming from an animal in the cohort

3.8.4.3 Hospital and Deads files

The Hospital and Deads Spreadsheets were sent to the data management team on a regular basis. Each entry was crossed checked against the Hospital Sessions or Deads Sessions files from the feedlot to confirm the date of sampling and that for hospital samples the animal did meet the BRD case definition on the day of sampling. Hospital samples were verified “A” if they could be linked to a particular animal on the first occasion that it met the BRD case definition, or “S” if they could be linked to a particular animal but the sample had been collected on a subsequent occasion. In some cases samples were labelled with the trial ear tag number rather than the Animal ID or Visual ID. This necessitated additional crosschecking with the Induction Sessions. Animal ID and Visual ID fields were completed for all entries to facilitate linking with other data.

Induction, Follow-up, Hospital and Deads files for all cohorts in the first selection batch were merged with the first selection batch dataset which consisted of animal-level data from the feedlots. This dataset included all the data required for the selection of cases and controls. At a later stage this was repeated for the cohorts in the second selection batch. The data from the Induction, Follow-up, Hospital and Deads Spreadsheets were stored in the main database in the Samples table.

3.8.4.4 Serology results

The results for each ELISA plate (see Section 3.6.6.5) were in exactly the same format for all plates such that the results of interest were arranged in two 8 by 12 tables (one with adjusted optical densities and one with derived categories) which corresponded to the layout of a single ELISA test plate. These results were extracted and merged using an automated Microsoft® Excel add-in, RDBMerge,[8] so that they were compiled in two single spreadsheets (one each for optical densities and categories). The 8 rows in the test result templates were labelled from "A" through to "H", while columns 1 through to 5 were additionally labelled with the 5 test agents, BoHV-1, BVDV, BRSV, BPI3 and *M. bovis*. This block of values (termed "block A" for data management purposes) was repeated for columns 6 through to 11 ("block B"). An individual test location could be identified by the plate number, row and column (e.g. 321, A5), while a set of test results (for 5 agents) from the same sample from a particular animal could be identified by the plate number, row and block. Negative control samples (provided by the manufacturer) were added to all wells in columns 6 and 12. Positive control samples were added to row B in block A. Other wells contained test samples.

Results were checked, compiled and allocated a unique test identification code (based on the plate, block and cell location) in Microsoft® Excel before being merged and linked to each animal's identification numbers in Stata® datasets. Data were cross-checked and any discrepancies (e.g. duplicated test locations or duplicated results) were followed up with laboratory staff. In this way, 100% resolution was achieved with each animal being linked to its unique test results. Data were then stored in the main database in the Serology table and merged with the Stata® dataset for analysis.

3.8.4.5 BVDV PCR results

Pooled test results from PCR testing for the presence of BVDV were supplied in the form of Excel spreadsheets which were cross validated by inspecting original laboratory records to confirm that positive pools were correctly allocated to the sample locations indicated. Animal-level results from each round of testing were compiled and merged with the pooled test results and the results from the testing of hospital samples that had been performed to determine which viruses were present in each cohort as described in the B.FLT.0225 'Epidemiology and management of BRD in feedlot cattle Part B: Virology' report. All results were compiled and stored in Stata® files.

3.8.5 Weather data

Interpolated weather data for all feedlots were compiled into a spreadsheet and imported into the Weather SILO table in the database. Similarly, wind data from the nearest Bureau of Meteorology stations were compiled into a spreadsheet and imported into the Weather Wind table in the database.

[8] http://www.rondebruin.nl/win/addins/rdbmerge.htm

3.8.6 NLIS data

The 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 99.9% of the 35,160 animals inducted into the study. For 340 of these 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 these animals. Records from the NLIS system were cross checked against the tail tag numbers recorded in the Induction Sessions. Often this allowed the research team 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 as “point to point” in the NLIS transfer type field, and source PIC, destination PIC, transfer date, transfer type and waybill number fields were also recorded. In contrast, a transfer via a saleyard was recorded as two transfers, the first with “saleyard in” as the NLIS transfer type and the second with “saleyard out” as the NLIS transfer type. An animal was classified as having a saleyard transfer within a time period of interest if it had such a pair of transfers on the same date.

The NLIS requires that each animal's transfer history has no missing steps i.e. that every transfer commences from the animal's most recent PIC. This requirement is breached when a transfer is entered into the NLIS database where the source PIC differs from the animal's most recent previous destination PIC, or in the case of the animal's first transfer, differs from its PIC of issue. In these situations, NLIS automatically imputes transfers with the date one day prior to the apparently illogical move. The unknown source or destination PIC is listed as “XXXXXXXX”, and the unknown waybill number is 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. Moves involving saleyard transfers or 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, transfers with an unknown source or destination were consolidated to form a single transfer between the two known PICs, with the transfer detail variable recording that the transfer was imputed by the NLIS system.

The number of days between the transfer and the induction date was determined and used to sequence transfers. For transfers imputed by NLIS, the dates were unlikely to be correct, so were changed to missing, but the transfer was retained in the animal’s sequence of moves. For animals that had valid transfer dates, the interval between the transfer and day 0 was used to determine the PIC location at the time points of interest. Where animals with an imputed transfer were part of a group of animals that shared common transfers, the missing PIC locations common to the group were allocated based on known transfer dates for common group animals. If no animals in the relevant group had recorded transfer dates, these transfer dates were allocated midway between the two surrounding known transfer dates. Where there was no move before the imputed move and no common group animals, the

date was changed to 180 days prior to the recorded 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 and to make the process repeatable. The vast majority of data were either validated or corrected in this manner. However, some of the checking process required examining individual (or group) move sequences to establish a “most likely” scenario.

An example of how the validation and correction process proceeded is illustrated in Table 3-2.

Table 3-2 Example of the data validation and correction process applied to a series of transfers common to seven animals. (A, B, C, D: unique PICs, FL: feedlot PIC, SY: saleyard, P2P: point to point)

Steps 1-6 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 arrival date
2. Determine whether the date of the move to the feedlot matches 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 interval between transfers and day 0 and the sequence of transfers
6. Change the imputed transfer date to a point midway between two surrounding dates
7. Examine records where the transfer interval remains at 1 day
8. Where records do not make sense, recheck against raw data and decide most logical sequence.

In this example, 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.

Table 3-2: Example of the data validation and correction process applied to a series of transfers common to seven animals. (A, B, C, D: unique PICs, FL: feedlot PIC, SY: saleyard, P2P: point to point).

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

After completion of the validation and correction process, it was determined that of the 35,160 animals inducted, 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 change of NLIS 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.

3.9 Derivation and definition of analysis variables

3.9.1 Time at risk of BRD and terminology for time-specific exposure variables

Many putative risk factors (exposure variables) used in the analyses were time-specific and described exposures prior to the animal's enrolment into the study.

Each animal was at risk of being recorded as having just acquired BRD only from the day after it was inducted and its identification details captured electronically, as this was the first opportunity that a pen rider (feedlot staff member responsible for checking the health of animals, usually on horseback) would have to pull this animal and record treatment details in the feedlot data management system. Further, the study aimed to identify risk factors for BRD occurrences only amongst cattle on feed i.e. when being fed a total mixed ration in a feedlot pen. At some feedlots, cattle were inducted and went on feed on the same day, whereas at other feedlots cattle may have been on feed for up to several days prior to induction. For feedlots that did not practice pre-assembly, each animal's first day at risk of being recorded as having just acquired BRD was the day after its induction date. For feedlots that practiced pre-assembly, each animal's first day at risk was the day after its movement from the pre-assembly facility and going on feed in a feedlot pen. The day before the animal's first day of risk was termed “day 0”. Time points for each animal were described relative to day 0 and were positive when referring to time points after day 0 (e.g. day 50 referred to the 50th day at risk) and negative when referring to time points prior to day 0 (e.g. day -13 referred to the 13th day before day 0).

As described earlier, a cohort was a collection of animals assembled during the induction period and placed on feed in the same feedlot pen. A group was a collection of study animals that were at the same PIC at the end of a given day and subsequently went into the same cohort. As PICs and groups could vary over time, these were identified for all study animals at particular time points of interest. The location and grouping of an animal at a particular time point (#) prior to day 0 were described by PIC-# and group-# variables, respectively, where # refers to the number of days prior to day 0. Hence, PIC-13 was the PIC where the animal was at the end of the 13th day before day 0, and group-13 identified the group that the animal was part of at that time point. Events occurring in particular time periods were described by day -# to -# variables, where the #s referred to the earliest and latest days in the period prior to day 0. Hence day -27 to -13 referred to the time period from 27 to 13 days before day 0, inclusive.

Where feedlots inducted cattle into a cohort over multiple days, day 0 differed among animals in the cohort. The cohort open date was defined as the earliest day 0 for an animal in that cohort and the cohort close date was the latest day 0.

3.9.2 Selection of clustering variables

The study data had a natural nested hierarchical structure with four levels: feedlot, cohort within feedlot, purchase group within cohort and animal within purchase group (Section 3.3.4). The two higher levels of clustering (feedlot and cohort) were defined by the data. However, there was no natural definition of the lowest level of clustering relating to the grouping structure of the animals prior to arrival at the feedlot. The group that any particular animal was in was not constant over time as, before arrival t the feedlot, it was common for animals to be added to groups over time. Purchase groups identified by Group ID were not suitable as all cattle purchased at one saleyard on one day that went into the same cohort were given the same Group ID and were clearly likely to have much less similarity to each other compared to cattle that had been together for an extended period of time rather than simply for a short period at a saleyard.

To address this issue, 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 was identified as the collection of study animals that had the same PIC of issue and subsequently went into the same cohort (group-issue), provided the PIC of issue was not the feedlot PIC.

Exploratory analyses were conducted using logistic regression part way through the study using records for 20,253 animals. These analyses compared the extent of clustering at the feedlot, cohort and group level using each of the groups listed earlier. The aim was to choose one of the group definitions as the lowest level of clustering to include in analyses to best account for clustering of BRD occurrence in the dataset. A null mixed effects model was fitted using the xtmelogit function in Stata® with feedlot, cohort and group-issue as random effects. This process was repeated for each of the group definitions. These models estimated the amount of variance at the feedlot, cohort and group level. The latent variable threshold approach, whereby the variance at the animal level was assumed to be $\pi^2/3$ (3.29), was then used to estimate the percentages of total variance that were at the feedlot, cohort, group and animal levels.[9] The sums of the percentages of total variance that were at feedlot, cohort and group level measured the correlations in BRD occurrence between any two animals from the same group, assuming that the correlation was the same for all pairs of animals within each group. These were treated as the intra-class correlation coefficients for correlations within group. The magnitude of the effect of clustering was then estimated by the design effect is given by the following formula: [10]

$$Design\ effect = (1 + (mean\ cluster\ size - 1) * intra - class\ correlation\ coefficient$$

Comparative results for the different group definitions are shown in Table 3-3. Based on these results, group-13 was selected as the identifier for the lowest level of clustering as it was thought to provide the best balance between a larger design effect, potential misclassification into groups where moves had been imputed and actual transfer dates were unknown, and the biological implications of the timing with respect to exposure to pathogens and formation of a stable social hierarchy. Specifically, risks of misclassification were probably greater with earlier grouping times and although later grouping times (group-7 and group-1) had larger design effects they were less likely to fully capture variance due to both exposure to pathogens and formation of a stable social hierarchy as effects of these would be expected to take more than 7 days to fully occur.

[9] Snijders, T., Bosker, R., 2012. Multilevel Analysis: An Introduction to Basic and Advanced Multilevel Modeling. SAGE Publications London.

[10] Dohoo, I., Martin, W., Stryhn, H., 2010. Veterinary Epidemiologic Research. VER Inc Charlottetown, Canada.

Table 3-3: 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. (ICC: intra-class correlation coefficient)

Group	No. of groups	Mean group size	Variance				Percentage of variance				ICC	Design effect
			Feedlot	Cohort	Group	Total	Feedlot	Cohort	Group	Animal		
group-issue	3,926	5.3	3.99	1.02	0.68	8.97	44.43%	11.32%	7.59%	36.66%	0.634	3.73
group-365	1,756	11.8	3.89	0.99	0.67	8.83	44.02%	11.18%	7.53%	37.27%	0.629	7.79
group-180	1,224	16.9	3.94	1.02	0.64	8.88	44.34%	11.44%	7.16%	37.06%	0.631	11.03
group-90	890	23.2	3.89	1.00	0.62	8.79	44.18%	11.33%	7.08%	37.41%	0.627	14.92
group-30	757	27.3	3.87	0.94	0.60	8.71	44.46%	10.84%	6.91%	37.79%	0.621	17.34
group-13	695	29.7	3.87	0.90	0.58	8.65	44.80%	10.43%	6.72%	38.05%	0.618	18.75
group-7	622	33.2	3.86	0.88	0.57	8.61	44.88%	10.23%	6.66%	38.23%	0.617	20.86
group-1	519	39.8	3.87	0.83	0.56	8.55	45.29%	9.73%	6.51%	38.48%	0.615	24.87

3.9.3 Exposure variables

3.9.3.1 Overview

A large quantity of data was collected during the course of the study. In developing exposure variables for use in the analyses the aim was to make use of the rich data and to consider associations that were biologically plausible. All continuous predictors were categorised to avoid invalidly assuming relationships were linear on the logit scale (the effect of a one unit change in the exposure variable not being consistent across the range of that variable). In categorising variables, the aim was to create categories with adequate numbers and variation among feedlots while taking into account prior knowledge and/or industry interest in particular categories. Often the form of the original data influenced the initial selection of categories. These were then modified based on the quality and distribution of the data. Where categories were sparse there was reduced power to identify relationships and where animals in a category were only from a small number of feedlots there was increased risk of bias in the estimates.

Variables considered for inclusion in analyses, along with their categories are described in detail below. Table 3-4 to Table 3-19 follow a common format which summarises this process. The original/intermediate variable column refers to raw data or intermediate variables used in the derivation of analysis variables. The examples or range column gives examples of categories for categorical variables and the range of values for continuous variables. The categories of the analysis variable are given in the categories column, and the analysis variable is described in the final column. For some variables, the analysis variable was used to derive further variables, so it also appears in the original/intermediate variable column.

The decision on the cut-points used and number of categories included in the variables used in the final analyses was often made after exploring the distributions, in part to avoid sparse categories, and associations with BRD occurrence with several different classifications. For example, induction weight was first categorised into eight categories, with cut points chosen at 25 kg intervals within the range containing 90% of values with the lower and upper 5% in separate categories. The distribution across categories was checked to determine that the numbers of animals in each category were not extremely imbalanced across feedlots. The variable was

then explored in univariable and multivariable models. There was a consistent pattern of decreasing BRD risk with increasing induction weight. To enable examination of possible interactions and simplify communication of results, the final analysis induction weight variable was collapsed into four categories as shown in Table 3-5.

3.9.3.2 Exposure variables relating to feedlot entry characteristics

Exposure variables relating to feedlot entry characteristics (mostly defined at the animal level) are described in Table 3-4 and Table 3-5. The interpretation for all feedlot-specific breed codes were obtained from feedlots and the data were re-coded to include all represented breeds and crosses. This classification was very extensive and is listed in the original variable column. Categories were combined as shown; the final breed variable had seven categories. The Tropical/Tropical cross breed category included any animals with feedlot specific breed codes that referred to a tropical cattle breed (e.g. Droughtmaster, Santa Gertrudis, Charbray, Braford, etc.), the European/European cross category to any European cattle breed (e.g. Charolais, Gelbvieh, Limousin and Simmental) but not a tropical breed and the British cross breed category referring to animals recorded by the feedlot as British breed crosses only.

With the exception of dentition, exposure variables relating to feedlot entry characteristics were recorded for nearly all animals. Sex was clustered by feedlot and cohort. Only 8% of animals were heifers and these were from only 6 of the 14 feedlots. Age data were not available for most cattle (only some of the vendor questionnaire dataset) so dentition was used as a proxy in the full cohort dataset. Dentition data were completely missing for one feedlot and were not recorded at the animal level in another. For the feedlot where dentition data were completely missing, the manager indicated that more than 99% of animals entering the feedlot had deciduous incisor teeth only, so dentition was imputed as zero (i.e. no permanent incisor teeth) for all animals in this feedlot. This was consistent with the observed low induction weight range and the practice of sending cattle to the feedlot soon after weaning in this region. Induction weight ranged between 196 and 756 kg and was split into four categories as described above. Weight difference (Weight diff) was a categorical variable derived from the difference between the induction weight of the animal and the mean cohort weight. Feedlot-specific cattle class codes varied considerably and are considered to be a composite of weight, breed, age, body condition, sex and intended days on feed. Only the intended days on feed component was used for analysis as this was not captured in other variables.

Table 3-4: Derivation and categories of the breed variable used in the final analyses.

Original variable	Examples	Category	Analysis variable
Breed			Breed category (Breed)
	Angus	Angus	
	Red Angus		
	Hereford	Hereford	
	Polled Hereford		
	Shorthorn	Shorthorn	
	Murray Grey	Murray Grey	
	British cross	British cross	
	Angus X		
	Hereford X		
	British X European	European/ European cross	
	Limousin		
	Simmental		
	Charolais		
	Gelbvieh		
	European X		
	Charbray	Tropical/Tropical cross	
	Santa Gertrudis		
	Droughtmaster		
	Braford		
	Brahman		
	Brangus		
	British/Tropical		
	British/Tropical/European		
	Tropical/European		
	Brahman / Brahman X		
	Wagyu		Excluded
	Unknown		Excluded

Table 3-5: Derivation and categories of variables for feedlot entry characteristics (except breed) used in the final analyses.

Original variable	Examples or range	Category	Analysis variable & notes
Sex			Animal-level sex (Sex)
	Steer	Male	92% were steers
	Heifer	Female	6 feedlots had no heifers
Dentition			Number of permanent incisors (Dentition)
	Deciduous only	0	Poor proxy for age if < 2 years
	2	2	
	4	≥ 4	Combined categories as
	6		very few '6 teeth'
Induction weight (kg)			Induction weight category (Weight) (kg)
	196 to 756	< 400	
		400 to < 440	
		440 to < 480	
		≥ 480	
Induction weight, Mean cohort weight (kg)			Difference between mean cohort weight and animal-level induction weight (Weight diff) (kg)
	-229 to 296		
		> 20 below	
		≤ 20 below	
		≤ 20 above	
		> 20 above	
Cattle class			Intended days on feed (Intended DOF)
	150D ox	≥ 120	
	BB	85 to < 120	
	60D domestic	≤ 85	

3.9.3.3 Exposure variables relating to mixing, moving, group size, saleyard exposure and transport prior to feedlot entry derived from NLIS data

Prior hypotheses

Previous research has identified market origin, number of cattle in the animal's 'group', and comingling with cattle from other sources close to the time of feedlot entry as risk factors for BRD.[11,12,13] In these studies, the data was not suitable for assessing the effects of more refined individual factors, and for assessing the effects of timing of exposure to comingling and saleyard transfers relative to when cattle commenced being at risk of BRD at the feedlot. The detailed movement histories recorded in the NLIS database enabled us to explore time specific effects of mixing, moving and saleyard exposures.

We hypothesised that the effect of mixing, moving and a saleyard transfer on the risk of developing BRD might differ depending on the timing of these events in relation to day 0. We hypothesised that mixing some time before day 0 might reduce risk as animals would be likely to have been exposed to more pathogens resulting in more effective immunity on arrival at the feedlot. Previously mixed cattle might also be more used to the changes in social hierarchy associated with mixing and thus less stressed by further mixing during the cohort induction period. We hypothesised that "some time" would probably be at least four weeks before day 0, but this was explored by splitting each animal's history from at least four weeks before day 0 into multiple time periods.

We also hypothesised that mixing in the interval from four to two weeks before day 0 could either increase or decrease the risk of BRD possibly depending on earlier and/or later mixing and on the timing and extent of mixing in this time period. Earlier mixing within this period might give time for pathogens to spread and some immunity to develop before further mixing when the cohort was formed. This is a scenario that may be more likely to be beneficial for cattle that have not been mixed previously compared to than those that have. Later mixing in this period may result in stressed cattle still with active infections going into the time interval leading up to cohort formation. This scenario might be slightly worse for those that have not been mixed previously compared to those that have and for those that are mixed further in the period leading up to cohort formation. We also hypothesised that mixing in the last two weeks before day 0 and during the period of cohort formation would increase risk due to increased exposure to pathogens and the stress associated with the changes to the social hierarchy.

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[11] Martin, S.W., Meek, A.H., Davis, D.G., Johnson, J.A., Curtis, R.A., 1982. Factors associated with mortality and treatment costs in feedlot calves: the Bruce County Beef Project, years 1978, 1979, 1980. Canadian Journal of Comparative Medicine 46, 341-349.

[12] Wilson, S.H., Church, T.L., Acres, S.D., 1985. The influence of feedlot management on an outbreak of bovine respiratory disease. Can Vet J 26, 335-341.

[13] Martin, S.W., Meek, A.H., 1986. A path model of factors influencing morbidity and mortality in Ontario feedlot calves. Can. J. Vet. Res. 50, 15-22.

We hypothesised that a saleyard transfer might reduce risk above and beyond any effects of being mixed and moved not through a saleyard if the saleyard transfer occurred a long time before day 0. We hypothesised this because a saleyard transfer may result in greater exposure to pathogens and subsequent development of immunity and animals being even more used to mixing. In contrast, we hypothesised that a saleyard transfer close to day 0 might increase risk above and beyond being mixed and moved but not through a saleyard because of greater exposure to pathogens and additional stress associated with extra mixing and handling at the saleyard.

We hypothesised that, in general, moves would not affect risk of BRD above and beyond any mixing associated with the move, but that moving to the feedlot earlier (i.e. more time before day 0) would decrease the risk of BRD as this stressful event would then be separated in time from the stressful period around day 0 and cohort formation (the process by which animals are selected to form a cohort). We also hypothesised that, for moves closer to day 0, longer transport times would increase risk through greater stress.

To test these hypotheses the following time points and time periods were chosen. Time points: day -365, day -91, day -28, day -13, day -7, day -2, day -1 and day 0 and time periods: prior to day -364, days -364 to -91, days -90 to -28, days -27 to -13, day -12 to -7, days -6 to -2 and days -1 to 0 or day -1 to cohort close date, or combinations thereof. PIC-# and group-# variables were determined for each of the time points.

Number of animals in a group

Groups were defined by the PIC location of animals at time points of interest where animals in the same group would subsequently be part of the same cohort. The numbers of animals in groups were then determined and categorised for analysis. Common categories were used for each time point (Table 3-6). Group-13N was defined as the number of animals in each group-13, group-28N was the number of animals in each group-28 and group-91N was the number of animals in group-91.

Moving between PICs

Animals were classified as having moved between PICs within a time period if the PIC at the end of the time period differed from the PIC at the start of the time period. Moves were sequenced and for animals with valid transfer dates, the time interval between the transfer and day 0 was determined for each transfer. A yes/no binary variable was derived for each time period indicating whether or not the animal had moved between PICs at least once during the time period. 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 moves to the feedlot were considered as these were of greatest interest to industry.

Mixing

Mixing 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 (no study animals added) were not considered to have mixed. In addition to direct moves from one PIC to another, 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. A mixing variable was derived for each time period describing the number of groups at the time point at the start that had been combined to form a single group by the time point at the end of the period. These variables were then categorised into two or three categories based on the distribution of number of mixing events for each time period.

Results of exploratory analyses using a large number of time periods supported the hypothesis that mixing on or before day -28 reduced the risk of BRD. However disaggregating the data into so many time periods resulted in sparse data in some categories for some periods. Based on prior hypotheses stated above and consistent patterns observed in the data, multiple time periods were amalgamated and variables for mixing prior to day -27 and between days -27 to -13 and day -12 to cohort close date were used for the next round of exploratory analyses (Table 3-6). Thus the extent of mixing prior to day -27 was determined by the number of group-issues that were combined to form group-28. Prior hypotheses suggested that the effect of mixing in particular time periods may not be independent of other time periods. Possible methods for assessing this were 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 whereas several combinations with very sparse data and therefore imprecise and potentially misleading estimates would have to have been retained in the model with the interaction terms.

The composite variable used in most analyses had twelve categories determined by various combinations of mixing prior to day -27, during days -27 to -13 and days -12 to cohort close date (Mix history, Table 3-7). The categories were selected after tabulating the mixing variables for all the time points to assess their distribution and by examining the effect of various combinations on the risk of BRD. Categories describing the extent of mixing (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 could not be made when mixing occurred during days -27 to -13 due to sparse data. Thus, although particular time periods and combinations thereof were partly defined based on results of associations with BRD, the general concept of the effects of mixing, including possible dependencies among time periods prior to day 0, was defined *a priori*.

A collapsed version of the mixing history variable was derived for use in subset analyses. This four category variable (Mix summary, Table 3-7) 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). To further evaluate mixing a variable describing the time of earliest mixing was derived (Mix first, Table 3-7). For animals with valid transfer dates, the time interval from the earliest transfer to day 0 was determined. This was combined with variables describing the number of groups mixed to form the group at the time point of interest to determine if the transfer interval corresponded with a mixing event. Animals with NLIS imputed transfer dates but with transfer sequences matching animals with known transfer dates were allocated to a common group earliest mixing category.

Move to the feedlot

For animals that did not have a NLIS record of the move to the feedlot, or that had a PIC of issue matching the feedlot PIC (i.e. NLIS device was replaced at the feedlot) the arrival date and tail tag recorded by the feedlot was used to impute the move and the time interval in which this occurred. A categorical variable (Arrival to day0, Table 3-8) was used in the pre-assembly subset analyses and was a component variable for the composite feedlot move variable.
Transport times were estimated for moves to the feedlot between days -12 and 0. Estimated road distances and travel times were established by entering the geographical coordinates of the source PIC, intervening PIC where relevant, and the feedlot PIC into Google maps.[14] 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[15], 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, so moves with an intervening PIC were allocated two hours. Estimated times for travel, driver rest time, and animal loading and unloading were summed to give move time variables in hours for days -12 to -2 and days -1 to 0. Composite categorical move time variables were developed for moves to the feedlot during these periods and combined with the categorical variable describing the numbers of days between arrival and day 0 to create a single composite feedlot move variable (Move_FL) which was used in the final analyses (Table 3-8).

[14] https://maps.google.com.au/

[15] http://www.ntc.gov.au/ViewPage.aspx?documentid=01499

Table 3-6: Derivation and categories of the number in group variables and intermediate variables used to derive mixing variables (# refers to 13, 28 and 91 in three separate variables).

Intermediate variable	Range	Category	Analysis variable & notes
Group-#	1 to 342		Number of cattle in the group defined # days before day 0 (Group-#N)
		< 50	
		50 to 99	
		≥ 100	
Number of group-issues 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-91s forming group-28			Mixed on or between days –90 and -28 (Mix-90 to -28)
		No	
		Yes	
Number of group-issues 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 on or between days -27 and -13 (Mix-27 to -13)
		No	
		Yes	
Number of group-28s forming cohort			Amount of mixing on or between day -27 and the cohort close date (Mix-27 to close)
		< 4	Less than 4 group-28s combine to form cohort
		≥ 4	4 or more group-28s combine to form cohort
Number of group-13s forming cohort	1 to 25		Amount of mixing on or 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 combine to form cohort
		4 to 9	4 to 9 group-13s combine to form cohort
		≥10	10 or more group-13s combine 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

Table 3-7: Derivation and categories of the mixing variables used in the final analyses.

Intermediate variable	Category	Analysis variable & notes
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-28s 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-28s form cohort
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)

Table 3-8: Derivation and categories of moving variables and the "move to the feedlot" variable (Move_FL) used in the final analyses.

Intermediate variables	Range	Category	Analysis variable
Number of days between day 0 and arrival at the feedlot PIC, number of days between day 0 and the transfer date from the NLIS database where the destination PIC is the feedlot PIC	0 to 228		Interval between arrival and day 0 (Arrival to day0)
		Pre day -27	Moved to the feedlot PIC prior to day -27
		Day -27 to day -13	Moved to the feedlot PIC between days -27 and -13
		Day -12 to day 0	Moved to the feedlot PIC between days -12 and 0
PIC geographic coordinates, estimated total transport time	0 to 41		Estimated total transport duration for animal's move to the feedlot
		< 6 hours	
		≥ 6 hours	
Days between arrival and day 0, transport duration			Feedlot move timing (Move_FL): composite variable which describes timing and duration of animal's move to the feedlot
		Pre day -27	Moved to feedlot prior to day -27
		Days -27 to -13	Moved to feedlot between days -27 and -13
		Days -12 to -2; < 6 hours	Moved to feedlot between days -12 and -2; transport time < 6 hours
		Days -12 to-2; ≥ 6 hours	Moved to feedlot between days -12 and -2; transport time ≥ 6 hours
		Days -1 to 0; < 6 hours	Moved to feedlot between days -2 and 0; transport time < 6 hours
		Days -1 to 0; ≥ 6 hours	Moved to feedlot move between days -2 and 0; transport time ≥ 6 hours

Saleyard transfers

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. Exploratory analyses examined several time periods; those used in the final analyses were pre day -27, days -27 to -13 and days -12 to 0 (Table 3-9).

Table 3-9: Derivation and categories of variables for movement through a saleyard used in the final analyses.

Original variables	Examples	Category	Analysis variable
Transfer type, transfer date	SY in, SY out		Saleyard transfer prior to day -27 (SYpre-27)
		No	
		Yes	
Transfer type, transfer date	SY in, SY out		Saleyard transfer in interval from day -27 to day -13 (SY-27 to -13)
		No	
		Yes	
Transfer type, transfer date	SY in, SY out		Saleyard transfer in interval from day -12 to day 0 (SY-12 to 0)
		No	
		Yes	

3.9.3.4 Exposure variables relating to formation of the cohort

A range of variables relating to formation of cohorts was derived from the Induction Sessions (Table 3-10). Only two categories were used for the number of animals inducted into each cohort (CohortN) as this was highly clustered by feedlot. Separation into additional categories would have further exacerbated this issue because models with cohort level risk factors that were markedly clustered by feedlot were unstable with binary variables and would have been even less stable with more categories. Although the range of the number of animals in the cohort was wide, only 5.8% of animals were in small cohorts (< 100 animals). Mean cohort weight (Weight cht) was a categorical variable derived from the mean induction weight of all animals in the cohort and cohort sex (Sex cht) identified whether the cohort was single sex (male or female) or mixed. Only four feedlots had mixed sex cohorts and only five had heifer only cohorts.

Cohort fill duration was defined as the number of days in the induction period. A binary variable (Cohort fill: 1 / >1) was used in the final analyses as for the majority of cohorts this was one day. Animals from several feedlots, representing 19% of the study population, 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 this time period was described using a three-category, animal-level variable (DOF1 to day0) which took the value zero when the first day on feed was day 0. The duration from day 0 to cohort close was captured in another three-category animal-level variable (Day0 to close). This variable took the value one for all animals in cohorts filled in one day.

Table 3-10: Derivation and categories of variables relating to cohort formation used in the final analyses.

Original variable	Range	Category	Analysis variable & notes
Cohort ID			Number of cattle inducted into cohort (CohortN)
	14 to 395	< 200	
		≥ 200	
Induction weight, number of cattle inducted into cohort	315 to 491		Cohort-level mean induction weight (Weight cht) (kg)
		< 425	
		425 to < 455	
		≥ 455	
Sex			Cohort-level sex (Sex cht)
		Male	
		Female	
		Mixed	
Induction date, Cohort ID			Pattern of cohort filling (Cohort fill) (days)
		1	Cohort filled on single day
		> 1	Cohort filled over more than one day
First day on feed (DOF1), induction date	0 to 13		Number of days between first day on feed and day 0 (DOF1 to day0) (days)
		0	
		1 to 2	
		≥ 3	
Induction date, Cohort ID	0 to 15		Number of days from day 0 to cohort close date (Day0 to close) (days)
		1	
		1 to 6	
		≥ 7	

3.9.3.5 Exposure variables relating to source and feedlot regions, timing of the induction date and weather in the first week after day 0

The source region for each animal was determined by the geographical coordinates of its PIC-28. Initially 12 categories were derived but these were later combined to six broad regions based on proximity and similarity in geography and weather patterns (Table 3-11, Figure 3.5). Feedlots were grouped into two broad categories: north consisting of all Queensland feedlots and south consisting of feedlots in New South Wales, South Australia and Western Australia (Table 3-11).

The calendar timing of induction date was categorised by calendar month, year-month, year and season. As 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. Categories for season and induction year are shown in

Table 3-12.

As 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. The data are available for such analyses but they were considered beyond the scope of the current study. It was however possible to include crude weather variables within the current modelling framework. As 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 based on observations during the first week after induction for each animal (i.e. observations from days 0 to 6). Using interpolated data, continuous temperature variables were derived by averaging the daily (midnight to midnight) maxima, minima and the differences between the two (temperature range) over the seven days, and a rain variable was derived from the total rainfall for all days in the time period. All variables were then categorised based on their distribution (Table 3-13).

Wind data were obtained from the nearest weather stations to each feedlot that recorded these data. For wind speed, direction and maximum gust speed, the weather station was between 6 and 94 km from the feedlot; these data were collected from a site within 30 km of the feedlot for only six of the 14 feedlots. Although these wind data may not have described what occurred on the feedlots, wind had been identified as a potential risk factor of interest so a categorical variable derived from the average maximum wind gust speed was derived from the observations from days 0 to 6 (Table 3-13). For wind run data (cumulative wind for 24 hours), weather stations were between 14 and 98 km from the feedlot, with only three feedlots having these data from a site within 30 km of the feedlot. As these data may have been markedly different from wind runs at the feedlots, and because much wind run data were missing, wind run data were not used for any analyses.

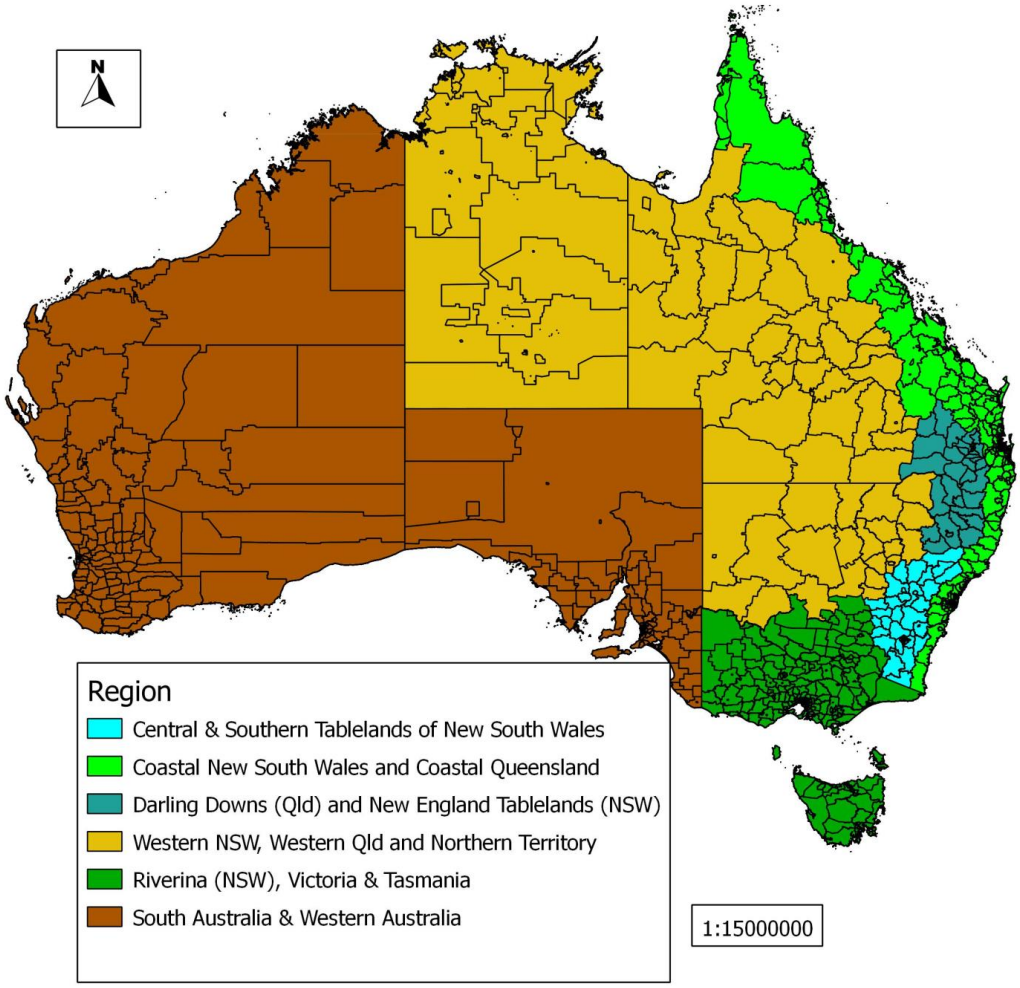

Figure 3.5: Geographic distribution of source regions

Table 3-11: Derivation and categories of source and feedlot region variables used in the final analyses.

Intermediate variable	Category	Analysis variable
PIC-28 Cohort ID		Source region: broad regions grouped by similar geography and climate describing location at day -28
	NSW Central & Southern Tablelands	
	Coastal NSW or Queensland	
	Darling Downs / New England	
	Western NSW/Qld or NT	
	NSW Riverina, Victoria & Tasmania	
	South Australia/ Western Australia	
Feedlot PIC		Feedlot region
	North	QLD feedlots
	South	NSW, SA and WA feedlots

Table 3-12: Derivation and categories of variables relating to timing of the induction date used in the final analyses.

Original variable	Range	Category	Analysis variable
Induction date	31/03/2009 to 14/12/2011		Season of induction (Season)
		Spring	September 1st - November 30th
		Summer	December 1st – February 28th
		Autumn	March 1st – May 31st
		Winter	June 1st – August 31st
Induction date			Year of induction (Induction year)
		2009	
		2010	
		2011	

Table 3-13: Derivation and categories of variables relating to weather during the first week of time after induction.

Data	Range	Category	Analysis variable
Daily maximum temperature from interpolated data	11.9 to 31.4		Mean maximum temperature in first week from day 0 (Temp max) Derived from averaging daily data (°C)
		< 17	
		17 to < 23	
		23 to < 30	
		≥ 30	
Daily minimum temperature from interpolated data	-2.1 to 21.8		Mean minimum temperature in first week from day 0 (Temp min). Derived from averaging daily data. (°C)
		< 5	
		5 to < 11	
		11 to <17	
		≥ 17	
Daily maximum and minimum temperature from interpolated data	6.9 to 20.9		Mean daily range in temperature in first week from day 0 (Temp range). Derived from averaging daily data. (°C)
		6 to < 11	
		11 to < 16	
		≥ 16	
Daily total rainfall from interpolated data	0 to 162		Total rainfall in first week from day 0 (Rain). Derived from totalling daily data from nearest weather station (mm)
		0	
		< 4	
		4 to < 25	
		≥ 25	
Daily maximum wind gust speed from nearest wind recording station	21 to 55		Mean maximum wind speed in first week from day 0 (Wind). Wind weather station 6-94km from feedlot (km/hour)
		< 35	
		35 to < 45	
		≥ 45	

3.9.3.6 Exposure variables relating to pen characteristics

All pen characteristic exposure variables described the home pen, which was defined as the pen where the cattle in a cohort spent most of their time during their first 50 days at risk. Details on the derivation and categories used in the final analyses are shown in Table 3-14. Stocking density (Pen density) was estimated as pen area per standard cattle unit (SCU). The approximate number of standard cattle units in the cohort was calculated using the number of animals in the cohort and the appropriate conversion factor for the mean weight of animals in the cohort.[16] The stocking density was then derived by dividing the pen area by the number of standard cattle units in the cohort. This was then categorised based on distribution to create the variable used in the final analyses. Descriptions of the extent and type of shade were often unique to individual feedlots, so a simple pen shade variable was used in the final analyses; this was a dichotomous variable indicating the presence or absence of any shade in the pen.

Data on pen slope, pen cleaning frequency, pen riding frequency and the distance to the hospital pen were not used in the analyses because of missing data, limited variation between feedlots and confounding by feedlot size.

[16] http://www.daff.qld.gov.au/business-trade/business-and-trade-services/oneplan/cattle-feedlot-plan/feedlot-management-terms-explained

Table 3-14: Derivation and categories of variables used in the final analyses relating to the home pen.

Original variables	Range	Category	Analysis variable & notes
Pen area, induction weight	12 to154		Number of standard cattle units per square meter of pen (Pen density): calculated from home pen area, total standard cattle units (SCU) estimated from the animal level induction weight and table of SCU values. (m^2 /SCU)
		< 14	
		14 to < 17	
		17 to < 25	
		≥ 25	
Pen shade		No	Presence of shade in pen (Pen shade): part of pen was shaded. Not enough variation between feedlots to assess effects of amount or type of shade cover
		Yes	
Outside access to water trough		No	Shared pen water (Pen water): pen water could be accessed by animals from an adjoining pen
		Yes	
Number of pens joining home pen		1	Number of pens adjoining home pen and separated by only a fence (Pen join)
		2	
Bunk space, number in cohort	0.13 - 0.93		Bunk space per head (Bunk space): linear meters per head, calculated from dimensions of feed bunk and number of animals in cohort at induction (m/head)
		< 0.18	
		0.18 to < 0.24	
		≥ 0.24	

3.9.3.7 Exposure variables relating to ration characteristics

Variables derived from the ration composition and ration routine data that were used in the final analyses are shown in Table 3-15. After discussion with some of the consulting veterinarians, we hypothesised that the higher the 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 variables for the percentage grain in the diet at day 0 and day 20 were derived and categorised (Grain1 and Grain21). For most animals, these time points corresponded to the first and 21st days on feed. For animals in some feedlots, day 0 was later than the first day on feed, but timing based on time at risk was chosen to ensure a consistent time interval between the two measurements. Wheat, corn, barley and sorghum were classified as grains.

A categorical variable for the time taken from the first day on feed for the diet to be at least 60% grain was also derived. Although times to higher grain percentages (e.g. 70%) were also of interest, diets did not reach these levels at all feedlots, so the variables could not be defined for many cattle.

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.

Table 3-15: Derivation and categories of ration variables used in the final analyses.

Original data	Examples or range	Category	Analysis variable
Ration composition	Corn, barley		Type of grain in ration (Grain type)
		Barley	
		Sorghum	
		Wheat mix	
		Other mix	
Ration composition	17 to 60%		Percentage of grain in ration on day 0 (Grain1)
		17 to < 35%	
		35 to < 40%	
		40 to < 45%	
		≥ 45%	
Ration composition	39 to 86%		Percentage of grain in ration on day 20 (Grain21)
		<60%	
		60 to <70%	
		≥70%	
Ration composition, ration routine	1 to 58		Number of days from first day on feed until 60% grain in ration (Grain 60%)
		0 to 6	
		7 to 13	
		14 to 20	
		≥ 21	

3.9.3.8 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 (Table 3-16). These treatments were typically administered to all or virtually all animals entering some feedlots and to no animals in remaining feedlots, so these were essentially feedlot-level variables.

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

Monthly data describing the total number of cattle on feed in the feedlot and the total number that were less than 40 days on feed were derived from the data described in Section 3.8.2.6 and were categorised as shown in Table 3-17. Following discussion with consulting veterinarians, several other variables were also derived from these data. These were the percentage of cattle on feed that were less than 40 days on feed, the percentage of cattle on feed in the 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 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 are many more cattle on feed compared to the preceding two months, the number of cattle per staff member was thought likely to increase. 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 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.

Table 3-16: Derivation and categories of variables relating to induction treatments and monthly summaries of numbers of cattle on feed

Original data	Range	Category	Analysis variable (abbreviation)
Rhinogard™			Rhinogard™ vaccination at Induction (Rhinogard)
		Yes	
		No	
VitADE			Vitamin A, D & E injection at induction (Vit ADE).
		Yes	
		No	
Number of cattle on feed in calendar month	950 to 42,229		Total number of cattle on feed in the animal's induction month (FeedlotN)
		< 10,000	
		10,000 to < 20,000	
		≥ 20,000	
Number of cattle on feed in calendar month	269 to 15,927		Number of cattle less than 40 days on feed in the animal's induction month (FeedlotN40)
		< 3,000	
		3,000 to < 6,000	
		≥ 6,000	

3.9.3.10 Exposure variables derived from the vendor questionnaire

All variables derived from vendor questionnaire data are described in Table 3-17. For all animals with vendor questionnaire data, responses to questions in the vendor questionnaire on ages of the study animals when various events (marking/weaning/purchase) occurred and associated dates were collated, cross-checked with each other, and used to derive an approximate average age for the animals in the purchase group at the time of induction. This was then categorised and used in the final analyses. Although there was a moderate amount of missing data for this variable (9.2% of animals with vendor questionnaire data), it provided an

estimate of age which better discriminated between categories of younger cattle than the dentition variable.

Other exposure variables derived from the vendor questionnaire data were restricted to particular subsets of enrolled animals. Variables describing weaning method, on-property mixing and prior feeding history were derived only for vendor questionnaire subset 1 data (animals that were born on the vendor's property), as full lifetime data for animals purchased by vendors was not known. All responses relating to feeding history were used to derive categorical variables indicating whether the animals had ever been fed grain and whether they had ever had any supplementary feeding (e.g. conserved forage). Responses relating to on-property mixing were combined to derive a variable indicating whether the animals had ever been mixed with cattle other than those that they were born with prior to leaving the property.

Variables for prior vaccination with Bovilis MH™ and Pestigard™ were derived for vendor questionnaire subset 2 data only (animals that were born on the vendor's property or had been purchased prior to ten 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.

Table 3-17: Derivation and categories of variables from the vendor questionnaire data.

Original data	Examples	Category	Analysis variable
Weaning method	Paddock, yard		Yard weaning and duration (Yard wean); involves keeping cattle in small yards after weaning for variable time periods. Restricted to vendor-bred cattle
		No	
		Yes; < 7	Less than 7 days
		Yes; ≥ 7	7 days or more
Grain feeding	Between weaning/purchase and sale		Cattle had been fed grain at any stage prior to feedlot entry (Grain pre). Restricted to vendor-bred cattle
		No	
		Yes	
Conserved forage or other supplements	Between weaning/purchase and sale		Cattle had been fed conserved forage or supplements at any stage prior to feedlot entry (Supp pre). Restricted to vendor-bred cattle
		No	
		Yes	
Mixing	At weaning, purchase		Cattle were mixed on-property at some stage prior to feedlot entry (Mix VQ). Restricted to vendor-bred cattle
		No	
		Yes	
Date of Bovilis MH™ vaccination			Vaccination with Bovilis MH™ administered prior to day -14 (BV_vacc). Restricted to vendor-bred & purchased by 10 months
		No	
		Yes	
Date of Pestigard™ vaccination			Vaccination with Pestigard™ administered prior to day -14 (PV_vacc). Restricted to vendor-bred & purchased by 10 months
		No	
		Yes	
Age/month of marking, weaning, purchase	8 months, Nov-08, 10 months		Estimated average age of group at feedlot entry (Age).
		< 16 months	
		16 to < 22 months	
		≥ 22 months	

3.9.3.11 Exposure variables relating to BVDV status

When assessing effects of BVDV on BRD risk, we hypothesised that animals persistently infected (PI) with BVDV would be at increased risk of BRD after induction, that exposure to a BVDV-PI animal would result in a protective effect against BRD in animals after induction if that exposure occurred at least 28 days prior to induction, and that animals that were immunologically naïve to BVDV at induction would experience increased risk of BRD following exposure to a BVDV-PI animal in the animal's cohort after induction or when there was evidence of transient infection in the animal's cohort.

Accordingly, we used the following four variables in models to assess the various effects of these exposures:

- whether or not the animal was persistently infected ("BVDV_PI_animal")
- whether or not BVDV was present in the cohort ("BVDV_cht")
- the combined BVDV status of animal's group-28 and the animal's cohort ("BVDV_grp_cht"):
    - BVDV not detected in any animals in the cohort
    - no persistently infected animals in the animal's group-28 but BVDV present in the cohort
    - at least one persistently infected animal in the animal's group-28 and BVDV present in the cohort
- of those cohorts with at least one persistently infected animal, whether or not at least one animal persistently infected with BVDV was in the animal's group-28 ("BVDV_grp_PIcht")
    - at least one persistently infected animal in the animal's cohort but no persistently infected animal in the animal's group-28
    - at least one persistently infected animal in the animal's cohort and in the animal's group-28

As not all animals were blood-sampled, and as sera were pooled for initial testing for presence of BVDV, a complex test protocol and algorithm was developed to identify each animal's status for these three variables. This is detailed below.

The derivation of the final analysis variables used to investigate the presence of animals persistently infected with BVDV (BVDV-PI) involved a number of stages and associated laboratory testing. A single sample may be positive for BVDV on PCR testing because of transient infection with BVDV, but a repeated positive test at least 14 days after an initial positive test is thought to be indicative of a persistently infected animal.[17] Hence, in identifying whether or not a cohort contained a BVDV-PI animal, repeated positive tests from the same animal were required. Initially we attempted to use the pooled test results to derive a cohort-level variable to describe

[17] Xue, W., Mattick, D., Smith, L., Umbaugh, J., Trigo, E., 2011. Vaccination with a modified-live bovine viral diarrhea virus (BVDV) type 1a vaccine completely protected calves against challenge with BVDV type 1b strains. Vaccine 29, 70-76.

whether or not a BVDV-PI animal was in the cohort. For this round of testing all cohorts had multiple pools. Positive pools containing induction and follow-up serum samples from the same animals were classified as positive and cohorts with all pools testing negative were classified as negative. Cohorts with a positive induction pool without a matching positive follow-up pool were classified as a separate category while those with positive follow-up pools without a positive induction pool were classified as missing, because this was not considered biologically plausible. However, this approach resulted in much missing data, and the accuracy of the classification was doubtful. Cross checking animal-level hospital and dead sample BVDV PCR results (Report B_FLT_0224 Bovine respiratory disease: Virology) against the pooled test results revealed that BVDV was present in many cohorts that had tested negative at induction, and it was likely that active infections circulating within cohorts were causing positive follow-up pool results. It was apparent that pooled testing would not allow the accurate determination of the presence of BVDV-PI animals. However, the pooled testing and hospital sample tests were used to derive a binary variable to describe whether or not BVDV was present in the cohort (BVDV_cht).

Additional testing was done at the animal level because this was logistically easier than multiple additional rounds of pooled testing. The first phase of this additional testing focused on induction samples from positive pools. Following compilation and cross-checking of animal-level induction results against pooled results and hospital sample results, animals were identified for further testing if they had a single positive test or if they did not have a sample sufficient for testing on the testing attempt. The second round of testing preferentially involved follow-up samples but hospital samples were eligible for inclusion provided the interval between sample collections (i.e. between the hospital sample and other available sample) was 14 days or more. Where an induction serum sample was not available induction swabs were tested to determine induction status for BVDV-PI suspect animals.

