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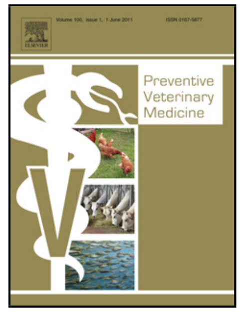

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Risk factors for bovine respiratory disease in Australian feedlot cattle: Use of a causal diagram-informed approach to estimate effects of animal mixing and movements before feedlot entry

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Abstract

A nationwide longitudinal study was conducted to investigate risk factors for bovine respiratory disease (BRD) in cattle in Australian feedlots. After induction (processing), cattle were placed in feedlot pens (cohorts) and monitored for occurrence of BRD over the first 50 days on feed. Data from a national cattle movement database were used to derive variables describing mixing of animals with cattle from other farms, numbers of animals in groups before arrival at the feedlot, exposure of animals to saleyards before arrival at the feedlot, and the timing and duration of the animal's move to the vicinity of the feedlot. Total and direct effects for each risk factor were estimated using a causal diagram-informed process to determine covariates to include in four-level Bayesian logistic regression models. Mixing, group size and timing of the animal's move to the feedlot were important predictors of BRD. Animals not mixed with cattle from other farms prior to 12 days before induction and then exposed to a high level of mixing (≥4 groups of animals mixed) had the highest risk of developing BRD (OR 3.7) compared to animals mixed at least 4 weeks before induction with less than 4 groups forming the cohort. Animals in groups formed at least 13 days before induction comprising 100 or more (OR 0.5) or 50 to 99 (OR 0.8) were at reduced risk compared to those in groups of less than 50 cattle. Animals moved to the vicinity of the feedlot at least 27 days before induction were at reduced risk (OR 0.4) compared to cattle undergoing short-haul transportation (<6 hours) to the feedlot within a day of induction, while those experiencing longer transportation durations (6 hours or more) within a day of induction were at slightly increased risk (OR 1.2). Knowledge of these risk factors could potentially be used to inform management decisions to reduce the risk of BRD in feedlot cattle.

Key words:

Bovine respiratory disease, feedlot, risk factors, total effects, causal diagram

1. Introduction

Bovine Respiratory Disease (BRD) describes a complex of diseases involving the respiratory system in cattle. BRD is particularly common where cattle are kept in intensive or confined conditions, such as in feedlots, and is a multifactorial disease; necessary factors include pathogenic organisms, environmental stressors and immunological susceptibility (Edwards, 2010). 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. Increased risk of BRD has been demonstrated in groups comprised of animals from multiple sources that were mixed at the feedlot compared to predominately singled-sourced groups (Martin et al., 1982; Wilson et al., 1985; Martin and Meek, 1986). Wide variation in incidence between source groups has been observed (Martin et al., 1988) as well as evidence of clustering of fatal BRD cases within truckloads and/or pens (Ribble et al., 1994). More recent studies agree that comingling of cattle from multiple sources around the time of feedlot entry increases risk of BRD (O'Connor et al., 2005; Sanderson et al., 2008; Step et al., 2008).

Routine practice in North American sale barns involves comingling of cattle from multiple farms immediately prior to sale (Macartney et al., 2003), which means that comingling and market source are interlinked. Cattle identified as coming from a single source such as a particular truckload or sale barn of origin may, in fact, have originated from several different farms, leading to potentially important misclassification bias. While some prior studies have reported that larger group size increases risk of BRD (Martin et al., 1982; Kilgore et al., 2005; O'Connor et al., 2005), 'groups' may have been assembled for varying lengths of time and larger groups may be a proxy for a larger number of sources. In determining the effect of the number of cattle in the animal's group, data indicating the date when the group was formed were usually not available. These studies lacked sufficient data to separate the effects of more refined individual factors, and to consider the effect of timing of exposure to comingling and auction sales relative to when cattle commenced being at risk of BRD at the feedlot.

While some North American studies returned equivocal results regarding the effect of transport distance on BRD risk (Cole et al., 1988; Ribble et al., 1995b; Schwartzkopf-Genswein et al., 2007), larger more recent studies suggest a positive association between distance transported and BRD incidence (Sanderson et al., 2008; Cernicchiaro et al., 2012).

Causal diagrams facilitate an informed approach to model building with postulated and potential relationships defined based on *a priori* knowledge and plausible biological pathways. Causal diagrams allow the separate estimation of total and direct effects, both of which may be of interest to researchers and industry stakeholders (Dohoo et al., 2009). In a causal diagram, the direct effect of an exposure is indicated by a single arrow directly linking the exposure and outcome variables. An indirect effect of an exposure is indicated by a pathway through a sequence of arrows passing through one or more intervening variables to the outcome variable. The total effect of an exposure variable is the sum of the direct and all indirect effects of that exposure on the outcome.

Many factors associated with the assembly and movement of animals from their home property to the feedlot may affect the risk of BRD in the feedlot, and it was postulated that the effects of these factors depend on the timing of the animal's exposure. This paper describes the development of a causal diagram and subsequent analyses with the aim of evaluating the total and direct effects of risk factors relating to mixing of animals with cattle from other farms before the latest induction date for the animal's pen ('cohort closure'), numbers of animals in groups before induction, exposure of animals to saleyards before arrival at the feedlot, and the timing and duration of the animal's move to the feedlot on the risk of BRD in Australian feedlot cattle.

