Final report

Monitoring health and welfare using emerging diagnostic technologies in the beef feedlot sector.

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Abstract

Bovine respiratory disease (BRD) is the most prevalent disease in feedlot cattle worldwide with Bovine alphaherpesvirus 1 (BoAHV1), *Histophilus somni, Mannheimia haemolytica, Mycoplasma bovis, Pasteurella multocida* and *Trueperella pyogenes* accepted to be common etiological agents associated with BRD. Whilst these agents are common in the upper and lower airways in clinical BRD cases, some also exist as normal flora suggesting their presence in the upper airways alone is not necessarily informative with respect to disease status or risk.

To determine the relationship between potential BRD pathogen presence, load and disease status, we investigated the correlation between load in the upper airways at induction and active BRD cases in feedlot cattle using efficiency-corrected (EC) PCR quantification. By this approach, we were able to accurately determine the prevalence and load of the key BRD agents in the upper respiratory tract showing that cattle in the hospital pen had a higher prevalence, and load, of these agents both singly and in combination compared to cattle sampled at feedlot induction.

Bayesian Network modelling indicated that the combination of agents and location was the most accurate indicator of BRD risk with cattle with four or more agents detected in the upper airway more likely to be treated during their time on feed, and more likely to be treated for BRD than non-BRD ailments. In addition, *M. bovis* was rarely detected at feedlot induction but was identified at high prevalence in cattle in the hospital pen. This study is the first to report on the practical application of efficiency-corrected quantification to determine accurate pathogen load of BRD associated organisms in the upper airways.

Review of this data suggests that the optimal pathogen panel for detection of animals suffering from, or at risk of developing BRD, should include Bovine herpesvirus 1, Bovine Corona Virus, Bovine Parainfluenza Virus 3, Bovine respiratory Syncytial Virus, *H. somni, M. haemolytica, M. bovis, P. multocida*, and *T. pyogenes*.

These findings present a potential new technological approach for the investigation, analysis and identification of BRD-associated viral and bacterial agents for Australian feedlot systems as well as for BRD disease management and treatment.

Finally, to determine the cost benefit of integration of a microorganism testing platform into feedlot management practices, a growth / intervention model was developed using data collected from four sites in 2021. The initial assumptions and grid frameworks are presented in this report.

Executive summary

Background

Bovine respiratory disease represents one of the largest animal health costs to the Australian feedlot industry. BRD incurs costs to the industry related to loss of production due to poor performance and / or death, time taken in management of sick cattle in this hospital system, cost of treatments, and reputational costs to the industry related to disease burden in intensive systems. Whilst all operations experience some level of BRD, not all operations experience the same level of disease, nor are management practices identical across all locations.

To better understand the pathognomonic relationship between disease agents known to be associated with risk of, or active BRD, this study profiled several feedlots across News South Wales and South Australia to determine the profile of BRD-causing agents in the upper airway of cattle at induction, compared to those within the hospital pen system. This approach needed to be rapid, scalable, and able to tests large numbers of animals with minimum handling and cost.

Results of this research may be used by the feedlot industry to determine disease risk as well as case diagnosis at feedlots across Australia.

Objectives

The objectives of this project were to:

- Determine the pathognomonic profile of BRD in a sample of Australian feedlots;
- Develop a rapid, scalable diagnostic testing platform that could be used to test within and between animals and cohorts and locations;
- To define an optimal agent test panel for the identification of BRD disease risk in Australian feedlot cattle.

Methodology

Both a qualitative and quantitative analysis was undertaken to determine if agent load, as well as agent presence was indicative of disease risk. A novel quantitative PCR approach was used to facilitate sampling and analysis, with generation of a commercially-viable syndromic diagnostic test panel fit for use in feedlot cattle. This test was applied at two timepoints during early time on feed: Day of induction (Day 0) and after 14 days on feed (Day 14), as well as to samples collected from hospital pen animals. Five feedlots across NSW and South Australia participated in the study with samples collected from more than 2000 cattle. Qualitative and quantitative PCR analysis was performed and results analysed using standard statistical methodologies and Bayesian Network Modelling.

A pathobioeconomic model was developed to interrogate the cost / benefit for various use cases of the testing protocol in a feedlot setting. Economic analysis was conducted by using trail data to calibrate CSIRO's AusFarm simulation model, with the outputs then used as growth parameters for multi-state Markov model which integrated BRD morbidity and mortality for bio-economic analysis. Sensitivity analysis was run on several the key model input parameters.

Results/key findings

Results showed that cattle identified to possess more than three agents at induction, or within the hospital system were more likely to be treated for BRD than other disorders. Quantitation showed that hospital pen cattle showed higher concentrations of agents than induction cattle, although

sample sizes were not large enough to show statistical significance relative to disease state. *Mycoplasma bovis* was found to be exhibit an infectious profile within the feedlot with very low, or no cattle testing positive for *M. bovis* at feedlot induction, but high proportions testing positive after 14 days on feed and in the hospital system. Bayesian Network modelling identified the number and combination of agents, distance travelled to feedlot and feedlot location to be the primary drivers of disease outcome by this analysis.

Bio-economic analysis determined that even at a relatively low predictive accuracy of 34%, the use of a risk-based test and treat strategy was profitable for feedlot cohorts with a higher proportion of high-risk animals (as classified by PCR), e.g. saleyard purchases. However, a risk-based test and treat strategy was not profitable when cohorts had a low risk profile ($\leq$15% of animals classified as low risk), as was the case for direct paddock purchased cohorts. Animal welfare, profitability and antimicrobial stewardship could all be improved with a higher accuracy test, particularly if the test can accurately identify subclinical BRD.

Benefits to industry

This project showed that an efficient, scalable PCR diagnostic testing platform can be used to inform individual feedlot operations of the underlying pathological causes of bovine respiratory disease incidence by mob, intake and/or location.

The platform developed is versatile and flexible, allowing rapid development of tests for other nucleic acid-based indicators of disease or performance. This could include RNA, DNA and eDNA targets of importance to the feedlot sector, including those indicative of emergency animal disease or other disease outbreaks of critical economic importance to the industry. The platform technology used in this project evidenced the ability to deploy a laboratory test in the field with a methodology that is directly comparable between those two sites.

The diagnostic test panel developed in this project represents the first objective and quantitative measure with the potential for use for risk analysis, as well as definitive diagnostic comparison to the current qualitative measures used for current BRD diagnosis in feedlot (pen rider identification and other behavioural measures of disease). This information can now be used to inform antimicrobial use and animal management to reduce disease burden using an evidence-based approach.

In addition, the test can be used to evaluate vaccines efficacy and other treatments, as demonstrated by pathogen load in relation to the use of *Mannheimia haemolytica* vaccination, and for the use of autogenous vaccines and other location-bespoke interventions in the future.

Use of the technology is ideally suited to, and ready to be adopted for reporting on, and audit of, antimicrobial stewardship practices by using an evidence-based treatment and / or management intervention for the control of bovine respiratory disease.

In feedlot intakes with high BRD risk by our measure (e.g. saleyard purchases), modelling of a risk-based treatment for BRD was found to increase profitability by \$5.44 – \$8.60 per head, while also improving animal welfare outcomes by treating animals identified to be sub-clinical (based on visual assessment). Our findings suggest that the ROI could further be improved by increasing the accuracy of the test and/or classification criteria, which would also further reduce antibiotic use.

Future research and recommendations

Recommendations arising from the outcomes of this study include:

- Further objective epidemiological testing and data collection should occur at additional sites across Australia, including in northern Australian feedlot systems, to examine BRD-casing agent profiles across the industry to correlate to disease incidence in different management systems, and to confirm the risk stratification identified in this report in a larger sample.
- Variability in data capture and used of health-related terminologies between locations through use of multiple data capture platforms, could be standardised across the feedlot sector to allow better data comparison between sites, states, and systems. A standardised minimum dataset of required fields should be recommended for inclusion in hospital and treatment data sheets to allow rigorous cross-sector analysis of disease in feedlot cattle into the future to evidence improved disease interventions and management practices. This should include easier data linkage between animal and carcase performance data tools.
- Further analysis needs to be undertaken to determine the role of *M. bovis* as a key opportunistic pathogen in Australian feedlot systems and to investigate the efficacy of vaccine interventions for this opportunistic pathogen.
- Further investigation is required into the role and impact of Bovine Herpes Virus-1 in the prevalence of BRD in Australian feedlot systems.
- The test developed in this project could be applied in all projects evaluating respiratory health in cattle to generate a database of disease profiles that can be used to refine an evidence-based risk approach for the management of BRD in Australian feedlot systems.
- The diagnostic test developed as part of this project should proceed to development of a commercially available kit or publication of the assays in the peer-review literature to allow widespread availability to industry either for location-specific profiling or individual animal diagnosis.

  Further research should be undertaken to assess the efficacy of PCR testing to diagnose subclinical BRD and implementation of this approach on AMR stewardship protocols in feedlot.

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1. Background

Despite significant research, bovine respiratory disease (BRD) still represents one of the greatest production costs to cattle production globally. BRD is a multifactorial syndrome with a number of bacterial and viral agents that can lead to disease (Chai, Capik et al. 2022). It can cause severe, and sometimes fatal, respiratory disease in cattle in both intensive and pasture systems (Fernández, Ferreras et al. 2020) with BRD being the most prevalent disease in veal calves, weaned dairy heifers and weaned/unweaned beef calves. It is also the leading cause of clinical disease and death in feedlot populations (Pardon, Hostens et al. 2013, Lubbers and Turnidge 2015, Hay, Morton et al. 2016). In Australian feedlot systems, BRD can account for more than 80% of morbidity and up to 78% of mortalities annually in a single feedlot operation (Gonzalez, Blakebrough-Hall et al. 2018). Globally the economic impact of BRD is estimated to be over $3 billion/year (DeDonder and Apley 2015).

The incidence and prevalence of BRD in cattle can be influenced by many factors. These include pre-exposure to viral or bacterial organisms (Hay, Clements et al. 2016); vaccination history (Cusack, Bergman et al. 2020); backgrounding (Cusack and Mahony 2016); co-mingling of non-consanguineous cohorts (Wheat, Chow et al. 2020); time on feed (Barnes, Hay et al. 2015); antimicrobial resistance (Alhamami, Trott et al. 2020); seroconversion (Barnes, Hay et al. 2015); transportation time to the feedlot (Melendez, Marti et al. 2020) and the presence of other co-morbidities and other undetermined factors (Sudaryatma, Mekata et al. 2019). Environmental risk factors such as the time of year of entry to the feedlot (also known as feedlot induction), the region, prevailing weather conditions (Cernicchiaro, Renter et al. 2012), source of the cattle, cattle breed and induction weight have all been positively correlated with BRD risk (Hay, Morton et al. 2016). Despite much research, the broad-ranging nature of these factors makes determining the underlying risk of BRD difficult which is further complicated by the absence of predictive tests for the disease.

In the Australian feedlot industry, BRD is most frequently diagnosed by designated animal care technicians, also known as ‘pen riders', by identification of cattle showing visual clinical signs, including changes in behaviour, respiratory pattern, appetite, and social interactions. This method is heavily reliant on the experience of the pen rider as cattle will often mask clinical signs of disease in the presence of humans due to the predator-prey hierarchy (Buczinski and Pardon 2020). Some other disease detection methods used in recent years include; the remote early disease identification (REDI) system (Abell 2018), disease biomarkers (Blakebrough-Hall, Dona et al. 2020) and thoracic ultrasound (TUS) (Cuevas-Gómez, McGee et al. 2021). However, none of these techniques correlate presence or absence of disease-causing agents back to clinical findings and lack the required level of accuracy to replace human intervention (Blakebrough-Hall, McMeniman et al. 2020), therefore leaving a gap between disease status and causality.

To investigate disease mechanisms in BRD, real-time PCR has been employed as a rapid and sensitive means of identifying the microbial organisms associated with BRD in affected animals (Loy, Leger et al. 2018, Pansri, Katholm et al. 2020). However, except for some true pathogens, many BRD-associated bacteria are part of the normal flora of the upper respiratory tract. Therefore, without quantitative analysis the use of traditional PCR is limited in determining disease causality or acting as a predictive tool. As it is hypothesised that the differentiation between normal and disease-causing agent load is likely to be one of increasing load, there is an inherent need to accurately compare agent load within and between animals to determine disease risk or status by this measure.

Emerging evidence of the importance of microbial load is now being reported for respiratory diseases in cattle. Recently, PCR threshold values were reported for *Pasteurella multocida, Mycoplasma bovis*

and Histophilus somni that showed a significant correlation with the risk of BRD (Klompmaker, Brydensholt et al. 2021). This supports a hypothesis that upper airway agent load could be a more useful predictor of BRD risk than presence or absence of an organism alone. However, PCR thresholds that are determined relative to clinical disease are often markedly different to those acquired in an analytical assay and can be affected by inherent inhibitory factors within the clinical sample. Without consideration of the amplification efficiency in association with the quantification cycle (Cq) value, the performance of the test on a biological sample is unknown (Ruijter, Barnewall et al. 2021) and can lead to misleading results.

While quantitative PCR (qPCR) is emerging as a useful diagnostic tool, most assays currently rely on the use of standard curves to establish the concentration in a clinical sample. This largely ignores the effect of the composition of the biological sample on the inherent efficiency of the reaction. As such, many 'quantitative' assays may only be considered as 'semi-quantitative'. If commensal agents need to be considered in a disease state, this becomes highly problematic. To overcome this, 'efficiency-corrected' (EC) quantification (Ruijter, Barnewall et al. 2021) was developed to provide accurate DNA concentrations relative to agent load for both human and animal clinical samples in a reproduceable manner. This technique can accommodate the inherent differences in efficiency found between individual animals and samples, each machine run, and time of analysis allowing both inter- and intra-animal comparisons.

To determine if load could be an important indicator of disease with relation to BRD in feedlots, a study was designed to compare the presence, load, and agent combinations of common BRD-associated agents (BoAHV1, *Histophilus somni, Mannheimia haemolytica, Mycoplasma bovis, Pasteurella multocida* and *Trueperella pyogenes)*. Efficiency-corrected quantitative PCR was applied to nasal swabs collected from asymptomatic cattle at feedlot induction and an independent cohort of animals receiving treatment in the hospital pen at two Australian feedlots. The term 'asymptomatic' was used to indicate cattle that were either subclinical or unaffected by respiratory disease at time of sampling.

2. Objectives

The objectives of this project were as follows:

1. To determine the cost-to-industry of BRD in Australian beef feedlots, and quantify feedlot production losses resulting from disease, carcass condemnation and lung abscesses.
2. To identify critical time points to monitor health and welfare with emerging diagnostic technologies. This will provide the grain-fed value chain with an objective and quantitative measure, from farm gate to meat hook. Fast decisions can be made to withhold entry to, manage, or remove commercially non-viable animals based on defined health and welfare grounds.
3. To generate quantitative data for feedback to the producer on the health and welfare status within the feedlot system.
4. To increase commercial stock value and outcome productivity in the beef feedlot sector by better decision making.

KPIs:

1. Development and validation of an 'in-house' diagnostic platform suitable for the beef feedlot industry.
2. Confirmation of an optimal pathogen panel for a one pass, or two pass diagnostic test for BRD for potential commercialisation.

All objectives of the proposal were met, including identification of an optimal syndromic diagnostic test panel for commercial use.

3. Methodology

3.1. Animals

This study was conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes (2013) and was approved by Charles Sturt University Animal Care and Ethics Committee (Protocols A18070 and A21902) and New South Wales Department of Primary Industries Elizabeth Macarthur Agricultural Institute Animal Care and Ethics Committee (Protocol M18/07 and M21/07). Informed consent for use of animals included in this study was obtained from the owner/manager of the cattle, in writing, at each location prior to sampling. The study was designed and reported using the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines (Percie du Sert, Hurst et al. 2020) for experiments involving live animals.

The sample size was calculated on the basis of predicted rates of BRD during the collection season (Autumn) for Australian feedlots (Barnes, Hay et al. 2015) to capture animals likely to be experiencing clinical disease, compared to those apparently unaffected. A total of 1850 asymptomatic animals were sampled upon presentation to five feedlot locations (Feedlot 1 -5) at feedlot induction. On the same day as cattle were sampled at induction, a convenience cohort of 380 animals was sampled at presentation to the hospital pen. Induction pens for sampling were selected randomly based on operational activities with no specific selection criteria applied. Induction and hospital cohort animals were independent of one another but were coincident in time. All hospital pen cattle presenting at the crush in a given session were selected for testing, regardless of diagnosis.

Upon induction, animals received a booster of the combined inactivated *M. haemolytica* and BoAHV-1 vaccine (Bovilis® MH + IBR, Coopers Animal Health), a 5 in 1 clostridial vaccine (TasVax® 5 in 1, Coopers Animal Health) and a broad spectrum anthelmintic (Bomectin™, Bayer). The addition of an intranasal live vaccine for BoAHV-1 (Rhinogard®, Zoetis) was used in locations 1, 3, and 4, but not in location 2 for the cohort collected. Location 5 used Rhingard® and Bovi-Shield® (Zoetis, Australia) at entry for vaccination against BoAHV1 and *M. haemolytica* respectively. In addition to prior treatments noted, Feedlot 1 utilised an autogenous *P. multocida* vaccine.

Bureau of Meteorology data was collected for minimum and maximum temperatures and mean monthly rainfall for all locations and collections (Appendix 1).

3.1.1. Disease definition

Diseased animals were identified by pen riders on horseback as cattle showing overt clinical signs of active disease or injury (respiratory disease, lameness) and were removed from their home pen to a hospital pen for treatment. Bovine respiratory disease was diagnosed by trained pen riders based on established criteria from other studies (Cusack 2004), namely, the absence of clinical signs linked to systems other than the respiratory system, and two or more of the clinical signs of depression, lethargy, altered respiratory pattern, discharge from the eyes, nose, and mouth, and inappetence (Cusack 2004). Pen riders also drafted steers to the hospital pen for treatment of non-BRD related ailments including lameness, bulling (dominance injuries), prolapse, necrotic laryngitis, rumen acidosis and scours. As such, the hospital pen at a given date could contain animals with varying diagnoses.

The reason for hospitalisation at an individual animal level was identified at each presentation to the hospital crush. Commonly, BRD was not noted as a specific pull reason, rather the term “respiratory” was used to cover all respiratory ailments (BRD and necrotic laryngitis) at all locations sampled. The treatment applied was therefore used to differentiate between these two potential diagnoses where

animals were assumed to have BRD if the reason for hospitalisation was categorised as “respiratory” and they received either tulathromycin, florfenicol or ceftiofur as treatment without the use of a corticosteroid. For example, at Feedlot 1, BRD was not noted as a specific pull reason, rather the term “respiratory” was used to cover all respiratory-like ailments which could include BRD and necrotic laryngitis. Therefore, animals pulled for “respiratory” were assumed to have had BRD if the treatment records included either tulathromycin, florfenicol or ceftiofur. On occasion, tulathromycin followed by florfenicol or ceftiofur as reported. Cattle at Feedlot 2 were classified as BRD cases if they received tulathromycin or ceftiofur on presentation to the hospital crush. The same approach was used across all sites to ensure consistency of disease identification.

3.2. Quantitative PCR for identification and quantification of respiratory disease agents in feedlot cattle.

3.2.1. Clinical sample collection

Nasal swabs were collected from entire pen cohorts (range: 88 – 224 head) presenting at feedlot induction and from a convenience sample of cattle presenting for treatment in the hospital pens on the same sampling date. Sampling was therefore opportunistic and in alignment with normal feedlot handling practices. All samples were collected while cattle were restrained in a crush with a head bail. Sterile, plastic shaft swabs were inserted gently into the external nares of the nasal cavity to approximately 5cm depth. Dual head swabs (Puritan Opti-Tranz® Plus duo HydraFlock® swabs, Mawson Lakes, SA, AUS) were used to facilitate the collection of multiple samples from the same animal without the need for multiple interventions at Feedlot 1. Single head swabs (PS, Viscose; Sarstedt, Mawson Lakes, SA, AUS) were used for the collection at Feedlot 2 due to the manufacturing discontinuation of the dual head swabs.

Post sampling, swabs were transferred to 5mls PBS pH 7.4 or for samples collected in 2019-20, and / or 5mls Viral Transport Fluid (VTF, Edwards Group Pty Ltd, Narellan, NSW, AUS) (all 2021 collections), and stored on ice for transportation to the laboratory. Samples were stored at -80°C before undergoing DNA / RNA extraction. Where swabs were analysed on-site (Feedlot 1, 2020 collection), these were transferred to the mobile laboratory on ice and processed immediately. VTF was used to ensure stability and facilitate the recovery of RNA viruses from nasal swab samples. All swabs in PBS were vortexed for 30 sec, boiled at 100˚C for 10 mins and centrifuged for 5 mins at 12,000 *xg*. The supernatant was transferred to a sterile Eppendorf tube for PCR analysis.

3.2.2. Quantitative PCR

3.2.2.1. Bacterial and viral standards

Bacterial standards were cultured from frozen pure isolates sourced from the New South Wales Department of Primary Industries, Elizabeth Macarthur Agricultural Institute (NSW DPI EMAI). All isolates for *H. somni, M. haemolytica, P. multocida* and *T. pyogenes* were cultured on Tryptic Soy Agar EH with Sheep Blood agar (MicroMedia; Edwards Group Pty Ltd, Narellan, NSW, AUS) at 35°C in 5% CO_2 for 24hours. *M. bovis* isolates were cultured on Mycoplasma Agar containing Supplement G (Oxoid; Thermo Fisher Scientific Australia Pty Ltd, Scoresby, VIC, AUS) at 35°C in 5% CO_2 for 7-10 days. Identification of bacterial standards was confirmed by MicroFlex MALDI-ToF Biotyper mass spectrometry (Bruker Pty Ltd; Prestons, VIC, AUS) and standard rapid biochemical testing: catalase, gram stain, indole, and oxidase tests.

Bacterial colonies from agar plates were washed twice in phosphate-buffered saline (PBS, pH 7.4) before DNA extraction with a QuickGene DNA tissue Kit S (Kurabo; Gene Target Solutions, Dural, NSW, AUS) using the Kurabo QuickGene-810 Nucleic Acid Isolation System according to the manufacturer's instructions. DNA was eluted with 20 µL Buffer AE (elution buffer) and purity and concentration were checked using standard spectrophotometry (Nanovue, GE Healthcare, Edwards Group Pty Ltd, Narellan, NSW, AUS) and fluorometry (Qubit™ 3.0, Thermo Fisher Scientific Australia Pty Ltd, Scoresby, VIC, AUS) using a Qubit™ dsDNA HS Assay Kit (Q32851; Thermo Fisher Scientific Australia Pty Ltd, Scoresby, VIC, AUS), respectively. G-Block and Ultramer control sequences for all targets are shown in Appendix 2.

3.2.1.1. Primer and probe sequences and internal controls

Primer / probe sequences, and g-block controls (synthesised double stranded DNA controls) are shown in Appendix 2. A previously described beta actin hydrolysis probe assay (Kishimoto, Tsuchiaka et al. 2017) was used as a positive internal control to ensure reagent activity.

The specificity of the primer and probe sequences used were evaluated *in silico* using NCBI BLASTn Nucleotide collection (nr/nt) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with the stringency set at ≤ 20,000 target sequences. All primer/probe sequences are shown in Appendix 2. Fluorescent reporter molecules and quencher molecules were allocated to each target, based on the manufacturer's recommendations (Mic™ thermocycler, Bio Molecular Systems, Upper Coomera, QLD, AUS).

For internal controls, BRD supermix control (100 copies per µL) was prepared from g-blocks controls (Integrated DNA Technologies, Iowa, United States) designed for each target in both Test 1 (BHV, TP, HsV2 & BA) and Test 2 (Mb, Mh, Pm & BA). The G-block sequences (Appendix 2) were only used for DNA targets, and the RNA targets used individual Ultramer synthetic controls (Integrated DNA Technologies, Iowa, United States).

Perfecta Multiplex QPcr Tough Mix reagent by Gene Target Solutions Pty Ltd, Dural, NSW, molecular grade water (Thermo Fisher Scientific Australia Pty Ltd, Scoresby, VIC, AUS) and 200 nM of all forward and reverse primers and 100nM of all probes were used in all reactions. Reactions were performed using the following cycling conditions: 95˚C for 2 minutes, 50 cycles of 95˚C for 10 seconds, 60˚C for 15 seconds and 72˚C for 20 seconds and preconditioning of 95˚C for 19 seconds. Product melt was completed from 72˚C to 95˚C at 0.3˚C/s for both test 1 and test 2[14].

SYBR Green assays (*H. somni* and all RNA viruses) contained PerfeCta® SYBR® Green FastMix® (Quanta Biosciences), molecular grade water (Sigma-Aldrich) and 200 nM of both forward and reverse primers. Reactions were performed using the following cycling conditions: 95˚C for 2 minutes, 45 cycles of 95˚C for 10 seconds, 60˚C for 15 seconds and 72˚C for 20 seconds and preconditioning of 95˚C for 19 seconds. Product melt was completed from 72˚C to 95˚C at 0.3˚C/s.

3.2.1.2. Test panel determination

Review of data presenting in previous milestone reports, indicated that a test panel using a two-pass approach may give optimal efficiency for epidemiological analysis and treatment decision making in feedlots. Specifically, a panel of BHV1, BCoV, BPIV3, BRSV, *H. somni, M. haemolytica, M. bovis, P. multocida*, and *T. pyogenes* should be used for disease risk correlation. This can be split in to two tiered assays, with Bovine herpesvirus 1, *H. somni, M. haemolytica, M. bovis, P. multocida*, and *T. pyogenes*

performed as a base multiplex assay (two reactions) and BCoV, BPIV3, and BRSV tested dependent on requirements and cost (Table 1).

The rationale for this approach is as follows: Bovine herpesvirus 1, *Histophilus somni, Mannheimia haemolytica, Mycoplasma bovis, Pasteurella multocida*, and *Trueperella pyogenes* consistently show the highest prevalence across all feedlots tested in both home pen animals and those referred to the hospital pen. These five organisms are likely contributing to the BRD risk at all locations tested. Equally, Day 14 on feed and hospital pen samples showed similar prevalence profiles in all locations tested, although BHV1, BPIV3 and BCoV were only identified in hospital animals, and not at all locations. This suggests that depending on pen location, a reduced viral panel may be optimal in hospitalised animals compared to tested those at induction.

Table 1. Optimal test panel for risk analysis and management of bovine respiratory disease in Australian feedlot cattle.

Panel	Test Number	Microorganisms
Multiplex test	1	Bovine herpesvirus 1 *Histophilus somni* *Trueperella pyogenes*
	2	*Mannheimia haemolytica* *Mycoplasma bovis* *Pasteurella multocida*
Singleplex tests (optional)	3	Bovine Corona Virus
	4	Bovine Parainfluenza Virus 3
	5	Bovine Respiratory Syncytial Virus

Real time quantitative PCR (qPCR) was conducted using a Myra Liquid Handling Station and Mic™ qPCR magnetic thermocycler (Bio Molecular Systems, Upper Coomera, QLD, AUS). PCR runs were recorded and analysed using the Mic PCR software (Bio Molecular Systems, Upper Coomera, QLD, AUS). DNA extraction process control (PC) and non-template controls (molecular grade water, NTC) were included in each experimental qPCR run. All reaction volumes totalled 20 µL. A schematic representation of the process for preparation of samples for Test 1, 2 and singleplex assays is shown in Figure 1.

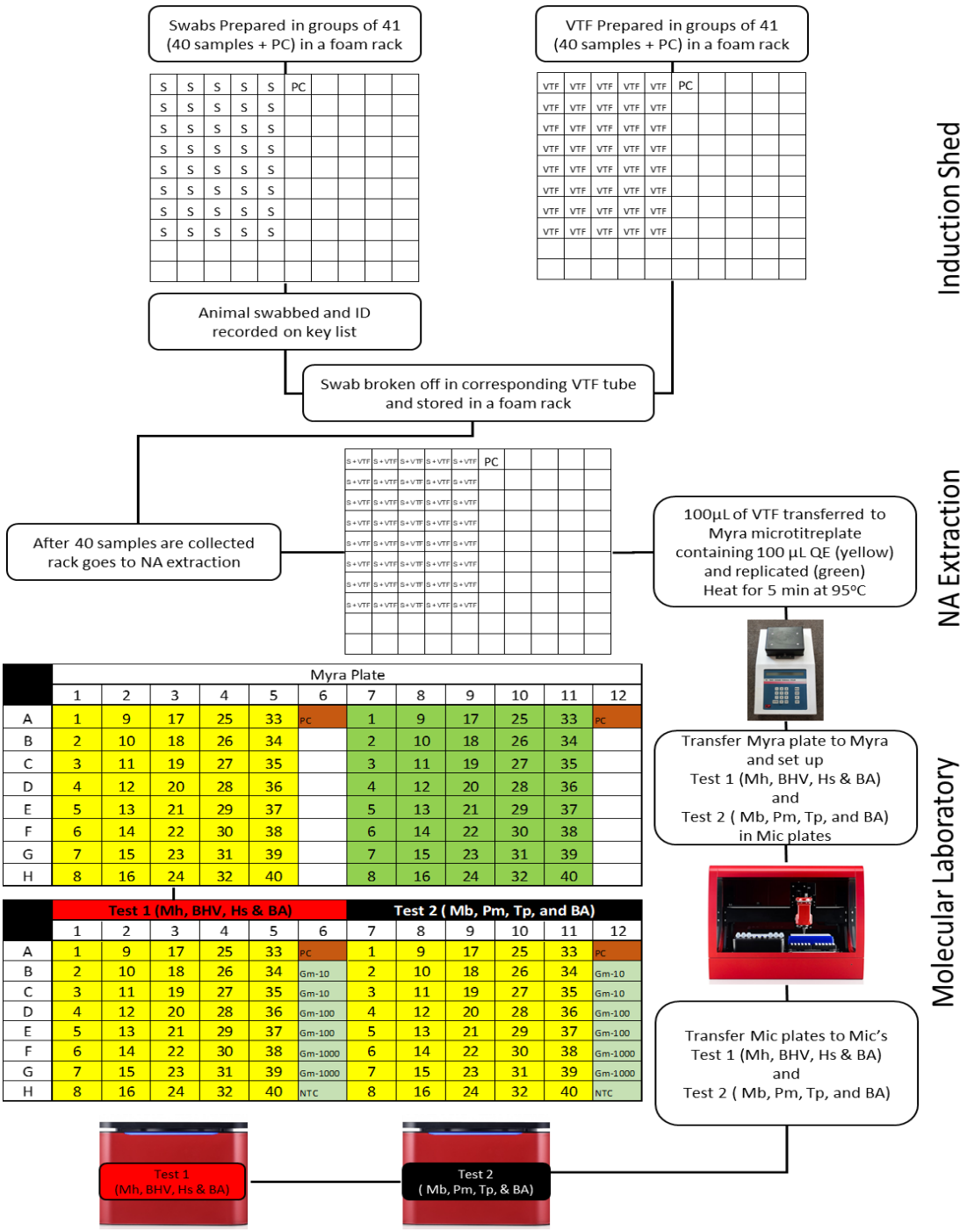


Figure 1. Process diagram for molecular analysis with BRD Panel 1 or 2, incorporating sample collection and organisation, nucleic acid extraction and quantitative PCR using one liquid handling station and two Mic thermocyclers. Beta actin (BA) is used as a positive internal control.

Abbreviations: NA, nucleic acid; Mh, *M. haemmolytica*; Hs, *H. somnii*; Mb, *M. bovis*; Pm, *P. multocida*; Tp, *T. pyogenese*; BHV, bovine α-herpes virus 1; BA, beta actin.