Where possible, two positive serum samples were used to diagnose BVDV-PIs. However, the status of a number of possible BVDV-PI animals could not be determined in this way because two serum samples were not available for testing. Samples that may have been of inadequate volume initially or that had already been used for case-control ELISA testing were sometimes not sufficient for repeat testing. Where only a single sample was available, additional criteria were applied in determining the BVDV-PI status. BVDV-PI animals typically do not develop antibodies against BVDV, so they would be expected to return a negative ELISA test to BVDV antibodies. Meanwhile, in-contact animals with a normal immune response would be expected to develop BVDV antibodies after a sufficient length of time following exposure. We therefore hypothesised that animals known to be in the same group as a BVDV-PI animal a month before feedlot entry would be expected to test seropositive at induction. In contrast, animals in a group-28 not previously exposed to BVDV or exposed more recently would be expected to be seronegative or show increasing antibody titres following exposure. From the cohort study dataset, we had derived variables to describe the length of time between arrival and day 0 and the mixing history for each animal and this information was of use in evaluating induction PCR results. We surmised that a positive PCR test at induction in an animal from a stable group-28 and in which the interval between arrival and induction sampling was very short (within 1 day) would be more likely to indicate a persistently infected animal than a positive test in an animal that had mixed in the weeks before induction sampling, or in a follow-up sample. Given that the prevalence of BVDV-PIs was expected to be very low, and that BVDV-PI animals tend to cluster within groups, we further surmised that the probability that an individual animal being a BVDV-PI

(without two samples for testing) would be extremely low if that animal was part of a large group-28 and all or most other animals in its group-28 tested negative.

The steps and rules applied in determining whether or not animals were BVDV-PIs were as follows:

1. Animals with samples received, verified and adequate, with a sample in a negative pool were negative
2. Animals with samples received and verified but not of adequate volume in a negative pool were probably negative
    - Induction or hospital samples from some of these were tested in the first round of animal-level testing
    - The remaining animals were classified as negative
3. Animals with samples received and verified in a positive pool, but without a sample in a negative pool were possible BVDV-PIs
    - Induction samples from all of these animals were identified for testing
    - Where induction sera samples were not available for testing, induction swabs were identified as an alternative.
4. All test results, including hospital and dead sample results were compiled and cross checked. Positive induction pool test plates were cross checked to see if at least one animal-level test tested positive.
5. Any animal-level negative test was used to classify animals as negative
6. Follow-up samples were identified and tested for animals with a single positive test without a negative test
7. Two positive animal-level tests were used to classify animals as BVDV-PI positive
8. Pooled test results, case-control serology results, animal-level results, and the ELISA and PCR results of animals in the same group-28 were cross-checked and considered in classifying animals that did not have 2 samples available for testing
    - If an animal had a sample received and verified in positive induction and follow-up pools and a strongly positive induction result but without an adequate sample for retesting and all other possible animals from the positive pool combination were classified as negative, that animal was classified as BVDV-PI positive
    - If an animal had only a single induction sample and had a mixing history and interval between arrival and induction consistent with being in a stable group for 28 days before induction, ELISA serology results for that animal were compared to other animals in the same group-28.
        - A positive PCR on a single sample in combination with a seronegative ELISA result, while common group-28 animals tested seropositive to BVDV, was used to classify some animals as BVDV_PI positive
        - A positive PCR on a single sample in combination with a seropositivity was not consistent with being a BVDV-PI animal and these were classified as negative

9. If an animal had only a single positive sample and the animal was part of a group-28 of at least 15 animals in which other animals were classified as BVDV-PI negative, and a positive PI animal had been identified in the animal's positive pooled tests, the animal was classified as negative. Serochange patterns of group-28 animals were also considered in some instances, with "seroincreases" being more consistent with active infection (where seroincrease is an increase in the ELISA result, described in Section 3.5.3, for a virus by two or more categories between the induction and follow up samples).
10. If an animal had a single positive test without serology results and other animals in the group-28 were positive BVDV-PIs, that animal's status was classified as missing
11. If an animal had a single positive test with fewer than 15 animals in its group-28, that animal's BVDV-PI status was classified as missing
12. If an animal had only a single untested sample or no samples and was part of a group-28 with 15 or more animals which had been classified as negative, that animal was classified as negative.

This process resulted in an animal-level variable describing whether or not the animal was a BVDV-PI (BVDV_PI_animal, Table 3-18). This variable was then combined with the group-28 variable to derive a variable to describe whether or not a BVDV-PI animal was present in the group-28. This was then combined with the cohort-level variable (BVDV_cht) to produce the final composite analysis variable (BVDV_grp_cht,) which classified animals as "no, no" if there was no BVDV-PI animal in the group-28 and no evidence of BVDV being present in the cohort, "yes, yes" if there was a BVDV-PI animal in the group-28 (and hence in the cohort) and "no, yes" If there was no BVDV-PI animal in the group-28 but BVDV was present in the cohort. A further variable (BVDV_grp_PIcht, Table 3-18) classified animals based on the presence of a BVDV-PI animal in their group-28; this was restricted to cohorts with at least one BVDV-PI animal.

Table 3-18: Derivation and categories of variables relating to the presence of BVDV in the cohort and animal(s) persistently infected with BVDV (BVDV-PI) in the group-28 and cohort.

Original data or Intermediate variable	Range	Category	Analysis variable
Cycle at which fluorescence reaches threshold level (C_T value) in real time PCR analyses of samples	23 to 45		BVDV PCR result
		Positive	C_T value ≤ 35
		Negative	Did not reach threshold
		Borderline	C_T value > 35
BVDV PCR result			BVDV present in cohort (BVDV_cht):
		No	No positive BVDV_PCR results for any animal in the cohort
		Yes	Positive or borderline BVDV_PCR result for any animal in the cohort
BVDV PCR result			Animal is persistently infected with BVDV (BVDV_PI_animal)
		No	
		Yes	
BVDV-PI animal ID, group-28			At least one animal persistently infected with BVDV in the animal's group-28 (BVDV_PI_group-28)
		No	
		Yes	
BVDV-PI animal in group-28, BVDV present in cohort			BVDV status of animal's group-28 and cohort (BVDV_grp_cht)
		No, no	BVDV not detected in any cohort animals
		No, yes	No persistently infected animals in the animal's group-28 but BVDV present in the cohort
		Yes, yes	At least one persistently infected animal in the animal's group-28 and BVDV present in the cohort
BVDV-PI animal in group-28, CohortID			Of those cohorts where a BVDV-PI animal was identified, the presence/absence of BVDV-PI animal in group-28 (BVDV_grp_PIcht)
		No	BVDV-PI animal in cohort and no BVDV-PI animal identified in group-28
		Yes	BVDV-PI animal in cohort and BVDV-PI animal identified in group-28

3.9.3.12 Exposure variables relating to serology results

The distributions of the categorical serology results for the induction samples for each virus were examined and some categories were combined to simplify analyses. The categorical induction serology results for each animal described in Section 3.5.3were combined so that there were only four categories used in the analyses: 0, 1, 2 or 3 and 4 or 5 (e.g. BoHV-1 ind).

Change in serostatus from induction to follow-up testing for each virus was assessed using results from the induction and follow-up samples (Figure 3.6). A change from an induction value of 0 to a follow-up value of at least 2 was classified as seroconversion. Increases from an induction category of 1, 2 or 3 to a follow-up category of at least 3 and 4 or 5, respectively, were classified as re-exposure. An induction sample with a value of 0, 1, 2 or 3 and a follow-up sample within one category of the induction sample was 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. If the follow-up sample was two or more categories less than the induction sample, serostatus change was coded as missing as this sequence was thought to be biologically implausible in the feedlot setting within the time frame. No further serological variables were defined for these animals.

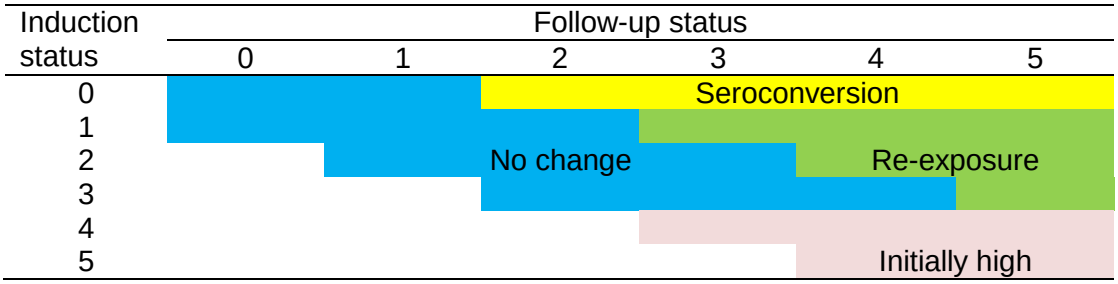

Figure 3.6: Classification of change in serostatus based on induction status and follow-up status.

In turn, three simpler variables to describe changes in serostatus were derived. A three-level composite variable for each virus (e.g. BoHV-1 comp, Table 3-19) was derived with the following categories:

- Initially high – if the change in serostatus was classified as “initially high”
- Up – if the change in serostatus was classified as “seroconversion” or “re-exposure” (i.e. follow-up value at least two categories greater than the induction value)
- No change – if the change in serostatus was classified as “no change”

A seroincrease variable (e.g. BoHV-1seroinc, Table 3-19) was derived as a collapsed version of the composite variable for each virus. This variable had two categories: yes for seroincrease (i.e. “seroconversion” or “re-exposure”), and no for “no change” or “initially high”.
A seroconversion variable (e.g. BoHV-1serocon, Table 3-19) 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.
Two variables combined data from all four viruses. The number of viruses to which each animal was seropositive at induction (VirusN_ind, Table 3-19) was calculated

as the number of viruses for which the induction category was at least 1. The number of viruses to which each animal had a seroincrease to (VirusN_seroinc, Table 3-19) was calculated as the number of viruses for which the animal was categorised as “yes” for the seroincrease variable.

Table 3-19: Derivation and categories of variables relating to the ELISA serology results.

Original data	Range	Category	Analysis variable
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	0 to 5		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 initially seronegative (e.g. BoHV-1 serocon)
		No	
		Yes	
Induction serology for each virus: (BoHV-1, BVDV, BRSV & BPI3)			Number of viruses animal is seropositive to at induction (VirusN_ind)
		0 to 4	
Seroincrease variable for each virus: (BoHV-1, BVDV, BRSV & BPI3)			Number of viruses animal seroincreases to between induction and follow-up (VirusN_seroinc)
		0 to 4	

*Equivalent variables were derived for each of the four viruses

3.10 Assessment of variable quality

The variables defined in Section 3.9 were further assessed to determine their suitability for inclusion in the analyses. Results of this assessment are shown in Table 3-20 to Table 3-22. Variables are listed by the level at which they were measured, where those at the group level relate to a range of groups depending on the timing to which the variable relates. A few variables were excluded from all but very preliminary analyses because of data quality or distribution issues (indicated by * in Table 3-20 to Table 3-22).

Missingness for a variable was considered a problem when data for all animals in particular cohorts were missing or if a large percentage of animals had missing values.

Potential inaccuracy was assessed based on whether it was thought that the derived variable was truly representative of the putative risk factor. For example, wind data from weather stations many kilometres from the feedlot were unlikely to be representative of wind conditions at the feedlot, and the pen cleaning data provided by most feedlots was usually reported as the number of times per year and did not provided specific information about the timing of cleaning of the home pen for a cohort.

Problems with 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 have the same value for these variables. As 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 would be likely to be biased due to confounding by other unmeasured exposure variables, for both feedlot-level variables and lower level variables that were clustered to some extent by feedlot. For example, the administration of Rhinogard™ at induction was likely to be more common in feedlots with high historical incidence of BRD in the feedlot, which, in turn, may have been a predictor of high risk in study animals. Less severe issues with distributions by feedlot occurred where some feedlots had no animals with particular exposure categories. Biased estimates may have resulted due to uncontrolled confounding at the feedlot level. Although inclusion of feedlot as a random effect would be expected to reduce the extent of this bias, it was possible that with extreme differences in distributions by feedlot and large variation in BRD risk between feedlots, some confounding may remain. This was of particular concern for the cohort-level variables that were highly clustered by feedlot.

Sparse categories referred to variables where one or more category contained less than 3% of animals. For example, relatively few animals (2.7%) had a saleyard transfer in the time periods close to day 0.

Correlations between exposure variables were also assessed. As most variables were ordinal, Spearman’s correlation coefficients were used. When variables were closely correlated, one variable was selected for inclusion based on the quality of the data from which the variables were derived and the extent and pattern of the missingness. Some nested variables were also excluded at this stage (e.g. all animals inducted in any one calendar month formed a subset of the animals inducted in a particular season). However, some correlated or nested variables were retained because they were of particular interest (e.g. although maximum and minimum temperatures were correlated, both were of *a priori* interest) and others were

intended for used in subset analyses or sensitivity analyses. Correlation and nesting were considered when constructing causal diagrams and fitting models (e.g. the mixing summary variable was nested within the mixing history variable, so these were not fitted in the same models).

Table 3-20: Assessment of the quality of animal-level and vendor questionnaire derived group-level exposure variables considered for inclusion in the final analyses**.**

	Criteria^					
Variable	A	B	C	D	E	Notes
Animal-Level Variables						
Breed						
Weight						
Sex			X			In 6 feedlots, all animals were male
Weight diff						
Dentition	X					1 feedlot had no data;1 had inferred data
Mix history					X	Correlated with Mix first & Mix summary
Mix first					X	Correlated with Mix history
Mix summary					X	Nested within Mix history
SYpre-27						
Group-Level Variables from Vendor Questionnaire						Restricted to vendor questionnaire subsets
Age	X					9% missing data
Mix VQ				X		94% of animals were mixed
Grain pre	X					
Supp pre	X					
Yard wean						
PV_vacc						
BV_vacc						

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

Table 3-21: Assessment of the quality of group-level exposure variables considered for inclusion in the final analyses.

	Criteria^					
Variable	A	B	C	D	E	Notes
Group-Level Variables						
Source region			X			Most categories have feedlots with no observations
Arrival to day0					X	Nested in Move_FL; used in pre-assembly subset
Move_FL			X			Only 4 feedlots had observations in first 2 categories
SY-27 to -13				X		Only 2.8% of animals were coded yes
SY-12 to 0				X		Only 2.7% of animals were coded yes
DOF1 to day0			X			8 feedlots had only zero values.
Day0 to close						
Group-91N					X	Correlated with group-13N & group-28N
Group-28N					X	Correlated with group-13N & group-91N
Group-13N					X	Correlated with group-28N & group-91N
Grain 60%			X			No observations for 7 feedlots in each of two categories
Grain1			X			Highly clustered by feedlot
Grain21			X			Highly clustered by feedlot
FeedlotN	X		X		X	Highly clustered by feedlot, correlated with feedlot capacity
FeedlotN40	X		X		X	Highly clustered by feedlot, correlated with FeedlotN
FeedlotN40prop*			X			7 feedlots with no observations in first category
FeedlotNprior*	X	X	X		X	Monthly data do not give required detail to estimate short-term changes, correlated with FeedlotN
FeedlotN40prior*	X	X	X		X	Monthly data do not give required resolution to estimate change
Season						
Induction month*					X	Nested within season
Induction year						
Temp max					X	Correlation between maximum & minimum temperature
Temp min					X	
Temp range						
Rain						
Wind		X			X	
Mean wind speed*		X			X	Wind data thought not representative as measured a long way from feedlots
Wind run below 3 meters*	X	X			X	

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

Table 3-22: Assessment of the quality of cohort-level and feedlot-level exposure variables considered for inclusion in the final analyses.

	Criteria^					
Variable	A	B	C	D	E	Notes
Cohort-Level Variables						
CohortN						
Intended DOF			X			8 feedlots have no animals < 85 DOF
Cohort fill						
Weight cht						
Sex cht			X			Only 2 feedlots had mixed sex cohorts and only 5 had female cohorts
Grain type			X			Highly clustered by feedlot; only 3 feedlots varied grain type between cohorts
Rumen Modifier*			X			Does not vary much between feedlots
Metabolisable Energy*	X		X			Missing values for many animals
Roughage percentage*	X		X		X	Inconsistent definitions used in original data; correlated with grain%
Grain: roughage ratio*	X		X		X	Correlated with grain%
Pen density				X		5-7 feedlots had no observations in some categories, but good distribution by feedlot overall
Pen shade			X			Highly clustered by feedlot; only 3 feedlots have disparate cohorts
Pens joining						
Bunk space	X			X		9 feedlots no observations in lowest category
Pen water			X			8 feedlots have no "nos"
Pen distance to hospital*			X		X	Correlated with feedlot capacity
Pen slope*	X					
Feedlot-Level Variables						
Feedlot region			X			Completely clustered by feedlot
Feedlot capacity*		X	X		X	Correlated with number on feed
Pen cleaning frequency*		X	X			Frequency per year does not measure intended cohort level timing
Pen riding frequency*			X			Little variation between feedlots
Rhinogard™			X			Completely clustered by feedlot
Vit ADE			X			Completely clustered by feedlot
Grain processing method*			X		X	Completely clustered by feedlot, correlated with grain type

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

* Not used in any analyses

3.11 Statistical analyses

3.11.1 Descriptive Analyses

3.11.1.1 BRD incidence risks

The BRD incidence risk was defined as the percentage of cattle in the population that were pulled and whose first pull was for BRD. BRD incidence risks were calculated for the study population, and by feedlot and cohort using the following equations:

$$BRD\ incidence\ risk = \frac{No.\ cattle\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ study\ population} * 100$$

$$BRD\ incidence\ risk\ for\ feedlot =$$

$$\frac{No.\ cattle\ in\ feedlot\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ feedlot} * 100$$

$$BRD\ incidence\ risk\ for\ cohort =$$

$$\frac{No.\ cattle\ in\ cohort\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ cohort} * 100$$

BRD incidence risks by day 50 were calculated for the study population, feedlot and cohort using the following equations:

$$BRD\ incidence\ risk\ by\ day\ 50 =$$

$$\frac{No.\ cattle\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ study\ population} * 100$$

$$BRD\ incidence\ risk\ for\ feedlot\ by\ day\ 50 =$$

$$\frac{No.\ cattle\ in\ feedlot\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ feedlot} * 100$$

$$BRD\ incidence\ risk\ for\ cohort\ by\ day\ 50 =$$

$$\frac{No.\ cattle\ in\ cohort\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ cohort} * 100$$

BRD incidence risks over time were calculated for cohorts for a range of different time periods. The time periods, in days from the start of time at risk, were: days 1 to 10, days 1 to 20, days 1 to 30, days 1 to 40, days 1 to 50, day 1 to the end of time at risk, days 11 to 20, days 21 to 30, days 31 to 40, days 41 to 50, day 50 to the end of time at risk. These incidence risks were calculated separately for all study cohorts and for cohorts with at least 20 BRD cases using the following equation:

$$BRD\ incidence\ risk\ for\ cohort\ in\ time\ period =$$

$$\frac{No.\ cattle\ in\ cohort\ first\ pulled\ within\ the\ time\ period\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ cohort} * 100$$

The proportion of the BRD incidence risk for each cohort that occurred in different time periods was calculated using the following time periods in days from the start of time at risk: days 1 to 10, days 11 to 20, days 21 to 30, days 31 to 40, days 41 to 50, day 50 to the end of time at risk. These incidence risks were calculated separately for all study cohorts with non-zero incidence risk and for cohorts with at least 20 BRD cases using the following equation:

$$Proportion\ of\ BRD\ incidence\ risk\ for\ cohort\ in\ time\ period =$$

$$\frac{No.\ cattle\ in\ cohort\ first\ pulled\ within\ the\ time\ period\ whose\ first\ pull\ was\ for\ BRD}{No.\ cattle\ in\ cohort\ that\ were\ pulled\ at\ any\ time\ and\ whose\ first\ pull\ was\ for\ BRD} * 100$$

3.11.1.2 BRD incidence rates

The BRD incidence rate was defined as the number of cattle in the population that were pulled whose first pull was for BRD per 1000 animal-days at risk where each animal contributed animal-days at risk from day 1 until it left the study cohort (i.e. it exited the feedlot, was first pulled, was transferred to another pen or died). BRD incidence rates were calculated for the study population, and by feedlot and cohort using the following equations:

$$BRD\ incidence\ rate =$$

$$\frac{No.\ cattle\ whose\ first\ pull\ was\ for\ BRD}{Total\ number\ of\ animal\text{-}days\ at\ risk\ for\ all\ cattle\ in\ the\ study\ population} * 1000$$

$$BRD\ incidence\ rate\ for\ feedlot =$$

$$\frac{No.\ cattle\ in\ feedlot\ whose\ first\ pull\ was\ for\ BRD}{Total\ number\ of\ animal\text{-}days\ at\ risk\ for\ all\ cattle\ in\ the\ feedlot} * 1000$$

$$BRD\ incidence\ rate\ for\ cohort =$$

$$\frac{No.\ cattle\ in\ cohort\ whose\ first\ pull\ was\ for\ BRD}{Total\ number\ of\ animal\text{-}days\ at\ risk\ for\ all\ cattle\ in\ the\ cohort} * 1000$$

BRD incidence rates by day 50 were calculated per 1000 animal-days at risk where each animal contributed animal-days at risk from day 1 until the sooner of day 50 or when it left the study cohort (i.e it exited the feedlot, was first pulled, was transferred to another pen or died). Incidence rates by day 50 were calculated for the study population, and by feedlot and cohort using the following equations:

$$BRD\ incidence\ rate\ by\ day\ 50 =$$

$$\frac{No.\ cattle\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD}{Total\ number\ of\ animal\text{-}days\ at\ risk\ prior\ to\ day\ 50\ for\ all\ cattle\ in\ the\ study} * 1000$$

$$BRD\ incidence\ rate\ for\ feedlot\ by\ day\ 50 =$$

$$\frac{No.\ cattle\ in\ feedlot\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD}{Total\ number\ of\ animal\text{-}days\ at\ risk\ prior\ to\ day\ 50\ for\ all\ cattle\ in\ the\ feedlot} * 1000$$

$$BRD\ incidence\ rate\ for\ cohort\ by\ day\ 50 =$$

$$\frac{No.\,cattle\ in\ cohort\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD}{Total\ number\ of\ animal\text{-}days\ at\ risk\ prior\ to\ day\ 50\ for\ all\ cattle\ in\ the\ cohort} * 1000$$

3.11.1.3 Case fatality risks

Case fatality risk for BRD pulls was defined as the percentage of cattle that met the BRD case definition when first pulled and the pull occurred within the first 50 days of the start of time at risk, which subsequently died for any reason within 50 days of the pull. Animals either died as a direct result of BRD or another condition, or were euthanased. The time frame for death was constrained to 50 days after the pull as it was likely that BRD was an important contributor to most deaths within this period but less so for later deaths. The time frame for the first pull was constrained to the first 50 days after the start of time at risk as some later pulls would not have remained in the feedlot for the full follow-up period of 50 days from first pull. Neither cattle that were pulled for BRD subsequent to a pull for another reason nor pen deaths were included. Deaths for all reasons were included as it was assumed that these cattle would not have died had they not been pulled for BRD in the first instance, and because it was not possible to accurately determine reasons for death.

Thus, case fatality risks were calculated for the study population, and by feedlot and cohort using the following equations:

$$Case\ fatality\ risk =$$

$$\frac{No.\,cattle\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD\ \&\ died\ within\ 50\ days\ of\ pull}{No.\,cattle\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD} * 100$$

$$Case\ fatality\ risk\ for\ feedlot =$$

$$\frac{No.\,cattle\ in\ feedlot\ pulled\ by\ day\ 50\ whose\ 1st\ pull\ was\ for\ BRD\ \&\ died\ within\ 50\ days\ of\ pull}{No.\,cattle\ in\ feedlot\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD} * 100$$

$$Case\ fatality\ risk\ for\ cohort =$$

$$\frac{No.\,cattle\ in\ cohort\ pulled\ by\ day\ 50\ whose\ 1st\ pull\ was\ for\ BRD\ \&\ died\ within\ 50\ days\ of\ pull}{No.\,cattle\ in\ cohort\ pulled\ by\ day\ 50\ whose\ first\ pull\ was\ for\ BRD} * 100$$

3.11.1.4 BRD mortality incidence risks

The BRD mortality incidence risk was defined as the percentage of cattle in the population that died from BRD using the definition in Section 3.7.2. BRD mortality incidence risks were calculated for the study population, and by feedlot and cohort using the following equations:

$$BRD\ mortality\ incidence\ risk = \frac{No.\,cattle\ that\ died\ from\ BRD}{No.\,cattle\ in\ study\ population} * 100$$

$$BRD\ mortality\ incidence\ risk\ for\ feedlot = \frac{No.\ cattle\ in\ feedlot\ that\ died\ from\ BRD}{No.\ cattle\ in\ feedlot} * 100$$

$$BRD\ mortality\ incidence\ risk\ for\ cohort = \frac{No.\ cattle\ in\ cohort\ that\ died\ from\ BRD}{No.\ cattle\ in\ cohort} * 100$$

3.11.1.5 Box plots

Horizontal box plots were used to display the distribution of BRD incidence risks and rates, case fatality risks and BRD mortality incidence risks for cohorts within feedlots. The lengths of the different parts of the box indicate the degree of variation in risk or rate between cohorts within feedlots. The central line in the box is the median (50th percentile or middle) value. The left-hand edge of the box is the 25th percentile value (25% of values recorded were less than this value) and the right-hand edge is the 75th percentile value (25% of values recorded were higher than this value). The extremities of the whiskers represent the upper and lower adjacent values (the most extreme values within 1.5 times the interquartile range of the nearer quartile). Values that are numerically quite different to the other values recorded (outliers) are represented with circles.

3.11.1.6 Epidemic curves

Epidemic curves for BRD cases can be produced in three ways: the time variable (the x-axis) can be the number of days from the start of time at risk (day 0), the cohort open date or the cohort close date. For a particular cohort, these three curves would differ if day 0 differed among cattle in the cohort. In this case, the first approach would group all BRD cases pulled on the same number of days after day 0 although these cattle may have been pulled on different calendar days whereas the other two curves group all BRD cases pulled on the same calendar day although these cattle may have been at risk for differing numbers of days. Epidemic curves were produced using each method, but most of those presented use the number of days from the start of time at risk. Epidemic curves show only numbers of animals whose first pull was for BRD.

3.11.1.7 Distributions of exposure variables and crude incidence risk

Descriptive results relating to exposure variables are presented in Section 4.2. Tables in Section 4.2 detail the distribution of animals by category (number and percentage) and the crude 50-day BRD incidence risks for each category of each exposure variable. For the serological variables, weighted seroprevalences are presented in these tables. These were calculated based on the percentages of eligible animals that were selected either as cases or controls. Of 28,081 animals that met the eligibility criteria to be selected either as a case or a control, 84% were eligible as controls and 16% were eligible as cases. Cases were over-represented in the case-control dataset because equal numbers of cases and controls were included. Thus, to describe the approximate distributions of serological statuses for the entire population, weighted seroprevalences for the serology results were calculated. Weighted seroprevalences were calculated using the following equation:

$$Weighted\ seroprevalence$$

$$= 0.84 * seroprevalence\ in\ controls + 0.16 * seroprevalence\ in\ cases$$

3.11.2 Overview of modelling methods

Effects of exposure variables on BRD risk were assessed using a causal-diagram informed approach (Section 3.11.4) to estimate total and direct effects (Section 3.11.5).

Total and direct effects can be conceptualised by considering the possible causal mechanism or "pathways" for a putative risk factor. A single risk factor can affect the risk of BRD through multiple pathways. For example, if cohort size affects the risk of BRD (with animals in larger cohorts at greater risk), this could be because larger cohorts are more likely to contain BVDV PI animals and these animals infect others in the cohort with BVDV, increasing their risk of BRD. However cohort size could also affect BRD risk in other ways. Suppose animals in larger cohorts have less bunk space per animal and higher pen densities. (This would occur, for example, if cohorts are sometimes small because pens are only partly full.) Then, cohort size may also affect BRD risk through changes in bunk space per animal and higher pen densities.) Cohort size may also "directly" affect BRD risk i.e. no intervening variable between cohort size and BRD risk is defined. From this example, the total effect of cohort size would be the sum of the effects via each of presence/absence of BVDV PI animals in the cohort, bunk space, pen densities, and the direct effect. In contrast, the direct effect is simply the effect described by that pathway.

From a practical point of view, the total effect is usually of greater interest. Using the above example, assuming relationships are causal and estimates are unbiased, and disregarding sampling variation, the total effect estimate describes the expected change in BRD risk if cohort size was changed by the specified amount. This is generally of primary interest because this is what a feedlot manager would want to know when assessing whether to alter cohort size.

For a small number of exposure variables, the direct effect is also of practical interest. For these variables, the reason for this is described and both total and direct effect estimates reported. Total and direct effects are discussed further in Section 3.11.5.

In contrast to total and direct effects approach, "traditional" automated variable selection to develop the parsimonious multivariable models are commonly used in veterinary epidemiology. The problems of "traditional"automated variable selection methods have been well-demonstrated.[18, 19, 20] Estimates of effects for variables

[18] Hurvich, C.M., Tsai, C.L., 1990. The impact of model selection on inference in linear regression. American Statistician 44, 214-217.

[19] Derksen, S., Keselman, H.J., 1992. Backward, forward and stepwise automated subset-selection algorithms - Frequency of obtaining authentic and noise variables. British Journal of Mathematical & Statistical Psychology 45, 265-282.

included in such models may be total, direct or partial. For this reason, and to minimise bias in effect estimates for variables of interest, Westreich and Greenland (2013) specifically recommend the use of separate models based on pathways in a plausible causal diagram to obtain total effect estimates for variables of interest and to repeat this process to obtain direct effect estimates when they are specifically required. [21]

Population effects of exposure variables were also assessed (Section 3.11.6). These estimated the change in BRD incidence across the entire population should exposure to a particular risk factor be removed. Consider a risk factor with large total effect to which a high proportion of animals are exposed. If exposure to this risk factor was removed, BRD incidence would be expected to decline markedly i.e. the risk factor would have a large population effect. In contrast, a risk factor with large total effect to which only a small proportion of animals are exposed will have only a small population effect.

Variance components were assessed after fitting a parsimonious multivariable model (Section 3.11.7). “Traditional” automated variable selection was used to develop this model (Section 3.11.7).

The individual animal was the unit of analysis for all models. As the outcome of interest for these models was binary (the animal either acquired BRD at least once by day 50 or did not acquire BRD by day 50), all models were fitted using logistic regression. The primary estimates of interest from such models are the ß-coefficients. When exponentiated these coefficients provide estimated odds ratios. Multilevel models were used to account for the clustering due to the hierarchical structure of the data (Section 3.3.4). Models with four levels were fitted where possible. These models included feedlot, cohort and group-13 as hierarchical random effects. In some instances four-level models could not be fitted so three-level models were fitted, with only feedlot and cohort fitted as random effects.

Most models were fitted using MLwiN® (Version 2.27) run from within Stata® using the runmlwin program.[22] This program facilitates the transfer of data between the two software packages and enabled the use of the more flexible multilevel modelling procedures in MLwiN and the wide range of post-estimation commands available in Stata®. For each model, estimates were first determined by fitting the model using second-order penalised quasi-likelihood methods. Estimates from models fitted in

[20] Steyerberg, E.W., Eijkemans, M.J.C., Habbema, J.D.F., 1999. Stepwise selection in small data sets: A simulation study of bias in logistic regression analysis. J. Clin. Epidemiol. 52, 935-942.

[21] Westreich, D., Greenland, S., 2013. The Table 2 Fallacy: Presenting and Interpreting Confounder and Modifier Coefficients. Am J Epidemiol 177, 292-298.

[22] Leckie, G., Charlton, C., 2013. runmlwin - A Program to Run the MLwiN Multilevel Modelling Software from within Stata. Journal of Statistical Software 52, 40.

this manner can be biased but these models can be fitted very quickly and estimates were of adequate quality for screening purposes.

To obtain more accurate estimates, and to obtain posterior probability distributions, all models of interest were re-run using Markov Chain Monte Carlo (MCMC) estimation with the MLwiN default priors and with the values from the models fitted using penalised quasi likelihood methods as starting values. This method enabled multilevel models to be fitted where alternative likelihood-based approaches do not fit. Such models were run for many iterations; with each iteration an estimate of the value of the parameter of interest was produced. These estimates provide a posterior distribution for each parameter value. The mean of this distribution was exponentiated to give the point estimate for the odds ratio, and the 2.5^{th} and 97.5^{th} percentiles were exponentiated to give the 95% credible interval, a range which includes the posterior results from 95% of all iterations.

MCMC models were initially run for 10,000 iterations after a burn-in of 500. The diagnostic output was then inspected (Section 3.11.3) and further chains were run using the estimates from the first 10,000 iterations as starting values. Diagnostics were reassessed and models re-run with more iterations if required. Convergence problems for some level 3 (cohort) or level 4 (feedlot) variables were addressed by re-categorising the variable of interest or re-parameterising the model. Techniques such as hierarchical centring and orthogonalisation improved convergence for these models.

3.11.3 Model diagnostics

If a model has not converged, the estimates are not reliable. Convergence in MCMC models must be assessed by the analyst by considering a number of model diagnostics. Models converge to a probability distribution rather than a single point estimate and the Rafferty Lewis diagnostic gives the estimated number of iterations required to provide accurate estimates for the 95% credible interval. Other standard diagnostics for MCMC models fitted using MLwiN® were also inspected to assess convergence for each MCMC model (time series plot of posterior predicted values, kernel density plot, autocorrelation and partial autocorrelation factors, estimated sample size, Monte Carlo standard error and Brooks Draper diagnostics).

The deviance information criterion (DIC), which gives an overall measure of model fit and complexity, was obtained and used to compare models. This comparison is only valid when both models are fitted using the same observations. A lower DIC indicates a better trade of between model fit and complexity, although small variations can occur due to the stochastic nature of the process. A difference of three is generally used for model selection.[23]

[23] 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.

3.11.4 Causal diagram

Causal diagrams have not been used extensively in veterinary studies, although Dohoo strongly recommends their use as part of model building strategies.[24] However, they have been increasing used in assessing causal relationships over the last 20 years and use is likely to increase with recent texts in epidemiology and causal analysis devoting chapters specifically to them.[25, 26] They have been used to inform multivariable models in numerous studies of risk factors for health disorders in humans. Work is continuing to improve the methodology, for example, through improved conceptualisation of interaction.[27, 28]

A causal diagram was developed with postulated interrelationships between proposed direct and indirect causes of BRD (Figure 3.7). This diagram visually depicts all proposed causal pathways between exposure variables of interest and between these and BRD. Some of the variables included in Figure 3.7 were assessed in subset analyses; those used in the vendor questionnaire subset are within ellipses and those used in the case-control analyses are within boxes.

Each arrow in the causal diagram depicts a hypothesised causal pathway in which one variable (the variable from which the arrow starts) might at least partly determine 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). As described in Section 3.11.2, direct pathways are those where the variables are linked by an arrow that passes directly from one to the other. A variable with a pathway directly to BRD depicts a direct effect of that variable. 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 one particular variable to any other particular variable. Effects mediated in this way are known as indirect effects. 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

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[24] Dohoo, I., Martin, W., Stryhn, H., 2010. Veterinary Epidemiologic Research. VER Inc Charlottetown, Canada.

[25] Law, G.R., Green, R., Ellison, G.T.H., 2012. Confounding and Causal Path Diagrams In: Tu, Y.-K., Greenwood, D.C. (Eds.), Modern Methods for Epidemiology. Springer, London.

[26] Elwert, F., 2013. Graphical Causal Models. In: Morgan, S.L. (Ed.), Handbook of Causal Analysis for Social Research. Springer, Dordrecht.

[27] VanderWeele, T.J., Robins, J.M., 2007. Four types of effect modification - A classification based on directed acyclic graphs. Epidemiology 18, 561-568.

[28] Weinberg, C.R., ibid.Can DAGs clarify effect modification? , 569-572.

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 3.7. However, feedlot personnel may decide first to feed a cohort with animals in a particular intended days on feed category, so would then chose 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 (Section 3.11.5). 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.[29] Accordingly, some pathways that were biologically plausible but for which there was little other evidence were included.

[29] Textor, J., 2013. Drawing and Analyzing Causal DAGs with DAGitty User Manual for Version 2.0. http://www.dagitty.net/manual-2.x.pdf.

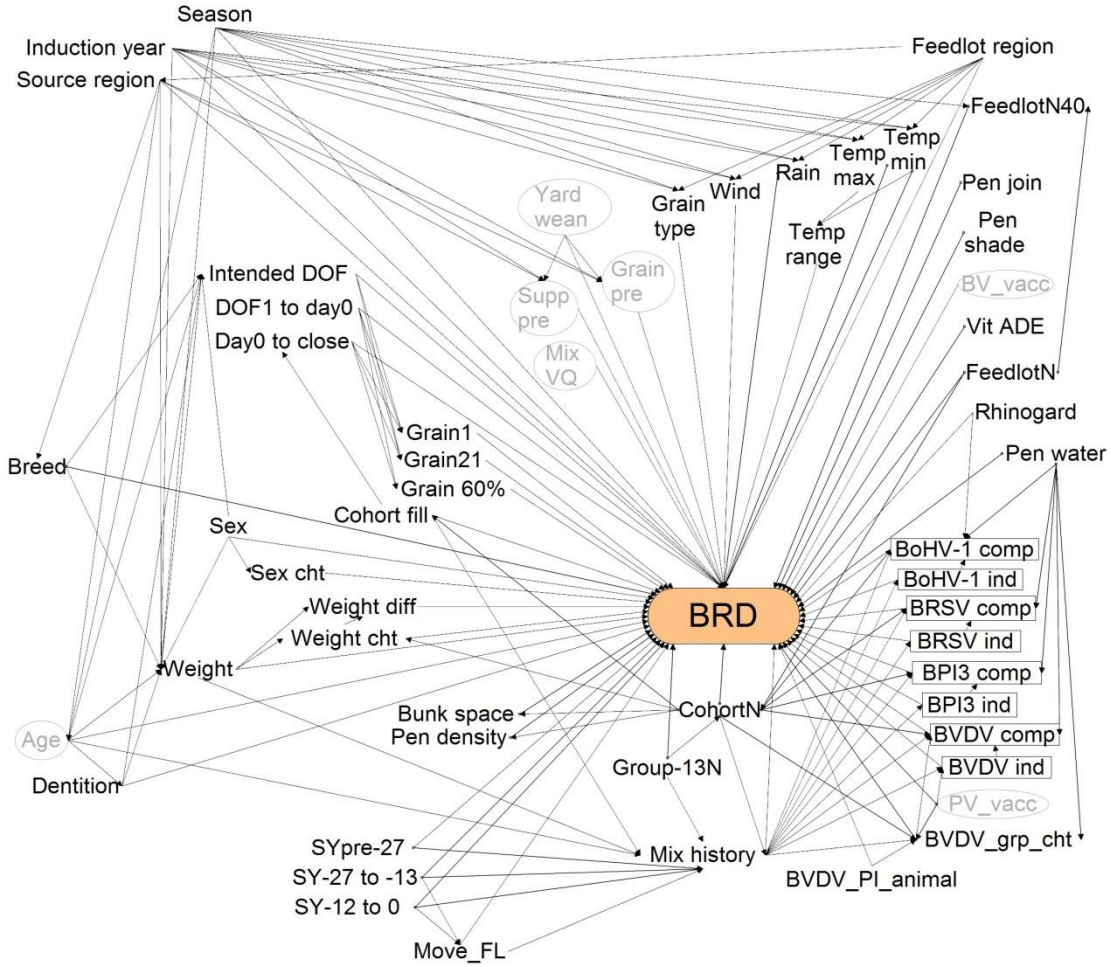

Figure 3.7: Causal diagram based on *a priori* knowledge and biologically plausible pathways interlinking the measured exposure variables with each other, as appropriate, and with the occurrence of BRD within the first 50 days of the start of time at risk. Variables included only in the vendor questionnaire subsets are enclosed in ellipses and those only included in the case-control subset are shown in boxes. For each virus, all change in serostatus variables have equivalent locations in the causal diagram. For example the location of BVDVseroinc and BVDVseroconv is the same as BVDV comp. A complete list of abbreviations of variables in this diagram is provided in the preliminary pages of this Report.

3.11.5 Total and direct effects

Models were fitted to estimate total effects for all exposure variables of interest. Models were also fitted to estimate direct effects for some exposure variables where this effect was also of particular interest.

To obtain correct estimates for the total effects for an exposure variable of interest, all confounding variables must be fitted but none of the intervening variables between the exposure variable of interest and BRD should be fitted. If confounders are not included, the estimate will be biased, and if one or more intervening variables are included, effects mediated via that indirect pathway to BRD will be removed so only a partial rather than total effect will be estimated. Identification of which variables might be possible confounders is complicated by conditional association (see below). Therefore selection of which variables to include in any model requires close inspection of the causal diagram.

Intervening variables can be identified from a causal diagram as they lie on a front door path between the exposure of interest and the outcome. A front door path is one that goes forwards from the exposure of interest to the outcome, so both direct and indirect paths are front door paths. For example, in Figure 3.7, considering the effect of sex on BRD, the sex of the cohort (Sex cht) is an intervening variable as it lies on a front door path from sex to BRD. Confounding variables can be identified as those which lie on a back door path between the exposure of interest and the outcome. A back door path is one that goes backwards one arrow from the exposure of interest (from the head backwards to the tail of the arrow) and then proceeds forwards to the outcome either directly or via one or more intervening variables. For example, considering the effect of induction weight (Weight) on BRD there is a back door path from induction weight to BRD via breed and another via breed and the difference between an animal's induction weight and the mean cohort weight (Weight diff). Each of the back door paths must be blocked by including a variable from the path in the model. Conditional association is induced if a variable is included in the model and it, in turn, is caused by two or more variables; conditional associations would occur between these latter variables. If any of these conditional associations open up other back door pathways then these also need to be blocked.

A set of all covariates that need to be included is known as a sufficient adjustment set. A minimal sufficient adjustment set is a sufficient adjustment set that would no longer be sufficient if any variable were removed. For example, the minimal sufficient adjustment set to estimate the total effect of intended days on feed consists of breed, induction weight, sex and dentition as these four variables block all back door paths to BRD. In this case, each variable blocks more than one path. For example, sex blocks the pathway "intended days on feed to sex to BRD" and the pathway "intended days on feed to sex to sex of the cohort (Sex cht) to BRD". If any of these variables (breed, induction weight, sex or dentition) were removed, one or more back door paths would be opened. In some circumstances, there may be more than one minimal sufficient adjustment set for a single variable. For example both of the following sets (breed, induction year, season, sex, dentition and source region) and (breed, grain type, rain, season, sex, maximum temperature, minimum temperature, wind, dentition and source region) are minimal sufficient adjustment sets for induction weight.

To obtain correct estimates for the direct effects for an exposure variable of interest all confounding variables must be fitted and sufficient intervening variables should be fitted such that all front door paths other than the direct pathway to BRD are blocked. If this process opens up additional back door paths, they too must be blocked. For example, the minimal sufficient adjustment set to estimate the direct effect of a saleyard transfer prior to day -27 is Mix history, Cohort fill, CohortN, Weight, SY-12 to day 0, SY-27 to -13, Group-13N and Move_FL. The addition of the single intervening variable (Mix history) opens up multiple back door paths which are blocked by the addition of the other variables in the set.

3.11.5.1 DAGitty

Manually identifying minimal sufficient adjustment sets to estimate the total and direct effects of the exposures of interest would have been prone to error given the complexity of the causal diagram. Instead, this was done using the DAGitty®

software which uses a series of algorithms to automate the process described above.[30]

The causal diagram was reproduced within the DAGitty web interface.[31] Each exposure variable of interest was sequentially identified as the exposure of interest and the variables listed in the minimal sufficient adjustment sets were copied into a spreadsheet. When assessing two-way interactions, both variables that contributed to the interaction term were simultaneously selected as exposure variables and the minimal sufficient adjustment sets identified in the same manner.

3.11.5.2 Modelling methodology

Separate multilevel models were fitted to estimate the total effects of each exposure variable as described in Section 3.11.2 using each minimal sufficient adjustment set. Direct effects of particular exposure variables of interest were also estimated with separate models for each variable. In a small number of instances, the models failed to converge with the more complex adjustment sets so these models were not reported.

3.11.5.3 Interactions

Two-way interaction terms investigated included those that were specified *a priori* based on prior literature and industry interest as well as those that were considered biologically plausible; interactions were investigated only if there were important associations between both main effects and BRD. Total effects models were fitted using second-order penalised quasi-likelihood methods. MCMC models were only fitted as described above where the joint Wald p-value for the interaction terms was < 0.05. Odds ratios and 95% credible intervals for effects with interactions were derived using the lincom command in Stata® following model convergence. Although these estimates may differ slightly from estimates obtained by rerunning the MCMC models with different reference categories, the estimates were considered adequate for comparisons across categories and visualising the effects through graphs. These estimates were compiled in a spreadsheet and imported into Stata® to produce graphical displays to decide on meaningful interactions to report. In some instances, despite significant interaction terms, the estimates were hugely imprecise and not conducive to meaningful interpretation.

3.11.6 Population attributable fractions and population attributable risks

Odds ratios as discussed above describe the odds of BRD should an animal be exposed to the risk factor relative to if the animal was not exposed. Odds ratios can thus identify factors that make animals at increased (or decreased) risk. It is also of interest to estimate how BRD incidence in the population would change, should a particular risk factor be avoided or its effects on BRD prevented. The effect of a particular risk factor at the population level depends on the prevalence of exposure to

[30] Textor, J., Hardt, J., Knuppel, S., 2011. DAGitty A Graphical Tool for Analyzing Causal Diagrams. Epidemiology 22, 745-745.

[31] http://www.dagitty.net/dags.html#

the risk factor in the population as well as the strength of association between the risk factor and BRD. A strong association is clearly important for those individuals exposed to the risk factor. However, if very few individuals in the population are exposed, removing the risk factor, or preventing the effects of the risk factor, will have very little impact in the disease frequency in the population. In such a case, 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 that quantify the effects of risk factors for the population; these can be used to gauge the relative importance of risk factors in the population represented by the study population.

The PAF for a particular risk factor estimates the proportion of the incidence risk of the disease of interest in a population that is due to some animals in the population being exposed to the risk factor, assuming that exposure is causal.[32] It is based on the effect estimate and the prevalence of the exposure in the population, and so is biased if either of these is biased. The PAF for a particular risk factor may be interpreted as the proportional reduction in the incidence risk of BRD that would occur in the population if all animals in that population in the higher risk categories were replaced with otherwise identical animals but in the lowest risk category. PAFs for multiple risk factors may sum to more than one because various risk factors may contribute to BRD risk in part via the same pathways.

The PAR for a particular risk factor describes the amount of incidence risk of the disease of interest in a population that is due to some animals in the population being exposed to the risk factor, assuming that exposure is causal. The PAR for a particular risk factor may be interpreted as the reduction in the incidence risk of BRD that would occur in the population if all animals in that population in the higher risk categories were replaced with otherwise identical animals but in the lowest risk category. Incidence risk of BRD may not decline by the sum of PARs for multiple risk factors after all risk factors were removed from the population because various risk factors may contribute to BRD risk in part via the same pathways.

PAFs and PARs were only estimated for those risk factors for which the results from the cohort study indicated either a protective or an adverse effect on the risk of BRD. PAFs and PARs were not estimated for serological risk factors because these variables are not directly manipulable by cattle vendors or feedlot managers.

3.11.6.1 Estimation with MLwiN® models

The total effect models described in Section 3.11.5 were each re-fitted with the lowest risk category as the reference category. Relative risks were estimated from the odds ratios. The observed crude percentage of individuals in the reference category that became BRD cases was used to calculate the odds of individuals in this category being a BRD case. The adjusted odds for all other categories were then estimated by multiplying the adjusted odds ratio estimate from the relevant model by the odds of BRD for the reference category. The adjusted relative risk was then

[32] Dohoo, I., Martin, W., Stryhn, H., 2010. Veterinary Epidemiologic Research. VER Inc Charlottetown, Canada.

obtained by dividing the adjusted percentages of individuals that became BRD cases for the category by the percentage that became BRD cases in the reference category.

The PAF for each risk factor was then estimated using the proportions of all BRD cases that were in each category for the particular risk factor and the adjusted relative risks for each category compared to the reference group.[33] The PAR was then obtained by multiplying the PAF by the crude incidence risk of BRD by day 50 in the population (17.63 to 17.66% depending on the animals included in each model). This method gave point estimates for PAFs and PARs for variables of interest.

3.11.6.2 Estimation with WinBUGs® models

To obtain estimates of PAFs and PARs with associated measures of uncertainty due to random variation, the modelling process was repeated using WinBUGs® with non-informative priors. For each risk factor of interest, the total effects model from Section 3.11.6.1 was refitted and nodes programmed to estimate the adjusted percentages of cases, adjusted relative risk and partial PAF for each category, and the total PAF and PAR. Models were run for a minimum of 10,000 iterations after a burn-in of 1000. Model diagnostics used to assess for convergence were the same as those described in Section 3.11.3. Mean values for PAFs and PARs for the risk factors of interest and associated 95% credible intervals were derived from the associated posterior distributions.

3.11.7 Variance components models

Models were fitted to estimate the proportions of variance in BRD occurrence at different levels (animal, group-13, cohort and feedlot). After identifying a parsimonious set of risk factors that were each significantly associated with BRD, the proportions of total variance in that model that were unexplained in total and at each of these levels were calculated.

In view of the large number of putative risk factors, it was necessary to screen variables. A variation on the backwards elimination process was used to select a parsimonious group of variables from which to estimate the proportions of outcome variance explained by risk factors that were associated with BRD at the 0.1 level. Models were initially fitted in MLwiN® (Version 2.27) run from within Stata® using second-order penalised quasi-likelihood methods. Overall joint Wald p values for each exposure variable were used for model building. All variables with a p value from univariable screening < 0.2 were simultaneously fitted in a multivariable model. Overall p values for each exposure variable were calculated; these were recalculated after each change in covariates included in the multivariable model. Variables with p values > 0.1 were progressively eliminated, in descending order of p values, until all remaining variables had a p value < 0.1. Each variable that had been eliminated was then re-tested and if the overall p value for the variable was < 0.1, it re-entered the model. The resulting model was the final main effects model.

[33] Hanley, J.A., 2001. A heuristic approach to the formulas for population attributable fraction. J. Epidemiol. Community Health 55, 508-514.

Two-way interactions for all risk factors in the final main effects model were then tested; each was included in the final model if the overall p value for the interaction terms was < 0.05. The final main effects model and the model with significant interaction terms were then fitted using MCMC methods and the DIC values were compared. The final model contained two interaction terms and resulted in a reduction in DIC (by at least three) compared to the main effects model or equivalent models with only one interaction term. All MCMC models were assessed for convergence as described in Section 3.11.3.

A four level null model (i.e. with no risk factors) was then fitted using the same animals as used in the final models. This was used to identify the total amount of variance and the proportion of variance at each level before accounting for any risk factors. For both the null and the final model, the animal-level variance was fixed at $\pi^2/3$ (3.29). The explained variance from the final model and the variances at each of the levels from the null and final models were obtained. These were used to determine the proportion of total variance that was unexplained at each level for the null and final model and the proportion of variance explained by the final model.[34]

Although effect estimates from models with a parsimonious set of risk factors such as this final model are commonly reported in the literature as “the effects”, this approach was not used for the key estimates reported in this study. The effect estimates for variables from such parsimonious models may be the total, partial or direct effects depending on which other variables remain in the model, and the estimates may be biased due to incomplete control of confounders.[35]

3.12 Analyses

3.12.1 Full cohort dataset

This dataset was used to estimate the following:

- Descriptive statistics e.g. BRD incidence risks (cumulative incidence)
- Total effects for all risk factors excluding those requiring serological results.
- The effect of BVDV in a cohort and the presence of a BVDV-PI animal in a group-28
- Direct effects for specific risk factors where direct effects were of particular interest, excluding those requiring serological results.
- Population attributable fractions and population attributable risks for total and direct effects for risk factors of interest
- Proportion of total variance in the parsimonious model that was explained by risk factors that were significantly associated with BRD.
- Proportions of total variance in BRD occurrence that was unexplained at each level (animal, group-13, cohort and feedlot)

[34] Snijders, T., Bosker, R., 2012. Multilevel Analysis: An Introduction to Basic and Advanced Multilevel Modeling. SAGE Publications London.