2. Materials and methods

2.1. Overview of study design

A nationwide prospective longitudinal study was conducted in Australia to evaluate many possible risk factors for BRD in feedlot cattle. Results for the subset of exposures relating to animal mixing, group size, exposure to saleyards and timing and duration of the animal's move to the feedlot are presented in this paper. Results for other exposures (e.g. animal entry characteristics, season, pen features) will be reported separately. Managers of feedlots with a minimum capacity of 1,000 cattle and the necessary resources to keep required records were invited to participate. After arrival at the feedlot, cattle were inducted (animal identity and other data recorded, and treatments applied), and enrolled in cohorts where a cohort consisted of all animals placed together in a feedlot pen following induction. Each cohort consisted of one of more 'group-13s' where a group-13 consisted of all animals that were together 13 days before induction that then went into the same cohort. Cohorts were generally selected at the convenience of the feedlot managers despite attempts to randomise the selection process. A total of 35,160 animals were inducted into study cohorts from March 2009 to December 2011, of which 35,131 animals had sufficient data for inclusion in this study. The study population had a nested hierarchical structure such that animals were clustered within 1,077 group-13s which, in turn, were clustered within 170 cohorts, which were clustered within 14 feedlots. The mean number of animals in a group-13 was 33 (range: 1 to 342) and the mean number of animals per cohort was 207 (range: 17 to 395). The number of animals and cohorts contributed per feedlot ranged from 466 animals in 3 cohorts to 6,114 animals in 22 cohorts. Of the 14 participating feedlots, three routinely practiced ‘pre-induction assembly’. This is a management practice whereby animals from different farms are assembled on pasture close to the feedlot for various periods of time prior to induction. Each animal was monitored from induction until it left the study cohort for any reason (i.e. removal to the hospital pen or another pen separate from the cohort, death or feedlot exit). Detailed data were recorded for each animal (e.g. identification number, arrival date, induction date, first day on feed, sex, dentition, breed, induction weight) and supplied as animal-level electronic files, while further data were supplied for animals that were hospitalised or died during the observation period.

2.2. National Livestock Identification System data

The Australian National Livestock Identification System (NLIS) requires that all cattle are individually identified with a unique identification string that may be applied as an ear tag or a rumen bolus, before they leave their farm of origin. Each farm, feedlot and saleyard is identified by a unique Property Identification Code (PIC). The system relies on registered users electronically scanning animals every time they are moved from one PIC location to another ('transfers') and uploading this data to an online national electronic database. The database records the PIC of issue (the animal's first lifetime PIC), and for each transfer, the source and destination PIC, transfer date and transfer type. Transfer type distinguishes between transfers to or from saleyards and ‘point to point’ (non-saleyard) transfers. NLIS transfer data were obtained for 98.8% of study animals. Transfer data were simplified to create a logical sequence from the PIC of issue to the feedlot for each animal. Multiple transfers occurring within a 48 hour period (e.g. PIC A to a saleyard or intermediate PIC and saleyard or intermediate PIC to PIC B) were consolidated to form a single record (PIC A to PIC B) while the transfer detail was retained as a separate variable. Time intervals between each transfer and the animal's induction date were used to determine the PIC at which the animal was located at various time points prior to induction date. NLIS imputes some transfers. For example, if an animal’s record contains a transfer from PIC A to PIC B and then a transfer from PIC C to PIC D, NLIS generates imputed transfers from B to ‘unknown’ and 'unknown' to C, along with associated imputed transfer dates. These imputed transfers were simplified and retained (PIC B to PIC C; imputed) but the imputed transfer dates were changed to missing. It was usually possible to determine the location of these cattle at the time points of interest by using the known transfer dates of other animals from the same cohort that shared a common move sequence. For transfers to the feedlot PIC, we used the arrival dates and tail tag numbers (identifying the most recent source PIC) supplied by the feedlots, to validate the data supplied by NLIS. We also determined the total number of lifetime transfers and the interval between transfers.

2.3. Case definition, exposure variables and causal diagrams

The unit of analysis was the individual animal. The outcome of interest was the development of BRD during the first 50 days following induction. The case definition was based on the clinical signs of disease recorded by feedlot staff in computerised hospital records after suspected ill animals were removed from their cohort for examination and treatment. Veterinarians servicing participating feedlots conducted regular training sessions for feedlot staff on the diagnosis of BRD and seven of the fourteen participating feedlots were serviced by the same veterinary group. Thus diagnosis of BRD was expected to be relatively consistent across participating feedlots. All animals with clinical signs indicating respiratory system involvement ("pneumonia", "respiratory", "BRD" and "IBR") were classified as BRD cases by the research team.