3.2.1.3. Quantitative PCR analysis

All nasal swabs were collected into viral transport medium prior to quantitative PCR. Samples were analysed for the presence and quantification of the following bacteria and viruses: *Histophilus somni*, (*H.somni); Trueperella pyogenes,* (*T. pyogenes*); Bovine Herpes Virus-1 (BHV1); *Mycoplasma bovis* (*M. bovis*); *Pasteurella multocida (P. multocida*); *Mannheimia haemolytica,* (*M. haemolytica*) and the RNA viruses Bovine Coronavirus (BCoV), Bovine Viral Diarrhoea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV) and Bovine ParaInfluenza Virus-3. Organisms were examined using two multiplex tests, and a series of singleplex analyses using hydrolysis probe assays partially based on assays described by Kishimoto et. al., 2017((Kishimoto, Tsuchiaka et al. 2017). Multiplex Test 1 contained primers and probes for *H. somni* (HS), *T. pyogenes* (TP), and Bovine α-Herpes Virus-1 (BoAHV-1); Multiplex Test 2 contained primers and probes for *M. bovis* (Mb), *P. multocida* (Pm) and *M. haemolytica* (Mh). BRSV, BCoV, BPIV-3 were run in singleplex assays as Test 3-5, with BVDV run as a further singleplex assay.

DNA targets were assessed for specificity and are reported in Barnewall et al., 2022 (Barnewall, Marsh et al. 2022). For DNA-detected agents, analytical limit of detection (LOD) was determined as the lowest concentration of a given agent at which 95% of known positive samples were detected (Bustin, Benes et al. 2009). Standard curves generated for each assay (Supplementary Figure S1) had R^2> 0.99 with efficiencies between 0.9 (90%) and 1.0 (100%). This efficiency only applies to the standard curve and not the individual sample amplification efficiencies. These were determined using the window of linearity (W-o-L) within the thermocycler software. Specifically, the LOD of *P. multocida* and *T. pyogenes* was 0.0175 pg/µL and 0.0161 pg/µL, respectively (Appendix 1). *H. somni* had the lowest LOD at 0.00152 pg/µL (Supplementary Figure S1B). BoAHV1 had the highest LOD at 0.177 pg/µL whilst *M. haemolytica* and *M. bovis* had LOD of 0.0610 pg/µL and 0.0206 pg/µL (Appendix 3).

To assess specificity of viral RNA PCR assays, standard curves were generated for each RNA target assay (Figure) that showed R^2> 0.99 with efficiencies between 0.85 (85%) and 0.96 (96%). Efficiency was determined using the W-o-L within the thermocycler software and only applies to the standard curve and not the individual amplification efficiencies. Specifically, the LOD of BCoV and BPIV-3 was 0.208 pg/µL and 0.0192 pg/µL, respectively Both BRSV and BVDV had LOD above 1 pg/µL, at 3.04 pg/µL and 1.6 pg/µL, respectively (Appendix 2).

To assess efficiency, known positive clinical samples were tested for each primer/probe pair and efficiency was determined. Eight biologically distinct samples were selected from Feedlot 1 animals that had demonstrated a variety of agent loads and combinations using traditional culture methods (Appendix 3). Six technical replicates of each biological sample were processed and analysed in singleplex PCR reactions. Some biological samples did not amplify in all six technical replicates: clinical sample 164 (n=2) for *M. haemolytica* and (n=3) for *H. somni,* clinical sample 217 (n=4) for BoAHV1 (Appendix 3), clinical sample 18 (n=4) for *P. multocida* and clinical samples 109 and 206 (n=4) for *T. pyogenes* (Appendix 4). As expected, all biological samples showed variation in Cq and amplification efficiencies but with limited variance in amplification efficiencies between technical replicates. Multiplex efficiencies were not significantly different from those identified for singleplex reactions, with improved efficiency in some cases.

3.2.1.4. Establishing cycle threshold (Cq) and efficiency (E) criteria

Individual sample efficiencies and Cq values were determined using MicPCR software (MicPCR v2.10.0, Bio Molecular Systems, AUS). Efficiency-corrected concentrations were established using the method

reported by Ruijter et al (Ruijter, Barnewall et al. 2021) using a template developed by R. Barnewall (Barnewall, Marsh et al. 2022).

Briefly the "dynamic method" used the average baseline value prior to detection (calculated using the second derivative maximum), subtracted from the average value of the measured values taking the slope into account to determine the corrected baseline (Figure 2). A corrected efficiency was then used for efficiency-corrected (EC) quantification PCR by adding 1 to the efficiency from the raw data, where 1 = no amplification and 2 = doubling every cycle. To accurately quantify each target pathogen using the EC method, efficiency cut-offs were determined, based on average efficiencies for each target ± 0.1 and a cycle threshold of 40 cycles was applied. Applying efficiency and Cq cut-offs allowed samples that sit outside of the ranges to be excluded from quantification due to inherent inaccuracy in the calculated concentration values. All samples that were excluded from quantification were reported qualitatively (detectable/not detectable).

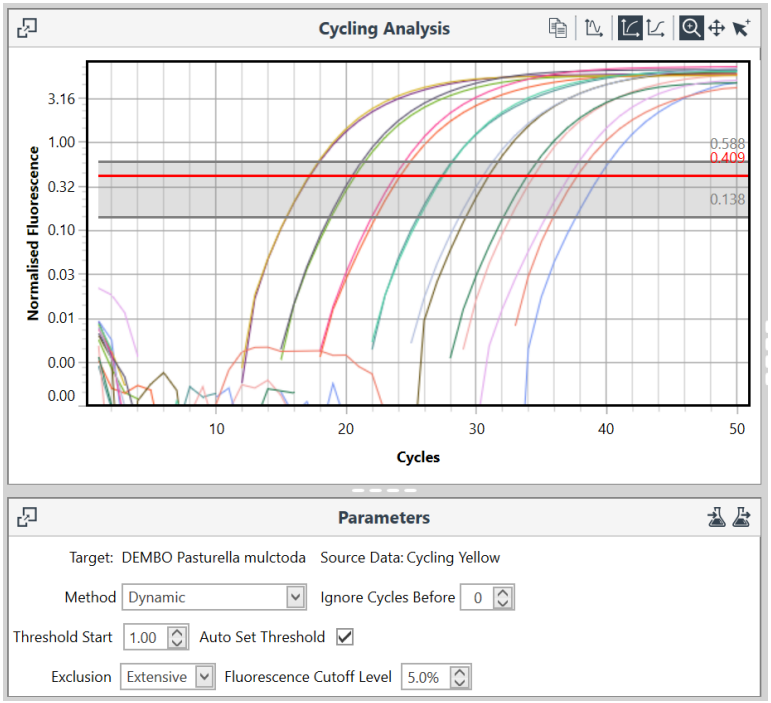


Figure 2. Example of PCR amplification curves from Mic™ PCR software. A corrected sample baseline value was determined using the "dynamic method". Threshold start was set automatically by the MicPCR software based on the window of linearity (the grey horizontal band). The pre-set fluorescence level of 5% was used as the cut-off value for all samples.

3.2.1.5. Efficiency-corrected quantitation of biological samples

Efficiency-correct quantification was undertaken to determine absolute DNA concentrations for BRD pathogens of interest using a previously published equation (Brankatschk, Bodenhausen et al. 2012, Ruijter, Barnewall et al. 2021):

$$N_{0,unk} = N_{0,std} \frac{E_{amc,std}^{C_{q,std}}}{E_{amc,unk}^{C_{q,unk}}}$$

Briefly, the starting concentration of target pathogen in a biological sample, $N_{0,unk}$, is calculated by defining one standard of known concentration for each target pathogen, $N_{0\ std}$. Calculation of the sample starting concentration is then obtained by filling in the equation with Cq and efficiency values derived from the standard and the sample.
For this study, the $Cq_{,std}$ and $E_{amc,std}$ were defined as the average of the standard for each pathogen from all qPCR runs. Whilst for each biological sample the Cq_{unk} and $E_{amc,unk}$ are the independent Cq and E values for each biological sample.

3.3. Animal production data and disease definitions

Production (induction and whole of time on feed) data and carcass data were sourced by agreement from each participating feedlot. Production and carcass data were aligned by individual animal and carcass IDs using Microsoft Excel®. Quantitative and qualitative PCR results by target were aligned to each animal by alignment of individual sample ID to animal ID.

Two cohorts were considered at each location: 1) animals undergoing induction to feedlot, and 2) a convenience cohort of hospital pen animals presented for treatment on the date of sampling. Production and health data were compiled for both groups for statistical analysis. All animal data including pull reason (reasons reported for treatment), dates and treatment were supplied by the respective feedlots.

At Feedlot 1, BRD was not noted as a pull reason, rather the term “respiratory” was used, which could cover all respiratory ailments not just BRD. Therefore, animals from Feedlot 1 were considered as having BRD if they were pulled for “respiratory” and received either tulathromycin and/or florfenicol. Florfenicol was not used at Feedlot 2 as a treatment for BRD therefore tulathromycin was identified as the BRD treatment if ‘BRD’ and where ‘respiratory’ was noted as the pull reason. If no pull reason was note, the identified treatment was used to determine categorisation. Feedlots 3-5 all conferred with Feedlot 1 in terms of data collection for pull reason for respiratory disease. All other diseases were considered as ‘non-BRD’ and treated together for the purposes of this analysis.

Total number of animals sampled and included in final analysis are shown in Tables 2 and 3.

Table 2. Number of induction animals sampled and rates of disease at five Australian feedlot locations sampled between 2019 – 2021.

	Feedlot 1			Feedlot 2	Feedlot 3		Feedlot 4	Feedlot 5
Location	South West Slopes NSW			Southeast SA	Southern NSW		Central NSW	North-western NSW
	Apr-2019	Oct-2020	Jun-2021	Jul-2019	Mar-2021	May-2021	Jun-2021	Aug-2021
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total induction animals sampled	220	221	236	241	0	384	194	354
Total animals excluded: n (%)	2 (0.91)	4 (1.81)	15 (6.36)	0 (0.0)	n/a	32 (8.33)	26 (13.40)	66 (18.64)
Incomplete performance data	2 (100.0)	0	0	0	n/a	1 (3.13)	1 (3.85)	50 (75.76)
Contaminated swab	0	2 (50.00)	0	0	n/a	0	0	0
Samples not collected at both timepoints (day 0 and day 14)	n/a	2 (50.00)	10 (66.67)	n/a	n/a	18 (56.25)	25 (96.15)	12 (18.18)
Wrong ID recorded	0	0	5 (33.33)	0	n/a	13 (40.63)	0	0
Missing hospital pull reason/treatment (but has a cost given)	0	0	0	0	n/a	0	0	4 (6.06)
Total induction animals included in analysis: n (%)	218 (99.09)	217 (98.19)	221 (93.64)	241 (100.00)	n/a	352 (91.67)	168 (86.60)	288 (81.36)
By pull reason:								
BRD	11 (5.05)	9 (4.15)	5 (2.26)	2 (0.83)	n/a	71 (20.17)	23 (13.69)	11 (3.82)
Non BRD	23 (10.55)	6 (2.76)	3 (1.36)	13 (5.39)	n/a	14 (3.98)	6 (3.57)	1 (0.35)
- Buller	9	0	0	0		0	0	0
- Lame	8	4	3	10		13	4	1
- Prolapse	1	0	0	0		0	0	0
- Necrotic laryngitis	3	1	0	1		0	0	0
- Observe	2	1	0	2		0	1	0
- Acidosis	0	0	0	0		1	0	0
- Bloat	0	0	0	0		0	1	0
No ailment	184 (84.40)	202 (93.09)	213 (96.38)	227 (94.19)	n/a	267 (75.85)	139 (82.84)	276 (95.83)

Table 3. Number of hospital pen animals and rates of disease at five Australian feedlot locations sampled between 2019 – 2022.

	Feedlot 1			Feedlot 2	Feedlot 3		Feedlot 4	Feedlot 5
Location	South West Slopes NSW			South East SA	Southern NSW		Central NSW	North-western NSW
BRD risk period	Low	High	High	High	Low	High	High	High
	Apr-2019	Oct-2020	Jun-2021	Jul-2019	Mar-2021	May-2021	Jun-2021	Aug-2021
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
c	54	44	30	98	40	44	57	13
Total excluded due to incomplete data, n (%)	0 (0.0)	10 (22.73)	1 (3.33)	9 (9.18)	34 (85.00)	2 (4.55)	6 (10.53)	6 (46.15)
Total hospital animals included in analysis, n (%)	54 (100.00)	34 (77.27)	29 (96.67)	89 (90.82)	6 (15.00)	42 (94.45)	51 (89.47)	7 (53.83)
By treatment reason:								
BRD	36 (66.67)	19 (55.88)	10 (34.48)	64 (71.91)	6 (100.0)	39 (92.86)	42 (82.35)	1 (14.29)
Non BRD	18 (33.33)	15 (44.11)	19 (65.52)	25 (28.09)	0 (0.0)	3 (7.14)	9 (17.65)	6 (85.71)
- Acidosis	2	0	0	0	n/a	0	0	0
- Necrotic laryngitis	10	2	3	9	n/a	0	2	0
- Lame	4	12	16	15	n/a	3	7	4
- Buller	1	0	0	0	n/a	0	0	0
- Observe	1	1	0	0	n/a	0	0	2
- Prolapse	0	0	0	1	n/a	0	0	0

3.4. Statistical analysis

The statistical software R (R Development Core Team 2020), was used for all statistical analyses. Fischer's exact test was used to determine the statistical significance (i.e., if $p < 0.05$) of an agent's prevalence between the induction and hospital cohorts, within both inter- and intra-feedlot comparison. Statistically significant differences in the agent load between cohorts, within the location, were investigated by the pairwise comparisons of least-square means using Tukey's honest significant differences method. Interactions between the cohort (induction or hospital) and location with respect to absolute agent load were modelled using generalised linear regression.

When agent concentration was considered, a Shapiro test was used to determine if data was normally distributed. As the majority of datasets (concentration grouped by feedlot and ailment) were not normally distributed or unable to be tested for normal distribution due to small sample size, the non-parametric Wilcox Un-Paired test was therefore used to determine if there was a significant difference in concentrations within feedlots between ailment type. Animals between ailment type were independent of one another therefore an un-paired Wilcox test was undertaken. Data was then log transformed prior to undertaking a paired Wilcox Test in R Studio.

GraphPad (GraphPad Prism 9 Version 9.1.1 for Windows, La Jolla California USA, www.graphpad.com) was used to transform the data by converting data into a number between 1-100 based on the highest and lowest value for each agent. GraphPad was then used to generate heatmaps for individual animals by location or pen type for visual comparison.

3.5. Bayesian Network analysis of qualitative and quantitative feedlot health and performance data

Bayesian network (BN) models were developed using Netica (Norsys Software Corp, Version 6.09). BN models provide insight into relationships between contributors and outcomes of an event by applying Bayes probabilistic reasoning (Chen and Pollino 2012, Butcher and Fenton 2020). It provides a graphical representation of conditional dependencies (arrows/arcs) between variables (nodes) of an event (Chen and Pollino 2012). Generally, the direction of an arrow roughly corresponds to causality, that is, nodes higher in the diagram tend to influence those below (Chen and Pollino 2012). This approach was included so that the interrelationships between variables and their impact on pull reason, for both induction and hospital cohorts, could be characterised/estimated.

A BN model using Netica Software (Norsys software Corp 2021) was developed to provide a coherent framework to investigate the complex associations between pull reason, agent load and combinations and pre- and post-slaughter production parameters. A BN model allows a level within multiple nodes to be selected as the target variable(s). Given the selected level of a target variable, the expected probability distributions of other variables can be assessed. For example, by fixing specific levels within the pull reason node we are able to estimate/predict the variables that have the most effect on pull reason (Norsys software Corp 2021).

All models, 'Full' and hybrid were populated using the following methodology. After selecting our response variable (pull reason), Tree Augmented Native Bayes Net algorithm (TAN) structure learning was then used to automatically learn the link structure between all nodes to best predict 'pull reason' for induction data and 'overall treatment reason' for hospital data. Following TAN learning all models

were assessed to ensure links between nodes made sense, and links were added between specific nodes, with our practical and expert knowledge in the field. This included, HGP to production factors (live exit weight, ADG, eye muscle area and carcass weight) and location to environmental factors (mean min and max temperatures, monthly rainfall and approx. distance travelled to feedlot). Learning algorithm, expectation-maximization (EM) was then used to populate the condition probability table (CTP) for each model (ie, induction and hospital). Briefly, EM learning takes a bayes net and repeatedly uses it to find a better one by completing an expectation followed by maximization step. During the expectation step, expected values of all missing data are computed using regular baye net inference along with the existing bayes net. The maximization step then finds the maximum likelihood bayes net given the now extended data (Norsys software Corp 2021).

An initial model was developed for induction (day 0 and day 14) collections. This involved importing all data, with each column representing a different node (i.e., ADG, number of agents detected). The bayes net was then graded using the 'test with cases' function, to see how well the model predicted the nodes when given the original data that was used to make the model. The error rate was relatively high for selected nodes (Table 4). Therefore, it was decided to trial a hybrid model. Two sub-models were chosen, one containing the agent data alone (ie, qualitative, quantitative and combination data) and the second model containing combination data and all pre- and post-slaughter production data. The net merge function in Netica was then applied to combine the two sub-models into one coherent BN model. The learning algorithm, EM, was then applied to the combined model to populate the CTP for the hybrid model. The hybrid model was then graded using 'test with cases' function and returned lower error rates for the majority of selected nodes (Table 4). Therefore, for the induction data, it was decided to progress with the hybrid model.

Table 4. Comparison of prediction error rates of selected nodes within the induction 'full' model and induction hybrid model.

Node	Prediction error Induction Full model	Induction Hybrid model
Overall pull reason	20.54%	10.88%
Agent combination	46.53%	0.1355%
Number of agents detected	29.39%	0.0678%
Eye muscle area	32.31%	35.30%
Fat depth	33.51%	32.31%
Live exit weight	44%	15.52%
Breed	45.07%	29.65%
ADG	53.99%	67.62%

Similar to the analysis of the induction data, an initial model was developed for hospital animals. This involved importing all data, with each column representing a different node (ie, ADG, number of agents detected). The Bayes net was then graded using the 'test with cases' function, to see how well the model predicted the nodes when given the original data that was used to make the model. The error rate was relatively high for agent qualitative nodes (Table 4). Therefore, it was decided to trial a hybrid model. Two sub-models were chosen, one containing just the agent data (ie, qualitative, quantitative and combination data) and the second model containing combination data and all pre and post slaughter production data. The net merge function in Netica® was then applied to combine the two sub-models into one coherent BN model. The learning algorithm, EM, was then applied to the

combined model to populate the CTP for the hybrid model. The hybrid model was then graded using ‘test with cases’ function and returned a lower error rate for the agent qualitative nodes (Table 5). Therefore, for the hospital data, it was decided to progress with the hybrid model.

Table 5. Comparison of prediction error rates of select nodes within the induction 'full' model and induction hybrid model.

Node	Prediction error Hospital Full model	Hospital Hybrid model
Overall hospital treatment reason	0.64%	1.60%
Agent combination	0%	0%
Number of agents detected	0%	0%
Eye muscle area	36.14%	36.14%
Fat depth	29.63%	29.63%
Live exit weight	37.07%	36.39%
Breed	34.94%	34.94%
ADG	33.67%	33.67%
M. haemolytica qualitative	1.60%	0%
T. pyogenes qualitative	6.73%	1.92%
BHV-1 qualitative	15.38%	6.41%
M. bovis qualitative	10.58%	2.56%
P. multocida qualitative	8.97%	2.89%
H. somni qualitative	8.01%	4.49%

The BN models developed were the predictive models that best partitioned the animal cohorts (e.g., BRD, non-BRD, or no ailment). For individual pathogen quantification, 0 represents an animal with no detectable pathogen while 10 was arbitrarily assigned when a pathogen was detectable but did not meet thresholds for EC quantification. Average daily gain (ADG) was included as a performance indicator. Animals that died whilst in the feedlot were assigned an ADG of 0. Four animals were excluded from the BN model, two from each feedlot location, due to missing animal information.

3.6. Bioeconomic modelling of BRD impact using a pathobiophysical model

3.6.1. Rationale for the pathobiophysical modelling approach

There is a long history of attempting to model cattle performance in a feedlot environment dating back to the 1970's (Ryan 1974). Approaches have changed over time due to increased understanding of biophysical processes, reductions in the cost of monitoring animal performance and advances in the computational power and data storage available to researchers and associated cost reductions of these enablers. This has led to a shift away from linear deterministic modelling environments and a move to dynamic and stochastic modelling approaches. Dynamics and stochastic approaches are better equipped to deal with population variability and temporal changes in dynamic variable interactions, which is pertinent for modelling the epidemiology of disease in animal populations.

Whilst there are several existing cattle feedlot models available including the Beef Cattle Nutrient Requirements Model (BCNRM) and Cattle Value Discovery Model (CVDS), these models are not fit for purpose for the current project due to their focus on dietary analysis and turnoff optimization, lacking the flexibility to model multiple animal production scenarios and variation in the duration and severity of disease and mortality.

Previous studies (McMeniman, Tedeschi et al. 2010) (Galyean, DiLorenzo et al. 2011) have developed robust models to predict dry matter intake (DMI) and animal growth and performance in feedlot

cattle, however the available datasets for these studies were orders of magnitude larger than the cattle sampled for the current study (over 100 times more animals), and rely on variables for which data was not collected in this study (e.g. DMI between 8-28 days).

Due to the limited datasets available in the current project, a dynamic process-based modelling approach has been taken using CSIROs AusFarm software modelling package to provide growth inputs to a Markov model. AusFarm is a daily time step model which simulates animal growth based on complex interactions between management systems, plant and animal performance and climatic data. AusFarm links the crop modelling capacity of ASPIM® (Keating 2003) with the pasture and livestock production modelling capacity of GRAZPLAN® (Donnelly 2002), using the CSIRO Common Modelling Protocol (CMP) (Moore et al., 2007). The modular architecture of the CMP allows respective individual components to be combined into an integrated whole farm system, with management systems configured using a rule-based language (Moore 2007, Robertson 2009). The CSIRO suite of modelling software also includes CattleExplorer, which allows the user to test the effect of different variable values and different parameters on the functions within the animal growth components in GRAZPLAN (Freer 1997). AusFarm has been widely validated in a number of production systems, however, whilst feedlot data was used in the development of the underlying animal performance equations in the model (Freer 1997), to date, AusFarm has not been used extensively for feedlot applications.

3.6.2. Summary of AusFarm simulation outputs

A step-wise model approach has been taken to model the impact of BRD on feedlot populations, with AusFarm simulations used to build growth curves for “healthy” feedlot animals. This data was then used to drive animal growth in the Markov model which overlayed the epidemiological impact of BRD (morbidity and mortality) based on observed data from the experimental dataset. This approach allows for direct control of epidemiology input variables and reduces the risk of cascading interactions between variables within the production model.

To build the “healthy” animal baseline in AusFarm, experiment data was filtered to remove animals that were removed to hospital pens, as well as suspected subclinical animals identified as having high pathogen loads from PCR testing at induction and day 14. The remaining “healthy” cohorts of animals were split into functional groups based on sex, breed, weight at induction, HGP status, feedlot location and purchase type. This resulted in a total of 9 functional groups (cohorts) which represented the paddock and saleyard purchase types for British breed cattle (Table 6). AusFarm simulation results showed a high level of accuracy for the 9 simulation groups, with simulated exit weights within a range of between +6% and -4% of the observed experimental exit weight data. Thus it was determined that these AusFarm outputs could be used as appropriate inputs to the economic model.

Table 6. Summary and description of sample simulation cohorts.

Sim group	Site	Sex	PurchaseType	HGP	InWeight	N	ExitWeight	Sim ExitWeight*	Diff %
1	**Feedlot 1**	**S**	**PADDOCK**	**N**	(380-420)	20	532.12	565.97	6%
2				**N**	(420 - 458)	13	551.52	586.44	6%
3				**N**	(458 - 544.0)	16	606.63	610.82	1%
4	**Feedlot 3**	**H**	**PADDOCK**	**Y**	(297 - 408)	12	507.08	509.04	0%
5				**Y**	(408 - 440)	12	537.83	524.85	-2%

6					Y	(440 - 548)	12	571.33	553.86	-3%
7	**Feedlot 3**	**H**	**SALEYARD**		Y	(338 - 399)	12	504.7	507.08	0%
8					Y	(399 - 449)	17	551.5	541.38	-2%
9					Y	(449 - 524)	15	619.1	595.36	-4%

* with HGP growth rate adjustment of 10% for HGP treated cohorts

3.6.3. Multi-state Markov model

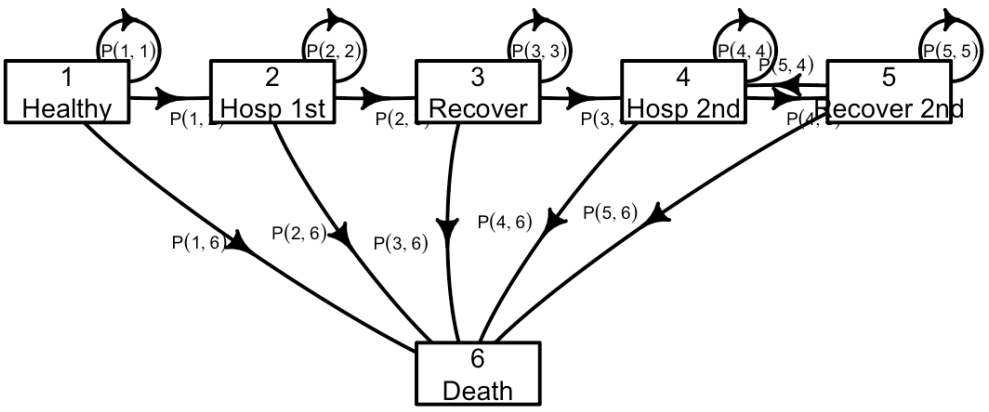
In order to simulate the population level dynamics of BRD in the case study feedlot, a multi-state Markov model was built to estimate the different probabilities of transitioning between different possible "health" states during 100 days in the feedlot. These health state transitions were used to simulate possible growth paths for a "high-risk" and "low-risk" animal cohorts within the paddock and saleyard purchase treatments. High-risk cohorts were classified as animals with four or more pathogens present in PCR testing, with low-risk cohort have less than four pathogen present.

The states in the model are as follows:

1. Healthy
2. Initial hospitalisation
3. Initial recovery
4. Subsequent hospitalistion
5. Subsequent recovery
6. Dead

Animals can go from any state to death.

Figure 3. A multistate model for bovine respiratory disease: states and allowable transitions, each transition is labeled P(i,j): the probability of going from state *i* to state *j* each day.



In this model animals can only move one state per day, that is if an animal is healthy, the next day they can either be hospitalised, or they can die. An animal cannot move from healthy to a second

hospitalisation or to recovered without having passed through the hospital state. In this model the first infection is treated as a different state to a subsequent infection (2nd or 3rd infection).

The transition probabilities are estimated from the data through the following process:

- data for southern feedlots combined together
- data rearranged in the format accepted by the package msm ie. subject, time, state
- some editing was required, for example if animal hospitalisation data and return date were the same. The return data was changed to the next day. This is because MSM model doesn't allow more than one state change in a time period (day). An example of the data format is given below in Table7.

Table 7. Example of animal transition status over time

animal_id	high_risk	days	state
900 093002161160	low	0	1
900 093002161160	low	13	1
900 093002161160	low	18	2
900 093002161160	low	19	3
900 093002161160	low	79	3

The probability matrix was generated using population data from the experimental dataset, and was formulated as follows:

$$P(from, to) = \begin{bmatrix} P(1,1) & P(1,2) & 0 & 0 & 0 & P(1,6) \\ 0 & P(2,2) & P(2,3) & 0 & 0 & P(2,6) \\ 0 & 0 & P(3,3) & P(3,4) & 0 & P(3,6) \\ 0 & 0 & 0 & P(4,4) & P(4,5) & P(4,6) \\ 0 & 0 & 0 & P(5,4) & P(5,5) & P(5,6) \\ 0 & 0 & 0 & 0 & 0 & P(6,6) \end{bmatrix}$$

As above P(i,j) denotes the probability of transitioning from state *i* to state *j* in the designated time period.

- P(1,1) is the probability of starting healthy and finishing healthy.
- P(1,2) is the probability of starting healthy and finishing in the first hospitalisation
- P(1,3) is the probability of starting healthy, transitioning through first hospitalisation and ending in first recovery

The estimated transition probabilities over a single day are as follows:

$$P(1)_{highrisk} = \begin{bmatrix} 0.996 & 0.003 & 0 & 0 & 0 & 0 \\ 0 & 0.887 & 0.112 & 0 & 0 & 0 \\ 0 & 0 & 0.999 & 0.001 & 0 & 0 \\ 0 & 0 & 0 & 0.835 & 0.165 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$P(1)_{lowrisk} = \begin{bmatrix} 0.999 & 0.001 & 0 & 0 & 0 & 0 \\ 0 & 0.878 & 0.122 & 0 & 0 & 0 \\ 0 & 0 & 0.998 & 0.001 & 0 & 0 \\ 0 & 0 & 0 & 0.838 & 0.091 & 0.07 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

Over a single day there is not a large difference between the transition probabilities, however if we look at the probabilities over a period of 100 days the difference becomes more obvious.

$$P(100)_{highrisk} = \begin{bmatrix} 0.67 & 0.02 & 0.254 & 0.002 & 0.013 & 0.041 \\ 0 & 0 & 0.901 & 0.006 & 0.093 & 0 \\ 0 & 0 & 0.893 & 0.006 & 0.102 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$P(100)_{lowrisk} = \begin{bmatrix} 0.908 & 0.006 & 0.068 & 0.001 & 0.002 & 0.016 \\ 0 & 0 & 0.853 & 0.007 & 0.063 & 0.077 \\ 0 & 0 & 0.842 & 0.007 & 0.068 & 0.083 \\ 0 & 0 & 0 & 0 & 0.565 & 0.435 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

In the high-risk group an animal who enters the feedlot health has a 67% probability of exiting without a BRD infection after 100 days. In the low-risk group an animal who enters the feedlot health has a 91% probability of exiting without a BRD infection after 100 days.

Note: for $P(100)_{highrisk}$ and $P(100)_{lowrisk}$ the entries for $P(2,2)$, $P(4,4)$ are very small and rounded to zero rather than being 'true' zeroes. This is because it is very unlikely an individual will start 'sick' and stay 'sick' for 100 days in a row.

3.6.4. Economic simulation experimental design

The proportion of high-risk animals in the feedlot cohorts varied primarily by purchase type (purchase type and location were associated management factors in the datasets). Saleyard purchases had much higher proportions of high-risk animals (as identified by the pathogen test) compared to paddock purchase. These results are similar to those in other Australian studies, e.g. In a large (36,160) animal trial Hay et al. (2014) found a marked increase in BRD risk associated with recent exposure to a saleyard environment.

Two primary simulation scenarios were designed to capture different risk profiles (informed by the risk profiles actually observed in the experimental data) of different purchasing strategies and the associated economic impact of a test and pre-emptively treat strategy for high-risk cohorts.

To assess profitability and breakeven return on investment of the testing intervention we compared:

- the status quo i.e. a proportion of high-risk animals come into the feedlot and aren't treated until they exhibit symptoms. Here we simulate x% of the animals with the high-risk transition probabilities and (1-x)% with the low-risk transition probabilities
- using early detection test and preemptive treatment (test and treat) - which we assume converts all the high-risk animals to low-risk. As such, we simulate a cohort where 100% of the animals are low-risk.

When conducted for each of the two purchase types (paddock and saleyard) this resulted in the following four treatments with associate proportions of high-risk and low-risk animals based on experimental data:

- status quo: Paddock purchase (95% low risk, 5% high risk)
- status quo: Saleyard purchase (70% low risk, 30% high risk)
- risk-based test and treat: Paddock purchase (95% low risk, 5% high risk*)
- risk-based test and treat: Saleyard purchase (70% low risk, 30% high risk*)

** note: high-risk animals that are tested and treated revert to the low-risk probability matrix*

For a proper 'counter factual' two identical cohorts were established on induction for status quo and test and treat cohorts (same starting weights) for the high-risk animals, with the treatment effect delivered through the different probability matrix driving growth, morbidity and mortality through the 100 day simulation.