[35] Westreich, D., Greenland, S., 2013. The Table 2 Fallacy: Presenting and Interpreting Confounder and Modifier Coefficients. Am J Epidemiol 177, 292-298.

3.12.1.1 Vendor questionnaire datasets

The entire vendor questionnaire dataset was used to estimate the total and direct effects of age. Subset 1 was used to estimate the total effects of yard weaning, on-property mixing and prior feeding with grain and conserved forage/supplement. Subset 2 was used to estimate the total effects of prior Bovilis MH™ and Pestigard™ vaccination.

3.12.1.2 Pre-assembly dataset

The pre-assembly dataset was used to evaluate the total effect of the timing of the move to the feedlot relative to induction.

3.12.2 Case-control dataset

This dataset was used for the following:

- To describe serostatuses at induction to BoHV-1, BVDV, BRSV, BPI3
- To describe incidences of change in serostatus to these agents between induction and day 42
- To estimate the total effect of risk factors based on serological results
- To evaluate the direct effect of shared pen water after accounting for serological change

Total effects were estimated as described above (Section 3.11.5) but with selection batch (whether the animal was selected for inclusion in the case-control study during the first or second selection process) and test batch forced into all models.

4 Results

4.1 Descriptive results from cohort study

4.1.1 General Summary

A total of 35,160 cattle derived from 1,077 group-13s and comprising 170 cohorts from 14 feedlots were enrolled in the study. Of these, 35,131 were included in the cohort study analyses. Five animals were ineligible because they died (N = 1) or were pulled (N = 4) on day 0. A further 24 animals were lost to follow-up. Numbers of cattle, group-13s and cohorts and variation in sizes of group-13s and cohorts by feedlot are shown in Table 4-1. Numbers of cattle, group-13s, cohorts and durations of enrolment period varied markedly among feedlots.

Table 4-1: Number of cattle enrolled in each participating feedlot, date range of enrolment and sizes of cohorts and group-13s by feedlot.

Feedlot	Enrolment date		No. animals	Group-13s				Cohorts			
	First	Last		No. of group-13s	No. animals per group-13			No. of cohorts	No. animals per cohort		
					Median	Min	Max		Median	Min	Max
1	Dec 2009	May 2010	633	35	10	1	141	4	156.5	141	179
2	Sep 2009	Nov 2011	5,364	189	17	1	342	19	350	63	350
3	May 2009	Apr 2010	539	24	11.5	1	100	5	105	75	145
4	Sep 2009	Dec 2011	6,114	262	15	1	137	22	272.5	105	395
5	Mar 2010	Sep 2011	2,193	77	5	1	239	17	127	56	239
6	Mar 2010	Sep 2010	466	3	157	149	160	3	157	149	160
7	Jun 2009	Dec 2011	2,999	87	29	1	130	21	90	80	285
8	Aug 2009	Dec 2011	2,982	56	32.5	1	180	20	148.5	130	180
9	Feb 2011	Nov 2011	2,569	38	43.5	1	241	14	229	17	241
10	Oct 2009	Dec 2011	5,616	212	18	1	165	18	355	62	355
11	Mar 2011	Nov 2011	1,536	41	14	1	181	9	184	87	252
12	Mar 2009	Jul 2009	500	5	129	8	186	3	163	129	208
13	Jul 2009	May 2011	1,927	12	180	1	280	8	273.5	180	280
14	Oct 2009	May 2011	1,693	36	38.5	1	244	7	240	229	250
Total	Mar 2009	Dec 2011	35,131	1,077	17	1	342	170	186	17	395

4.1.2 Feedlot distribution

The locations of Australian feedlots and participating feedlots are shown in Figure 4.1. The feedlots were located in four states: New South Wales (N = 7), Queensland (N = 5), South Australia (N = 1) and Western Australia (N = 1). The two regions where feedlot density is highest, the Riverina and the Darling Downs, contributed most feedlots, with six and five participating feedlots, respectively.

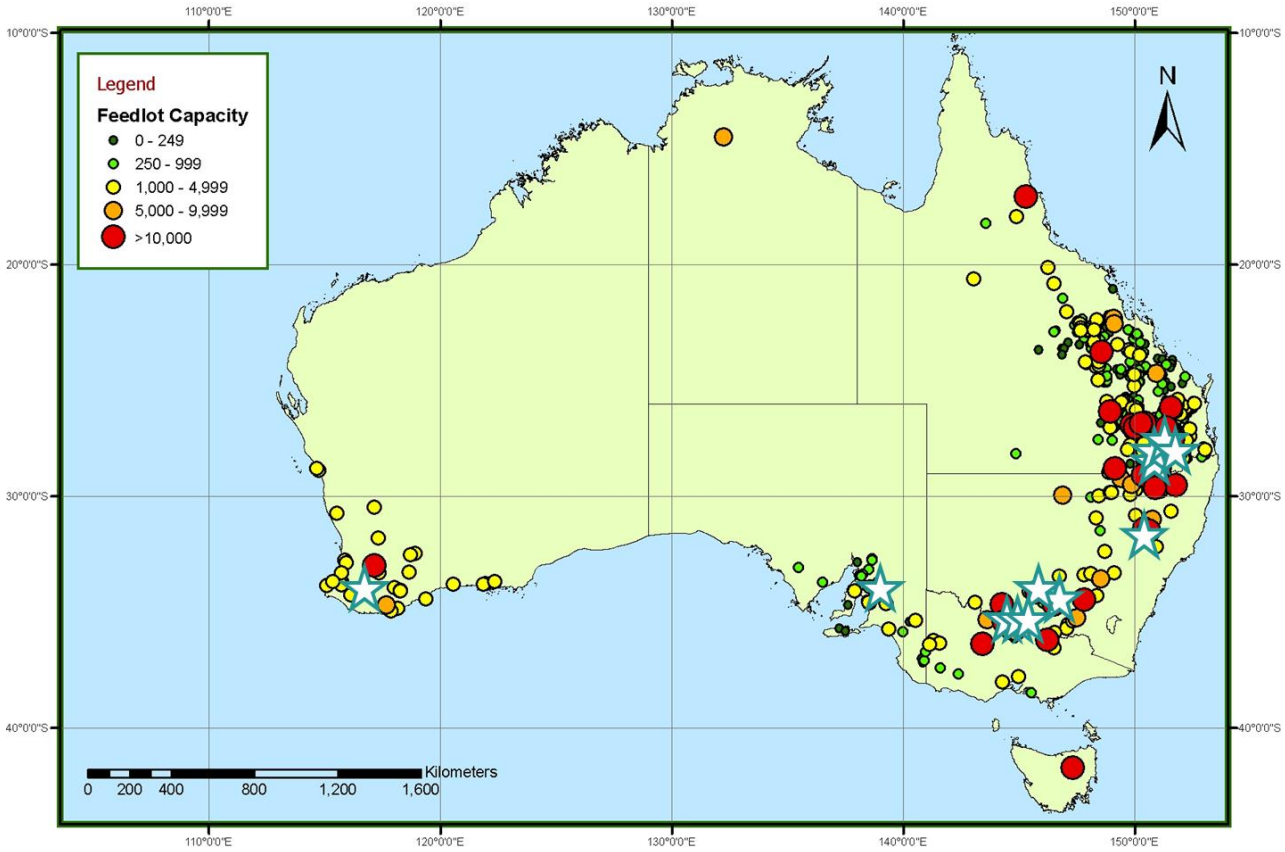

Figure 4.1: Distribution of accredited feedlots in Australia by size (Source: FSA Consulting, 2011) overlaid with the location of participating feedlots (white stars).

4.1.3 BRD cases

4.1.3.1 BRD incidence risk

In total, 23.6% (8,285/35,131) of study cattle were pulled at least once whilst on feed and 21.4% (7,517/35,131) were first pulled within the first 50 days at risk. BRD was the most common reason for pulling amongst first pulls. The BRD incidence risk (the percentage of cattle whose first pull was for BRD) was 18.2% (6,406/35,131) and the BRD incidence risk by day 50 was 17.7% (6,200/35,131, 96.8% of all BRD cases). Amongst first pulls, the proportional morbidity rate for BRD was 77.3% (6,406 animals with BRD at first pull/8,285 animals pulled at least once) overall and 82.5% (6,200/7,517) for the first 50 days at risk.

4.1.3.2 BRD incidence risks by feedlot and cohort

There was considerable variability among feedlots and among cohorts within feedlots in BRD incidence risk (the percentage of cattle whose first pull was for BRD, feedlot mean: 14.1%, median: 6.4%, range: 0.1 to 45.3%, cohort mean: 16.2%, median: 8.5%, range: 0.0 to 72.1%). Some feedlots had high variability at the cohort level whereas others had a consistently low BRD incidence risk (Figure 4.2). Cohort-level incidence risks were right-skewed, with most cohorts having a relatively low incidence risk and a small number of cohorts having a relatively high incidence risk (Figure 4.3).

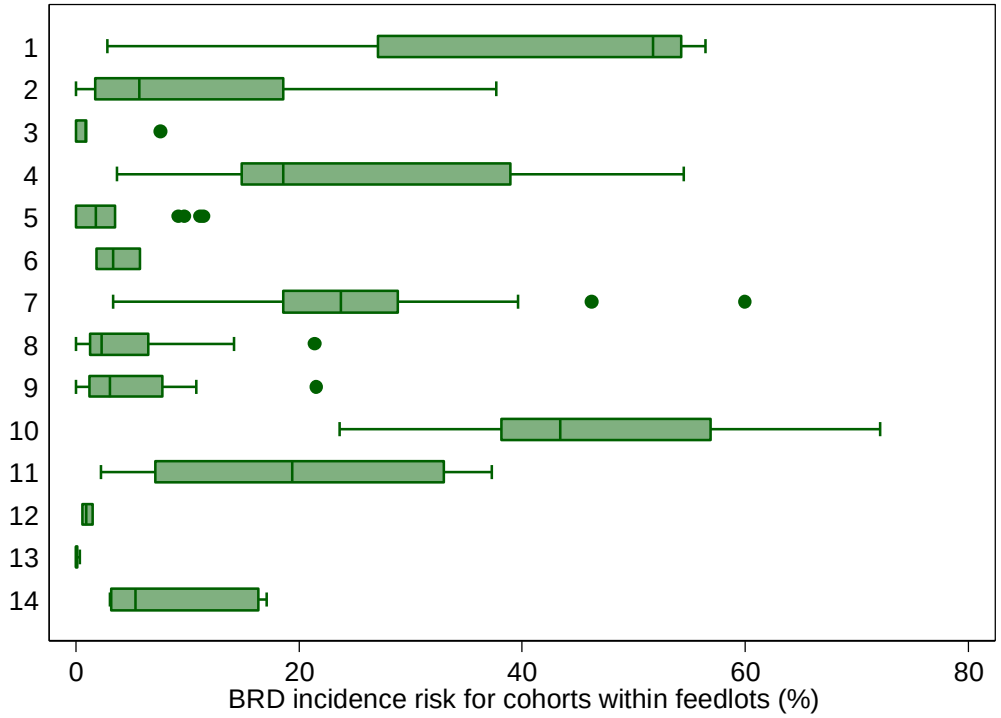

Figure 4.2: Distributions of BRD incidence risks for cohorts within feedlots. (Y-axis shows feedlot numbers)

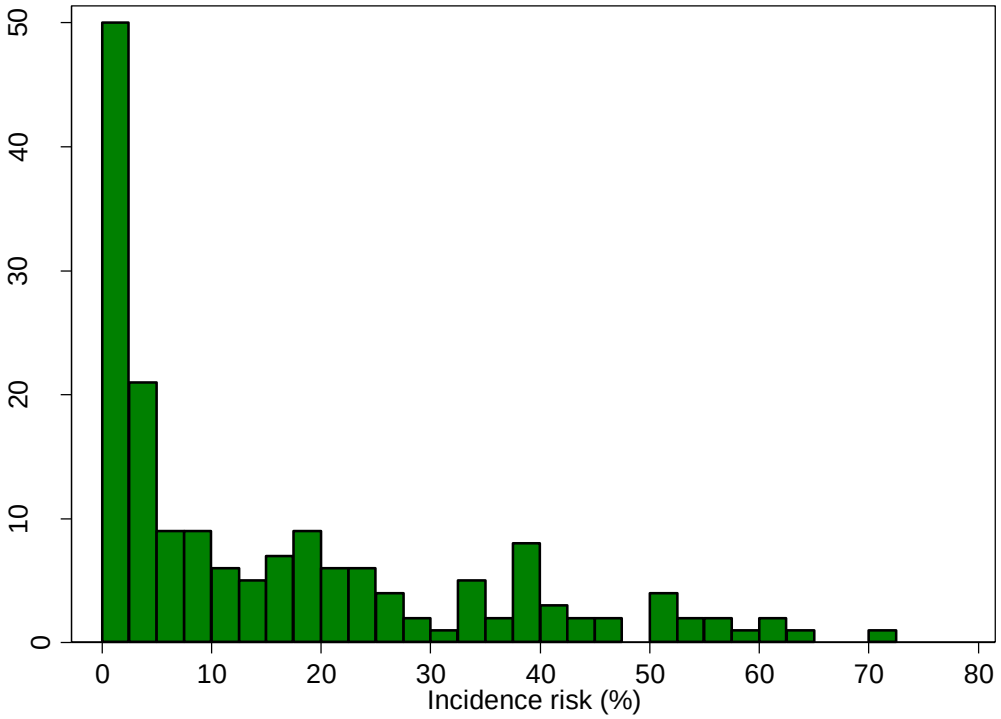

Figure 4.3: Distributions of cohort-level incidence risks.

4.1.3.3 BRD incidence risks by day 50 by feedlot and cohort

There was considerable variability among feedlots and among cohorts within feedlots in BRD incidence risk by day 50 (the percentage of cattle whose first pull was for BRD by day 50, feedlot mean: 13.6%, median: 5.6%, range: 0.1 to 44.9%, cohort mean: 15.4%, median: 7.7%, range: 0.0 to 72.1%). Some feedlots had high variability at the cohort level whereas others had a consistently low BRD incidence risk by day 50 (Figure 4.4).

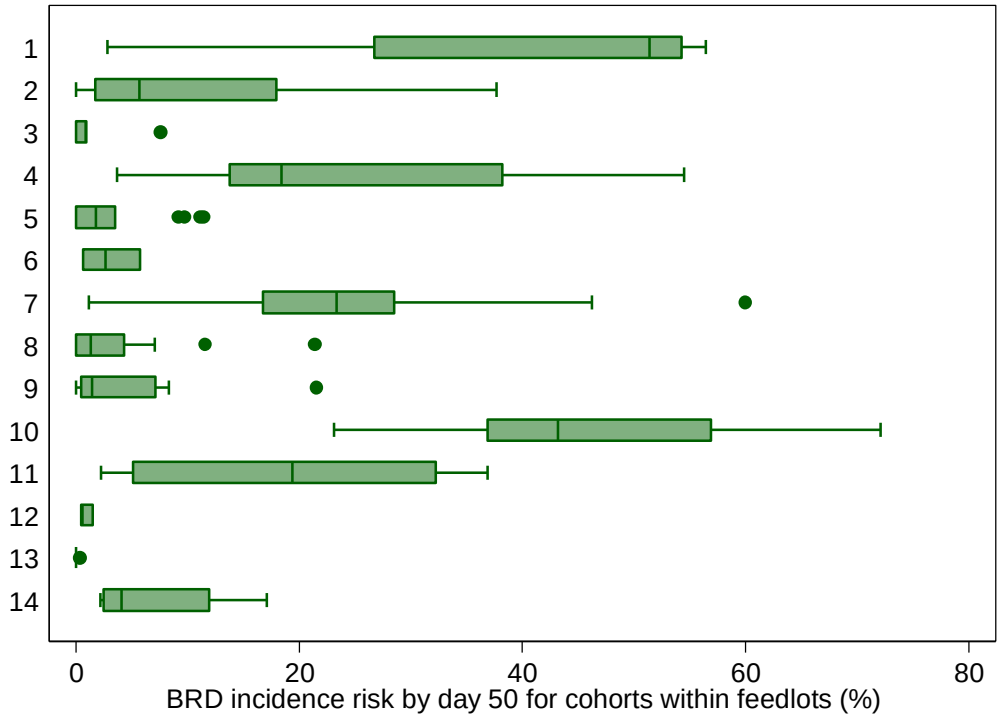

Figure 4.4: Distributions of BRD incidence risks by day 50 for cohorts within feedlots. (Y-axis shows feedlot numbers).

4.1.3.4 BRD incidence rates by feedlot and cohort

There was considerable variability among feedlots and among cohorts within feedlots in BRD incidence rate (the number of cattle of cattle whose first pull was for BRD per 1,000 animal-days at risk, feedlot mean: 1.5, median: 0.7, range: 0.01 to 6.4, cohort mean: 1.8, median: 0.8, range: 0.0 to 11.5). Some feedlots had high variability at the cohort level whereas others had a consistently low BRD incidence rate (Figure 4.5).

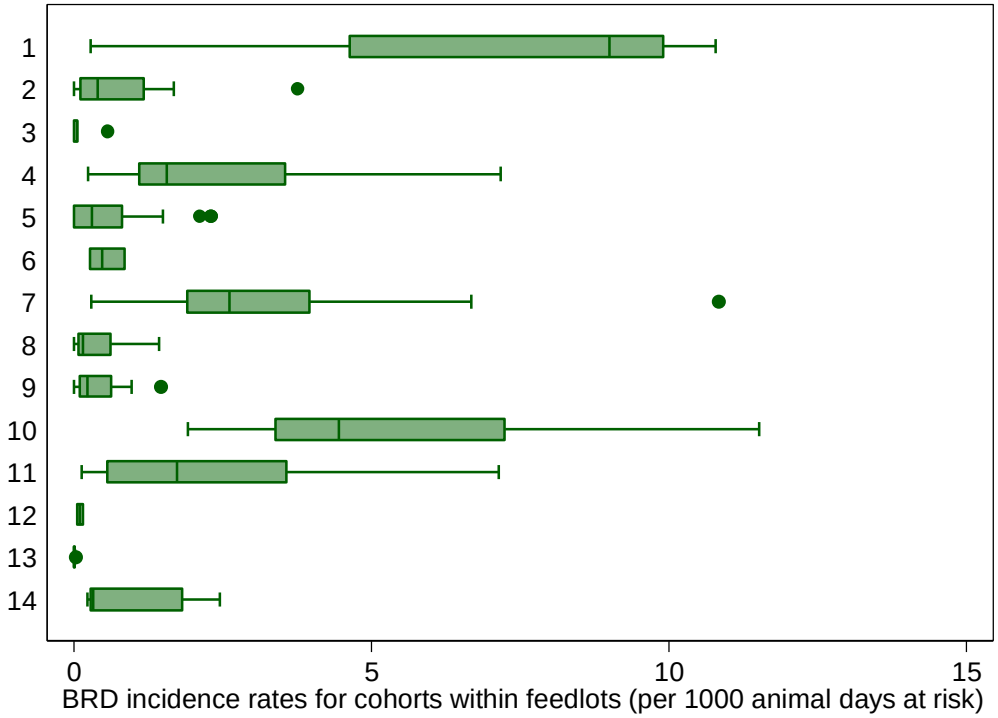

Figure 4.5: Distributions of BRD incidence rates (number of cattle whose first pull was for BRD/1,000 animal-days at risk) for cohorts within feedlots. (Y-axis shows feedlot numbers).

4.1.3.5 BRD incidence rates by day 50 by feedlot and cohort

There was considerable variability among feedlots and among cohorts within feedlots in BRD incidence rate by day 50 (the number of cattle whose first pull was for BRD by day 50 per 1,000 animal-days at risk, feedlot mean: 3.4, median: 1.2, range: 0.01 to 12.1, cohort mean: 4.0, median: 1.7, range: 0.0 to 25.6). Some feedlots had high variability at the cohort level whereas others had a consistently low BRD incidence rate (Figure 4.6).

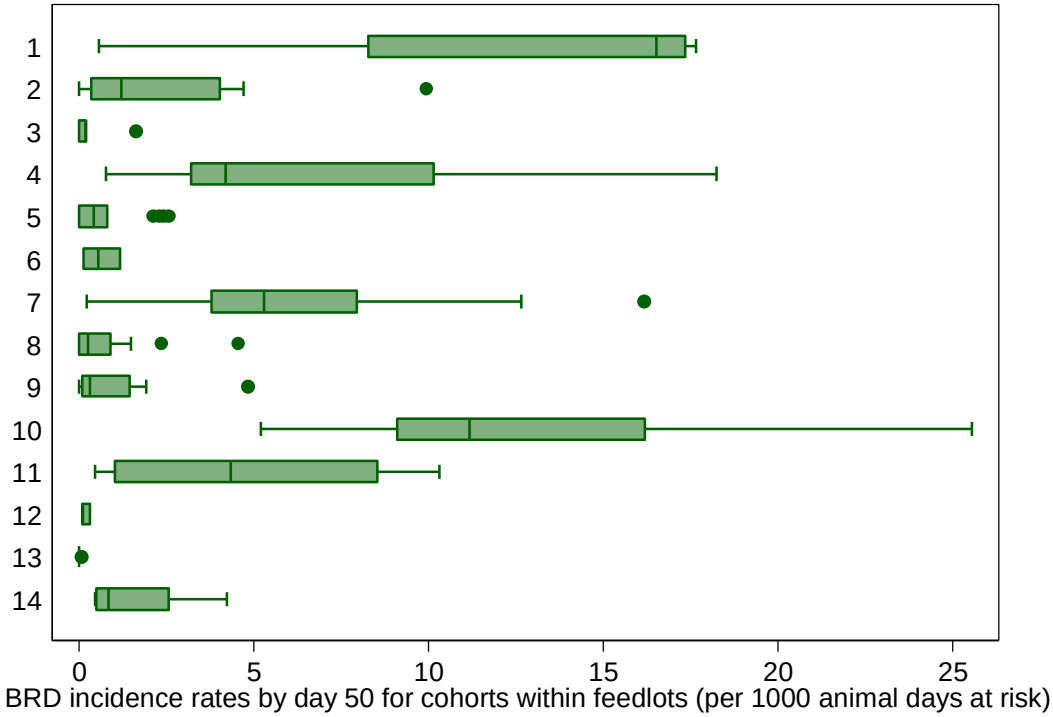

Figure 4.6: Distributions of BRD incidence rates (number of cattle number whose first pull was for BRD/1,000 animal-days at risk) by day 50 for cohorts within feedlots. (Y-axis shows feedlot numbers).

4.1.3.6 Epidemic curves

The epidemic curves for the whole study population are shown in Figure 4.7 (based on number of days from the start of time at risk, i.e. day 0), Figure 4.8 (based on the number of days from cohort open date) and Figure 4.9 (based on the number of days from cohort close date). BRD cases were most common from 15 to 30 days after the start of time at risk.

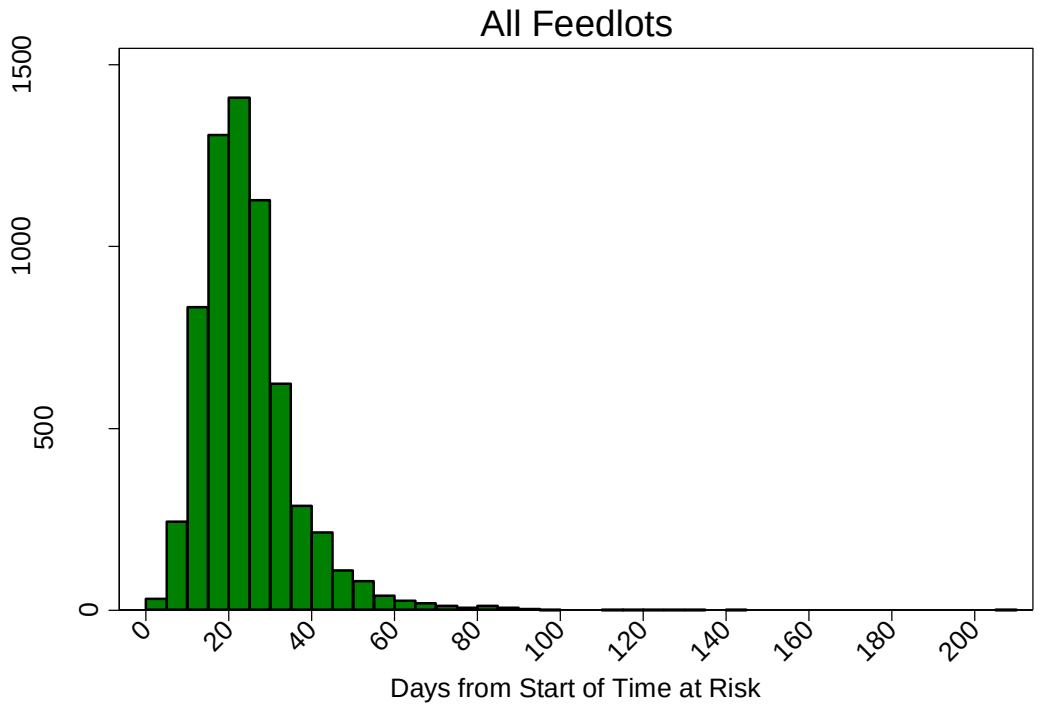

Figure 4.7: Epidemic curve for BRD using times from the start of time at risk (day 0) to first pull for all cattle in the study population whose first pull was for BRD (bin width = 5 days).

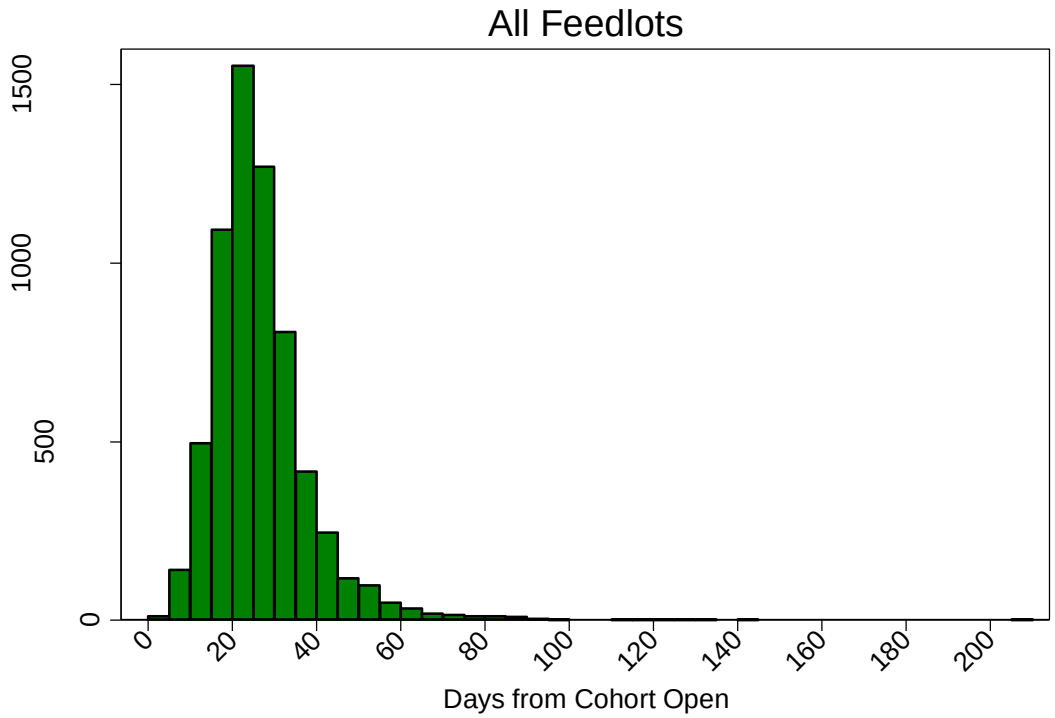

Figure 4.8: Epidemic curve for BRD using times from cohort open date to first pull for all cattle in the study population whose first pull was for BRD (bin width = 5 days).

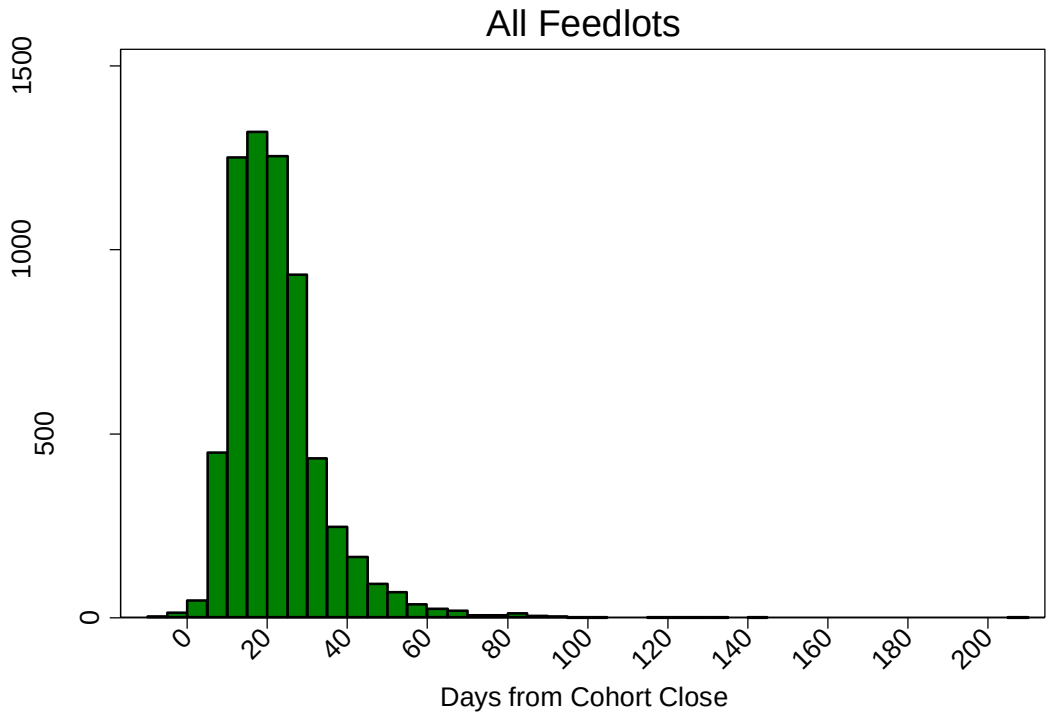

Figure 4.9: Epidemic curve for BRD using times from cohort close date to first pull for all cattle in the study population whose first pull was for BRD (bin width = 5 days).

Where there were sufficient BRD cases for a pattern to be apparent, the epidemic curves for individual cohorts typically showed a peak in the number of BRD cases of 5 to 15 days duration in the period from 15 to 30 days after the start of time at risk (Figure 4.10). There were a few cohorts where most BRD cases were either earlier (Figure 4.11) or later (Figure 4.12) than this or where BRD cases occurred over an extended period of time (Figure 4.13).

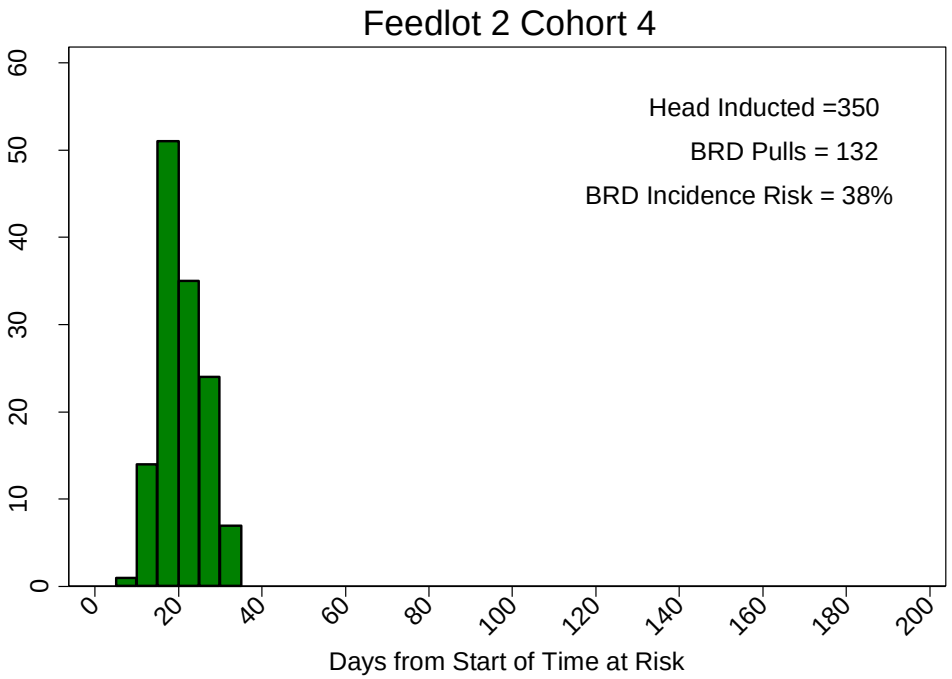

Figure 4.10: Example of a typical epidemic curve for BRD; the peak number of animals pulled for BRD was commonly between 15 and 30 days after the start of time at risk and 5 to 15 days in duration**.**

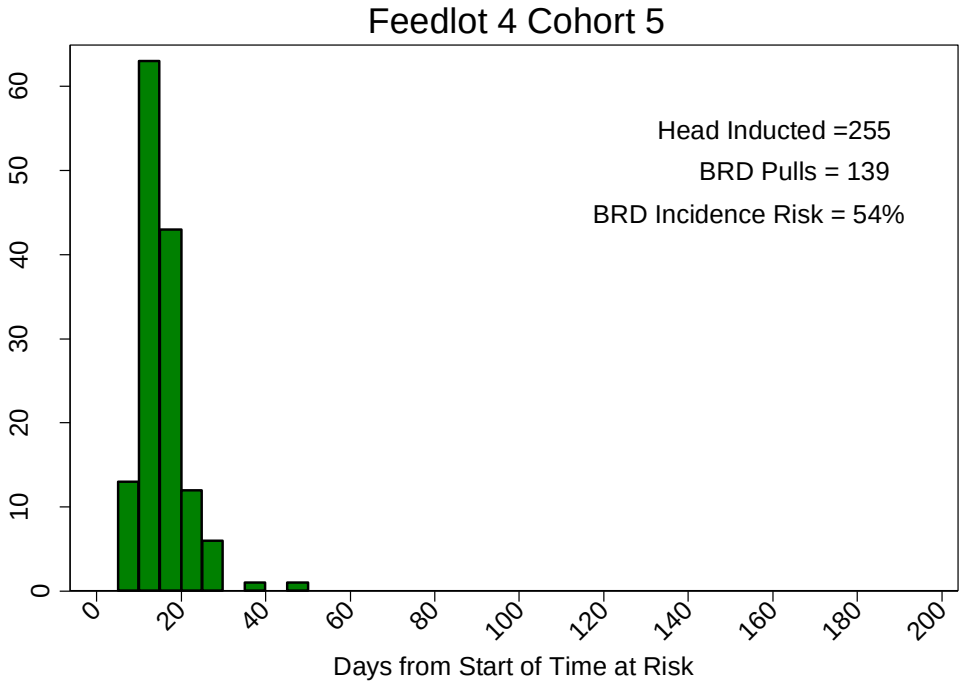

Figure 4.11: Example of an epidemic curve for BRD with an early peak in the number of animals pulled for BRD; in this example, this occurred from 10 to 20 days after the start of time at risk.

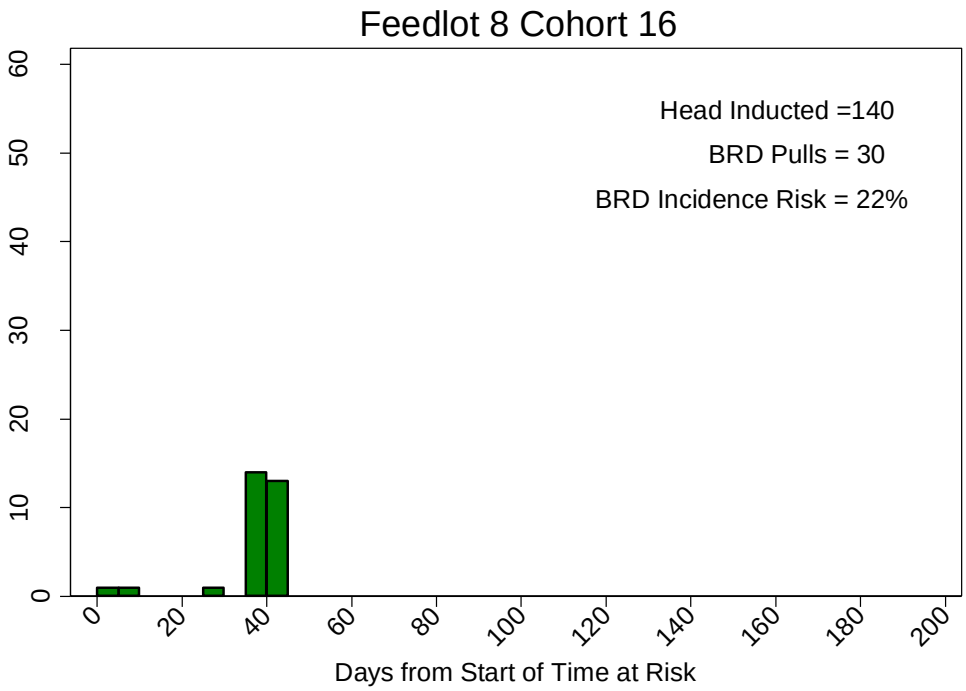

Figure 4.12: An example of an epidemic curve for BRD with a late peak in the number of animals pulled for BRD; in this example, this occurred from 35 to 40 days after the start of time at risk.

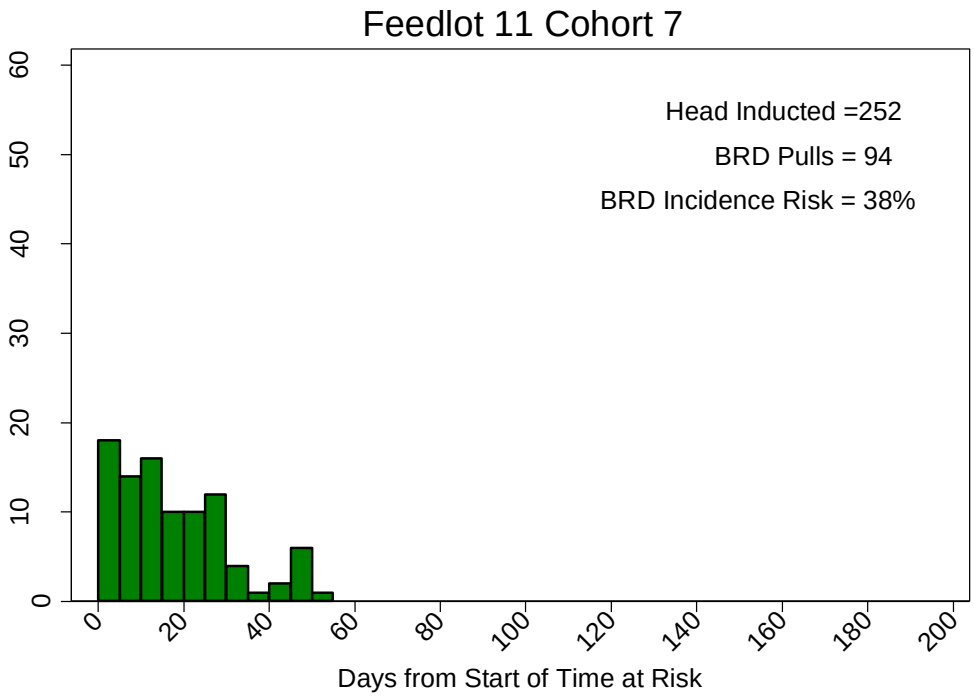

Figure 4.13: An example of an epidemic curve for BRD where cases occurred over an extended period of time, in this case with steadily declining numbers of animals pulled for BRD from day 0**.**

4.1.3.7 Variation in incidence risk over time

Cohort-level incidence risks for various time periods are shown in Table 4-2, Table 4-3 and Table 4-4. Table 4-2 shows the descriptive statistics for incidence risks from day 0 to days 10, 20, 30, 40, 50 and the end of time at risk. Table 4-3 shows the descriptive statistics for the incremental incidence risks within each 10-day period for all cohorts and for cohorts with at least 20 BRD cases. The 10-day periods relate to time-at-risk from day 0 for each animal, rather than from the cohort open or close date. Incidence risks from day 0 to days 10, 20, 30, 40, 50 and the end of time at risk varied widely between cohorts. Means were markedly higher than medians, indicating that cohort-level incidence risks were right-skewed as shown in Figure 4.3. Although the mean incidence risks within 10-day periods were highest within the 11 to 20 day and 21 to 30 day periods, the maximum incidence risk was at least 10% within each of the time periods considered.

Table 4-4 shows the descriptive statistics for the proportions of BRD incidence risks occurring within 10-day periods for all cohorts with non-zero incidence risk and for cohorts with at least 20 BRD cases. The mean proportions of BRD incidence risks showed that about one third of cases occurred in each of the 11 to 20 day and 21 to 30 day periods indicating that in the average cohort, about two thirds of cases occurred within that 20 day window. However, there were cohorts with at least 20 cases where cases mostly occurred within a shorter time frame. For example, there were cohorts where at least 50% of cases occurred within each 10-day time period, or after 50 days and where 80% of cases occurred within a single 10-day period (11 to 20 days and 21 to 30 days).

Collectively, these results indicate that BRD incidences are typically highest between 11 and 30 days after day 0 but that there is large variation in incidences, and in temporal patterns of occurrence, between cohorts.

Table 4-2: Descriptive statistics for the incidence risks for cohorts for the first 10, 20, 30, 40 and 50 days of time at risk and for the total time at risk ("overall") for all cohorts and for cohorts with at least 20 BRD cases

Time at Risk	Number	Mean	Median	Std. Dev.	Min	Max
All cohorts						
Days 1 to 10	170	1.16	0.00	2.67	0.00	19.86
Days 1 to 20	170	6.71	1.99	9.86	0.00	49.02
Days 1 to 30	170	12.26	4.00	15.41	0.00	70.09
Days 1 to 40	170	14.40	5.97	17.05	0.00	72.08
Days 1 to 50	170	15.35	7.71	17.61	0.00	72.08
Overall	170	16.01	8.54	17.61	0.00	72.08
Cohorts with ≥ 20 BRD cases						
Days 1 to 10	79	1.96	0.73	3.54	0.00	19.86
Days 1 to 20	79	12.97	8.89	11.48	0.00	49.02
Days 1 to 30	79	24.14	22.25	15.38	0.65	70.09
Days 1 to 40	79	28.29	25.52	15.86	2.17	72.08
Days 1 to 50	79	29.82	25.56	16.05	5.71	72.08
Overall	79	30.62	25.56	15.77	5.71	72.08

Table 4-3: Descriptive statistics for the incidence risks for cohorts for 10-day periods up to day 50 and from day 50 onwards for all cohorts and for cohorts with at least 20 BRD cases

Time at Risk	Number	Mean	Median	Std. Dev.	Min	Max
All cohorts						
Days 1 to 10	170	2.22	0.00	5.20	0.00	38.00
Days 11 to 20	170	5.55	1.62	8.80	0.00	45.51
Days 21 to 30	170	5.55	1.67	7.62	0.00	37.04
Days 31 to 40	170	2.13	0.68	3.30	0.00	15.00
Days 41 to 50	170	0.95	0.00	2.18	0.00	16.13
Day 50 onwards	170	0.66	0.00	1.49	0.00	10.81
Cohorts with ≥ 20 BRD cases						
Days 1 to 10	79	4.08	2.00	7.00	0.00	38.00
Days 11 to 20	79	11.01	7.59	10.45	0.00	45.51
Days 21 to 30	79	11.18	9.59	7.92	0.00	37.04
Days 31 to 40	79	4.15	2.82	3.90	0.00	15.00
Days 41 to 50	79	1.52	0.50	2.78	0.00	16.13
Day 50 onwards	79	0.80	0.28	1.47	0.00	10.33

Table 4-4: Descriptive statistics for the proportions of the total BRD incidence risks that occurred in 10-day intervals up to day 50 and from day 50 onwards by cohort, for all cohorts with a non-zero incidence risk and for cohorts with at least 20 BRD cases

Time at Risk	Number	Mean	Median	Std. Dev.	Min	Max
All cohorts with non-zero incidence risk						
Days 1 to 10	153	9.01	0.47	17.40	0.00	100.00
Days 11 to 20	153	29.27	23.53	27.51	0.00	100.00
Days 21 to 30	153	27.01	24.32	24.11	0.00	100.00
Days 31 to 40	153	13.90	8.86	18.16	0.00	100.00
Days 41 to 50	153	9.07	1.33	17.13	0.00	100.00
Day 50 onwards	153	11.74	0.00	25.23	0.00	100.00
Cohorts with ≥ 20 BRD cases						
Days 1 to 10	79	6.92	2.72	11.07	0.00	52.17
Days 11 to 20	79	32.55	28.71	20.78	0.00	87.36
Days 21 to 30	79	35.98	37.88	18.23	0.00	82.93
Days 31 to 40	79	15.02	13.10	13.61	0.00	70.00
Days 41 to 50	79	5.70	1.59	9.37	0.00	59.09
Day 50 onwards	79	3.83	0.83	8.57	0.00	61.29

4.1.4 Deaths

4.1.4.1 Case fatality risk

Overall case fatality risk and times to death

The overall case fatality risk for first pulls due to BRD within 50 days of the start of time at risk was 3.4% (212/6,200) when all deaths within 50 days of first pulling were included. Among these deaths, 64.1% (152/212) were attributed to BRD using the definition described in Section 3.7.2. Numbers of fatalities for BRD pulls peaked 25 to 40 days after the start of time at risk (Figure 4.14, mean time to death: 38.7 days, median: 37, range: 9 to 92), and within 10 days of pulling (Figure 4.15, mean: 15.3 days, median: 12.5, range: 0 to 50).

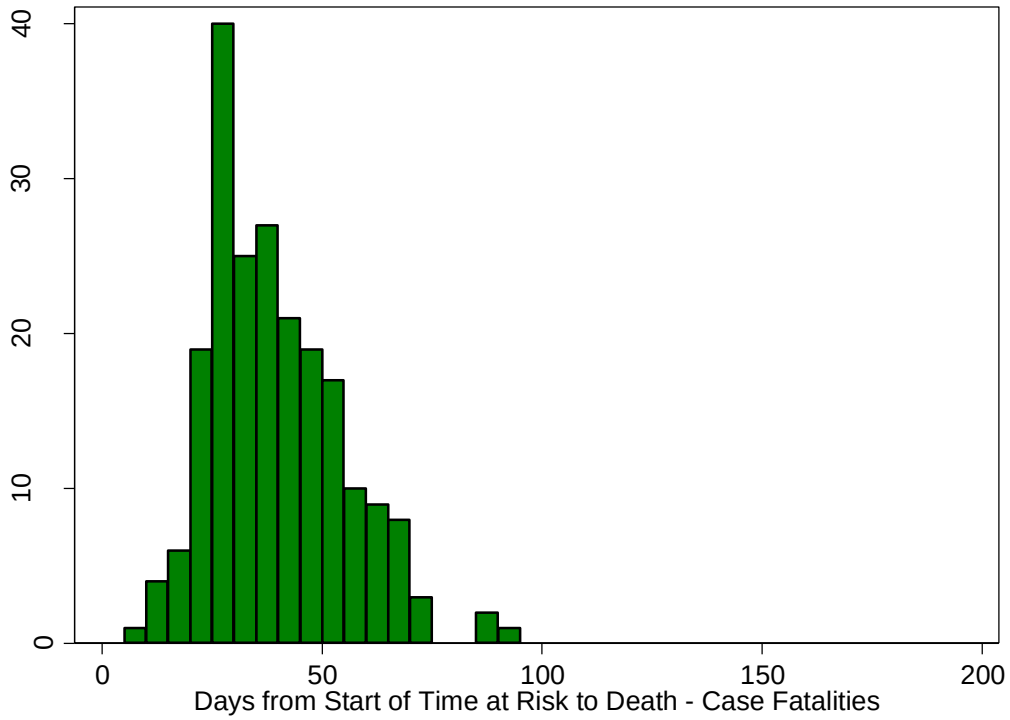

Figure 4.14: Distribution of durations in days between the start of time at risk and death for case fatalities

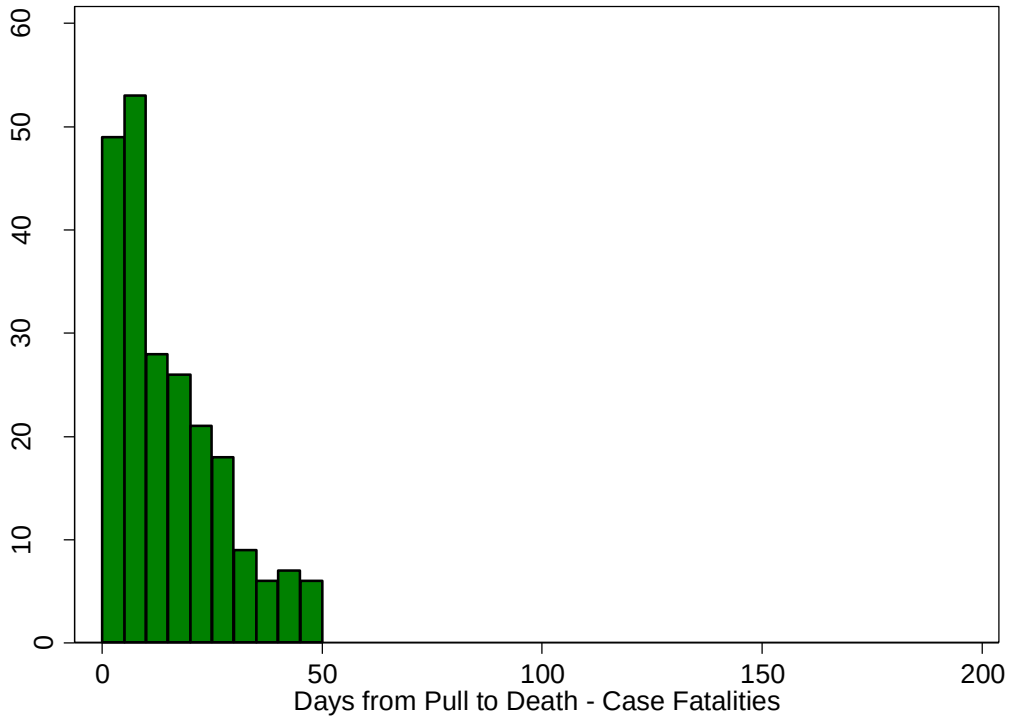

Figure 4.15: Durations in days between diagnosis with BRD and death for case fatalities

Case fatality risk by feedlot and cohort

Variability in case fatality risk among feedlots and cohorts was examined for feedlots and cohorts where at least 20 cattle that met the BRD case definition when first pulled and the pull occurred within the first 50 days of the start of time at risk. There was considerable variability in case fatality risk among these 10 feedlots (mean: 4.1%, median: 3.6% range: 1.5 **–** 8.0%) and 74 cohorts within feedlots (Figure 4.16, mean: 3.2%, median: 3.1%, range: 0.0 to 16.1%).