All exposure variables were categorised for use in analyses, with definitions of categories based on prior hypotheses and distributions. Variables were derived from the NLIS data to determine each animal's PIC and the number of animals in its group at particular time points of interest. Each animal's time at risk began the day after induction into a study cohort; hence the induction date was designated "day 0". Days prior to this date were identified using negative values, and time points of particular interest (days -91, -28, -13 and -2) were chosen based on *a priori* hypotheses. It was hypothesised that the effects of mixing, group size, exposure to a saleyard and timing of the animal's move to the feedlot on the risk of developing BRD would differ depending on the timing of these events in relation to day 0. Early studies describing the epidemic curves for BRD in feedlot calves indicated that the majority of cases occurred in the first 4 weeks following arrival at the feedlot (Martin, 1983). Assuming a similar epidemic curve would apply to animals exposed to stress and pathogens through mixing, saleyard exposure or transport, we postulated that 28 days would be the minimum amount of time required for the majority of susceptible animals in a group to develop signs and recover from infection. More recently, researchers identified several different temporal patterns in the cumulative incidence of BRD in feedlot populations (Babcock et al., 2010). For the majority of cohorts in their study, the cumulative incidence of BRD was more than 50% by 28 days. However, different patterns were identified and later onset and more gradual rises in cumulative incidence were also observed. In all of these patterns, the cumulative incidence was above 80% by day 90, and in 95% of cohorts it was above 95% by day 90. Thus, day -91 was chosen to further evaluate the timing of mixing. At the animal level, uncomplicated respiratory viral infections (e.g. with bovine herpesvirus type 1; (Ellis, 2009)) may resolve within 2 weeks, so day -13 was chosen as a comparative time point to evaluate the effects of all exposures. Day -2 was only used to derive the variable describing the timing and duration of transport to the feedlot so that transport within a day of day 0 would be in a separate category to transport at earlier times.

Each animal's group was derived based on which cattle from its cohort were at the same PIC at each time point. For example, "group-28" identified the animal's group 28 days before induction. Changes in each animal's group between time points were used to describe mixing. Mixing before day -27, and between day -27 and day -13, were described as binary variables (mixed or not mixed within period). Because the majority of animals were moved to the vicinity of the feedlot within the 12 days prior to their induction, the amount of mixing between day -12 and cohort closure (the latest induction date for the animal's pen) was described by a categorical variable based on the number of group-13s forming the cohort (1, 2–3, 4–9 and ≥10). These three variables were combined to form a single variable ('mixing history') to describe the animal's lifetime mixing history. A further variable, ('mix_first') consisted of three categories which described the time interval of the earliest mixing event. Numbers of animals in each animals group were also defined; for example, "group-28N" indicated the number of animals in the animal's group-28.

Three binary saleyard variables were derived to describe whether or not animals had been exposed to saleyards in the intervals before day -27, day -27 to day -13, and day -12 to induction. The timing and duration of an animal's move to the feedlot was described by a composite variable ('feedlot move timing') based on the number of days between arrival at the feedlot vicinity and induction, and the estimated duration of transport for those animal's arriving within 12 days before induction. The duration of transport was determined by estimating the travel time between the source and feedlot PICs. Additional time was included for transfers via saleyard or intermediate PICs or for driver rest periods for long-haul transportation. The time interval between arrival at the vicinity of the feedlot and induction ('Arrival_day0') and other collapsed versions of variables described above were used in analyses restricted to animals from the three feedlots routinely using pre-induction assembly.

Causal diagrams were constructed to describe postulated links between measured exposure variables and between exposure variables and occurrence of BRD in the first 50 days at risk. As this resulted in a very complex diagram, a simplified version (only including variables relevant to the assessment of the risk factors included in the analyses reported in this paper) is shown in Figure 1. Figure 2 shows the causal diagram used to inform the analyses restricted to the three feedlots that routinely used pre-induction assembly. Additional variables included as potential confounders in either of these diagrams were cohort fill duration (all animals added to their cohort within a single day or over a longer period), total number of animals on feed in the animal's feedlot (average for the animal's induction month), number of animals in the animal’s cohort, induction weight, breed and season in which the animal was inducted.

2.4. Data management and statistical modelling

The Stata® statistical software package (version 12) was used for all data management and preliminary analyses and MLwiN® (version 2.27) was used to fit final four-level models.

In estimating total effects, care needs to be taken to adjust appropriately for confounders, including variables that become confounders through conditional associations (Dohoo et al., 2009). Various methods have been proposed for determining which covariates to fit when estimating total effects, but they all have similar features (Greenland et al., 1999; Shrier and Platt, 2008; Dohoo et al., 2009; Textor and Liskiewicz, 2011). The DAGitty® software (Textor et al., 2011) was used to identify minimal sufficient adjustment sets to assess total and direct effects of the exposure variable of interest on the occurrence of BRD. A sufficient adjustment set is a set of variables that appropriately controls confounding of the association between the exposure variable of interest and the outcome. When direct effects are required, the sufficient adjustment set also includes all intervening variables between the exposure variable of interest and the outcome. The causal diagram was reproduced within the DAGitty® user interface. Each variable of interest was sequentially identified as the exposure of interest, and the minimal sufficient adjustment sets for both total and direct effects as defined by DAGitty® were noted.

The multilevel modelling software package MLwiN® (version 2.27) was used for modelling. For each exposure of interest, a model containing covariates determined by the minimal sufficient adjustment set for the total effects was fitted using second-order penalised quasi-likelihood methods to produce starting values for the second model using Bayesian Markov chain Monte Carlo (MCMC) methods. The process was then repeated using the adjustment set for direct effects, where these were desired. For all models, random effects of feedlot, cohort nested within feedlot and group-13 nested within cohort were included, and Metropolis Hastings sampling methods were used. Gaussian prior distributions with extremely large variances, the default for multilevel logistic models fitted in MLwiN® (Browne, 2012), were used. After a burn-in of 1000 iterations, 10,000 further iterations were run and diagnostic trajectory plots and summary statistics (Browne, 2012) assessed to estimate the required chain length. Further iterations were run and models were reassessed until convergence was achieved. Animal-level variables such as exposure to a saleyard prior to day -27 displayed good mixing and low autocorrelation, while mixing history and feedlot move timing displayed higher autocorrelation and were slower to converge. Consequently, MCMC chains were run for between 50,000 and 300,000 iterations to obtain final posterior parameter estimates of mean odds ratios (ORs) and 95% credible intervals.

