Bovine respiratory disease: Treatment outcomes, prevalence of antimicrobial resistance and systematic review of control methods

by

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Abstract

Bovine respiratory disease (BRD) is an economically important disease in feedyards influencing both animal welfare and use of antimicrobials. While much research has been completed there are still knowledge gaps regarding the treatment plans and optimal ways to manage cattle with BRD.

The objective of the first chapter of the thesis was to identify potential associations between nasopharyngeal microbiota and antimicrobial resistance patterns of clinical cases that lived or died compared to non-diseased controls. Enrolled animals were subdivided based on clinical disease status and case outcome (subsequent mortality or finishing the feeding phase). Deep nasopharyngeal swabs were collected from enrolled animals and submitted for bacterial isolation, antimicrobial susceptibility determination, and metagenomics analysis. Enrolled cattle were represented in three groups: animals at first treatment for BRD that subsequently died (BRDM, n=9), animals at first treatment for BRD that subsequently lived (BRDL, n=15), and animals that were never treated for BRD during the feeding phase (CONT, n=11). Antimicrobial resistance patterns for *Pasteurella multocida* illustrated that cattle in each outcome category had isolates that were pan-susceptible or only showing resistance to oxytetracycline. Nasal metagenomics analysis showed relative abundance of species and genera with few differences among the three outcomes. Higher alpha diversity was identified in BRDL compared to CONT at the species level and both BRDL and BRDM showed increased alpha diversity compared to CONT at the genera level. Overall, this work illustrated nasopharyngeal microbiota showed relatively few differences among BRD cases that lived or died compared to animals without BRD.

The second chapter objective was to identify potential relationships between risk factors known at the time of initial BRD treatment with three post-treatment outcomes: first treatment success (FTS; finishing feeding phase with no further treatments), non-cause specific case fatality risk (CFRALL; post-treatment mortalities from any cause), and cause-specific case fatality risk (CFRBRD; mortalities attributed to BRD). This retrospective analysis used generalized linear mixed models to evaluate relationships between covariates of interest (arrival: sex, weight, month; treatment event: days-on-feed (DOF), rectal temperature, day-of-week, antibiotic drug class) with each outcome (FTS, CFRALL, CFRBRD). Analysis included 132,521 individual-animal initial BRD treatment records from 14 central U.S. feedyards (May 2017 to Dec 2020) with overall FTS of 67.8%, CFRALL of 10.0%, and CFRBRD of 6.3%. The FTS was associated ($P < 0.05$) with all covariates except sex, CFRALL was associated with all covariates, and CFRBRD was associated with all covariates except day-of-week treated. Treatment early in the feeding phase (DOF 0-10, 11-20) was associated with lower FTS (49.2% ± 0.8; 55.3% ± 0.8), higher CFRALL (12.5% ± 1.3; 12.6% ± 1.4) and higher CFRBRD (6.3% ± 1.1; 6.1% ± 1.0) compared to cattle treated on days 21-70. Rectal temperature in the 39.4-40.0°C category had higher CFRALL (15.3% ± 1.5) and CFRBRD (9.2% ± 1.5) compared to other rectal temperature categories. Increased knowledge of risk factors associated with treatment outcomes has the potential to help inform therapeutic decisions.

This systematic review in the third thesis chapter sought to identify published information relative to antimicrobial use in BRD control and metaphylaxis. Specific research questions addressed case fatality risks, first treatment success, switching drug classes between treatments, switching drug classes between metaphylaxis and first treatment, post-metaphylactic or post-treatment intervals, as well as concomitant therapies. Databases searched included PubMed,

CAB Direct, and Agricola, resulting in 418 publications meeting the search criteria. Manuscripts were then sorted into topic categories for review. Case fatality risk decreases with treatments of tulathromycin when compared to other antimicrobials. Similar trends were seen with first treatment success and included other macrolides as well, resulting in higher success. Studies showed longer post-treatment intervals had better morbidity resolution when compared to shorter post-treatment intervals. No prospective manuscripts were found regarding switching drug classes between metaphylaxis and first treatment or first and subsequent treatments. The research that evaluated concomitant therapy for BRD treatment did not show an advantage compared to single-antimicrobial treatment. This literature review identified several knowledge gaps related to specific application methods of treatment; and more research in these areas could be conducted to understand the optimal management practices and treatment strategies for BRD in cattle.

By looking into more treatment options and outcomes there is hope to improve future BRD treatment outcomes. While much remains to be learned, research is always being conducted and new information leading to BRD successes can be found.

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Acknowledgements

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Chapter 1 - Nasopharyngeal bacterial prevalence and microbial diversity at first treatment for bovine respiratory disease (BRD) and its associations with health and mortality outcomes in feedyard cattle

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Abstract: Bovine respiratory disease (BRD) is an economically important disease in feedyards influencing both animal welfare and antimicrobial utilization. Major pathogens associated with BRD have been identified in previous research, but little information is available on the relationship of nasopharyngeal microbiota and health outcomes. The objective of this study was to identify potential associations between nasopharyngeal microbiota and antimicrobial resistance patterns of clinical cases that lived or died compared to non-diseased controls. Enrolled animals were subdivided based on clinical disease status and case outcome (subsequent mortality). Deep nasopharyngeal swabs were collected on enrolled animals and submitted for bacterial isolation, antimicrobial susceptibility determination, and metagenomics analysis. Enrolled cattle were represented in three groups: animals at first treatment for BRD that subsequently died (BRDM, n=9), animals at first treatment for BRD that subsequently lived (BRDL, n=15), and animals that were never treated for BRD during the feeding phase (CONT, n=11). Antimicrobial resistance patterns for *Pasteurella multocida* illustrated cattle in each outcome category had isolates pan-susceptible or only showing resistance to oxytetracycline. Relative abundance of species and genera illustrated few differences among the three outcomes. Higher alpha diversity was identified in BRDL compared to CONT at the species level and both BRDL and BRDM showed increased alpha diversity compared to CONT at the genera level.

Overall, this work illustrated nasopharyngeal microbiota showed relatively few differences among BRD cases that lived or died compared to animals without BRD.

1. Introduction

Bovine respiratory disease (BRD) is a major cause of morbidity and mortality in beef cattle feedyards (Schneider et. al., 2009) (Smith et. al., 2009) (Babcock et. al., 2013) (Blakebrough-Hall et. al., 2020) (White et. al., 2020). This syndrome can have significant economic impacts on feedyards by lowering an animal's average daily gain, thus increasing feed and housing costs associated with that animal Griffen et. al., 1997) (Cernicchiaro et. al., 2013) (Johnson et. al., 2017). Economically, mortality is severe as it results in a total loss of all resources invested in that animal. Thus, understanding factors that could result in fatality following treatment for disease is important (Chai et. al., 2022). While common pathogens associated with BRD cases have been identified, little research has been done evaluating differences in case outcomes based on deep nasopharyngeal microbiota at the time of treatment (Confer et. al., 2009) (Griffen et. al., 2010) (Zhang et. al., 2020).