Growth assumptions

- The starting weights for the animals are drawn from a normal distribution with $mu = 370$ and $sd. = 20$.
- Each individual is given an induction weight on day 0
- Each individual is assigned a "growth state" for each day i.e. whether they are 'normal' or 'sick' or 'dead'.
- When an animal is in state 2 or 4 (first BRD infection and subsequent BRD infection) they are assigned 'sick', in addition to this, in the days prior to illness they are assigned a 'sick' state such that the total time in 'sick' growth phase is 14 days based on estimates from Jackson *et al.* (2016). With data extrapolated from Jackson et al. (2016) the model assumes that on average over the 14 day sick period animal intake declines to 75% of "healthy" intake, and the animals make a net gain of 0kg over the same period. These assumptions achieve the same net outcome as the recovery profile in Jackson et al., (2016), however they are a simplification of reality, as animals will initially reduce growth, lose weigh, slowly gain weight and then recover to their new "recovered" intake (Jackson, 2016) (Jackson, Carstens et al. 2016) .
- Outside "sick" state days, if the animal is in state 1 (healthy), 2 or 5 (first and subsequent recoveries) they are assigned a 'normal' growth state.
- Once recovered, animals do not grow at a reduced rate (assumes no long-term residual morbidity). Additional sensitivity analysis was also conducted to assess the impact of a 10% decline in production and a 15% decline in production due to post BRD morbidity.
- If the animal dies, their growth state is 'dead'

Calculating feed intakes and weight gain is then an iterative process based on a daily time-step:

- The feed intake at time 0 (induction) is calculated from an AusFarm generated curve based on liveweight. Each liveweight increment also has an associated feed conversation percentage (also generated from AusFarm outputs) which changes depending on current liveweight.

e.g. A healthy animal with a starting weight of 370kg will consume 2.79% of their body weight in dry matter (10.34kg) and will gain 2.07kg of livewieght on that day (10.34kg intake x 0.2 feed conversion).

A sick 370kg animal will consume only 75% of the feed intake of a healthy animal, (so for a 370kg animal 7.76kg) and weight remains constant for a 14 day sickness period (as described above).

- The calculations are repeated for day 2, until the animal dies or exits the feedlot at day 100.

Summarising cohorts

Key variables calculated for each animal:

- total feed consumed
- whether they were from the high-risk subset or low-risk (hence whether they received treatment in the preemptive treat scenario)
- induction weight
- exit weight
- whether the animal died, and on what day
- whether the animal had a first BRD infection, and on what day
- whether the animal had a second BRD infection, and on what day

These output variables are then used to calculate revenue, expenditure and net return for that simulated cohort on an average per animal basis. Calculations for scenarios with different high-risk:low-risk ratios were then calculated by multiplying the per animal return for the cohort by the proportion of the population that cohort represented.

Eg:

$$\text{Paddock purchase system} = \frac{(\text{per animal low-risk return} \times 95) + (\text{per animal high-risk return} \times 5)}{100}$$

$$\text{Saleyard purchase system} = \frac{(\text{per animal low-risk return} \times 70) + (\text{per animal high-risk return} \times 30)}{100}$$

Economic assumptions

For each base simulation the following costs and prices were used, with associated sensitivity analysis (in brackets) for feed price, purchase & carcass price (in matching pairs to represent low, medium and high price cycles).

Days on feed	100	
Feed Price (t)	$350	($300, $400)
Purchase Price (LW)	$4.75	($3.00, $6.00)
Carcass Price (Cwt)	$8.00	($6.50, $9.50)
Cost of BRD treatment	$30.00	
Cost of BRD Test	$8.00	

Cost of dead Disposal	$60.00
Feedlot processing cost	$10.00
Yardage Cost	$1.20
Levies	$5.00
Transport cost to feedlot	$15.00
Transport cost to abattoir	$15.00

Breakeven return on investment for the test and treat scenarios was determined by calculating the net return for each percentage of low-risk animals in a treatment population in 1% increments between 95%-70% and recording the inflection point were returns became positive.

Sensitivity analysis

In addition to the base analysis, sensitivity analyses were run to assess the impact of the presence and impact of subclinical BRD, and the presence and absence of a carcass price discount based on the probability of BRD and healthy animals having a carcass downgrade (based on a simple 10% price discount). Due to the complexity of factors that contribute to carcasses failing to hit grade specifications on a grid, and the lack of definitive relationship to BRD, a simple price discount was applied to assess the general impact of carcass downgrades. The probably of a 10% price discount varied between healthy and BRD affect animals, with 5% of healthy animals being discounted, and BRD cohorts receiving a 10% or 20% discount depending on the scenario.

For the subclinical sensitivity analysis, it was assumed that subclinical BRD was as prevalent in cohorts as clinical BRD. As subclinical BRD was not assessed in the current study, this assumption was based results from a feedlot BRD study conducted in southern NSW (Gonzalez et al., 2018), which found that subclinical BRD was as prevalent as clinical BRD (Gonzalez et. al, 2018). Studies in the US have found similar results with Thompson et al., (2006) reporting subclinical BRD occurring in 29.7% of animals and clinical BRD occurring in 22.6% of animals

In the simulation populations, clinical BRD was present at a rate of 34% in high-risk cohorts and 8% in low-risk cohorts. Therefore, when subclinical cases were simulated at a same rate (34% and 8%) this gave a total cohort prevalence of BRD 68% for high-risk cohorts and 16% for low-risk cohorts. It should be noted that clinical cases of BRD were treated in the status quo and test and treat scenarios, however subclinical BRD was only treated in the test and treat scenario, as they are treated pre-emptively based on PCR risk assessment. Subclinical BRD was assumed to have the following impact on animal performance based on data from Gonzalez et al. (2018), untreated subclinical BRD cases resulted in a 5% reduction in DMI and a 10% reduction in ADG, with a second run of simulations assuming a 10% reduction in DMI and a 15% reduction in ADG (Gonzalez et. al., 2018).

4. Results

4.1. Sample collection and molecular testing

Between 2019 and 2022, 1850 induction cattle and 380 hospital cattle were tested at 5 commercial locations using a two-pass PCR panel containing primer / probes for detection and quantification of: (Pass 1-Multiplex Test 1) Bovine alpha herpesvirus 1 (BoAHV1), *Histophilus somni,* and *Trueperella pyogenes*, (Pass 1- Multiplex Test 2) *Mannheimia haemolytica, Mycoplasma bovis* and *Pasteurella multocida*, (Pass 2 individual qPCR tests for RNA viruses) Bovine Corona Virus (BCoV), Bovine Parainfluenza Virus 3 (BPIV3), Bovine Respiratory Syncytial Virus (BRSV), Bovine Viral Diarrhoea Virus (BVDV), . Two timepoints for home pen sampling were investigated (Day of induction, Day 0, and after 14 days on feed, Day 14) in addition to sampling of all animals presenting at the hospital pen for treatment coincident with induction sample collection.

Molecular tests targeting the presence of BCoV, BPIV-3, BRSV and BVDV were not run for induction and hospital samples collected at; Feedlot 1 April 2019, Feedlot 2 July 2019 and Feedlot 3 March 2021 samples.. Viral RNA targets (BCoV, BRSV, BPIV-3 and BVDV) were only considered as qualitative results for this study as the assays were not optimised for quantitation.

4.2. Induction cohorts

A total of 1706 Day 0 induction samples and 1246 Day 14 samples were included in the analysis (Table 2). Of the total number of animals sampled, 7.84% (n= 148) were excluded, mostly due to incomplete data sets. Samples were only collected at day 0 for collections undertaken in 2019 (Feedlot 1 April-2019 and Feedlot 2 July-2019, Table 3). All other collections involved Day 0 and Day 14 on feed alongside a concurrent convenience hospital pen sampling.

Initial collections were undertaken at 2 feedlots on day of induction (Day 0) to test for all bacterial targets and BoAHV1 to establish a minimal, and most direct testing method that did not require a second analytical step for cDNA preparation (2019 collections). Testing during this phase also included assessment of methods for storage, DNA extraction and stability, confirming both Quick Extract DNA extraction and the novel PBS-boil method as suitable methods for fast and efficient DNA extraction.

Data from this early analysis indicated the presence of all agents tested at two locations (Feedlots 1 and 2, Figure 4). This analysis showed varying prevalence of agents across the locations tested ranging from 0% of cattle testing positive for presence of a particular agent, *M. bovis,* Feedlot 1, to 67% of cattle positive for *P. multocida* at the same location (Figure 3). Relatively low prevalence of most organisms was detected at the two locations, except for *P. multocida* at Feedlot 1 that showed a high prevalence compared with the other agents tested.

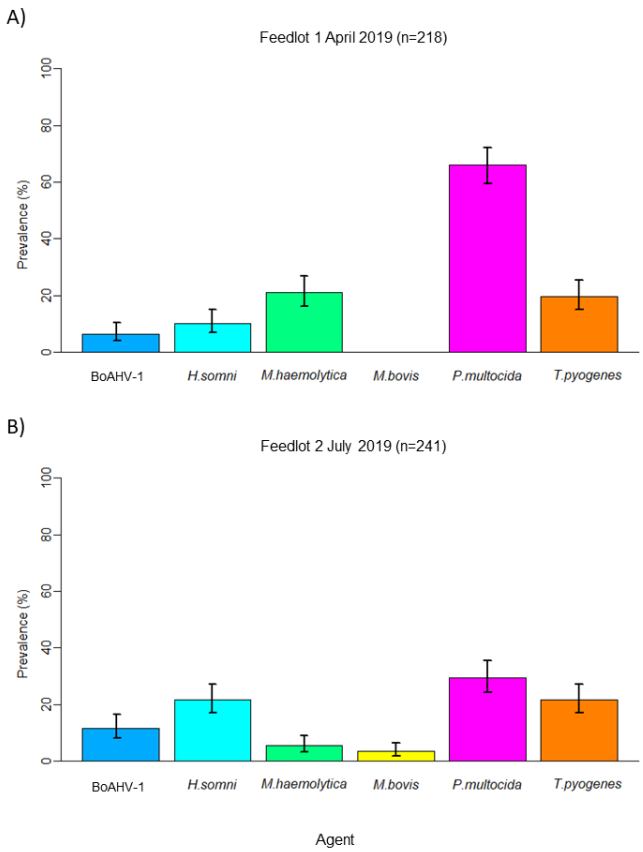


Figure 4. Prevalence (% +/- CI) of BRD associated DNA agents from nasal swabs collected from induction cohort animals at feedlot induction to two Australian feedlots, A) Feedlot 1 and, B) Feedlot 2).

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; *M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes*

To determine if prevalence of BRD agents increased over time during the early feeding period, and to investigate the relationships between bacterial and viral agents present in nasal swab samples, 1311 cattle were tested at 4 commercial feedlots using two multiplextests which contained primer / probes for detection and quantification of both bacterial and viral agents, specifically (Multiplex Test 1) Bovine alpha herpesvirus 1 (BoAHV1), *Histophilus somni,* and *Trueperella pyogenes*, (Multiplex Test 2) *Mannheimia haemolytica, Mycoplasma bovis* and *Pasteurella multocida*. In addition, individual qPCR tests for RNA viruses were run for each samples, specifically: Bovine Corona Virus (BCoV), Bovine Parainfluenza Virus 3 (BPIV3), Bovine Respiratory Syncytial Virus (BRSV), Bovine Viral Diarrhoea Virus (BVDV). Two timepoints for home pen sampling were investigated (Day of induction, Day 0, and after 14 days on feed, Day 14, Figure 4). In each location two induction pens were sampled; this was accommodated by two collection dates at Feedlot 1. Pen size varied in each location between approximately 80 head to 240 head per pen.

Significant variation was observed in agent prevalence, for the majority of agents tested, between Day 0 and 14 at each location and between locations. There was significant variation in prevalence of *M. bovis*, *P. multocida*, and *T. pyogenes* at Feedlots 1, 3 and 5, with *M. haemolytica* showing a significant increase in prevalence at Feedlots 1 and 5, and *P. multocida* significantly decreased at Feedlot 1 on the second sampling occasion (June 2021) but increased at the first (October 2020) (Figures 5A – D).

Except for Feedlot 5, *M. bovis* showed a significant increase between Day 0 and 14 at all other locations (Figure 5). Conversely, Feedlots 1 (June 2021) and 5, second sampling were the only locations to show a significant increase in BoAHV1 prevalence (Figure 5C and E). The DNA virus BoAHV1 was identified at all locations with highest prevalence noted at locations 1 and 5 where significant increases were noted between Day 0 and 14 (Figure 5C and E). RNA viral prevalence was low, or absent, at all locations tested with only Feedlot 1 June collection showing moderate levels of two viral pathogens, BCoV and BRSV in addition to BoAHV1 (Figure 5A). BVDV was not detected in nasal swabs in any animals sampled regardless of location or timing. Detailed prevalence percentages and p values are shown in Appendix 5.

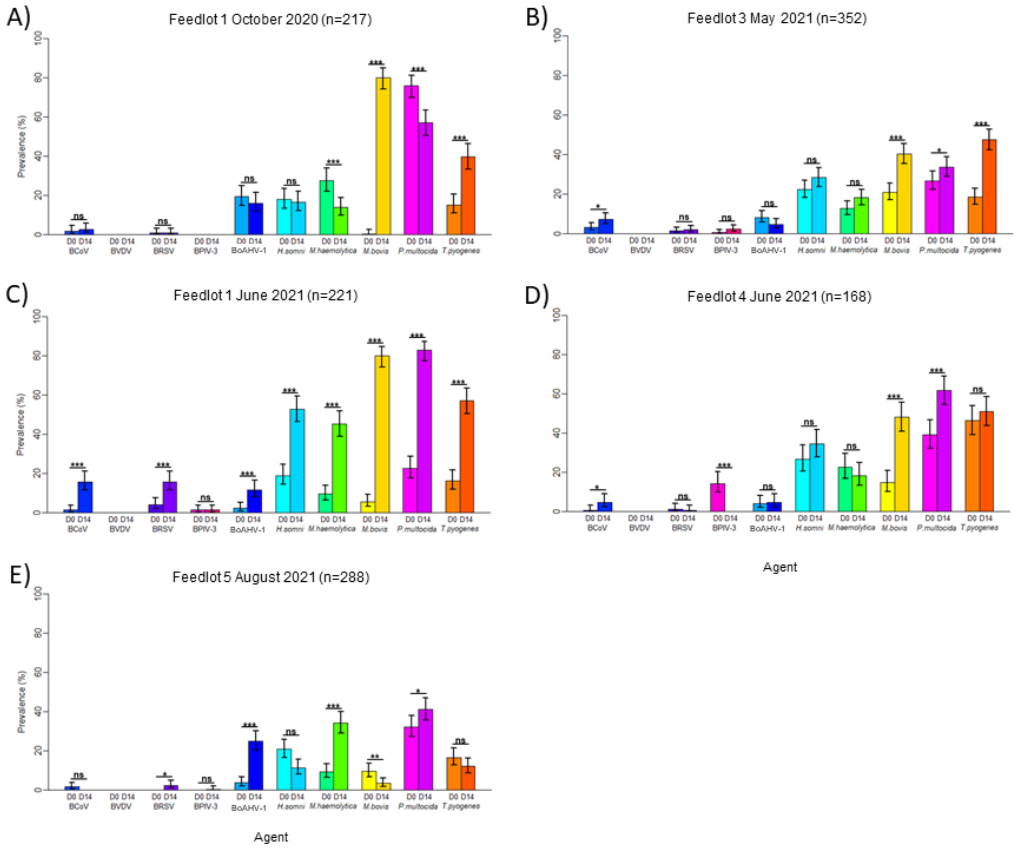


Figure 5. Prevalence (% +/- CI) of BRD associated DNA and RNA agents in nasal swabs collected from induction cohort animals at feedlot entry (D0) and after 14 days in their respective feedlot system (D14). Fischer's exact test: ns, not significant; * = p ≤ 0.05; ** = p ≤0.01, *** = p≤0.001

Abbreviations: BCoV, Bovine coronavirus; BVDV, Bovine viral diarrhea virus; BRSV, Bovine respiratory syncytial virus; BPIV-3, Bovine parainfluenza virus 3; BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

When absolute concentration using efficiency-corrected quantitation was considered (Figure 6), again, there was considerable variation between agents and locations, noting that most agents tested fell within consistent concentration ranges across all locations tested. Only agents that had undergone efficiency analysis of primer / probe sequences were considered, specifically BoAHV1, *H. somnii, M. bovis, M. haemolytica, P. multocida and T. pyyogenes.*

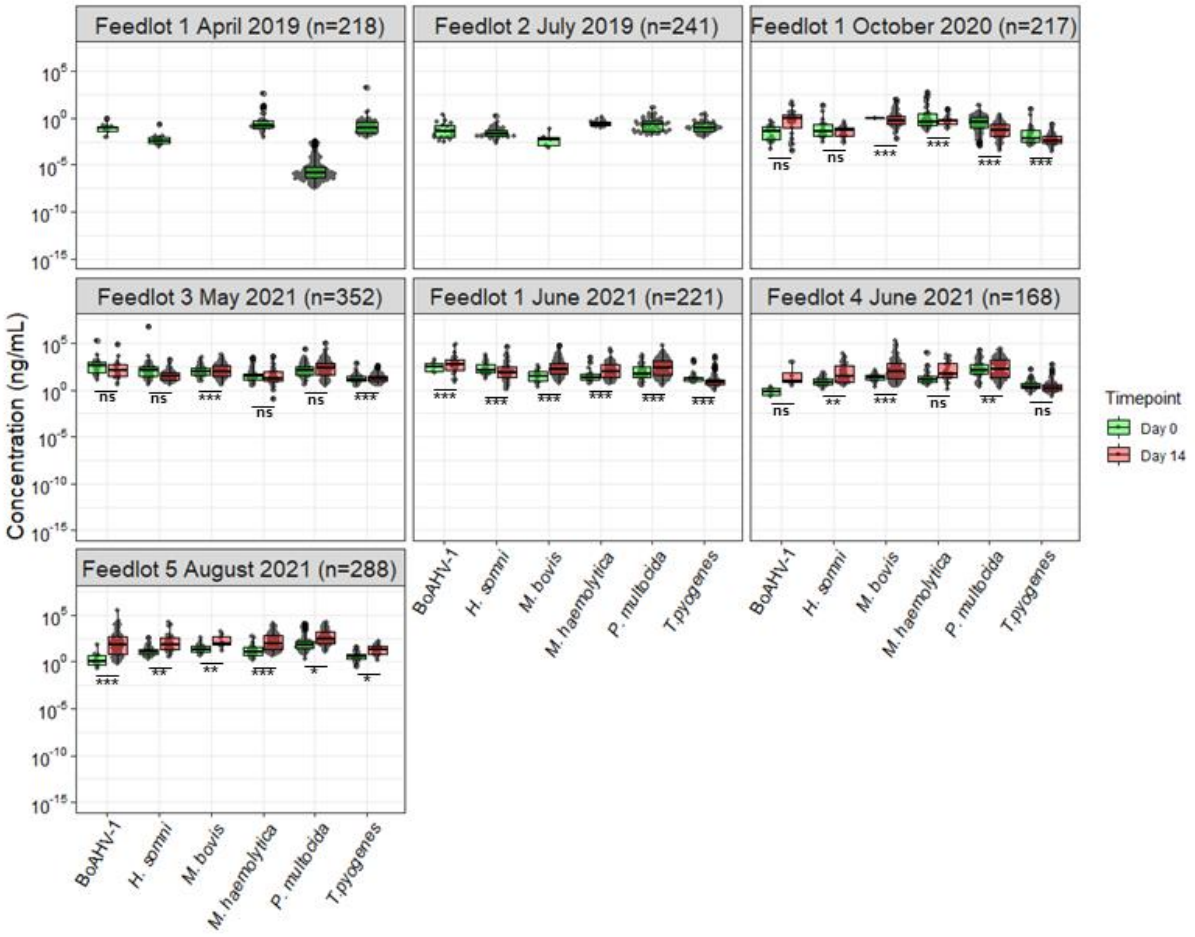


Figure 6. Absolute concentrations of agents commonly assoicated with bovine respiratory disease in nasal swabs collected from induction cohort animals at feedlot entry (Day 0) and after 14 days in their respective feedlot systems (Day 14), grouped by feedlot location. Only Day 0 samples were collected at Feedlot 1, April 2019 and Feedlot 2, July 2019. Wilcox rank sum test: ns, not significant; * = p ≤ 0.05; ** = p ≤0.01, *** = p≤0.001

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

Significant increases in agent concentration were observed for *M. bovis* (Feedlots 1, 3, 4 and 5), *H. somnii (*Feedlots 1 Oct 2020, 4 and 5), *M. haemolytica* (Feedlots 1 and 5), P*. multocida* (Feedlots 1, 4 and 5) and *T. pyogenes* (Feedlots 1, 3 and 5) (Figure 5). Feedlot 5 and Feedlot 1 (June 2021) were the only locations that showed a significant increase in the concentration of BoAHV1 between Day 0 and Day 14. Overall, higher concentrations of all agents were observed during 2021 collections (range 10^0 – 10^5 ng/ mL) compared to 2019 and 2020 collection dates (10^{-7} – 10^2 ng/ml) (Figure 5).

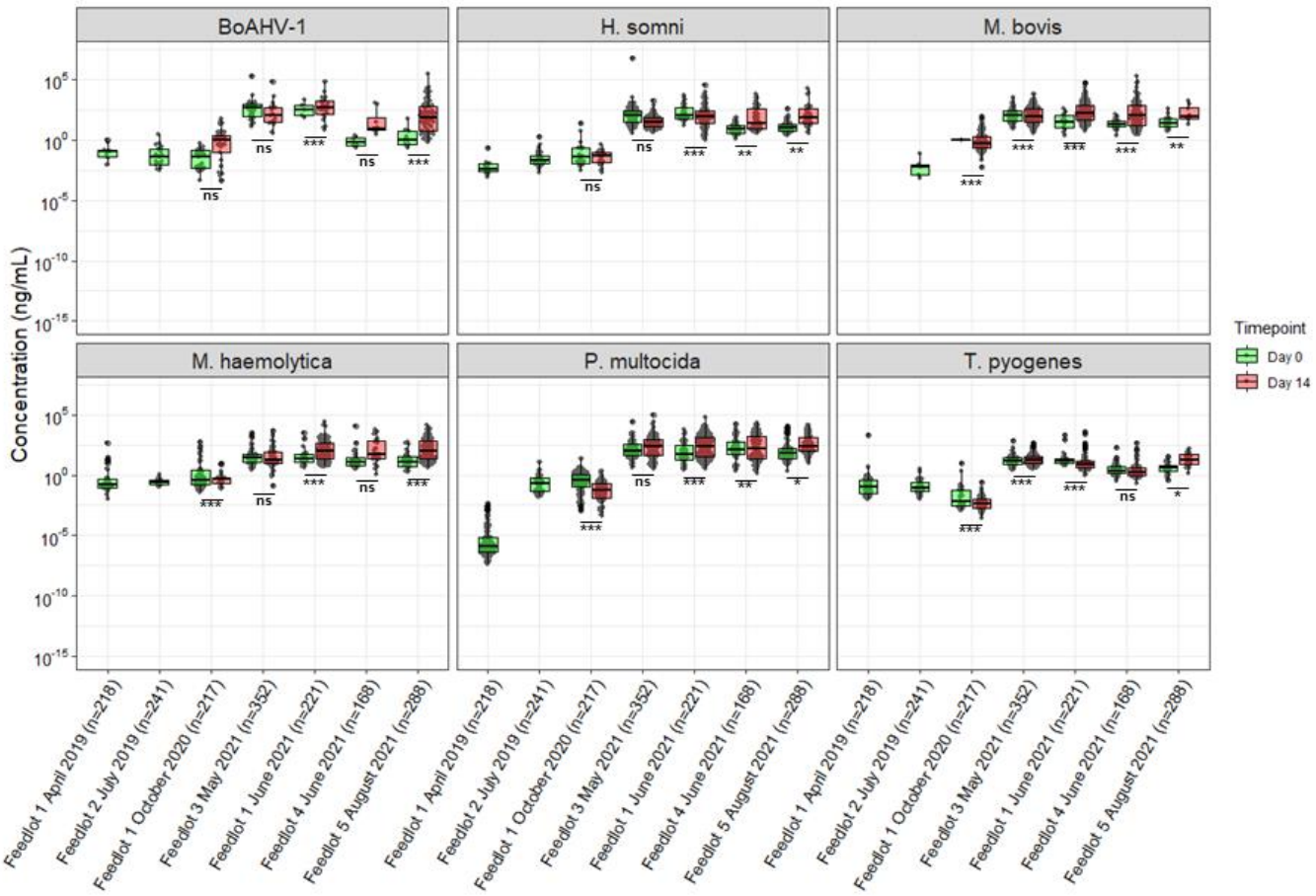


Figure 7. **Absolute concentrations of agents commonly assoicated with bovine respiratory disease in nasal swabs collected from induction cohort animals at feedlot entry (Day 0) and after 14 days in their respective feedlot systems (Day 14), grouped by feedlot agent. Wilcox rank sum test: ns, not significant; * = p ≤ 0.05; ** = p ≤0.01, *** = p≤0.001**

Abbreviations: BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

When concentration was considered by agent between locations and days of collection (Figure 7), *M. bovis* showed the most significant increases in concentration between Day 0 and Day 14 across all locations and timepoints tested, with variation between concentration and location across sampling times and sites for all other agents. The comparative difference in concentration of agent detected between 2019 and 2021 cohorts was most clearly observed for *P. multocida*, *M. bovis* and *T. pyogenes* where concentrations between 2019 and 2021 collections differed by up to 7 logarithmic points (*P. multocida*, Figure 7). Detailed p values are shown in Appendix 6.

To determine if the number of agents present during induction could be indicative of disease risk, the number of agents detected per induction animal was then considered in relation to their prospective health outcomes. Prevalence data had indicated that there were a number of animals tested that showed detectable levels of more than one agent (Barnewall, Marsh et al. 2022) with a preliminary analysis indicating that cattle presenting with more than 3 agents were potentially more likely to be at risk of development of respiratory disease. The minimum number of agents detected in any individual animal was zero (0) and the maximum number was seven (7). Therefore, comparison of the

occurrence of 0 -7 agents being detected in any individual animal, and the percentage of animals presenting with either no ailment, BRD or another ailment was considered for all cohorts together and at each timepoint (Figure 8).

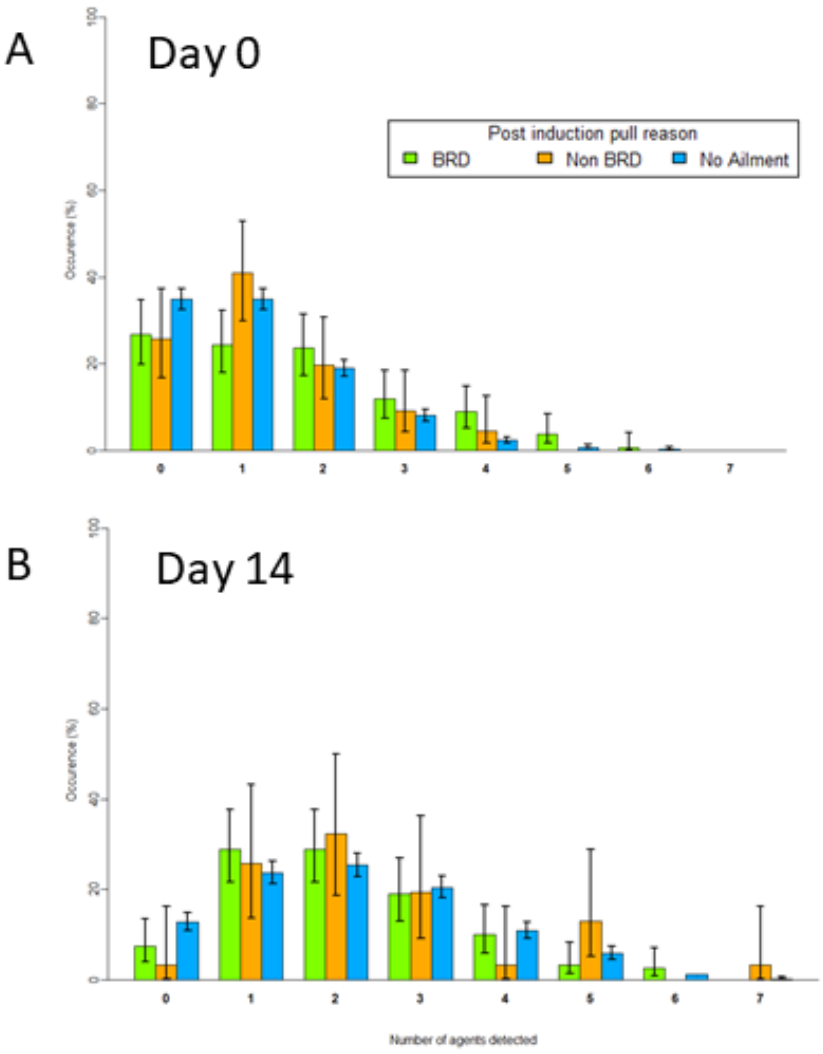


Figure 8. The mean occurrence (%) of the number of agents detected in induction cohort animals sampled at A) Day 0 during 2019 - 2021 (No ailment: n = 1505; Non-BRD: n = 66; BRD: n = 135) or, B) Day 14 during 2020 – 2021 (No ailment: n = 1094; Non-BRD: n = 31; BRD: n = 121) at the 5 feedlot locations. Data are pooled from all locations. Day 0 and Day 14 sampling was only undertaken in 2020 and 2021, therefore there are no 2019 animals represented in panel B).

When the two timepoints were considered, the distribution of number of agents detected was shifted to the right, with more agents detected in combination at Day 14 than at Day 0 when all locations were considered together (Figure 8). Notably, more cattle were observed to have 5 -7 agents detected at Day 14 than at Day 0, and less animals showed zero agents for all categories examined (No ailment, Non-BRD, and BRD). Early findings from this study reported in Barnewall 2022 (Barnewall, Marsh et

al. 2022) identified an increase in the relative number of agents in hospital compared to induction animals..

To compare both the number of agents present and their relative concentration by location, heat maps were generated for all animals sampled across all locations comparing reason for pull during time on feed for all induction animals sampled (Figures 9 – 15), comparing between Day 0 and Day 14 where both collections had been undertaken.