3. Results

3.1. Descriptive statistics

Induction weights ranged from 196 to 756 kg; 20% of the study population were <400kg, 31% were 400–439kg, 34% were 440–479 kg and 15% were ≥480kg. The most common breeds in the study population were Angus (56%), tropical breeds or crosses (16%), British breed crosses (12%) and Hereford (6%). The study population comprised 92% steers and 8% heifers. An estimated 41% of animals had a single lifetime transfer (from the source property to the feedlot). For animals that had at least 2 lifetime transfers with known transfer dates, the transfer prior to the feedlot move occurred an average of 280 days (about 9 months) before induction, and 80% of these transfers occurred between 16 and 3 months before induction.

Of all suspected ill study animals removed from their cohort for examination and treatment, 77.3% (6,406/8,285) met the BRD case definition at first examination, comprising 18.2% (6,406/35,131) of the study population. The majority of animals that had BRD when first examined were examined during their first 50 days at risk, giving a 50-day BRD cumulative incidence of 17.6%. This cumulative incidence does not include BRD occurrences subsequent to diagnosis with another condition at the time of first examination.

Descriptive statistics for exposure variables of interest are shown in Table 1. The most common mixing history involved animals mixed prior to day -27 joining cohorts formed by 10 or more group-13s (labelled 'Yes, no, ≥10'; 22%) or 4–9 group-13s ('Yes, no, 4–9'; 16%). A high level of mixing within 12 days of induction was also common in animals not mixed prior to day -27 ('No, no, ≥10'; 15%; 'No, no, 4–9'; 10%). The majority (62%) of animals had been mixed prior to day -90 and 5% were mixed for the first time between days -90 and -28.

Smaller groups defined at day -13 (<50 animals) were the most common (39% of animals), but nearly a third of animals (33%) came from groups defined at day-13 with at least 100 animals. About a third (36%) of the study population had been exposed to saleyards prior to day -27, while relatively few had been through saleyards within 27 days of induction (3% between days -27 and -13 and 3% within 12 days of induction). Most cattle (76%) were moved to the feedlot within a day before induction, with 36% of these being transported for 6 hours or longer.

Those cattle that were moved to the vicinity of the feedlot prior to day -12 and mixed between day -27 and day -13 were mostly from the three feedlots that practiced pre-induction assembly. The pre-induction assembly subset comprised 5,641 animals from 3 feedlots, 297 group-28s, 136 group-13s and 40 cohorts. The 50-day BRD cumulative incidence was 3.3% in this subset. As shown in Table 2, 31% of these animals arrived at the vicinity of the feedlot more than 27 days before induction and 31% arrived in the interval between days -27 and -13. More than half (57%) were in cohorts formed by four or more groups defined at day -28.

3.2. Multilevel logistic regression models

Estimated total effects of variables of interest on 50-day BRD cumulative incidence for the whole study population are shown in Table 1. Mixing history had a marked effect. Compared to the reference category of animals that had been mixed prior to day-27 from cohorts formed by 2 or 3 group-13s ('Yes, no, 2-3'), a similar risk level was observed for those not mixed between day -27 and cohort close (‘Yes, no, no’: OR 1.2), but risk was substantially increased if more than 4 group-13s formed the cohort ('Yes, no, 4-9': OR 2.8; 'Yes, no, ≥10': OR 2.2). Risk was also markedly increased for animals not mixed before day -27 (‘No, no, no’ and ‘No, no, 2-3: OR:2.3), with the highest risk for animals subjected to a high level of mixing between day -12 and cohort close ('No, no, 4-9' and 'No, no, ≥10': OR 3.7). Based on the causal diagram (Fig. 1), direct and total effects for mixing history were equivalent. Results from the analysis of the first mix timing variable indicated a similar marked protective effect of prior mixing, both for animals first mixed before day -90 (OR 0.6) and for those first mixed between day -90 and day -28 (OR 0.6) compared to animals first mixed between day -27 and cohort close. Estimated odds ratios for mixing between days -27 and -13 were consistent with increased risk but estimates were imprecise. Subset analysis (Table 2), used because mixing during this time interval was an uncommon practice across the whole study population, showed a markedly increased risk for animals in cohorts formed by mixing 4 or more group -28s (OR:5.5) compared with less than four.

Animals in larger groups defined at day -13 were at reduced risk of BRD. Compared to animals from groups with less than 50 animals, animals from groups with 50 to 99 animals were at somewhat reduced risk (OR 0.8) and animals from groups with 100 or more animals were at markedly reduced risk (OR 0.5) of developing BRD (Table 1). The direct effects of the number of animals in the group-13 (Table 3) were of a similar magnitude to the total effects, indicating that these effects are not due to differences in mixing history, cohort fill duration or the number of animals in the cohort.