Various factors can predispose cattle to BRD, including: host, environmental, and agent factors. Some of the BRD causative agents include Mannheimia haemolytica, Histophilus somni, Mycoplasma bovis, and Pasteurella multocida. Many different pathogens play a role in development of BRD which creates complex interactions among microbial populations (Nobrega et. al., 2021). Other bacteria, which may or may not be associated with BRD, commonly found from nasopharyngeal samples of cattle, both healthy and with respiratory disease, include the genera, Moraxella, Acinetobacter, Corynebacterium, Clostridium, Solibacillus, Turicibacter, Bacteroides and Blautia. In one study, the most prevalent bacteria isolated from nasopharyngeal samples taken upon entry into the feedyard belonged to the Mycoplasma, Moraxella, and

Acinetobacter genera (Holman et. al., 2019). However, it was observed that cattle with clinical BRD had a higher prevalence of Acinetobacter, Solibacillus and Pasteurella compared to clinically healthy cattle. Furthermore, even though Mannheimia is commonly recognized as a part of the BRD complex, there was no observed increase in relative prevalence of Mannheimia in BRD affected cattle compared to healthy cattle (Zeinedin et. al., 2017). While prevalence of bacteria in the nasopharynx has been studied, little work has been done evaluating outcomes associated with different spectrums of nasopharyngeal microbial populations.

Understanding the exact relationship between BRD and the nasopharyngeal microbiome requires more research as it is difficult to ascertain whether the microbiome at time of sampling leads to clinical BRD cases, or if the immune system response to clinical BRD in turn alters the microbiome (Jobman et. al., 2023). Studies of cattle at the time of first BRD treatment in relation to bacterial prevalence, antimicrobial resistance and nasopharyngeal microbiome are sparse. The objective of this study was to identify potential associations between nasopharyngeal microbiota and antimicrobial resistance patterns with clinical cases that lived or died compared to non-diseased controls.

2. Materials and Methods

All research and procedures were approved prior to trial initiation by the Kansas State University Institutional Animal Care and Use Committee (IACUC#4588. 99).

2.1. Animals and nasopharyngeal swab collection

Deep nasopharyngeal swabs were collected from cattle located at a collaborating feedyard in southwest Kansas at the time of first treatment for BRD and from a non-BRD counterpart for comparison. Individual animals were identified as potential BRD cases by evaluation of clinical signs in the housing pen (depression, anorexia, in-creased respiration), then

further evaluated in the treatment chute (rectal temperature). Non-diseased cases were selected as cattle not showing clinical signs of BRD and not subsequently treated for BRD during the rest of the feeding phase. Each BRD case was monitored to determine post-treatment mortality during the feeding phase. Samples were collected in a manner to create three cattle groups for comparison: cattle treated for BRD with subsequent mortality (BRDM), cattle treated for BRD with no subsequent mortality (BRDL), and a control group of cattle not treated for BRD (CONT).

Swabs were placed in 2 ml cryovials, labeled with the animal number, and stored immediately in liquid nitrogen until shipped to Kansas State University. On arrival swab samples were stored in a -80 °C freezer until analysis. Following collection of all samples, subsets of samples were created including first treatment BRD cases that resulted in fatality, first treatment BRD cases that did not result in fatality, and non-BRD case comparisons.

2.2. Bacterial isolation and identification

From the nasopharyngeal swab samples, the isolation of Pasteurella spp. and M. haemolytica was carried out as described respectively in (DeRosa et. al., 2000) (Zaheer et. al., 2013). Two isolates of each nasopharyngeal pathogen exhibiting a typical colony morphology were subjected to catalase and oxidase biochemical tests. Genus and species confirmation of P. multocida and M. hemolytica was done by PCR (Deressa et. al., 2010). The confirmed isolates (two isolates of each species per sample) were stored in Cryocare-protect® beads (Key Scientific Products, Stamford, TX) at -80 ºC for future use.

2.3. Antimicrobial susceptibility determinations

Minimal inhibitory concentrations (MIC) were determined by broth-microdilution method as per CLSI guidelines (2023) using the Sensititre® automated antimicrobial system

(Trek Diagnostics Systems, Cleveland, OH). A commercially available Bovine Tulathromycin MIC format Sensititre® (BOPO-7F) panel plate was used with the aid of the Sensititre® automated inoculation delivery system (Trek Diagnostics Systems, Cleveland, OH). Appropriate ATCC (American Type Culture Collection, Manassas, VA) quality control strains, Escherichia coli ATCC 25922 and Enterococcus faecalis ATCC 29212, were used as reference standards for susceptibility testing. The MIC for each isolate was recorded and classified as resistant, intermediate or susceptible based on the guidelines given by (CLSI, 2023).

2.4. DNA extraction from nasopharyngeal samples

Total DNA was extracted using the "PowerSoil® DNA isolation kit" (MO BIO Laboratories; Carlsbad, CA) according to the manufacturer's protocol. The isolated DNA was stored at -20°C until used for metagenome shotgun sequencing.

2.5. Library preparation and Sequencing

Genomic DNA samples were quality checked with HS dsDNA Qubit assay for quantification and Agilent TapeStation gel analysis for gDNA quality. Sequencing libraries were constructed using 100-500 ng of gDNA using the Illumina DNA Prep sequencing library kit (Illumina, San Diego, CA). The sequencing library construction included tagmentation of the gDNA using a bead-based transposome complex to simultaneously fragment and tag the DNA with adapter sequences. Following tagmentation, unique dual index adapters are added in a PCR amplification step to the ends of the DNA fragments. The constructed sequencing libraries were quantified and validated with Qubit and TapeStation assays. After pooling the sequencing library preps by ng amount, the nM concentration of the pool was verified with an Illumina Library Quant qPCR assay (Roche, Indianapolis, IN). An Illumina NextSeq2000 system at the University of Kansas Genome Sequencing Core was used to generate paired-end, 150-base

sequence reads from the multiplexed libraries. Base calling was carried out by the instrument's Real Time Analysis (RTA) software. The base call files (bcl files) were demultiplexed and converted to compressed FASTQ files using the on-board DRAGEN BCL Convert software.

2.6. Bioinformatics analysis

Software for sequence analysis was installed and run on Beocat, the Kansas State University High Performance Computing Cluster. Using the AMRPlusPlus v2.0 pipeline (Doster et. al., 2020), raw reads were subjected to adapter and quality trimming with Trimmomatic (Bolger et. al., 2014). These trimmed reads were aligned to the *Bos taurus* reference genome (GCF_002263795.2_ARS-UCD1.3_genomic.fna.gz) plus the Y chromosome sequence (Bos_taurus_Y_CM001061.2.fasta) using Burrows-Wheeler Alignment (BWA) (Li et. al., 2009). More than 99% of reads aligned to the host genome and were removed from further analysis using BEDTools (Quinlan et. al., 2010). The taxonomic classification of the remaining non-host reads was assigned using Kraken 2 (Wood et. al., 2019). AMRPlusPlus v3.0 (Bonin et. al., 2023) with the MEGARes v3.0 database and ARIBA (Hunt et. al., 2017) with input from the CARD database (Alcock et. al., 2020) were used to identify reads mapping to potential Antimicrobial Resistance (AMR) genes and to check for SNPs in those reads.

Statistical analysis was performed in R (R Core Team, 2022) using species level read counts produced by Kraken2. Linear models (using the *lm* function with *anova* from the R *stats* package) were used to test for significant effect of group (CONT, BRDM, BRDL) on raw read counts and non-host read counts. Post hoc pairwise testing was performed using the TukeyHSD () function. Before normalization and additional analysis, the raw read counts were filtered to keep only bacterial taxa with at least 10 total counts across all samples. Counts were then normalized by the Cumulative Sum Scaling method using the *cumNorm* function of

metagenomeSeq (Paulson et. al., 2013). The *tax_glom* function of *phyloseq* (McMurdie et. al., 2013) was used to aggregate the normalized counts to the species, genus, and phylum levels.