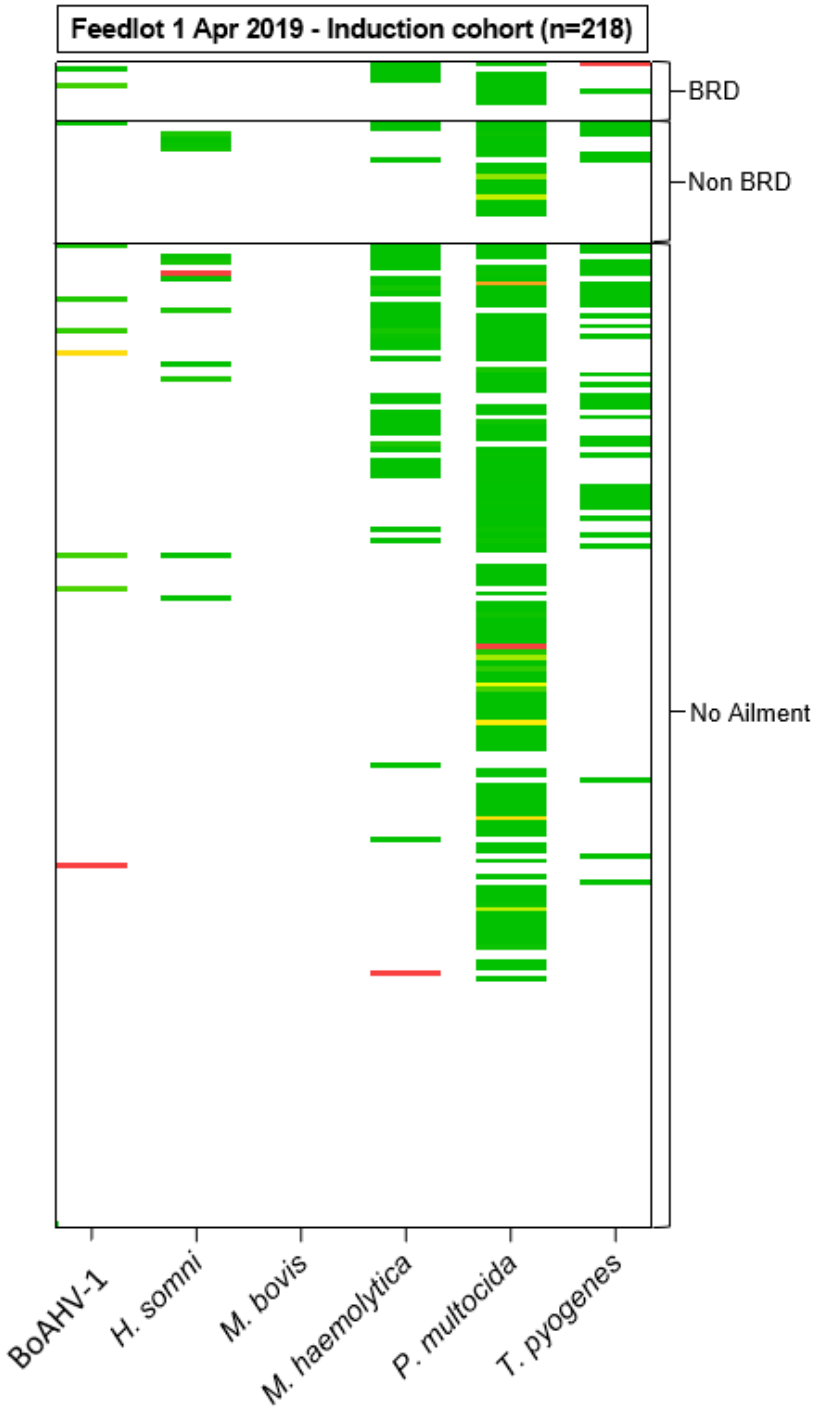


Figure 9. Upper airway load determined by EC quantification, of individual animals sampled at feedlot induction from Feedlot 1 (April 2019). Heatmap is sorted based on post induction pull

reason (No ailment, Non-BRD or BRD), and number of agents present per animal (most to least). Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

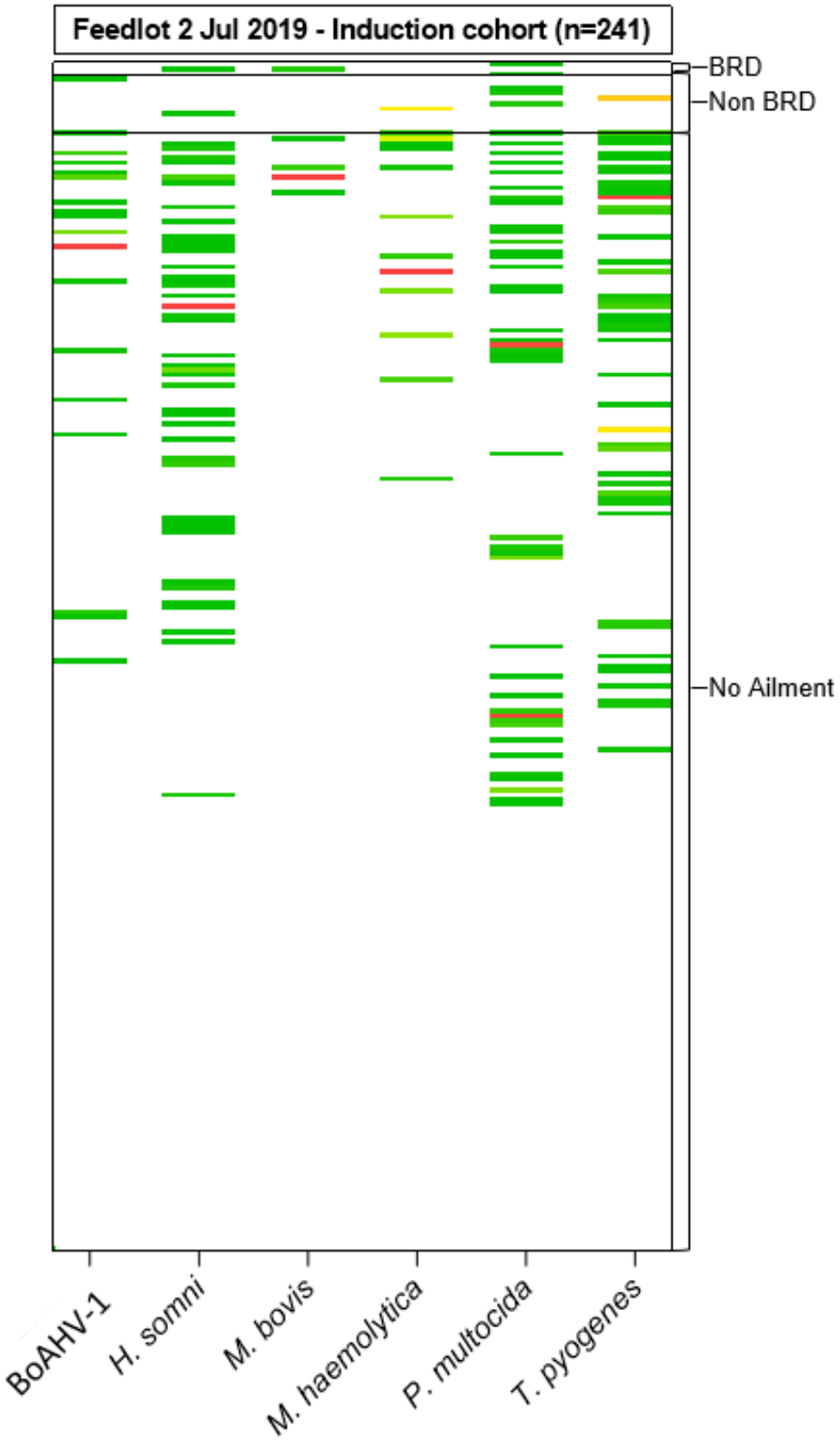


Figure 10. Upper airway load determined by EC quantification, of individual animals sampled at feedlot induction from Feedlot 2 (July 2019). Heatmap sorted based on post induction pull reason (No ailment, Non-BRD or BRD), and number of agents present per animal (most to least). Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

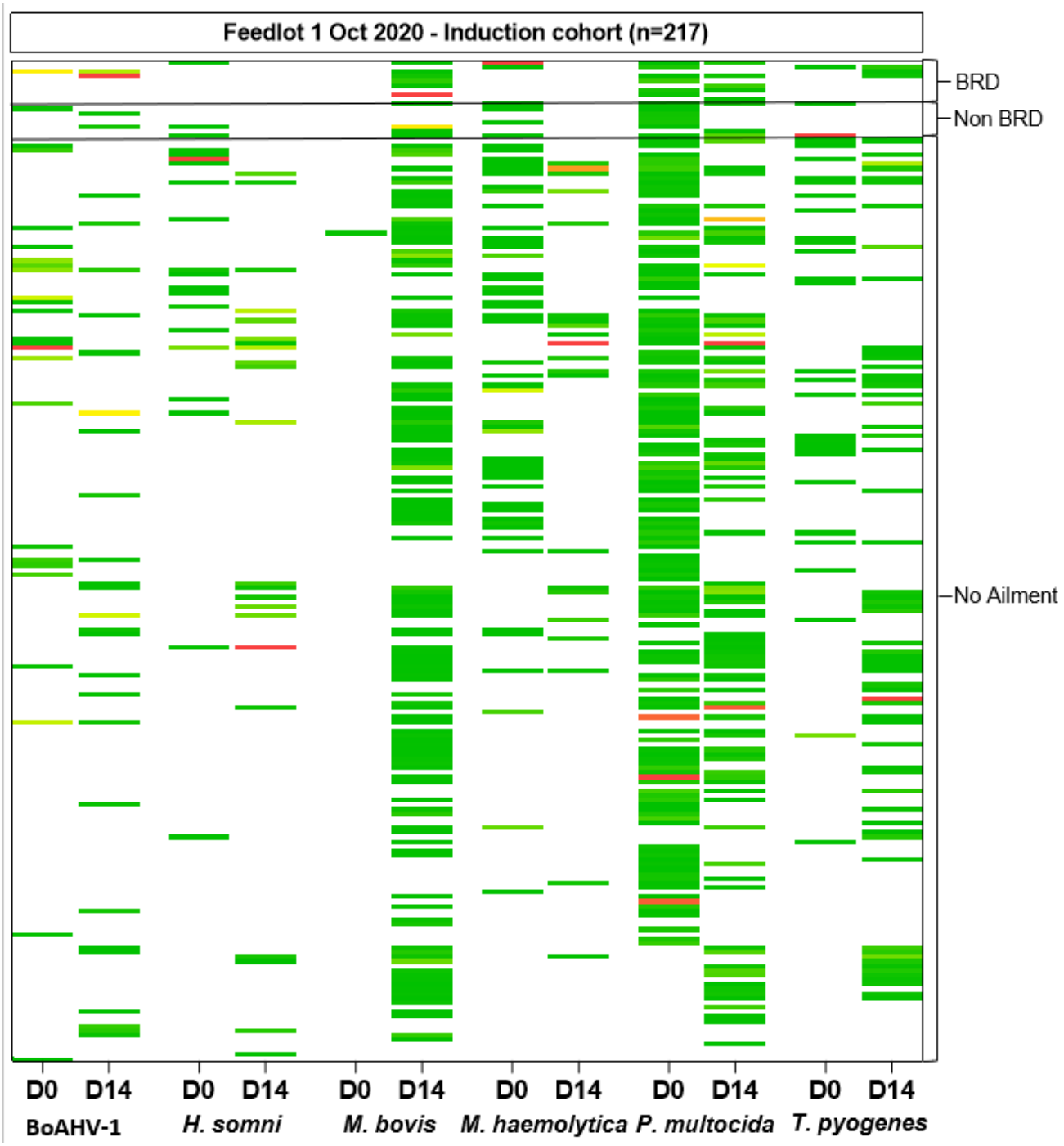


Figure 11. Upper airway load determined by EC quantification, of individual animals from Feedlot 1 (Oct 2020) sampled at feedlot entry (D0) and again at revaccination (D14). Heatmap sorted based on post induction pull reason (No ailment, Non-BRD or BRD) and number of agents present per animal (most to least). Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

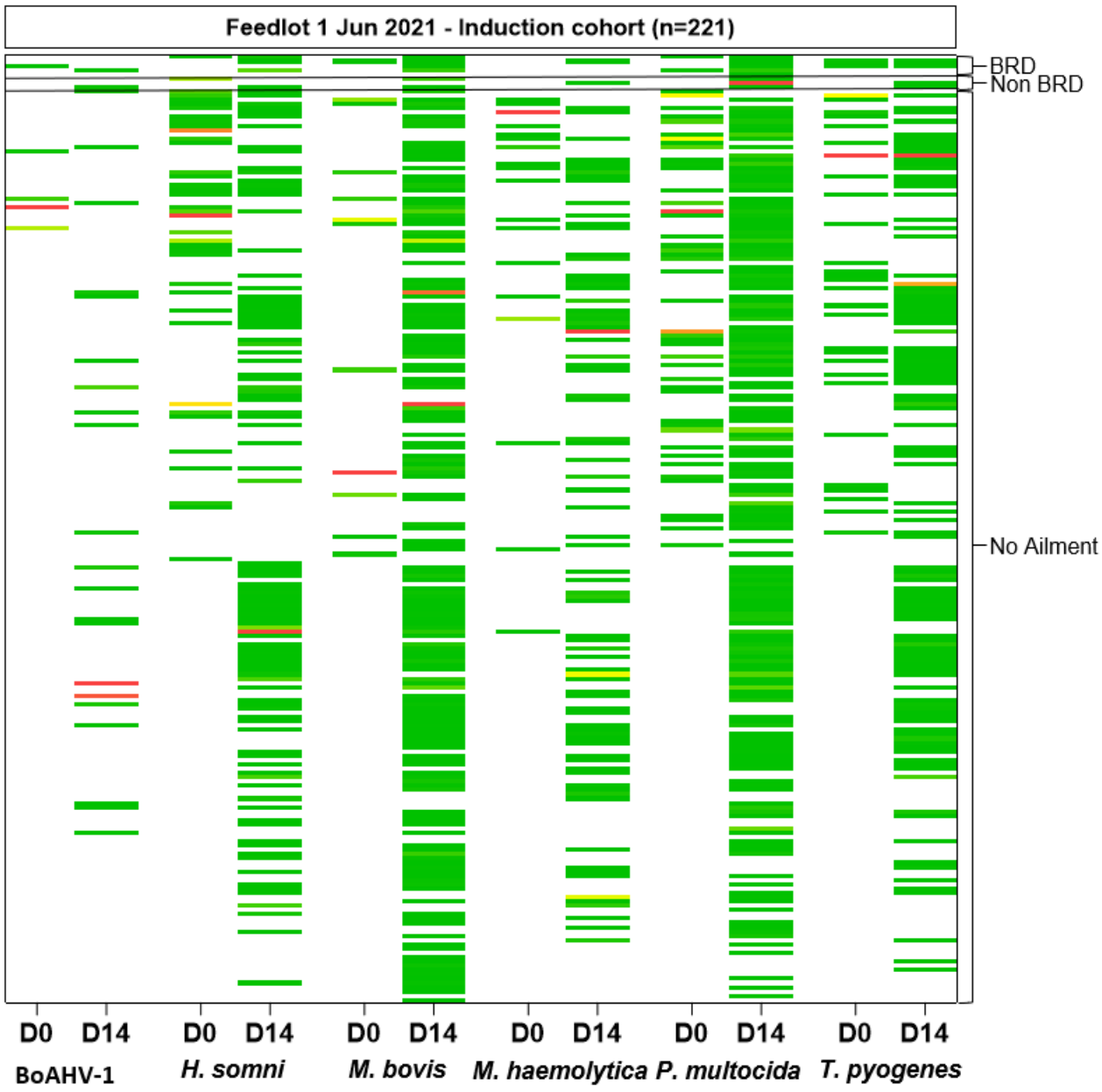


Figure 12. Upper airway load determined by EC quantification, of individual animals from Feedlot 1 (June 2021) sampled at feedlot entry (D0) and again at revaccination (D14). Heatmap sorted based on post induction pull reason (No ailment, Non-BRD or BRD) and number of agents present per animal (most to least). Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

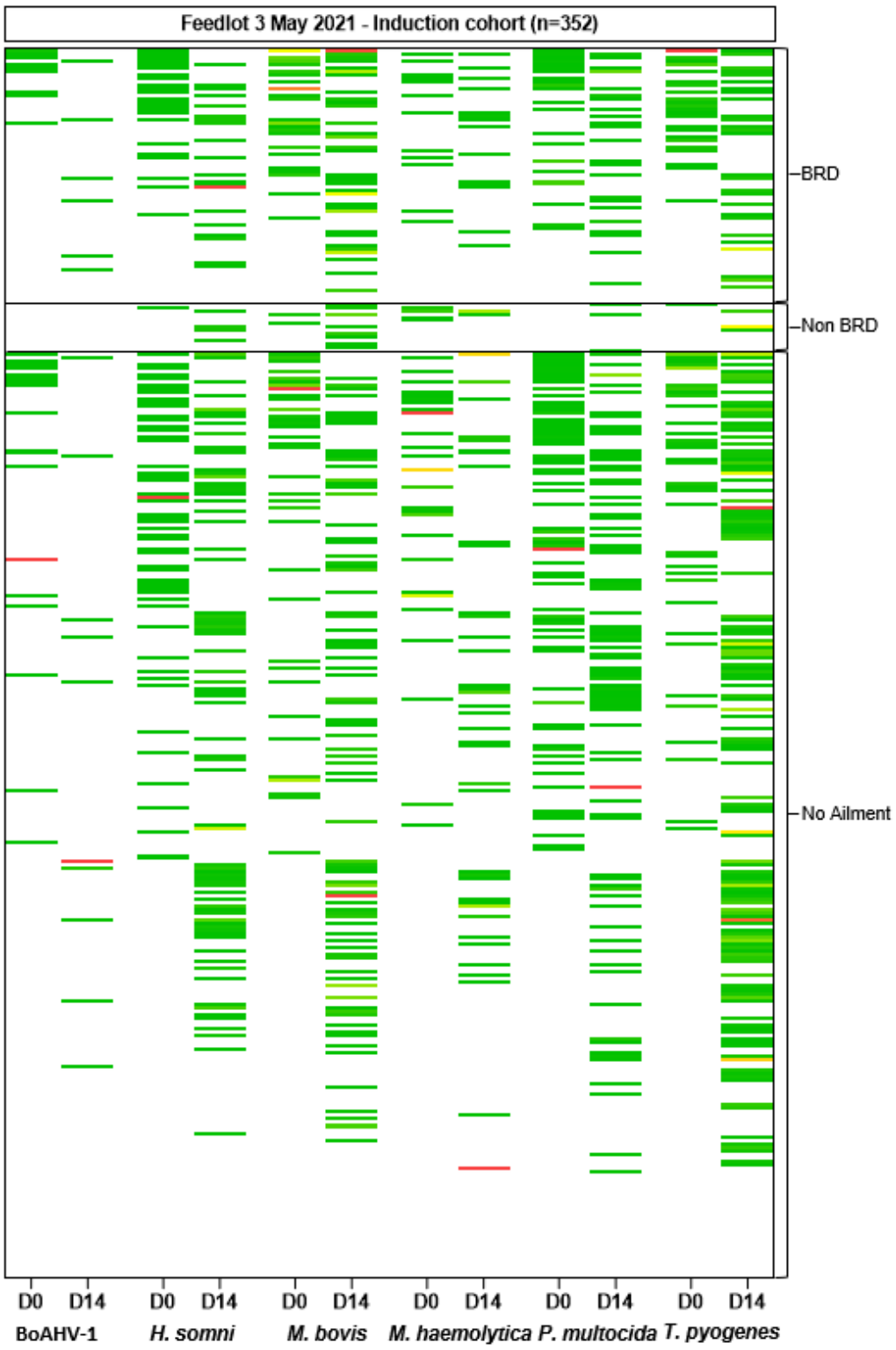


Figure 13. Upper airway load determined by EC quantification, of individual animals from Feedlot 3 (May 2021) sampled at feedlot entry (D0) and again at revaccination (D14). Heatmap sorted based on post induction pull reason (No ailment, Non-BRD or BRD), and number of agents present per animal (most to least). Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

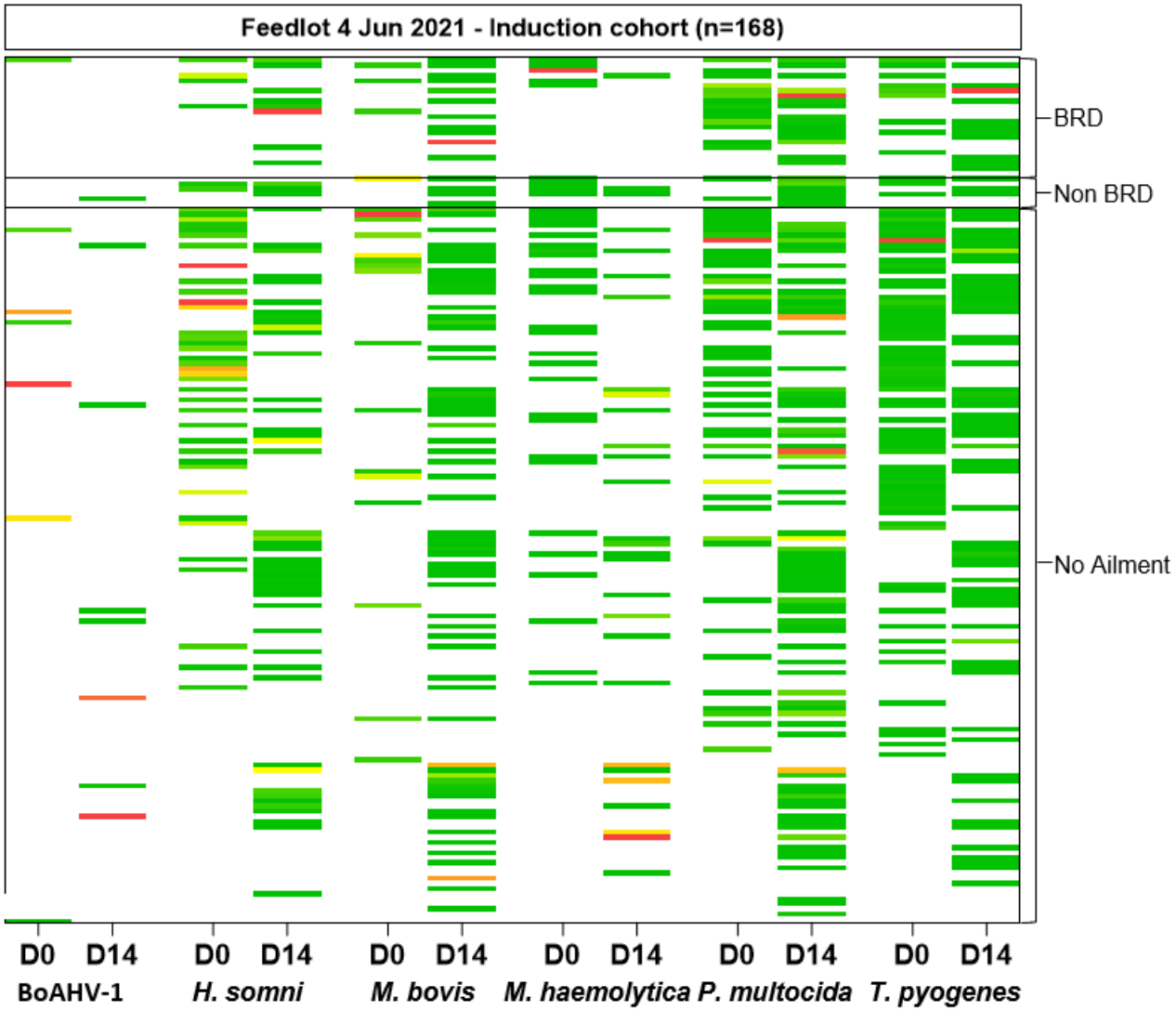


Figure 14. Upper airway load determined by EC quantification, of individual animals from Feedlot 4 (June 2021) sampled at feedlot entry (D0) and again at revaccination (D14). Heatmap sorted based on post induction pull reason (No ailment, Non-BRD or BRD), and number of agents present per animal (most to least). Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

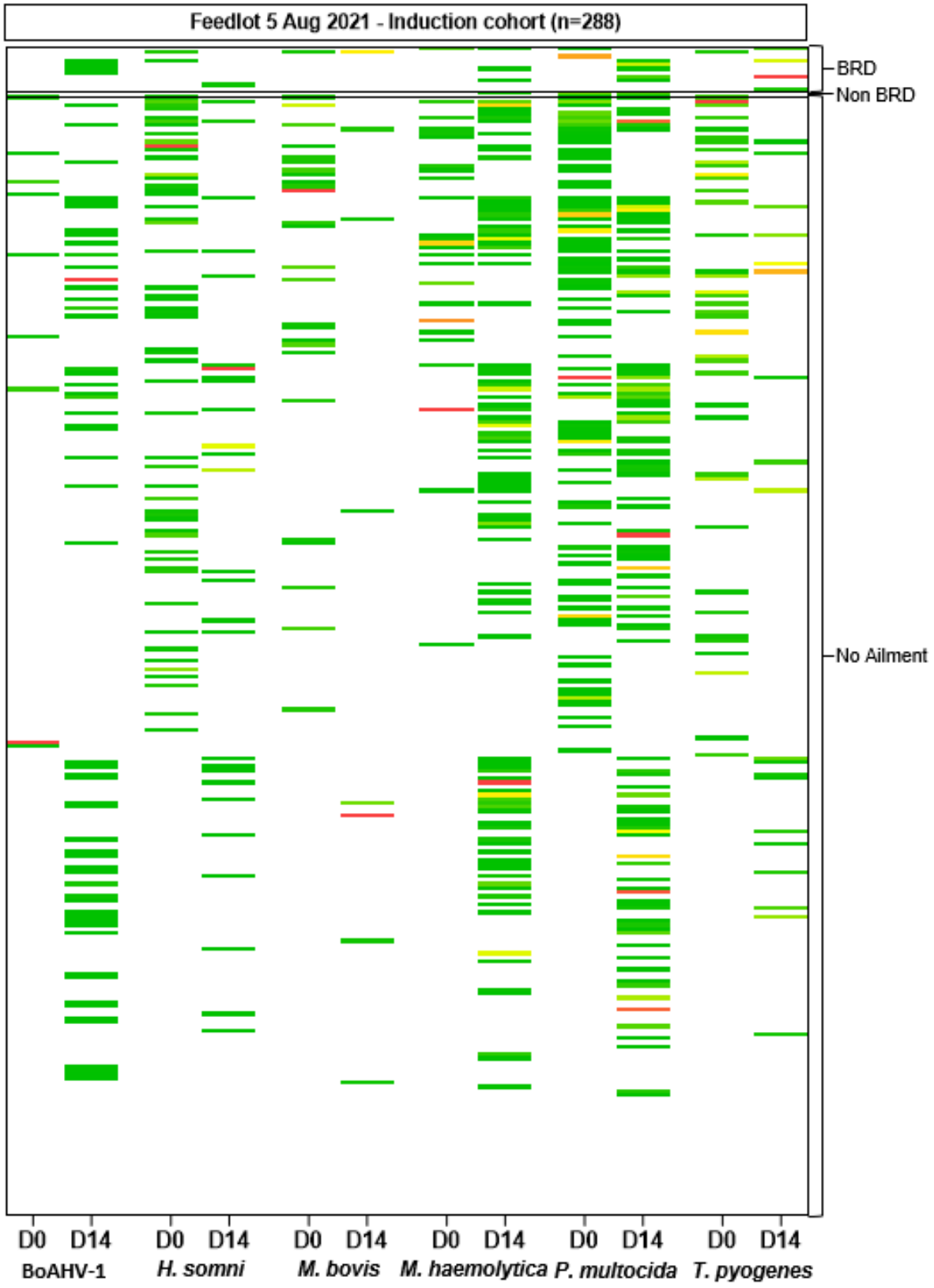


Figure 15. Upper airway load determined by EC quantification, of individual animals from Feedlot 5 (August 2021) sampled at feedlot entry (D0) and again at revaccination (D14). Heatmap sorted based on post induction pull reason (No ailment, Non-BRD or BRD), and number of agents present per animal (most to least). Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

When microorganism prevalence was considered at an individual animal level, similar trends could be observed as reported for prevalence for each feedlot where *M. bovis* was entirely absent (Feedlot 1, Figure 9) or only sparsely present at induction (Feedlot 2, Figure 10; Feedlot 1, Figure 12; Feedlot 4, Figure 14). Exceptions were Feedlot 3 where moderate numbers of animals were identified to be positive for *M. bovis* at Day 0 (Figure 13, Feedlot 3) or where numbers reduced between Day 0 and Day 14 (Feedlot 5, Figure 15). As suggested previously, Feedlot 1 showed consistently high numbers of animals testing positive for *P. multocida* at both Day 0 and Day 14 (Figure 9, 11 and 12). The visual proportion of numbers of cattle testing positive for any particular agent followed trends in prevalence previously reported, but without any particular trends in concentration. Specifically, cattle pulled for BRD or other ailments were not consistently observed to have higher concentrations of BRD-related agents than their No Ailment counterparts, with the vast majority of cattle tested showing no or low levels of agents tested. Cattle identified to have the highest loads of agents tested could be identified within No Ailment, or BRD groups, but only one animal with a high load of an agent (*T. pyogenes*, Feedlot 1, Figure 11) could be identified in the Non-BRD group. In each case, there appeared to be no consistent trend in concentration between cattle pulled for BRD or those fed for 14 days without any identified ailment, at any location, by this analysis.

Next, agent combination was considered for all induction animals, at all locations. The ability to test multiple agents in swabs from individual animals allowed, for the first time, the combination of agents to be determined across cohorts and within each sampling, Day 0 and Day 14 (Figs 16 – 21).