The total effect of exposure to saleyards varied with time of the exposure (Table 1), with markedly increased risk associated with saleyard exposure closer to induction (exposed between days -27 to -13: OR 1.9; days -12 to 0: OR 2.6) and modestly decreased risk for saleyard transfers prior to day -27 (OR 0.8). However, there was no evidence for an important direct effect of having been through a saleyard prior to day -27 after accounting for the intervening variable, mixing history (OR 1.0, Table 3). The direct effects of saleyard transfer between days -27 and -13, and day -12 to induction were also much attenuated, after accounting for mixing history and feedlot move timing as intervening variables, suggesting that total effects of exposure to saleyards during these periods were probably largely due to the effect of mixing. However, an important adverse direct effect of exposure to saleyards between days -12 and induction was evident (OR 1.8) indicating that exposure within this period has a negative effect over and above the effects of mixing and feedlot move timing.

Compared to animals undergoing transport of less than 6 hours within a day before induction, animals having longer transport times (6 hours or more) within a day of induction were at slightly increased risk (OR 1.2), while animals that moved to the vicinity of the feedlot at least 27 days before induction were at substantially reduced risk (OR 0.4). The direct effects of the feedlot move timing were generally similar to the total effects, with greater differences in the estimates for exposure categories with very unbalanced distributions across feedlots (Table 3). Subset analyses restricted to the three feedlots practicing pre-induction assembly (Table 2) also provided evidence that animals arriving prior to day -27 were at reduced risk compared to those arriving within 12 days before induction.

4. Discussion

From this study, we identified important differences in the effects of risk factors relating to animal mixing and moving depending on the timing of these events in relation to the animal's induction at the feedlot. We found that there was a protective effect of mixing prior to 27 days before induction and an adverse effect of mixing 4 or more groups compared to less than 4 groups within 12 days of induction. Moving to the vicinity of the feedlot at least 27 days prior to induction was protective and longer transport duration within a day of induction slightly increased risk. The effect of saleyard exposure varied depending on the timing of exposure, and the effect was largely mediated through mixing but saleyard exposure within 12 days of induction increased risk over and above the effect mediated through mixing and the move to the feedlot. Being part of a larger group (more than 50 animals) established at least 13 days prior to induction was protective.

Comingling of animals from multiple sources immediately prior to arrival at the feedlot has been consistently shown to be associated with increased risk of BRD (Martin et al., 1982; Ribble et al., 1995a; O'Connor et al., 2005; Sanderson et al., 2008; Step et al., 2008). Results from the current study demonstrated that the effect of comingling depends on prior mixing history; important differences were observed between categories of cattle with differing mixing histories. By utilising lifetime animal-level data we have been able to examine mixing history in a way that has not, to our knowledge, previously been described. Mixing prior to

27 days before induction was protective and comingling with cattle from less than 4 groups within the 12 days preceding induction did not increase risk provided cattle had been mixed prior to 27 days before feedlot entry. A high level of mixing (defined by the combination of 4 or more group-13s forming a cohort) close to induction markedly increased the risk of BRD.

Cattle transported for 6 hours or more within one day of induction were at slightly increased risk of BRD compared to those undergoing shorter duration transport in this period, which is consistent with findings from recent North American studies (Sanderson et al., 2008; Cernicchiaro et al., 2012). To our knowledge, prior studies have not investigated the effect of time interval between arrival at the vicinity of the feedlot and induction on BRD risk after induction. Our results showed that cattle arriving at the feedlot vicinity more than 27 days before induction were at reduced risk of BRD. We speculated that this may have been overestimated as only three feedlots in the study moved cattle to the vicinity of the feedlot prior to day -12 and there may have been uncontrolled confounding despite having fitted feedlot as a random effect. However, results of analyses restricted to animals from these three feedlots were consistent with a large protective effect, although the odds ratio estimate for cattle moved prior to day -27 was imprecise. We postulated that the total effect of the feedlot move timing was likely to be partially mediated through mixing (the intervening variable in the causal diagram (Figure 1). Our results indicated that the timing of the move to the vicinity of the feedlot was an important contributor to the risk of BRD over and above effects of mixing.

In the current study, animals that were part of a larger group 13 days prior to induction were at reduced risk of BRD. A larger number of animals in a group has been associated with increased risk of BRD in prior studies, but this may be due to the effects of more comingling in larger groups (Martin et al., 1982; Martin, 1983; Martin and Meek, 1986). The interpretation of the effects of 'group size' in prior research is problematic because the length of time the group has been assembled was usually not able to be determined. The number of animals in the cohort aligns more closely with group size investigated in other studies, but we do not draw a conclusion about cohort size because it tended to be clustered by feedlot, limiting the power to detect an effect and possibly leading to uncontrolled feedlot-level confounding. We defined group size at a consistent time point for comparison of all study animals, potentially avoiding misclassification bias if effects of group size depend on time before induction when group size is assessed. However, group sizes were often stable for extended periods of time before the move to the feedlot and for the majority of animals the grouping structure did not change dramatically between 3 months and 13 days before induction. Hence, our conclusion is that group size is very important, but the stability of group sizes observed in our study means that the duration of time that the group is formed should be considered alongside the effects of mixing history and feedlot move timing. As a consequence of being in a larger group, fewer such groups are likely to be mixed to form a cohort, but the similar effect in both total and direct effects models, indicates an important effect over and above that mediated through mixing. Possible additional reasons for the protective effect could relate to a lower level of stress associated with the disruption of their social hierarchy, and if the group is of sufficient size, animals may be exposed to fewer novel pathogens in the feedlot pen.