Relative taxa abundance by group was calculated at the species, genus, and phylum levels using normalized, aggregated counts. For the species and genus levels, taxa with relative abundance less than 1% were grouped together as "low abundance" taxa. Relative Abundance bar plots were generated with the *geom_bar* function of *ggplot2* (Wickham et. al., 2016). The *estimate_richness* function of *phyloseq* was used to calculate alpha diversity for species, genus, and phylum levels. The Observed, Shannon, and Inverse Simpson indices were compared between groups using ANOVA of linear models (*lm* function) to test for significant differences. The TukeyHSD function was used for post hoc pairwise testing. The *ordinate* function of the *vegan* (Oksanen et. al., 2022) package was used to create non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis distance. The *stat_ellipse* function of *ggplot2* was used to add ellipses showing 95% confidence intervals for a multivariate t-distribution. PERMANOVA testing (Anderson et. al., 2017) via the *adonis* function of *vegan* was used to check for significant differences in beta diversity between sample groups. The ANCOMBC package (Lin et. al., 2020) (Lin et. al., 2022) was used to test for significant differences in species and genus abundance between sample groups with the default Holm-Bonferroni method used for multiple testing correction. Before heatmap creation, additional filtering was applied with the *phyloseq_filter_taxa_rel_abund* function of *metagMisc* (Mikryukov et. al., 2023) to remove species with relative abundance less than 0.005. Aggregated counts for species with relative abundance greater than 0.005 were used to create a heatmap using the *plot_heatmap* function of *phyloseq*. Ordering of rows (species) was determined by NMDS ordination and columns (samples) were ordered by group.

3. Results

3.1. Bacterial prevalence

A total of 99 samples were collected for potential participation in the study. The BRDM group (n=9) consisted of cases sampled at first treatment for BRD that subsequently died. Samples for BRDL group were collected from cattle at the time of first treatment for BRD that did not die (n=76). Additional cattle not treated for BRD during their feeding period were sampled as CONT (n=11). Due to resource allocation, only limited samples were submitted for culture, susceptibility testing and sequencing procedures. Final submissions included all BRDM (n=9) cases and randomly selected samples from BRDL (n=15) and CONT (n=11). Of the 35 samples tested, only one sample (from BRDL group) was positive on culture and confirmed by PCR for *M. haemolytica*. The sole isolate of *M. haemolytica* was susceptible to all antimicrobials except penicillin, to which it was resistant. Nine of our 35 samples were positive via culture and confirmed by PCR for *P. multocida*, and this was distributed among groups: CONT (n=3), BRDL (n=4), and BRDM (n=2).

3.2. Antimicrobial Minimal Inhibitory Concentrations

The nine isolates of *P. multocida* showed varying drug resistance (Table 1). The bacteria from Sample 1 (BRDM) and sample 2 (BRDL) were susceptible to all antimicrobials. Sample 3 (CONT) and sample 4 (BRDL) produced isolates with an intermediate susceptibility to penicillin and susceptibility to all other antimicrobials tested. *P. multocida* isolated from sample 5 (BRDL) was resistant to oxytetracycline but susceptible to all others. Isolates from samples 6 and 7 (CONT) showed resistance to oxytetracycline, intermediate susceptibility to chlortetracycline, and was susceptible to all other antimicrobials. Sample 8 (BRDM) contained *P. multocida* resistant to oxytetracycline, intermediate to spectinomycin, and susceptible to all other

antimicrobials. *P. multocida* from sample 9 (BRDL) was resistant to oxytetracycline, intermediate to penicillin, and susceptible to all other antimicrobials.

Antimicrobial	MIC Susceptible	MIC Intermediate	MIC Resistant	Sample 1 BRDM	Sample 2 BRDL	Sample 3 CONT	Sample 4 BRDL	Sample 5 BRDL	Sample 6 CONT	Sample 7 CONT	Sample 8 BRDM	Sample 9 BRDL
Ampicillin	≤ 0.03	0.06–0.12	≥ 0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
Ceftiofur	≤ 2	4	≥ 8	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
Chlortetracycline	≤ 2	4	≥ 8	2	<0.5	<0.5	<0.5	1	4	4	2	2
Danofloxacin	≤ 0.25	0.5	≥ 1	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	0.25	<0.12	<0.12
Enrofloxacin	≤ 0.25	0.5–1	≥ 2	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12
Florfenicol	≤ 2	4	≥ 8	<0.25	0.5	1	1	0.5	0.5	0.5	0.5	1
Oxytetracycline	≤ 2	4	≥ 8	<0.5	<0.5	<0.5	<0.5	>8	>8	>8	>8	>8
Penicillin	≤ 0.25	0.5	≥ 1	<0.12	<0.12	0.5	0.5	<0.12	<0.12	0.25	<0.12	0.5
Spectinomycin	≤ 32	64	≥ 128	<8	16	32	32	16	16	32	>64	32
Tulathromycin	≤ 16	32	≥ 64	2	<1	16	16	<1	2	16	<1	2

Table 1 *Pasteurella multocida* susceptibilities.

Pasteurella multocida isolated from first treatment bovine respiratory disease (BRD) cattle in comparison to healthy cattle. Samples are labeled with classification group for study: CONT (non-BRD cattle), BRDL (cattle treated for BRD that lived), and BRDM (cattle treated for BRD that died).

3.3. Shotgun metagenomics

Of the 35 samples, 8 samples did not yield enough DNA for further testing. We obtained shotgun metagenomic sequences from 27 samples (which were assigned sample IDs 91-117 for sequencing). Of these, eight were CONT samples, 11 were from animals that survived BRD (BRDL), and eight were from animals that died (BRDM). A total of 670.9 million reads (335.45 million paired reads) were sequenced for an average of 24.8 million reads per sample. Significantly fewer raw reads were obtained from the CONT samples than from the BRDL and BRDM samples. Less than 1% of reads were removed because of low quality, but over 99 % of

the remaining reads were bovine host reads which were removed from further analysis. Non-host read counts ranged from 6,408 to 97,130 reads per sample with an average of 22,510 reads. Despite the difference in raw reads between sample groups, the difference in non-host read counts between sample groups was not significant. Non-host reads were taxonomically classified with Kraken 2 (Wood et .al., 2019) within the AMRPlusPlus v2.0 pipeline (Doster et. al., 2020). After removal of non-bacterial and very low abundance taxa, 328 taxa remained, of which 252 were classified to the species level. These taxa represented 96 genera and 6 phyla. We calculated the relative abundance of taxa in each sample and used stacked bar graphs and heatmaps to visualize the results (Figure 1 and Figure 2). No species or genera showed statistically significant differences in relative abundance between the CONT, BRDM and BRDL groups. After ANCOMBC2 (Lin et. al., 2020) (Lin et. al., 2022) global testing with Holm-Bonferroni correction for multiple comparisons, all taxa had q value of 1.