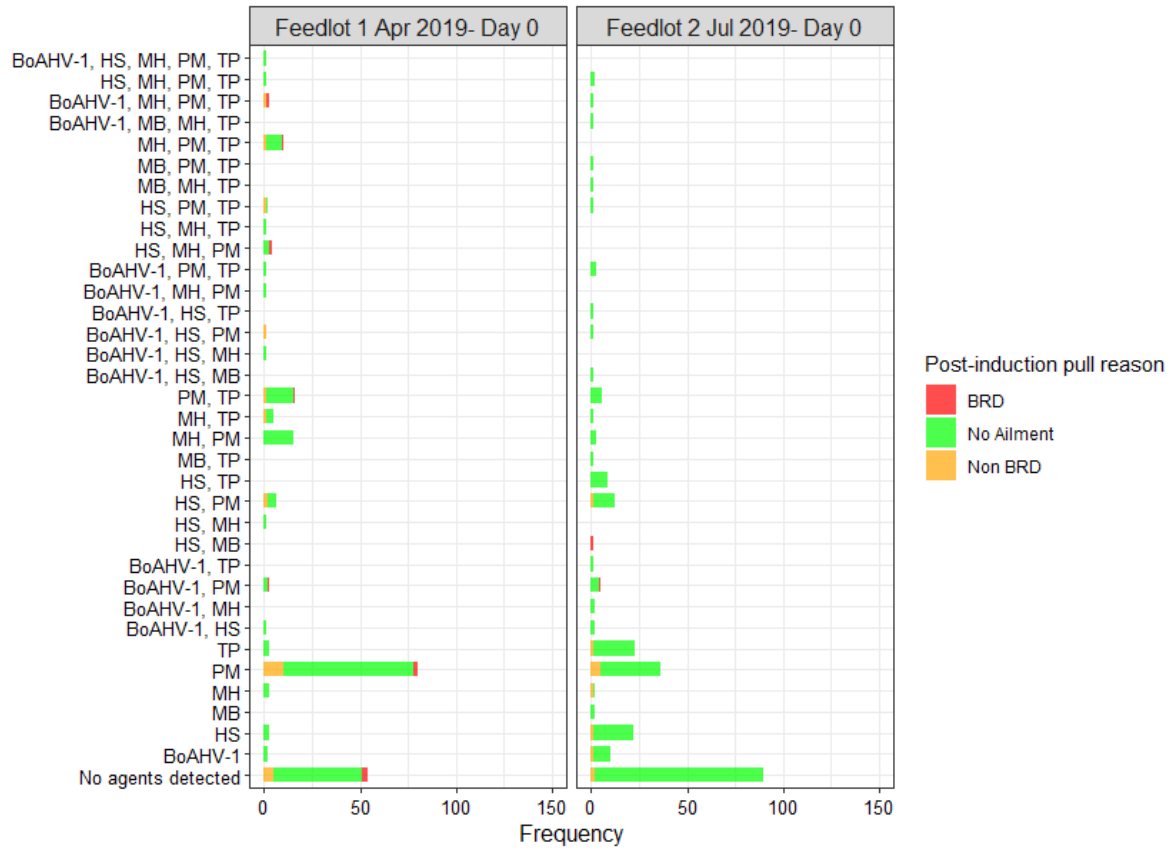


Figure 16. Frequency of BRD-associated agent combinations in induction animals sampled from two independent feedlot locations with frequencies coloured by post-induction pull reason. Feedlot 1, Apr 2019: n = 218; Feedlot 2, July 2019: n = 241). Agents are ordered by

cumulative number with the number of animals with the greatest number of agents at the top, and those with the fewest or no agents at the bottom of the chart. *Abbreviations: BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes*

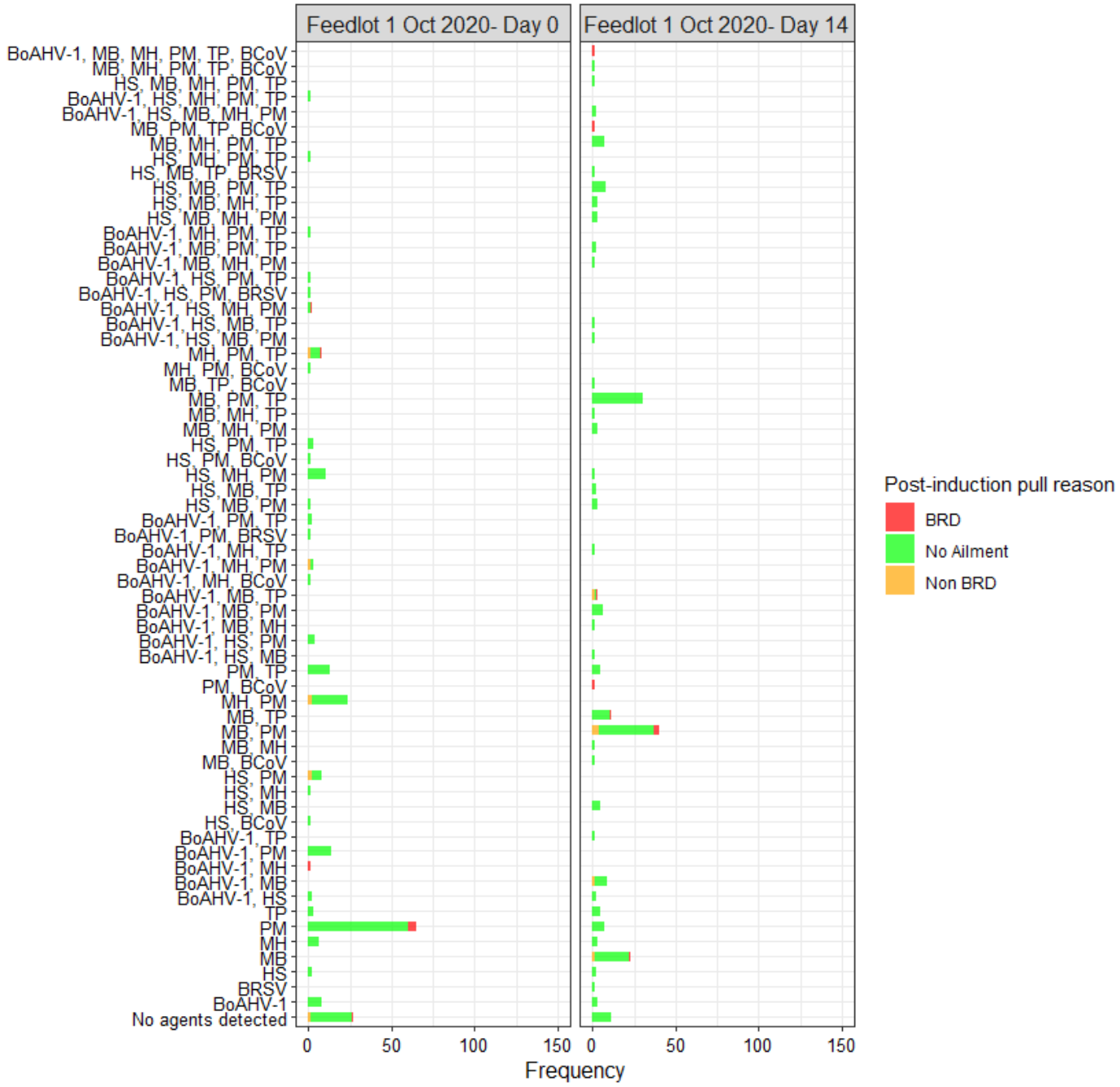


Figure 17. Frequency of BRD-associated agent combinations in induction animals sampled at two time-points from an Australian feedlot in the South-West Slopes of NSW (Feedlot 1 Oct 2020: BRD n = 217). Agents are ordered by cumulative number with the number of animals with the greatest number of agents at the top, and those with the fewest or no agents at the bottom of the chart.

Abbreviations: BCoV, Bovine Coronavirus, BRSV, Bovine Respiratory Syncytial Virus, BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

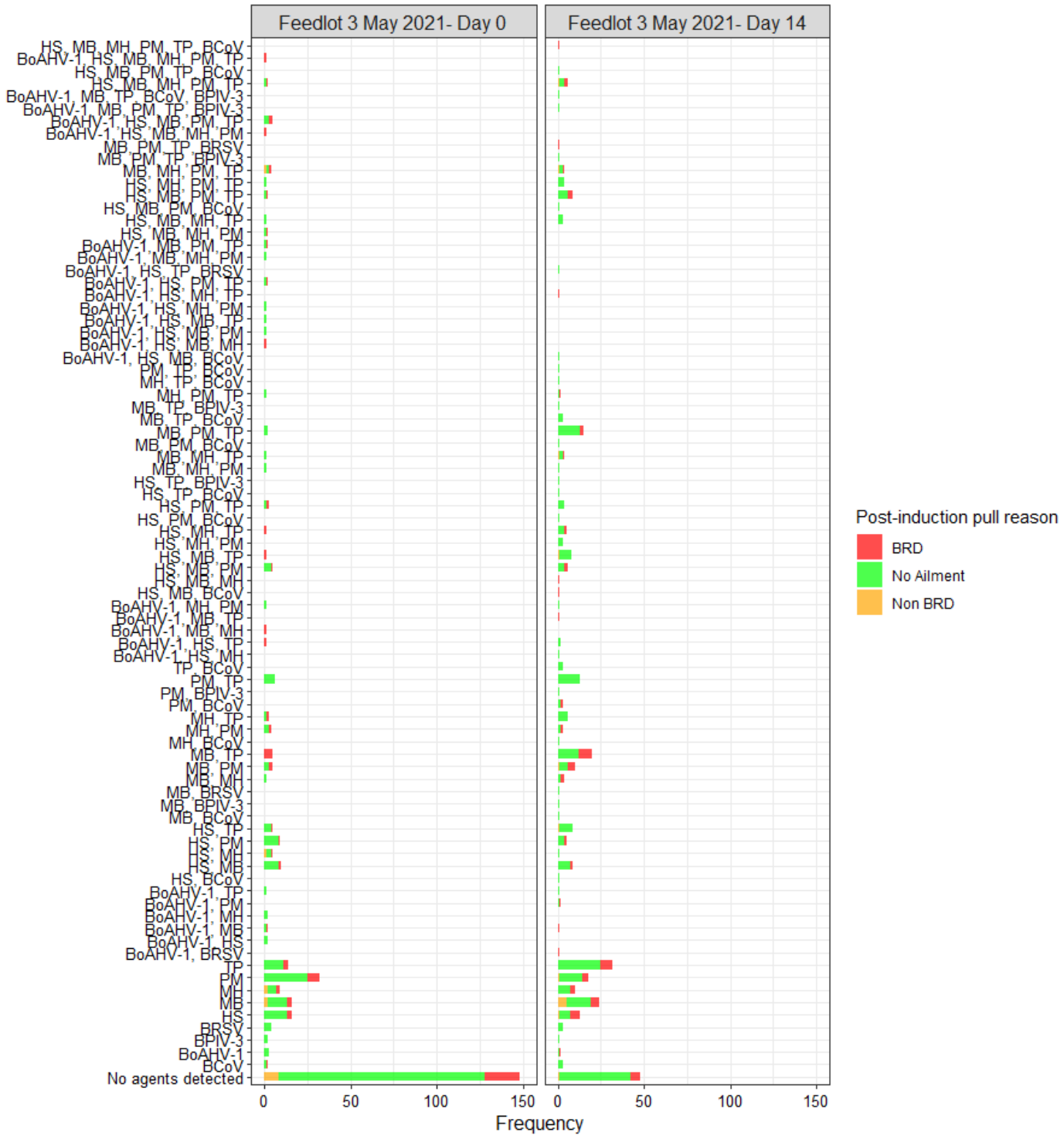


Figure 18. Frequency of BRD-associated agent combinations in induction animals sampled at two time-points from an Australian feedlot in Southern NSW (Feedlot 3 May 2021, n=352). Agents are ordered by cumulative number with the number of animals with the greatest number of agents at the top, and those with the fewest or no agents at the bottom of the chart.

Abbreviations: BCoV, Bovine Coronavirus, BRSV, Bovine Respiratory Syncytial Virus, BoAHV-1, BPIV3, Bovine Parainfluenza Virus 3, BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

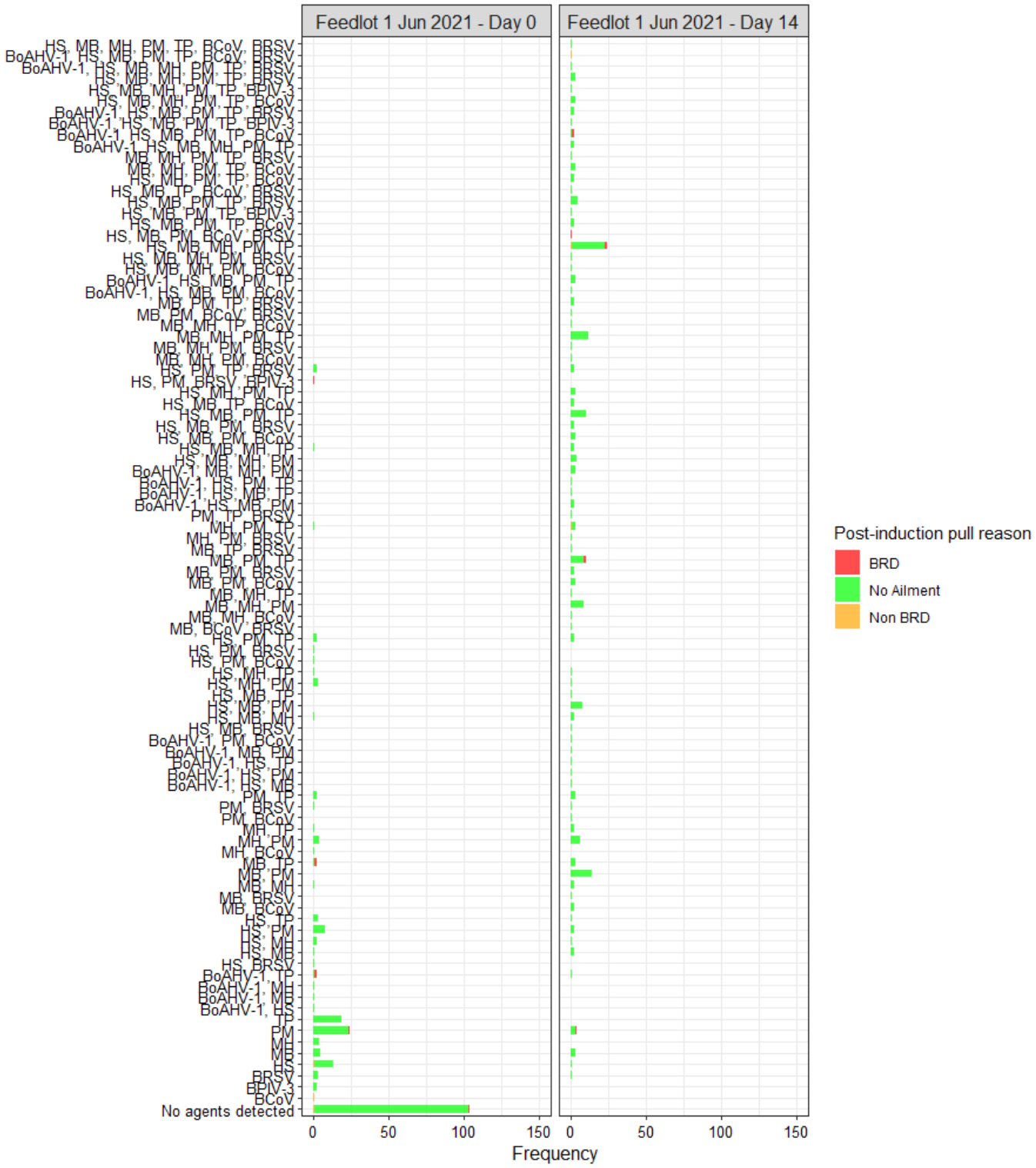


Figure 19. Frequency of BRD-associated agent combinations in induction animals sampled at two time-points from an Australian feedlot in the South-West Slopes of NSW (Feedlot 1 June 2021, n= 221). Agents are ordered by cumulative number with the number of animals with the greatest number of agents at the top, and those with the fewest or no agents at the bottom of the chart.

Abbreviations: BCoV, Bovine Coronavirus, BRSV, Bovine Respiratory Syncytial Virus, BoAHV-1, BPIV3, Bovine Parainfluenza Virus 3, BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

Figure 20. Frequency of BRD-associated agent combinations in induction animals sampled at two time-points from an Australian feedlot in Central NSW (Feedlot 4 June 2021, BRD n= 168). Agents are ordered by cumulative number with the number of animals with the greatest number of agents at the top, and those with the fewest or no agents at the bottom of the chart.

Abbreviations: BCoV, Bovine Coronavirus, BRSV, Bovine Respiratory Syncytial Virus, BoAHV-1, BPIV3, Bovine Parainfluenza Virus 3, BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

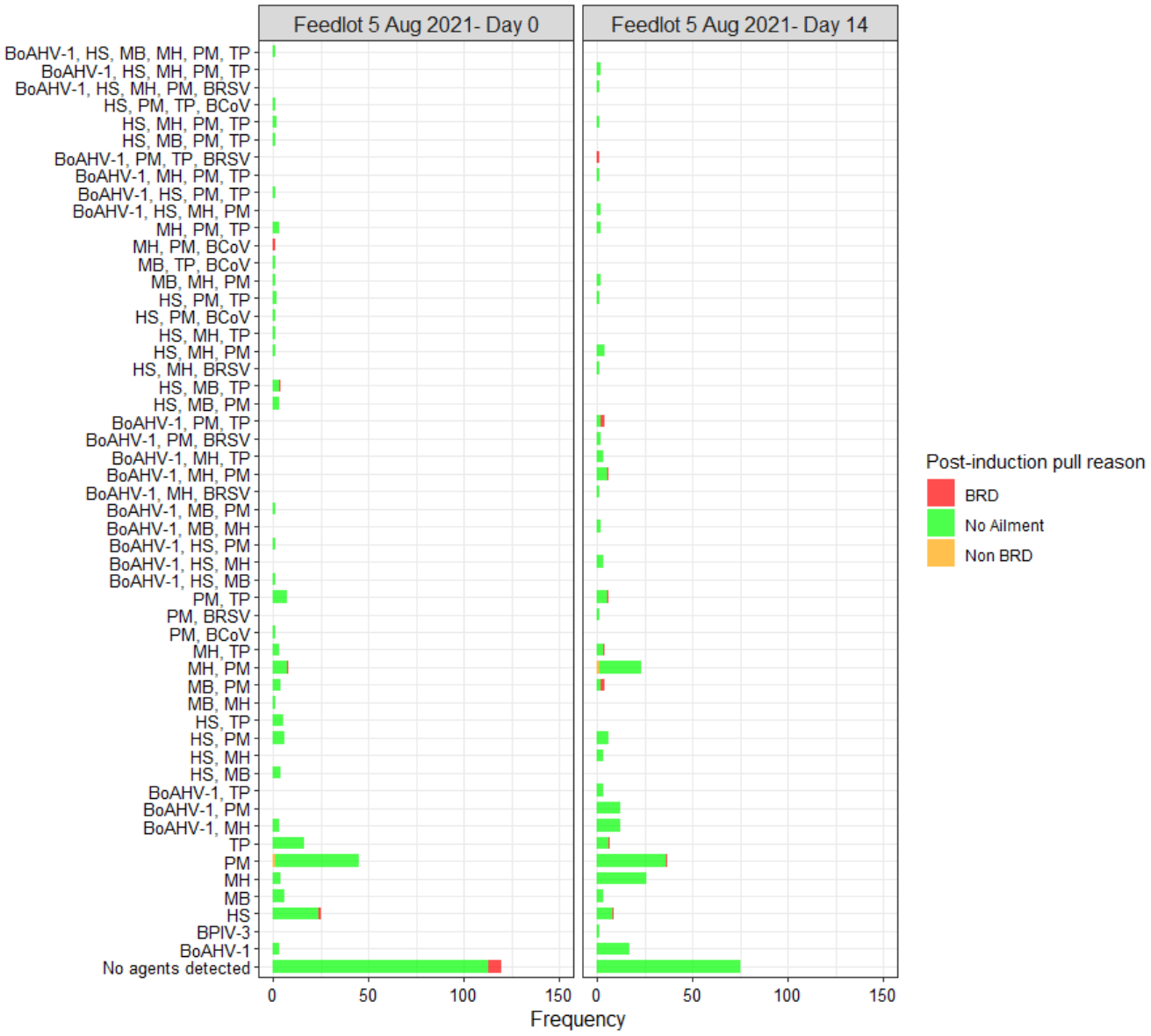


Figure 21. Frequency of BRD-associated agent combinations in induction animals sampled at two time-points from an Australian feedlot in North-western NSW (Feedlot 5 August 2021, n = 288). Agents are ordered by cumulative number with the number of animals with the greatest number of agents at the top, and those with the fewest or no agents at the bottom of the chart.

Abbreviations: BCoV, Bovine Coronavirus, BRSV, Bovine Respiratory Syncytial Virus, BoAHV-1, BPIV3, Bovine Parainfluenza Virus 3, BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

In all cases, low numbers of animals were identified with most agent combinations, with the exception of 'no agent detected' which comprised the largest number of animals at most locations and times tested (Figs 16-21). When considering animals presenting with BRD during time on feed there was no consistent pattern relative to agent combination. There were, however, differences between feedlots. Feedlots 1 and 3 (Figs 18 and 19 respectively) showed the greatest shift from no agents detected to a spread of agents identified at Day 14. Feedlot 4 showed a greater spread of BRD cases across all

combinations identified, at both Day 0 and Day 14, and Feedlot 5 showed the fewest identified cases of BRD at both timepoints tested. The other locations (Feedlot 1 (June), Fig. 17; Feedlot 4, Fig 18 and Feedlot 1 (June), Fig. 19) showed a greater spread of agent combinations identified at Day 14 than Day 0, with Feedlot 1 (June) showing no animals with no agents detected at Day 14. Day 0 showed fewer cattle with combinations of 4 or less agents compared with Day 14, and cattle with no agents detected at Day 0 could be identified to be pulled later for BRD across all sites investigated.

4.3 Hospital pen cattle

To consider the agent profile, prevalence and combination in cattle known to be suffering from BRD, convenience samples of hospital pen animals (total n = 380) were swabbed at each location coincident with days on which induction cattle were sampled (Table 3). After excluding animals with incomplete hospital data (total n = 58; 15.68% of total animals sampled) 312 hospital pen animals were included in the analysis (BRD n = 217, Non BRD n = 95).

Initial panels were run using only the two-pass test without addition of RNA viruses. Prevalence data for these collections and locations is shown in Fig. 22, Table A5.2. There was no significant difference in hospital animals tested by disease status for the prevalence of any agent tested, except for BoAHV1 at Feedlot 2 which was observed to be significantly greater in BRD treated animals compared with their non-BRD counterparts (Fig.2B). However, in all cases, the prevalence of potential BRD agents were observed to be greater in hospital than for induction cohorts.

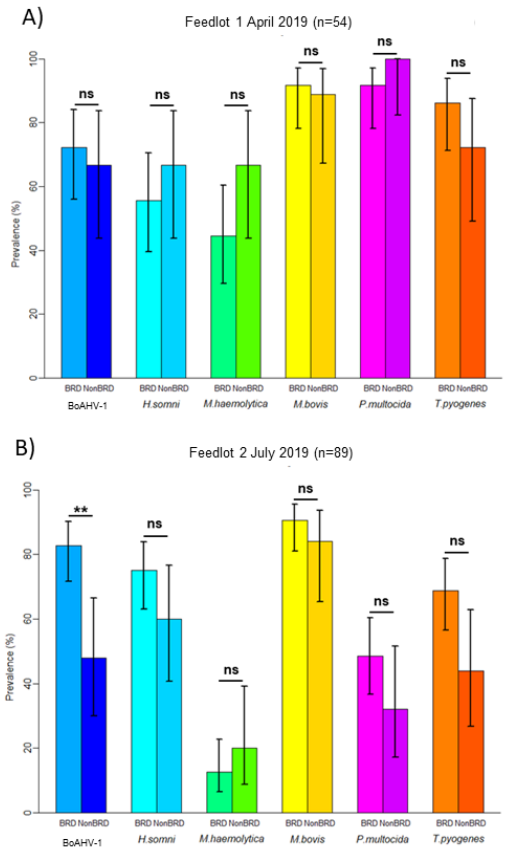


Figure 22. Prevalence (% +/- CI) of BRD associated DNA agents from nasal swabs collected from animals presenting to the hospital pen for treatment of respiratory disease (BRD) or other ailments (non-BRD). A) Feedlot 1, BRD, n = 36, Non-BRD n = 18; B) Feedlot 2, BRD n = 64, non-BRD n = 25. Fischer's exact test: ns, not significant; * p ≤ 0.05; ** p ≤0.01; *** p≤0.001.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

When hospital pen cohorts were considered from 2020-2021 with the inclusion of RNA viruses, a similar trend was observed. In most cases there was no significant difference in the prevalence of agents between BRD and non-BRD treatment groups for all agents tested (Fig. 23, Table A5.2). The exception was *M. bovis* in Feedlot 1 (October collection) which showed a significant increase in BRD-treated compared to Non-BRD cattle (Fig 23A).

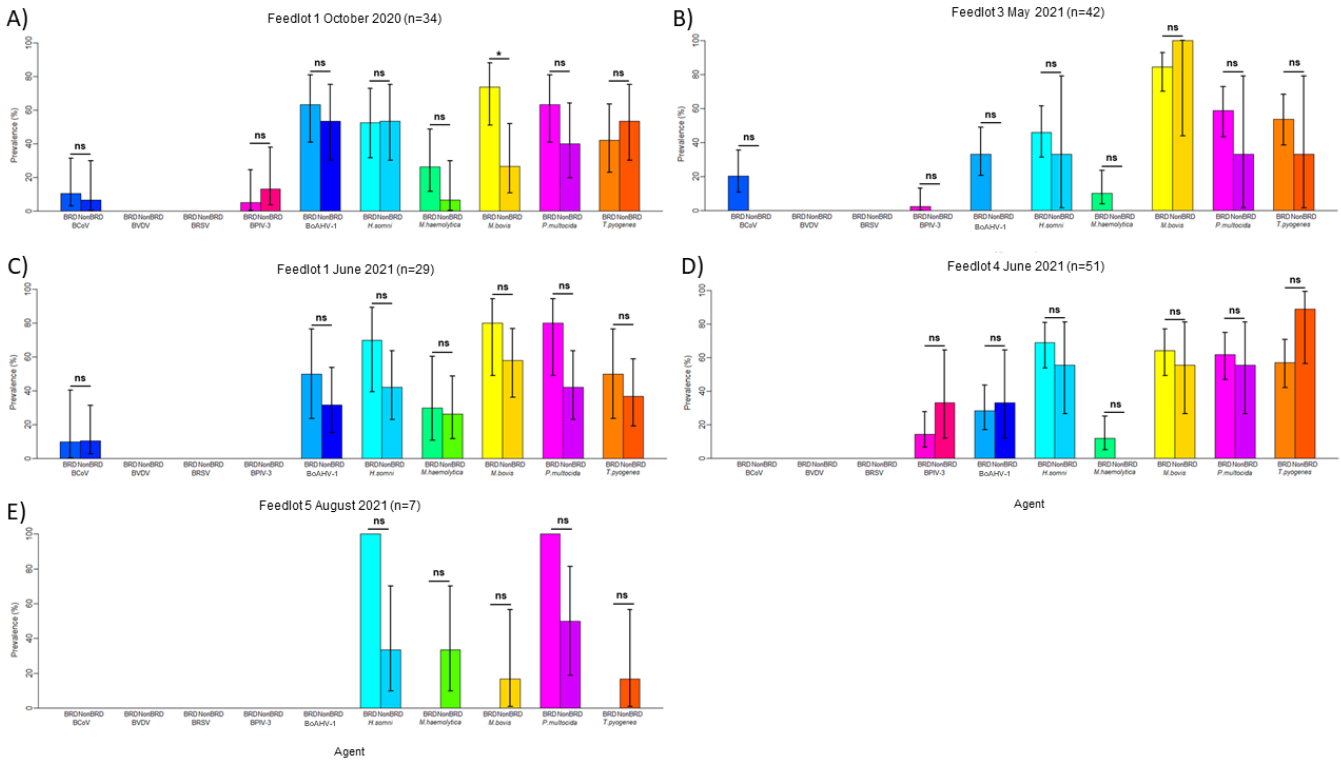


Figure 23. Prevalence (% +/- CI) of BRD associated DNA and RNA agents from nasal swabs collected from animals presenting to the hospital pen for treatment by location. Feedlot 1: BRD n = 19; Non-BRD, N = 16; Feedlot 2: BRD n = 39, Non-BRD n = 3; Feedlot 3: BRD n = 10, Non-BRD n = 19; Feedlot 4: BRD, n = 42, Non-BRD n = 9; Feedlot 5: BRD n = 9, Non-BRD n = 6. Fischer's exact test: ns, not significant; * p ≤ 0.05.

Abbreviations: BCoV = Bovine coronavirus; BVDV = Bovine viral diarrhea virus; BRSV = Bovine respiratory syncytial virus; BPIV-3 = Bovine parainfluenza virus 3; Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

There were no significant differences between treatment groups (Fig. 24) in terms of agent concentrations. Further, when agent concentration was considered by agent, location and date of testing (Fig. 25), no significant differences were observed. It is possible that this outcome is due to the relatively low number of agents, and large concentration range, observed within and between cohorts. Similar to induction cohorts (Fig. 7), there a trend to lower concentration ranges for *P. multocida* and *H. somni* during 2019 collections at Feedlot 1 (Fig. 25), compared to 2020 and 2021 which may be evidence of a seasonal effect for these agents.

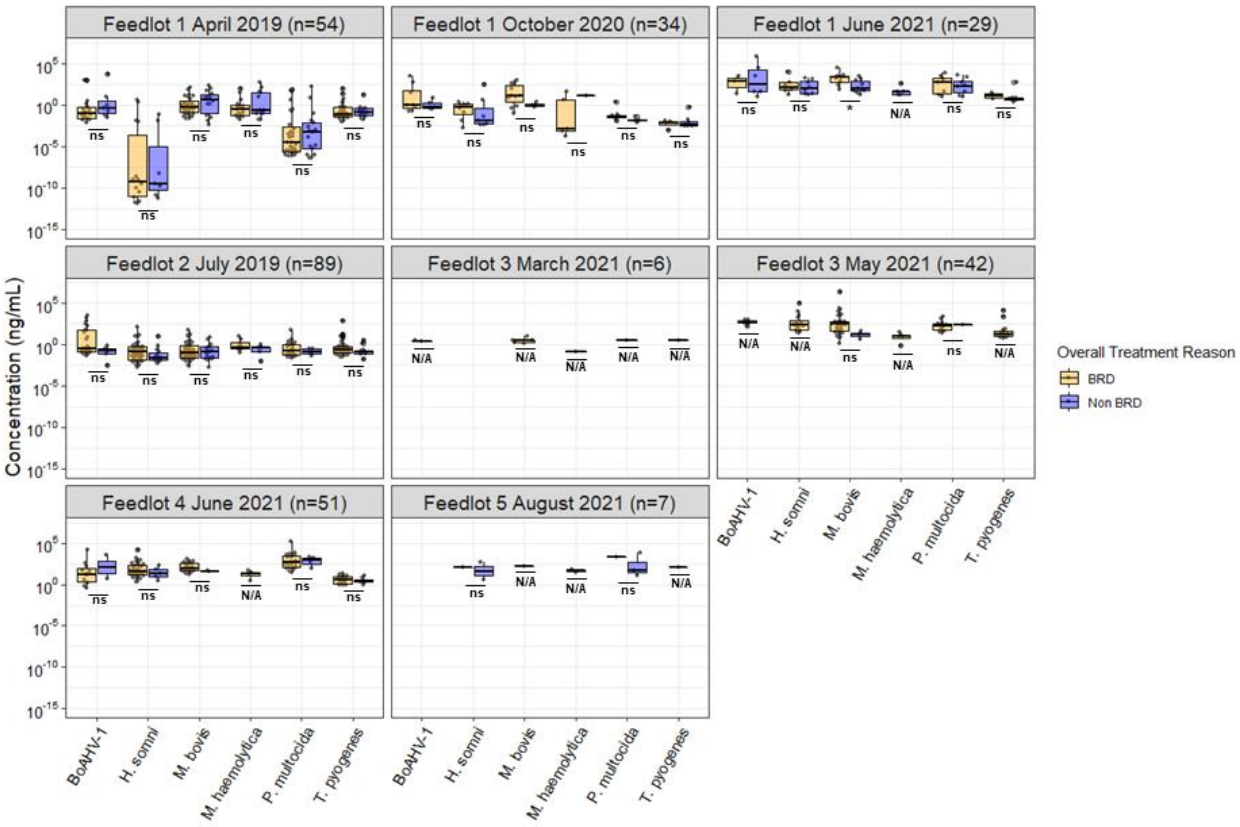


Figure 24. Absolute concentrations of agents commonly associated with bovine respiratory disease from nasal swabs collected from hosptial pen animals treated for bovine respiratory disease, grouped by feedlot location, sorted by BRD risk period. Wilcox rank sum test: ns, not significant; * p ≤ 0.05.