Conclusions are supported by secondary analyses. Although first mixing in the interval from day -90 to day -28 occurred in only 5% of the full study population, it was associated with a similar level of reduced risk as prior mixing before day -90. Because mixing from day -28 to day -13 and moving to the feedlot prior to day -12, and to a lesser extent prior to day -2, were generally restricted to feedlots that practised pre-induction assembly, the analyses restricted to these feedlots may be more appropriate for drawing inference about these practices. Although consistent results indicative of a protective effect of moving animals to the vicinity of the feedlot prior to day -27, and the harmful effect of mixing four or more group-28s to form a cohort support our conclusions, further research is needed to better understand the effects of mixing and moving associated with pre-induction assembly.

An important finding from this study is that the effects of exposure to saleyards differ depending on the timing of exposure relative to induction. Our results show that cattle exposed to saleyards more than 27 days before induction are at lower risk but this protective effect is primarily mediated by factors other than the process of unloading, yarding, holding then reloading at saleyards. This was demonstrated by separately estimating total and direct effects. Similarly, the detrimental total effect of saleyard exposure within 27 days of induction should be interpreted in combination with the much attenuated direct effect estimates. Our results showed that saleyard exposure within 27 days of induction increased risk of BRD but this is also due to factors other than the actual saleyard processes. However, our results indicate that exposure to saleyards within 12 days prior to induction further increased risk of BRD over and above effects of mixing and feedlot move timing.

There were several potential sources of bias in this study. Despite training of feedlot staff by veterinarians, there may have been differences in detection and/or recording of clinical signs between feedlots. Feedlot was fitted as a random effect in all models, and this will have at least partially removed any confounding by feedlot. In using the PICs to determine whether cattle were mixed, misclassification errors may have occurred in the classification of animals' mixing histories and group sizes. Cattle with the same PIC were assumed to have mixed with each other while on that farm when, in fact, some they may have been maintained on that farm separately from other cattle. Similarly, cattle moved from multiple sources to a common PIC were assumed to have been mixed on that new farm. However, these assumptions were supported by additional data obtained from farms selling study cattle directly to feedlots (about 30% of the study population) which indicated that 94% of study groups were mixed on these farms. Under-recording of mixing and group sizes may also have occurred. Each animal's groups were defined based only on the cattle entering the same cohort, and additional animals may have been mixed with study animals prior to induction. Provided any misclassification error patterns were the same across all true values of the variables, the resulting misclassification biases for mixing history and group size variables would be expected to be towards the null. The finding that cattle with a history of having being through a saleyard or mixed prior to day -27 are at reduced risk would be expected to be largely explained by the protective immunity expected to develop following exposure to viruses prior to feedlot entry.

Beef cattle management practices in Australia differ from those in North America and Europe in some key aspects. Cattle in Australia enter feedlots at an older average age, often many months after weaning; and it is common for recently weaned cattle to be sold through saleyards or weaner sales and then spend 6 months or more on an intermediate farm before being sold to a feedlot (Walker et al., 2007). Accordingly, results of this study may not reflect causal relationships in feedlot cattle in other countries. In addition, larger capacity feedlots were more likely to participate in the study, so conclusions may not be generalizable to all Australian feedlots. However, results should be generalizable to moderate to large feedlot operations in Australia because feedlots from most major feedlot regions participated in the study and enrolled cattle would be expected to be representative of the Australian feedlot population as they came from throughout the wide geographical beef-cattle producing regions and had a broad range of entry characteristics.

The use of a causal diagram and the determination and comparison of separate direct and total effects provides informative estimates of effect from complex datasets. The adjusted effects estimated in a multivariable model built using an automated model building process may be direct, partial or total effects, and therefore do not necessarily reflect the total causal effect of the exposure variable on the outcome variable (Westreich and Greenland, 2013). This is because with automated model building processes, variable selection is not based on whether variables may be potential confounders or intervening variables for particular exposure-outcome relationships, so some variables that are important confounders may not be included and intervening variables may be included in the model. However, the use of a causal diagram to inform model building can result in uncontrolled confounding if the causal diagram does not accurately capture causal pathways or important confounders are missing from the diagram. It also relies on some assumptions about the directionality of associations and this is not always clear.

5. Conclusions

The risk of BRD in feedlot cattle varied markedly with prior mixing history; there was a protective effect of mixing prior to 27 days before induction and an adverse effect of mixing 4 or more groups compared to less than 4 groups within 12 days of induction. Moving to the vicinity of the feedlot at least 27 days prior to induction was protective. Longer transport duration (6 hours or more compared to less than 6 hours) within a day of induction slightly increased risk of BRD. The effect of saleyard exposure varied depending on the timing of exposure, and the effect was largely mediated through mixing but saleyard exposure within 12 days of induction increased risk. Being part of a larger group (more than 50 animals) established at least 13 days prior to induction was protective. Management decisions regarding these factors have the potential to markedly reduce the incidence of BRD in feedlot cattle.

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Table 1. Distribution of variables and estimated odds ratios for their total effects on the occurrence of BRD by day 50 based on models derived from the causal diagram shown in Figure 1.