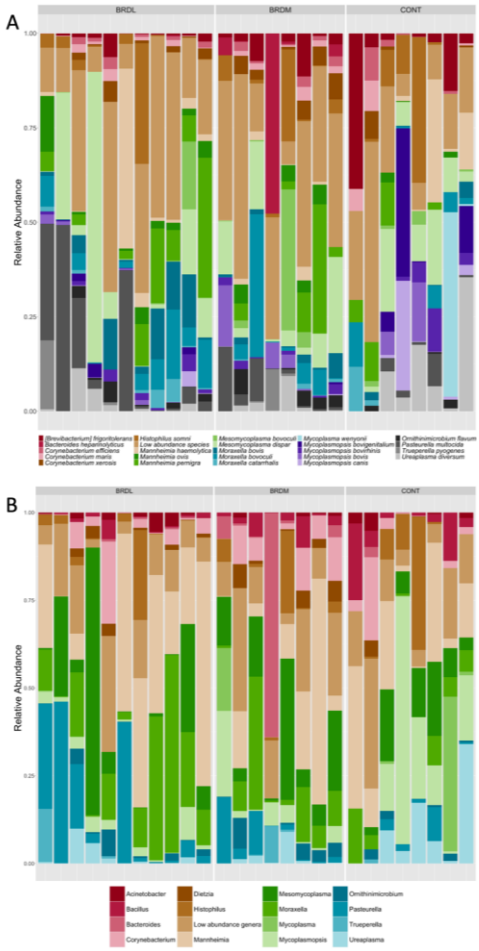


Figure 1 Species Relative Abundance.

Relative abundance of A) species or B) genera in samples grouped by Classification group (CONT (non-BRD cattle), BRDL (cattle treated for BRD that lived), and BRDM (cattle treated for BRD that died)). Species and genera with relative abundance under one percent are grouped as Low abundance taxa.

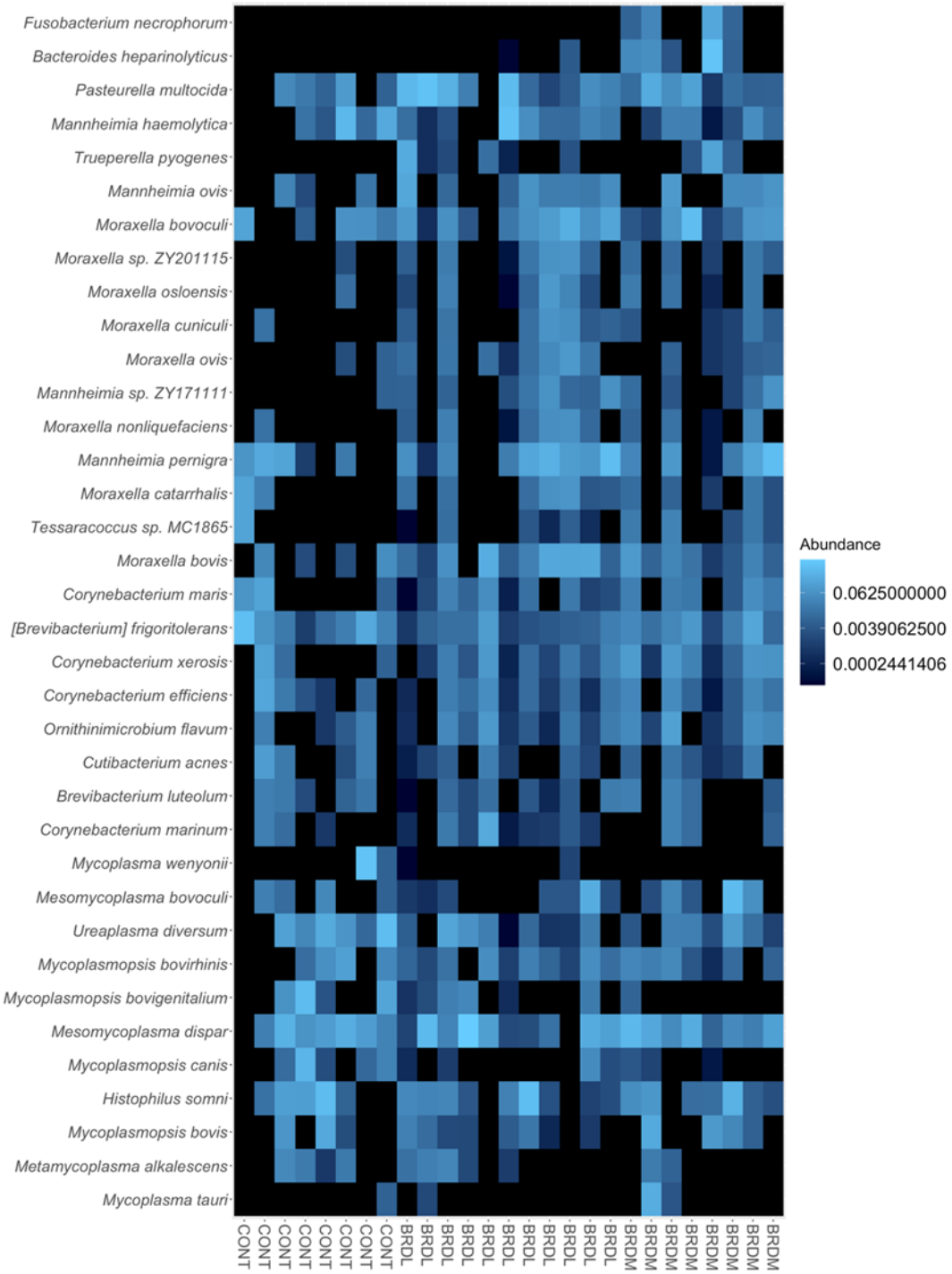


Figure 2 Heatmap of species relative abundance.
Species with less than 0.5% relative abundance are not shown. Species on the Y-axis are clustered by NMDS ordination. Samples are arranged on the X-axis by group (CONT (non-BRD cattle), BRDL (cattle treated for BRD that lived), and BRDM (cattle treated for BRD that died).

Although we found no significant difference in relative abundance of individual species between groups, we decided to look more closely at the prevalence of four bacteria commonly associated with BRD: *Histophilus somni*, *Mannheimia hemolytica*, *Mycoplasmopsis bovis*, and *Pasteurella multocida*. With respect to the raw read count number for each of these species, at least one read classified as *M. haemolytica* was present in 21 of the 27 samples, while 23 had at least one read classified as *P. multocida*. The samples with the highest counts of *P. multicide* and/or *M. hemolytica* were all in the BRDL group of samples. At least one read was classified as *H. somni* in 22 of the 27 samples. Samples with some *H. somni* reads were found in all groups, but none had extremely high counts. *M. bovis* was represented by at least one read in 15 of the 27 samples, but like *H. somni* did not have high counts in any samples and was found in a few samples in all three groups.

Alpha diversity for each sample was measured at the species and genus levels by calculating Observed, Shannon, and Inverse Simpson indices. The observed (richness) metric was significantly higher in BRDM samples than in the CONT samples at the species level, and in both BRDL and BRDM samples compared to CONT samples at the genus level in Figure 3. We visualized beta diversity using NMDS plots of Figure 4 and used PERMANOVA testing (Anderson et. al., 2017) to assess statistical significance of differences between our sample groups. We found no significant differences in beta diversity between sample groups at either the species or genus level.

Figure 3 Observed (richness) metric of alpha diversity at A) species level and B) genus level for CONT (non-BRD cattle), BRDL (cattle treated for BRD that lived), and BRDM (cattle treated for BRD that died) groups. The Y axis shows the number of taxa present. Boxes denote the 25th to 75th percentile (interquartile range) for the group, with the horizontal line within the box indicating the median value. Post hoc pairwise comparisons with significant adjusted P values are indicated by horizontal lines with asterisks (* = < 0.05, ** = <0.01 and *** = <0.001).