Abbreviations: BoAHV-1, Bovine a-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

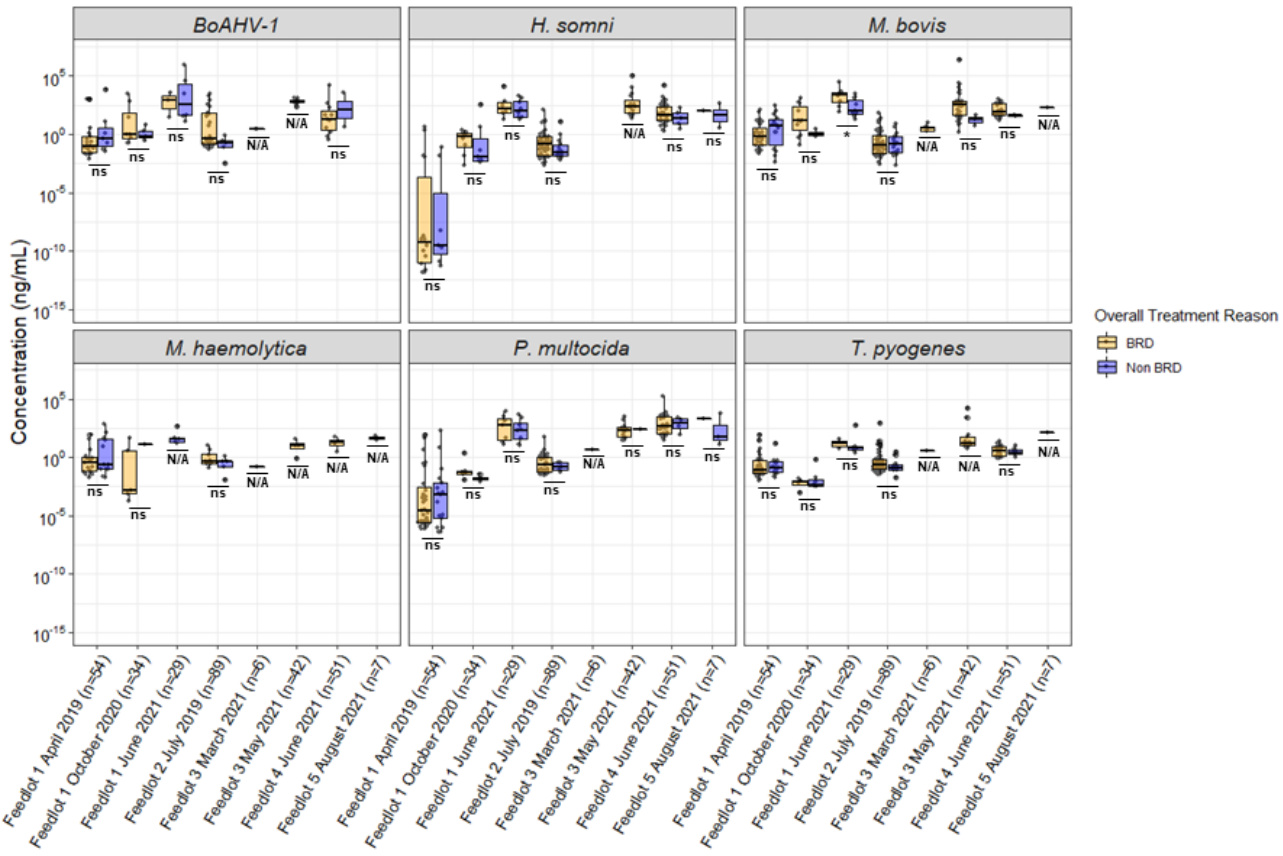


Figure 25. Absolute concentrations of agents commonly associated with bovine respiratory disease from nasal swabs collected from hosptial pen animals treated for bovine respiratory disease, grouped by agent and collection date. Wilcox rank sum test: ns, not significant; * p ≤ 0.05.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

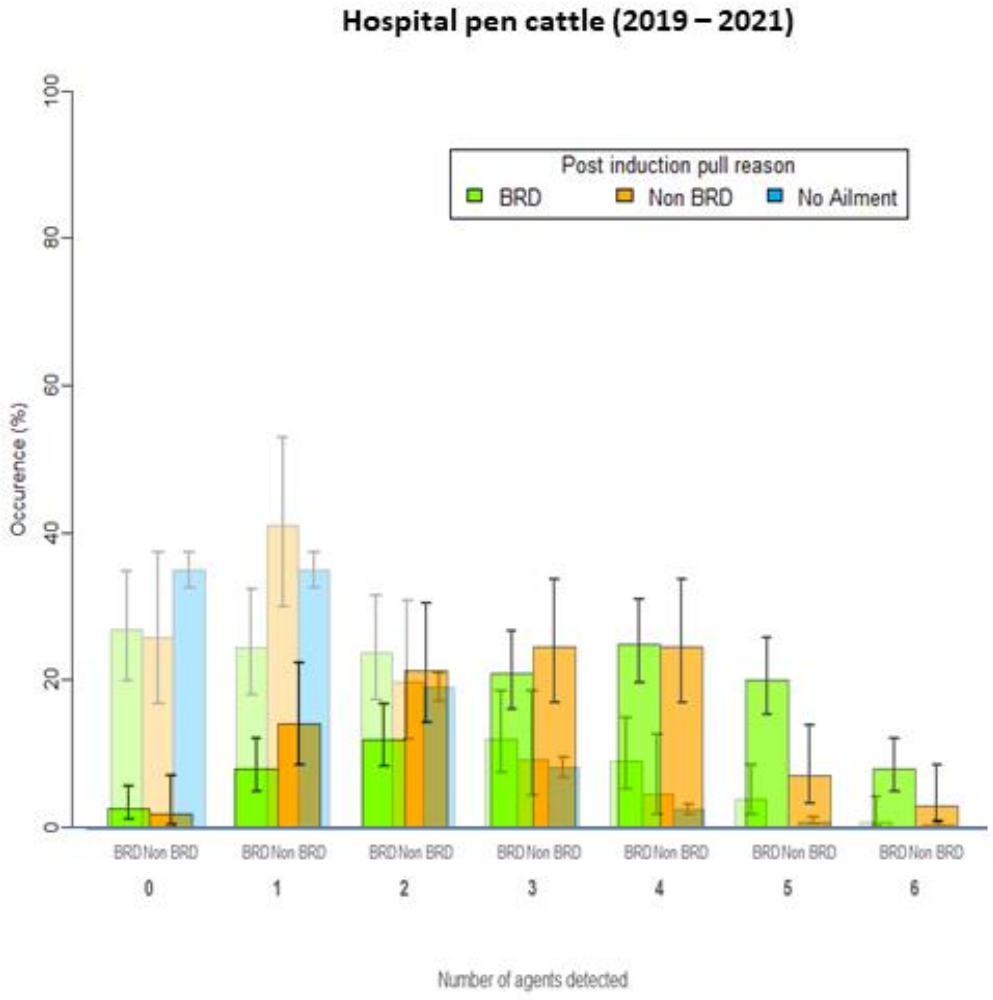


Figure 26. The occurrence of the number of agents detected in hospital pen animals sampled between 2019 -2021, grouped by ailment; BRD (n=217) or Non BRD (n=95). Data for induction (Day 0) animals is shown underneath for comparison.

When occurrence of agents within a single animal was considered relative to treatment reason, few animals (3-5%) in the hospital pen had no detectable agents present, compared to between 24-36% of those sampled in concurrent induction (Day 0) cohorts, with more animals identified with 3 or more agents. This right shift towards a greater number of agents identified per animal was more pronounced than that observed between Day 0 and Day 14 as previously described (Fig. 8).

Concentration of agent was considered relative to treatment reason compared to agent combination by location. As inferred from occurrence data, more individual animals presented with concurrent identification of more agents, with few agents being undetectable relative to induction cohorts, however, the number of cattle with high loads was not significantly altered nor was there an obvious trend towards a particular agent or agent combination being present at high load. Overall, *M. haemolytica* was detected less frequently at all locations, with no significant increase in numbers of cattle with high concentrations (Fig. 27), whilst more individual animals with moderate concentrations of *M. bovis* were observed at Feedlot 1 (Oct 2020, Fig. 6) and Feedlot 4 (June 2021,

Fig. 27A) compared with other locations. Feedlot 4 (Jun 2021) also appeared to have more cattle with a moderate load of *T. pyogenes* than was observed in other locations (Fig 27B) with Feedlot 3 showing more animals with high concentrations of BoAHV1 than others (Fig. 27B). Although the overall numbers of animals sampled from the Feedlot 3 hospital pen (March 2021) and Feedlot 5 hospital pen (August) was low compared to other locations, these showed the greatest proportion of cattle with high concentrations across all agents tested by location (Fig. 27B).

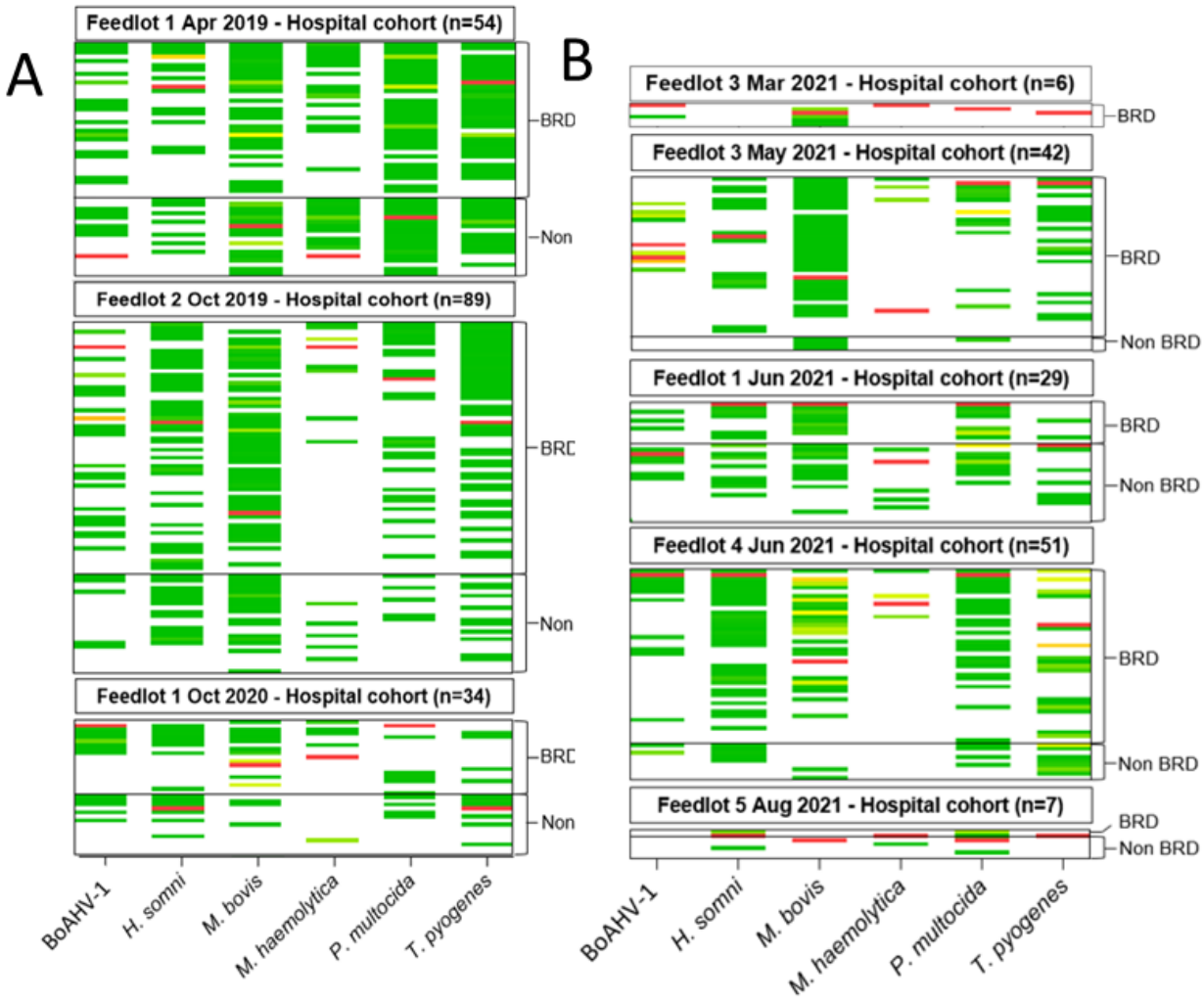


Figure 27. Upper airway agent load determined by EC quantification, of individual animals from hospital cohorts from two Australian feedlots sampled during A) 2019 and 2020 and B) 2021. Heatmap sorted based on pull reason and the number of agents present per animal (most to least) and grouped by feedlot location Normalised absolute agent load is on a colour scale for each agent; Red – high agent load, Yellow – medium agent load, Green – low agent load, White – agent not detected or not quantifiable.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

Figure 28. Frequency of agent combinations in hospital pen animals sampled between 2019 and 2021, grouped by overall treatment reason. Agents are ordered by cumulative number with the number of animals with the greatest number of agents at the top, and those with the fewest or no agents at the bottom of the chart.

Abbreviations: BoAHV-1, Bovine α-herpesvirus 1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes

To investigate whether specific combinations of agents were occurring with greater frequency in hospital pen cattle, agent combinations were considered by treatment group across all locations (Fig. 28). Hospital pen cattle were not selected by treatment, but simply by presentation to the hospital crush. This analysis showed several combinations at Feedlots 1 and 2 to be more associated with treatment for BRD than 'other' ailments specifically: Feedlot 1 April 2019: BoHV1 + HS + MB + MH + PM + TP, 13 animals, and BoHV1 + HS + MB + PM + TP: 5 animals, and BoHV1 + MH + MB + PM + TP: 4 animals; Feedlot 2 July 2019: BoHV1 + HS + MB + PM + BPIV3: 10 animals; BoHV1 + HS + MB + TP: 6 animals; BoHV1 + HS + MB: 5 animals. At Feedlot 4 (June 2021), the combination of HS + MB + PM + TP: 5 animals) was the most frequent combination. Feedlot 3 was the only location where individual cattle were noted to be only detected with a single agent (HS, 3 animals). Generally, BRD-treatment cattle tended to be more prevalent, and show more combinations of agents present that their non-BRD treatment counterparts and in many locations were the majority of cattle tested in the hospital pens.

4.4 Bayesian modelling of cohort demographics, BRD agent and disease

4.4.1. Network models

Two hybrid Bayesian Network (BN) models were generated: one for animals sampled at induction, and one for animals sampled at presentation in the hospital system. As probabilistic, graphical models, BNs allow for modelling of complex interdependencies, such as occur with syndromic diseases such as bovine respiratory disease, where there are multiple influencing factors. The model defines the optimal way to categorise known and unknown interdependencies based on the information provided to the model. In this case, the two hybrid models contained a series of independent and dependent nodes, Identified by colour in the two BN models (Figs 29 and 31). The following categorical domains were established; location dependent variables, induction and lifetime on feed data, environmental data, induction treatment data, carcass data and BRD agent data. Interdependencies between the nodes are shown, with all hierarchies for BRD agents feeding to the top-level hierarchy of 'Number of agents'. The central node in each BN model is identified in pink.

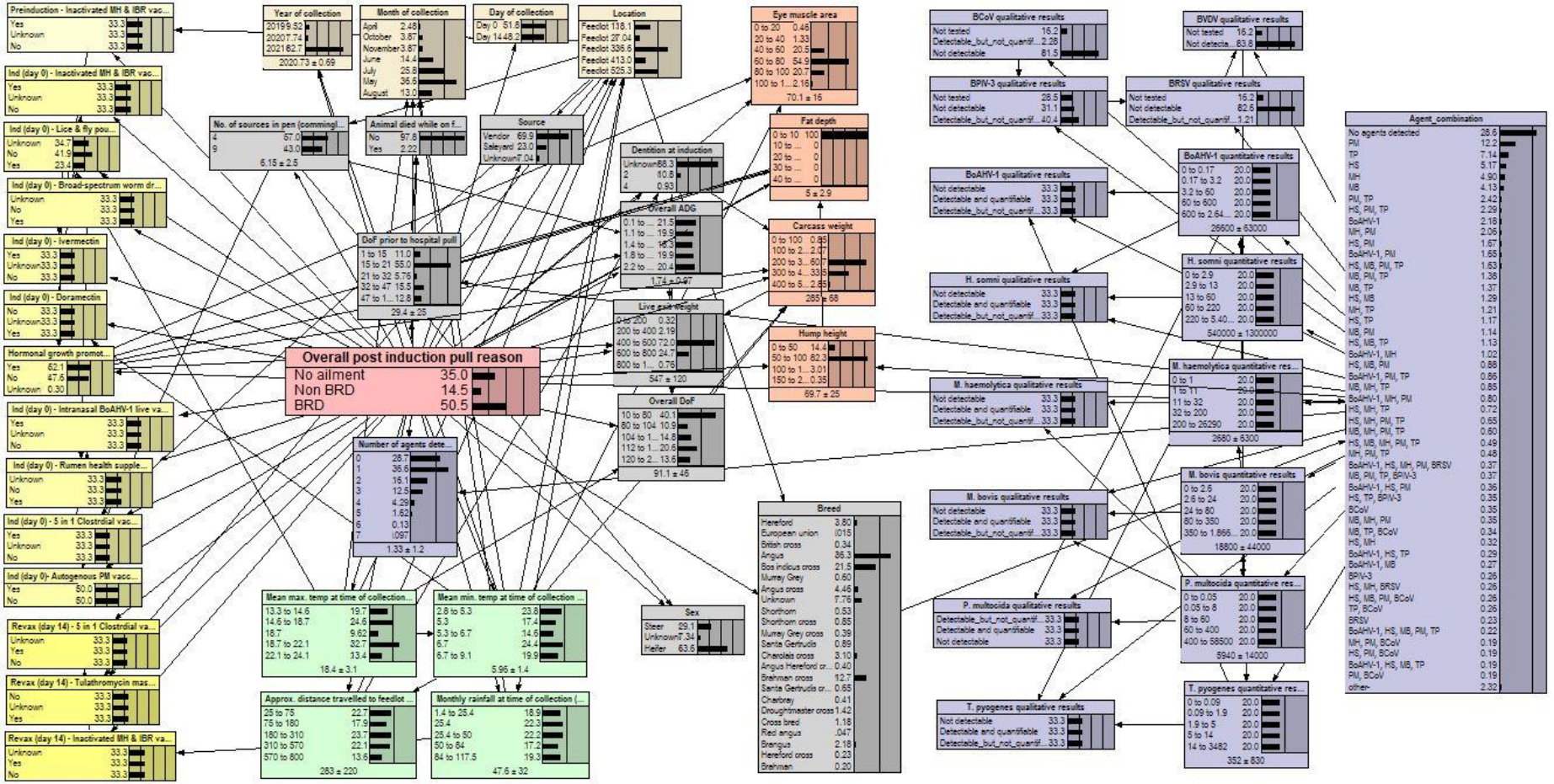


Figure 29. Schematic representation of the BN hybrid induction model. The following categorical domains are identified by colour: location dependent variables (light yellow), induction and lifetime on feed data (grey), environmental data (green), induction treatment data (yellow), carcass data (orange), BRD agent data (purple) and post-induction pull reason (pink). No node selected.

4.4.2. Induction model

When interdependencies between the major nodes related to pull reason was considered for the induction model, the following findings were apparent.

4.4.2.1. Induction cattle 'No ailment'

When pull reason was considered, unsurprisingly the 'no ailment' group was the largest of all groups represented in the induction cohort. When this category was considered, the majority of animals contributing to this finding were located at Feedlot 1 (44.6%), 2 (20.1% and Feedlot 5 (35.3%). The majority of animals had no pathogens or 1 pathogen detected (29% and 51.1% respectively, mean pathogens detected: 1.1 ± 0.95), with steers contributing at a higher rate than heifers (44.7% and 34.3%).

Exit liveweight was between 440-800kg, mean 587kg ± 120kg, with an average of 111 days on feed. Most of these animals were HGP-free (99.1%). The category of 'no agents detected' contributed the largest influence (28.9%) with *Pasteurella multocida* (20.9%), *Histophilus somni* (10.3%), *Trueperella pyogenes* (5.43%) and Bovine Alpha Herpes Virus 1 (5.38%) ranked highest among the agent combinations detected (Figure 28). Agent concentration did not exert a significant effect.

4.4.2.2. Induction cattle progressing to disease: 'Non-BRD'

When 'non-BRD' was considered as the pull reason for animals sampled at induction, the majority of animals contributing to this category were located at Feedlot 5 (53%) with Feedlots 1, 3 and 4 showing similar proportions (17.1%, 16.2% and 13.7% respectively). The number of agents detected increased slightly to a mean of 1.43 ±1.3, with most animals showing 0-2 pathogens detected.

Exit liveweight ranged again between 440-800kg but most animals fell within the 400-600kg category, without a significant impact on mean live exit weight (583 ± 150kg), with an average of 94 days on feed. There were equal proportions of HGP-free (53.0%) and HGP-treated cattle (47%). Angus and Hereford breeds contributed equally to exit liveweight without other significant breed representation.

Similar to the 'no ailment' cattle, the category of 'no agents detected' (30.1%) contributed the largest influence to induction cattle pulled for 'other' treatment reasons with *P. multocida* (9.2)%, BoAHV1 plus *P. multocida* (6.27%) and *Mycoplasma bovis* (4.41%) identified as the top four combinations (Figure 29). Agent concentration did not play a significant role.

4.4.2.3. Induction cattle progressing to disease: 'BRD'

Bayesian Network modelling showed a similar pattern for cattle sampled at induction and pulled for BRD compared with cattle pulled for other reasons. Most cattle contributing to this category were located at Feedlot 3 (67.9%) with Feedlots 4 and 5 exerting the remainder of the effect (21% and 10.3% respectively). The number of agents detected were greater, similar to the findings with 'Non-BRD' cattle, with a mean 1.52 ±1.4, with most cattle showing 0-3 pathogens detected. This right shift included a small contribution of cattle with 4 or more pathogens detected at induction compared to the other groups (4 or more pathogens: 'No ailment 0%; Non-BRD, 5.4%; BRD, 9.86%) (Figure 30).

In this category, the majority of cattle sampled at induction that had been pulled for BRD during time on feed, showed a lower exit live weight range with nearly all animals showing between 440-600kg as

live exit weight (90.4%). Mean live exit weight was reduced compared to the other two groups showing a mean of 509kg ± 86kg. The majority of BRD pulls had received HGPs, an effect likely linked to their location. Angus and *Bos indicus* X contributed equally to this outcome without other significant breed representation.

Similar to the previous categories, when agent combination was considered 'no agents detected' contributed the largest influence to this group (28.0%), whilst in this category *Trueperella pyogenes* (10.5%) exerted greater influence than *P. multocida* (7.15%), with *Mycoplasma bovis* (5.87%) and the the combination of *H.somni* , *P. multocida* and *T. pyogenes* (4.25%) as the fifth greatest influence. As before, concentration did not play a significant role (Figure 30).

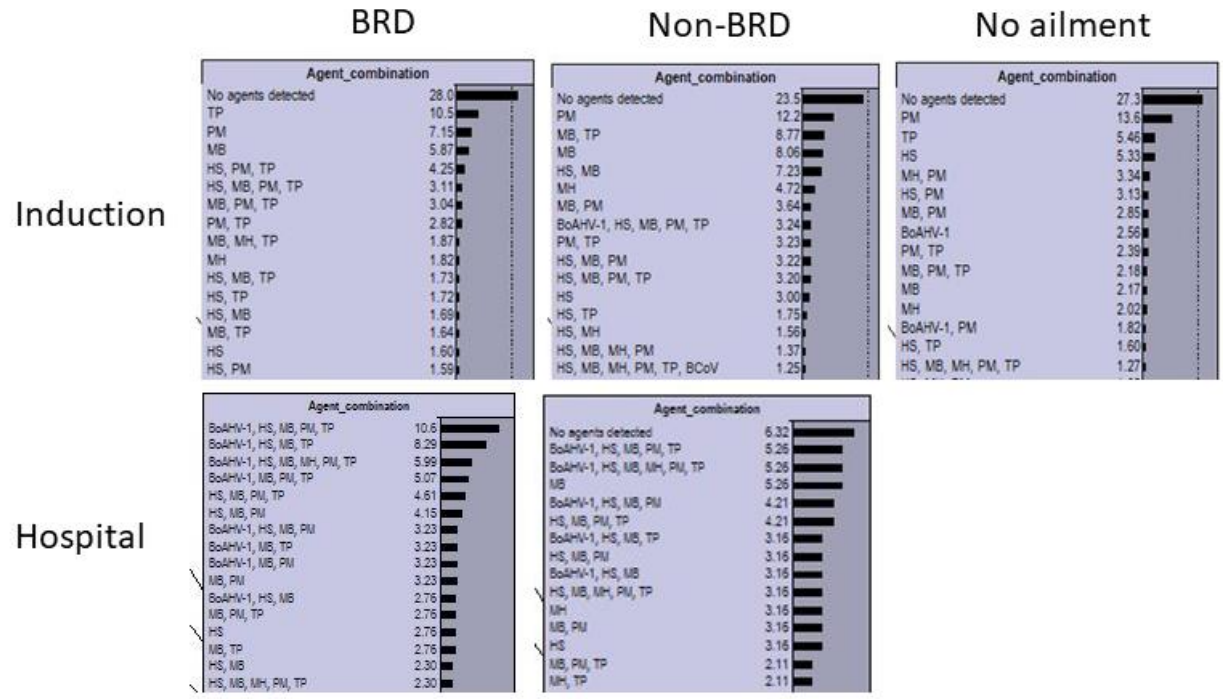


Figure 30. Agent combination nodes showing categories and % for induction and hospital-sampled feedlot steers relative to the categories of 'BRD', 'Non-BRD' or 'No ailment'. For induction cattle, BRD or Non-BRD identifies cattle that were pulled and treated for an ailment during their time on feed; for hospital cattle the categories apply to identified reason for treatment in the hospital pen.

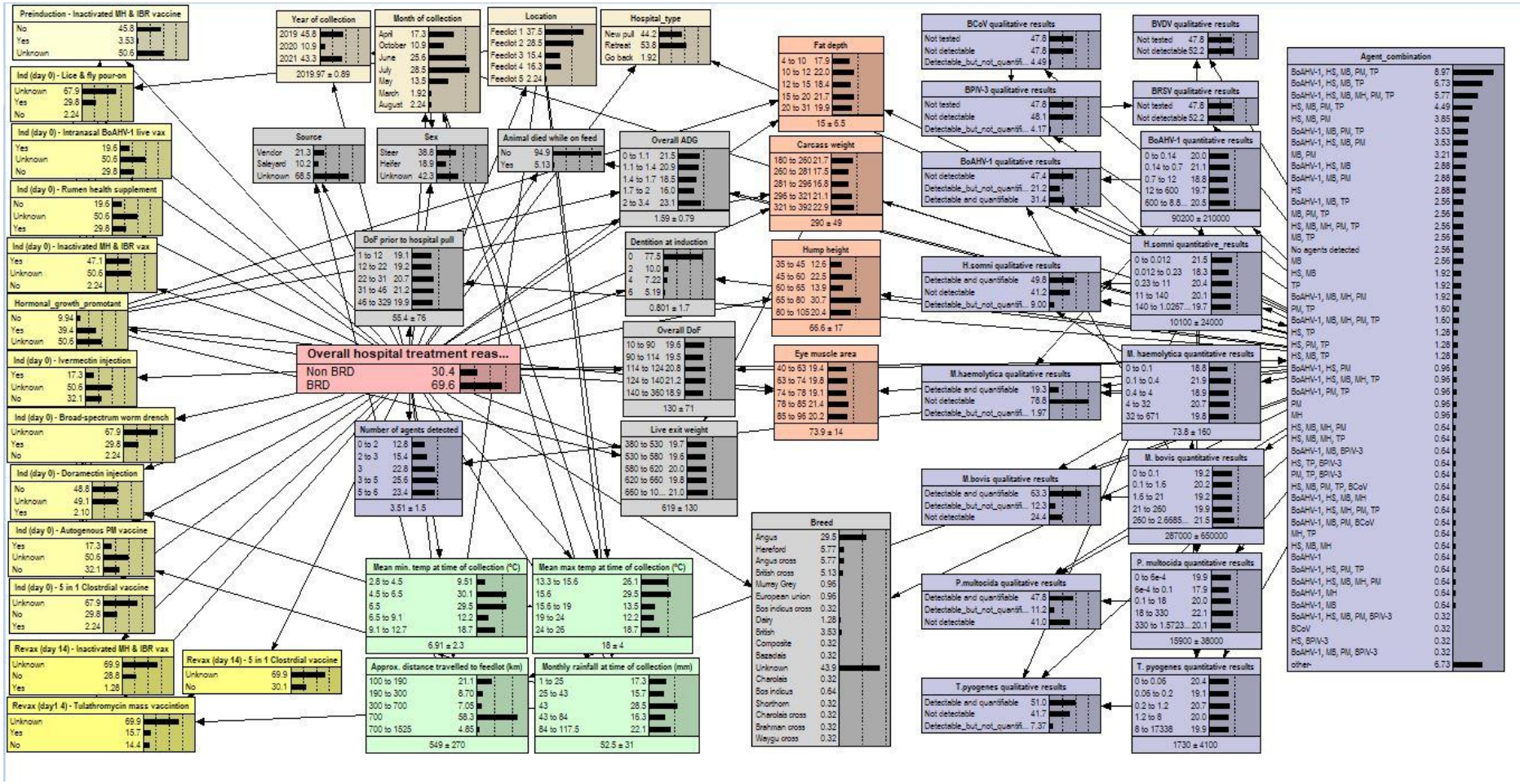


Figure 31. **Schematic representation of BN hybrid Hospital model. The following categorical domains are identified by colour: location dependent variables (light yellow), induction and lifetime on feed data (grey), environmental data (green), induction treatment data (yellow), carcass data (orange), BRD agent data (purple) and post-induction pull reason (pink). No node selected.**

4.4.3. Hospital Bayesian Network model

A hybrid model was also developed to consider the interrelationships between categories for animals already identified as sick and therefore presenting at the hospital pen. When interdependencies between the nodes were considered, the following findings were apparent (Fig. 31).

4.4.3.1. Hospital cattle treated for 'Non-BRD' reasons

Most cattle contributing to the category of hospital cattle treated for non-BRD ailments were located at Feedlot 1 (54.7%) with less influence from Feedlot 2 (26.3%) compared with the other 3 locations. Retreats were represented in slightly higher proportion by this analysis (new pulls: 40.0%; retreats 60.0%).

The majority of non-BRD ailment cattle were categorised as attaining a live exit weight of 662-660kg as live exit weight (mean live exit weight 619 ± 120kg), a weight very similar to their induction sampled counterparts and a relatively small influence was exerted by HGPs (21.2%). Angus was the greatest breed influence (32.6%) although a small number of other breeds were identified in this category (Hereford, 3.16%; Other British breeds 8.2%). *Bos indicus* cattle did not contribute to this outcome.

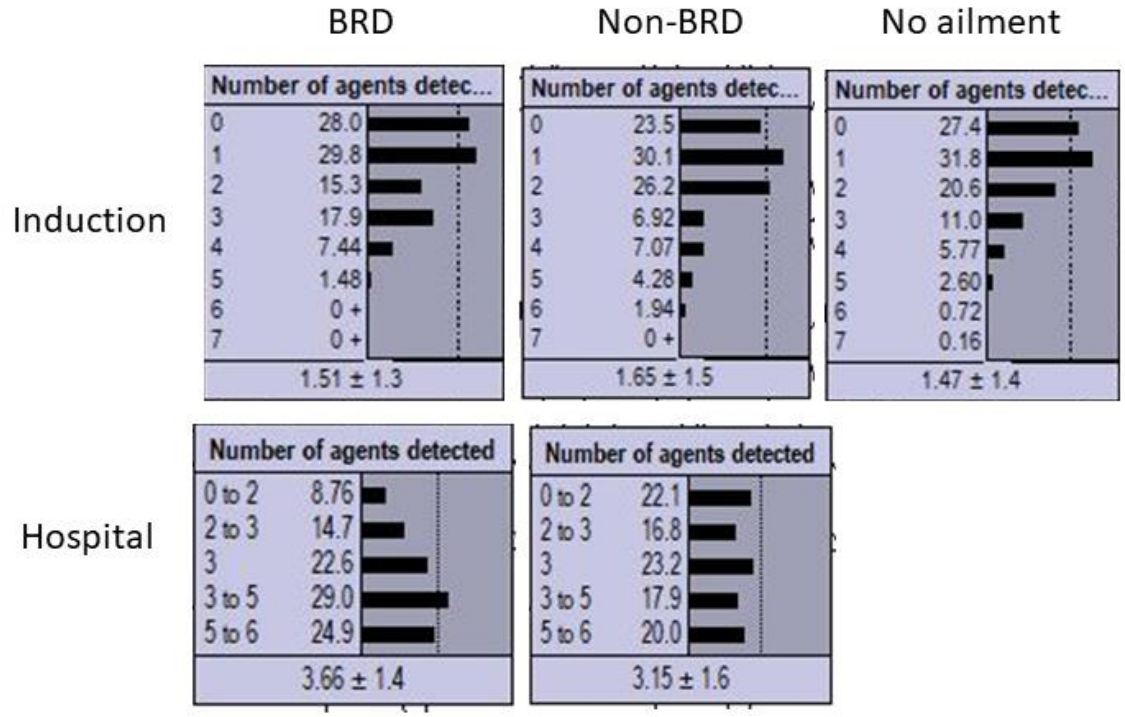


Figure 32. Agent number nodes showing categories and % for induction and hospital-sampled feedlot steers relative to the categories of 'BRD', 'Non-BRD' or 'No ailment' for number of agents detected. For induction sampled cattle, BRD or Non-BRD identifies cattle that were pulled and treated for an ailment during their time on feed; for hospital cattle the categories apply to identified reason for treatment in the hospital pen. Mean number of agents with SD is shown at the bottom of each node.