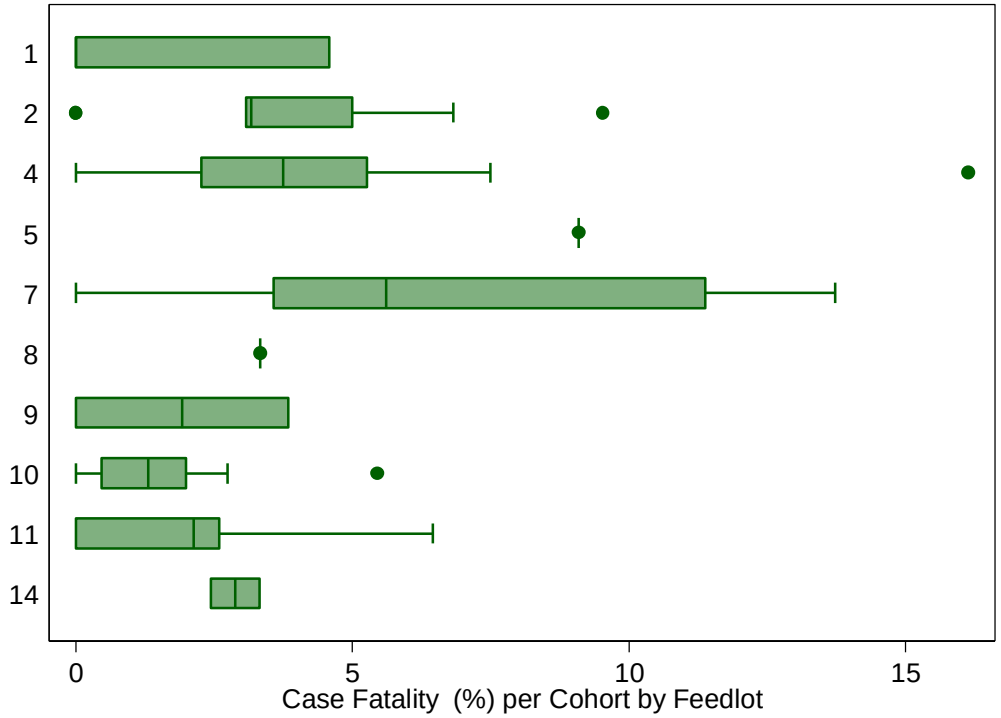

Figure 4.16: Distributions of case fatality risks for cohorts within feedlot. Only cohorts (n = 74) where at least 20 cattle that met the BRD case definition when first pulled and the pull occurred within the first 50 days of the start of time at risk are included.

4.1.4.2 Pen deaths

A pen death was defined as a death without a hospital record. Of all study cattle, 0.1% (44/35,131) died as a pen death where the cause of death was attributed to BRD using the definition described in Section 3.7.2. Of those cattle that died from BRD using this definition, 18.6% (44/237) were pen deaths. Pen deaths where the cause of death was attributed to BRD were most common from 15 to 45 days after the start of time at risk (Figure 4.17). There was a small amount of variability among the 14 feedlots in the incidence risk of pen deaths attributed to BRD (mean: 0.10%, median: 0.06%, range: 0.00 - 0.23%) and considerable variability amongst feedlots in the proportion of cattle deaths attributed to BRD that were pen deaths (in 11 feedlots where there was at least one death attributed to BRD, mean: 28.3%, median: 25.0%, range: 0.0 - 100%). Variability among cohorts was not examined due to the small number of pen deaths.

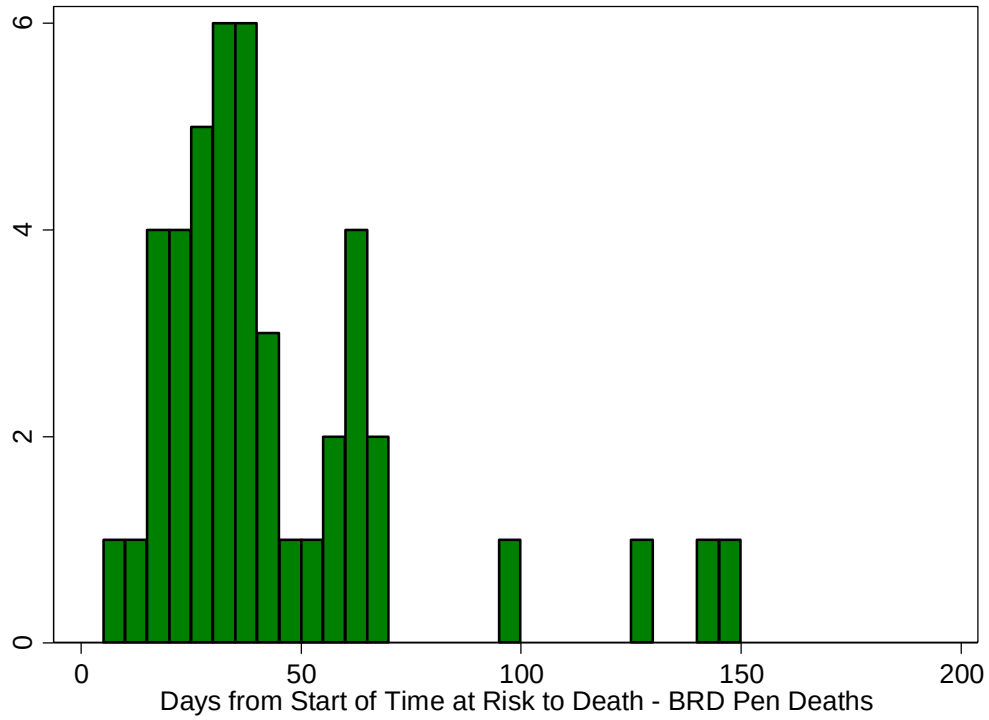

Figure 4.17: Durations in days between the start of time at risk and death for pen deaths attributed to BRD.

4.1.4.3 Proportion of deaths that were due to BRD

A total of 1.3% (460/35,131) of study cattle died whilst on feed and 0.7% (237/35,131) died from BRD using the definition described in Section 3.7.2. Thus, 51.5% of all deaths were attributed to BRD. Of the 237 BRD deaths, 72.2% (N = 171) were pulled for BRD when first pulled, 18.6% (N = 44) did not have a record of a visit to the hospital (pen deaths), 8.4% (20/237) were pulled for another reason when first pulled and 0.8% (2/237) did not have a pull reason recorded.

4.1.4.4 BRD mortality incidence risk

There was considerable variability among feedlots and among cohorts within feedlots in the percentage of cattle dying from BRD (feedlot mean: 0.52%, median: 0.49, range: 0.00 to 2.00%, cohort mean: 0.65%, median: 0.00%, range: 0.00 to 7.06%, Figure 4.18). Some feedlots had high variability at the cohort level whereas some others had a consistently low percentage of cattle that died from BRD. Three feedlots reported no deaths due to BRD.

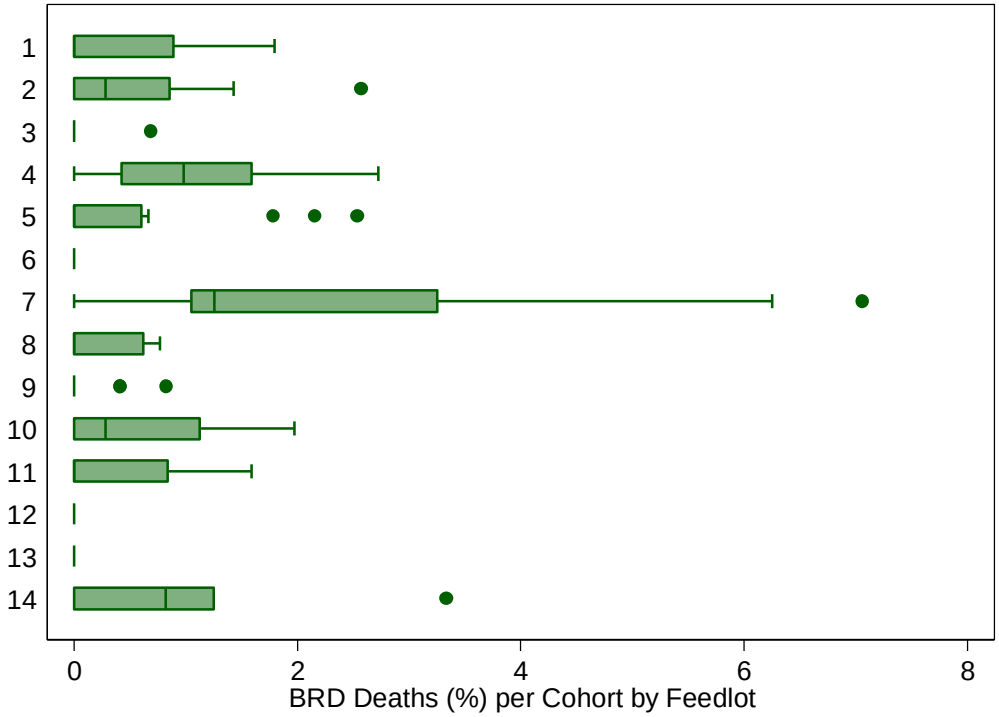

Figure 4.18: Distributions of BRD mortality incidence risk for cohorts within feedlot

4.1.4.5 Timing of deaths attributed to BRD

The majority of deaths attributed to BRD occurred within 100 days of the start of time at risk, with a peak from 20 to 40 days after the start of time at risk (Figure 4.19, mean: 41 days, median: 36, range: 8 to 153). Most deaths of cattle whose first pull was due to BRD occurred within 50 days of the animal's first pull, with the highest numbers of these cattle dying during the first 10 days (Figure 4.20). Deaths were typically sooner after pulling if the death was attributed to BRD (mean: 15 days, median: 9, range 0 to 108) compared to all deaths (mean: 25 days, median: 14, range: 0 to 176).

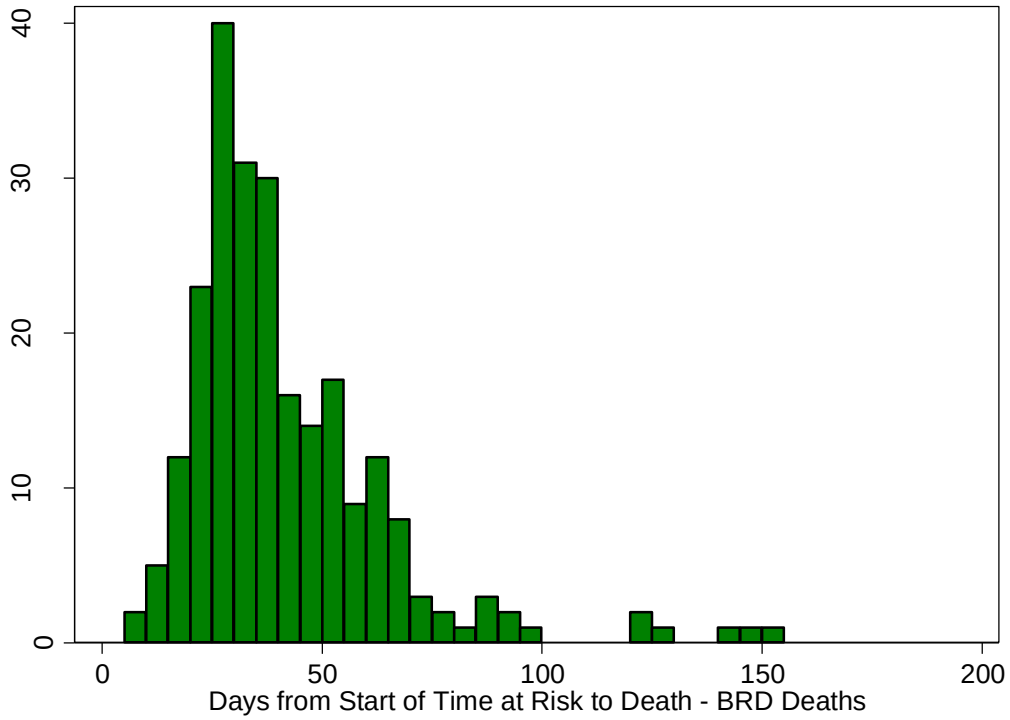

Figure 4.19: Durations in days between the start of time at risk and death for deaths attributed to BRD.

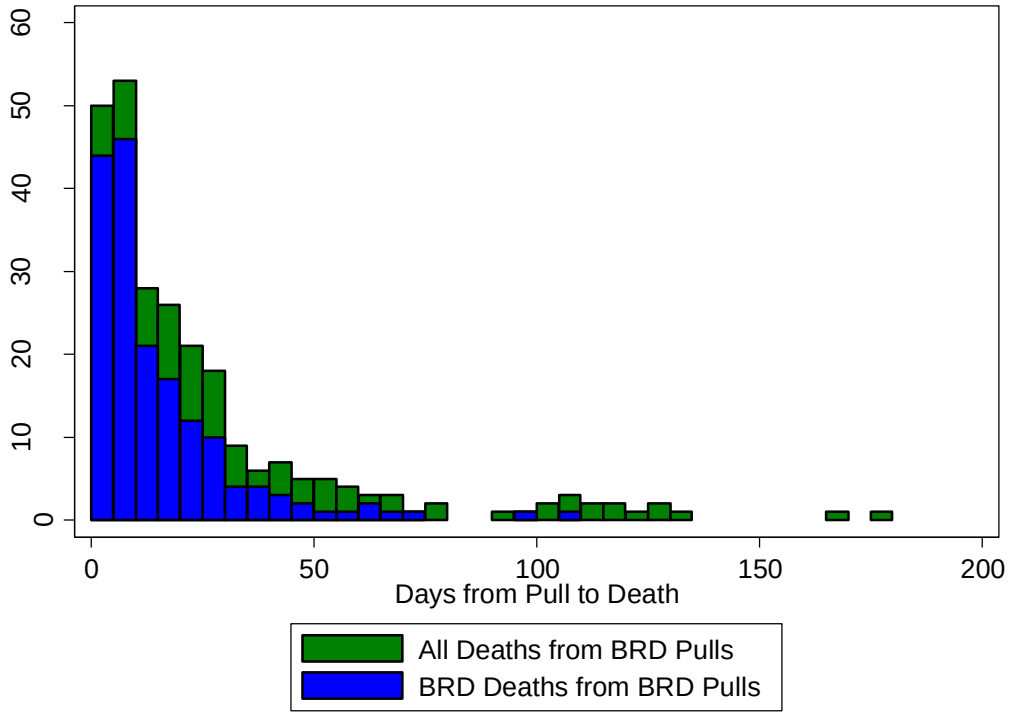

Figure 4.20: Durations in days between diagnosis with BRD and death for all deaths and deaths attributed to BRD.

4.2 Analyses of putative risk factors

Causal diagrams

Causal diagrams relevant to specific analysis datasets were constructed based on the complete causal diagram shown in Figure 3.7 (Section 3.11.4). Thus, the causal diagram shown in Figure 4.21 was used to derive models for variables analysed in the full cohort dataset. The causal diagram displayed in Figure 4.22 shows additional variables analysed in the vendor questionnaire datasets along with any covariates that were included in adjustment sets. The variables relevant to the case-control dataset are shown in Figure 4.23 and Figure 4.24 shows the diagram used for the combined number of virus variables. Selection batch and test batch were additional variables forced into the case-control analyses where possible, but which are not shown in the causal diagrams.

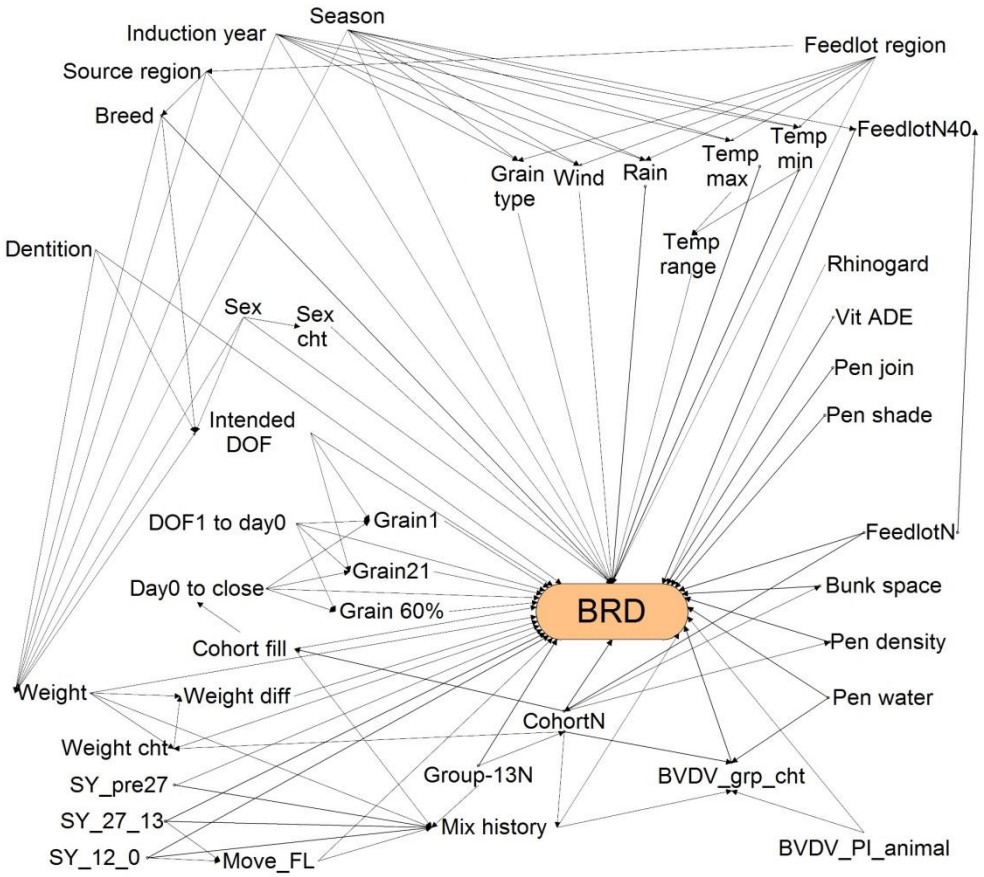

Figure 4.21 Causal diagram depicting pathways relevant for the determination of total and direct effects of putative risk factors investigated in the full cohort dataset. Group-28N or Group-91N were substituted for Group-13N to determine the models for these variables. A complete list of abbreviations of variables in this diagram is provided in the preliminary pages of this Report.

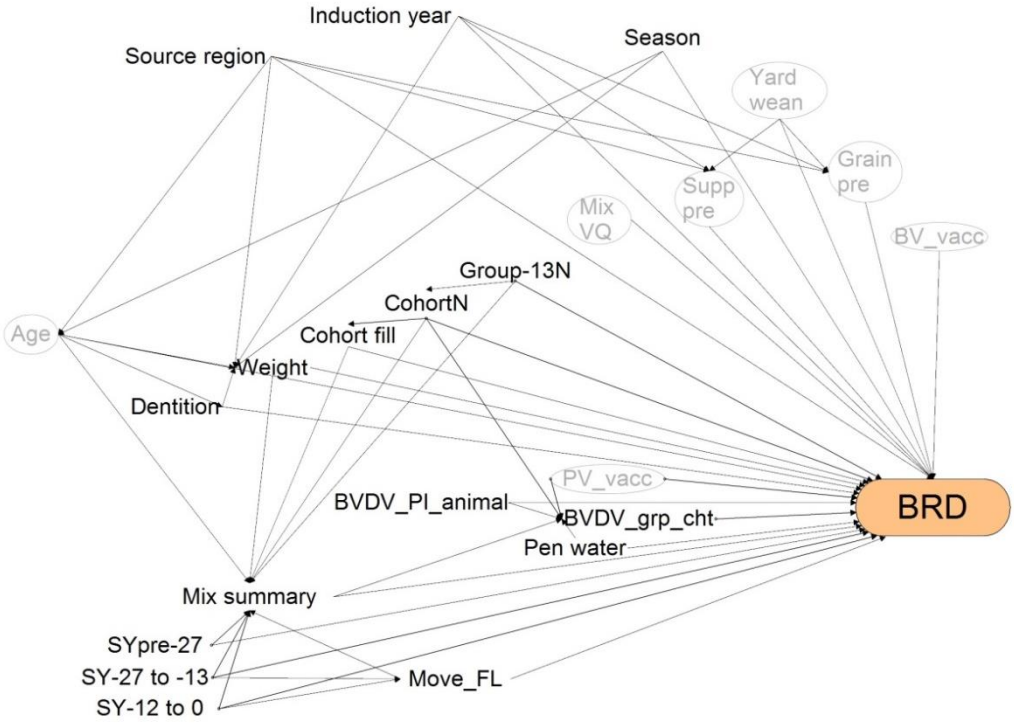

Figure 4.22: Causal diagram depicting pathways relevant for the determination of total and direct effects of putative risk factors investigated in the vendor questionnaire datasets. A complete list of abbreviations of variables in this diagram is provided in the preliminary pages of this Report.

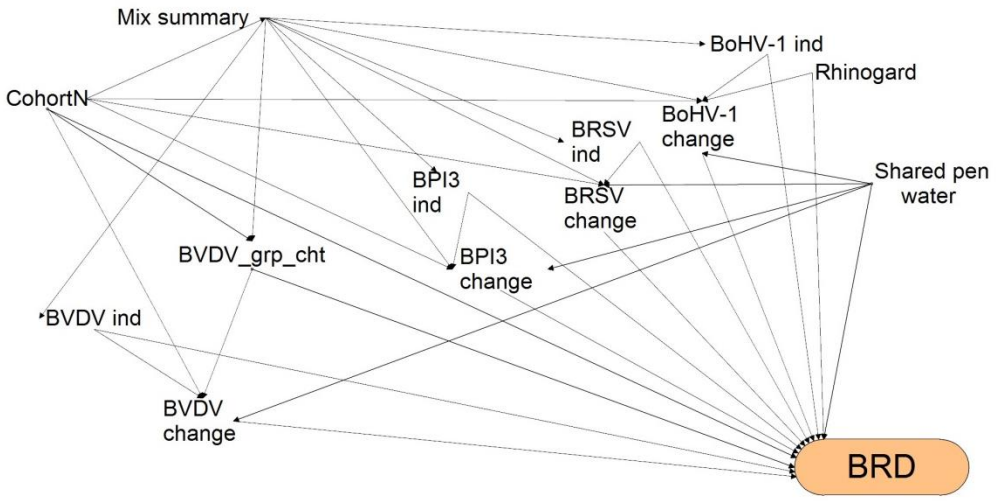

Figure 4.23: Causal diagram showing variables relevant to the case-control analyses; “change” variables represent one of the three variables that measured change in serostatus between induction and follow-up, (e.g. BVDV comp, BVDVseroinc or BVDVserocon). A complete list of abbreviations of variables in this diagram is provided in the preliminary pages of this Report.

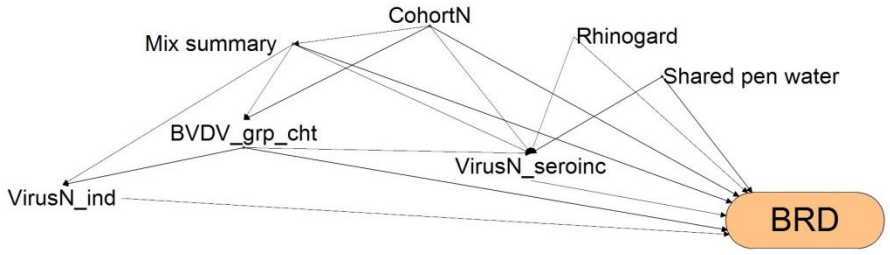

Figure 4.24: Causal diagram showing variables relevant to estimating the effects of "number of virus" variables in the case-control analyses. A complete list of abbreviations of variables in this diagram is provided in the preliminary pages of this Report.

4.2.1 Interpretation of associations between putative risk factors and BRD

Associations between each putative risk factor and BRD are reported in Sections 4.2.2 to 4.2.12. Several results are presented for each putative risk factor. The estimated odds ratios for the total effects of the risk factor were estimated from models fitted using MLwiN, and population attributable fractions and population attributable risks were estimated from models fitted using MLwiN and WinBUGs. For those putative risk factors where the direct effects were also of interest, these are also presented. Risk factors were grouped in the same way as in Section 3.9.3.

Example interpretation

Details of the association between the number of animals in group-13 and BRD are presented in Table 4-5. Results in this table are interpreted as follows:

- The risk factor column lists the risk factors presented in the table and the abbreviation used in the causal diagram, where appropriate. In this example only the results for one risk factor, the number of animals in group-13, are reported.
- The category column lists the categories of the risk factor. These categories are the same as those derived in Section 3.9.3. In this example, there are three categories: less than 50 animals, 50 to 99 animals and at least 100 animals.
- The odds ratio column states which of the categories is the reference group ("Ref. cat.") and gives the point estimates of the odds ratios for the other categories relative to the reference group. In this example, the odds of an animal in a group-13 of at least 100 animals developing BRD by day 50 were estimated as being 0.5 times that for an animal if it was in a group-13 of less than 50 animals, and the odds of an animal in a group-13 of 50 to 99 animals developing BRD by day 50 were estimated as being 0.8 times that for an animal if it was in a group-13 of less than 50 animals. These estimates are for the total effects of the number of animals in group-13, so include the combined effects of all direct and indirect pathways.
- These estimated odds ratios are the best estimate of the true odds ratio (the odds ratio for all feedlot cattle, past and future, as represented by the study cattle). A true odds ratio of less than one would indicate a protective effect; animals in a category of a risk factor where the true odds ratio is less than one would be less likely to develop BRD compared to the reference group. A true odds ratios of more than one would indicate a detrimental effect, so

animals in a category of a risk factor where the true odds ratio is more than one would be more likely to develop BRD compared to the reference group. A true odds ratio of one would indicates no effect, and so no association would be present.

- The 95% credible intervals (reported under “95% cred int”) describe the range which is likely to include the true odds ratio. (These were calculated as the ranges in which 95% of the estimates of the odds ratio fell from all iterations of the model.) In this example, the 95% credible interval for at least 100 animals in group-13 is 0.4 to 0.7. This indicates that the true odds ratio is probably between 0.4 and 0.7, assuming no bias. The upper limit of this range (0.7) is markedly less than one. Hence having at least 100 animals in group-13 is almost certainly quite strongly protective, relative to less than 50 animals (the reference category). In contrast, if the 95% credible interval includes one, it is possible that the true odds ratio could be one or in the opposite direction to that indicated by the point estimate, and hence there is more uncertainty about whether there is an association and the direction of that association.
- The values in the “Prob </>1” column are estimates of the probabilities that the odds ratios are greater than one (if the point estimate was less than one), or less than one (if the point estimate was greater than one). In this example, the probability that the odds ratio for a group-13 of at least 100 animals compared to less than 50 animals is greater than one is less than 0.001 (i.e. less than one in a thousand). So assuming no bias, we can be almost certain that there is a protective effect of being in a group-13 of at least 100 animals. These probabilities are quite different from, and more informative than, the more commonly used p values. Like p values, they are used when deciding whether to reject the null hypothesis (i.e. whether to conclude that the true odds ratio differs from one, i.e. that there is an association). A low probability provides basis for rejecting the null hypothesis, and instead concluding that the effect is in the direction indicated by the observed odds ratio.
- The adjustment set column lists the covariates that were included in the model. In this example, the minimal sufficient adjustment set (identified based on the causal diagram and using DAGitty®) to estimate the total effects was an empty set. In other words, it was not necessary to include any covariates in the model.
- The final column shows the number of animals included in the analysis and the number of hierarchical levels that were fitted in the model. In this example, all animals were included (N = 35,131). When some animals had missing data for one or more of the variables in the model, those animals were automatically dropped from the model. This model was fitted with all four levels, so feedlot, cohort (within feedlot) and group-13 (within cohort) were all included as random effects. “3 level” indicates that only feedlot and cohort (within feedlot) were included as random effects. Where there was more than one minimal sufficient adjustment set, the DIC is also reported in this column. Where the datasets are the same for two models, the model with the lower DIC is a better model.

Table 4-5: Estimated odds ratios for the total effects of the number of animals in group-13 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)					()	N = 35,131 4 level
	< 50	Ref. cat.				
	50 to 99	0.8	(0.7 to 0.9)	0.002		
	≥ 100	0.5	(0.4 to 0.7)	< 0.001		

Details of the association between the number of animals in group-13 and BRD for estimating direct effects are presented in Table 4-6. The format of this table is the same as for the total effects table. In this example, the minimal sufficient adjustment set required to estimate the direct effect identified using DAGitty® consisted of the nine variables listed. The abbreviations for variable names used in the causal diagram have been used for brevity. The odds ratios, 95% credible interval and probability </> 1 are interpreted in the same manner as for the total effects estimate except the estimate is for the direct effects of the number of animals in group-13.

From the causal diagram for the full cohort dataset (Figure 4.21), effects of the number of animals in group-13 on BRD were postulated as occurring a) directly (i.e. through no measured intervening variable), b) indirectly through the number of animals in the cohort, and c) indirectly through mix history. In this example, the odds ratios and 95% credible intervals for the direct effects were very similar to the total effects estimate. Assuming there are no other causal pathways, this indicates that most of the effect of this risk factor is mediated through the direct pathway, rather than through either the number of animals in the cohort or mix history (the only postulated indirect pathways).

Table 4-6: Estimated odds ratios for the direct effects of the number of animals in group-13 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, SYPre-27, FeedlotN, Mix history, Move_FL)	N = 34,726 4 level
	< 50	Ref. cat.				
	50 to 99	0.8	(0.7 to 1.0)	0.009		
	≥ 100	0.6	(0.4 to 0.8)	0.001		

Estimated PAFs and PARs are presented in Table 4-7. As odds ratios for both total and direct effects are reported for the number of animals in group-13 and the results indicate a protective effect, estimates for PAFs and PARs for total and direct effects are also reported. Both were estimated using models fitted in both MLwiN and WinBUGs. The point estimates differed slightly between these two software packages as there were some differences in the algorithms and the prior distributions used.

PARs and PAFs described the importance of the risk factor at the population level. They estimated the change in BRD frequency across the entire population represented by the study population if all animals in the higher risk categories instead had the same risk of BRD as those in the lowest risk category (in this example, at least 100 animals). In this example, the estimated PAFs for the total effects of the number of animals in group-13 from MLwiN and WinBUGs models were 0.35 and 0.39, respectively. For the entire population represented by the study population, if the PAF is 0.39, the incidence of BRD would be reduced by 39% if the risk of BRD in all animals from group-13s of less than 100 animals was reduced to that for animals from group-13s of at least 100 animals. (This could occur, for example, if all animals from group-13s of less than 100 animals were replaced with otherwise identical animals but from group-13s of at least 100 animals.) If the BRD incidence by day 50 across all animals before this reduction was 17.65%, a 39% reduction would equate to this being reduced by an absolute amount of 6.9 percentage points (17.65%*39%), to 10.8%. Thus PAFs describe proportional reduction in incidence risk whereas PARs describe absolute reductions and so are in the same units as incidence risk itself. In Table 4-7, the WinBUGs PAR is shown as 6.9%. This assumes that there is a causal relationship between the number of animals in group-13 and the risk of BRD, that the odds ratios were unbiased, and that the proportions of these pooled animals that were in group-13s of < 50, 50 to 99 and ≥ 100 animals were as observed in the study animals.

Ninety-five percent credible intervals for PAFs and PARs were also available from the WinBUGs models. In this example, the 95% credible interval for the PAF of 0.23 to 0.51 (Table 4-7) indicates that the true proportional reduction in BRD incidence is unlikely to be less than 23% or more than 51%. Thus, if the BRD incidence across all animals before BRD risk was reduced to that for animals from group-13s of at least 100 animals was 17.65%, the absolute reduction in BRD incidence is unlikely to be less than 4.1% (17.65%*23%) or more than 9.1% (17.65%*51%). Thus the PAR credible interval in Table 4-7 is 4.1% to 9.1%. The estimates of the PAFs and PARs for the direct effects are slightly lower, corresponding to the slightly reduced odds ratios for the strength of the protective effects observed in the direct effect model reported in Table 4-6. Where the 95% credible interval for a PAF and PAR does not include zero, there is likely to be a true population-level effect of the risk factor. In contrast, if the 95% credible interval for a PAF and PAR includes zero, the probability that the true population effect is zero or even an increase (rather than reduction) in BRD incidence risk is higher, and hence there is more uncertainty about whether there is a population effect and the direction of that effect.

Table 4-7: Estimated population attributable fractions (PAFs) and population attributable risks (PARs) for the total and direct effects of the number of animals in group-13 on the risk of BRD by day 50. Estimates are derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR %	WinBUGS PAR %	WinBUGS 95% cred int
Total effects						
No. of animals in group-13 (Group-13N)	0.35	0.39	(0.23 to 0.51)	6.20	6.90	(4.1 to 9.1)
Direct effects						
No. of animals in group-13 (Group-13N)	0.30	0.30	(0.10 to 0.44)	5.25	5.22	(1.78 to 7.80)

4.2.2 Putative risk factors relating to feedlot entry characteristics

4.2.2.1 Breed

The most common breed was Angus (56% of animals, Table 4-8). Tropical breeds and tropical crosses comprised about 16% of the population, European breeds about 4% and the remainder were of British origin or derivation.

The risk of BRD varied considerably between different breeds (Table 4-9). Compared to Angus cattle, Herefords were at markedly increased risk (OR: 2.0, 95% credible interval: 1.5 to 2.6) and British 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.

The PAFs and PARs for total effects from the MLwiN model were 0.53 and 9.3%. The estimates from the WinBUGS model were similar at 0.67 (95% credible interval: 0.54 to 0.77) and 11.8% (95% credible interval: 9.6 to 13.5%) (Table 4-12). Thus, overall BRD incidence was estimated to decline by an absolute amount of 9.3% or 11.8% if it were possible to ensure that all cattle were at the same risk as tropical breed and tropical crossbred cattle. These results indicate that breed was a very important risk factor at the population level.

There was a significant interaction between breed and season (i.e. the effect of breed differed with season, Figure 4.25). Most notably, the adverse effect of Hereford breed was compounded in autumn. However, estimates of interaction terms were very imprecise, so conclusions focus on the main effects.

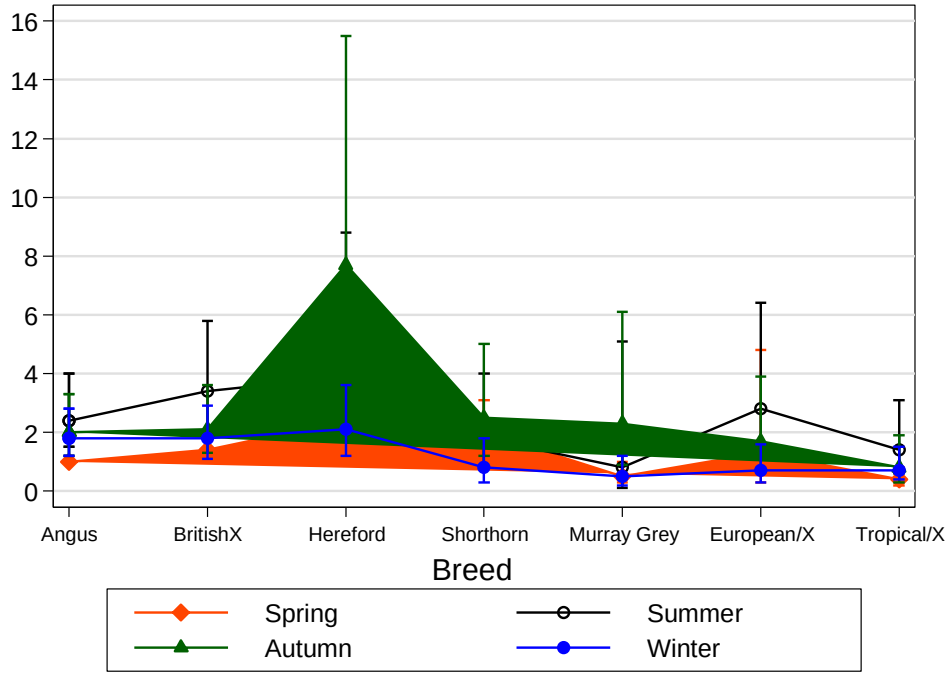

Figure 4.25: Estimates for odds ratios and 95% credible intervals for breed-season combinations derived from a model including an interaction between breed and season.

4.2.2.2 Sex

Most cattle in the study population were steers (92% of animals, Table 4-8). The total effect estimates suggest that heifers were probably at reduced risk compared to steers (OR: 0.7, 95% credible interval: 0.4 to 1.1, Table 4-9).

The total effects estimates for the PAF and PAR were 0.27 and 6.3% 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 4-12). The estimates of PAF and PAR for sex were very imprecise, as indicated by the wide credible intervals.

4.2.2.3 Dentition

Most cattle in the study population had no permanent incisors (81% of animals, Table 4-8); 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 4-9).

4.2.2.4 Age

Just over half of animals with vendor questionnaire data were aged 16 to < 22 months at the start of time at risk (55%,Table 4-8). 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 4-9). Direct effect estimates were similar (Table 4-11). The cause of this increased risk in older animals is unknown but it may be because of uncontrolled confounding due, for example, to factors causing delayed sale of animals to feedlots also causing increased risk of BRD.

4.2.2.5 Induction weight

Most cattle in the study population were either 400 to < 440 kg (31%) or the 440 to < 480 kg (34%,Table 4-8) at induction. Compared to light cattle (< 400kg), 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 – 0.7 in both models, Table 4-10).

The PAFs and PARs for total effects were 0.17 and 3.0% 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 4-12). Thus, overall BRD incidence was estimated to decline by an absolute amount of 2.9 to 3.0% if it were possible to ensure that all cattle were at the same risk as cattle ≥ 480 kg. These results indicate that induction weight was a moderately important risk factor at the population level.

There was a significant interaction between induction weight and the number of animals in the group-13 (Figure 4.26). 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.

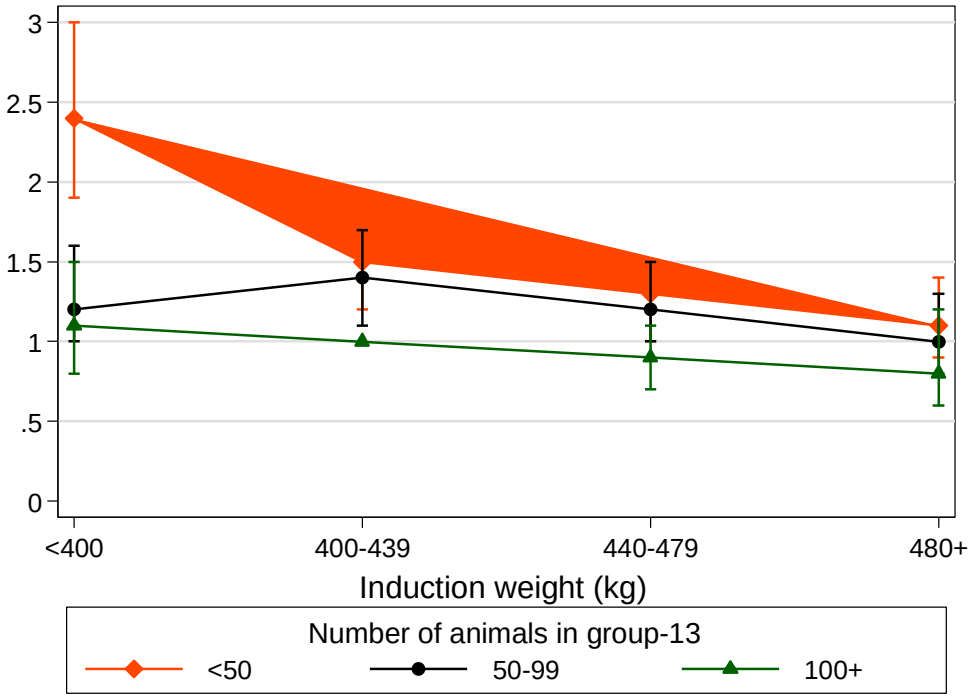

Figure 4.26: Estimates for odds ratios and 95% credible intervals for induction weight-number of animals in group-13 combinations derived from a model including an interaction between induction weight and number on animals in group-13.

4.2.2.6 Weight difference from mean cohort weight

The study population was evenly distributed among the four categories of weight difference from the mean cohort weight (Table 4-8). After adjusting for individual animal weight, there was no evidence of a moderate or large effect of the difference in weight from the mean cohort weight and risk of BRD (Table 4-10).

4.2.2.7 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 4-8). There was no evidence of a large effect of intended days on feed on the risk of BRD (Table 4-9).

Table 4-8: Putative risk factors relating to feedlot entry characteristics; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing %	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Breed		0.2			
	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		0.0			
	Male		32,260	91.8	18.8
	Female		2,871	8.2	5.3
Dentition		1.9			
	0		27,812	80.7	19.3
	2		5,560	16.1	12.9
	≥ 4		1,082	3.1	10.1
Age* (months)		9.2			
	<16^		1,598	16.4	12.5
	16 to < 22		5,326	54.7	23.3
	≥ 22		2,807	28.9	17.1
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
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 above		8,552	24.2	17.3
Intended days on feed		0.0			
	≥ 120		18,561	52.8	22.3
	85 to <120		12,615	35.9	15.3
	≤ 85^		3,955	11.3	3.6

^ Categories where 7 or more feedlots have no observations

* Age was analysed in the vendor questionnaire dataset

Table 4-9: Estimated odds ratios for the total effects of putative risk factors relating to feedlot entry characteristics on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level, DIC
Breed					(Source region)	N = 35,049 4 level
	Angus	Ref. cat.				
	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. cat.				
	Female	0.7	(0.4 to 1.1)	0.063		
Dentition					()	N = 34,454 3 level
	0	Ref. cat.				
	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. cat.				
	≥ 22	1.6	(1.2 to 2.0)	<0.001		
Intended days on feed					(Breed, Weight, Sex, Dentition)	N = 34,361 3 level
	≥ 120	Ref. cat.				
	85 to <120	1.2	(0.7 to 2.0)	0.269		
	≤ 85^	1.1	(0.4 to 2.6)	0.493		

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

Table 4-10: 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, DIC
Induction weight (kg) Model A					(Dentition Breed, Grain type, Rain, Wind, Season, Sex, Temp max, Temp min, Source region)	N = 34,361 3 level DIC = 24,256
	< 400	Ref. cat.				
	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 DIC = 24,291
	< 400	Ref. cat.				
	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		
Weight difference from mean cohort weight (kg)					(Breed, Weight cht, Weight)	N = 35,044 4 level
	> 20 below	Ref. cat.				
	≤ 20 below	0.9	(0.8 to 1.0)	0.110		
	≤ 20 above	0.9	(0.8 to 1.1)	0.093		
	> 20 above	1.0	(0.8 to 1.2)	0.357		

Table 4-11: 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, SYpre-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. cat.				
	≥ 22	1.6	(1.3 to 2.1)	<0.001		

* Age was analysed in the vendor questionnaire dataset

Table 4-12: Estimated population attributable fractions (PAF) and population attributable risks (PAR) for the total effects of putative risk factors relating to feedlot entry characteristics where study results indicated either a protective or an adverse effect on the risk of BRD by day 50. Estimates were derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR	WinBUGS PAR	WinBUGS 95% cred int
Breed	0.53	0.67	(0.54 to 0.77)	9.30	11.80	(9.6 to 13.5)
Sex	0.27	0.36	(0.00 to 0.59)	4.70	6.30	(-0.1 to 10.5)
Weight	0.17	0.16	(0.09 to 0.23)	3.00	2.90	(1.6 to 4.1)

4.2.3 Putative risk factors relating to mixing, moving, group size, saleyard exposure and transport prior to feedlot entry

4.2.3.1 Lifetime movement and mixing patterns

The NLIS database was used to determine lifetime movement and mixing patterns for study animals as described in section 3.8.6. The mean number of lifetime transfers for study animals, including the transfer to the feedlot PIC was 1.9 (median: 2, range: 1 to 12). A total of 41% (14,463/35,160) of animals had only one move (to the feedlot) and 57% (20,175/35,160) of animals had between two and four transfers. For all transfers, the mean time was 148 days before day 0, but the distribution was negatively skewed (median: 13, range: 0 to 2,140). For transfers prior to the transfer to the feedlot PIC, the mean time was 327 days before day 0 (median: 319, interquartile range: 199 to 440). Our definition of mixing required at least some animals to change PIC (i.e. move), but not all moves resulted in mixing. Thus, for the majority of animals that had been mixed prior to arrival at the feedlot PIC, it was apparent that, the earliest mixing event was likely to occur many months prior to day 0.

4.2.3.2 On-property mixing

The majority of animals with vendor questionnaire data that were born on the vendor's property had been mixed on the property before leaving the property (94%,Table 4-13). There was no evidence of a large effect on the risk of BRD associated with on-property mixing (

Table 4-17).

4.2.3.3 Mixing history

Cattle in the study had widely varying mixing histories. Just over one-third of animals were mixed prior to day -27 and went into cohorts formed by 10 or more group-13s (22% of animals) or 4 to 9 group-13s (16%,Table 4-13). A high level of mixing between day -12 and cohort close was also common in animals not mixed prior to day -27 (25%10%). The majority of animals were mixed prior to day -27 (67%), and/or during the period from day -12 to cohort close (82%), whereas mixing in the period from days -27 to -13 was much less common (11%).

Group-13 was a key variable because of its use (i) as the lowest clustering variable in the data hierarchy (ii) to define mixing categories and (iii) to draw conclusions about effects of group size before induction. To interpret associations between group-13 variables and risk of BRD, it was important to consider the stability of groups over time. The distribution of animals by time since other animals were added to their group-13 before day -13 is shown in Table 4-16 A and the distribution of animals by time since other animals were added to their group-13 after day -13 is shown in Table 4-16 B. For most cattle (74.9%), other animals had not been added to their group-13 for at least 120 days prior to the start of time at risk and the majority of mixing after day -13 occurred on day 0. Hence, the majority of groups as at day -13 had been stable for at least 3.5 months prior, and remained stable until day 0.

Risk of BRD varied considerably between the different categories of mixing history (Table 4-17). 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. The direct effects of mixing were similar to those observed for the total effects estimates (Table 4-21). Assuming there are no other pathways, this indicates that most of the effect of this risk factor is mediated through the direct pathway, rather than through the presence of a BVDV-PI in the group-28 and BVDV activity in the cohort (the only postulated indirect pathway).
The mixing summary variable was a collapsed version of the mixing history variable and effect estimates (Table 4-17) were consistent with those seen in the 12 category variable.

To further explore the effects of mixing in different time periods, multiple pair-wise comparisons were made (Table 4-18). Comparing mixing and not mixing prior to day -27, if animals were not mixed during days -27 to -13, their risk of BRD was increased if they were not mixed prior to day -27 and they were mixed with 2 or 3, 4 to 9 or ≥ 10 group-13s compared to those with similar mixing of group-13s. The point estimate for those not mixed between day -12 and cohort close was also suggestive of increased risk. However, the credible interval was wide and included one and only some feedlots had animals with the “No, no, no” category. Again comparing mixing and not mixing prior to day -27, if animals were mixed during days -27 to -13 and mixed again between day -12 and cohort close, results were suggestive of increased risk if they were not mixed prior to day -27 but the credible intervals were wide and included one. The estimate comparing those mixed and not mixed prior to day -27 when mixed during days -27 to -13 and not mixed between day -12 and cohort close

was imprecise and the point estimate close to one. Estimates directly comparing mixing or not mixing during days -27 to -13 were imprecise; relatively few animals were mixed in this time period and they were highly clustered by feedlot.

Further comparisons were made for animals not mixed during days -27 to -13 where the categories that were compared differed only in the extent of mixing from day -12 to cohort close date (Table 4-18). Of these animals, those mixed prior to day -27 had higher risk of BRD if the cohort consisted of more than 4 group-13s (4 to 9 group-13s: OR 2.7, 95% credible interval: 1.3 to 4.6, ≥ 10 group-13s: OR 2.1, 95% credible interval: 1.1 to 3.7) compared to 2 or 3 group-13s. A similar trend was observed with animals not mixed prior to day -27 or during days -27 to -13, but the point estimate for the increase in risk was smaller and the credible interval wider and included one.

The PAFs and PARs for total effects were, respectively, 0.57 and 10.1% 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 4-23). Thus, overall BRD incidence was estimated to decline by an absolute amount of 9.7% or 10.1% if it were possible to ensure that all cattle were at the same risk as those whose mixing history was “yes, no, 2 or 3”. The estimates of the PAFs and PARs for the direct effects are slightly lower and less precise.

4.2.3.4 Time of first mixing

To further investigate the importance of time in relation to mixing, we hypothesised that the earliest time point that mixing occurred may be important. The composite mixing history variable gave a clear indication that mixing prior to day -27 was protective and a high level mixing between day -12 and 0 was harmful, but there was a large amount of uncertainty associated with estimates for mixing between days -27 and -13. The time to first mixing variable gave more insight into this. 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 4-13). Animals that were first mixed prior to day -90 (OR 0.6, 95% credible interval: 0.5 to 0.7, Table 4-17) or between day -90 and day -28 (OR 0.6, 95% credible interval: 0.4 to 0.8, Table 4-17) were at moderate to markedly reduced risk compared to animals first mixed between days -12 and cohort closure. 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 4-17) and for those not mixed ever the effect estimate was highly imprecise so no conclusion was possible.

4.2.3.5 Number of animals in a group

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 a group defined close to feedlot entry (e.g. day -13 or day -28) were highly correlated and largely reflected stable group sizes for several months prior to that. The effects of group size therefore need to be compared at different time points and interpreted alongside results for mixing and feedlot move timing. 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. Group size defined at other time points (day -28 or day -91) were included instead in some models where appropriate.

Numbers of animals in group-13s were fairly evenly distributed (Table 4-14). 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) 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 4-19). The direct effects of the number of animals in group-13 were of a similar magnitude to the total effects (Table 4-22). Assuming there are no other pathways, this indicates that most of the effect of this risk factor 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).

The PAFs and PARs for total effects were 0.35 and 6.2% 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 4-23). Thus, overall BRD incidence was estimated to decline by an absolute amount of 6.2 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. The PAFs and PARs for direct effects were slightly lower, corresponding to the slightly reduced protective effect observed in the direct effect model. These results indicated that the number of animals in group-13 was an important risk factor at the population level.

However, as groups are relatively stable over time, it should not be inferred that establishing a large group on (or even shortly before) day -13 will confer the observed protective effect. The distributions and 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 4-14 and Table 4-19). 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) as were animals from group-91s with 100 or more animals (OR: 0.7, 95% credible interval: 0.5 to 1.0). These results suggest that group size should be as large as is practical for at least 28 days before day 0.

There was a significant interaction between induction weight and the number of animals in the group-13 (Figure 4.26). The adverse effect of small group size was compounded in animals in the lowest induction weight category. However, estimates of interaction terms were imprecise, so conclusions focus on the main effects.

4.2.3.6 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 4-14).

Compared to animals transported for less than six 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 4-19). 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). Point estimates for the effects of transporting to the vicinity of the feedlot between days -27 and -13 and

between days -12 to -2 relative to transporting 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 4-21).

The PAFs and PARs for total effects 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 4-23). The estimates of the PAFs and PARs for the direct effects are slightly lower and less precise. Thus, overall BRD incidence was estimated to decline by an absolute amount of 12.1% or 13.3% if it were possible to ensure that all cattle were at the same risk as those moved to the vicinity of the feedlot at least 27 days before day 0.