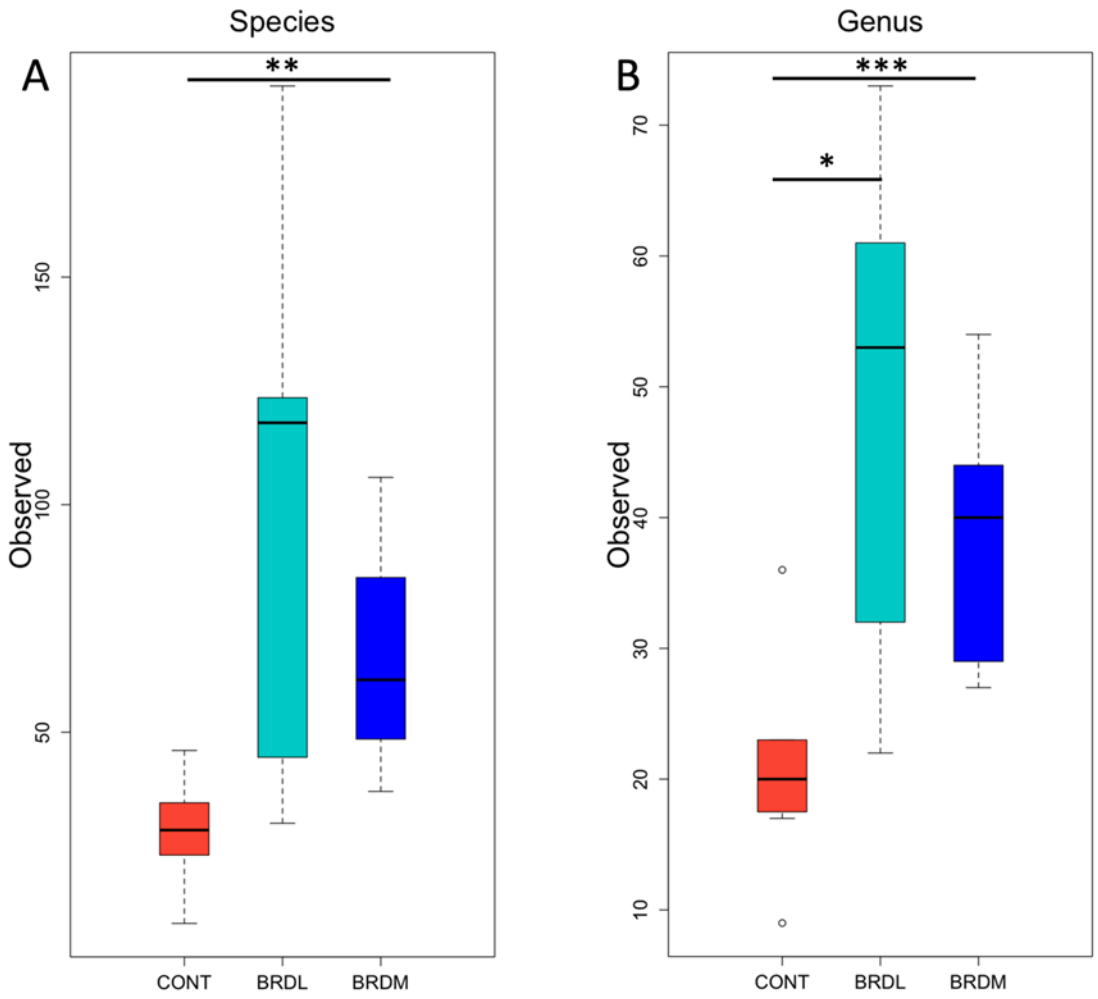
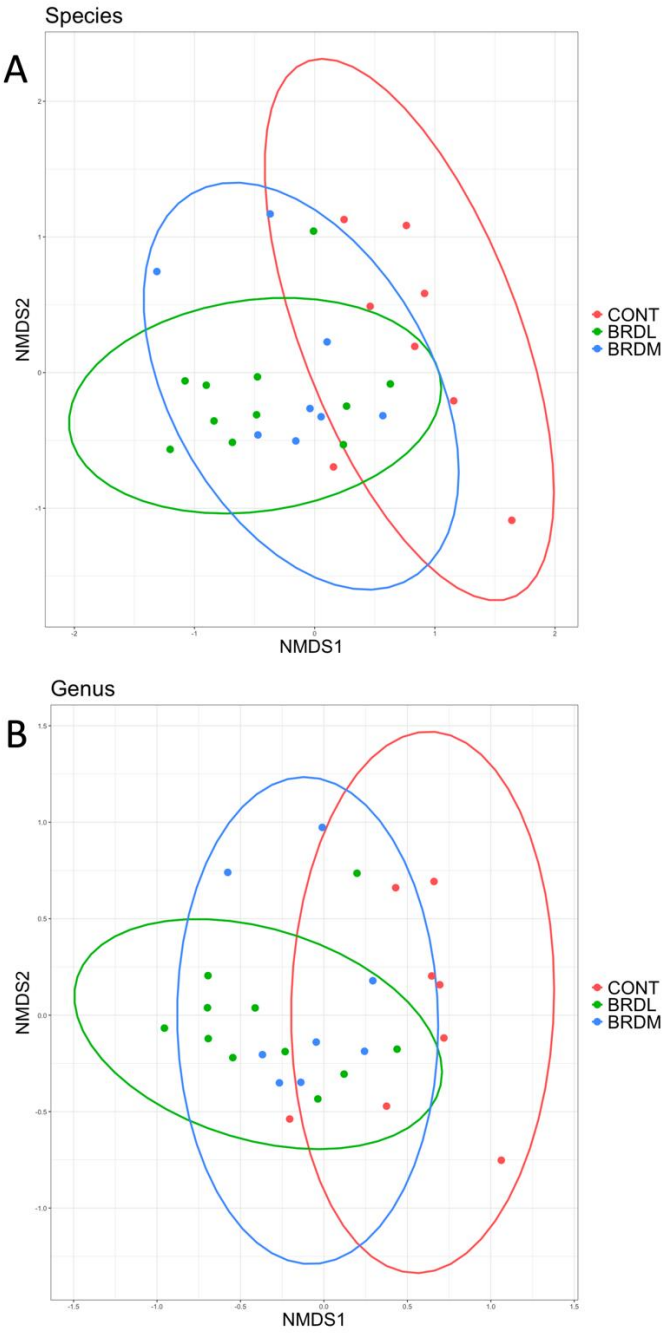


Figure 4 NMDS plot of beta diversity at the A) species and B) genus level
No significant differences were found between sample groups: CONT (non-BRD cattle), BRDL (cattle treated for BRD that lived), and BRDM (cattle treated for BRD that died).



3.4. AMR genes

To search for antimicrobial resistance (AMR) genes in the shotgun metagenomic sequences, we used AMRPlusPlus v3.0 (Bonin et. al., 2023) and ARIBA (Hunt et. al., 2017) to map reads to known AMR genes. AMRPlusPlus v3.0 identified eight potential AMR genes

across all samples, but only five of these were found to carry sequences known to be associated with AMR as shown in Table 2. Three samples, one CONT (94) and two BRDL (102 and 111) had at least one of the verified AMR alleles.