When the number and combination of agents were considered, the mean number was increased compared to induction sampled cattle showing a mean of 3.15 ±1.6 agents and equal distribution

across all categories (0-6 agents). The influence of 3 agents or more was 54% with 20% of cattle in the cohort identified with 5-6 agents (Fig. 32).

The 'no agents detected' category exerted the greatest effect on this group, but its effect was less compared with induction sampled cohorts (6.32%). Combinations of BOAHV1 + *M. bovis* + *P. multocida* + *T. pyogenes* + *M haemolytica* contributed 10.62% and *M. bovis* alone contributed 5.56% of the effect (Fig. 30). As before, concentration did not exert a significant effect on the finding.

4.4.3.1. Hospital cattle treated for 'BRD'

Of hospital cattle treated for BRD ailments, cattle contributing to this category were located at Feedlot 1 (30.0%), Feedlot 2 (29.2%), Feedlot 3 (20.7%) and Feedlot 4 (19.4). Feedlot 5 exerted no influence on this finding. New pulls and retreats were equally represented (new pulls: 46.1%; retreats 51.2%).

BRD hospital cattle showed the widest variation in live exit weight with cattle contributing equally to all weight categories (380-530; 530-580; 580-620 and above 620kg). Despite this range, mean live exit weight was not different to hospital non-BRD ailment cattle, with a mean of 619 ± 140kg. HGP treated animals exerted 39.4% of the influence. Younger cattle were more represented with 83.3% of BRD-treated animals identified as 0-tooth compared to 64.3% of 'other' ailment hospital animals.

Angus was the major breed influence (28.1%) and again, a small number of other British breeds were identified in this category (16.59%). *Bos indicus* was not identified to be a major contributor to this finding.

When the number and combination of agents was considered, the mean number was also increased compared to induction sampled cattle showing a mean of 3.66 ± 1.4 agents with distribution weighted towards the higher agent number categories (3-6 agents) with 24.9% of cattle in the cohort identified with 5-6 agents.

Dissimilar to all other groups examined in both the induction and hospital models, agent combination was not a major influence. The greatest influence was exerted by BOAHV1 + *M. bovis* + *P. multocida* + *T. pyogenes* + *H. somni* in combination, which contributed 10.6% of the effect with the combination of and BOAHV1 + *M. bovis* + *P. multocida* + *T. pyogenese* as the next most influential combination (8.29%). BOHV1, *M. bovis*, *H. somni,* T. *pyogenes* and *P. multicida* appear in combination in all the top 5 combinations, with BoAHV1 appearing in 8/10. As before, concentration did not exert a significant effect on the finding for any agent examined although quantitation and detection were influential for both *M. bovis*, *T. pyogenes* and *P. multocida* with this analysis (Fig. 32).

4.4.4. Sensitivity analysis

To determine the key data influencing outcomes in the dataset, establishment of hierarchical criteria within the Bayesian Network model was applied and reason for treatment in either cohort (induction or hospital) identified as the critical node in the model. Sensitivity analysis allows the ranking of other interrelated or independent variables regarding a selected target variable. In this case, influencing variables were considered alongside unknown interrelationships to determine the level of influence on a disease finding in either the induction or hospital cohorts (Norsys software Corp 2021) (Tables 8 and 9). Agent combination and location were found to have the greatest effect on post induction pull reason for induction sampled animals, exerting 11.9% and 9.22% of the overall effect respectively (Table 8). While for the hospital animals approximate distance travelled to the feedlot and agent

combination had the greatest effect on overall hospital treatment reason, exerting 32.9% and 25.1% of the effect respectively (Table 9). Although the ranking of various induction treatments is highlighted by this analysis, this is likely to be correlated with location and therefore confounded.

Table 8. Sensitivity to findings for Induction BN hybrid model of overall pull reason post induction for feedlot cattle sampled at feedlot induction. Mutual information is a measure of the co-dependence between two random variables.

Node	Mutual information	%
Overall pull reason post induction	0.62926	100
Agent combination	0.07489	11.9
Location	0.05799	9.22
Induction Day 0 _ broad spectrum antibiotic	0.05722	9.09
Induction Day 0_Rumensin	0.05722	9.09
Induction Day 0_BHV1 vaccine	0.05721	9.09
HGP	0.05146	8. 08
Breed	0.04812	7.65

Table 9. Sensitivity to findings for Hospital hybrid BN model of overall treatment reason for feedlot cattle sampled from the hospital pen. Mutual information is a measure of the co-dependence between two random variables.

Node	Mutual information	%
Overall hospital treatment	0.88671	100
Approximate distance travelled	0.29207	32.9
Agent combination	0.22266	25.1
Induction Day 0 _ 5 in 1 vaccination	0.11433	12.9
Induction Day 0 _ broad spectrum antibiotic	0.11433	12.9
Induction Day 0_ insecticide treatment	0.11433	12.9
Induction Day 0_Rumen modifier	0.09990	11.3
Induction Day 0_ BHV1 vaccine	0.09990	10.8
Location	0.9562	9.03

Together the BN model analysis indicates that location and agent combination exerted significant effects for cattle sampled both at induction and at hospital processing, whilst only BRD-hospital treatment animals showed a strong relationship with multiple agent combinations. Other factors, such as induction protocol, are likely strongly linked to location, rather than being causal to disease outcomes. When agent combinations were considered, BoAHV1 in combination appears to be exerting a significant effect from this analysis being identified in 8/10 of the top combinations associated with BRD-treated animals. Combinations of greater than 3 agents for animals sampled at

induction, and 4 agents for animals sampled in the hospital pen were potentially indicative of disease risk or active infection.

4.5. Comparative analysis of BRD diagnostic testing options

4.5.1. Definition of an optimum test panel

Analysis of prevalence in 2021 Day 0, Day 14 and hospital pen data reported in this study indicated that a test panel using a two-pass approach may give optimal efficiency for epidemiological analysis of BRD risk in feedlot cattle. To achieve testing at low cost and scale, a panel of BoAHV1, BCoV, BPIV3, BRSV, *H. somni, M. haemolytica, M. bovis, P. multocida*, and *T. pyogenes* has been identified to be optimal for disease risk correlation. This can be split into two tiered assays, with BHV1, *H. somni, M. haemolytica, M. bovis, P. multocida*, and *T. pyogenes* performed as a base assay (two reactions) and BCoV, BPIV3, BRSV tested dependent on data requirements, cost and location (Table 10).

The rationale for this approach is as follows: Bovine α-herpesvirus 1, *H. somni, M. haemolytica, M. bovis, P. multocida*, and *T.pyogenes* consistently showed the highest prevalence across all feedlots tested in both home pen animals and those referred to the hospital pen. These five organisms are likely contributing to the disease risk profile at all locations tested. Equally, Day 14 on feed and hospital pen show similar prevalence profiles in all locations tested although only BoAHV1, BPIV3 and BCoV were identified in hospital animals, and not at all locations. Largescale testing has not shown analysis of viral agents, other than BoAHV1, to be informative in this project relative to disease risk or management practice.

Table 10. Optimal multiplex test panel for risk analysis and management of bovine respiratory disease in Australian feedlot steers.

Panel	Test Number	Microorganisms
Multiplex	Multiplex 1	Bovine α-herpesvirus 1 *Histophilus somni* *Trueperella pyogenes*
	Multiplex 2	*Mannheimia haemolytica* *Mycoplasma bovis* *Pasteurella multocida*
Singleplex (optional / bespoke)	3	Bovine Corona Virus
	4	Bovine Parainfluenza Virus 3
	5	Bovine Respiratory Syncytial Virus

BCoV, BPIV3 and BRSV show significantly lower prevalence than all bacteria and BoAHV1, indicating that these might contribute to a lower risk profile than the other microorganisms tested. The value of testing for viral load of these organisms may only be necessary for epidemiological analysis and might therefore be less relevant for management decision making. This is particularly the case for viruses for which no vaccine currently exists, or where treatment with antivirals or steroids is not an option.

Bovine Viral Diarrhoea Virus was not detected in nasal swabs taken from any animal at any location and therefore utility of this test in this setting is unproven by our analysis.

4.5.2. Current diagnostic options for the beef feedlot industry

Current diagnostic testing modalities for identification of disease-causing organisms in the animal industries consist of four main options: serology, microbiological culture, conventional PCR and real-time PCR (Figure 32). Although certain methods are available, the utility of these is variable and their application to diagnosis and analysis of disease risk in feedlots have been variable.

Other advanced diagnostics are also in existence and are comparable to those more routinely used in human clinical medicine (e.g. whole genome sequencing, MALDI-ToF, metabolomic analysis using Liquid Gas Chromatography and Mass spectroscopy Time of Flight or other high end analytical techniques) but these have largely not made their way into routine use in the feedlot sector due to limited availability and high cost of use. Other, less invasive and faster diagnostic modalities are more likely to be useful in disease risk management and prevention.

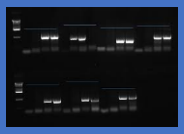
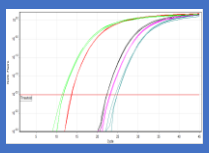
					
Method	**Serology**	**Culture**	**Conventional PCR**	**Next Generation Sequencing**	**Real time PCR**
Detects	Antibodies / antigen	Organism	Organism	Organism (genus and species)	Organism (Species)
Sensitivity	Variable	High	Medium	High	High
Specificity	Medium – High	High	High	High	High
Cost	Low	High	High	High	High
Speed (off-site)	Slow-Medium	Slow – Medium – Fast (organism dependent)	Medium	Medium	Medium
Speed (on-site)	N/A	N/A	N/A	N/A	Same day
Suited to BRD diagnostics	Undetermined	Useful post-mortem	May be useful post-mortem	Currently useful research only	Highly suited

Figure 33. Current testing options for disease microorganism identification for bovine respiratory pathogens and other disease-causing agents.

4.5.3. Scalability of current molecular diagnostic approach compared to existing options

The technology we have chosen for this project satisfies three critical criteria (1) that Biomolecular Systems is an Australian owned and operated company and the Mic and Myra technology (Fig. 34) is Australian designed and manufactured; (2) the Mic and Myra are fully relocatable without the need for expensive recalibration and, (3) the scalability of the technology lends itself to a range of enterprises whilst requiring minimal operator changes. The scalability capability allows for increased testing capacity by the addition of mic and Myra units as described in Fig. 35. This operation can be as simple as a single operator, e.g. local veterinary practice, consultant, small scale operation, to the large scale analytical laboratory or large feedlot operation. However large or small the scale, the footprint required to run this technology remains very small by comparison to other industry standard technology.

Figure 34. Top panels: Mic thermocycler being used directly in the induction facility. Bottom photograph Mic thermocycler x4 (top left) and Myra liquid handling x1 (bottom right) platforms located in the mobile laboratory shown.

4.6. Considerations for on-site / off-site platform approaches for PCR-based rapid diagnostics for the feedlot industry

To interrogate options for use of PCR-based rapid diagnostic platforms for use in the Australian feedlot sector, several approaches were applied.

1. Onsite or closely co-located testing was undertaken in a commercial setting;
2. Comparative analysis of alternative testing paradigms was considered, and;
3. A scalable platform approach was considered where use models were applied between different professions and sectors (feedlot / local provider / regional provider).

Tests	Details	
No. of cattle tested	40 per run	
No. of tests per animal	2	
No. of PCR tubes per animal	2	
No. of pathogens tested	6	
Test 1 (Multiplex)	1 Viral, 2 bacterial and 1 endogenous control	
Test 2 (Multiplex)	3 bacterial and 1 endogenous control	
Test Protocol	**Details**	**Operator time**
No. Operators	1	
DNA extraction	20 mins	20 mins
PCR reaction preparation Automated reaction preparation Automated sample addition	20 mins	5 mins
PCR	75 mins	5 mins
Analysis		20-30 min
Total time per run	2 hrs	1 hr
Scalable	**Details**	**Samples per 2 hours**
Option 1 (1 operator)	1 Liquid Handling station 2 Thermocyclers	First run – 40 Subsequent runs – 40
Option 2 (1 operator)	1 Liquid Handling station 4 Thermocyclers	First run – 40 Subsequent runs – 80
Option 3 (2 operators)	2 Liquid Handling station 4 Thermocyclers	First run – 80 Subsequent runs – 80
Option 3 (2 operators)	2 Liquid Handling station 8 Thermocyclers	First run – 80 Subsequent runs – 160
Deployable		
Can entire protocol be deployed in field	Yes The entire protocol can be run identically in field or in the laboratory	
Results	**Field**	**Laboratory**
Qualitative (presence absence)	Yes	Yes
Semi-Quantitative (standard curve)	Yes	Yes
True Quantitative (efficiency corrected single point calibration)	Yes (only platform at present to do this) Critical for accurate differentiation of disease or expression at high Cq values	Yes (only platform at present to do this) Critical for accurate differentiation of disease or expression at high Cq values

Figure 35. Scalability of Mic thermocycler and Myra liquid handling platforms relative to number of tests and operators.

The process used in this study was configured in alignment with a 96 well plate preparation protocol, giving rise to 40 animal samples per thermocycler (x 2) plus controls. It was also based on a minimum

of 2 and maximum of 4 thermocyclers in use at any one time. The time taken to deliver results to the user / producer was maximised when an in-field testing paradigm was used. In this case, time from sample collection to testing is minimised with an increased number of operators / thermocycler units (Figure 35), returning a result on which a management decision can be made in the shortest timeframe. On-site testing requires 1-2 operators depending on the throughput required, where individual runs of 40 animals can be included at one time, with subsequent runs either occurring sequentially (one operator) or in parallel (two operators). Where additional thermocycler units are deployed, the number of animal samples considered within a specific timeframe can be increased (Figure 35, see also Figure 1).

4.6.1. On site analysis – the mobile laboratory

On-site testing analysis and establishment of a single step DNA extraction technique in 2019 validated that pen data could be returned to the feedlot manager on the same day as feedlot induction. To further validate this approach a second on-site trial sample analysis was planned for October 2020. A necessary change in planning was required due to the widespread impact of COVID19 at that time which severely limited access to all commercial locations due to significant safety concerns related to the spread of COVID19 across regional Australia. To overcome this limitation, we recruited assistance from Gene Target Solutions, the Australian distributor of the Mic PCR hardware, who allowed the team use of their mobile 'laboratory' (Fig. 34, 36). This approach enabled the research team to co-locate very close to the collection site (<2km distance) to allow fast 'on-site' sample processing on the date of collection.

Figure 36. Mobile 'laboratory' containing molecular testing platform for on-site sample processing and analysis. This mobile facility was used on-site co-located to a commercial feedlot in 2020.

The mobile laboratory contained four Mic thermocyclers, one liquid handling station and two preparation hoods to allow the full sample process and analysis pipeline from DNA extraction to sample analysis and reporting.

4.7. Bio-economic analysis

4.7.1. Biophysical model data

The multi-state Markov model generated biophysical data outputs which aligned with parameterisation variables. The average induction weight for each cohort was 370kg, BRD rates were 34% and 8% respectively for high-risk and low-risk cohorts, and subclinical BRD resulted in a 10% and 15% decline in weight gain, with associated DMI intake declines of 5% and 10%. Clinical BRD resulted in an average reduction in weight gain of between 12-13% across the cohorts, with an associated reduction in DMI of 4%. Gain to Feed (G:F) ratio was highest for clinical BRD groups (6.30-6.40), followed by sub-clinical groups (6.10-6.15), with healthy groups (5.77-5.84) having the lowest G:F ratio (Table 11).

Healthy, clinical BRD, sub-clinical BRD health status groups within each of the cohorts returned similar DMI and weight gain results, with a variation of >1.5% between similar groups in each cohort. This is to be expected given that the differentiator at a cohort level was the proportion of each health status group within a cohort, rather than variation within health status groups (e.g. healthy animals grow the same irrespective of the cohort they are in).

Cohort level averages where similar between high-risk test and treat (T&T) and low-risk cohorts. As outlined in the methods section, this is expected because when tested and treated, high-risk cohorts revert to a low-risk BRD probability matrix, and as a result returned similar cohort percentages of 8% and 92% for scenarios without subclinical BRD and 8%, 8% and 84% for scenarios with subclinical BRD (Table 11). At a cohort level, when compared to the no-subclinical scenario, a 10% reduction in growth for sub-clinical BRD resulted in 3.6% average reduction in weight gain due to the proportion of healthy animals in the cohort. At a cohort level, the same comparison with the 15% growth penalty for sub-clinical BRD scenarios resulted in a 5.4% reduction in weight gain.

Table 11: Summary of biophysical model data used to populate the economic model.

Cohort	Health status	% of cohort	DMI (kg)	Exit wgt (kg)	Gain (kg)	G:F
			No subclinical BRD			
	BRD	34%	1031.5	531.6	162.0	6.37
High-risk	Healthy	66%	1070.3	555.4	185.5	5.77
	Average	100%	1057.1	547.3	177.6	5.95
	BRD	8%	1029.6	532.5	163.3	6.31
High-risk T&T	Healthy	92%	1085.0	555.7	185.9	5.84
	Average	100%	1080.2	553.7	183.9	5.87
	BRD	8%	1043.3	533.5	163.0	6.40
Low-risk	Healthy	92%	1082.7	555.8	185.8	5.83
	Average	100%	1079.7	554.2	184.1	5.87
	Subclinical BRD - 10% reduction in growth, 5% reduction in DMI					
	BRD	34%	1031.5	531.6	162.0	6.37
High-risk	Sub-clinical	34%	1016.6	536.9	166.8	6.10
	Healthy	32%	1070.5	555.4	185.8	5.76
	Average	100%	1038.8	540.9	171.2	6.07
	BRD	8%	1029.6	532.5	163.3	6.31
High-risk T&T	Sub-clinical	8%	1026.1	536.5	167.4	6.13
	Healthy	84%	1085.5	555.8	185.9	5.84
	Average	100%	1075.4	552.1	182.3	5.90
	BRD	8%	1043.3	533.5	163.0	6.40
Low-risk	Sub-clinical	8%	1026.7	536.9	167.5	6.13
	Healthy	84%	1082.9	555.9	185.7	5.83
	Average	100%	1075.8	552.7	182.6	5.89
	Subclinical BRD - 15% reduction in growth, 10% reduction in DMI					
High-risk	BRD	34%	1031.5	531.6	162.0	6.37
	Sub-clinical	34%	963.1	527.6	157.5	6.11
	Healthy	32%	1070.5	555.4	185.8	5.76
	Average	100%	1020.5	537.8	168.0	6.07
	BRD	8%	1028.3	532.3	163.3	6.30
High-risk T&T	Sub-clinical	8%	972.4	527.3	158.1	6.15
	Healthy	84%	1085.6	555.8	185.9	5.84
	Average	100%	1071.5	551.2	181.4	5.91
	BRD	8%	1041.2	533.0	162.7	6.40
Low-risk	Sub-clinical	8%	973.2	527.6	158.3	6.15
	Healthy	84%	1083.0	555.9	185.8	5.83
	Average	100%	1071.9	552.1	182.0	5.89

4.7.2. Economic model data

The economic viability of the test and treat scenario was driven primarily by the proportion of high-risk animals in the feedlot population for the respective paddock and saleyard purchase treatments. As the percentage of high-risk animals in the treatment declined, the cost of testing outweighed the benefits of reducing the prevalence of BRD in high-risk animals. Thus, under the main scenarios analysed, testing cohorts with a high percentage of low-risk animals (paddock purchase treatments) reduced marginal profitability by between $3.75-$5.50 per head (Table 12). This is to be expected, as low risk animals that are tested incur the cost of the test but get none of the benefit of pre-emptive treatment (as they are not likely to get BRD and are subsequently not treated). In the scenarios assessed, the breakeven percentage of low-risk animals in the population was between 84%-90%. Due to the cost of the test and the unknown efficacy of the test with respect to identifying probable sub-clinic BRD, the test is not likely to be cost effective in feedlots that have low rates of visually assessed clinical BRD (<10%), as was the case for the treatments with animals purchased directly from the paddock.

When price discounts were applied, the marginal return per head for the test and treat scenarios improved, however the return was still negative for the paddock purchase treatment. A 10% price discount, with 10% of BRD animals and 95% of health animals having a carcass price discount, only improved the marginal profitability by $0.02 for the paddock treatment and $0.12 for the saleyard treatment when compared to the no discount analysis. When the proportion of BRD animals discounted was increased to 20%, marginal profitability increased by $0.31 and $1.90 for the respective paddock and saleyard treatments when compared to the no discount analysis. As the impact of discounting is greater for BRD cohorts, the benefit (marginal return) of reducing BRD related discounting by pre-emptively treating high-risk animals is greater for systems with a high incidence of BRD, e.g. the saleyard treatment in this study.

When the impacts of subclinical BRD were applied, the marginal return per head for the test and treat scenarios improved again, however the return remained negative for the paddock purchase treatment. Profitability improved by between $0.90 - $1.44/head for the paddock treatment and between $5.44 – $8.60/head for the saleyard treatment, depending on the applied impact of BRD (10% or 15% reduction in weight gain). While paddock treatments had a negative marginal return for test and treat under all BRD scenarios, saleyard test and treat profitability increased over the status quo as the impact of sub-clinical BRD on animal growth increased.

It should be noted that the parameters used in the model which are reported here are relatively conservative with respect to the impact of BRD and BRD related carcass downgrades (discounting). This was due to the preliminary results showing a general trend in positive marginal profitability in the saleyard T&T treatments, and a consistently negative marginal returns for the paddock purchased T&T treatments. Thus, it was assumed that increasing the impact of BRD would continue to increase the marginal return of T&T treatments and that additional analysis at high rates would not add value to the project. Additional sensitivity analysis (Appendix 6) on animal purchase costs, carcass prices and the cost of feed, showed that while increasing the price level from $4.75/kg (purchase) and $8.00/kg (carcass) to $6.00/kg and $9.50/kg respectively increased the marginal return of testing and treating, as did decreasing feed costs from $350/t to $300/t, marginal returns were still negative for the

paddock purchase T&T treatment. While marginal return of T&T could be positive (at least breakeven) in some scenarios with ≤90% of the cohort being low-risk, none of these breached the 95% threshold for the paddock purchase treatments in this case-study.

The economic results show that even with an assumed relatively low predictive power (34% of animals identified as high-risk going on to develop clinical BRD) using PCR testing as a tool to manage BRD via risk-based pre-emptive treatment can improve profitability. The level of profitability is primarily determined by proportion of high-risk animals in a cohort. The economic analysis suggests that using a test and treat strategy for cohorts of animals purchased from high-risk environment (e.g. through saleyard or other instances where prior management practices or cattle type might increase BRD risk) increased profitability and reduced the overall incidence of BRD. Theoretically this could also apply for higher-risk times of year/seasonal conditions, assuming that the ROI for PCR testing is driven by reducing disease rates and treatment costs during periods of higher instances of BRD.

The ROI for use of the testing platform on risk-based treatment would be even higher if current practice for managing high-risk cohorts in a feedlot was to mass treat. In this scenario, cost would be reduced by not treating animals in the cohort that are low risk (as defined by the testing protocol), which would further reduce overall antibiotic use. However, if the current practice was not to treat until animals showed visual clinical signs of BRD, a risk-based treatment with a test accuracy of 34% leads to 66% of high-risk animals receiving antibiotics when they may not have gone on to develop BRD. If the PCR test was also identifying sub-clinical BRD in these cohorts, the number would drop to 32% of high-risk animals being unnecessarily treated. When you also consider that saleyard purchased populations still had a large number of low-risk animals in the total population (70%), the number of unnecessarily treated animals drops to 19.8% (visual clinical) and 9.6% (visual clinical and subclinical) respectively at a population level. If subclinical efficacy was established, this would reduce unnecessary antibiotic use, however there would still be a trade-off between profitability and animal welfare gains (for animal that would have otherwise developed BRD) and the risk of antimicrobial resistance. It may be possible to profitability improve this trade-off by reducing the number of false positives returned by the test, a factor that could be established by larger datasets and increased cohort analysis. Improvement in use case here would be returned either by improving the test sensitivity or changing the parameters for a “high-risk” classification, or both. If a higher proportion of animals now identified as low-risk would go on to develop BRD, the cost saving of not over treating with antibiotics while still reducing the impact of BRD for a smaller number animals (more accurately classified as high-risk of developing BRD) need to offset this cost. This evidence-based improvement in AMR stewardship practice would also be industry leading globally.

Table 12. Summary of impact of pathobiological parameters on economic performance.

	no discount 0% discount			10% BRD discounted, 5% healthy discounted 10% carcass price discount			20% BRD discounted, 5% healthy discounted 10% carcass price discount		
	SubCl 0%	**SubCl 10%**	**SubCl 15%**	**SubCl 0%**	**SubCl 10%**	**SubCl 15%**	**SubCl 0%**	**SubCl 10%**	**SubCl 15%**
Net Return per head									
High risk	$17.21	-$1.17	-$8.20	$3.75	-$20.57	-$27.44	-$4.00	-$36.14	-$42.87
High - Test & Treat	$59.16	$53.75	$51.87	$46.08	$39.74	$37.90	$44.26	$35.86	$34.05
Low risk	$92.73	$88.09	$86.49	$79.89	$74.28	$72.70	$78.22	$70.82	$69.27
Low - Test (no-treat)	$84.73	$80.09	$78.49	$71.89	$66.28	$64.70	$70.22	$62.82	$61.27
High-risk test return	$41.94	$54.92	$60.07	$42.33	$60.30	$65.34	$48.26	$73.00	$76.93
Low-risk test return	-$8.00	-$8.00	-$8.00	-$8.00	-$8.00	-$8.00	-$8.00	-$8.00	-$8.00
Scenario 1 Status Quo									
Paddock (L95H5)	$88.96	$83.63	$81.75	$76.08	$69.54	$67.70	$74.11	$65.47	$63.67
Saleyard (L70H30)	$70.08	$61.31	$58.08	$57.05	$45.82	$42.66	$53.55	$38.73	$35.63
Scenario 2 Test and Treat									
Paddock (L95H5)	$83.45	$78.78	$77.16	$70.60	$64.95	$63.36	$68.92	$61.47	$59.91
Saleyard (L70H30)	$77.06	$72.19	$70.50	$64.15	$58.32	$56.66	$62.43	$54.73	$53.11
Marginal change									
Paddock	-$5.50	-$4.85	-$4.60	-$5.48	-$4.58	-$4.33	-$5.19	-$4.00	-$3.75
Saleyard	$6.98	$10.88	$12.42	$7.10	$12.49	$14.00	$8.88	$16.00	$17.48
Breakeven (% of low-risk animals)	84%	87%	88%	84%	88%	89%	85%	90%	90%

5. Conclusions

5.1 Key findings

- Use of a multiplex PCR diagnostic platform is beneficial for mob-level syndromic testing of disease agent prevalence in feedlot cattle to determine pathobiont profile at different timepoints on feed, and in hospital cattle undergoing treatment for disease.
- A two-pass multiplex test for the major BRD agents was validated in the laboratory and in field situations.
- Whilst the project was able to reliably and accurately detect microorganism concentrations in biological samples, this data has yet to be confirmed as predictive for BRD risk due to the relatively small sample size. Despite this, concentrations were found to be greater in hospital pen cattle compared to cattle sampled at induction at the same location, with a certainly at similar levels to other syndromic diagnoses.
- Microbiological agent and BHV1 in combination with other agents, were important in terms of risk of BRD in both induction and hospital pen cattle. Distance travelled prior to feedlot entry emerged as an influencing factor for disease risk by Bayesian Network modelling.
- *Mycoplasma bovis* was identified to be at highest prevalence after 14 days on feed, in hospital pen cattle, but not at cattle at induction. This is suggestive that this microorganism is either acting as a true pathogen inside the feedlot, or that this agent is able to act as an ultimate opportunist in the feedlot setting.
- Location exerted a strong influence on disease risk, with pathogen combination exerting a second level risk of development of BRD in feedlot cattle. This data indicates that better management of cattle travelling through saleyards is necessary to reduce disease risk in feedlot.
- Testing and treating animals identified as being at high-risk developing BRD based on a PCR is profitable in scenarios with a high proportion ($\geq$15%) of high risk animals in a feedlot intake. This is likely to be the case for animals purchased from saleyards, however this is unlikely for animals purchased directly from the paddock.
- Establishing testing efficacy of detecting subclinical BRD would significantly increase the profitability of risk-based treatment with confirmation of findings through longitudinal sampling of cohorts, and / or, broadscale testing to generate cross-industry risk profiles. Differences between northern and southern systems could also be evaluated by further testing..
- Point-of-Decision diagnostics coupled with evidence-based risk management of anti-microbial treatment could improve animal welfare outcomes and antimicrobial stewardships practices in the feedlot industry.

5.2 Benefits to industry

The platform developed in this project is versatile and flexible, allowing rapid development of tests for other nucleic acid-based indicators of disease or performance. This could include RNA, DNA and eDNA targets of importance to the feedlot sector, including those indicative of emergency animal disease or other disease outbreaks of critical economic importance to the industry. The project places the Australian intensive beef industry at the forefront of advanced in quantitative PCR diagnostics (Ruitjer, 2021; Barnewall, 2022a), and the call for greater integrity in the reporting of qPCR data across all health industries, human and animal (Barnewall, 2022b; Untergasser, 2022).

The platform technology used in this project evidenced the ability to deploy a laboratory test in the field with a methodology that is directly comparable between those two sites. In addition, the technology developed in this project is the groundwork for larger, affordable multiplex panels (for example, 50 targets or more) that can be developed for widespread screening for multiple health disorders from a single sample.

The diagnostic test panel developed in this project represents a objective and quantitative measure with the potential for use for risk analysis, as well as definitive diagnostic comparison to the current qualitative measures used for current BRD diagnosis in feedlot (pen rider identification and other behavioural measures of disease). This information can now be used to inform antimicrobial use and animal management to reduce disease burden using an evidence-based approach.

In addition, the test can be used to evaluate vaccines efficacy and other treatments, as demonstrated by pathogen load in relation to the use of *Mannheimia haemolytica* vaccination, and for the use of autogenous vaccines and other location-bespoke interventions in the future.

In addition to use in live animals for disease identification and epidemiological investigations, the developed test panel could also be applied in the meat processing sector for evaluation and diagnosis of respiratory disease and impacts on reason for condemnation, carcase characteristics, quality, and yield.

Finally, this test is applicable to any cattle breed or system both nationally and internationally. In particular, the diagnostic test developed in this study is also highly applicable to the identification and management of respiratory disease in the dairy sector for development of a better understanding of the aetiology and pathogenesis of BRD in calves, and evaluation of vaccine efficacy for BRD prevention in both calves and cows.

This project showed that an efficient, scalable PCR diagnostic testing platform can be used to inform individual feedlot operations of the underlying pathological causes of bovine respiratory disease incidence by mob, intake and/or location. Data captured from multiple locations in this study indicates the importance of site-specific, objective and quantifiable data collection on microbiological prevalence and disease risk in the feedlot sector. The technology being used in this study has now been linked to cloud-based data collection platforms to allow large scale data capture and disease surveillance and analysis subsequent to its use for global COVID testing and reporting. This capability could be extended to the livestock sectors. This type of metadata analysis could be utilised by the

Australian feedlot sector, in real time, on disease outbreaks, risks, and to improve disease surveillance and reporting for the industry.

In modelled scenarios for feedlot intakes with high BRD prevenance (e.g. saleyard purchases), risk-based treatment for BRD increased profitability by $5.44 – $8.60 per head, while also improving animal welfare outcomes by treating animals while sub-clinical. The ROI could further be improved by increasing the accuracy of the test and/or classification criteria, which would also reduce antibiotic use.