Variable & category	Number of animals (%)	Crude BRD 50-day cumulative incidence (%)	Adjusted odds ratio	95% credible interval
Mixing history[a]	34,730			
No, no, no[e]	418 (1)	20.6	2.3	(0.4–7.4)
No, no, 2–3	1,489 (4)	19.5	2.3	(1.3–3.8)
No, no, 4–9	3,334 (10)	30.3	3.7	(1.7–7.2)
No, no, ≥10	5,114 (15)	31.4	3.7	(1.7–7.4)
No, yes, yes	627 (2)	17.2	3.4	(1.4–7.2)
No, yes, no[e]	407 (1)	2.5	2.3	(0.5–6.9)
Yes, no, 2–3	3,893 (11)	5.7	Ref. cat.	
Yes, no, 4–9	5,409 (16)	16.4	2.8	(1.3–5.4)
Yes, no, ≥10	7,793 (22)	20.7	2.2	(1.0–4.5)
Yes, yes, yes[e]	946 (3)	13.7	2.2	(0.9–4.5)
Yes, yes, no[e]	1,958 (6)	3.3	2.5	(0.7–6.6)
Yes, no, no	3,342 (10)	3.4	1.2	(0.5–2.6)
Number of animals in group-13[b]	35,131			
< 50	13,782 (39)	24.1	Ref. cat.	
50 to 99	9,783 (28)	21.3	0.8	(0.7 - 0.9)
≥ 100	11,566 (33)	6.9	0.5	(0.4 - 0.7)
Saleyard transfer prior to day-27 [b]	34,730			
No	22,223 (64)	18.7	Ref. cat.	
Yes	12,507 (36)	15.7	0.8	(0.7 - 0.9)
Saleyard transfer between day-27 and day-13 [b]	35,131			
No	34,162 (97)	17.8	Ref. cat.	
Yes	969 (3)	11.2	1.9	(1.3 - 2.7)
Saleyard transfer between day-12 and day 0 [b]	35,131			
No	34,200 (97)	17.6	Ref. cat.	
Yes	931 (3)	21.4	2.6	(1.6 - 4.1)
Feedlot move timing[c]	35,131			
Pre day-27[e]	1,880 (5)	1.5	0.4	(0.2 - 0.8)
Day -27 to -13[e]	2,000 (6)	4.6	1.0	(0.4 – 1.9)
Day -12 to -2; <6 hours	2,183 (6)	10.9	0.9	(0.6 - 1.2)
Day -12 to -2; ≥6 hours	2,339 (7)	8.0	0.9	(0.5 - 1.4)
Day -1 to 0; <6 hours	17,139 (49)	19.9	Ref. cat.	
Day -1 to 0; ≥6 hours	9,590 (27)	23.5	1.2	(1.0 - 1.5)
First mix timing[d]	34,730			
Pre day -90	21,559 (62)	13.5	0.6	(0.5 – 0.7)
Day -90 to day -28	1,713 (5)	4.6	0.6	(0.4 – 0.9)
Day -27 to 0	11,458 (33)	27.4	Ref. cat.	

[a]Mixing history: pre day-27, day-27 to day-13and day-12 to cohort close; covariates include Fill, Weight, SY-12_0, SY-27_13, SYpre-27, CohortN, Move_FL, Group-13N, N=34,726
[b]Models have no additional covariates as they have empty adjustment sets
[c]Feedlot move time interval and transport duration (within 12 days); covariates: SY-12_0, SY-27_13, N=35,131
[d]First mix timing describes the earliest time period that the animal was mixed with cattle from other PICs; model derived from a variation of the causal diagram: covariates include Fill, Weight, SY-12_0, SY-27_13, SYpre-27, CohortN, Move_FL, Group-13N, N=34,726
[e]Categories where 7 or more feedlots have no observations

Table 2 Distribution of variables and estimated odds ratios for their total effects on the occurrence of BRD by day 50 in the pre-induction assembly subset based on models derived from the causal diagram shown in Figure. 2.

Variable & category	Number of animals (%)	Crude BRD 50-day cumulative incidence (%)	Adjusted odds ratio	95% credible interval
Days between arrival at vicinity of feedlot and induction[a]	5,641			
> 27	1,747 (31)	1.5	0.6	(0.2 - 1.5)
27 to 13	1,723 (31)	5.3	1.2	(0.4 - 2.7)
12 to 0	2,171 (38)	3.3	Ref. cat.	
Number of animals in group-28[b]	5,641			
< 50	1,962 (35)	5.3	Ref. cat.	
50 to 99	962 (17)	2.0	0.6	(0.2 –1.2)
≥ 100	2,717 (48)	2.4	0.8	(0.3 – 1.8)
Number of group-28s forming cohort[c]	5,641			
< 4	2,421 (43)	2.1	Ref. cat.	
≥ 4	3,220 (57)	4.3	5.5	(1.0 –18.7)

[a] Covariates: Breed, Induction weight, Season, SY-27_ 0 and SYpre_27. N=5,551
[b] Covariate: Arrival_day0. N=5,590
[c] Covariates: SY-27_0, Arrival_day0 and Group-28N. N=5,589

Table 3. Estimated odds ratios for direct effects of selected variables on the occurrence of BRD by day 50 based on models derived from the causal diagram shown in Figure.1