4.2.3.7 Time from arrival to day 0 in pre-assembly subset

The variable describing the time interval between arrival and day 0 was a component variable of the composite variable describing the timing and transport duration of the move to the feedlot. 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 we hypothesized that in pre-assembly 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, we conducted a subset analysis restricted to animals in the pre-assembly 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 4-14). Compared to animals moved between day -12 and cohort close, those moved prior to day -27 were probably at reduced risk of developing BRD but estimates were imprecise (OR: 0.6, 95% credible interval: 0.2 to 1.5, Table 4-19) and 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 4-19).

4.2.3.8 Saleyard transfer prior to day -27

About a third of the cattle in the study had at least one saleyard transfer prior to day -27 (36%, Table 4-15). The total effect estimate suggested that animals that had been exposed to a saleyard during this time period were at reduced risk compared to those that had not (OR 0.8, 95% credible interval: 0.7 to 0.9, Table 4-20). However, there was no evidence of a moderate or large direct effect (OR: 1.0, 95% credible interval: 0.9 to 1.1, Table 4-22) indicating that most of the effect of this risk factor is mediated through mixing history (the only postulated indirect pathway) rather than through the direct pathway.

4.2.3.9 Saleyard transfer between days -27 and -13

Only 3% of the cattle in the study were exposed to saleyards between days -27 and -13 (Table 4-15). The total effect estimate suggested that animals that had been exposed to a saleyard during this time period were at moderate to markedly higher risk compared to those that had not (OR 1.9, 95% credible interval: 1.3 to 2.7, Table 4-20). However, the direct estimate was reduced and suggested only a probable slight to moderate adverse (OR: 1.3, 95% credible interval: 0.8 to 2.0, Table 4-22). Although this estimate is highly imprecise, it indicates that most, but probably not all,

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

4.2.3.10 Saleyard transfer between days -12 and 0

Only 3% of the cattle in the study were exposed to saleyards from days -12 to 0 (Table 4-15). The total effect estimate indicated that animals that had been exposed to a saleyard during this time period were at markedly increased risk compared to those that had not (OR 2.6, 95% credible interval: 1.6 to 4.1, Table 4-20). The direct effect was attenuated (OR: 1.6, 95% credible interval: 0.9 to 2.6, Table 4-22) but still important, indicating that exposure to saleyards during this time period has a negative effect over and above the effects of mixing history (the only postulated indirect pathway).

The PAFs and PARs for total effects 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 4-23). The PAFs and PARs for direct effects were even lower, corresponding to the reduced effect observed in the direct effect model. These results indicate that exposure to saleyards from days -12 to 0 was not an important risk factor at the population level.

Table 4-13: Putative risk factors relating to mixing; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
On-property mixing (Mix_VQ)*		0.6			
	No^		322	6.4	27.3
	Yes		4,711	93.6	20.9
Mix history prior to day -27, days -27 to -13, day -12 to cohort close		1.1			
	No, no, no^		418	1.2	20.6
	No, no, 2 or 3		1,489	4.3	19.5
	No, no, 4 to 9		3,334	9.6	30.3
	No, no, ≥ 10		5,215	15.0	31.8
	No, yes, yes		629	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		5,409	15.6	16.4
	Yes, no, ≥ 10		7,690	22.1	20.3
	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
Mix summary prior to day -27, group-28s in cohort		1.1			
	No, 1 to 3		2,314	6.7	16.7
	No, ≥ 4		9,178	26.4	30.2
	Yes, 1 to 3		8,195	23.6	4.6
	Yes, ≥ 4		15,043	43.3	17.3
Time of earliest mixing (Mix first)		0.9			
	Prior to day -90		21,623	62.1	13.5
	Day -90 to -28		1,725	5.0	4.6
	Day -27 to -13		1,053	3.0	11.2
	Day -12 to 0		10,009	28.7	29.4
	Not mixed^		418	1.2	20.6

^ Categories where 7 or more feedlots have no observations
*Vendor questionnaire subset 1

Table 4-14: 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 incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
No. animals in group-91 (Group-91N)		1.1			
	< 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)		0.0			
	< 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)		0.0			
	< 50		13,782	39.2	24.1
	50 to 99		9,783	27.9	21.3
	≥ 100		11,566	32.9	6.9
Move to feedlot Days before day 0 and hours of transport		0.0			
	Prior to day -27^		1,893	5.4	1.5
	Days -27 to -13^		1,987	5.7	4.6
	Days -12 to -2; < 6 hours		2,183	6.2	10.9
	Days -12 to-2; ≥6 hours		2,339	6.7	8.0
	Days -1 to 0; < 6 hours		17,139	48.8	19.9
	Days -1 to 0; ≥ 6 hours		9,590	27.3	23.5
Arrival to day 0*		0.0			
	≥ 28		1,733	30.7	1.5
	27 to 13		1,713	30.4	5.3
	12 to 0		2,195	38.9	3.3

^ Categories where 7 or more feedlots have no observations

* Restricted to pre-assembly subset, N=5,641

Table 4-15: Putative risk factors relating to transfers through a saleyard; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Saleyard prior to day -27		1.1			
	No		22,223	64.0	18.7
	Yes		12,507	36.0	15.7
Saleyard days -27 to -13		0.0			
	No		34,162	97.2	17.8
	Yes		969	2.8	11.2
Saleyard days -12 to 0		0.0			
	No		34,200	97.4	17.6
	Yes		931	2.7	21.4

Table 4-16: A: Distribution of animals by last day before day -12 that other animals were added to their group-13 (i.e. no other animals added to the group after this time point and before the start of day - 12). B: Distribution of animals by first day after day -13 that other animals were added to their group-13 (i.e. no other animals added to the group after day -13 before this time point).

A

Last day before day -12 that animals were added to group	% animals
Day -27 to day -13	12.1
Day -44 to -28	3.5
Day -59 to -45	1.4
Day -89 to -60	3.1
Day -119 to -90	5.0
Day -364 to -120	37.4
Origin to day -365	37.5

B

First day after day -13 that animals were added to group	% animals
Day -12 to -6	5.2
Day -5 or -4	2.2
Day -3	2.4
Day -2	4.7
Day -1	16.8
Day 0	68.7

Table 4-17: 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, DIC
On-property mixing (Mix_VQ)*					()	N = 5,033 3 level
	No^	Ref. cat.				
	Yes	1.1	(0.7 to 1.6)	0.463		
Mix history prior to day -27, days -27 to -13, day -12 to cohort close					(Cohort fill, Weight, SY-12 to 0, SY-27 to -13, SYpre-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. cat.				
	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		
Mix summary prior to day -27, group-28s in cohort					(Cohort fill, Weight, SY-12 to 0, SY-27 to -13, SYpre-27, CohortN, Move_FL, Group-13N)	N = 34,726 4 level
	No, 1 to 3	2.1	(1.3 to 3.3)	0.001		
	No, ≥ 4	3.6	(2.1 to 5.7)	< 0.001		
	Yes, 1 to 3	Ref. cat.				
	Yes, ≥ 4	2.3	(1.3 to 3.6)	0.001		
Time of earliest mixing (Mix first)					Weight, SY-12 to 0, SY-27 to -13, SYpre-27, Group-91N, Arrival to day0)	N=34,725, 4 level
	Prior to 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. cat.				
	Not mixed	1.0	(0.2 to 2.9)	0.350		

^ Categories where 7 or more feedlots have no observations

* Vendor questionnaire subset 1

Table 4-18: Putative risk factors relating to mixing – comparison of mixing categories within time periods. Adjustment set (Cohort fill, Weight, SY-12 to 0, SY-27 to -13, SY pre27, CohortN, Move_FL, Group-13N).

Mix history category	Odds ratio	95% Cred Int	Prob </>1
Yes, no, 2 or 3	Ref. cat.		
No, no, 2 or 3	2.3	(1.3 to 3.7)	0.003
Yes, no, 4 to 9	Ref. cat.		
No, no, 4 to 9	1.3	(1.0 to 1.8)	0.026
Yes, no, ≥ 10	Ref. cat.		
No, no, ≥ 10	1.7	(1.4 to 2.0)	< 0.001
Yes, no, no	Ref. cat.		
No, no, no^	2.4	(0.3 to 8.9)	0.248
Yes, yes, yes^	Ref. cat.		
No, yes, yes	1.6	(0.9 to 2.5)	0.044
Yes, yes, no^	Ref. cat.		
No, yes, no^	1.0	(0.3 to 2.2)	0.382
Yes, no, no	Ref. cat.		
Yes, yes, no^	2.8	(0.6 to 8.7)	0.118
No, no, no^	Ref. cat.		
No, yes, no^	1.8	(0.3 to 5.8)	0.346
Yes, no, no	1.1	(0.5 to 2.4)	0.455
Yes, no, 2 or 3	Ref. cat.		
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
No, no, no^	1.0	(0.2 to 3.3)	0.349
No, no, 2 or 3	Ref. cat.		
No, no, 4 to 9	1.5	(0.7 to 2.8)	0.138
No, no, ≥ 10	1.6	(0.7 to 3.0)	0.118
Yes, yes, yes^	Ref. cat.		
Yes, yes, no^	1.4	(0.4 to 3.3)	0.376
No, yes, yes	Ref. cat.		
No, yes, no^	0.8	(0.2 to 2.4)	0.254

^ Categories where 7 or more feedlots have no observations

Table 4-19: 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. cat.				
	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. cat.				
	50 to 99	0.8	(0.6 to 0.9)	0.002		
	≥ 100	0.5	(0.3 to 0.6)	< 0.001		
No. animals in group-13 (Group-13N)					()	N = 35,131 4 level
	< 50	Ref. cat.				
	50 to 99	0.8	(0.7 to 0.9)	0.002		
	≥ 100	0.5	(0.4 to 0.7)	< 0.001		
Move to feedlot Days before day 0 and hours of transport					(SY -12 to 0, SY-27 to 13)	N = 35,131 4 level
	Prior to 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 hours	0.9	(0.6 to 1.3)	0.217		
	Days -12 to -2; ≥ 6 hours	0.9	(0.5 to 1.4)	0.305		
	Days -1 to 0; < 6 hours	Ref. cat.				
	Days -1 to 0; ≥ 6 hours	1.2	(1.0 to 1.5)	0.016		
Days from arrival to day 0*					(Breed, Weight, Season, SY-27 to 0, SYpre-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. cat				

^ Categories where 7 or more feedlots have no observations

* Restricted to preassembly feedlots

Table 4-20: 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, DIC
Saleyard transfer prior to day -27					()	N = 34,730 4 level
	No	Ref. cat.				
	Yes	0.8	(0.7 to 0.9)	< 0.001		
Saleyard transfer days -27 to -13					()	N = 35,131 4 level
	No	Ref. cat.				
	Yes	1.9	(1.3 to 2.7)	0.001		
Saleyard transfer days -12 to 0					()	N = 35,131 4 level
	No	Ref. cat.				
	Yes	2.6	(1.6 to 4.1)	<0.001		

Table 4-21: Estimated odds ratios for the direct effects of mixing history and move to the feedlot on the risk of BRD by day 50

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level, DIC
Mix history prior to day -27, days -27 to -13, day -12 to cohort close					(Cohort fill, Weight, SY-12 to 0, SY-27 to -13, SYpre-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. cat.				
	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		
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
	Prior to 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 hours	0.9	(0.6 to 1.3)	0.275		
	Days -12 to-2; ≥6 hours	0.9	(0.6 to 1.5)	0.346		
	Days -1 to 0; < 6 hours	Ref. cat.				
	Days -1 to 0; ≥ 6 hours	1.2	(1.0 to 1.5)	0.012		

Table 4-22: Estimated odds ratios for the direct effects of moving through a saleyard and the number of animals in group-13 on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level, DIC
Animals in group-13 (Group-13N)					(CohortN, Cohort fill, Weight, SY-12 to 0, SY-27 to -13, SYpre-27, FeedlotN, Mix history, Move_FL)	N = 34,726 4 level
	< 50	Ref. cat.				
	50 to 99	0.8	(0.7 to 1.0)	0.009		
	≥ 100	0.6	(0.4 to 0.8)	0.001		
Saleyard transfer prior to 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. cat.				
	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. cat.				
	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. cat.				
	Yes	1.6	(0.9 to 2.6)	0.049		

Table 4-23: Estimated population attributable fractions (PAF) and population attributable risks (PAR) for the total and direct effects of mixing, moving through a saleyard, move to the feedlot and number of animals in a group-13 on the risk of BRD by day 50. Estimates are derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR	WinBUGS PAR	WinBUGS 95% cred int
Total effects						
Mixing history	0.57	0.55	(0.30 to 0.72)	10.10	9.70	(5.3 to 12.7)
No. animals in group-13 (Group-13N)	0.35	0.39	(0.23 to 0.51)	6.20	6.90	(4.1 to 9.1)
Move to feedlot	0.69	0.75	(0.57 to 0.88)	12.09	13.30	(10.1 to 15.5)
Saleyard transfer days -12 to 0	0.02	0.02	(0.02 to 0.02)	0.30	0.30	(0.3 to 0.3)
Direct effects						
Mixing history	0.52	0.46	(0.17 to 0.69)	9.14	8.18	(2.93 to 12.10)
No. animals in group-13 (Group-13N)	0.30	0.30	(0.10 to 0.44)	5.25	5.22	(1.78 to 7.80)
Move to feedlot	0.59	0.48	(-0.14 to 0.81)	10.40	8.52	(-2.38 to 14.35)
Saleyard transfer days -12 to 0	0.01	0.00	(0.00 to 0.01)	0.09	0.08	(-0.03 to 0.15)

4.2.4 Putative risk factors relating to formation of the cohort

4.2.4.1 Number of animals in cohort

Two-thirds of the cattle in the study population were in cohorts of at least 200 animals (65% of animals, Table 4-24). 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 4-25).

4.2.4.2 Mean cohort weight

About half of the cattle in the study population were in cohorts where the mean weight was 425 to < 455 kg (50% of animals, Table 4-24). After adjusting for individual animal weight, there was no evidence of a large effect of the mean cohort weight on feed and risk of BRD (Table 4-25).

4.2.4.3 Sex of cohort

Most of the cattle in the study were in cohorts of steers only (91%, Table 4-24). Heifer only and mixed-sex cohorts were restricted to a small number of feedlots. The estimates for the effect of the sex of the cohort on the risk of BRD were consistent with a moderate effect but were very imprecise so no conclusion is possible, probably because the distribution of the categories was clustered by feedlot (Table 4-25).

4.2.4.4 Cohort fill duration

The majority of the cattle in the study were in cohorts that were filled over more than one day (66% of animals, Table 4-24). 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). 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 4-26) indicating that most of the effect of this risk factor is 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.

The PAFs and PARs for total effects 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 4-27). Thus, overall BRD incidence was estimated to decline by an absolute amount of 6.2 to 6.4% if it were possible to ensure that all cattle were at the same risk as those cohort fill duration was one day. The PAFs and PARs for the direct effects were much lower and the credible intervals include zero, corresponding to the much reduced effect observed in the direct effect model.

4.2.4.5 Days from DOF1 to day 0

For the majority of the cattle in the study, the first day on feed was the same date as the induction date (81% of animals, Table 4-24). Animals for which the first day on feed occurred earlier than the induction date were restricted to a small number of feedlots. 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 at least three days was very imprecise probably because this was a sparse category and was restricted to a few feedlots (Table 4-25).

4.2.4.6 Days from day 0 to cohort close

For more than half of the cattle in the study, day 0 was the cohort close date (57%) but for a small proportion of cattle (8%) the cohort close date was at least 7 days after day 0 (Table 4-24). 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 same date as the cohort close date (Table 4-25). The direct effect of the number of days from day 0 to cohort close was slightly lower than the total effects (Table 4-26), indicating that most of the effect of this risk factor is 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).

The PAFs and PARs for total effects 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 4-27). Thus, overall BRD incidence was estimated to decline by an absolute amount of 2.8% if it were possible to ensure that all cattle were at the same risk as those whose cohort close date was day 0. The PAFs and PARs for direct effects were slightly lower, corresponding to the slightly reduced protective effect observed in the direct effect model.

Table 4-24: Putative risk factors relating to cohort formation; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing % / Distribution^	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
No. animals in cohort (CohortN)		0.0			
	< 200		12,243	34.8	11.5
	≥ 200		22,888	65.2	20.9
Mean cohort weight (kg)		0.0			
	< 425		8,615	24.5	14.0
	425 to < 455		17,694	50.4	20.7
	≥ 455		8,822	25.1	15.2
Sex cohort		0.0			
	Male		31,854	90.7	18.7
	Female^		2,405	6.8	6.0
	Mixed^		872	2.5	11.6
Cohort fill duration (days)		0.0			
	1		12,051	34.3	7.4
	> 1		23,080	65.7	23.0
Days from DOF1 to day 0		0.0			
	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		0.0			
	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 have no observations

Table 4-25: Estimated odds ratios for the total effects of putative risk factors relating to cohort formation 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 cohort (CohortN)					(Group-13N, FeedlotN)	N = 35,131 4 level
	< 200	Ref. cat.				
	≥ 200	1.2	(0.7 to 1.8)	0.254		
Mean cohort weight (kg)					(CohortN, Weight)	N = 35,126 4 level
	< 425kg	Ref. cat.				
	425 to < 455kg	0.8	(0.6 to 1.1)	0.117		
	≥ 455kg	1.0	(0.6 to 1.6)	0.489		
Sex cohort					(Sex)	N = 35,131 4 level
	Male	Ref. cat.				
	Female^	0.7	(0.2 to 1.9)	0.266		
	Mixed^	0.6	(0.05 to 2.9)	0.166		
Cohort fill duration (days)					(CohortN, DOF1 to day0)	N = 35,131 4 level
	1	Ref. cat.				
	> 1	1.9	(1.2 to 2.8)	0.005		
Days from DOF1 to day 0					()	N = 35,131 4 level
	0	Ref. cat.				
	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
	1	Ref.cat.				
	1 to 6	0.8	(0.7 to 1.0)	0.008		
	≥ 7	0.7	(0.5 to 0.9)	0.004		

Table 4-26: Estimated odds ratios for the direct effects of putative risk factors relating to cohort formation on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level, DIC
Cohort fill duration (days) Model A					(CohortN, Day0 to close, DOF1 to day0, Weight, SY-12 to 0, SY-27 to -13, SYpre-27, Group-13N, Mix history, Move_FL)	N=34,726 4 level DIC:23,434
	1	Ref. cat.				
	> 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, SYpre-27, Group-13N, Mix history, Move_FL)	N=34,726 4 level DIC:23,440
	1	Ref. cat.				
	> 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. cat.				
	1 to 6	0.8	(0.7 to 1.0)	0.026		
	≥ 7	0.8	(0.5 to 1.1)	0.067		

Table 4-27: Estimated population attributable fractions (PAF) and population attributable risks (PAR) for the total and direct effects of risk factors relating to cohort formation where study results indicated either a protective or an adverse effect on the risk of BRD by day 50. Estimates are derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR	WinBUGS PAR	WinBUGS 95% cred int
Total effects						
Cohort fill duration (days)	0.37	0.35	(0.09 to 0.53)	6.40	6.19	(1.65 to 9.36)
Days from day 0 to cohort close	0.16	0.16	(-0.01 to 0.31)	2.80	2.84	(-0.11 to 5.42)
Direct effects						
Cohort fill duration (days)	0.12	0.26	(-0.15 to 0.53)	2.06	4.60	(-2.60 to 9.28)
Days from day 0 to cohort close	0.14	0.13	(-0.03 to 0.32)	2.49	2.2	(- 0.53 to 5.64)

4.2.5 Putative risk factors relating to source region, feedlot region, the timing of the induction period and weather in the first week after day 0

4.2.5.1 Source region

The most common regions from which cattle were sourced were Darling Downs/New England (25%) and Western NSW/QLD/NT (24%,Table 4-28). Cattle from five of the six source regions went to seven or less of the participating feedlots. 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 4-29).

4.2.5.2 Feedlot region

The majority of cattle in the study were inducted into southern feedlots (62%, Table 4-28). 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 4-29). 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 4-30). As these estimates were very imprecise and, from a biological perspective, as feedlot region is a crude variable that captures a large number of causal factors collectively, no causal inference about the effects of region should be made. However, the PAFs and PARs were massive indicating that this series of causal factors collectively has enormous effects (Table 4-36). As the direct effect PAF and PAR were also large, there are large effects of region over and above breed, weight and sex (the high quality adjustment variables).

4.2.5.3 Induction season

The distribution of the induction season for the cattle in the study was fairly balanced across seasons (21 to 29% of animals, Table 4-31). 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 4-32). The PAFs and PARs for the total effects 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 4-36). Thus, overall BRD incidence was estimated to decline by an absolute amount of 5.0% or 5.3% if it were possible to ensure that all cattle were at the same risk as those inducted during spring.

There was a significant interaction between breed and season displayed graphically in Figure 4.25. The adverse effect of autumn was compounded in Herefords. However, estimates of interaction terms were very imprecise, so conclusions are based on the main effects.

4.2.5.4 Induction year

The majority of the cattle in the study were inducted in 2011 (54%, Table 4-31). The estimates for the total effect of year on the risk of BRD were imprecise so no conclusion was possible.

4.2.5.5 Mean of daily maximum temperatures in week one

The means of the daily maximum temperatures in week one were commonly 17 to < 23°C (32% of animals) or 23 to < 30°C (36%, Table 4-31). There was no consistent evidence of a large effect of maximum temperature on the risk of BRD across the models fitted using the three minimal sufficient adjustment sets (Table 4-32).

4.2.5.6 Mean of daily minimum temperatures in week one

The means of the daily minimum temperatures in week one were commonly 5 to < 11°C (36%) or 11 to < 17°C (27%, Table 4-31). There was no consistent evidence of a large effect of minimum temperature on the risk of BRD across the models fitted using the three minimal sufficient adjustment sets. 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 4-33).

4.2.5.7 Mean of daily temperature ranges in week one

The means of the daily temperature ranges in week one were commonly 11 to <16°C (63%, Table 4-31). There was no evidence of a large effect of temperature range on the risk of BRD (Table 4-33).

4.2.5.8 Total rainfall in week one

The total rainfall in week one was most commonly 0.1 to < 4 mm (28%) or 4 to < 25 mm (37%, Table 4-31). 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 4-34) but in all three models there was a possible adverse effect of 4 to < 25mm rain compared to no rain.

4.2.5.9 Mean of daily maximum windspeeds in week one

The means of the daily maximum windspeeds in week one were most commonly 35 to < 45km/h (56%, Table 4-31). There was no consistent evidence of a large effect of mean maximum windspeed on the risk of BRD across the models fitted using the three minimal sufficient adjustment sets (Table 4-35).

Table 4-28: Putative risk factors relating to move to the feedlot, source region and feedlot region; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Source region		0.02			
	NSW Central & Southern Tablelands ^		6,251	17.8	21.5
	Coastal NSW or Queensland^		1,224	3.5	18.3
	Darling Downs / New England^		8,900	25.3	6.9
	Western NSW /Qld or NT		8,452	24.1	19.1
	NSW Riverina, Victoria & Tasmania^		6,188	17.6	33.2
	South Australia/ Western Australia^		4,110	11.7	13.2
Feedlot region		0.0			
	North^		13,342	38.0	5.4
	South^		21,789	62.0	25.1

^ Categories where 7 or more feedlots have no observations

Table 4-29: 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, DIC
Source region Model A					(Feedlot region)	N = 35,125 4 level DIC = 23,762
	NSW Central & Southern Tablelands ^	Ref. cat.				
	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 DIC = 23,760
	NSW Central & Southern Tablelands ^	Ref. cat.				
	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. cat.				
	South^	22.1	(1.6 to 99.3)	0.011		

^ Categories where 7 or more feedlots have no observations

Table 4-30: 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, DIC
Feedlot region Model A					(Breed, Grain type, Weight, Rain, Season, Sex, Temp max, Temp min, Wind, Dentition, Source region)	N=34,361 3 level DIC = 24,258
	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 DIC =23,760
	North^					
	South^	23.8	(0.8 to 132.6)	0.041		

^ Categories where 7 or more feedlots have no observations

Table 4-31: 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 incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Induction season		0.0			
	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		0.0			
	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)		0.0			
	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)		0.0			
	< 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)		0.0			
	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)		0.0			
	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/hr)		0.0			
	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 have no observations

Table 4-32: 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, DIC
Induction season					()	N = 35,131 4 level
	Spring	Ref. cat.				
	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. cat.				
	2010	0.8	(0.4 to 1.4)	0.200		
	2011	0.8	(0.5 to 1.4)	0.239		
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 DIC = 24,256
	11 to < 17	Ref. cat.				
	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 DIC = 23,760
	11 to < 17	Ref. cat.				
	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 DIC = 23,765
	11 to < 17	Ref. cat.				
	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 4-33: 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, DIC
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 DIC = 24,256
	< 5	Ref. cat.				
	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 DIC = 23,760
	< 5	Ref. cat.				
	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 DIC = 23764
	< 5	Ref. cat.				
	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. cat.				
	11 to < 16	1.1	(0.7 to 1.6)	0.404		
	≥16	1.0	(0.6 to 1.6)	0.448		

Table 4-34: 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, DIC
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 DIC = 24,256
	0	Ref. cat.				
	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 DIC = 23,760
	0	Ref. cat.				
	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 DIC = 23,766
	0	Ref. cat.				
	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 4-35: 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, DIC
Mean of daily maximum wind speeds in week 1 (km/hr) Model A					(Dentition, Breed, Grain type, Weight, Rain, Season, Sex, Temp max, Temp min, Source region)	N = 34,361 3 level DIC = 24,256
	20 to < 35	Ref. cat.				
	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/hr) Model B					(Grain type, Induction year, Rain, Season, Temp max, Temp min, Source region)	N = 35,125 4 level DIC = 23,760
	20 to < 35	Ref. cat.				
	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/hr) Model C					(Feedlot region, Induction year, Season)	N = 35,131 4 level DIC = 23,765
	20 to < 35	Ref. cat.				
	35 to < 45	0.9	(0.7 to 1.2)	0.239		
	≥ 45	0.9	(0.6 to 1.3)	0.278		

Table 4-36: Estimated population attributable fractions (PAF) and population attributable risks (PAR) for the total effects of season and feedlot region on the risk of BRD by day 50. Estimates are derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR	WinBUGS PAR	WinBUGS 95% cred int
Total effects						
Season	0.30	0.28	(0.12 to 0.40)	5.3	5.0	(2.2 to 7.0)
Feedlot region	0.80	0.74	(0.62 to 0.81)	14.0	13.1	(10.9 to 14.2)
Direct effects						
Feedlot region*	0.76	0.72	(0.47 to 0.82)	13.4	12.8	(8.8 to 14.4)

* Using model A from Table 4-30

4.2.6 Putative risk factors relating to pen characteristics

4.2.6.1 Stocking density

The stocking density was commonly 11 to < 14m^2/SCU (41% of animals) or 14 to < 17m^2/SCU (31%,Table 4-37). 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 4-38).

4.2.6.2 Pen shade

About two-thirds of the cattle in the study were in pens with some shade (69%,Table 4-37). Pen shade did not vary between study cohorts in eleven of the fourteen feedlots. 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 4-38).

4.2.6.3 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 4-37).

Shared pen water was associated with a markedly increased risk of BRD (OR 3.6, 95% credible interval: 1.3 to 8.8,Table 4-38). Pen water access status did not vary between study cohorts in ten of the fourteen feedlots. Results from subset analysis using only data from the four feedlots with disparate values for study cohorts were similar (OR 4.2, 95% credible interval: 1.5 to 9.3), indicating that the observed increase in risk was not confounded in any important way by feedlot.

The PAFs and PARs (using the full dataset) were 0.67 and 11.8% 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 (Table 4-40). Thus, overall BRD incidence was estimated to decline by an absolute amount of 11.8% or 12.3% if it were possible to ensure that all cattle were at the same risk as those whose pen water was not accessible by cattle in another pen.

In further analyses, total and direct effect estimates at the animal level of having shared pen water were compared within the case-control dataset, in which change in serostatus to each of the four viruses were postulated intervening variables between shared pen water and BRD. The total effect estimate from this subset of data was similar to the full dataset (OR 5.0, 95% credible interval: 1.4 to 14.6, Table 4-38). The direct effect was estimated by adjusting for change in serostatus (up/no change/initially high) to each of the four viruses (the only postulated indirect pathways) at animal level. The direct effect estimate was attenuated (OR: 3.1, 95% credible interval: 1.0 to 7.7,Table 4-39) supporting the hypothesised mechanism but also suggesting that exposure to pen water accessible to another pen has a negative effect over and above the effects of increase in serostatus to one or more of the four viruses.

4.2.6.4 Number of adjoining pens

Of the cattle in the study, 70% were in pens that had two (rather than one) other pens adjoining (Table 4-37). 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).

4.2.6.5 Bunk space

Forty-five percent of the cattle in the study were in pens with bunk spaces of 0.18 to < 0.24 m/head (Table 4-37). 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 4-38).

Table 4-37: Putative risk factors relating to pen characteristics; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Stocking density (m^2/standard cattle unit)		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
Pen shade		0.00			
	None		11,141	31.7	9.6
	Any		23,990	68.3	21.4
Shared pen water		0.00			
	No^		6,453	18.4	3.9
	Yes		28,678	81.6	20.7
Number of adjoining pens		0.00			
	1		10,394	29.9	14.7
	2		24,391	70.1	19.1
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 have no observations

Table 4-38: 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. cat.				
	14 to < 17	1.1	(0.5 to 2.0)	0.489		
	17 to < 25	0.8	(0.3 to 1.5)	0.210		
	≥ 25^	1.2	(0.5 to 2.5)	0.394		
Pen shade					()	N = 35,131 4 level
	No	Ref. cat.				
	Yes	1.7	(0.8 to 3.4)	0.108		
Shared pen water					()	N = 35,131 4 level
	No^	Ref. cat.				
	Yes	3.6	(1.3 to 8.8)	0.006		
Shared pen water (only feedlots with both categories)					()	N = 14,210 4 level
	No	Ref. cat.				
	Yes	4.2	(1.5 to 9.3)	0.001		
Shared pen water (case-control study)					()	N = 7,314 3 level
	No	Ref. cat.				
	Yes	5.0	(1.4 to 14.6)	0.001		
Number of adjoining pens					()	N = 34,785 3 level
	1	Ref. cat.				
	2	1.1	(0.6 to 1.6)	0.394		
Bunk space (m/head)					(CohortN)	N=33,967 3 level
	< 0.18^	Ref. cat.				
	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		

^ Categories where 7 or more feedlots have no observations

Table 4-39: Estimated odds ratios for the direct effect of shared pen water on the risk of BRD by day 50 estimated from the case-control dataset.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level, DIC
Shared pen water (case-control study)					(BPI3seroinc BRSVseroinc, BVDVseroinc, BVDV_grp_cht, BVDV_PI_animal BHV1seroinc, CohortN, Rhinogard, Mix summary)	N = 6,477 3 level
	No	Ref. cat.				
	Yes	3.1	(1.0 to 7.5)	0.027		
Shared pen water (case-control study)					VirusN_seroinc, BVDV_grp_cht, BVDV_PI_animal BHV1seroinc, CohortN, Rhinogard, Mix summary	N=6,477 3 level
	No	Ref. cat.				
	Yes	3.3	(1.1 to 7.7)	0.018		

Table 4-40: Estimated population attributable fractions (PAF) and population attributable risks (PAR) for the total effects of shared pen water on the risk of BRD by day 50. Estimates are derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR	WinBUGS PAR	WinBUGS 95% cred int
Shared pen water	0.67	0.70	(0.45 to 0.83)	11.8	12.3	(7.9 to 14.7)

4.2.7 Putative risk factors relating to ration characteristics

4.2.7.1 Prior grain feeding

The majority of animals with vendor questionnaire data that were born on the vendor's property had not been fed grain before leaving the property (77%, Table 4-41). Estimates were suggestive of a possible decrease in the risk of BRD associated with prior feeding of grain, but the estimates were imprecise (OR 0.7, 95% credible interval: 0.4 to 1.4, Table 4-42).

4.2.7.2 Prior conserved forage or supplement

The majority of animals with vendor questionnaire data that were born on the vendor's property had been fed conserved forage or supplement before leaving the property (84%, Table 4-41). There was no evidence of a large effect on the risk of BRD associated with prior feeding of conserved forage or supplement (Table 4-42).

4.2.7.3 Grain type

The most commonly fed grain types were barley (48%) and wheat mix (40%, Table 4.41). Grain type was highly clustered by feedlot. 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 4-42). Such inconsistencies were likely to be due to confounding by feedlot. The models were supportive of a protective effect of sorghum but the estimates were very imprecise so no conclusion can be reached.

4.2.7.4 Percentage grain on day 0

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 4-41). Estimates for the total effect of the percentage of grain in the ration fed on day 0 on the risk of BRD were imprecise probably because the distribution of the categories was clustered by feedlot (Table 4-43).

4.2.7.5 Percentage grain on day 20

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 4-41). Estimates for the total effect of the percentage of grain in the ration fed on day 20 on the risk of BRD were imprecise probably because the distribution of the categories was clustered by feedlot (Table 4-43). Estimates were consistent across the models fitted using the two minimal sufficient adjustment sets.

4.2.7.6 Days to 60% grain

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 4-41). Estimates for the total effect of 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 4-43). Estimates were fairly consistent across the models fitted using the two minimal sufficient adjustment sets.

Table 4-41: Putative risk factors relating to prior feeding and ration characteristics; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Prior grain feeding (Grain pre)*		20.6			
	No		3,082	76.6	24.9
	Yes		940	23.4	16.4
Prior conserved forage or supplement (Supp pre)*		20.6			
	No		659	16.4	28.8
	Yes		3,363	83.6	21.7
Grain type		0.0			
	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		0.0			
	< 35%		7,762	22.1	16.5
	35 to < 40%		8,322	23.9	32.0
	40 to < 45%		9,007	25.6	9.5
	≥ 45%		10,040	28.6	14.0
Grain % on day 20		0.0			
	< 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		0.0			
	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 have no observations
*Analysed in the vendor questionnaire subset1 dataset

Table 4-42: 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, DIC
Prior grain feeding (Grain pre)*					(Yard weaning, Induction year, Source region)	N = 4,022 3 level
	No	Ref. cat.				
	Yes	0.7	(0.4 to 1.2)	0.109		
Prior conserved forage/supplement (Supp pre)*					(Yard weaning, Induction year, Source region)	N = 4,022 3 level
	No	Ref. cat.				
	Yes	0.8	(0.4 to 1.4)	0.150		
Grain type Model A					(Dentition, Breed, Weight, Rain, Wind, Season, Sex, Temp max, Temp min, Source region)	N = 34,361 3 level DIC = 24,256
	Barley	Ref. cat.				
	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 DIC = 23,760
	Barley	Ref. cat.				
	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 DIC = 23,762
	Barley	Ref. cat.				
	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 have no observations
*Analysed using the vendor questionnaire subset1 dataset

Table 4-43: 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, DIC
Grain % on day 0					(Day 0_close, DOF1_day 0, Intended DOF)	N = 35,131 4 level
	< 35%^	Ref. cat.				
	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 DIC=23,771
	< 60%^	Ref. cat.				
	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 DIC = 23,770
	< 60%^	Ref. cat.				
	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 DIC=23,769
	0 to 6^	Ref. cat.				
	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 DIC=23,770
	0 to 6^	Ref. cat.				
	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 have no observations

4.2.8 Putative risk factors relating to induction treatments

4.2.8.1 Rhinogard™ at induction

Most of the cattle in the study were vaccinated with Rhinogard™ at induction (79%, Table 4-44) and Rhinogard™ use was completely clustered by feedlot (i.e. within feedlots, either all animals or no animals received Rhinogard™). Although the estimate was imprecise, vaccination with Rhinogard™ was associated with a markedly increased risk of BRD (OR 5.3, 95% credible interval: 0.4 to 17.0,Table 4-45). 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. This confounding could be minimised if effects of Rhinogard™ were assessed using a randomised controlled trial, to control for confounding at the feedlot level.

4.2.8.2 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 (Table 4-44). There was no evidence of a large effect on the risk of BRD (Table 4-45).

Table 4-44: Putative risk factors relating to induction treatments; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Rhinogard™ at induction		0.0			
	No^		7,365	21.0	2.8
	Yes		27,766	79.0	21.6
Vitamin ADE at induction		0.0			
	No		24,518	69.8	17.1
	Yes^		10,613	30.2	18.9

^ Categories where 7 or more feedlots have no observations

Table 4-45: 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. cat.				
	Yes	5.3	(0.4 to 17.0)	0.090		
Vitamin ADE at induction (Vit ADE)					()	N = 35,131 4 level
	No	Ref. cat.				
	Yes^	1.1	(0.6 to 1.9)	0.364		

^ Categories where 7 or more feedlots have no observations

4.2.9 Putative risk factors relating to numbers of animals on feed in the feedlot

4.2.9.1 Number of animals on feed in the feedlot in the animal's month of induction

Nearly 40% of the cattle in the study were at feedlots where there were 10,000 to < 20,000 cattle on feed at the start of or during the animal's induction month (Table 4-46). Estimates for the total effect on the risk of BRD were imprecise probably because the distribution of the categories was clustered by feedlot (Table 4-47).

4.2.9.2 Number of animals less than 40 days on feed in the month of induction

Nearly 40% of study animals were at 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 4-46). Estimates for the total effect on the risk of BRD were imprecise probably because the distribution of the categories was clustered by feedlot (Table 4-47).

Table 4-46: 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 incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
Number on feed in animal's induction month (FeedlotN)		0.0			
	< 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)		0.0			
	< 3,000^		11,240	32.8	6.5
	3,000 to < 6,000^		12,793	37.3	18.6
	≥ 6,000^		10,269	29.9	29.3

^ Categories where 7 or more feedlots have no observations

Table 4-47: 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^					
	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. cat.				
	3,000 to < 6,000^	1.3	(0.4 to 2.9)	0.353		
	≥ 6,000^	1.1	(0.3 to 2.6)	0.531		

^ Categories where 7 or more feedlots have no observations

4.2.10 Other putative risk factors derived from the vendor questionnaire

4.2.10.1 Weaning method

The majority of animals with vendor questionnaire data that were born on the vendor's property were yard weaned (80%, Table 4-48) 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 4-49). The effect was similar for those weaned over less than seven and at least seven days.

The PAFs and PARs were 0.08 and 1.6% 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 4-50). Thus, overall BRD incidence was estimated to decline by an absolute amount of 1.6% or 1.7% if it were possible to ensure that all cattle were at the same risk as those that were yard weaned.

4.2.10.2 Prior Bovilis MH™ vaccination

The majority of animals with vendor questionnaire data that were born on the vendor's property or were purchased prior to 10 months of age had not been vaccinated with Bovilis MH™ prior to day -14 (85%,Table 4-48). 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 4-49).

The PAFs and PARs 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 4-50). Thus, overall BRD incidence was estimated to decline by an absolute amount of 3.3% if it were possible to ensure that all cattle were at the same risk as those that were vaccinated with Bovilis MH™ prior to day -14.

4.2.10.3 Prior Pestigard™ vaccination

The majority of animals with vendor questionnaire data that were born on the vendor's property or were purchased prior to 10 months of age had not been vaccinated with Pestigard™ prior to day -14 (88%,Table 4-48). 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 4-49).

The PAFs and PARs 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 4-50). Thus, overall BRD incidence was estimated to decline by an absolute amount of 3.2% if it were possible to ensure that all cattle were at the same risk as those that were vaccinated with Pestigard™ prior to day -14.

Table 4-48: Putative risk factors relating to the vendor questionnaire data; distribution by category, percentage missing and crude 50-day BRD incidence risk.

Variable	Category	Missing % / Distribution^	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
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

^ Categories where 7 or more feedlots have no observations
*Analysed in the vendor questionnaire subset 1 dataset
#Analysed in the vendor questionnaire subset 2 dataset

Table 4-49: Estimated odds ratios for the total effects of risk factors relating to the vendor questionnaire 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. cat.				
	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. cat.				
	Yes	0.8	(0.6 to 1.0)	0.020		
Prior Pestigard™ vaccination (PV_vacc)#					()	N = 8,045 3 level
	No	Ref. cat.				
	Yes	0.8	(0.5 to 1.1)	0.054		

*Analysed in the vendor questionnaire subset 1 dataset
#Analysed in the vendor questionnaire subset 2 dataset

Table 4-50: Estimated population attributable fractions (PAF) and population attributable risks (PAR) for the total effects of yard weaning, prior Bovilis MH™ and prior Pestigard™ vaccination on the risk of BRD by day 50. Estimates are derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR	WinBUGS PAR	WinBUGS 95% cred int
Yard weaning	0.08	0.08	(0.01 to 0.13)	1.60	1.67	(0.18 to 2.78)
Prior Bovilis MH™ vaccination	0.18	0.18	(0.01 to 0.32)	3.30	3.27	(0.25 to 5.99)
Prior Pestigard™ vaccination	0.17	0.17	(-0.03 to 0.34)	3.20	3.21	(-0.59 to 6.34)

4.2.11 Putative BVDV risk factors

4.2.11.1 BVDV-PI animals

A flow chart depicting the detection of BVDV-PI animals within the study population is shown in Figure 4.27. 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, while 32,536 animals had both induction and follow-up samples received and verified.

Any animal with a sample received, verified and adequate in a negative pool was deemed negative (N = 33,189) and the status was probably negative for 302 animals that had samples of inadequate volume received and verified in negative pools. A total of 1,606 animals with samples received and verified had a sample in a positive pool but without a sample in a negative pool. These were classified as possible BVDV-PI animals and were the subject of further testing. Only 63 animals had neither induction nor follow-up serum samples, although 41 of these did have a single other sample (induction swab or hospital sample).

Of the 1,606 animals with at least one sample in a positive pool, but without a sample in a negative pool, 1,292 returned negative induction sera tests, while 124 tested positive. An additional 6/58 returned positive induction swab tests. Of the 130 animals with a positive induction sample test, 74 also had positive follow-up (N = 66) or hospital (N = 8) sampling tests, while 33 returned negative follow-up or hospital sample tests. The remaining 23 animals with a positive induction test and one animal with a positive hospital sample test did not have an adequate second sample for testing. The steps described in Section 3.9.3.11 were applied so that 8 of the 24 animals were classified as BVDV-PI positive as they were the only possible PI in paired positive plates (N = 2) or their serological profiles compared to those of animals in the same group-28 supported this classification (N = 6). An estimated total of 82 animals from the 35,160 animals inducted (0.23%) were persistently infected with BVDV (Table 4-51).

BVDV-PI animals were at increased risk of developing BRD compared to animals that were not PI (OR 1.9, 95% credible interval: 1.0 to 3.2,Table 4-52).

4.2.11.2 BVDV-PI animal in the group-28 and cohort.

Of a total of 1,274 group-28s, 67 contained at least one BVDV-PI positive animal; a single animal was identified in 56 group-28s, two in eight group-28s, three in two group-28s and four in one group-28. The BVDV-PIs were distributed among 54 of the 170 cohorts, from 12 of the 14 feedlots. BVDV was detected in at least one animal from 101 cohorts (59%). At the animal level, 34% were in negative group-28s and cohorts, compared to 10% in positive BVDV-PI group-28s and cohorts and the remaining 57% of animals were in group-28s where no BVDV-PI animal was identified, but BVDV was present in the cohort (Table 4-51).

Compared to animals in cohorts where BVDV was not identified, animals in cohorts where BVDV was present but from group-28s where no BVDV-PI animal was identified were at increased risk of developing BRD (OR 1.7, 95% credible interval: 1.1 to 2.6, Table 4-52), while there was some evidence of a similarly increased risk for animals that were in the same group-28 as a BVDV-PI animal (OR 1.6, 95% credible interval: 0.9 to 2.4, Table 4-52). The effect of a BVDV-PI animal in a group-28 and cohort was explored further in secondary analyses. In addition to equivalent adjustment set variable, the model in the vendor questionnaire subset 2 dataset also contained prior vaccination with Pestigard^{TM}. The total and direct effects were equivalent and the results (Table 4-53) were consistent with those obtained from the full cohort dataset. When restricted to cohorts containing a positively identified BVDV-PI animal, there was no evidence of a large difference in risk of BRD between animals that were in the same group-28 as the BVDV-PI animal and those that were

in a group-28 where no BVDV-PI animals were identified (OR 1.0, 95% credible interval: 0.8 to 1.1, Table 4-53).

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 0.89) from the WinBUGs model (Table 4-54). Thus, overall BRD incidence was estimated to decline by an absolute amount of 5.6% or 5.3% if it were possible to ensure that all cattle were at the same risk as those without a PI in the group-28 or evidence of BVDV circulating in the cohort.

Table 4-51: 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 incidence risk.

Variable	Category	Missing (%)	Number	Distribution by category (%)	Crude 50-day BRD incidence risk (%)
BVDV-PI animal		0.1			
	No		35,008	99.8	17.6
	Yes		82	0.2	26.8
BVDV present in cohort		0.0			
	No		11,896	33.9	8.7
	Yes		23,235	66.1	22.2
PI animal in group-28 and BVDV present in cohort		0.0			
	No, no		11,896	33.9	8.7
	Yes, yes		3,379	9.6	17.9
	No, yes		19,856	56.5	23.0

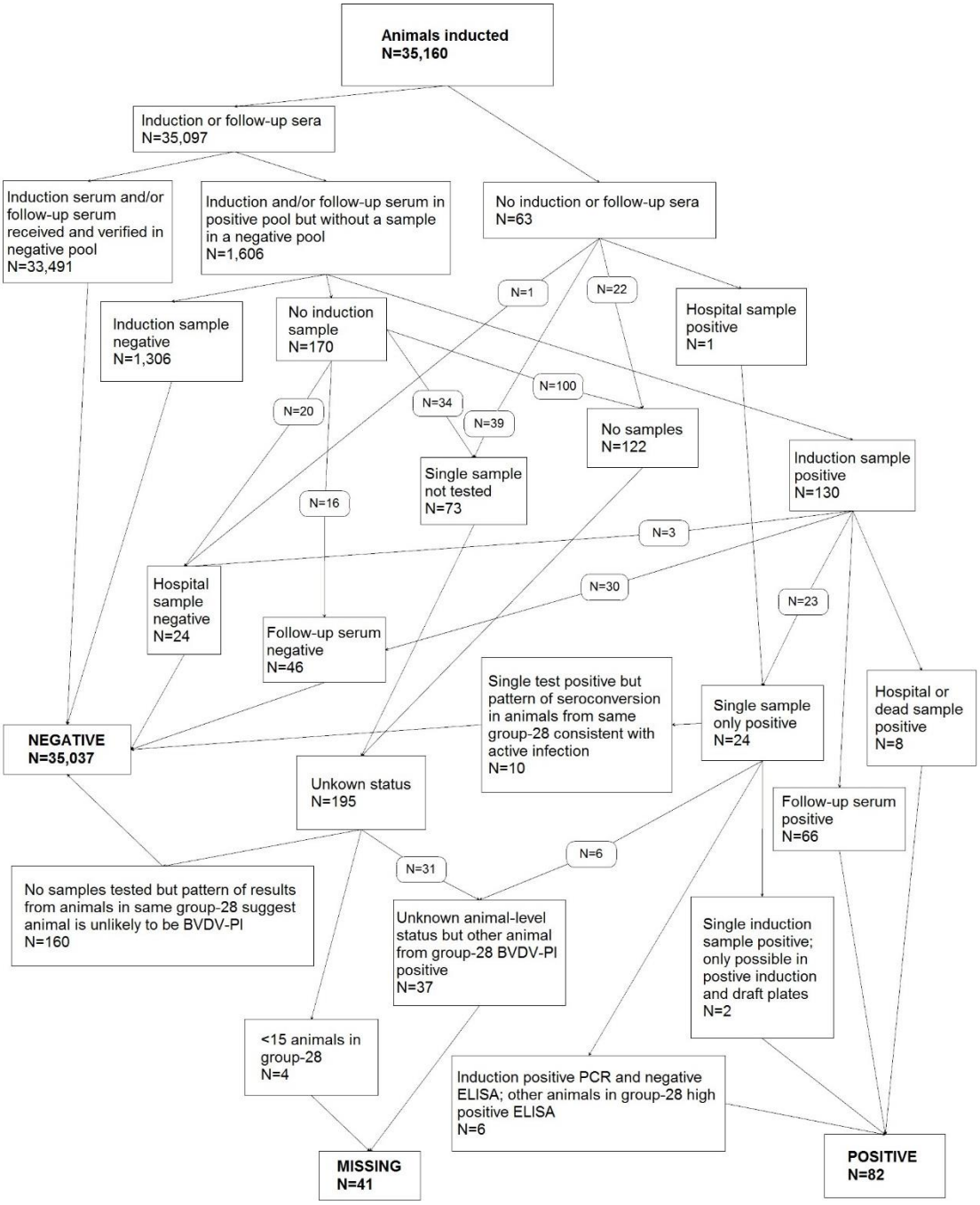

Figure 4.27: Flow chart depicting the determination of animal-level BVDV-PI status in the full cohort dataset.

Table 4-52: 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
BVDV-PI animal						
	No	Ref. cat.				
	Yes	1.9	(1.0 to 3.2)	0.030	()	N=35,090
PI animal in group-28 and BVDV present in cohort					(BVDV_PI_animal, CohortN, Shared pen water, Mix history)	N=34,693
	No, no	Ref. cat.				
	Yes, yes	1.6	(0.9 to 2.4)	0.041		
	No, yes	1.7	(1.1 to 2.6)	0.009		

Table 4-53: Estimated odds ratios for the total effects of the presence of animals persistently infected with BVDV in the group-28 (restricted to BVDV_PI positive cohorts) and in the group-28 and cohort (restricted to the vendor questionnaire subset 2 dataset) on the risk of BRD by day 50.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
PI animal in group-28 (restricted to cohorts where BVDV was present)					(BVDV_PI_animal, CohortN, PenWater, Pest_vacc, mix_summ)	N=13,392 3 level
	No	Ref. cat.				
	Yes	1.0	(0.8 to 1.1)	0.243		
PI animal in group-28 and BVDV present in cohort (restricted to vendor questionnaire subset 2)					(BVDV_PI_animal, CohortN, Shared pen water, Mix summary, PV_vacc)	N=7,981 3 level
	No, no	Ref. cat.				
	Yes, yes	1.7	(0.7 to 3.5)	0.147		
	No, yes	1.7	(0.8 to 3.4)	0.105		

Table 4-54: Estimated population attributable fractions (PAF) and population attributable risks (PAR) for the total effects of PI animal in group-28 and BVDV present in cohort on the risk of BRD by day 50. Estimates are derived from models fitted in both MLwiN and WinBUGs.

Risk factor	MLwiN PAF	WinBUGS PAF	WinBUGS 95% cred int	MLwiN PAR	WinBUGS PAR	WinBUGS 95% cred int
PI animal in group-28 and BVDV present in cohort	0.32	0.30	(0.04 to 0.50)	5.62	5.30	(0.73 to 8.89)

4.2.12 Putative risk factors relating to serology results

4.2.12.1 BoHV-1

From the case-control study, the estimated weighted average seroprevalence for antibodies against BoHV-1 was 24% (Table 4-55). At induction, very few animals were categorised as 4 or 5 (2%).