6. Future research and recommendations

Recommendations arising from the outcomes of this study include:

- Further objective epidemiological testing and data collection should occur at additional sites across Australia, including in northern Australian feedlot systems, to examine BRD-casing agent profiles across the industry to correlate to disease incidence in different management systems. This should include a longitudinal study of cattle across their time on feed to identify peak risk periods of microbiological infection.
- Variability in data capture and used of health-related terminologies between locations through use of multiple data capture platforms, should be standardised across the feedlot sector to allow better data comparison between sites, states, and systems. A standardised minimum dataset of required fields should be recommended for inclusion in hospital and treatment data sheets to allow rigorous cross-sector analysis of disease in feedlot cattle into the future to evidence improved disease interventions and management practices. This should include easier data linkage between animal and carcase performance data tools.
- Further analysis needs to be undertaken to determine the role of *M. bovis* as a key opportunistic pathogen in Australian feedlot systems and to investigate the efficacy of vaccine interventions for this microorganism. Involvement of environmental reservoirs as a risk factor, or for use as indictors of *M. bovis* prevalence should be investigated.
- Further investigation is required into the role and impact of Bovine Herpes Virus-1 in the prevalence of BRD in Australian feedlot systems.
- The test developed in this project could be applied in all current and future projects investigating respiratory health in cattle to generate a database of disease profiles that can be used to refine an evidence-based risk approach for the management of BRD in Australian feedlot systems.
- The diagnostic test developed as part of this project could proceed to development of a commercially available kit to allow widespread availability to industry either for location-specific profiling or individual animal diagnosis for cattle production industries.
- Additional research to validate the efficacy of the PCR test in detecting clinical and subclinical BRD and impacts on antimicrobial usage.
- Finer scale data collection (daily per animal intake and weight gain) to improve the accuracy of the biophysical production model. This data would also aid early identification of subclinical BRD based on feed intake and weight gain.
- Standardisation of data collection and reporting across projects to aid in developing larger standardised datasets for feedlot model development for wider industry research and practical management applications.

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8. Appendices

Appendix 1. Bureau of Meteorology mean / max temperature and rainfall for all collection sites

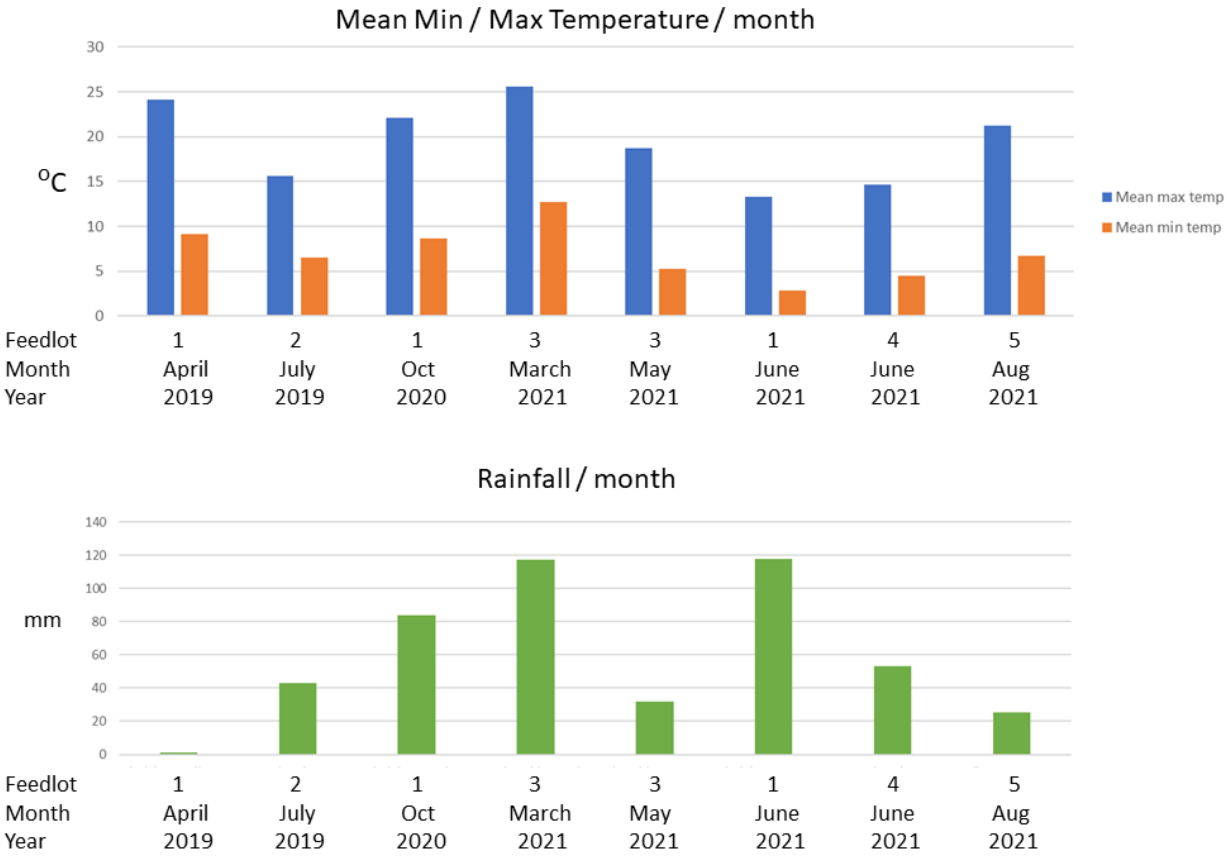

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Figure A1.1. Bureau of Meteorology data for minimum and maximum temperature, and rain fall by month for each collection location and month of collection for all Feedlots (1-5).

Appendix 2. G-Block and ultramer control, primer and probe sequences for all targets.

Table A2.1. G-block and Ultramer control sequences used in Test 1, Test 2 and SYBR green assays.

Target	Name	Sequence
Mannheimia haemolytica	**EMAI_MH**	ATTATGAGCAATAAGCAAACACTTTCTACTGTAACTTCTGCTATTTTAGAAAAAATTCAACCTGTTATTGCCGAATACAAACC AACTACTATTTTTGTTCATGGCGATACAGCAACCACTCTTGCCTCTTCATTAGCAGCCTATTACAAC
Pasteurella multocida	**EMAI _PM**	GGCTCGTTGTGAGTGGGCTTGTCGGTAGTCTTTTATTTGGCTTGTGGCAAAGAAAAGCACAGTTTTGTTGGGCGGAGTTTGGT GTGTTGAGCCAATCTGCTTCCTTGACAACGGCGCAACTGATTGGACGTTATTTATTACTCAGCTTATTGTTATTT
Trueperella pyogenes	**DEMBO_TP**	CACGTGCTCCGCAAACTGTCAGCGTCGACTTGCCAGGATTAGTTGACGGTAAGAGTAAGGTCGTCATCAACAATCCCACGAA GAGTTCCGTGACTCAAGGAATGAACGGCCTTCTCGACGGTTGGATTCAGCGCAATAGCAAGTATCCTGACCATGCTGCAAAG ATCTTCTACGATGAGACTATGGTGACGT
Mycoplasma bovis	**DEMBO_MB**	TGATGATGAGAGATTATTCTCAATTCAAGGAACCCCACCAGATATGGCAAACTTACCTATCGGTGACCCTTTTGCACCTAGAA ATGACTTTGCCTTAGAAATTGACTATGAAAAAGAACCACCATTAATTGAAATTAATAGTCATCATAA
Histophilus somni	**EMAI_Hs V2**	CTGACCCAAGAAAAATTTGCTTTGCCTCGGTATTGGCGATTTACGGACAAATTACCTCGCAATAACCAATCTAAAATCAGCCG TTTAGATTTTGAAAAAATTTGTACCGCACTTGAGTATGAGGTATTTGCATGACAGCGTTTAATCCCATTGCGATCATTCCCCAT TATAACCATTCTGCAACGGTAGGTAGCGT
Bovine Alpha herpes virus-1 (BoAHV-1)	**DEMBO_Bo AHV1**	TCCCGCCAATAACAGCGTAGACCTGGTCTTTGCCGACGCGCCGGCTGCGGCCTCCGGGCTTTACGTCTTTGTGCTGCAGTACA ACGGCCACGTGGAAGCTTGGGACTACAGCCTAGTCGTTACTTCGGACCGTTTGGTGCGCGCGGTCACCGACCA

Beta Actin	**DEMBO_Beta Actin**	CAATGAAGATCAAGATCATCGCGCCCCCTGAGCGCAAGTACTCCGTGTGGATTGGCGGCTCCATCCTGGCCTCGCTGTCCACC TTCCAGCAGATGTGGATCAGCAAGCAGGAGTACGATGAGTCCGGCCCCTCCATCGTCCACCGCAAAT
Bovine Corona Virus (BCoV)	***BCoV***	GGACCCAAGTAGCGATGAGGCTATTCCGACTAGGTTTCCGCCTGGTACGGTACTCCCTCAGGGTTATTATATTGAAGGCTCAG GAAGGTC
Bovine Viral Diarrhoea Virus (BVDV)	***BVDV***	GAAAACGGTTTGATCAACCGCTACGAATACAGCCTGATAGGGTGCTGCAGAGGCCCACTGTATTGCTACTAAAAATCTCTGCT GTACATGGCAC
Bovine Respiratory Syncytial Virus (BRSV)	***BRSV***	GCAATGCTGCAGGACTAGGTATAATGGGTGAGTATAGAGGTACACCAAGAAACCAAGACTTGTATGATGCTGCCAAAGCATA TGCGGAACAATT AAAAGAGAATGGGGTCATCAATTACAGTGT
Bovine Para-Influenza Virus-3 (BPIV3)	***BPIV3***	TGTCTTCCACTAGATAGAGGGATAAAATTCAGGGTGATATTCGTGAATTGCACAGCAATTGGATCAATAACTCTATTTAAAAT CCCCAAATCCATGGCATTGTTATCA

Table A2.2. Primer and probe sequences for PCR amplification quantification of microbial organisms and viruses associated with bovine respiratory disease. (F: forward primer, P: probe, R: reverse primer, abbreviations within primer sequences: R = A or G; W = A or T; N = any base).

Target Pathogen	Name	Primer/Probe sequence 5'-3'
Bovine Alpha herpes virus-1 (BoAHV-1)	Dembo-BHV1	F: CAATAACAGCGTAGACCTGGTC
		R: GCTGTAGTCCCAAGCTTCCAC
		P: FAM-TGCGGCCTCCGGGCTTTACGTCT-BHQ1
Histophilus somni	EMAI-HsV2	F: ACTTGGATTTAGCCACGCTATT
		R: TCCGCTTGTTCGAGCATTT
		P: CAL Fluor Red 610-CAAGTAGATGCAGATGGGCAGCAT-BHQ2
Trueperella pyogenes	Dembo-Tp	F: ATCAACAATCCCACGAAGAG
		R: TTGCAGCATGGTCAGGATAC
		P: CAL Fluor Orange 560-TCGACGGTTGGATTCAGCGCAATA-BHQ1
Pasteurella multocida	EMAI-Pm	F: GGGCTTGTCGGTAGTCTTT
		R: CGTTGTCAAGGAAGCAGATTG
		P: CAL Fluor Red 610 TTTGTTGGGCGGAGTTTGGTGTG
Mycoplasma bovis	Dembo-Mb	F: TCAAGGAACCCCACCAGAT
		R: AGGCAAAGTCATTTCTAGGTGCAA
		P: FAM-TGGCAAACTTACCTATCGGTGACCCT-BHQ1

Mannheimia haemolytica	EMAI-Mh	F: GCA AAC ACT TTC TAC TGT AAC TTC T
		R: GTT GCT GTA TCG CCA TGA AC
		P: CAL Fluor Orange 560-CAACCTGTTATTGCCGAATACAAACCAACT_BHQ1
Beta Actin	Dembo_ β-Actin	F: AGC GCA AGT ACT CCG TGT G
		R: CGG ACT CAT CGT ACT CCT GCT T
		P: Quasar 670-TCGCTGTCCACCTTCCAGCAGATGT-BHQ2
Bovine Corona Virus (BCoV)	Nucleocapsid	F: GGACCCAAGTAGCGATGAG
		R: GACCTTCCTGAGCCTTCAATA
Bovine Viral Diarrhoea Virus (BVDV)	5'UTR	F: GGGNAGTCGTCARTGGTTCG
		R: GTGCCATGTACAGCAGAGWTTTT
Bovine Respiratory Syncytial Virus (BRSV)	Nucleocapsid	F: GCAATGCTGCAGGACTAGGTATAAT
		R: ACACTGTAATTGATGACCCCATTCT
Bovine Para-Influenza Virus-3 (BPIV3)	Matric (M) protein	F: TGTCTTCCACTAGATAGAGGGATAAAATT
		R: GCAATGATAACAATGCCATGGA

Appendix 3. Analytical sensitivity of bacterial and viral assays.

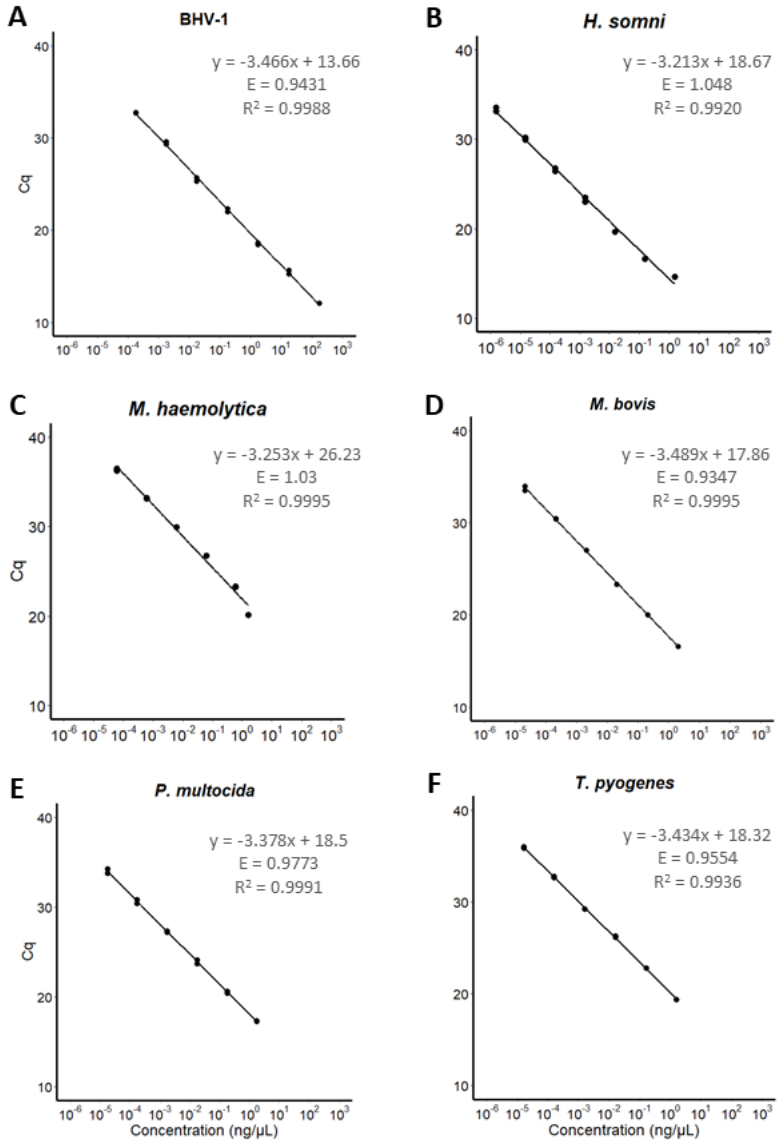

Figure A3.1. Analytical sensitivity of BRD associated agents determined using a serial dilution of DNA of known concentration.

Abbreviations: BoAHV1, Bovine α-herpesvirus 1; *M. haemolytica*, *Mannheimia haemolytica*; *H. somni*, *Histophilus somni*; *M. bovis*, *Mycoplasma bovis*; *P. multocida*, *Pasteurella multocida*; *T. pyogenes*, *Trueperella pyogenes*

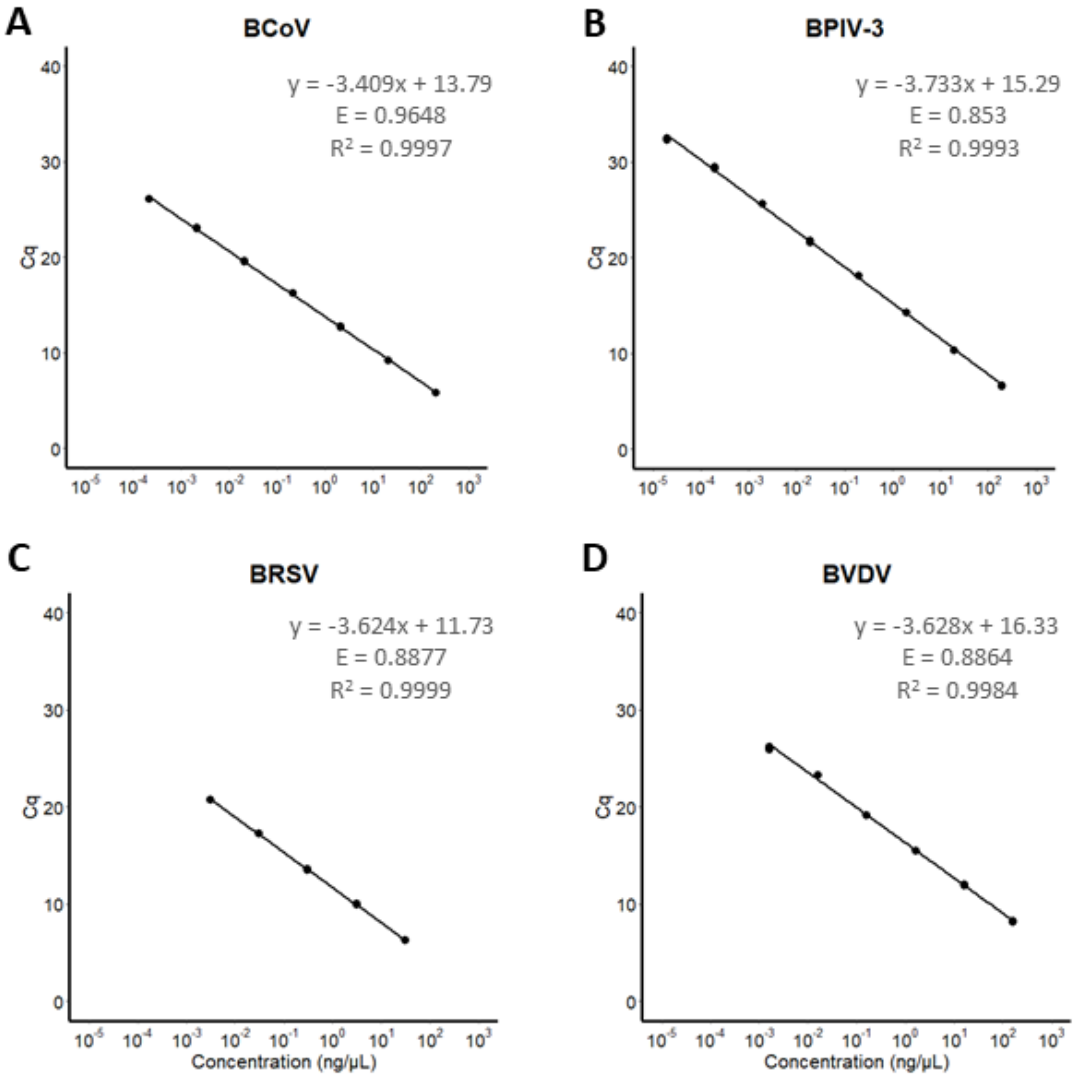

Figure A3.2. Analytical sensitivity of BRD associated RNA viral agents determined using a serial dilution of synthetic RNA controls of known concentration.

Abbreviations: BCoV, Bovine coronavirus; BVDV, Bovine viral diarrhea virus; BRSV, Bovine respiratory syncytial virus; BPIV-3, Bovine parainfluenza virus 3.

Appendix 4. Analytical efficiency of bacterial PCR assays for quantification.

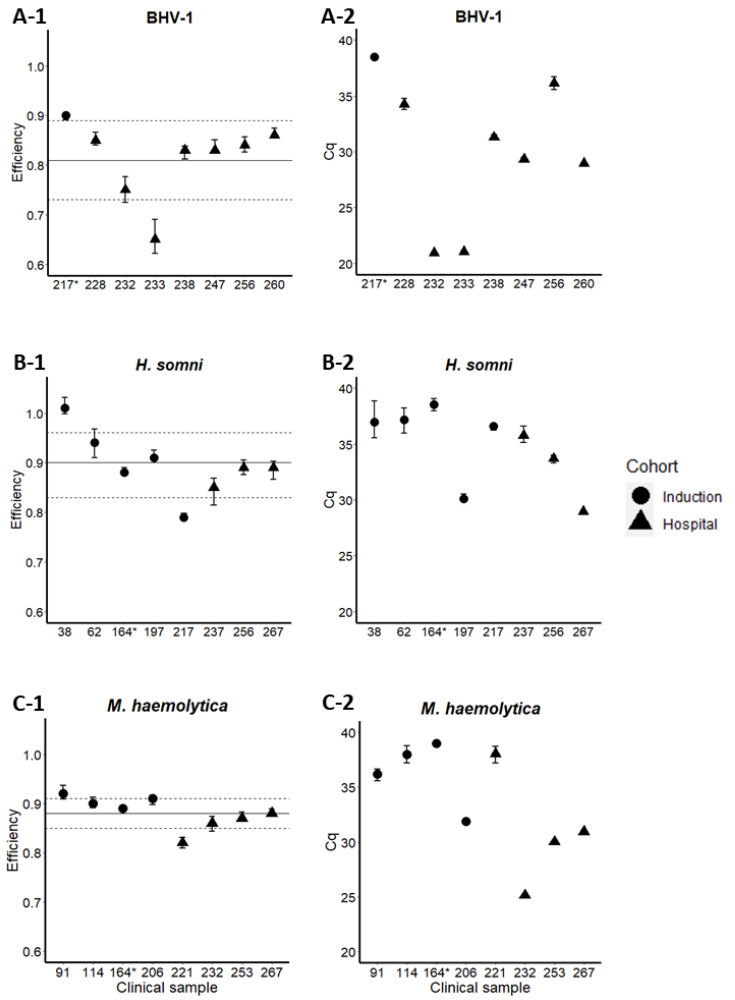

Figure A4.1. Reproducibility of singleplex qPCR efficiency and Cq for key DNA agents associated with bovine respiratory disease (Bovine alpha herpesvirus 1, *Histophilus somni* and *Mannheimia haemolytica*) from multiple clinical samples. qPCR efficiency and Cq are displayed as the average ± SD. Mean (solid horizontal line) and SD variance (dashed line) for each agent is shown.

Abbreviations: BoAHV1, Bovine α-herpesvirus *1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes*

* less than 6 technical replicates amplified.

Appendix 5. Statistical significance for analysis of prevalence of all agents tested at five feedlot locations between 2019 and 2021.

Table A5.1. Induction animals Prevalence and P-values for Figures 3 & 4 – Feedlots 1-5.

Location	Collection Month/Year	BCoV			BVDV			BRSV			BPIV-3			BHV-1			*H. somni*			*M. haemolytica*			*M. bovis*			*P. multocida*			*T. pyogenes*		
		Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value
		D0	D14		D0	D14		D0	D14		D0	D14		D0	D14		D0	D14		D0	D14		D0	D14		D0	D14		D0	D14	
Feedlot 1	Apr 2019	NT	N/A	N/A	NT	N/A	N/A	NT	N/A	N/A	NT	N/A	N/A	14 (6.4)	N/A	N/A	22 (10.1)	N/A	N/A	46 (21.1)	N/A	N/A	0 (0.0)	N/A	N/A	144 (66.1)	N/A	N/A	43 (19.7)	N/A	N/A
	Oct 2020	4 (1.8)	6 (2.8)	0.751	0 (0.0)	0 (0.0)	n/a	2 (0.9)	2 (0.9)	1	0 (0.0)	0 (0.0)	n/a	42 (19.4)	35 (16.1)	0.451 1	39 (18.0)	36 (16.6)	0.79 97	60 (27.6)	30 (13.8)	0.000 55	1 (0.46)	174 (80.2)	<2.2E-16	165 (76.0)	124 (57.1)	4.33E-05	33 (15.2)	86 (39.6)	1.46E-08
	Jun 2021	3 (1.4)	35 (15.8)	1.95E-08	0 (0.0)	0 (0.0)	n/a	9 (4.1)	35 (15.8)	4.61E-05	3 (1.4)	3 (1.4)	1	5 (2.3)	26 (11.8)	0.000 011	43 (19.5)	117 (52.9)	1.01E-13	21 (9.5)	100 (45.2)	<2.2E-16	12 (5.4)	177 (80.1)	<2.2E-16	50 (22.6)	183 (82.8)	<2.2E-16	36 (16.3)	126 (57.0)	<2.2E-16
Feedlot 2	Jul 2019	NT	N/A	N/A	NT	N/A	N/A	NT	N/A	N/A	NT	N/A	N/A	28 (11.6)	N/A	N/A	52 (21.6)	N/A	N/A	13 (5.4)	N/A	N/A	8 (3.3)	N/A	N/A	71 (29.5)	N/A	N/A	52 (21.6)	N/A	N/A
Feedlot 3	May 2021	11 (3.1)	26 (7.4)	0.001 696	0 (0.0)	0 (0.0)	n/a	5 (1.4)	7 (2.0)	0.772 5	2 (0.6)	8 (2.3)	0.106 9	29 (8.2)	17 (4.8)	0.092 448	79 (22.4)	100 (28.4)	0.083 328	45 (12.8)	64 (18.2)	0.060 38	74 (21.0)	142 (40.3)	3.62E-08	94 (26.7)	119 (33.8)	0.048 881	65 (18.5)	167 (47.4)	2.42E-16
Feedlot 4	Jun 2021	1 (0.6)	8 (4.8)	0.036 57	0 (0.0)	0 (0.0)	n/a	2 (1.2)	1 (0.6)	1	2 (1.9)	0 (0.0)	4.93E-08	7 (4.2)	8 (4.8)	1	4 (2.2)	5 (3.1)	0.155 5	3 (2.2)	3 (1.8)	0.417 9	2 (1.9)	8 (4.8)	4.89E-11	6 (3.9)	1 (0.6)	5.06E-05	7 (4.5)	8 (5.1)	0.444 9
Feedlot 5	Aug 2021	5 (1.7)	0 (0.0)	0.061 42	0 (0.0)	0 (0.0)	n/a	0 (0.0)	7 (2.4)	0.015 06	0 (0.0)	1 (0.3)	1	1 (3.8)	7 (25.3)	9.35E-14	6 (2.8)	3 (1.5)	0.003 09	2 (9.7)	9 (34.4)	2.39E-13	2 (9.7)	1 (3.5)	0.003 79	9 (32.6)	1 (0.4)	0.030 67	4 (16.7)	3 (12.2)	0.154 2

Abbreviations: NT = Not tested; N/A = Not applicable*;* BoAHV1, Bovine a-herpesvirus *1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes; BCoV, Bovine coronavirus; BVDV, Bovine viral diarrhea virus; BRSV, Bovine respiratory syncytial virus; BPIV-3, Bovine parainfluenza virus 3.*

Table A5.2. Hospital animals Prevalence and P-values for Figures 21 and 22 – Feedlots 1-5.

Location	Collection Month/Year	BCoV			BVDV			BRSV			BPIV-3			BHV-1			H. somni			M. haemolytica			M. bovis			P. multocida			T. pyogenes		
		Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value	Prevalence		p-value
		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD		BRD	Non_BRD	
Feedlot 1	Apr 2019	NT	NT	N/A	NT	NT	N/A	NT	NT	N/A	NT	NT	N/A	26 (72.2)	12 (66.7)	0.756	20 (55.6)	12 (66.7)	0.5601	16 (44.4)	12 (66.7)	0.1554	33 (91.7)	16 (88.9)	1	33 (91.7)	18 (100.0)	0.5428	31 (86.1)	13 (72.2)	0.2728
	Oct 2020	2 (10.5)	1 (6.7)	1	0 (0.00)	0 (0.00)	N/A	0 (0.00)	0 (0.00)	N/A	1 (5.3)	2 (13.3)	0.5714	12 (63.2)	8 (53.3)	0.72822	10 (52.6)	8 (53.3)	1	5 (26.3)	1 (6.7)	0.1936	14 (73.7)	4 (26.7)	0.014123	12 (63.2)	6 (40.0)	0.2988	8 (42.1)	8 (53.33)	0.73033
	Jun 2021	1 (10.0)	2 (10.5)	1	0 (0.00)	0 (0.00)	N/A	0 (0.00)	0 (0.00)	N/A	0 (0.00)	0 (0.00)	N/A	5 (50.0)	6 (31.6)	0.4332	7 (70.0)	8 (42.1)	0.245 51	3 (30.0)	5 (26.3)	1	8 (80.0)	11 (57.9)	0.413 37	8 (80.0)	8 (42.1)	0.11422	5 (50.0)	7 (36.8)	0.69422
Feedlot 2	Jul 2019	NT	NT	N/A	NT	NT	N/A	NT	NT	N/A	NT	NT	N/A	53 (82.8)	12 (48.0)	0.002 665	48 (75.0)	15 (60.0)	0.19777	8 (12.5)	5 (20.0)	0.5044	58 (90.6)	21 (84.0)	0.45799	31 (48.4)	8 (32.0)	0.23446	44 (68.8)	11 (44.0)	0.05087
Feedlot 3	Mar 2021	NT	NT	N/A	NT	NT	N/A	NT	NT	N/A	NT	NT	N/A	2 (33.3)	0 (0.00)	1	0 (0.000)	0 (0.00)	N/A	1 (16.7)	0 (0.00)	1	5 (83.3)	0 (0.00)	1	1 (16.67)	0 (0.00)	1	1 (16.7)	0 (0.00)	1
	May 2021	8 (20.5)	0 (0.00)	1	0 (0.00)	0 (0.00)	N/A	0.00	0 (0.00)	N/A	1 (2.6)	0 (0.00)	1	13 (33.3)	0 (0.00)	0.5402	18 (46.2)	1 (3.3)	1	4 (10.3)	0 (0.00)	1	33 (84.6)	3 (100.0)	1	23 (59.0)	1 (3.3)	0.56722	21 (53.9)	1 (3.3)	0.5976
Feedlot 4	Jun 2021	0 (0.00)	0 (0.00)	N/A	0 (0.00)	0 (0.00)	N/A	0.00	0 (0.00)	N/A	6 (14.3)	3 (33.3)	0.1855	12 (28.6)	3 (33.3)	1	29 (69.1)	5 (55.6)	0.45889	5 (11.9)	0 (0.00)	0.57112	27 (64.3)	5 (55.6)	0.71144	26 (61.9)	5 (55.6)	0.72411	24 (57.1)	8 (88.9)	0.12822
Feedlot 5	Aug 2021	0 (0.00)	0 (0.00)	N/A	0 (0.00)	0 (0.00)	N/A	0.00	0 (0.00)	N/A	0 (0.00)	0 (0.00)	N/A	0 (0.00)	0 (0.00)	N/A	1 (100.0)	2 (33.3)	0.42866	0 (0.00)	2 (33.3)	1	0 (0.00)	1 (16.7)	1	1 (100.0)	3 (50.0)	1	0 (0.00)	1 (16.7)	1

Abbreviations: NT = Not tested; N/A = Not applicable*;* BoAHV1, Bovine a-herpesvirus *1; M. haemolytica, Mannheimia haemolytica; H. somni, Histophilus somni; M. bovis, Mycoplasma bovis; P. multocida, Pasteurella multocida; T. pyogenes, Trueperella pyogenes; BCoV, Bovine coronavirus; BVDV, Bovine viral diarrhea virus; BRSV, Bovine respiratory syncytial virus; BPIV-3, Bovine parainfluenza virus 3.*