Variable & category	Adjusted odds ratio	95% credible interval
Number of animals in group-13[a]		
< 50	Ref. cat.	
50 to 99	0.8	(0.7 - 1.0)
≥ 100	0.6	(0.4 - 0.8)
Saleyard transfer prior to day-27[b]		
No	Ref. cat.	
Yes	1.0	(0.9 - 1.1)
Saleyard transfer between day-27 and day-13[b]		
No	Ref. cat.	
Yes	1.3	(0.8 - 2.0)
Saleyard transfer between day-12 and day0[b]		
No	Ref. cat.	
Yes	1.8	(1.0 - 2.9)
Feedlot move timing[b]		
Pre day-27[c]	0.6	(0.2 - 1.3)
Day-27 to -13 [c]	1.4	(0.6 – 3.0)
Day-12 to -2; <6 hours	0.9	(0.6 - 1.3)
Day-12 to -2; ≥6 hours	1.0	(0.6 - 1.5)
Day-1 to 0; <6 hours	Ref. cat.	
Day-1 to 0; ≥6 hours	1.2	(1.0 - 1.5)

[a]Covariates: Group-13N, CohortN, Fill, Weight, SY-12_0, SY-27_13, SYpre-27, FeedlotN, Mix and MoveFL. N=34,726

[b]Model: Mix, Move_FL, SY-12_0, SY-27_13, SYpre-27, CohortN, Group-13N, Fill and Weight. N=34,726

[c]Categories where 7 or more feedlots have no observations

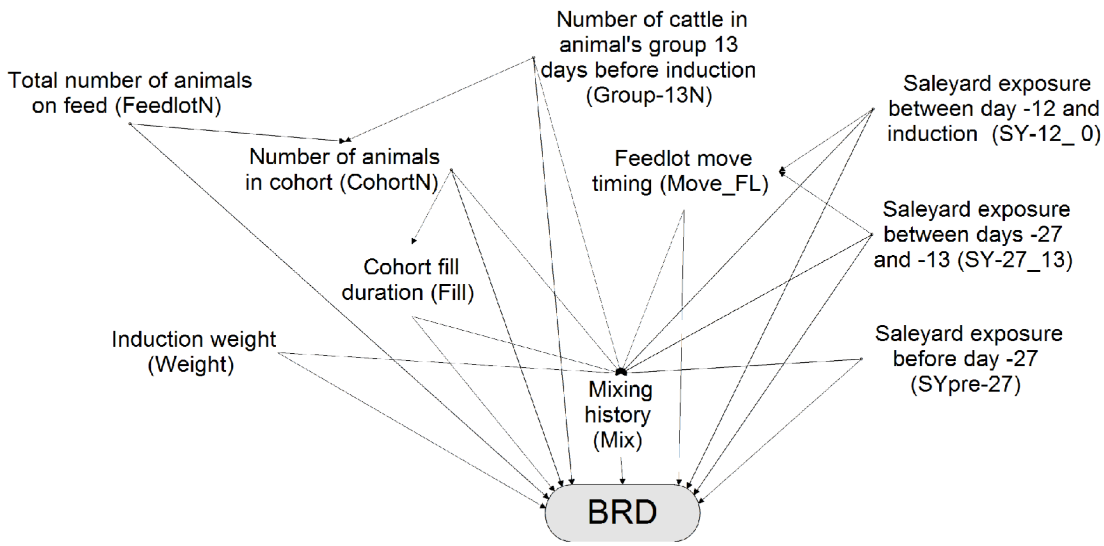

Figure 1: Causal diagram showing postulated causal paths linking variables related to mixing history, group size, exposure to saleyards and timing of the move to the feedlot to occurrence of bovine respiratory disease (BRD) in the first 50 days on feed.

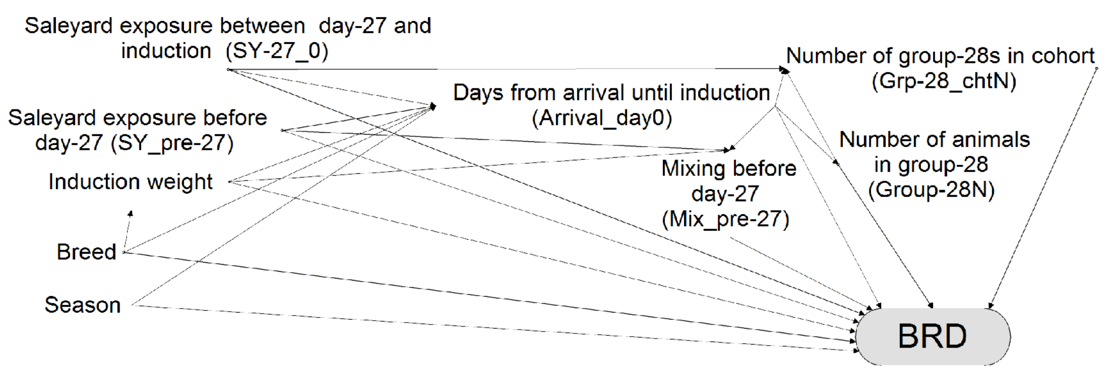

Figure 2: Causal diagram showing postulated causal paths linking variables related to the interval between arrival and induction, group size and number of groups combined to occurrence of bovine respiratory disease (BRD) in the first 50 days on feed in three feedlots where pre-induction assembly was implemented routinely.