Table 2 Presence of antimicrobial resistance genes in total community DNA isolated from nasopharyngeal swabs from a first treatment bovine respiratory disease (BRD) cattle in comparison to healthy cattle.
For each potential AMR gene identified by AMRPlusPlus v3.0 in at least one sample, counts of AMR-verified reads are given as the numerator and total read counts for that gene as the denominator. Genes and samples with verified AMR-related reads are shown in bold.

MEGARes database accession	**Sequenced samples**					
	94 CONT	**102 BRDL**	**111 BRDL**	**112 BRDL**	**113 BRDL**	**114 BRDL**
MEG_2860|Drugs|MLS|23S_rRNA_methyltransferases|ERMX	0	13/13	0	0	0	0
MEG_3977|Drugs|MLS|Macrolide-resistant_23S_rRNA_mutation|MLS23S|RequiresSNPConfirmation	0	0	0/341	0	0	0
MEG_3978|Drugs|MLS|Macrolide-resistant_23S_rRNA_mutation MLS23S|RequiresSNPConfirmation	0	0/219	0	0	0/290	0/64
MEG_3979|Drugs|MLS|Macrolide-resistant_23S_rRNA_mutation|MLS23S|RequiresSNPConfirmation	0	17/158	0	0	0	0
MEG_3983|Drugs|MLS|Macrolide-resistant_23S_rRNA_mutation|MLS23S|RequiresSNPConfirmation	4/37	0	0	0	0	0
MEG_6|Drugs|Aminoglycosides|Aminoglycoside-resistant_16S_ribosomal_subunit_protein|A16S|RequiresSNPConfirmation	0	0/319	0/401	0/36	0/63	0
MEG_7310|Drugs|Elfamycins|EF-Tu_inhibition|TUFAB|RequiresSNPConfirmation	0	4/55	11/102	0	0	0
MEG_8249|Drugs|MLS|Macrolide-resistant_23S_rRNA_mutation|MLS23S|RequiresSNPConfirmation	0	196/196	248/248	0	0	0

4. Discussion

Samples were evaluated from cattle based on outcomes following BRD treatment (BRDM, BRDL) or cattle never treated for BRD (CONT) to determine potential differences in bacterial profiles, antimicrobial resistance patterns, relative abundance of species/genera, and alpha and beta diversity indices. Common BRD pathogens were identified with relatively minor changes in antimicrobial susceptibility among cattle outcome classification groups. The relative abundance of species and genera did not illustrate large visual differences among the outcome groups; however, alpha diversity index illustrated differences between BRD treated animals (BRDM and BRDL) compared to cattle never treated (CONT).

Mannheimia haemolytica is often reported as a primary pathogen identified in fatal BRD cases but was rarely identified by culture in this study (Booker et. al., 20008) (Fulton et. al., 2009). *Pasteurella multocida* was cultured from 9 samples allowing for antimicrobial susceptibility testing. While not enough numbers of cattle were present in each category for statistical analysis, cattle in each outcome category had isolates either pan-susceptible or only showing resistance to oxytetracycline. In BRDM, 1 isolate was pan-susceptible and one illustrated oxytetracycline resistance. Similarly, BRDL had 2 isolates pan-susceptible and 2 isolates resistant to oxytetracycline. The CONT cattle had a similar ratio with 1 isolate pan-susceptible and 2 resistant to oxytetracycline. No resistance to commonly used macrolides was identified, and each outcome category had animals possessing both susceptible and resistant isolates. This finding is logical as samples were collected prior to the BRD antimicrobial treatment and resistance patterns may have been influenced by previous antimicrobial treatment programs which would be similar among cattle in all three groups. Few samples had known AMR genes identified with shotgun metagenomics: only three animals (1 CONT and 2 BRDL)

were able to be evaluated. In this small study differences in antimicrobial resistance patterns were not identified in BRD cases that lived or died.

Relative abundance of species and genera were compared among all three outcomes. Cattle in each class showed presence of common pathogenic (*Mannheimia haemolytica, Pastuerella multocida, Histophilus somni,* and *Mycoplasma*) species. Finding *Mannheimia* species and genera on shotgun metagenomics, but not on culture, is not surprising due to the increased sensitivity of the metagenomics testing compared to culture. Few major differences were identified visually or statistically using relative abundance to compare BRDM, BRDL, and CONT groups. This finding is consistent with previous research comparing BRD treated animals to non-BRD treated animals (McMullen et. al., 2020). McMullen et al. noted more changes in bacterial populations over time compared to bacterial population differences among BRD and non-BRD cattle.

The alpha diversity was higher in BRDL compared to CONT at the species level and both BRDL and BRDM showed higher alpha diversity compared to CONT at the genus level. While differences in alpha diversity were identified relative to CONT cattle, no differences were found among the outcomes of BRD treated cattle. Increased alpha diversity in cattle treated for BRD differs from previous research that illustrated higher species richness and Shannon diversity in cattle not treated for BRD. One potential difference is in the previous study, cattle raised without antimicrobials were used as the study population and in the current study cattle could have received previous antimicrobial treatment (McMullen et. al., 2019). Another study identified less diverse bacterial species in cattle that died of BRD (Li et. al., 2022); however, this study was evaluating cattle with previous treatments and in the current study cattle were evaluated at first

treatment for BRD. Antimicrobial treatment would likely impact the diversity of bacterial species.

Limitations of this study include a relatively small sample size from each outcome (BRDL, BRDM, and CONT) submitted for full testing; however, relatively few differences were identified in the evaluations. Cattle enrolled in the study were from a commercial facility and no history prior to arrival at the facility was available; therefore, previous antimicrobial or disease treatments could not be included in the analysis.

Results from this study illustrated relatively few major differences in bacterial populations, antimicrobial resistance patterns, or relative abundance among cattle treated for BRD that lived, cattle treated for BRD that died, or animals never treated for BRD. Alpha diversity index was higher at both species and genera level for BRD treated animals compared to CONT. While some differences were identified relative to CONT cattle, no apparent differences were identified between BRDL and BRDM groups.

Chapter 2 - Risk factors associated with case fatality and treatment success following initial bovine respiratory disease treatment in feedyard cattle

As submitted to *Bovine Practitioner*

Abstract

Bovine respiratory disease (BRD) is an important disease of fed cattle and knowledge gaps exist regarding factors predicting treatment success. The study objective was to identify potential relationships between risk factors known at the time of initial BRD treatment with three post-treatment outcomes: first treatment success (FTS; finishing feeding phase with no further treatments for any disease), non-cause specific case fatality risk (CFRALL; post-treatment deaths from any cause), and cause-specific case fatality risk (CFRBRD; deaths attributed to BRD). This retrospective analysis used generalized linear mixed models to evaluate relationships between covariates of interest (arrival: sex, weight, month; treatment event: days on feed (DOF), rectal temperature, day-of-week, antibiotic drug class) with each outcome (FTS, CFRALL, CFRBRD). Analysis included 132,521 individual-animal initial BRD treatment records from 14 central U.S. feedyards (May 2017 to Dec 2020) with overall FTS of 67.8%, CFRALL of 10.0%, and CFRBRD of 6.3%. The FTS was associated ($P<0.05$) with all covariates except sex, CFRALL was associated with all covariates, and CFRBRD was associated with all covariates except day-of-week treated. Treatments early in the feeding phase (DOF 0-10, 11-20) resulted in lower ($P < 0.05$) FTS (49.2% ± 0.8; 55.3% ± 0.8), higher ($P < 0.05$) CFRALL (12.5% ± 1.3; 12.6% ± 1.4) and higher ($P < 0.05$) CFRBRD (6.3% ± 1.1; 6.1% ± 1.0) compared to cattle treated on days 21-70. Rectal temperature in the 103.1-104.0 (39.4-40.0°C) category had higher ($P < 0.05$)

CFRALL (15.3% ± 1.5) and CFRBRD (9.2% ± 1.5) compared to other rectal temperature categories. Specific risk factors including days on feed at treatment, sex, and weight at the time of treatment were associated with treatment outcomes. Results can be useful for defining expectations following first treatment for BRD.