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 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

Prior exposure to BoHV-1 (induction categories 2 or 3) was associated with a reduced risk of BRD relative to induction category 0 (OR 0.7, 95% credible interval: 0.6 to 0.9, Table 4-59). There was no evidence of a large effect of induction categories 4 or 5 but the estimate was imprecise due to the very small proportion of animals in this category. An increase in BoHV-1 antibody category ("up" category) was associated with an increased risk of BRD (OR 1.4, 95% credible interval: 1.2 to 2.6, Table 4-60) as was seroconversion (OR 1.3, 95% credible interval: 1.1 to 1.5, Table 4-61). However, this effect was not apparent in a subset analysis of animals not given Rhinogard™ at induction; the odds ratio was consistent with a protective effect but the estimate was imprecise (OR 0.7, 95% credible interval: 0.3 to 1.4, Table 4-61).

4.2.12.2 BVDV

From the case-control study, the estimated weighted average seroprevalence for antibodies against BVDV was 69% (Table 4-56). At induction, nearly half of the study population were categorised as 4 or 5 (49%). It was not possible to make a meaningful comparison between animals that had and had not been previously vaccinated with Pestigard™ as only 982/7,063 (12.2%) of animals with data vendor questionnaire subset 2 dataset had been vaccinated.

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 4-59). The estimated effect of induction category 1 was indicative of a possible increase in risk but the estimate was imprecise. An increase in BVDV antibody category ("up" category) was associated with an increased risk of BRD (OR 1.3, 95% credible

interval: 1.1 to 1.6, Table 4-60) as was seroconversion (OR 1.6, 95% credible interval: 1.2 to 2.1, Table 4-61).

4.2.12.3 BPI3

From the case-control study, the estimated weighted average seroprevalence for antibodies against BPI3 was 91% (Table 4-57).

BPI3 antibody category increased from induction to follow-up sampling in 17% of animals and 52% of initially seronegative animals seroconverted.

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 4-59). An increase in BPI3 antibody category ("up" category) was associated with an increased risk of BRD (OR 1.4, 95% credible interval: 1.2 to 1.7, Table 4-60). 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 4-61).

4.2.12.4 BRSV

From the case-control study, the estimated weighted average seroprevalence for antibodies against BRSV was 89% (Table 4-58).

BRSV antibody category increased from induction to follow-up sampling in 28% of animals and 63% 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 4-59). An increase in BRSV antibody category ("up" category) was associated with an increased risk of BRD (OR 1.4, 95% credible interval: 1.2 to 1.7, Table 4-60) as was seroconversion (OR 1.5, 95% credible interval: 1.0 to 2.2, Table 4-61).

Table 4-55: 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	3253	45.1	51.1
	Up	44.9	62.8	3886	53.9	47.8
	Initially high	1.1	0.9	73	1.0	1.1
	Missing			102		
BoHV-1 seroincrease						
	No	55.1	37.2	3,326	46.1	52.2
	Yes	44.9	62.8	3,886	53.9	47.8
	Missing			102		
BoHV-1 seroconversion						
	No	48.3	32	2267	39.9	45.7
	Yes	51.7	68	3414	60.1	54.3
	Missing			0		

Table 4-56: 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	29.9	22.6	1,844	26.21	28.7
	Up	21.9	34.9	1,999	28.42	24
	Initially high	48.2	42.6	3,192	45.37	47.3
	Missing			279		
BVDV seroincrease						
	No	78.1	65.1	5,036	71.6	76.1
	Yes	21.9	34.9	7,035	28.4	24
	Missing			279		
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 4-57: 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	25.6	1,962	26.8	27.6
	Missing			0		
BPI3 composite						
	No change	57.95	52.1	3,817	55	57
	Up	15.95	23.5	1,370	19.7	17.2
	Initially high	26.1	24.4	1,752	25.3	25.8
	Missing			375		
BPI3 seroincrease						
	No	84.1	76.5	5,569	80.3	82.8
	Yes	15.9	23.5	1,370	19.7	17.2
	Missing			375		
BPI3 seroconversion						
	No	48.6	31.6	278	39	45.8
	Yes	51.5	68.4	435	61	54.2
	Missing			0		

Table 4-58: 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
	4 or 5	16.8	15.7	1,189	16.3	16.7
	Missing			0		
BRSV composite						
	No change	55.86	50.04	3,712	52.9	54.9
	Up	28.49	35.6	2,247	32.1	29.6
	Initially high	15.66	14.35	1,052	15.0	15.5
	Missing			303		
BRSV seroincrease						
	No	71.5	64.4	4,764	67.9	70.4
	Yes	28.5	35.6	2,247	32.1	29.6
	Missing			303		
BRSV seroconversion						
	No	36.8	26.1	282	30.7	35.1
	Yes	63.2	74	637	69.3	64.9
	Missing			0		

Table 4-59: Estimated odds ratios for the total effects of induction serostatus 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. cat.				
	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		
BVDV induction category					(Mix summary, Test batch, Selection batch, BVDV_grp_cht)	N=7,240 3 level
	0	Ref. cat.				
	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		
BPI3 induction category					(Mix summary, Test batch, Selection batch)	N=7,240 3 level
	0	Ref. cat.				
	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		
BRSV induction category					(Mix summary, Test batch, Selection batch)	N=7,240 3 level
	0	Ref. cat.				
	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		

Table 4-60: Estimated odds ratios for the total effects of change in serostatus on the risk of being a BRD case.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
BoHV-1 composite					(Mix summary, Test batch, Selection batch, CohortN, Rhinogard)	N=7,139 3 level
	No change	Ref. cat.				
	Up	1.4	(1.2 to 1.6)	< 0.001		
	Initially high	1.5	(0.7 to 2.9)	0.107		
BVDV composite					(Mix summary, Test batch, Selection batch, CohortN, BVDV_grp_cht)	N=6,620 3 level
	No change	Ref. cat.				
	Up	1.3	(1.1 to 1.6)	0.001		
	Initially high	1.0	(0.9 to 1.2)	0.462		
BPI3 composite					(Mix summary, Test batch, Selection batch, CohortN	N=6,938 3 level
	No change	Ref. cat.				
	Up	1.4	(1.2 to 1.7)	< 0.001		
	Initially high	1.1	(0.9 to 1.3)	0.155		
BRSV composite					(Mix summary, Test batch, Selection batch, CohortN	N=6,938 3 level
	No change	Ref. cat.				
	Up	1.4	(1.3 to 1.7)	< 0.001		
	Initially high	1.2	(1.0 to 1.5)	0.025		

Table 4-61: Estimated odds ratios for the total effects of seroconversion on the risk of being a BRD case.

Risk factor	Category	Odds ratio	95% cred int	Prob </>1	Adjustment set	N, level
BoHV-1 seroconversion					(Mix summary, CohortN, Shared pen water, Rhinogard)	N=5,623
	No	Ref. cat.				
	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
	No	Ref. cat.				
	Yes	0.8	(0.3 to 1.6)	0.205		
BVDV seroconversion					(Mix summary, CohortN, Shared pen water, BVDV_grp_cht)	N=2,446
	No	Ref. cat.				
	Yes	1.7	(1.3 to 2.1)	< 0.001		
BPI3 seroconversion					(Mix summary, CohortN, Shared pen water)	N=709
	No	Ref. cat.				
	Yes	1.4	(0.9 to 2.2)	0.088		
BRSV seroconversion					(Mix summary, CohortN, Shared pen water)	N=914
	No	Ref. cat.				
	Yes	1.5	(1.0 to 2.3)	0.036		

4.2.12.5 Exposure to multiple viruses

Using the results from the case-control study it was estimated that the majority of study animals were seropositive to at least two viruses prior to induction (93%, Table 4-62) and most seroincreased to one or more viruses prior to follow-up sampling (74%). 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 4-63). Those animals seroincreasing to at least one virus prior to the follow-up sample 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 4-62: 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	28.2	14.0	1,376	21.0	25.9
	1	41.0	35.4	2,498	38.2	40.1
	2	21.3	32.0	1,744	26.7	23.0
	3	7.7	15.0	745	11.4	8.8
	4	1.8	3.2	178	2.7	2.1

Table 4-63: 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. cat.				
Number of viruses animal seroincreased to by follow-up					(Mix summary, CohortN, BVDV_grp_cht, Shared pen water, Test batch, Selection batch, Rhinogard)	6,477
	0	Ref. cat.				
	1	1.3	(1.1 to 1.6)	0.003		
	2	1.9	(1.5 to 2.3)	< 0.001		
	3	2.1	(1.6 to 2.6)	< 0.001		
	4	1.8	(1.1 to 2.7)	0.006		

4.3 Variance components results

The parsimonious group of variables included in the final variance components model were: sex, breed, induction weight, mixing history, move to the feedlot, number of animals in group-13, number of days from day 0 to cohort close, shared pen water, BVDV group-28 and cohort status and season. All possible two-way interactions between these variables were assessed. Two interactions (breed*season and induction weight*number of animals in group-13) had overall p-values <0.05 and reduced the DIC by more than 3 so were included in the final model. A comparison of variance components between the null model and the final variance components model is shown in Table 4-64. The fixed effects in this model explained 14.1% of the variance in BRD. Of the unexplained variance, 36.8% was at the feedlot level, 10.1 % was at the cohort level, 5.9% was at the group-13 level and 47.2% was at the animal level. In the null model (no explanatory variables added) the majority of the variance (51.9%) was at the feedlot level, 9.7% was at cohort level, 5.5% at the group-13 level and 32.9% at the animal level. However, the absolute feedlot-level variance was unstable, and the values changed upon repeated runs (ranging from 4.6 to 5.2). Investigation revealed that this was probably due to one feedlot having an extremely low BRD incidence and therefore not behaving in an analogous way to the rest of the population of feedlots. The feedlot-level variance was more stable when the null model was run excluding this feedlot, while cohort and group-13 level variances remained stable. In this model the feedlot-level variance estimate was much lower at 2.7, so that the proportion of variance at the feedlot level would be estimated at 36%.

The sum of the percentages of total variance that were at feedlot and cohort level estimates the correlation in BRD occurrence between any two animals from the same cohort, assuming that the correlation was the same for all pairs of animals within each cohort. Thus the intra-class correlation coefficient for cohort was approximately 0.47. This value is much larger than the value of 0.1 that derived from retrospective data that was used to estimate the required number of cohorts to be enrolled in the study in Section 3.3.5.

The percentage of unexplained variance at the animal level has increased in the final model because the total variance is reduced and as animal-level variance is a constant value the relative percentage has increased. Many explanatory factors at lower levels (animal, group and cohort) tend to cluster at the feedlot level, and so would contribute to the substantial reduction in the proportion of unexplained variance at the feedlot level.

Table 4-64: Partitioning of variance at each of the four levels in the null and final models and the percentage of the variance explained by the final model overall and at each of the four levels.

Partition	Null model	% of unexplained variance	Final model	% of unexplained variance
Feedlot level variance	5.19	51.9	2.56	36.8
Cohort level variance	0.97	9.7	0.71	10.1
Group-13 level variance	0.55	5.5	0.41	5.9
Animal level variance	3.29	32.9	3.29	47.2
Total unexplained variance	10.00		6.97	
Fixed effect variance	n/a		1.14	
Total variance	10.00		8.11	

5 Conclusions/discussion

This project investigated the occurrence of BRD in cattle that were on feed at predominantly medium-sized and large feedlots across several Australian states. This population is probably representative of cattle at feedlots of this size, but the conclusions may not be generalizable to smaller feedlots. Only two study feedlots in the study had a physically-constructed capacity of less than 5,000 head and it is possible that effects of these risk factors, particularly population-level effects (as assessed by PAFs and PARs) may differ between small and larger feedlots.

Our estimates of population-level effects are based, in part, on the percentage of animals exposed to particular risk factors within this study population. Percentages of animals exposed to some risk factors vary between individual feedlots, so the risk factors with the largest population-level effects causing BRD will also differ between feedlots. This must be addressed in any extension process designed to reduce BRD incidence in Australian feedlots. Our estimates of population-level effects will also change over time if percentages of animals exposed to various risk factors change.

Many factors that increase or decrease the risk of BRD have been identified. It is important that stakeholders consider other issues when selecting control strategies based on these results. Animal welfare should be considered, and economic evaluations specific to individual feedlots and considering current market demands are recommended.

The main purpose of this section is to present conclusions based on interpretation of study findings. Key issues arising from these results are also discussed.

5.1 Descriptive statistics from the cohort study

5.1.1 BRD cases

BRD is common and is the reason for most pulls across medium to large Australian feedlots. (In the study population, 18.2% of animals were pulled for BRD and of all pulls, 77.3% were for BRD.)

There is variability in BRD incidence between cohorts within feedlots. This variability is not random. Some feedlots have highly variable incidences of BRD at the cohort level whereas some others show consistently low incidence.

Peak daily BRD incidence is generally between 15 and 30 days after induction. (In the study population, 90% of BRD cases occurred between 5 and 35 days after induction and 96.8% by day 50and a second peak later in the feeding period was generally not seen).

Epidemic curves vary markedly between cohorts.

5.1.2 Deaths from BRD

BRD is the most common cause of mortality. (Within the study population, 0.7% of cattle inducted were reported to have died from BRD and of all deaths, 51.5% were attributed to BRD. The case fatality risk was 3.4% i.e. of BRD pulls, 3.4% died within 50 days of diagnosis.

BRD mortality risk (i.e. % of inducted animals that die where the death is attributed to BRD) varies markedly between cohorts within feedlots.

5.2 Risk factor results from the cohort study and associated subsets

5.2.1 Putative risk factors relating to feedlot entry characteristics

The risk of BRD varies markedly by breed. Tropical breeds cross breeds are at lowest risk, Angus, Shorthorn and British cross are at higher risk, Herefords are at highest risk. Further investigation into Murray Greys is warranted as the study found that this breed was at low risk, but this finding has not been documented previously. Males are probably at higher risk of BRD but results from this study were not definitive. Animals with two or more teeth are not at markedly different risk from those with only milk teeth.

Induction weight is an important risk factor. Risk of BRD reduces with increasing induction weight such that animals weighing at least 480kg are at markedly reduced risk compared those weighing less than 400kg. Animals that are well below cohort average are increased risk because they are light not because of their weight relative to the cohort average.

Notes: The observed range of within-cohort variability in weight was relatively small, so conclusions cannot be drawn on the possible effect of extreme differences from average cohort weight. Due to lack of age data for most of the study population and body condition data for the whole study population, it was not possible to tease apart the relative contributions of these components on the observed effect of induction weight. Furthermore, findings from the vendor questionnaire subset, that risk increases with age is contrary to biological plausibility and we think that these results may be confounded e.g. some vendor bred cattle may have been sold at an older age due to unfavourable conditions during the growth period that may have increased their subsequent susceptibility to BRD.

5.2.2 Putative risk factors relating to mixing, moving, group size, saleyard exposure and transport prior to feedlot entry

Mixing history is a very important risk factor. There is a protective effect of mixing prior to day -27. (This was consistent across all pairwise comparisons where there were no distribution problems, the effect was consistent in direction but less precisely estimated for 2 comparisons where there are distribution issues, and there was no evidence of a strong effect for one comparison where animals in each category were very unevenly distributed across feedlots.)

Furthermore, animals that are first mixed prior to day -27 are at substantially reduced risk compared to those first mixed between days -12 and 0.

There is an adverse effect of mixing four or more group-13s together rather than less than four if cattle have mixed prior to day-27 and not been mixed between day –27 and day -13. There may be a similar but less marked effect for cattle that have never been mixed prior to day -12.

Notes: It was not possible to reach a general conclusion about mixing in the day -27 to day -13 time period because this occurred rarely except at feedlots that practice pre-assembly.

Risk is markedly increased when four or more group-28s are mixed together and this appears to be more important than the timing of arrival at the feedlot in relation to day 0. Animals first mixed between days -27 and -13 appear to be at similar risk to those first mixed between days -12 and 0. (Estimates for this effect were imprecise as mixing between days -12 and 0 was uncommon. This result was not likely to be biased in any important way due to feedlot as mixing between days -12 and 0 was not clustered by feedlot.)

Notes: Neither mixing of animals within any given PIC nor mixing of study animals with animals that were not enrolled in the study could be assessed as the necessary data were not available. As a result, some animals classified as not mixed may in fact have mixed, but not vice versa. This may have caused our estimated effects to be smaller than is actually the case.

The timing of the move to the feedlot is an important risk factor. Risk is much lower if the move is at least 27 days before day 0 compared to later moves. This reduction in risk is independent of mixing history. For animals that move on induction day or the day before induction day, travel six hours or more in duration is associated with higher risk compared to travel of less than six hours duration.

There is a protective total effect of saleyard transfer prior to day -27 but this effect is predominantly due to protective effect of mixing prior to day -27. There is an adverse total effect of saleyard transfer between 27 and 13 days before induction but the direct effect is not significant so this is predominantly due to mixing. A saleyard transfer between 12 to 0 days before the start of time at risk increases the risk of BRD over and above mixing history and the timing and duration of the move to the feedlot. There is a need for the reasons for this increased risk to be defined.

Animals in large group-13s are at less risk, partly because of less subsequent mixing. The adverse effects of small group-13s may be worse in light animals. As most group-13s are stable for at least 120 days prior to day 0, the time prior to day 0 by which larger groups should be formed to confer this protective effect cannot be inferred. However, the less apparent protective effect of large group-91s compared to group-28s and group-13s suggests that establishment of larger groups by day -28 is likely to confer the majority of the protective effect. This risk factor is closely related to and should be considered in conjunction with mixing history and the timing of the move to the feedlot.

5.2.3 Putative risk factors relating to formation of the cohort

Animals with a longer period between day 0 and cohort close date are at reduced risk of BRD. While this is beneficial for those animals, cohort fill durations of greater than one day have an adverse effect on the cohort as a whole. After accounting for individual animal weights, there is no apparent effect of mean cohort weight.

5.2.4 Putative risk factors relating to source region, feedlot region, the timing of the induction period and weather in the first week after day 0

Southern feedlots are at considerably higher risk. This is probably due to effects over and above those due to breed, weight, sex but other possible contributing factors remain unknown. Relative to spring, risk is increased in winter and markedly increased in summer and autumn. Reasons for the large effects of region and season are not understood. 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 as they may play a role. Fluctuations in weather may be important to consider along with lag times; weather may affect BRD risk up to two weeks later.

5.2.5 Putative risk factors relating to pen characteristics

Animals in pens with water troughs that are shared with an adjacent pen are at markedly increased risk. This effect is partly, but not entirely, due to exposure to one or more of BoHV-1, BVDV, BPI3 or BRSV. As this is a relatively common practice, the population effect is large. This risk factor has not been identified previously and further investigation is recommended to enhance understanding of how this effect is mediated.

It is possible that risk is increased with some pen shade rather than none but estimates are imprecise. Further investigation is required to establish whether such an effect does exist and if it varies with the amount of shade available and/or is influenced by prevailing weather conditions.

It is possible that risk is decreased with larger bunk space but estimates are imprecise so further investigation would be required if provision of more bunk space (> 0.24m/head) is feasible.

5.2.6 Putative risk factors relating to ration characteristics

Notes: No conclusions were reached in relation to effects of ration characteristics. These were clustered by feedlot and so estimates were both imprecise and prone to confounding by unmeasured feedlot-level or feedlot-associated variables e.g. pen riding practices, unmeasured attributes of purchase animals. This is a limitation of the observational study approach. Specific hypotheses about rations would be better tested in randomised controlled trials.

5.2.7 Putative risk factors relating to induction treatments and numbers on feed

Notes: The use of Rhinogard™ was clustered by feedlot and was associated with an increase in risk of BRD. Assuming Rhinogard™ does not cause BRD. This was probably because feedlots with past high BRD incidences preferentially used Rhinogard™ for study cattle and these cattle were also at increased risk. Hence effects of Rhinogard™ could not be assessed, and a randomised controlled trial is required to assess the effects of Rhinogard™ in commercial feedlots.

5.2.8 Other putative risk factors derived from the vendor questionnaire

Across the relatively unmixed population of vendor bred cattle and those purchased when aged less than ten months, both Pestigard™ and Bovilis MH™ given at least 14 days before induction reduce risk, probably by a modest amount. Across the relatively unmixed population of vendor bred cattle, yard weaning reduces risk, probably by a modest amount and it is possible that there is also a modest beneficial effect of prior feeding of grain.

5.2.9 Putative risk factors relating to the presence of BVDV in the cohort and exposure to BVDV-PI animals

About one third of all cohorts include at least one BVDV-PI. BVDV activity in the cohort (i.e. BVDV-PI(s) or just transiently infected animals), increases risk. This effect appears consistent whether there is or is not a persistently infected animal in the animal's group-28.

5.3 Descriptive statistics from case-control study

Substantial proportions of incoming cattle have had prior exposure to BPI3, BRSV and BVDV. In contrast, the majority of incoming cattle have not had prior exposure to BoHV-1.

Of those animals that were initially seronegative to BoHV-1, about half seroconvert in the first 5 to 7.5 weeks on feed. Seroincreases are also very common. Of those that are initially seronegative to BVDV, the majority seroconvert in the first 5 to 7.5 weeks on feed. Seroincreases are common. For each of BPI3 and BRSV, of those that are initially seronegative about half seroconvert in the first 5 to 7.5 weeks on feed. Seroincreases are quite common. Of those that showed any seroincrease, about half seroincreased to only one agent. Of those animals exposed at the feedlot to any virus, it is usually only one or two viruses. It is rare for animals to be exposed to more

than two viruses during around induction and in the few weeks after. There is evidence that all four viruses are present in moderate to large Australian feedlots. However, not all four viruses are likely to be present at any one feedlot at any given time.

5.4 Risk factor results from case-control study

Past exposure to each of BoHV-1, BPI3, BRSV and BVDV reduces risk of BRD. The more viruses individual animals have had prior exposure to, the more their risk of BRD is decreased. For each virus, animals that seroincrease are only at modestly increased risk of BRD. Exposure to a single agent increases the risk of BRD but does not do so dramatically. The more viruses individual animals seroincrease to the more their risk of BRD is increased.

5.5 Variance components

Half the variability in BRD occurrence is at the feedlot level. Known risk factors for BRD account for a substantial amount of the variability in occurrence. Although none of these variables are feedlot-level variables, when they are taken into consideration, only just over one third of the unexplained variability in BRD occurrence is at the feedlot level. Future studies to better understand collective risk factors for BRD should focus on differences between feedlots including attributes of their animals, groups and cohorts rather than studies of animals within feedlots. These studies would require a large number of feedlots.

5.6 Ranking and grouping of risk factors

The putative risk factors explored in the NBRDI were grouped into animal, management and environmental risk factors with moderate to high PAFs, those with small to moderate PAFs, serological risk factors, risk factors with some evidence of an effect but imprecise estimates, putative risk factors assessed but estimates too imprecise to reach a conclusion, risk factors with important total effects but with effects mediated through or correlated with other risk factors investigated and putative risk factors with unexpected effects that may be due to uncontrolled confounding.

5.6.1 The main animal, management and environmental risk factors

Risk factors that are important to industry as a whole because they have moderate to large effects on the risk of BRD (i.e. a moderate to high PAF) are:

- Timing of the move to the feedlot (PAF: 0.69 from MLwiN model/0.75 from WinBUGs model, indicating that the incidence of BRD would be reduced by 69%/75% if the risk of BRD in all animals that moved to the feedlot 27 or fewer days before day 0 was reduced to that for animals that moved to the feedlot at least 27 days before day 0)
- Shared pen water (PAF: 0.67/0.70)
- Mixing history/time of first mixing (PAF: 0.57/0.55)
- Breed (PAF: 0.53/0.67)
- Number of animals in a group established at least 13 days before induction (PAF: 0.35/0.39)
- BVDV present in cohort (0.32/0.30)

- Season (PAF: 0.30/0.28)

Notes: The majority of these risk factors relate to management decisions, so are potentially amenable to intervention. Replacement of water troughs that are shared by two pens, with ones that are accessible only by animals in one pen can be implemented relatively easily at individual feedlots. In contrast, ensuring that (i) animals have been mixed at least 28 days prior to feedlot entry, (ii) have been in stable groups for this period, and (iii) feedlot personnel to have access to movement and mixing history to confirm mixing history clearly requires co-operation between vendors, feedlot personnel and other industry stakeholders. We recommend enhancing the functionality of the NLIS database and increasing the availability of the data. Ideally, purchasers should be able to interrogate the movement history for animals in real time prior to purchasing, to establish not just the movement history but also the likely mixing history. This would require algorithms to query the movement of the animals in question in relation to all other animals in the database. Movement of animals to the feedlot location at least 28 days prior to induction is not currently feasible for many feedlots due to absence of grazing land on/near the feedlot premises. Ensuring access to nearby grazing land should be a consideration for feedlots established in the future and purchasing nearby land is worth the consideration of management of existing feedlots.

5.6.2 Other important animal and management risk factors

The next group is risk factors that have small to moderate PAFs. The relative importance of these risk factors to individual feedlots will be greater where exposure to the risk factors is more common. These risk factors are:

- Prior vaccination with Bovilis MH™ (PAF: 0.18/0.18)
- Induction weight (PAF: 0.17/0.16)
- Prior Pestigard™ vaccination (PAF: 0.17/0.17)
- Yard weaning (PAF: 0.08/0.08)
- Saleyard exposure within 12 days of induction (PAF: 0.02/0.02)

Notes: The majority of risk factors in this group relate to management of animals prior to arrival at the feedlots. Widespread uptake of these practices clearly requires co-operation between vendors and feedlots. To achieve this, we recommend that a panel of key industry stakeholders work together with the research team to drive out and own practical and evidence-based messages that can be widely adopted.

5.6.3 Important serological risk factors

Prior exposure to viral pathogens is generally protective, whereas exposure while on feed increases risk of BRD. Prior vaccination with both Bovilis MH™ and Pestigard™ is protective. On this basis it would be reasonable to conclude that safe and efficacious vaccines used correctly for the other pathogens involved in BRD would also be protective. The strength of the contribution of each virus to BRD is relatively low compared to the main animal, management and environmental risk factors. These risk factors are:

- Serostatus at induction to BoHV-1
- Serostatus at induction to BVDV
- Serostatus at induction to BPI3

- Serostatus at induction to BRSV
- Number of viruses animal is seropositive to at induction
- Change in serostatus to BoHV-1 by 6 weeks after induction
- Change in serostatus to BVDV by 6 weeks after induction
- Change in serostatus to BPI3 by 6 weeks after induction
- Change in serostatus to BRSV by 6 weeks after induction
- Number of viruses animal seroincreases to by 6 weeks after induction

5.6.4 Exposures unlikely to have an important effect in exposed animals

We conclude that the following risk factors are unlikely to have important effects in exposed animals. They are:

- Dentition (as distinct from age at induction)
- Mean cohort weight
- Weight difference from mean cohort weight
- Intended days on feed
- Number of adjoining pens
- Vitamin A,D and E at induction
- Prior supplementary feeding (e.g. conserved forage)
- On-property mixing (vendor bred animals)

Notes: We do not recommend any further specific research on these risk factors.

5.6.5 Putative risk factors with some evidence of an effect but imprecise estimates

There are several risk factors where some evidence of an association with BRD was found, but the estimates were imprecise. These are:

- Sex
- Pen shade
- Bunk space
- Prior grain feeding

Notes: We recommend further research on these risk factors, particularly those that are amenable to intervention. If retrospective data could be made available from a large number of feedlots where animals had differing exposure to pen shade and bunk space and were of differing sex, it is likely that these risk factors could be explored in more detail at limited additional cost. Clearly this would require widespread co-operation throughout the feedlot industry, so is only feasible if there is sufficient industry interest.

5.6.6 Putative risk factors assessed but estimates too imprecise to reach a conclusion

There is a group of putative cohort- and feedlot-level risk factors for which the estimates were too imprecise to reach a conclusion nor to even identify any evidence of an effect. This was due to clustering of many of these risk factors by feedlot and the small number of feedlots in the study. These are:

- Number of animals in cohort
- Sex of cohort
- Days from DOF1 to day 0
- Source region
- Induction year
- Weather variables
- Stocking density
- Grain type
- Time until ration contains at least 60% grain
- Grain percentage in ration at induction
- Grain percentage in ration 20 days after induction
- Number of cattle on feed in induction month
- Number of cattle < 40DOF in induction month

Notes: Associations between each of these risk factors and BRD are biologically plausible. Data for most of these factors are routinely recorded by many feedlots. It would therefore be possible to investigate these factors if routinely recorded data from a large number of feedlots could be made available. With respect to wind data, the data from the Bureau of Meteorology stations closest to the study feedlots were not suitable to determine BRD associations. The current push within the industry for feedlots to install weather stations to aid in other issues such as heat load management could also be used for future research into BRD risk factors. Clearly further studies on weather or any of these other factors would require widespread co-operation throughout the feedlot industry, so is only feasible if there is sufficient industry interest.

5.6.7 Risk factors with important total effects but with effects mediated through or correlated with other risk factors investigated

There is a group of risk factors with important total effects but these effects are mediated primarily through other risk factors, and/or the risk factor is highly correlated with another. These are:

- Saleyard transfer prior to day -27
- Saleyard transfer between day -27 and day -13
- Cohort fill duration
- Days from day 0 to cohort close
- Feedlot region

Notes: Management decisions relating to risk factors in this group should not consider the specific risk factor in isolation, rather they should consider both the specific risk factor and the risk factor(s) through which the risk factor is mediated. For example, avoiding saleyard transfer in the period between day -27 and day -13, but not avoiding mixing would not be expected to reduce risk to the extent estimated by the total effect.

5.6.8 Risk factors with unexpected effects that may be due to uncontrolled confounding

Estimated effects for each of Rhinogard™ and age were contrary to biological plausibility. It is likely that these results are due to uncontrolled confounding. A randomised controlled trial is recommended to assess the effects of Rhinogard™ in commercial feedlots.

5.7 Study strengths and limitations

A large number of important risk factors for BRD were identified, their effects quantified, and their relative importance at the population level assessed. The majority of these associations are biologically plausible as causes of BRD, and are consistent with industry beliefs. 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 “traditional” automated parsimonious model had been reported. In addition, these effect estimates represent the total expected change in BRD risk if the risk factor is modified (assuming the relationship is causal and the estimates unbiased). This contrasts with effect estimates from “traditional” parsimonious models; these may be total, partial or direct. They represent the expected change in BRD risk if all other variables in the model are held constant, which is commonly not a realistic scenario.

The use of the causal diagram informed approach 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. Removing exposure to an important risk factor, but failing to remove exposure to an important intervening variables through which it is mediated will not lead to a beneficial effect. For example, avoiding a saleyard transfer between day -27 and day -13 but still mixing an animal with other animals in this time period would not result in a beneficial effect of the magnitude indicated by the total effect estimate. Such detailed understanding of causal mechanisms cannot be inferred from a single “traditional” parsimonious model.

There was initial concern amongst consulting veterinarians about formulation of an appropriate case definition, primarily in regards to the inclusion/exclusion of pulls with non-specific signs. We are, however, confident that the case definition used for analyses was robust. The number of non-specific pulls was small so their classification as BRD cases or not would have had little impact on the analyses.

At the start of the study there were concerns about the follow-up blood sampling because of additional stress to the animals and the additional labour required to perform the task. In addition, difficulties were observed in the pilot study in matching blood samples to individual animals. We believe these issues were adequately overcome as 92.5% animals had both induction and follow-up blood samples that were received and verified at the animal level.

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 data from three feedlots. This is markedly lower than the observed cohort intra-class correlation coefficient 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) the actual required sample size to have had the desired precision to estimate cohort-level effects would have been four times greater. Clearly such as study would have been neither logistically feasible, nor financially viable. However, this does in part explain the inability to reach conclusions about some of the putative cohort-level risk factors. That many of these were largely clustered by feedlot further exacerbated this issue.

The mortality results from this study did not distinguish between natural deaths directly from disease and management decisions to euthanase chronically diseased animals on welfare and/or economic grounds. As a result, estimates for mortality incidence risk may not reflect the true percentage that would have died had treatment been continued to late stage disease at all feedlots.

The observed effect of weight may be due in part to age and/or body condition. Age data were not available for most of the study population and body condition data were not recorded as this was deemed unfeasible during the pilot study.

The study definition of mixing referred specifically to between-PIC mixing among animals enrolled in the study. It is possible that animals in the study may have mixed with animals from the same or other PICs that were not in the study. There is therefore likely to have been some misclassification of animals as not mixed when they were in fact mixed. In contrast, we assumed that all animals on one PIC at any given time that went into the same cohort were mixed with each other but this might not have been the case. Provided such misclassification errors were non-differential, 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.

5.8 Recommendations for future research and extension

This study has identified many management based risk factors that influence the risk of BRD. Translation of the findings from this research into practices that can and will be adopted by industry requires input from vendors, feedlot personnel and other industry stakeholders. A participatory approach involving representatives across the breadth of the industry working in co-operation with the research team is likely to have the greatest chance of success. To achieve this, we recommend that a panel of key stakeholders work together with the research team to drive out and own practical and evidence-based messages that can be widely adopted. Depending on industry interest, this may involve development of a decision support tool. An action research model is proposed whereby the stakeholders reflect regularly on (i) the extent to which adoption of each message has occurred and (ii) whether it has been successful. These reflections can be used to revise key messages and the cycle repeated.

We also recommend enhancing the functionality of the NLIS database and increasing the availability of the data. Ideally, purchasers should be able to interrogate the movement history for animals in real time prior to purchasing, to establish not just the movement history but also the likely mixing history. Critical to this being adopted by industry will be the development of an interface which distils this complex data into a form that allows purchasers to make informed decisions with respect to BRD risk. In the hypothetical situation where a group of cattle are deemed to have a high risk, prior knowledge of this will allow feedlot operators to adjust management to minimise the impact on their enterprise.

There are several putative cohort-level risk factors where this study identified either some evidence of an effect, such as shade and stocking density, or the estimate was too imprecise to reach a definitive conclusion, such as cohort size and ration content/routine. If retrospective data could be made available from a large number of feedlots where animals had differing exposures to these risk factors it is likely that they could be explored in more detail at limited additional cost. Clearly this would require widespread co-operation throughout the feedlot industry, so is only feasible if there is sufficient industry interest.

Broad environmental risk factors such as feedlot region and induction season are unlikely to be directly "causal" or closely linked to causality in the biological sense because they are likely to be proxy measures for other unmeasured or unknown factors. Thus, while an important total effect of these risk factors has been shown, this is useful in prompting further investigation into why the association may occur rather than in suggesting strategies to reduce risk. Overseas studies have shown associations between weather variables and BRD, and in the Australian context these are the most obvious possible explanatory factors for the effects of region and season. These factors can be investigated further within a model framework more suited to time varying covariates such as in survival analysis models.

The strong association between shared access to pen water and risk of BRD has not been identified previously. 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. Although shared access to pen water was clustered by feedlot, conclusions were the same when only feedlots where cohorts varied in exposure, so it is unlikely that the observed effect is due to unmeasured cohort- or feedlot-level confounders. Further investigation is recommended to enhance understanding of how this effect is mediated. Further investigation is also recommended to explain the low risk of BRD that was observed in Murray greys.

Finally, a randomised controlled trial is recommended to assess the effects of Rhinogard™ in commercial feedlots.

6 Communication

6.1 Published papers

Horwood, P.F., Gravel, J.L. Mahony, T.J 2008. Identification of two distinct Bovine Parainfluenza Virus type 3 genotypes. Journal of General Virology. 89(7):1643-1648.

Horwood, P.F., Schibrowski, M.L., Fowler, E.V., Barnes, T.S., Mahony, T.J. 2014 Is *Mycoplasma bovis* a missing component of the bovine respiratory disease complex in Australia? Australian Veterinary Journal. 92:6,185.

Hay, K.E., Barnes, T.S., Morton, J.M.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

Goldspink, L.K, Mollinger, J.L., Barnes, T.S., Groves, M., Mahony, T.J., Gibson, J.S. Antimicrobial susceptibility of *Histophilus somni* isolated from clinically infected cattle in Australia. The Veterinary Journal (In press)

6.2 Oral conference presentations

Mahony T.J., Gravel, J.L., Hall, R.N., Horwood, P.F., West, L., Robinson, K.E., Fowler, E.V. 2011 Characterisation of old, new and emerging viruses with next generation sequencing technologies. 6th Australasian Virology Society Meeting. Kingscliff, NSW 4-8 December 2011.

Hay, K.E., 2013. Use of National Livestock Identification System Data to Determine Lifetime Movement and Mixing History Predictors of Bovine Respiratory Disease in Australian Feedlot Cattle. Australian and New Zealand College of Veterinary Scientists Epidemiology Chapter Meeting, Gold Coast, Australia

Barnes, T.S., 2013. Comparing an "Automated" Model Building Selection Procedure with a Causal Diagram-Informed Technique to Identify and Quantify Risk Factors for Bovine Respiratory Disease in Feedlot Cattle. Australian and New Zealand College of Veterinary Scientists Epidemiology Chapter Meeting, Gold Coast, Australia

Mahony, T.J., Morton, J.M.M., Schibrowski, M.L., Hay, K.E., Gravel, J., Commins, M., Ambrose, R., Barnes, T.S. 2013. The National Bovine Respiratory Disease Initiative. Beef Works, Jondaryan, Australia

Hay, K.E., Barnes, T.S., Morton, J.M.M., Clements, A.C.A., Mahony, T.J., 2014. Risk factors for bovine respiratory disease in Australian feedlot cattle: using a causal diagram-based approach to estimate total effects, Society for Veterinary Epidemiology and Preventive Medicine Conference, Dublin, Ireland

Hay, K.E., Barnes, T.S., Morton, J.M.M., Clements, A.C.A., Mahony, T.J., 2014. Risk factors for bovine respiratory disease in Australian feedlot cattle: using a causal diagram-based approach to estimate total and direct effects of mixing and group size before induction, World Buiatrics Congress, Cairns, Australia

Schibrowski, M.L., Barnes, T.S., Gibson, J.S. Mahony, T.J., 2014. Assessment of sero-diagnosis options for infection with *Mycoplasma bovis,* World Buiatrics Congress, Cairns, Australia

6.3 Poster presentations

Hay, K.E., 2013. Epidemiology of Bovine Respiratory Disease: lifetime mixing, moving and saleyard risk factors. Animal Science Olympics, Queensland Alliance for Agriculture and Food Innovation, Brisbane

Mahony, T.J., Morton, J.M.M., Schibrowski, M.L., Hay, K.E., Gravel, J., Commins, M., Ambrose, R., Barnes, T.S. 2013. Cattle producers have a key role to play in strategies to reduce bovine respiratory disease in feedlots. Northern Beef Research Update Conference, Cairns

Hay, K.E., Barnes, T.S., Morton, J.M.M., Clements, A.C.A., Mahony, T.J., 2014. Management-related risk factors for bovine respiratory disease in Australian feedlot cattle with important population level effects. Animal Science Olympics, Queensland Alliance for Agriculture and Food Innovation, Brisbane

6.4 Workshops and seminars

Practitioners Symposium, School of Veterinary Science, University of Queensland, July 2010

Workshop for Consulting Veterinarians, Riverview Hotel, Brisbane, October 2012

School of Veterinary Science Staff Seminar, School of Veterinary Science, University of Queensland, November 2012

Feedlot Co-operator Workshop, Riverview Hotel, Brisbane, March 2013

Queensland Alliance for Agriculture and Food Innovation Seminar, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, April 2013

6.5 Theses

Goldspink, L. Antimicrobial resistance of *Histophilus somni* isolated from infections of cattle in Queensland and New South Wales, Australia. Honours Class I November 2012

Gestier, S., Methodology for the measurement of HIF-1α in bovine leukocytes and assessment of the molecule as a biomarker for bovine respiratory disease outcome. MPhil thesis conferred October 2014

Schibrowski, M.L. *Mycoplasma bovis* in Australian feeder cattle. PhD thesis submitted October 2014

Hay, K.E. Epidemiology of Bovine Respiratory Disease in Australian Feedlot Cattle. PhD thesis submitted December 2014

7 Bibliography

Derksen, S., Keselman, H.J., 1992. Backward, forward and stepwise automated subset-selection algorithms - Frequency of obtaining authentic and noise variables. British Journal of Mathematical & Statistical Psychology 45, 265-282.

Dohoo, I., Martin, W., Stryhn, H., 2010. Veterinary Epidemiologic Research. VER Inc Charlottetown, Canada.

Dunn, S., Godwin, J., Hoare, R., Kirkland, P.D., 1993. Diseases of Feedlot Cattle. Meat Research Corporation.

Elwert, F., 2013. Graphical Causal Models. In: Morgan, S.L. (Ed.), Handbook of Causal Analysis for Social Research. Springer, Dordrecht.

Hanley, J.A., 2001. A heuristic approach to the formulas for population attributable fraction. J. Epidemiol. Community Health 55, 508-514.

Horwood, P.F., Mahony, T.J., 2007. Rapid detection of bovine respiratory disease pathogens. B_FLOT_219 Final Report. Meat and Livestock Australia.

Hurvich, C.M., Tsai, C.L., 1990. The impact of model selection on inference in linear regression. American Statistician 44, 214-217.

Law, G.R., Green, R., Ellison, G.T.H., 2012. Confounding and Causal Path Diagrams In: Tu, Y.-K., Greenwood, D.C. (Eds.), Modern Methods for Epidemiology. Springer, London.

Leckie, G., Charlton, C., 2013. runmlwin - A Program to Run the MLwiN Multilevel Modelling Software from within Stata. Journal of Statistical Software 52, 40.

Martin, S.W., Meek, A.H., 1986. A path model of factors influencing morbidity and mortality in Ontario feedlot calves. Can. J. Vet. Res. 50, 15-22.

Martin, S.W., Meek, A.H., Davis, D.G., Johnson, J.A., Curtis, R.A., 1982. Factors associated with mortality and treatment costs in feedlot calves: the Bruce County Beef Project, years 1978, 1979, 1980. Canadian Journal of Comparative Medicine 46, 341-349.

Sackett, P., Holmes, P., Abbott, K., Jephcott, S., Barber, M., 2006. Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers. Meat and Livestock Australia Limited, North Sydney, NSW.

Sergeant, E., 2001. Survey of Feedlot Diseases in Australia. Meat and Livestock Australia Limited, North Sydney, NSW.

Snijders, T., Bosker, R., 2012. Multilevel Analysis: An Introduction to Basic and Advanced Multilevel Modeling. SAGE Publications London.

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.

Steyerberg, E.W., Eijkemans, M.J.C., Habbema, J.D.F., 1999. Stepwise selection in small data sets: A simulation study of bias in logistic regression analysis. J. Clin. Epidemiol. 52, 935-942.

Textor, J., 2013. Drawing and Analyzing Causal DAGs with DAGitty User Manual for Version 2.0. http://www.dagitty.net/manual-2.x.pdf.

Textor, J., Hardt, J., Knuppel, S., 2011. DAGitty A Graphical Tool for Analyzing Causal Diagrams. Epidemiology 22, 745-745.

VanderWeele, T.J., Robins, J.M., 2007. Four types of effect modification - A classification based on directed acyclic graphs. Epidemiology 18, 561-568.

Weinberg, C.R., 2007. Can DAGs clarify effect modification? Epidemiology 18, 569-572.

Westreich, D., Greenland, S., 2013. The Table 2 Fallacy: Presenting and Interpreting Confounder and Modifier Coefficients. Am J Epidemiol 177, 292-298.

Wilson, S.H., Church, T.L., Acres, S.D., 1985. The influence of feedlot management on an outbreak of bovine respiratory disease. Can Vet J 26, 335-341.

Xue, W., Mattick, D., Smith, L., Umbaugh, J., Trigo, E., 2011. Vaccination with a modified-live bovine viral diarrhea virus (BVDV) type 1a vaccine completely protected calves against challenge with BVDV type 1b strains. Vaccine 29, 70-76.

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Appendices (supplied as separate documents)

Appendix 1

Evaluation of practices used to reduce the incidence of bovine respiratory disease in Australian feedlots

Appendix 2

National BRD Initiative – Information for Feedlot Managers

Appendix 3

National BRD Initiative – Protocols

Appendix 4

National BRD Initiative – Vendor Questionnaire

Appendix 5

National BRD Initiative – Information for Vendors

Appendix 1

Evaluation of practices used to reduce the incidence of bovine respiratory disease in Australian feedlots

Evaluation of practices used to reduce the incidence of bovine respiratory disease in Australian feedlots

(September 2011)

Evaluation of practices used to reduce the incidence of bovine respiratory disease in Australian feedlots

(September 2011)

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Introduction

Bovine respiratory disease (BRD) has been identified as the most significant infectious disease of feedlot cattle in eastern Australia (MLA Project DAN.064).

Annual losses to the feedlot sector have been estimated at $60 per head, placing total industry losses at a minimum of $60 million per year. BRD causes economic loss due to medication costs, mortalities, excessive feed inputs associated with increased time on feed, reduced sale prices and associated labour costs.

BRD is a complex multifactorial condition with a number of animal, environmental and management risk factors predisposing cattle to illness. A range of microorganisms are implicated in BRD with at least four viral and three bacterial species involved singly or in combination.

The four viruses most commonly associated with BRD in Australia are:

- bovine herpesvirus 1 (BHV-1)
- bovine viral diarrhoea virus (BVDV or bovine pestivirus)
- bovine parainfluenza 3 virus (BPI3)
- bovine respiratory syncytial virus (BRSV).

Serological surveys have shown that all of these viruses infect feedlot cattle in Australia (MLA Project DAN.064 and unpublished data). A number of bacterial species have also been recognised as important to the BRD complex; these include *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*.

Though one or more of the pathogens listed above can be isolated from clinical cases of BRD, there is no evidence that infection alone causes serious illness. This indicates that, in addition to specific infectious agents, other factors are crucial for the development of BRD under field conditions.

These can be categorised as animal, environmental and management risk factors.

These risk factors are likely to exert their effects through a number of pathways including reductions in systemic and possibly local immunity. For example, stressors such as weaning, handling at saleyards, transport, dehydration, weather conditions, dietary changes, commingling, and pen competition may reduce the effectiveness of the immune system, allowing infection with pathogens to lead to the development of BRD. In overseas research, environmental and management risk factors have been identified as contributing to the development of BRD.

To date, there have been few Australian feedlot studies that have critically evaluated the practices currently used to reduce the incidence of BRD. Many of the BRD preventative practices are based on overseas research or anecdotal information.

This manual summarises the practices currently used by the Australian feedlot sector to reduce the incidence of BRD, and evaluates the evidence supporting these practices.

The current practices are classified as either 'animal preparation' or 'feedlot management'. Under these headings, the practices have been categorised into one of five categories based on the evidence which underpins their use:

- Australian evidence – A practice that is supported by published peer-reviewed research conducted in Australia. Typically, the supporting evidence would be published in a scientific journal but could also include studies submitted for higher degrees.
- Overseas evidence – A practice that is supported by published peer-reviewed research conducted overseas. This distinction has been made as there are some intrinsic differences between the industries in different countries or continents that may or may not enable direct transfer of this evidence into the Australian sector.
- Registration evidence – A practice supported by research done in Australia and submitted to the Australian Pesticides and Veterinary Medicines Authority (APVMA) for product registration. These studies frequently use small numbers in experimental settings, and are not subject to broader review and publication.

- No evidence – This might include practices that have been adopted in the past but the basis for this adoption is now unknown. This classification also includes practices that have not been subjected to scientific evaluation.
- Anecdotal evidence – The reliability of this evidence is variable. It can be based on controlled research that has not been published or observations of population data.

Table 1. Summary of the animal preparation practices currently used to minimise BRD in Australian feedlots – and their supporting evidence

Animal preparation practice	Evidence	Comments
Yard weaning	Australian	
Pre-vaccination against infectious bovine rhinotracheitis	Registration	
Pre-vaccination against *Mannheimia haemolytica* with Bovilis MH™ vaccine	Registration anecdotal	
Reducing transport time	Australian, overseas	Australian data based on immunological function, not BRD incidence.
Recognising Hereford cattle as having a higher BRD risk	Australian, overseas and anecdotal	
Resting on pasture in stable social group for six weeks before going to feedlot	Anecdotal	
Distance travelled (as opposed to time in transport)	Overseas	US multivariate analysis of survey data.
Truck design/exhaust fumes	Anecdotal	Overseas results published as conference proceedings.
Hydration status on arrival at feedlot	Overseas	
Higher pre-feedlot growth rate	No evidence	

Table 2. Summary of the feedlot management practices currently used to minimise BRD in Australian feedlots – and their supporting evidence

Feedlot management practice	Evidence	Comments
Reduction in purchase groups per pen	Australian, overseas	
Vaccination with modified live BHV1 at feedlot entry	Registration	
Mass medication with antibiotics at feedlot entry	Australian, overseas	
Climate/season	Australian, overseas	Limited Australian data.
Introductory diet	Overseas	
Dietary vitamin E	Overseas	Australian meta-analysis using data from North America.
Vaccination against *Mannheimia haemolytica* at feedlot entry	Anecdotal	Single injection at feedlot entry, reduced BRD mortality, not BRD morbidity.
In feed antibiotics	Overseas, anecdotal	North American meta-analysis showed no effect; Australian anecdotal study found reduced mortality, no effect on morbidity.
Dust concentration	Overseas	Evidence from sheep exposure that may translate to cattle.
Mixing cattle during the feeding period	Overseas	Indirect evidence based on metabolic measures of stress.
Vaccination with two injections against BVDV before feedlot entry	Anecdotal	Pestigard has not been specifically tested for reducing BRD.
Vaccination with a single injection against BVDV at feedlot entry	No evidence	
Large BW range within a pen (ie >100 kg)	No evidence	
Liquid supplements in receival or starting pens (ie urea/molasses)	No evidence	Study currently being done.
Concurrent disease (disease referable to a system other than the respiratory system)	No evidence	
Staffing levels ie pen riders per 10,000 head	No evidence	
Low-stress cattle handling	No evidence	
Rainfall/mud	No evidence	
Use of growth implants	No evidence	
Electrolytes in the water on arrival	No evidence	
Low pen density/bunk space	No evidence	
Reducing time between feedlot arrival and induction	No evidence	
Removal of cattle persistently infected with BVDV	Overseas, anecdotal	Equivocal research outcomes.
Gender	Australian, overseas and anecdotal	Equivocal research outcomes.
Vitamins A, D and E at feedlot entry	Australian	Found not to be effective in Australian published study.

Animal preparation

Practices with robust supporting evidence:

Yard weaning and pre-feedlot vaccination

(MLA project DAN.069; Fell et al., 1998; Walker et al., 2007 (Australian data))

Fell and co-workers (1998) examined the effects of different weaning procedures and vaccination regimens in the preparation of cattle for feedlots on subsequent health outcomes over a three-year period. Vaccines were administered at least one month before feedlot delivery to ensure that vaccinated animals had developed immunity by the time they arrived at the feedlot.

This study examined the performance and health outcomes for groups of cattle that were weaned in one of three ways:

- Paddock weaning – no supplemental feeding or handling for 21 days (PW, control group).
- Yard weaning with good quality hay or silage with minimal handling for 10 days (YW).
- Yard weaning with good quality hay or silage with novel training procedures to increase capacity to adapt to feedlot (YW-T).

British breed calves from two herds (one experimental and one commercial) were weaned at seven to nine months of age. Following weaning, the calves were held on pasture for six to nine months and then transferred to a commercial feedlot. At the feedlot, the study cattle were mixed in a pen with cattle sourced using standard feedlot practices. Within these experimental treatments, a variety of experimental vaccines were applied to the experimental groups with similar numbers of controls.

Calves YW and YW-T had higher average daily gains and reduced morbidity compared with PW. A similar, but lesser, effect was observed with vaccination with YW compared with PW, with average daily gain and morbidity for YW-T being intermediate. The effect of vaccination was somewhat complicated with a variety of vaccines and regimens applied over the course of the three experiments; this may have reduced the effectiveness of this treatment.

While training in combination with YW showed some benefit, it was not as beneficial as YW alone. This finding led the authors to conclude that the establishment of social groups within weaning groups is a critical component for improving health outcomes and productivity of feedlot cattle.

In summary, management of weaning alone or in combination with vaccination at least one month before feedlot delivery yielded an economic benefit in reduced disease incidence and increased weight gain during the feedlot phase.

Pre-vaccination against *Mannheimia haemolytica* with Bovilis MHTM (Intervet) vaccine (two vaccine injections)

(Controlled experiment registration data with anecdotal support)

There have been no formal studies published regarding the effectiveness of pre-vaccination against *Mannheimia haemolytica* with the commercially available vaccine Bovilis MH™ (Intervet). Efficacy was demonstrated by CSIRO for registration of the vaccine with the Australian Pesticides and Veterinary Medicines Authority (APVMA) using a pen study with experimental challenge (n = 8). A field experiment was also conducted at three sites (n = 100 at each site), with no significant difference in morbidity or mortality found in response to two injections of vaccine at a four-week interval. However, the incidence of BRD at all three sites was reported as being very low for the duration of the experiment, making the detection of vaccination effects unlikely. With subsequent commercial use of Bovilis MH™ in a two-dose programme, Sullivan found a reduction in the incidence of BRD (unpublished data). The first dose of vaccine was given not less than two weeks before feedlot entry with the second dose at feedlot entry. Bovine respiratory disease morbidity was reduced by 40%, and BRD mortality was reduced by 58% (*P* values not quoted).

The vaccine is an inactivated preparation; the manufacturer currently recommends two injections of vaccine at a four to six week interval for optimal protection. A current experiment is evaluating the effectiveness of delaying the second injection of vaccine for up to six months. Greater flexibility with the

timing of the second vaccine injection would be advantageous to the management of cattle during backgrounding.

Recognising Hereford cattle as having a higher BRD risk

(Australian data supported by North American data)

Breed can be related to the incidence of BRD (Cusack et al., 2007). British breeds were more likely to develop clinical BRD than *Bos indicus* breeds. Compared to the base population, the development of clinical BRD over time was ten times higher in Herefords, six times higher in Murray Greys, and five times higher in Angus feedlot cattle. The finding of a higher incidence of BRD in the Hereford breed is supported by unpublished Australian data from Sullivan (pers. comm., 2007). A relationship between breed and BRD incidence is supported by North American studies (Muggli-Cockett, 1992; Snowder et al., 2005), with greater susceptibility of the Hereford breed identified by Snowder et al. (2006).

Reducing time taken for transport of cattle to the feedlot

(Indirect Australian and overseas data)

While no studies in Australia have specifically investigated the relationship between increased transport times and subsequent BRD outcomes, one study did assess metabolic changes in cattle subjected to transportation. Stanger et al. (2005) indicated a degree of dysfunction in the immune status of *Bos indicus* steers for six days after 72 hours of road transportation. They concluded this could increase susceptibility to infectious agents for six days after transport though this was not actually tested in the study. In keeping with this, a Polish study (Urban-Chmiel, 2006) found transport duration of 72 hours (1,700km) resulted in significantly reduced ($P<0.05$) leukocyte viability with samples exposed to leukotoxin from *M. haemolytica.*

Most of the stress of transport of less than 24 hour duration appears to be related to the loading and unloading process (Cole et al., 1988). Conversely, increasing transport time for trips longer than 24 hours was associated with higher BRD incidence in US cattle (Johnson, 1985).

The effects of the time cattle are held in saleyards or holding yards before transport to the feedlot have not been evaluated.

Distance travelled (as opposed to time in transport)

(Inadequately defined European data and robust US data)

Mormede et al. (1982) found a higher incidence of BRD in cattle held overnight in a holding yard, and transported a longer distance (300km) than in cattle transported a short distance, from the same property of origin, directly to the feedlot. The study design does not allow the separation of the effects on BRD incidence of transport distance from transport duration. Another possible confounding factor in the higher incidence of BRD is the increased handling due to being held overnight in holding yards. However, a US study (Sanderson, et al., 2008) found an increase in BRD morbidity with increased transport distance (Incidence Rate Ratio [IRR] = 1.001, P <0.001), with the data indicating a 10% increase in initial BRD morbidity risk for each 160 km increase in transport distance.

Practices with anecdotal evidence

Resting on pasture in stable social group for six weeks before feedlot entry

There are no published Australian studies to support this practice, but there are anecdotal reports of effective use of this practice in some Queensland feedlots (attributed to David Brown). The importance of the establishment of stable social groups is supported by the results of yard-weaning in MLA report DAN.069.

Practices with minimal evidence or untested

Truck design/exhaust fumes

(Extrapolation from North American unpublished data (conference proceedings))

Exposure to exhaust fumes was found to reduce subsequent feedlot growth rate (Cole, et al., 1989). When the exhaust stack on a prime-mover was lower than the top of the trailer, calves that travelled on the top deck tended to have lower subsequent feedlot

growth rates than calves that travelled on the lower deck. When the exhaust stack was higher than the trailer, calves from the top deck had higher feedlot growth rates than calves from the bottom deck. An expectation of an increase in the incidence of BRD in calves exposed to exhaust fumes is based on this recorded effect on growth rate.

A more recent study (White et al., 2009), which assessed the effects of location within the transport vehicle on growth rates and health, supported the previous finding that animals located closer to the front of the trailer had lower growth rates. Again the assumption was that the findings were due to exposure to exhaust fumes.

Both of these studies have been published as conference proceedings and not subjected to peer review.

Hydration status on arrival at the feedlot

(Inadequately defined European data)

Mormede et al. (1982) found a higher BRD incidence in cattle transported for longer distances that were dehydrated, but the effects of hydration status were not isolated from the effects of transport distance and duration. Dehydration can be a result of prolonged transport and might be one of the mechanisms by which transport could increase the incidence of BRD. These effects and their relative contributions to BRD incidence have not been adequately defined.

Higher pre-feedlot growth rate

(Untested)

Feedlot management

Practices with robust supporting evidence

Reduction in purchase groups per pen

(Extrapolation from Australian findings measuring growth rate, with support from North American studies measuring morbidity and mortality)

Australian cattle maintained as a group from weaning until feedlot entry adapted more rapidly to the feedlot diet and had higher growth rates over the first 37 days compared with cattle purchased through saleyards from a variety of sources (Fell et al., 1998). It is not possible to separate the effects of mixing in this study from the potential effects of exposure to saleyards. However, in the Canadian Bruce County Project, morbidity and mortality from BRD were greater with mixing of calves from different sources and assembly of calves from widely separated geographic locations (Martin et al., 1982). More recently, O'Connor et al. (2005) found a strong relationship between commingling and BRD (odds ratio = 3; 95% CI = 2.5 to 3.6), and Sanderson et al. (2008) also found an increase in BRD morbidity (Incidence Rate Ratio [IRR] = 2.0, P <0.001) with cattle from multiple sources.

Vaccination against infectious bovine rhinotracheitis (IBR) at feedlot entry

(Australian data used to register the vaccine Rhinogard™ (Q-vax))

Seven trials with a live attenuated Australian strain of BHV-1 administered intranasally resulted in a significant improvement in growth rate and feed conversion ratio (P <0.05) without a significant reduction in the percentage of cattle treated for all feedlot diseases (P >0.05) during the first 30 days on feed (P. Young, unpublished registration data submitted to the APVMA). It is possible vaccination might have had a significant effect on the incidence of BRD or, more specifically, IBR, had these diagnoses been recorded. Field observations by feedlot veterinarians support the effectiveness of vaccination at feedlot entry with Rhinogard™ in the prevention of IBR. The onset of activity of this modified live vaccine is rapid, with local production of immunoglobulin A in the upper airways conferring protection against the development of IBR. Infectious bovine rhinotracheitis is caused by a single organism, BHV-1, and there is a vaccine that is effective against this organism. It is therefore a preventable disease and should be viewed separately to the pneumonia of BRD.

Mass medication with antibiotics at feedlot entry

(Australian data with numerous supporting North American studies)

An Australian study examined the effects on cattle destined for the domestic market of mass medication at feedlot entry with long-acting oxytetracycline and tilmicosin (Cusack, 2004). Cattle mass medicated with tilmicosin

had significantly fewer treatments for all illnesses (P = 0.0004) and BRD specifically (P = 0.0001), compared with cattle not given antibiotic at feedlot entry and compared with cattle mass medicated with oxytetracyline (P = 0.004). There was no significant difference in treatments for all diseases (P = 0.47) and treatments for BRD (P = 0.26) between oxytetracycline-treated cattle and cattle not given antibiotic at feedlot entry. The cattle treated with tilmicosin at feedlot entry had a significantly higher mean daily body weight gain (1.67 v. 1.59 kg/day) than cattle not medicated with antibiotic at feedlot entry (P = 0.03) and cattle medicated with oxytetracycline at feedlot entry (P = 0.05).

Unpublished financial analysis of this study showed mass medication was profitable, even with a relativlely low incidence of BRD, mainly due to the higher growth rate of the tilmicosin-medicated cattle. Field observations show the lower body weight cattle fed for the Australian domestic market have a higher incidence of BRD than the heavier cattle fed for the Japanese market. This would presumably affect the response to mass medication of cattle in the different weight ranges, and therefore the profitability of the practice. Further research into responses to antibiotic mass medication of cattle over a range of feedlot entry weights under a variety of Australian feedlot production systems is warranted.

North American studies have illustrated reductions in the incidence of BRD in response to mass medication with injectable antimicrobials. Positive responses to mass medication have been found following adminstration to all cattle at feedlot entry of benzathine penicillin (King et al., 1955), long-acting oxytetracycline (Lofgreen, 1983; Harland et al., 1991; Van Donkersgoed et al., 1994), sulfadimethoxine (Lofgreen, 1983), and tilmicosin (Schumann et al., 1990; Schumann et al., 1991; Galyean et al., 1995; McClary and Vogel, 1999); selective administration on the basis of rectal temperature at feedlot entry of tilmicosin (Galyean et al., 1995); administration of long-acting oxytetracycline to all cattle in a pen once BRD incidence exceeded 5% (no time frame reported, Janzen and McManus, 1980); and delayed administration of tilmicosin to all cattle in a pen (Schumann et al., 1991; McClary and Vogel, 1999). In addition to a reduction in BRD morbidity, four of these experiments (Janzen and McManus, 1980; Schumann et al., 1990; Schumann et al., 1991; Galyean *et al.*, 1995) also showed a positive growth rate response to treatment.

Van Donkersgoed (1992) used meta-analysis to examine the effect of antimicrobial mass medication on morbidity, mortality and growth rate as these related to BRD. Of 107 field trials, only ten were randomised controlled field trials deemed suitable for meta-analysis. The results indicated that parenteral mass medication with long-acting oxytetracycline or tilmicosin on feedlot arrival would significantly reduce BRD morbidity in feedlot cattle. However, the author concluded that data on the effects of mass medication on mortality and performance were unreliable, that there were insufficient data on the most effective treatment regimens, and that there were no valid data on the efficacy of mass medication delivered in feed or water for prevention of BRD. Subsequently, Hellwig et al. (1999) found mass medication with injectable tilmicosin at feedlot arrival was superior to chlortetracycline added to the ration in terms of BRD morbidity and treatment costs.

Tulathromycin has recently become available for the treatment and prevention of BRD. As yet, there are no published Australian studies on the efficacy of mass medication with tulathromycin, but north American studies have shown it to be more effective in reducing the incidence of BRD than tilmicosin (Kilgore et al., 2005; Rooney et al., 2005) or florfenicol (Rooney et al., 2005).

Climate/season

(Limited Australian and North American data)

The peak incidence of BRD usually occurs in autumn and early winter in Australia and the USA (Irwin et al., 1979). Whereas the association between season and BRD incidence in the USA could be confounded by the influx of light-weight calves in autumn, feedlot cattle numbers do not consistently vary with season in eastern Australia. More rapid and severe temperature changes and greater

weather extremes in the USA contribute to higher BRD morbidity and mortality rates than in Australia (Irwin et al., 1979).

This observation prompted Australian reviewers to propose that rapid change in temperature, rather than temperature per se, is responsible for an increase in the incidence of BRD (Cusack et al., 2003). However, a subsequent Australian study showed a stronger correlation between minimum temperature and BRD incidence ($r = 54\%$, $P = 0.002$) than temperature range and BRD incidence ($r = 25\%$, $P = 0.05$) during the winter months (Cusack et al., 2007). The findings from Cusack et al. (2007) do not preclude the possibility that temperature range is strongly correlated with the incidence of BRD during autumn. Further research into this issue is warranted.

Introductory diet

(North American data)

There is a strong association between feeding corn silage during the first month in the feedlot and increased incidence of BRD (Martin et al., 1982). In the Bruce County Beef Project's analysis of introductory feeding practices, mortality due to BRD was five times higher in calves fed corn silage as a major portion of their diet during the first week in the feedlot than in calves that were not fed substantial amounts of corn silage until the fourth week. Feeding grain with the silage appeared to reduce some of the negative effects of silage consumption.

Inclusion of non-protein nitrogen in the introductory diet in addition to that in the silage was also associated with increased mortality. Although analyses of the diets were not provided in this study it appears that feeding excessive amounts of non-protein nitrogen with inadequate rumen degradable true protein and inadequate starch and sugars may be responsible for the observed increase in the incidence of BRD rather than silage feeding per se. The relationship between dietary crude protein and BRD incidence is unclear (Duff and Galyean, 2007). Crude protein is derived from dietary nitrogen concentration and does not adequately describe the characteristics of the protein provided by a diet. The relationship between protein and BRD incidence can only be accurately assessed by evaluating the relative contributions to diets of true protein, non-protein nitrogen, rumen degradable protein, rumen undegradable protein and unavailable protein (from acid detergent insoluble nitrogen).

Lofgreen (1983) reported a reduction in morbidity and mortality when newly-arrived calves were fed grass hay only, but this feeding practice resulted in a decrease in growth rate. If hay was provided for longer than three days in the receiving pen, it tended to inhibit intake of mixed ration, thereby reducing energy intake (Johnson, 1985). Cattle purchased in saleyards and introduced to diets containing 20–30% high-moisture barley were 4.9 times more likely to be treated for BRD, and 6.7 times more likely to die from BRD, than cattle assembled on their farm of origin and started on a diet containing 10% high-moisture barley (Wilson et al., 1985), but this study does not isolate the effects of saleyard purchase from diet.

Cattle with low blood glucose concentrations on arrival at the feedlot had a greater chance of subsequently developing severe BRD, and morbidity and mortality were reduced in calves fed a diet containing 55% concentrate rather than good-quality hay at the saleyards before transport to the feedlot (Cope, 1978). Conversely, Rivera et al. (2005) found a slight increase in BRD morbidity with diets with increasing concentrates over a range from zero to 75% concentrate [morbidity % = 49.59 – (0.0675 x roughage %); $P = 0.003$]. However, higher roughage diets were associated with lower daily weight gain ($P < 0.001$); lower BRD morbidity with such diets did not offset the financial loss due to lower growth rate. Although rumen pH was not measured in these studies, the effects of higher grain diets on the incidence of BRD may be mediated by the development of lactic acidosis, a disorder which is influenced by feed milling and delivery in addition to diet formulation. It may be that diets with at least 50% concentrates can reduce the incidence of BRD in cattle newly arrived at the feedlot provided they do not result in lactic acidosis. The appropriate formulation of the initial diet for cattle on

arrival at feedlots requires further research. The potential for inappropriately processed or limit-fed higher concentrate introductory diets to have adverse health effects due to lactic acidosis should be measured in research on the relationship between introductory diet and BRD by monitoring rumen pH, total volatile fatty acid yield and lactate concentration.

In summary, published studies indicate that introductory diets should not provide a high proportion of crude protein as non-protein nitrogen, particularly where fermentable carbohydrate is limiting. Further, it appears that higher concentrate introductory diets are appropriate provided their milling and delivery does not cause lactic acidosis. Formulation targets for introductory diets to minimise the incidence of BRD are yet to be established, and research to determine them will require full description of dietary protein and monitoring of rumen fermentation characteristics. The relationship between lactic acidosis and the incidence of BRD should also be clarified.

Dietary Vitamin E

(Australian meta-analysis of North American data)

Delivery of supplemental antioxidant vitamins to cattle placed in feedlots might be expected to improve health and performance outcomes by reducing the effects of oxidative stress to which these cattle are exposed (Chirase et al., 2004). Meta-analytic procedures were used by Cusack et al. (2008) to assess published experiments on the effects of vitamin E supplementation in feedlot cattle. The health outcome of morbidity, and the production outcomes of average daily gain (ADG) and gain to feed ratio (G:F) were analysed. The authors concluded that supplemental dietary vitamin E should be fed within the range recommended by NRC (1996) and that higher dietary inclusion rates do not consistently reduce BRD morbidity, and are not profitable.

Practices with anecdotal evidence

Vaccination with Bovilis MH™ against Mannheimia haemolytica at feedlot entry

(Unpublished Australian data)

Sullivan (unpublished data, 2008) found single injection of Bovilis MH™ at feedlot entry reduced mortality from BRD by 12–15%. During periods of high risk of respiratory disease (cattle entering the feedlot from January to April), Sullivan reported a 30% reduction in BRD mortalities. No reduction in BRD morbidity was measured.

In-feed antibiotics

(Unpublished Australian data)

Addition of oxytetracycline to feed at 25 mg/kg body weight daily from day 5 to day 10 of the feeding period reduced ($P < 0.06$) mortalities (Sullivan, unpublished data, 2008). The treatment did not reduce morbidity or have any effect on feed intake or daily gain. This finding is not consistent with the results of the meta-analysis of North American data by van Donkersgoed (1992) where in-feed antibiotics were not found to be effective in reducing the incidence of BRD.

Practices with minimal evidence or untested

Dust concentration

(Indirect supporting evidence from North America using experimental exposure of small ruminants to dust)

Feedlot dust can contain viable microbes and, more importantly, endotoxin (Purdy et al., 2002). Repeated exposure of sheep to feedlot dust containing endotoxin for four-hour periods induced temporary pyrexia and leukocytosis, and generalised alveolar septal thickening and hypercellularity (Purdy et al., 2002). Repeated exposure of goats to feedlot dust resulted in a mild, acute exudative bronchointerstitial pneumonia (Purdy et al., 2002).

Mixing cattle during the feeding period

(Limited indirect North American data)

Gupta et al. (2005) found steers mixed and relocated at two-week intervals had increased plasma cortisol, albumin, urea and non-esterified fatty acids. There was also a trend ($P = 0.10$) for lower growth rate in the mixed and relocated steers. However, this study only had 6 steers in each pen. This small number of animals in each pen would presumably reduce the effects of social stress compared with commercial feedlot pens considering the observation of Taylor et al. (1997) that social

hierarchy becomes unstable with more than about 100 animals in a pen.

Vaccination with two injections against BVDV at a four-week interval before feedlot delivery

(Unpublished Australian data)

The role of BVDV in the pathogenesis of BRD has been subject to much conjecture due to a lack of evidence implicating it as a primary BRD pathogen. BVDV may facilitate colonisation of the lungs by other pathogens (Richer et al., 1988).

Experimental infection of immunocompetent, seronegative calves with BVDV type 1d induced primary BRD in the absence of concurrent infection with other BRD pathogens (Baule et al., 2001); this suggests a possible primary role for the virus in the pathogenesis of BRD. It appears, therefore, that BVDV might enhance the development of BRD by immunosuppression and as a primary respiratory pathogen. The effects of vaccination against BVDV with two injections of Pestigard™ at a four-week interval in backgrounding programmes have not been separated from the presumed positive effects of backgrounding itself.

Unpublished Australian data (Batterham) showed vaccination of backgrounded cattle with two injections of Pestigard™ had no significant effect on the incidence of BRD, but the serological status of the study cattle at the time of vaccination was not known.

Vaccination with a single injection against BVDV at feedlot entry

(Untested)

Large body weight range (ie >100 kg) within a pen

(Untested)

Liquid supplements in receival or starting pens (ie urea/molasses)

(Untested)

Concurrent disease

(Untested)

Staffing levels (ie pen riders per 10,000 head)

(Untested)

Low-stress cattle handling

(Untested)

Rainfall/mud

(Untested)

Use of growth implants

(Untested)

Electrolytes in the water on arrival

(Untested)

High pen density

(Untested)

Low bunk space

(Untested)

Reducing time between feedlot arrival and induction

(Untested)

Practices with equivocal research outcomes

Removal of cattle persistently infected with BVDV

Whilst the prevalence of cattle entering the feedlot persistently infected with BVDV is low (0.3% in a US study; Loneragan et al., 2005), cattle in the same and adjoining pens have been found to have an increased risk of BRD (0.5 cases per 1000 head days vs 0.35 cases per 1000 head days; RR = 1.43, 1.0 to 2.0; P = 0.04). Conversely, O'Connor et al. (2005) found the presence of an animal persistently infected with BVDV did not increase the incidence of BRD in the same pen. However, the serological status of the pen-mates at the start of the feeding period was not determined in either the Loneragan (2003) experiment or the O'Connor (2005) experiment so the susceptibility of the populations of interest to infection with BVDV was unknown.

Preliminary research from Batterham (unpublished data) found cattle in a pen with a persistently infected animal had a 2.3 times greater likelihood of being treated for BRD, but there was no effect on growth rate or FCR. There was also no effect on the BRD treatment rate in adjacent pens. From these data, Batterham suggested that identification and removal of persistently infected animals from cattle newly arrived at a feedlot would be profitable only where pen size is greater

than 200 animals and the incidence of BRD exceeds 10% of mean feedlot occupancy on a monthly basis.

Gender

(Australian and North American data)

Cusack et al. (2007) found an association (P = 0.03) between gender and mortality due to BRD, with steers being slightly more likely to die during the feeding period than heifers. However, the effect was small, and Sullivan (unpublished data) has found the relationship to be variable. In North America, Snowder et al. (2006), found steers were more likely to be diagnosed with BRD than heifers.

Practices that we know do not work

Vitamins A, D and E at feedlot entry

(Australian data)

Cusack et al. (2008) examined the effects of injectable vitamins A, D and E at feedlot entry on health and growth rate. 2,465 cattle were allocated systematically at feedlot entry to: a commercial vitamin A, D and E preparation at the label dose rate; commercial vitamin A, D and E at twice the label dose rate; a formulation with no vitamin D, a lower concentration of vitamin A and a higher concentration of vitamin E; and the oil-based carrier alone at volumes corresponding to the above treatments. Growth rates, disease and mortality were compared between the groups at the conclusion of the feeding period. There were no differences between cattle administered vitamin A, D and E at feedlot entry and the controls in growth rate (P = 0.11), in all diseases (P = 0.99), in BRD (P = 0.60) or in mortalities (P = 0.95). Cattle treated with the higher vitamin E and lower vitamin A preparation had a higher (P = 0.02) incidence of anorexia than the other groups. The routine injection of cattle with vitamins A, D and E at feedlot entry is unlikely to result in improvements in health and growth rate where cattle are provided with these vitamins in their diets at concentrations equal to the recommendations by the National Research Council (1996). In addition, a meta-analytic review by Cusack et al. (2008) found that the currently available data do not support the use of supplemental vitamin E administered as an injection (morbidity risk ratio = 1.17; P = 0.165).

Recommendations

In summary, we have *substantial* evidence to recommend the following BRD prevention practices (as at September 2011):

- Yard weaning with or without two injections of Bovilis MH at four-week intervals before feedlot delivery.
- Minimise the distance cattle are transported to the feedlot and the time taken for delivery.
- Vaccination with modified live BHV1 vaccine at feedlot entry.
- Reduce the number of purchase groups per pen with cattle placed directly in the feedlot.
- Avoid high concentrations of non-protein nitrogen in starter diets.
- Mass medication of high-risk cattle where the other preventative measures have not been possible.
- Provide dietary vitamin E at the upper range of the National Research Council recommendation of 60 IU/kg diet DM.

These recommendations will be added to and refined over time as more research findings are published. Studies are currently being done in Australia to assess the effects on BRD incidence of: short-term local backgrounding (6 to 12 weeks) with or without vaccination against *M. haemolytica* and/or BVDV; and the provision of a urea/molasses liquid supplement in addition to starter ration for the first three days in the feedlot.

References

Chirase, N.K., L.W. Greene, C.W. Purdy, R.W. Loan, B.W. Auvermann, D.B. Parker, E.F. Walborg, D.E. Stevenson, Y. Xu and J.E. Klaunig. Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. Am. J. Vet. Res. 2004. 65 (6):860-864.

Cole, N.A. 1989. Prearrival factors affecting feeder calf health and performance. Page 1 in Proc. Joint Meeting of the Academy of Veterinary Consultants/Plains Nutrition Council, Amarillo, Texas.

Cole, N.A., C.W. Purdy and D.M. Hallford. 1988. Influence of fasting and postfast diet energy level on feed intake, feeding pattern and blood variables of lambs. Am. J. Vet. Res. 49:178-183.

Cope, G.E. 1978. For better health ship them full. Page 116 in Proc. Convention of American Association of Bovine Practitioners, Baltimore, USA.

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. Aust. Vet. J. 82:154-156.

Cusack, P.M.V., N.P. McMeniman, and I.J. Lean. 2003. The medicine and epidemiology of bovine respiratory disease in feedlots. Aust. Vet. J. 81:480-487.

Cusack, P.M.V., N.P. McMeniman, and I.J. Lean. 2007. Feedlot entry characteristics and climate: their relationship with cattle growth rate, bovine respiratory disease and mortality. Aust. Vet. J. 85:311-316.

Cusack, P.M.V., N.P. McMeniman, and I.J. Lean. 2008. Effects of injectable vitamins A, D, E and C on the health and growth rate of feedlot cattle destined for the Australian domestic market. Aust. Vet. J. 86:81-87.

Cusack, P.M.V., N.P. McMeniman, A.R. Rabiee and I.J. Lean. 2008. Assessment of the effects of supplementation with vitamin E on health and production of feedlot cattle using meta-analysis. Prev. Vet. Med. (in press).

Duff, G.C. and M.L. Galyean. 2007. Recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823-840.

Fell, L.R., K.H. Walker, L.A. Reddacliff, L. Davies, H.J. Vallance, J.R. House and S.C. Wilson. 1998. Effects of yard weaning and pre-feedlot vaccination on feedlot performance of *Bos taurus* steers. Anim. Prod. In Aust. 22:173-176.

Galyean, M.L., S.A. Gunter and K.J. Malcolm-Callis. 1995. Effects of arrival medication with tilmicosin phosphate on health and performance of newly received beef cattle. J. Anim. Sci. 73:1219-1226.

Gupta, S., B. Earley, S.T.L. Ting and M.A. Crowe. 2005. Effect of repeated regrouping and relocation on the physiological, immunological, and hematological variables and performance of steers. J. Anim. Sci. 83:1948-1958.

Harland, R.J., G.K. Jim, P.T. Guichon, H.G.G. Townsend and E.D. Janzen. 1991. Efficacy of parenteral antibiotics for disease prophylaxis in feedlot calves. Can. Vet. J. 32:163-168.

Hellwig, D., B. Kegley and S. Silzell. 1999. A comparison of mass antibiotic treatments on arrival with regards to morbidity, mortality and performance in Arkansas stocker cattle. Arkansas Agricultural Experiment Station, University of Arkansas, Fayetteville, USA. No. 464:96-97.

Irwin, M.R., S. McConnell, J.D. Coleman and G.E. Wilcox. 1979. Bovine respiratory disease complex: a comparison of potential predisposing and aetiologic factors in Australia and the United States. J. Am. Vet. Med. Assoc. 175:1095-1099.

Janzen, E.D. and R.F. McManus. 1980. Observations on the use of a long-acting oxytetracycline for in-contact prophylaxis of undifferentiated bovine respiratory disease in feedlot steers under Canadian conditions. Bovine Practitioner. 15:87-90.

Johnson, E.G. 1985. Feedlot management practices and bovine respiratory disease. Vet. Clin. Nth Am. Food Anim. Pract. 1:413-419.

Kilgore, W.R., M.S. Spensley, F. Sun, R.G. Nutsch, K.A. Rooney and T.L. Skogerboe. 2005. Clinical effectiveness of tulathromycin, a novel triamilide antimicrobial, for the control of respiratory disease in cattle at high risk for developing bovine respiratory disease. Vet. Therapeutics 6:136-142.

King, N.B., B.H. Edgington, L.C. Ferguson, D.L. Thomas, W.D. Pounden, E. Klosterman. 1955. Preliminary results in the control and treatment of shipping fever complex in beef cattle. J. Am. Vet. Med. Assoc. October:320-323.

Lofgreen, G.P. 1983. Mass medication in reducing shipping fever-bovine respiratory disease complex in highly stressed calves. J. Anim. Sci. 56:529-536.

Lofgreen, G.P. 1983. Nutrition and management of stressed beef calves. Vet. Clin. Nth Am. Food Anim. Pract. 5:87-101.

Loneragan, G.H., D.U. Thomson, D.L. Montgomery, G.L. Mason and R.L. Larson. 2005. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. J. Am.Vet. Med. Assoc. 4:595-601.

Martin, S.W., A.H. Meek, D.G. Davis. 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.

McClary, D. and G. Vogel. 1999. Effect of timing of tilmicosin metaphylaxis on control of bovine respiratory disease and performance in feeder cattle. Bovine Practitioner 33:155-161.

Mormede, P., J. Soissons, R.M. Bluthe, J. Raoult, G. Legarff, D. Levieux and R. Dantzer. 1982. Effect of transportation on blood serum composition, disease incidence, and production traits in young calves. Influence of the journey duration. Ann. Rech. Vet. Ann. Vet. Res. 13:369-384.

Muggli-Cockett, N.E., L.V. Cundiff and K.E. Gregory. 1992. Genetic analysis of bovine respiratory disease in beef calves during the first year of life. J. Anim. Sci. 70:2013-2019.

National Research Council (NRC).1996. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

O'Connor, A.M., S.D. Sorden and M.D. Apley. 2005. Association between the existence of calves persistently infected with bovine viral diarrhea virus and commingling on pen morbidity in feedlot cattle. Am. J. Vet. Res. 66:2130-2134.

Purdy, C.W., D.C. Straus, N. Chirase, D.B. Parker, J.R. Ayers and M.D. Hoover. 2002. Effects of aerosolised endotoxin in feedyard dust on weanling goats. Small Rum. Res. 46: 133-147.

Purdy, C.W., D.C. Straus, N. Chirase, D.B. Parker, J.R. Ayers and M.D. Hoover. 2002. Effects of aerosolised feedyard dust that contains natural endotoxins on adult sheep. Am. J. Vet. Res. 63: 28-34.

Purdy, C.W., D.C. Straus, D.B. Parker, J.R. Ayers and M.D. Hoover. 2002. Treatment of feedyard dust containing endotoxin and its effect on weanling goats. Small Rum. Res. 46: 123-132.

Rivera, J.D., M.L. Galyean and W.T. Nichols. 2005. Review: Dietary roughage concentration and health of newly received cattle. Prof. Anim. Sci. 21:345-351.

Rooney, K.A., R.G. Nutsch, T.L. Skogerboe, D.J. Weigel, K. Gajewski and W.R. Kilgore. 2005. Efficacy of tulathromycin compared with tilmicosin and florfenicol for the control of respiratory disease in cattle at high risk of developing bovine respiratory disease. Vet. Therapeutics 6:154-166.

Sanderson, M.W., D.A. Dargatz and B.A. Wagner. 2008. Risk factors for initial respiratory disease in United States' feedlots based on producer-collected morbidity counts.

Schumann, F.J., E.D. Janzen and J.J. McKinnon. 1991. Prophylactic medication of feedlot calves with tilmicosin. Vet. Rec. 128:278-280.

Snowder, G.D., L.D. Van Vleck, L.V. Cundiff and G.L. Bennett. 2005. Influence of breed, heterozygosity, and disease incidence on estimates of variance components of respiratory disease in preweaned beef calves. J. Anim. Sci. 83:1247-1261.

Snowder, G.D., L.D. Van Vleck, L.V. Cundiff and G.L. Bennett. 2006. Bovine respiratory disease in feedlot cattle: Environmental, genetic, and economic factors. J. Anim. Sci. 84:1999-2008.

Stanger, K.J., N. Ketheesan, A.J. Parker, C.J. Coleman, S.M. Lazzaroni and L.A. Fitzpatrick. 2005. The effect of transportation on the immune status of Bos indicus steers. J. Anim. Sci. 83(11):2632-2636.

Taylor, L.F., C.W. Booker, G.K. Jim and P.T. Guichon. 1997. Epidemiological investigation of the buller steer syndrome (riding behaviour) in a western Canadian feedlot. Aust. Vet. J. 75:45-51.

Urban-Chmiel, R., A. Puchalski and A. Wernicki. Influence of transport stress on susceptibility of bovine leukocytes to the cytotoxic effect of M. haemolytica leukotoxin. Medycyna Weterynaryjna. Polskiego Towarzystwa Nauk Weterynaryjnych, Lublin, Poland: 2006. 62(9):1045-1047.

Van Donkersgoed, J. 1992. Meta-analysis of field trials of antimicrobial mass medication for prophylaxis of bovine respiratory disease in feedlot cattle.Can. Vet. J. 33:786-795.

Van Donkersgoed, J., E.D. Janzen, A.A. Potter and R.J. Harland. 1994. The occurrence of *Haemophilus somnus* in feedlot calves and its control by postarrival prophylactic mass medication. Can. Vet. J. 35:573-580.

Wilson, S.H., T.L. Church and S.D. Acres. 1985. The influence of feedlot management on an outbreak of bovine respiratory disease. Can. Vet. J. 26:335-341.

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Appendix 2

National BRD Initiative – Information for Feedlot Managers

The National BRD Initiative

Information for Feedlot Managers

Table of Contents

29 May 2007

To: Feedlot Operator

Dear Sir/Madam

RE: National BRD Initiative

Animal health surveys have consistently identified the bovine respiratory disease complex (BRD) as the most significant infectious disease of feedlot cattle in eastern Australia (MRC Project DAN.064 (1991) and 'Survey of feedlot diseases in Australia' (2001)). BRD is responsible for over 60% of all morbidities and mortalities in feedlots and causes economic loss due to medication costs, mortalities, excessive feed inputs associated with increased time on feed, reduced sale prices and labour costs associated with the detection, handling and treatment of sick animals. Annual losses to the feedlot sector have been estimated at $20 per head across all animals, placing total industry losses at a minimum of $40 million per year (MLA Project AHW.087).

Since the time of the 2001 survey, three vaccines have become available and are routinely utilised by a significant proportion of industry operators. Despite the benefit that the vaccines offer, BRD continues to be a major problem for the industry with many operators believing that the viruses have become more virulent. While there is no scientific evidence to support this assertion, there is anecdotal evidence that particular pathogens have become endemic in many of the older feedlots.

The need to undertake further research to address the BRD problem was identified as a major priority in the feedlot program strategic planning process undertaken during 2005. Since that time, significant consultation has taken place between MLA and industry in developing a project to address the problem.

A specific BRD workshop, involving industry, veterinary, research and animal health company representatives, was held in May 2006 to identify the researchable issues. This workshop identified that it was necessary to first identify and understand the critical risk factors leading to BRD development before management strategies could be developed to

intervene in the disease development process. As such, the central platform of the project proposal is the conduct of an epidemiological study to identify, and quantify the impact of, the critical risk factors associated with BRD development. Additional components of the project will examine the role that animals that are persistently infected with BVDV play in the occurrence of BRD and the potential for use of new technologies for detecting sick animals.

The major project output will be a best practice manual for the management of BRD in feedlot cattle, incorporating a decision support tool for feedlot managers and advisors. This tool will calculate the economic impacts associated with the various management decisions and practices that affect BRD incidence.

A successful project outcome will provide the feedlot sector with improved strategies for managing BRD in a feedlot situation, underpinned by a comprehensive understanding of the critical risk factors involved in BRD development, and the opportunity to minimise the economic impact of BRD on feedlot cattle performance.

We seek your assistance with this research and ask that you give favourable consideration to being one of our co-operator feedlots. Further details on what is involved in being a co-operator feedlot are attached. Please peruse these and come back to me if you have any questions about the project. My contact details are shown below

Yours sincerely

Des Rinehart

Feedlot R&D Project Manager

Phone: 07 5464 2277 or 0417 728785

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The National BRD Initiative

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Swans Veterinary Services

Overview and Study Design

Bovine Respiratory Disease (BRD) has been identified as an ongoing issue for Australian feedlots and a priority for current MLA research and development activities. Annual losses attributable to BRD are estimated at over AUD $60 million with the potential for further losses in "peak" years (1996, 2005). Recent industry consultation has identified the need for Australia specific research into management practices which may impact on BRD and their cost-effectiveness. To meet this end, Meat and Livestock Australia, Queensland DPI&F and the University of Queensland have come together to form the National Bovine Respiratory Disease Initiative (National BRD Initiative).

The Initiative comprises six inter-related projects (see Table 1). Results will be used to create a decision support tool and best practice manual to aid cost-effective management of BRD.

The major initiative is a prospective observational study of 32 - 40,000 cattle entering Australian feedlots during 2009 - 2011. Data on potential risk factors for the development of BRD will be collected from feedlot databases, vendors, the National Livestock Identification System (NLIS), dust monitors (where available) and feedlot weather stations (Component 2). In addition, exposure of these cattle to known BRD pathogens will be monitored through blood serology and nasal swabs (Component 3). A subset of cattle will be followed through until slaughter enabling correlation between lung lesions at slaughter and BRD diagnosis at the lot (Component 5). A pilot study involving three feedlots was conducted during 2007 – 2008. The main study consisting of up to 16 feedlots will commence in 2009. Component 4 is a parallel intervention study being carried out on selected feedlots.

What is required:

- Enthusiasm and commitment for duration of the study.
- A computerised database.
- The enrolment of approximately 13 cohorts (groups of animals that comprise a "filled pen" at the time of induction) during the study period. We envisage that feedlots will enrol cohorts at approximately 8 week intervals over an enrolment period of about 24 months.
- Bleeding and nasal swabbing of all study animals at induction and of any study animals that are pulled for suspected BRD (taken at the time of pulling). Bleeding of all study animals at 42 days post induction. You will need to have a crush suitable for bleeding animals.
- Fresh post mortem samples of trachea and lung from all study animals that die while on feed.

- With the exception of the additional sample collection, it is most important for the study that you maintain normal feedlot management practices (movement of animals between pens, splitting of groups of animals inducted into study pens, etc.). The only possible exception to this is that we request that study cohorts remain together until the 42 day bleed as splitting prior to this would create logistical difficulties.
- Completion of an animal health survey.
- Data collection - initial set up: Some fields may have to be added to the database to ensure adequate data collection. Feedlots using StockaID will receive an upgraded version which will enable feedlot staff to send the required data to the Operations Manager with minimal effort. Most data from AMH feedlots will be collected centrally.
- Access to the feedlot database and records for the data listed on pages 8 – 10 – from induction until the end of time on feed.
- Permission to access the NLIS database for movement records of study cattle.
- Collection of data from feedlot weather stations and, for a sub-set of feedlots, recording of daily particulate matter (dust) data.
- For a sub-set of feedlots: an interview with the feedlot manager and some staff for the better diagnosis study.
- Organisation (and payment if required) of a competent bleeder – either a trained member of feedlot staff or a contract bleeder such as a local vet.
- When animals are bought directly from a vendor, contacting the vendor to obtain permission for National BRD Initiative staff to conduct a telephone questionnaire. Provision of contact details of vendor if permission is granted.
- Nomination of appropriate feedlot staff member to be responsible for the project at the feedlot (e.g. Animal Health Manager)

What is provided:

- Compensation of **$30/head** will be paid to the feedlot on receipt of data at the end of each cohort's time on feed. Bleeder costs are to be paid by feedlots and this compensation is, in part, to compensate for those costs.
- Reports at approximately 6 monthly intervals. These will include virological results from BRD pulls and deads and a comparison of evidence of virus exposure between BRD cases and some animals from the cohort not pulled for BRD.
- An interim visit to discuss reports and study progress.
- Results from the analyses will be available to each enrolled feedlot and the consulting veterinarian of their choice at the completion of the study, well ahead of the rest of industry. Results specific to each feedlot and benchmarked against other non-identified participating feedlots will be provided.

Table 1: The National BRD Initiative – component projects

Component 1: Best practice Manual.	Component 2: Epidemiology of BRD in Australian feedlots.	Component 3: Pathogen exposure	Component 4: The role of BVDV in Australian BRD	Component 5: Towards better diagnosis of BRD	Component 6: Development of a decision support tool
Objectives: 1. To allow practical implementation of research findings relevant to cattle producers and feedlot operators.	**Objectives:** 1. Define typical and achievable performance for BRD incidence 2. Identify priority preventive strategies. 3. Describe health and production outcomes for BRD cases	**Objectives:** 1. Assessment of pathogen exposure at induction. 2. Incidence of seroconversion 3. Association between serology and subsequent BRD incidence and mortality 4. Determine predominant pathogens	**Objectives:** 1. To clarify the role of pestivirus in BRD in Australian feedlots. 2. To determine the incidence of PI animals arriving at feedlots. 3. Clarify whether removing PI animals will give better outcomes.	**Objectives:** 1. To determine the sensitivity and specificity of BRD case detection 2. Examine linkages between BRD incidence and performance	**Objectives:** 1. Provide a support tool for decision making in BRD management which incorporates relative cost-benefits and is relevant to each section of the feedlot industry.

Data to be collected at feedlots

1. Data to be collected from feedlot database for each animal:

StockaID users will have an update supplied that will facilitate data transfer.

After induction bleed:
NLIS number
Feedlot tag number
Tailtag
Vendor
Breed
Sex
Lot no
SAN
Cattle Class
Date arrived at feedlot
Co-mingling prior to induction (Y/N)
Date inducted
Order of induction
Pen inducted into
Dentition at induction (number of permanent incisors erupted)
Weight at induction (kg)
Vaccines given at induction (none, Bovilis-MH®, Pestigard®, Rhinogard®, Ultravac™ 5in1, Ultravac™ 7in1, other)
Other treatments at induction (back-liner, drench, implants, antibiotics, ADE, footbath etc) – product used and quantity

Immediate origin – (saleyard, property of origin, backgrounding or resting property)
Where animals have arrived direct from a vendor, feedlots will be asked to contact the vendor to obtain permission for National BRD Initiative to contact them to obtain pre-feedlot history for study animals
Enrolment in Feeder Guard/Feedlot Ready
Vaccination prior to feedlot entry (yes/no/unknown)
If yes, which vaccines (Bovilis-MH®, Pestigard®, Rhinogard®, Ultravac™ 5in1, Ultravac™ 7in1, other). This information may be obtained directly from the vendor (see earlier)

After 42 day bleed:
NLIS number
Date of 42 day bleed
Order of 42 day bleed
Weight at 42 day bleed (if available)

After slaughter:
NLIS number
Last date on feed
Weight on last date on feed
Replacement NLIS ID (if applicable)

All pen movements (Both individual movements (e.g. to hospital pen) and group movements (e.g. movement of all or part of cohort to a different pen).
For each movement:
New pen ID
Previous pen ID
Date pen entered
If pulled while on feed:
For each time examined:
Date of examination
Diagnosis/Ailment/Course/Sequence Description/Treatment protocol
New pull/retreat/repull
Weight (if available)
Temperature (if available)
For each treatment administered:
Date
Product
Dose
If died while on feed:
Date of death
Reason for death/Post mortem report
Pen died in

2. Data to be collected for each cohort:

Range of dates of induction of cattle
Dates when mixing occurred between component groups of cohort
Is the cohort stable i.e. are new animals introduced to the group while on feed (add-ons)?
If no - date of "adding-on", details of additional animals
Number of days to introduction of final diet

For each ration fed:
Date of commencement of ration
Metabolisable energy
Crude protein
Undegraded dietary protein
Rumen modifier – Ionophore (Y/N)
Rumen modifier – Virginiamycin (Y/N)
% starch based concentrate
Method of grain processing
Steam flaking – flake density
Dry rolling – number of fragments
Intake as % body weight on each ration

For each pen occupied (movement of whole cohort):
Pen ID
Date entered pen
Dates that pen was cleaned (immediately prior to and during cohorts time in pen)
For each day cohort on feed:

Penrider ID (this can be an anonymous unique ID – we are not judging penriders but we do need to take possible variation into consideration
Penrider in pen/rides by pen/pen not checked
Number of surrounding pens with cattle present (0, 1, 2, 3, 4?)

3. Data to be collected for each pen (i.e. physical pen):

Pen ID/number
Location in feedlot (map)
Typical capacity (including variation by season)
Length (m)
Width (m)
Surface area of pen (m^2)
Bunk space (m)
Water trough space (m)
Water trough accessed by other pens
Water trough location relative to feed trough
Shade cloth – y/n, percentage cover, type of shade
Proximity to hospital pen
Aspect – direction and steepness of slope
Pen cleaning protocol

4. Data to be collected from feedlot weather stations:

At a minimum:
- Daily rainfall (mm)
- Daily maximum temperature
- Daily minimum temperature
- Wind speed

Where available:
- Relative humidity or wet bulb temperature
- Black globe temperature
- Thermal heat index
- Particulate matter (for feedlots with dust analysers)

5. Carcass data

If available this will be collected after animals are processed at the abattoir, either from the feedlot or from the abattoir.

Kill body number
Date slaughter
Hot carcass weight
P8 fat depth
Fat colour
Marbling score
Meat colour
Ribeye 5/6 or 10/11 area
Firmness

Suggested procedure for enrolment in study

- ✓ Initial approach made by consultant veterinarian or through MLA and expression of interest from feedlot.
- ✓ Contact made by Operations Manager. Feedlot agrees in principle to involvement. "Information for Feedlot Managers" sent to feedlot.
- ✓ Feedlot visit by Operations Manager and possibly consultant veterinarian.
    - Presentation to feedlot staff involved in the project or discussion based on "Information for Feedlot Managers" manual.
    - Information gathered on typical management practices in feedlot. Tailoring of study design to each feedlot to ensure that animals enrolled in the study are in cohorts representative of those fed at the feedlot.
    - Consent form (pages 19 – 20) completed and returned to Operations Manager – agreement to participate in the study and participate in MLA animal health survey
    - Feedlot background information (pages 21 - 24) returned to the Operations Manager.
    - Obtain/discuss availability of pen data listed on page 10. If available, a copy of map of feedlot may be useful.
- ✓ Data management:
    - Extra data fields to be added to database if required.
    - StockaID users – installation of upgraded version from Elynx to facilitate data transfer to Operations Manager.
    - Trial of data acquisition from feedlot database to Operations Manager.
    - Trial of data acquisition from feedlot weather station to project database.
- ✓ Bleeder identified by feedlot – either feedlot staff member or contract bleeder such as local vet.
    - Animal ethics and consent forms signed by bleeder.
    - Logbook of bleeder names and dates of bleeding must be maintained for the purposes of the animal ethics committee.
- ✓ Feedlot contact and bleeder organise date for induction of first study cohort.

- ✓ Bleeding & swabbing equipment sent to feedlot/bleeder.
- ✓ First cohort inducted, samples taken and sent to project laboratory. Induction data sent to Operations Manager.
- ✓ Pen questionnaire completed for pen(s) occupied by study cattle (pages 25 – 32). More forms will be provided and an electronic form is available. These forms need to be completed for all pens occupied by study cattle during their time on feed.
- ✓ Feedlot contact and bleeder organise date for 42-day bleed of first study cohort.
- ✓ Bleeding equipment sent to feedlot/bleeder
- ✓ 42-day bleed for first study cohort, samples taken and sent to project laboratory. 42 day data sent to Operations Manager.
- ✓ Procedures repeated for approximately 13 study cohorts, at ~8 week intervals, during the data collection period.
- ✓ Throughout study cohorts' time on feed, samples are taken from study animals pulled for BRD and samples from dead cattle in study pens sent to project laboratory.
- ✓ At end of time on feed, remaining data sent to project database.
- ✓ Compensation to feedlot paid on receipt of quality data.

Appendices

APPENDIX 1

RESEARCH PROJECT INVESTIGATING RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF BOVINE RESPIRATORY DISEASE COMPLEX (BRD) IN AUSTRALIAN FEEDLOT CATTLE

INFORMATION FOR MANAGERS OF FEEDLOTS PARTICIPATING IN THIS STUDY

Objectives

1. Describe BRD incidence for cohorts of cattle over time and by feedlot
2. Define typical and achievable 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, cohort and pen level.
4. Assess the strength of association between ('known'/potential) risk factors and BRD occurrence
5. Identify priority preventive strategies and areas for further research/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 health and production outcomes for BRD cases

Overview

This is a prospective observational study of 32 - 40,000 cattle entering Australian feedlots during 2009 - 2011 aimed at identifying and quantifying risk factors for the development of BRD in Australian feedlots. Data will come from several sources including: feedlot databases, the National Livestock Identification System, weather stations and Queensland DPI&F (virological monitoring). This project ties in with other components of the National BRD Initiative, namely Component 3 (Intensive virological monitoring) and Component 4 (Improving BRD diagnosis). A pilot study comprising 3 feedlots commenced in July 2007. The main data collection period will begin during early 2009 and cohorts will continue to be inducted over the subsequent 24 months.

Study population

Study sites

Approximately 16 feedlots representative of the industry will be enrolled in the study. Feedlots will be selected from throughout Australia. Approximately 13 cohorts will be enrolled at each feedlot over the 24 month enrolment period. The study design will be tailored to each feedlot following the visit to the feedlot by the operations

manager. This will ensure that the cohorts are representative of those fed on the feedlot (e.g. different classes of cattle fed, different degrees of mixing around the induction period, different pen sizes). Data will be collected on each animal and on all pens occupied by study cattle during their time on feed. For this study a "cohort" is the group of animals residing in a pen when that pen is said to have been "filled". A "pen" refers to the physical pen animals are kept in. If cohorts, parts of cohorts or individual animals are moved to other pens (including hospital pens) during the feeding period it is important that dates of movement are recorded for each animal and pen data are obtained for the new pen.

Virological Monitoring

Blood samples, nasal swabs from all study animals and post-mortem tissue from "deads" will be analysed for evidence of exposure to Bovine herpesvirus 1 (BHV1) (also known as Infectious Bovine Rhinotracheitis virus, IBR), Bovine parainfluenza virus 3 (PIV3), Bovine Viral Diarrhoea Virus (BVDV or pestivirus) and Bovine Syncytial Virus (BRSV). This will allow us to characterise what pathogens cattle are immune to at feedlot entry and what they are exposed to during transport and the early feeding period. The dynamics of viral infection on specific feedlots and the relationship between pathogen exposure, other risk factors and BRD may therefore be determined.

Blood samples

Will be collected from all study cattle at induction and at 42 days on feed. Cattle "pulled" for suspected BRD will also be bled at the time of pulling.

Nasal swabs

Will be collected from all study cattle at induction. Cattle "pulled" for suspected BRD will also be swabbed at the time of pulling.

Post-mortem samples

Any study cattle that die while on feed will undergo a post-mortem examination. Fresh samples of trachea and lung will be sent for analysis.

Animal Health Data

Data stored in the feedlot database will be sent to the operations manager at the end of each cohort's time on feed. A detailed list of data to be collected is attached. Before animals are enrolled a trial of data acquisition will occur. Most feedlots already collect the data required; occasionally small changes to the feedlot database may be necessary.