Introduction

Bovine respiratory disease (BRD) remains a significant challenge for the beef industry. The syndrome is common, with approximately 97% of feedyards administering antimicrobial treatments to cattle affected with BRD (NAHMS, 2011) resulting in significant economic cost to the beef industry (Johnson et. al., 2017) (Miles, 2009). Welfare and financial impacts of BRD emphasize the importance of designing and managing appropriate therapeutic interventions. Multiple studies have evaluated factors associated with BRD morbidity and mortality risk, (Rojas et. al., 2022) (Reinhardt et. al., 2009) (Babcock et. al., 2013) (Cernicchiaro et. al., 2012) and economic impacts; (Blakebrough-Hall et. al., 2020) (Cernicchiaro et. al., 2013) (Smith, 1998) however, few studies have focused on identifying factors associated with post-BRD treatment success or failure.

Common post-treatment evaluation metrics are case fatality risk and first treatment success. Several variables influence the likelihood of treatment success, and improved understanding of potential causes of treatment failures facilitates refining therapeutic protocols (Booker et. al., 2020). Previous studies have evaluated physiological (e.g., rectal temperature) or pathological (e.g., blood metabolites) parameters associated with BRD case outcomes (Blakebrough-Hall et. al., 2022) (Blakebrough-Hall et. al., 2020) (Theurer et. al., 2014) (Li et. al., 2022) (Baruch et. al., 2019). Cattle demographic factors (e.g., gender, arrival weight) have been infrequently evaluated combined with information known at initial treatment (e.g. days on

feed (DOF), rectal temperature, and selected antimicrobial) when evaluating differences in case outcomes. Avra et al. evaluated initial BRD treatment success and determined arrival factors including risk classification, DOF, quarter of arrival, and rectal temperature were associated with the likelihood of treatment failure; however, potential differences between treatment failure resulting in retreatment versus death were not elucidated (Avra et. al., 2017). Theurer et al. evaluated the likelihood of cattle not finishing the feeding phase, but no evaluation of retreatments were performed (Theurer et. al., 2014). Identifying risk factors associated with treatment success as gauged by retreatment or cause-specific mortality allows feedyard personnel to better anticipate likely outcomes following initial treatment for BRD.

The objective of this retrospective analysis was to identify potential association between risk factors known at the time of first BRD treatment with three post treatment outcomes: first treatment success, (FTS; percent of animals requiring no further treatment and finishing the feeding phase), non-cause specific case fatality risk (CFRALL; mortalities following treatment from any cause), and cause-specific case fatality risk (CFRBRD; mortalities attributed to BRD).

Materials and Methods

This retrospective analysis used existing feedyard operational data and was exempt from Institutional Animal Care and Use Committee approval as no animals were used specifically for this study. The project included data from 14 commercial U.S. feedyards from May 31, 2017 through December 28, 2020. Included feedyards were located in the U.S. central high plains region. Data were included from native (beef) as well as dairy-influenced or mixed breeds of cattle. However, breed of animal was not available in the records to be included in the statistical analyses as a potential risk factor.

Data management

The initial dataset contained data 269,683 animals at the time of first treatment for any disease. Observations were filtered to meet ensuing inclusion criteria. Metaphylactic status was not included in this analysis as it was not known for all feedyards. Only cattle with a clinical diagnosis of BRD at first treatment that received an antimicrobial were included. The BRD diagnosis was performed by personnel at each feedyard. Diagnostic criteria varied slightly between feedyards, but included clinical signs consistent with respiratory disease namely depression, anorexia, increased respiratory rate/effort. Average cohort arrival weight was limited to cohorts between 500 to 1100 lbs (226.8- 499kg) and only cohorts with gender described as either steer or heifer were included (excluding mixed sex and non-labeled cohorts). Cohort size was restricted to ≤ 400 with no minimum number of animals received per group. The days on feed (DOF) at the initial BRD treatment event was limited to between 0 and 300 days. Finally, rectal temperature at the time of initial BRD treatment was limited to between 100.4 and 109.4 °F (38 and 43°C) to minimize potential temperature recording errors.

After filtering, data were imported into statistical software[a] for management and analysis. Continuous variables were categorized to avoid violation of assumption of linear associations with outcomes of interest. Arrival dates were categorized to represent the month cattle entered the feedyard. Arrival weight categories were assigned in 100 lb (45 kg) increments to represent weight ranges commonly used in cattle sales transactions (500-599, 600-699, 700-799, 800-899, 900-999, 1000-1100 lb; 227-272, 273-317, 318-362, 363-407, 408-452, 453-497kg). Categories for DOF at initial treatment ranged from 0 to 100 days in 10-day increments, with a single additional group for >100 days. Recorded treatment dates were categorized by day of the week at the time of treatment. Rectal temperature measurements were categorized based on

measurements as recorded in °F and converted to °C. The categorization was based on rectal temperatures less than or equal to 103, 103.1-104.0, 104.1-105, 105.1-016, and greater than 106°F. This resulted in categories in °C of < 39.4, 39.4 – 40.0, 40.1-40.6, 40.7 – 41.1, and > 41.1°C. Antimicrobial treatment for the first BRD diagnosis was classified by antimicrobial types into beta lactams, fluoroquinolones, macrolides, phenicols, and tetracyclines.

Risk factors known at the time of initial BRD treatment fall into two broad categories: arrival cohort-level information and individual-animal treatment event data. Arrival information includes gender, arrival month, and cohort average arrival weight. Individual-animal information at initial BRD treatment event includes the DOF at treatment, rectal temperature, day-of-week treatment occurred, and antibiotic drug class used.

Outcomes of interest

Measured outcomes were first treatment success, mortality risk (CFRALL; deaths from any cause following treatment), and cause-specific case fatality risk (CFRBRD; deaths attributed to BRD).

The FTS was calculated as animals treated for the first time for BRD that received no subsequent treatments for BRD and finished the feeding phase divided by total animals initially treated for BRD with no subsequent treatments for BRD. All cause case fatality risk (CFRALL) was calculated as animals treated for BRD only once that later died of any cause divided by all animals initially treated for BRD only once. The BRD specific case fatality risk definition (CFRBRD) was calculated as animals initially treated for BRD and died with a mortality diagnosis of BRD (as defined by the feedyard personnel via necropsy), divided by all animals initially treated for BRD. Morbidity and mortality diagnoses for individual animals were made at the feedyard level by trained personnel.