Weather

Temperature, rainfall and wind speed data will be collected from feedlot weather stations. If additional data is available, this will also be collected. Weather data from feedlots with dust monitors will be analysed to assess associations between weather and dust.

Other data

A history of all movements for each study animal will be obtained from the NLIS database.

Where possible, vendors will be contacted (feedlots will be asked to seek permission from vendors for the National BRD Initiative to contact them) and asked to provide as full a history on study animals as possible, such as details of weaning, backgrounding and pre-feedlot vaccinations.

A subset of study cattle will be followed through until slaughter and lung lesions scored as part of the National BRD Initiative Component 4 (Improving BRD diagnosis). A face to face interview with the feedlot manager and selected staff will be conducted to define the criteria used for diagnosing cases of BRD and to identify factors which may be affecting the accuracy of these diagnoses.

Schedule

Time	Treatment
Prior to enrolment of study animals	Visit from Operations Manager to discuss the project, tailor study design to each feedlot and check data availability and accessibility
Induction of cohort of study cattle	Bleeding and nasal swabbing. Induction data sent to Operations Manager.
Throughout time on feed	Bleeding and nasal swabbing of all study animals pulled for suspected BRD. Fresh post-mortem samples of lung and trachea from all study animals which die (or are euthanased) while on feed.
42 days post induction	Re-bleeding of study cattle. 42 day data sent to Operations Manager.
End of time on feed	Remaining data for cohort sent to Operations Manager Daily weather records from feedlot weather station for the duration of time on feed will also be sent at this time.
Slaughter	Carcass data will be collected when available from either feedlot or abattoir and a subset of animals will have their lungs scored for lesions by pathologists.

Outcomes

Sero-conversion and diagnosis of "deads"

Analysis of blood samples, nasal swabs and post-mortem tissue may permit the identification of viral pathogens:

a) Encountered during transport or the early feeding period,

b) Associated with the development of BRD,

c) Identification of viral infections at "pulling" or death.

Risk Factor Analysis

Analysis of animal health data, NLIS data, weather data and virological data will enable the accurate determination of risk factors for the development of BRD in Australia and will allow us to identify the most important factors to be incorporated into extension programs and decision making tools.

Correlation between "pulls" and lung lesions at slaughter

Accurate diagnosis of BRD is essential for positive treatment outcomes. Comparison animal health records and lung lesions at slaughter will determine the accuracy of BRD diagnosis on a subset of feedlots.

Compensation

All equipment and consumables required for sample collection will be provided free of charge.

It is recognised that there will be a time cost to the feedlot for partaking in the study and potentially lost production associated with re-handling cattle. Monetary compensation of **$30/head** to cover time, potential production loss and bleeder fees will be paid on receipt of data at the end of each study cohort's time on feed. Invoice forms will be provided.

Contact for general information:

1. Dr. Tamsin Barnes MA, Vet MB, PhD, Operations Manager
University of Queensland
Phone Until 15/2/10 - (07) 3365 3203
From 15/2/10 - (07) 5460 1965
Mobile - 0422 980499
Fax (07) 5460 1922
Email t.barnes@uq.edu.au

2. Dr. Tim Mahony BSc (Hons), PhD, Project Leader
Agri-Science Queensland
Department of Employment, Economic Development and Innovation
Phone (07) 3346 6505 / 0434 076 196
Facsimile (07) 3346 6501
Email Timothy.Mahony@deedi.qld.gov.au

Contact for information regarding sample collection supplies (blood tubes, swabs etc) and sending samples:

Dr Rebecca Kann or Dr Shannon Waldron
Virology Co-ordinators
Agri-Science Queensland
Department of Employment, Economic Development and Innovation
Phone (07) 3346 6517
Fax (07) 3346 6501
Email brdinitiative@deedi.qld.gov.au

RESEARCH PROJECT INVESTIGATING RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF BOVINE RESPIRATORY DISEASE COMPLEX (BRD) IN AUSTRALIAN FEEDLOT CATTLE

CONSENT AND AGREEMENT TO PARTICIPATE

(Please note this is not intended to serve as a legally binding document but rather to acknowledge your understanding of the nature of this study)

I have read the accompanying "Information for managers of feedlots participating in this study" and I understand the nature of the study.

- I understand that this study will involve:
    - The enrolment of approximately 13 cohorts over a period of up to two years.
    - The collection of blood samples and nasal swabs from all study cattle at induction and collection of blood samples at approximately 42 days post induction.
    - The collection of blood samples and nasal swabs from all study animals that are pulled for suspected BRD and post-mortem samples from all "deads" among study cattle.
    - The daily collection of weather data and pen dust data (if applicable).
    - The collection of animal health data into the feedlot database which may require some fields to be added or modified.
    - The collection of carcass data when animals are sent to slaughter (if available).
    - A face-to-face interview for some feedlot managers and staff.

- I understand that monetary compensation will be paid on the receipt of data at the end of each cohort's time on feed.

- I grant permission for:
    - Blood sample collection and analysis.
    - Nasal swab collection and analysis.
    - Post-mortem sample collection and analysis.
    - Access to the feedlot database for the purposes of this study (this does not include financial records).

    - Access to the NLIS database for information regarding cattle enrolled in this study.

- I agree to participate in an MLA animal health survey to develop baseline data on the incidence of BRD and other diseases.

I understand that all the data pertaining to my cattle and my business will be treated in a strictly confidential manner and that participating feedlots will not be identifiable in any resulting published work or public forums.

Manager: _____ _____

(Name) (Signature)

Date: _____ / _____ / _____

APPENDIX 2

Background information required in order to set up the National BRD Initiative on each feedlot

1. Please nominate the person who will be responsible for the project at the feedlot e.g. Animal Health Manager:

   Name _____

   Phone _____

   Mobile _____

   Email _____

   Fax _____

   Nominate preferred hours and method of communication

   _____

2. Please provide some basic details about the feedlot

   What is the feedlot's NFAS accreditation number? _____

   Size of feedlot (number of animals at full capacity) _____

   Number of pens _____

   Size of pens (e.g. 20x200head + 25x300head) _____

   _____

   _____

   _____

   If you have a map of the feedlot please could you send us a copy?

   This will help us gather information that we need about the study pens and tailor the study design to the feedlot.

   What are the typical cattle classes fed and their expected time on feed?

   _____

   _____

   _____

   _____

   Where do animals go to slaughter? _____

   _____

   _____

3. Contract bleeders

   The National BRD Initiative suggests that bleeding is done by a trained member of feedlot staff as this will provide the greatest flexibility when cohorts are inducted over a period of several days. Feedlot consultants will assist in training of feedlot staff. The alternative option is for the feedlot to arrange for a contract bleeder, such as a local vet, to visit and bleed study cattle at induction and at 42 days on feed. We expect that feedlots will cover this cost from the compensation provided. Please provide us with names and training/experience of all bleeders. A log of all bleeders, dates and numbers of animals bled is required for animal ethics.

Name	**Contact no.**	**Experience/Job description**	**Will need training?**

4. Nominate a preferred courier for blood samples, nasal swabs and post-mortem samples to allow us to track any missing samples.

   Courier 1_____

   Phone : _____ Fax _____

   Email _____

   Courier 2: _____

   Phone : _____ Fax _____

   Email _____

5. Data collection system

   a. Program type _____

   b. Looking at the attached list of data to be collected (pages 8 – 9), please list any which are **not** currently collected.

      _____

      _____

      _____

      _____

      _____

      _____

   c. Do you foresee any problems in adding these factors to the database?

_____

_____

_____

_____

_____

6. BRD cases:
    a. What criteria do you/penrider use to decide when to treat an animal for BRD?

_____

_____

_____

_____

_____

_____

    b. What is your typical treatment protocol for an animal diagnosed with BRD?

_____

_____

_____

_____

_____

_____

7. Induction practices:
    a. How will you identify study cattle? Do you wish us to provide ear tags?

_____

_____

    b. Are animals inducted on arrival, in groups that form a cohort or on set days? (We will ask more questions about induction procedures during the visit to the feedlot.)

_____

_____

_____

c. What is the average cohort (number of animals per pen) size _____

_____

d. On average, how long does it take to “fill” a pen (animals will need to be sampled at induction) _____

_____

_____

e. Is there a crush side NLIS tag reader which will allow us to identify the order in which NLIS tags passed through the induction crush? This will enable us to correlate NLIS numbers with blood tube numbers.

_____

f. Please list other processes which typically occur at induction e.g. vaccinating, branding etc. We will gather data specific to each cohort at the time of induction.

_____

_____

_____

_____

8. Weather stations
   a. What type of weather station is used? _____
   b. Please list data currently recorded by the feedlot weather station?

_____

_____

_____

_____

_____

_____

Background Information on Study Pens

National BRD Initiative

In addition to the information below it would be really helpful if you are able to give us a copy of a map of the feedlot. Additional copies of this form, including one that can be filled in electronically, will be provided by the Operations Manager

Please fill out one form for each pen used in the study.

Name of Feedlot _____

Pen number/ID (identifier used by feedlot staff) _____

Typical capacity of pen (e.g. 200 head)

_____

Length of pen (m) _____

Width of pen (m) _____

Bunk space (m) _____

Water trough space (m) _____

Location of water trough relative to feed trough (eg adjacent/opposite ends of pen)

_____

_____

Can water trough be accessed by animals from other pens? Yes / No

Is part of the pen shaded? Yes / No

If so please describe (eg shade cloth over half pen) _____

_____

_____

_____

How many pens adjoin the study pen? 0 / 1 / 2 / 3 / 4

(When each study cohort is on feed we will ask you how many of these pens are occupied.)

How far is the pen from the hospital pen (m approx)? _____

What is the direction of slope of the pen (eg north – south (north=higher ground))?

_____

What is the angle of pen slope (eg 2^{o} or drop of 0.5m over 100m)? _____

_____

_____

How frequently is the pen cleaned? _____

(When each cohort is on feed we will ask you for the dates the pen was cleaned while occupied by study cattle)

Please describe your pen cleaning protocol (only do this for one study pen, assuming protocol is consistent)

_____

_____

_____

_____

_____

Background Information on Study Pens

National BRD Initiative

In addition to the information below it would be really helpful if you are able to give us a copy of a map of the feedlot. Additional copies of this form, including one that can be filled in electronically, will be provided by the Operations Manager

Please fill out one form for each pen used in the study.

Name of Feedlot _____

Pen number/ID (identifier used by feedlot staff) _____

Typical capacity of pen (e.g. 200 head)

_____

Length of pen (m) _____

Width of pen (m) _____

Bunk space (m) _____

Water trough space (m) _____

Location of water trough relative to feed trough (eg adjacent/opposite ends of pen)

_____

_____

Can water trough be accessed by animals from other pens? Yes / No

Is part of the pen shaded? Yes / No

If so please describe (eg shade cloth over half pen) _____

_____

_____

_____

How many pens adjoin the study pen? 0 / 1 / 2 / 3 / 4

(When each study cohort is on feed we will ask you how many of these pens are occupied.)

How far is the pen from the hospital pen (m approx)? _____

What is the direction of slope of the pen (eg north – south (north=higher ground))?

_____

What is the angle of pen slope (eg 2^{o} or drop of 0.5m over 100m)? _____

_____

_____

How frequently is the pen cleaned? _____

(When each cohort is on feed we will ask you for the dates the pen was cleaned while occupied by study cattle)

Please describe your pen cleaning protocol (only do this for one study pen, assuming protocol is consistent)

_____

_____

_____

_____

_____

Background Information on Study Pens

National BRD Initiative

In addition to the information below it would be really helpful if you are able to give us a copy of a map of the feedlot. Additional copies of this form, including one that can be filled in electronically, will be provided by the Operations Manager

Please fill out one form for each pen used in the study.

Name of Feedlot _____

Pen number/ID (identifier used by feedlot staff) _____

Typical capacity of pen (e.g. 200 head)

_____

Length of pen (m) _____

Width of pen (m) _____

Bunk space (m) _____

Water trough space (m) _____

Location of water trough relative to feed trough (eg adjacent/opposite ends of pen)

_____

_____

Can water trough be accessed by animals from other pens? Yes / No

Is part of the pen shaded? Yes / No

If so please describe (eg shade cloth over half pen) _____

_____

_____

_____

How many pens adjoin the study pen? 0 / 1 / 2 / 3 / 4

(When each study cohort is on feed we will ask you how many of these pens are occupied.)

How far is the pen from the hospital pen (m approx)?_____

What is the direction of slope of the pen (eg north – south (north=higher ground))?

_____

What is the angle of pen slope (eg 2^{o} or drop of 0.5m over 100m)? _____

_____

_____

How frequently is the pen cleaned? _____

(When each cohort is on feed we will ask you for the dates the pen was cleaned while occupied by study cattle)

Please describe your pen cleaning protocol (only do this for one study pen, assuming protocol is consistent)

_____

_____

_____

_____

_____

Background Information on Study Pens

National BRD Initiative

In addition to the information below it would be really helpful if you are able to give us a copy of a map of the feedlot. Additional copies of this form, including one that can be filled in electronically, will be provided by the Operations Manager

Please fill out one form for each pen used in the study.

Name of Feedlot _____

Pen number/ID (identifier used by feedlot staff) _____

Typical capacity of pen (e.g. 200 head)

_____

Length of pen (m) _____

Width of pen (m) _____

Bunk space (m) _____

Water trough space (m) _____

Location of water trough relative to feed trough (eg adjacent/opposite ends of pen)

_____

_____

Can water trough be accessed by animals from other pens? Yes / No

Is part of the pen shaded? Yes / No

If so please describe (eg shade cloth over half pen) _____

_____

_____

_____

How many pens adjoin the study pen? 0 / 1 / 2 / 3 / 4

(When each study cohort is on feed we will ask you how many of these pens are occupied.)

How far is the pen from the hospital pen (m approx)? _____

What is the direction of slope of the pen (eg north – south (north=higher ground))?

_____

What is the angle of pen slope (eg 2^{o} or drop of 0.5m over 100m)? _____

_____

_____

How frequently is the pen cleaned? _____

(When each cohort is on feed we will ask you for the dates the pen was cleaned while occupied by study cattle)

Please describe your pen cleaning protocol (only do this for one study pen, assuming protocol is consistent)

_____

_____

_____

_____

_____

APPENDIX 3

Sample handling and storage.

Below is a summary of the sample collection, handling and storage required. Detailed reference sheets which can be placed at handling and storage sites will be sent with bleeding supplies and equipment prior to induction of the first cohorts into study pens. If the bleeder has a specific requests for needles or tubes that differs from those provided as standard, please let us know. If possible we will try to accommodate such requests.

Samples required

- Induction : Blood and nasal swab
- 42 days: Blood only
- BRD Pulls: Blood and nasal swabs immediately when pulled
    - (repeat samples required for repulls, but not for retreats for animals in hospital pen)
- BRD Deads: Abnormal lung and/or trachea

Blood samples

Collection

- Samples are collected from each animal in each cohort at induction (nasal swabs also collected) and after 42 days (blood only) on feed. These samples are to be collected by a trained member of feedlot staff, or a contract bleeder organised by the feedlot.
- Study animals that are “pulled” for suspected BRD will also have blood and nasal samples taken. Feedlot staff will take these samples.
- Depending on crush set-up, bleeding will be done from either the jugular or tail veins.
- 5ml of blood in a serum tube (“red top”) is required for the induction bleed, 42-day bleed and bleeds from all animals pulled for suspected BRD.

Handling

- For induction and 42-day bleeds blood tubes are pre-labelled (from 1 to expected size of cohort). They are then filled in the order animals are run through the crush which is recorded in feedlot database and then sent to the Operations Manager.

- Approximately every 10 animals please cross check to ensure the tube number matches with the number in the handling order. It is essential that you are confident about the numbering process. Please record the last 5 digits of the animal's NLIS number on every 10th tube to enable us to cross-check. If there is any doubt about the order of samples, details must be recorded on the form provided.
- Prior to packaging, please record the date and number of animals bled on the form provided.
- For blood samples from pulled animals the NLIS number and date of bleeding are required.

Storage

- Blood samples must be refrigerated.
- If refrigerated transport is not available, bloods must be transported in eskies with ice-packs wrapped in newspaper to avoid condensation ruining labels.
- Samples collected from pulled animals need not be sent individually on the day they are collected. We recommend sending these samples on a weekly basis.

Nasal swabs

Collection

- Samples are collected for each cohort at induction. Feedlot staff will collect these samples.
- Study animals that are "pulled" for suspected BRD will also have nasal swabs taken. Feedlot staff will take these samples.
- Nasal swabs are collected by wiping the cotton tip inside the nostril to collect any discharge. The cotton tip is placed into the media. Care must be taken not to contaminate the tip with fingers or parts of the animal other than the inner nostril. After the swab is taken the bottom of the tube needs to be squeezed so that the liquid media washes over the swab. Probably two squeezes would be sufficient.

Handling

- For induction swabs please label each swab consecutively and ensure that the swab and blood tube from each animal is labelled with the same number. This can then be traced to the NLIS number via the feedlot database (as with blood sample).
- Approximately every 10 animals please cross check to ensure the swab number matches with the number in the handling order and that of the

blood tube. It is essential that you are confident about the numbering process. Please record the last 5 digits of the animal's NLIS number on every 10th swab to enable us to cross-check. If there is any doubt about the numbering of samples, details must be recorded on the form provided.

- Prior to packaging, please record the date and number of animals swabbed on the form provided.
- For swabs from pulled animals the NLIS number and date of swabbing are required.

Storage

- Nasal swabs must be refrigerated.
- If refrigerated transport is not available, swabs must be transported in eskies with ice-packs wrapped in newspaper to avoid condensation ruining labels.
- Samples collected from pulled animals need not be sent individually on the day they are collected. We recommend sending these samples on a weekly basis.

Post-mortem samples

Collection

- Samples of lung and trachea are to be collected from all animals in study pens which die while on feed.
- Approximately 5cm^3 lung and/or trachea taken from margins of obvious abnormalities. If there are no obvious lesions please send ~5cm from the middle of the trachea and 5cm^3 piece of the right cranial lung lobe (lobe nearest to the head). A separate sheet on sampling will be provided.

Handling

- Samples should be collected as aseptically as possible under field conditions. Searing is not required if 5cm^3 of tissue is provided.
- Samples should be placed immediately in the sterile jars provided.
- The NLIS number of the dead animal, date of death and date of sampling must be clearly labelled on the jar.

Storage

- Samples must be refrigerated immediately and sent by courier within a week of collection. Samples must be transported in eskies on ice packs and wrapped in newspaper to avoid condensation ruining the labels.

The following packs will be supplied with quantities of serum tubes and swabs appropriate to expected cohort size:

Induction Pack:

- Cohort number x 6ml serum tubes (pre-labelled) in foam racks
- 50 x spare 6ml serum tubes
- 18G x 1" needles – required number plus spares
- 10 x vacuette needle holders
- 1 x roll of cling wrap (for wrapping up the trays for sending)
- Cohort number x nasal swabs (unlabelled, plus some spares)
- 1 x sharps bin
- 2 x marker pens
- 8 x freezer blocks
- Name and address label for sending the samples back to us
- Form to provide date and number of animals bled and swabbed

Pack for BRD pulls:

- 50 x 6ml serum tubes (unlabelled)
- 50 x 18G 1" needles and 10 needle holders
- 50 x nasal swabs
- 20 x yellow topped jars for post-mortem samples
- 2 x small eskies
- 4 x freezer blocks

Day 42 Pack:

- Cohort number x 6ml serum tubes (160 labelled) in 2 foam racks
- 50 x spare 6ml serum tubes
- 18G x 1" needles – required number plus spares
- 10 x vacuette needle holders
- 1 x roll of cling wrap
- 1 x sharps bin
- 1 x marker pen
- 8 x freezer blocks
- Name and address label for sending the samples back to us
- Form to provide date and number of animals bled

Appendix 3

National BRD Initiative – Protocols

The National BRD Initiative Protocols

TABLE OF CONTENTS

CONTACT LIST

CONTACTS FOR GENERAL INFORMATION:

1. Dr. Tamsin Barnes MA, Vet MB, PhD, Operations Manager
   University of Queensland
   Phone Until 15/2/10 - (07) 3365 3203
   From 15/2/10 - (07) 5460 1834
   Mobile 0422 980499
   Fax (07) 5460 1922
   Email t.barnes@uq.edu.au

2. Dr. Tim Mahony BSc (Hons), PhD, Project Leader
   Agri-Science Queensland
   Department of Employment, Economic Development
   and Innovation
   Phone (07) 3346 6505 / 0434 076 196
   Fax (07) 3346 6501
   Email Timothy.Mahony@deedi.qld.gov.au

CONTACT FOR INFORMATION REGARDING SAMPLES AND SUPPLIES:

Dr Rebecca Kann or Dr Shannon Waldron
Virology Co-ordinators
Agri-Science Queensland
Department of Employment, Economic Development
and Innovation
Phone (07) 3346 6517
Fax (07) 3346 6501
Email brdinitiative@deedi.qld.gov.au

PRIOR TO INDUCTION

- Induction pack with sufficient blood tubes and nasal swabs will be sent to feedlot by the Virology Co-ordinator
- Organise a competent bleeder (from feedlot or contract bleeder). If induction will occur over >1 day, ensure bleeder is available to bleed all cattle in study cohort/lot
- Inform Operations Manager of the name of the bleeder.

AT INDUCTION

- Standard induction procedure
- Ensure order of animals going through the crush is recorded in some manner
    - Sequential numbering in StockaID
    - Use of sequentially numbered trial tags (provided on request)
- Collect blood sample
    - Must be done by a competent bleeder and name of bleeder must be recorded on sample form (Animal Ethics requirement)
    - Tubes arrive pre-labelled from 1 – expected size of cohort
    - Ensure that number on blood tube used matches sequential number for animal
    - Ideally collect a full tube. Minimum requirement – 5ml (half tube)
    - If the vacuum is lost/tube breaks use one of the spare unlabelled tubes and label immediately with the sequential number of the animal
    - Every 10th animal record the last 5 digits of the animal's NLIS number on the blood tube to enable us to cross-check the sequential numbering system. If there is any doubt about the order of samples, details must be recorded on the form provided.

- Collect nasal swab
    - Before administration of Rhinogard® (if applicable)
    - Ideally swab both nostrils using one swab for each animal
    - Scrub the tip of swab against the inside of the nose as far back as possible and place cotton tip in media
    - Take care not to contaminate tip with fingers or other parts of the animal. If contamination is suspected please collect another sample.
    - Squeeze the bottom of the tube twice to ensure the media covers the swab
    - Label each swab consecutively ensuring that the number matches the sequential number for the animal and the number on the blood tube
    - Every 10th animal record the last 5 digits of the animal's NLIS number on the swab to enable us to cross-check the sequential numbering system. If there is any doubt about the order of samples, details must be recorded on the form provided.
- Sample storage
    - Swabs and blood tubes must be refrigerated until transported
- Inductions over more than one day
    - Continue numbering animals from last number from previous session. In StockaID manually enter the correct number for the first animal. Subsequent animals will continue to be numbered sequentially
    - Return samples to Virology Co-ordinator only after all animals have been inducted

AFTER INDUCTION

- Packaging and Transport
    - Complete sample form included with pack.
    - Pack up samples in box in which they arrived

    - If refrigerated transport is not available, samples must be transported with ice-packs wrapped in newspaper
    - Arrange courier to transport samples to Virology Co-ordinator (address labels provided)
    - Samples must be sent within 5 days of collection
- Send induction data from feedlot database to Operations Manager (see admin section)

42 DAY BLEED

PRIOR TO 42 DAY BLEED

- 42 day pack with sufficient blood tubes will be sent to feedlot
- Organise bleeder (from feedlot or contract bleeder)
- Timing
    - Ideally animals will be bled 42 days after induction
    - Animals must be bled between 37 – 47 days after induction. If this is not possible please contact the Operations Manager

AT 42 DAY BLEED

- Ensure order of animals going through the crush is recorded in some manner
    - E.g. Draft session with sequential numbering in StockaID
- Record weights of animals as they go through the crush (if possible)
- Collect blood sample
    - Must be done by a competent bleeder and name of bleeder must be recorded on sample form (Animal Ethics requirement)
    - Tubes arrive pre-labelled from 1 – size of cohort
    - Ensure that number on blood tube used matches sequential number for animal
    - Ideally collect a full tube. Minimum requirement – 5ml (half tube)
    - If the vacuum is lost/tube breaks use one of the spare unlabelled tubes and label immediately with the sequential number of the animal
    - Every 10th animal record the last 5 digits of the animal's NLIS number on the blood tube to enable us to cross-check the sequential numbering system. If there is any doubt about the order of samples, details must be recorded on the form provided.

- Cross check for missing animals
    - Animals in hospital
        - Those not bled in hospital crush (i.e. hospitalised for reasons other than BRD) need to be bled
        - Those bled in hospital crush more than 5 days prior to 42 day bleed need to be re-bled
        - Those bled in hospital crush within the 5 days prior to 42 day bleed do not need to be re-bled, unless it is convenient to do so.
    - Animals in other pens
        - Track down and bleed if possible.
- Sample storage
    - Blood tubes must be refrigerated until transported

AFTER 42 DAY BLEED

- Packaging and Transport
    - Complete sample form included with pack.
    - Pack up samples in box in which they arrived
    - If refrigerated transport is not available, samples must be transported with ice-packs wrapped in newspaper
    - Arrange courier to transport samples to Virology Co-ordinator (address labels provided)
    - Samples must be sent within 5 days of collection
- Send 42 day data from feedlot database to Operations Manager (see admin section, include AnimalID Replacements export)

HOSPITAL SAMPLING OF BRD PULLS

HOSPITAL SAMPLE PACKS

- Hospital packs will be provided at the start of the study.
- Additional packs will be sent on request – please contact the Virology Co-ordinator

IN THE HOSPITAL

- Identify all study animals pulled from pen and examined in hospital crush
    - Warning will display on StockaID screen
    - Use of trial tags
- If animal is pulled for reason other than BRD, treat as per standard protocol
- If animal is pulled for BRD (new case)
    - Collect blood sample
        - Ideally collect a full tube. Minimum requirement – 5ml (half tube)
    - Collect nasal swab
        - Ideally swab both nostrils using one swab for each animal
        - Scrub the tip of swab against the inside of the nose as far back as possible and place cotton tip in media
        - Take care not to contaminate tip with fingers or other parts of the animal. If contamination is suspected please collect another sample.
        - Squeeze the bottom of the tube twice to ensure the media covers the swab
    - Label tube and swab with:
        - Animal ID (NLIS ID or visual tag)

        - Date of sampling
        - Name of feedlot
    - Continue with standard treatment protocol
- If animal is re-treated for BRD (i.e. is receiving a course of treatment and is still in a hospital pen and has not been back to the home pen)
    - There is no need to collect further samples.
    - Treat as usual
- If animal is re-pulled for BRD (has returned to home pen and been pulled again)
    - Collect samples as above for a new case
- Sample storage
    - Swabs and blood tubes must be refrigerated until transported
    - Samples should be stored for a maximum of 1 week before sending to Virology Co-ordinator
- Packaging and transport
    - Complete sample form included with pack.
    - Pack up samples in small esky provided
    - If refrigerated transport is not available, samples must be transported with ice-packs wrapped in newspaper
    - Arrange courier to transport samples to Virology Co-ordinator (address labels provided)

PM SAMPLING OF STUDY ANIMALS

POST MORTEM SAMPLE PACKS

- Post mortem packs will be provided at the start of the study.
- Additional packs will be sent on request – please contact the Virology Co-ordinator

POST MORTEM PROTOCOL

- All study cattle that die on feed must be post mortemed
- Examine both lungs and the trachea
- Trachea
    - If there are any obvious lesions collect ~5cm length that includes the abnormality
    - If there are not obvious lesions collect ~5cm length from the mid-trachea
- Lungs
    - If there are any obvious lesions collect at least one piece ~5cm^3. Ideally select a sample from the edge rather than the middle of lesion
    - If there are no obvious lesions collect ~5cm^3 sample from the right cranial lung lobe (lobe nearest the head)
- Sample storage
    - Place each sample in a separate yellow pot (provided)
    - Label each pot with:
        - Animal ID (NLIS ID or visual tag)
        - Date of sampling
        - Type of sample (lung lesions/lung no lesions/trachea lesions/trachea no lesions)
        - Name of feedlot

    - Samples must be refrigerated until transported
    - Samples should be stored for a maximum of 1 week before sending to Virology Co-ordinator
- Packaging and transport
    - Complete sample form included with pack.
    - Pack up samples in small esky provided
    - If refrigerated transport is not available, samples must be transported with ice-packs wrapped in newspaper
    - Arrange courier to transport samples to Virology Co-ordinator (address labels provided)
- Record results of post mortem on database and post mortem form

GENERAL ADMINISTRATION

COHORT SELECTION

- Method for cohort selection will be worked out after Operations Manager has visited feedlot. For the results of the study to be meaningful it is essential that cohorts enrolled are **REPRESENTATIVE** of animals fed at the feedlot.
- One Cohort will be enrolled at ~8 week intervals for a 2 year period

PRE-INDUCTION

- Discuss next cohort to be enrolled with Operations Manager at least one week before induction
- Ensure that a bleeder is available to bleed all animals in the cohort.
- The details of bleeder must be provided to the Operations Manager prior to collection of any samples.

AFTER INDUCTION

- Arrange for samples to be sent to Virology Co-ordinator
- Send Induction Export to Operations manager
- Advise Operations Manager of dates when groups of cattle were mixed (if applicable)
- Contact vendors of all groups of animals in cohorts and ask if NBRDI can call to ask a few simple questions about management practices/vaccinations given etc.
- Send vendor contact details to Operations Manager

PRE-42 DAY BLEED

- Ensure that a bleeder is available to bleed all animals

AFTER 42 DAY BLEED

- Arrange for samples to be sent to Virology Co-ordinator
- Send Draft Export to Operations Manager

HOSPITAL/PM SAMPLES

- Arrange for samples to be sent to Virology Co-ordinator on a regular basis
- Samples should not be kept for more than 1 week

AFTER SLAUGHTER

- Send the following to Operations Manager:
    - Remaining Data Exports
    - Dates when pens occupied by study cohort were cleaned
    - Pen rider diary (anonymous ID for who rode pen on each day – if available)
    - Copies of any post mortem reports
    - Details for each ration fed (see separate forms)
- Advise whether carcass data will be available

RATION & PEN FORMS

- Complete a Ration Form for each ration fed to animals from each study cohort
- Complete a Pen Form for each pen used by animals from each study cohort

WEATHER

- Send Weather Export to Operations Manager every 6 months

STOCKAID SETUP, DATA ENTRY AND EXPORT

SET UP

Mark Courses as Trial Courses:

- Go to "Administration"
- Select "Courses" from bottom buttons
- "Select Course" – highlight each course that could be used on an animal with BRD and mark as "Trial Course" (check box at top right of screen).
- Repeat for all courses

You will only need to do this once unless new respiratory courses are added during the duration of the trial

Mark Lot as Trial Lot:

- When a new Lot is going to be a Trial Lot (i.e. Cohort)
- Go to "Lots"
- At any point after the Lot has been added mark it as a "Trial Lot" (check box in last column)

You will need to do this for each Trial Lot before exporting any data.

DATA ENTRY

On arrival:

- Data entry as usual

At induction bleed:

- Use default Induction Session
- If Notes field is not enabled and auto-incremented on Induction Measurements Screen
    - Click on Set Form Button for Default Induction Measurements Template (yellow diamond with black arrow near top right of screen)
    - Click on checkbox to enable Notes field (no. 30)
    - From Drop down box for Notes Increment (near top of screen) select Sequential
    - This field will now increase by 1 as sequential animals are measured
- Induct animals as usual
- For each animal ensure that Notes field, blood tube and nasal swab all have the same number

At 42 day bleed:

- Use default Draft Session
- If Notes field is not enabled and auto-incremented on Draft Measurements Screen
    - Click on Set Form Button for Default Draft Measurements Template (yellow diamond with black arrow near top right of screen)
    - Click on checkbox to enable Notes field (no. 24)
    - From Drop down box for Notes Increment (near top of screen) select Sequential
    - This field will now increase by 1 as sequential animals are measured
- Bleed animals
- If possible record weight

- For each animal ensure that Notes field and blood tube have the same number

In hospital:

- When an animal from a Trial Lot is examined in the hospital you will see the following pop-up when its ID is entered “This Animal is from a Trial Lot and will need special treatment”
- Animals pulled/treated for BRD need to be bled and swabbed at this stage
- If possible record weight and temperature
- Data entry as usual

At post mortem:

- Use Dead Session as usual
- Conduct post mortem and record results

Draft Sessions:

- As usual

Exit Sessions:

- As usual

Carcass Data:

- Enter as usual if applicable

Animal ID replacements:

- Enter as usual if applicable

DATA EXPORT

There are 10 standard exports to be sent to Operations Manager for each Trial Lot

Standard export procedure:

- Ensure Lot is marked as a Trial Lot (see Set Up)
- Go to “Administration”
- Select “Trial Data Export” from bottom buttons
- From list of Trial Lots select the relevant Lot (check box)
- Ensure that the “Session Dates” includes all the dates relevant to the exports
- Select the relevant export(s) (check box(es))
- Click export
- Pop-up will say that export is complete
- Files will be saved in “Outgoing” folder in StockaID directory (this may be C:\elynx\StockaId\Outgoing – but may differ between computers)
- File name format “ExportName_YYYYMMDD_YYYYMMDD.csv”. Dates represent Session Dates from and to that you selected
- Send file(s) to Operations Manager by email

Extra details for specific exports

Induction Sessions

- File saved as: “InductionSessions_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: On day of induction/day after induction

Draft Sessions

- Ensure that “Session Dates” is set specifically to date of 42 day bleed to avoid including additional Draft Sessions for that Lot
- File saved as: “DraftSessions_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: On day of 42 day bleed/day after

Exit Sessions

- File saved as: “ExitSessions_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: Immediately after all Lot slaughtered

Carcass Details

- Only include if carcass details have been entered to database after slaughter. If there are no details the file will not be saved
- File saved as: “CarcassDetails_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: After carcass details entered (if applicable)

Hospital Sessions

- File saved as: “HospitalSessions_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: Immediately after all Lot slaughtered

Hospital Treatments

- Ensure that all courses used to treat BRD are marked as Trial Courses before running this export (see Set Up)
- File saved as: “HospitalTreatments_ YYYYMMDD_YYYYMMDD.csv”

- Time of sending: Immediately after all Lot slaughtered

Induction Treatments

- File saved as: “InductionTreatments_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: On day of induction/day after induction

Dead Sessions

- File saved as: “DraftSessions_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: Immediately after all Lot slaughtered

Pen Movements

- File saved as: “PenMovements_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: Immediately after all Lot slaughtered

Animal ID replacements

- If you run this export and no file is saved it means that there were no Animal ID replacements
- File saved as: “AnimalIDReplacements_ YYYYMMDD_YYYYMMDD.csv”
- Time of sending: With DraftSessions after 42 day bleed AND immediately after all Lot slaughtered

If you have any problems contact the Operations Manager

Appendix 4

National BRD Initiative – Vendor Questionnaire

The National BRD Initiative

Information for Vendors

1st July 2010

To: Feedlot Cattle Supplier

Dear Sir/Madam

RE: National BRD Initiative

Animal health surveys have consistently identified the bovine respiratory disease complex (BRD) as the most significant infectious disease of feedlot cattle in eastern Australia (MRC Project DAN.064 (1991) and 'Survey of feedlot diseases in Australia' (2001)). BRD is responsible for over 60% of all sickness and deaths in feedlots and costs the Australian feedlot sector an estimate of $20 per head across all animals on feed; this has been equated to a minimum total industry loss of $40 million per year (MLA Project AHW.087). These losses are attributed to medication costs, mortalities, excessive feed inputs due to increased time on feed, reduced sale prices and labour costs associated with the detection, handling and treatment of sick animals.

MLA is currently funding a project to better understand the critical risk factors associated with BRD development. An integral part of this work involves understanding the on-farm and feedlot factors that reduce the susceptibility to BRD of animals that enter feedlots. The major project output will be a best practice manual for the management of BRD in feedlot cattle, incorporating a decision support tool for feedlot managers and advisors. This tool will calculate the economic impacts associated with the various management decisions and practices that affect BRD incidence.

The project outcomes will provide the feedlot sector supply chain with a comprehensive understanding of what drives the development of BRD in Australian feedlot cattle. The outcomes will provide cattle producers, like you, with information on how best to prepare feeder cattle for the industry. The outcomes will also provide feedlot operators with decision making tools to minimise the economic impact of BRD on feedlot cattle performance.

If you have received this information package, you have recently supplied cattle to one of the feedlots cooperating with this important industry project. We seek your assistance with this research and ask that you give favourable consideration to completing the attached vendor questionnaire. Further details on what is involved in completing the vendor questionnaire are attached. Please peruse these and come back to me if you have any questions about the project. My contact details are shown below.

Yours sincerely

Des Rinehart

Feedlot R&D Project Manager

Phone: 07 5464 2277 or 0417 728785

Email: drinehart@mla.com.au

The National Bovine Respiratory Disease Initiative

The National Bovine Respiratory Disease Initiative (NBRDI) is a research project funded by Meat & Livestock Australia (MLA) through the Australian Lot Feeders Association (ALFA). The NBRDI was established in response to ALFA members continued concerns of industry losses associated with BRD in this beef industry sector. The study will collect samples from, and management data for 32 - 40,000 cattle entering Australian feedlots during 2009 – 2011 with the aim of identifying risk factors associated with the development of BRD in Australian feeder cattle.

Project data will come from several sources including: feedlot databases, cattle vendors, the National Livestock Identification System, weather stations and monitoring of infectious agents. A critical component of this project is developing an understanding of the role that pre-feedlot management has in preventing BRD development, which is why we need your help by completing the Vendor Questionnaire.

Q. What is The Vendor Questionnaire?

The Vendor Questionnaire is a short survey based on the management of the cattle you recently sold from marking, weaning or purchase to transportation to the feedlot. It is not a comprehensive or detailed survey. The questionnaire is based around pre-feedlot respiratory disease vaccination status, nutrition, handling, feeding and the timing of management practices.

Q. Is Participation Compulsory?

The Questionnaire is by no means compulsory; however your input will be very important for development of future pre-feedlot management regimes. Any information you provide is strictly confidential. While the information you supply will be used to develop a comparative database you, your property and your cattle will not be identifiable in anyway to people outside the project team. The purpose of the database is to look at what management practices vendors, like you, are using and how these practices help to prepare cattle at the feedlot level.

Q. Will I be contacted more than once by the Project Team?

If you supply multiple groups of cattle to the participating feedlots you may be contacted more than once.

As part of the study it is important that we identify any differences in the management of the cattle arriving at the feedlots. So even if groups of cattle come from the same vendor we can not assume that all management practices were the same.

If you would not like to be contacted again, please let us know.

Q. What other information are you collecting during the study?

Apart from the vendor questionnaire we have asked you to complete, we are collecting information about what happens to your cattle once they enter the feedlot as outlined below:

Study sites

Feedlots (11 to 13) throughout Australia considered representative of the feedlot sector will be enrolled in the study. Approximately 13 groups of cattle will be enrolled at each feedlot over the 24 month study period. Data will be collected on each animal and on all pens occupied by the study cattle during their time on feed. Depending on the number of cattle you have supplied, your cattle could be a whole group or grouped with cattle from other vendors to make up a group.

Animal Health Data

Data stored in the feedlot database will be provided to the project at the end of each study group's time on feed. This will include information on entry weight, treatments at the feedlot, health data, ration information, exit data, pen details and carcass data.

Weather

Temperature, rainfall and wind speed data will be collected from feedlot weather stations. Weather data from selected feedlots with dust monitors will be analysed to assess associations between weather and dust.

Cattle movement data

A history of all property movements for each study animal will be obtained from the National Livestock Identification System database.

Cattle preparation data

For feeder cattle purchased direct from the paddock, like yours, vendors will be contacted by mail, phone, fax or email and asked to provide general information on study animals relating to details of weaning, backgrounding and pre-feedlot respiratory disease vaccinations.

Other Sources of Data

To determine the role of virus exposure in BRD development we will be collecting blood samples and nasal swabs from all study animals and tissue samples from any study animals that die while in the feedlot. These samples will be analysed for evidence of exposure to a number of the viruses that are known to be involved in the development of BRD. This will allow us to characterise what pathogens cattle are immune to at feedlot entry, and, what they are exposed to during transport and the early feeding period.

Project Outcomes

By combining all of this data in a comprehensive database we will be able to quantify what role these risk factors play in BRD development. The quantification of these risks will enable industry to determine which risk factors can be effectively managed in an economically viable manner to reduce BRD incidence. These project outcomes will be incorporated into extension programs that enable the optimal preparation of cattle for finishing at feedlots by minimising the risk of these cattle developing BRD.

Q. Where can I find more information on this project?

If you would like to know more about the project, details for project contacts are available at the end of this document.

Q. Will I be provided with any feedback on the information I supply?

Unfortunately, due to the nature of this type of study we will not be able to provide any feedback until we complete the analyses of all project data once the final group of cattle finish their time on feed. The reason for this is to ensure that any project findings are sound and based on all of the data. Conducting “as we go analyses” could be misleading with project findings regarding BRD risk factors changing as more data is added.

We appreciate that this requires you to have a degree of faith in the project and the project team that we are not wasting your valuable time. We would encourage you to contact a member of the project team if you have any doubts or reservations about your participation.

NBRD Initiative contacts for further information:

1. 
Dr. Tamsin Barnes MA, Vet MB, PhD, Operations Manager
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2. 
Dr. Meghan Schibrowski BVSc (Hons), Project Officer
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3. 
Dr. Tim Mahony BSc (Hons), PhD, Project Leader
Agri-Science Queensland
Department of Employment, Economic Development
and Innovation (formerly DPI)
Phone (07) 3346 6505 / 0434 076 196
Fax (07) 3346 6501
Email Timothy.Mahony@deedi.qld.gov.au / t.mahony@uq.edu.au

4. **Des Rinehart**
Feedlot R&D Project Manager
Meat & Livestock Australia
Phone (07) 5464 2277 or 0417 728 785
Email drinehart@mla.com.au

Appendix 5

National BRD Initiative – Information for Vendors

BRD Initiative Vendor Questionnaire V2

Section A: All respondents to complete

1. Vendor's name*

2. Cattle group ID (provided in covering email/letter)*
   All questions in this survey relate specifically to this group of cattle

3. Property name and address

Name	
Address Line 1	
Address Line 2	
Town	
State	
Postcode	
Telephone	
Fax	
Email	

4. Preferred method of contact for follow up

   ○ Email ○ Telephone ○ Fax ○ Mail

5. Were the cattle running together as a single group prior to yarding for transport/sale to the feedlot?
   If yes go to Question 6. If no go to Question 7

   ○ Yes ○ No

6. If you answered "Yes" to Question 5; Approximately how long were the cattle running together as a single group? *Please indicate approximate time in months*

7. If you answered "No" to Question 5; How many mobs were mixed together around the time of yarding for transport/sale? ***Please complete the rest of the questionnaire for the largest mob and indicate any major differences between mobs in the section provided at the end.***

8. What was the main breed of cattle in this group?

Management from Weaning or Purchase until Yarding Prior to Transport or Sale

9. Were the cattle mixed with others between weaning or purchase and yarding prior to transport/sale?

○ Yes ○ No

10. Approximately how many times between weaning/purchase and yarding prior to transport/sale did you yard and handle the cattle?

11. What was the main reason for yarding/handling?

12. Were the cattle given any of the following vaccinations for respiratory disease between weaning/purchase and yarding prior to transport?
Please enter the approximate month and year the vaccinations were given in the check boxes.

	Pestigard	Rhinogard	Bovilis MH
Date Vaccination 1 (mm/yy)			
Date Vaccination 2 (mm/yy)			

13. Were the cattle on any pre-feedlot preparation program?

○ Feedergard I ○ Feedergard II ○ Feedlot Ready ○ None

○ Other, please specify

14. Were the cattle on native or improved pasture between weaning/purchase and yarding prior to transport/sale?

○ Yes ○ No

15. If you answered "Yes" to Question 14, please indicate the type of pasture and the start/finish months that pasture was available.

	From date (mm/yy)	To date (mm/yy)	Type
Native Pasture			
Improved Pasture			

16. Were the cattle EVER given any supplementary feeding such as grain, conserved forage or mineral supplements between weaning/purchase and yarding prior to transport/sale? If "Yes" please go to Question 17.

○ Yes ○ No

17. If you answered "Yes" to Question 16, please indicate the type of feed supplements other than pasture offered in the 2 months prior to sale.
If known, please indicate the approximate from and to dates for each feed supplement fed.
For method of feeding please indicate trough/rack/self-feeder/on ground etc.

	From date (mm/yy)	To date (mm/yy)	Type	Method of feeding
Grain concentrate 1				
Grain concentrate 2				
Conserved forage 1				
Conserved forage 2				
Mineral Supplement 1				
Mineral Supplement 2				
Other Feed Supplement 1				
Other Feed Supplement 2				

Handling and transport to the Feedlot

18. For how long were the cattle yarded prior to transport?

○ < 2 hrs ○ 2 - 4 hrs ○ 4 - 6 hrs ○ 6 - 8 hrs ○ > 8 hrs

19. Was water provided in the yards?

○ Yes ○ No

20. If water was provided in the yards, were electrolytes added?

○ Yes ○ No

21. Were the cattle fed in the yards?

○ Yes ○ No

22. If the cattle were fed in the yards, please indicate the type of feed available
Please select all that apply.

☐ Pasture Hay

☐ Forage Hay

☐ Straw

☐ Mineral Supplement

☐ Other, please specify

Section B: Vendor Bred Cattle Only

Marking or branding management

23. What was the APPROXIMATE age range of this group at marking/branding?

24. In which month (approximately) was this group marked/branded?

25. Were the cattle castrated at the time of marking or branding?

Yes No

26. Were the cattle dehorned at marking/branding?

	Yes	No
Were your cattle dehorned at this time?		
If No, are the cattle a polled breed?		

27. Were the cattle mixed with other cattle at marking or branding?

Yes No

Weaning Management (Vendor Bred Cattle Only)

28. What was the APPROXIMATE age range of this group at weaning?
If unknown for this specific group, please indicate the approximate age you generally wean calves at.

29. In which month (approximately) was this group weaned?
Again, the general month you wean at is appropriate if specifics for this group are unknown.

30. How were the cattle weaned?
If the cattle were yard weaned please go to Question 31, otherwise go to Question 33.

Yard weaned Paddock weaned

Other, please specify

31. If the cattle were yard weaned, for how long were the cattle kept in the yards?
Please enter approximate time in days

32. If the cattle were yard weaned, please indicate the type(s) of feed provided in the yards.
For method of feeding please indicate if fed on the ground/trough/rack/self-feeder etc.

	Yes/No	Type	Method of Feeding
Conserved Forage			
Grain Concentrate			
Other			

33. Were the cattle dehorned at the time of weaning?

	Yes	No
Were the cattle dehorned at the time of weaning?		
If No, were the cattle already dehorned?		

34. Were the cattle castrated at the time of weaning?

Yes No

35. Were the cattle mixed with other cattle at the time of weaning?

Yes No

36. Were the cattle given any of the following vaccinations for respiratory disease during this period?
Please enter the APPROXIMATE month and year the vaccinations were given in the check boxes.

	Pestigard	Bovilis MH
Vaccination Date (mm/yy)		

Section C: Purchased Cattle Only

Purchase of Cattle

37. In which year and month (approximately) did you purchase the cattle?

38. Were the cattle purchased from one or more vendors?

Single vendor Multiple vendors

39. Where were the cattle purchased from?
Please indicate all that apply and provide location(s) for each

	Yes/No	Location
Weaner sales		
Saleyard		
Paddock		

40. Approximately how old were the cattle when purchased?

41. What was the average weight of cattle when purchased?

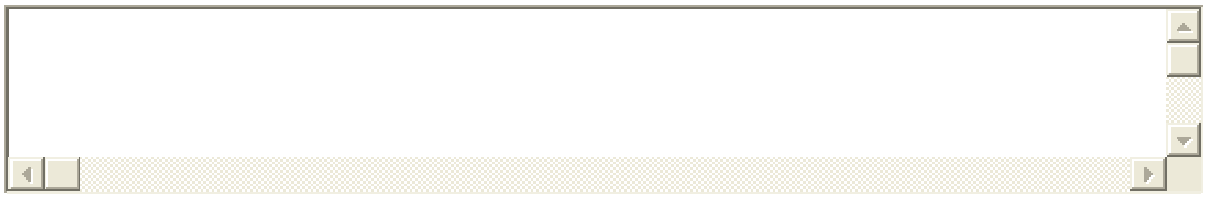
42. Do you keep purchased cattle in the yards for any period of time after arrival at your property?

Yes / No

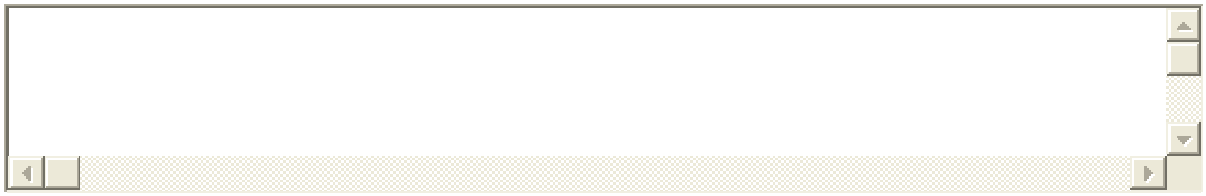
If yes, how long do you generally keep purchased cattle in the yards?

Section D: All respondents to complete

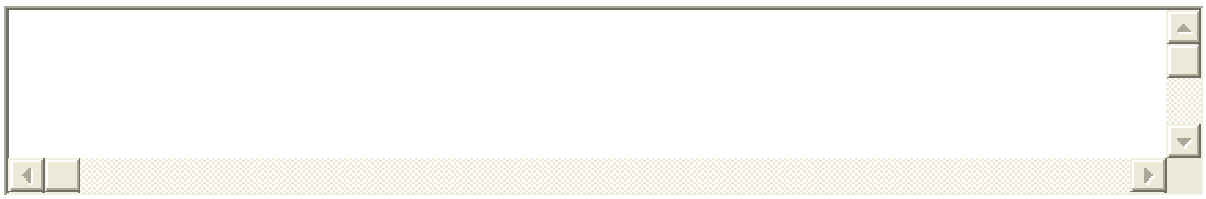
43. If there was more than one mob of cattle in this group please indicate how the management of the other groups differed from that described above.



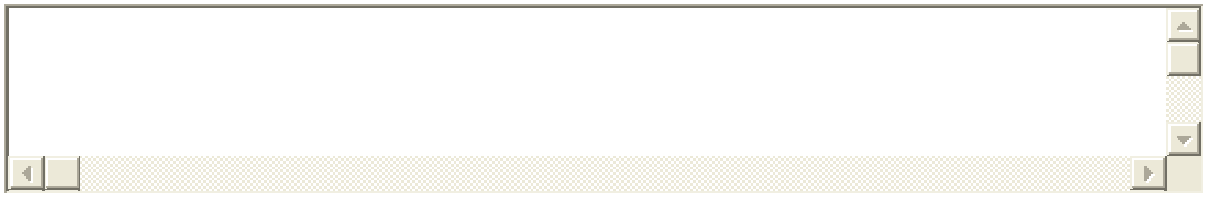
44. Do you have any additional comments about this group of cattle?



45. In your opinion what are the three most critical factors in preparing cattle for feedlots?



46. Do you have any comments about this survey?



Thank you for completing this survey.