Statistical Analysis

Statistical models were created using generalized linear mixed models[a] using the glmer function. Separate models were created for each outcome (FTS, CFRALL, CFRBRD) to evaluate potential associations with study factors of interest including arrival factors (sex, average cohort weight category, arrival month) and initial treatment event factors (DOF at treatment, rectal temperature category, day-of-week treated, and antibiotic class). Random effects for each cohort nested within feedyard and arrival year were incorporated in each model to account for the lack of independence for cattle from the same cohort and feedyard. Multivariable models were constructed through a backward iterative process until only variables significantly ($P \leq 0.05$) associated with the outcome remained in each model. Potential differences among model least squares mean probabilities among levels of each covariate were evaluated and adjusted for multiple comparisons using Tukey HSD with $P \leq 0.05$ considered significant.

Results

Initial data consisted of individual-animal treatment records (n=269,683) for cattle treated for all causes. After filtering criteria (Figure 1) were applied, 132,521 individual-animal initial BRD treatment records were available for analysis. Overall FTS in the final dataset was 67.8% (89,885/132,521) with CFRALL of 10.0% (13,248/132,527) and CFRBRD of 6.3% (8,414/132,521).

The study population consisted of 74.3% steers with 81.8% of the study population arriving with average cohort arrival weight between 700 and 1,000 lbs (317.6 and 453.6 kg). Study animals arrived throughout the year with 43.3% of the arrivals between September and December. Most BRD treatment events occurred early in the feeding phase with 73.3% of initial treatments prior to 50 DOF. The majority (68.9%) of rectal temperatures were above 104.0°F

(40.0°C) at the time of initial treatment. Cattle were initially treated for BRD at similar frequency on each day-of-week with the percent of BRD treatments ranging from 13.1% to 15.1% of all treatments occurring on each day. Macrolides were the most common (68.0%) antimicrobial class at first treatment. Descriptive statistics by each potential risk classification variable are provided in Table 1.

First Treatment Success (FTS)

The final multivariable model for FTS included significant associations ($P \leq 0.05$) with arrival (cohort average weight category, month), and initial treatment factors (DOF at treatment, rectal temperature, day-of-week and antimicrobial class). Arrival year was not able to be included in the model due to a lack of convergence. Cohort gender was not ($P = 0.13$) associated with FTS. Model-adjusted probabilities for each covariate are displayed in Table 2.

Cattle with average cohort arrival weight of 600-699 lbs (272.3-317.5kg) had the lowest ($P \leq 0.05$) probability of FTS (53.5% ± 1.2) compared to all other weight classes. Each subsequent weight class had higher ($P \leq 0.05$) probability of success through the 1,000-1,100 lb (453.7-499.0 kg) category (66.0% ± 0.8). The lowest weight class (226.8-272.2) did not differ ($P > 0.05$) from the highest two weight classes in the model-adjusted likelihood of FTS. Cattle arriving in September (66.2% ± 0.8) did not differ from cattle arriving in October, but had higher FTS compared to all other arrival months. Cattle treated at early DOF (0-10: 49.2% ± 0.8; 11-20: 55.3%± 0.8) or late in the period (>100: 54.0% ± 0.9) had lower FTS compared to all other categories. Cattle with rectal temperatures below 103°F (39.4°C) had the best first treatment success (66.3% ± 0.7) compared to all other categories. While day-of-week at initial treatment was statistically associated with FTS, few clinically meaningful differences were identified. Cattle initially treated with antibiotics from macrolide or tetracycline classes had higher FTS

(69.9% ± 0.4; 69.1% ± 0.4, respectively) compared to other antibiotic classes, but did not significantly differ from each other.

Case Fatality Risk All

The non-cause specific post-treatment mortality model revealed all arrival and initial BRD treatment event factors were significantly ($P \leq 0.01$) associated with CFRALL and model estimates for each level of the covariates are included in Table 2. Arrival year was not able to be included in the model due to a lack of convergence. Heifers had higher CFRALL (11.8% ± 1.2) compared to steers (10.3% ± 1.0). Few differences were identified based on arrival average cohort weight category; however, cattle in the 800-899 lb (363.0-408.2 kg) category had higher CFRALL (11.7 ± 1.2) compared to cattle in the 1000-1099 lb (453.7-499.0 kg) category (10.0 ± 1.1). Cattle arriving in January and February had higher CFRALL compared to cattle arriving in August, September, and October, and the remaining months did not differ from either of these periods. Cattle treated at > 100 DOF had the overall highest CFRALL (19.1% ± 1.2); however, cattle treated in the first 30 DOF had higher CFRALL compared to cattle treated d31-50. Cattle treated on Sundays had higher CFRALL (11.7% ± 1.2) compared to cattle treated on Fridays (10.3 ± 1.1), but neither day differed from any other day of the week. Cattle treated with tetracyclines had a lower CFRALL (7.7% ± 0.8) compared to most drug classes but did not differ from beta lactams.

Case Fatality Risk for BRD

For the CFRBRD model, all variables except initial BRD treatment day-of-week ($P = 0.12$) were significantly ($P \leq 0.01$) associated with the BRD-specific post-treatment deaths (Table 2). Heifers had higher CFRBRD (6.1 ± 1.0) compared to steers (5.1% ± 0.8). Cattle in the two

heaviest arrival weight categories (900-999, 1,000 – 1,100 lbs; 408.3-453.6, 453.7-499.0 kg) had lower CFRBRD when compared to all categories except 500-599 lbs (226.8-272.2 kg) which did not differ in CFRBRD risk from any category. The month cattle arrived at the feedyard was associated with CFRBRD but no statistical ($P \leq 0.05$) differences among arrival months were identified. Cattle treated in the first 10 days on feed had higher CFRBRD (8.2% ± 1.3) compared to other treatment DOF categories but did not differ from cattle treated at > 100 DOF (7.2% ± 1.2). Rectal temperature at initial treatment < 103 °F (39.4°C) had the lowest CFRBRD (3.3% ± 0.6) compared to all other categories. Initial treatment with tetracyclines was associated with the lowest CFRBRD (3.5% ± 0.6) compared to all other categories.

Discussion

Outcomes following treatment influence refining therapeutic and disease management plans. The study objective was to identify potential arrival and initial BRD factors that may be associated with three major outcomes: FTS, CFRALL, and CFRBRD. Overall calculations of each outcome identified a baseline expectation for each outcome. The baseline risk for CFRBRD was lower than CFRALL as expected when utilizing a more specific case definition. Multivariable analysis of the retrospective data revealed many risk factors significantly associated with each outcome of interest. Improved understanding of expected outcomes based on cattle demographic and treatment information allows the inclusion of more variables that impact BRD treatment success when evaluating therapeutic protocols after initial BRD treatment in specific types of cattle.

Risk of death following treatment (CFRALL and CFRBRD) was higher in heifers, but FTS was not associated with gender. This finding agrees with research illustrating that overall feedyard mortality risk may be higher in heifers than steers; (Vogel et. al., 2015) (Loneragan et. al., 2001) however, some research shows the actual level of mortality risk by gender is also

influenced by arrival weight and time of year of arrival (Babcock et. al., 2013). These previous studies evaluated the overall mortality risk and the current work is only evaluating the post-BRD treatment mortality risk. Heifers have been associated with higher risk of frequently fatal diseases such as acute interstitial pneumonia, (Woolums et. al., 2005) but it is unknown the role this disease syndrome may have played in findings from the current study which focused on BRD. Results indicate expectations for fatality risk should be higher in heifers compared to steers after first treatment for BRD.

Mean cohort arrival weight is a commonly used risk factor to predict level of morbidity in groups of cattle with lower arrival weights generally considered to be at higher risk for BRD. (Cernicchiaro et. al., 2012) (Taylor et .al., 2010). This study evaluated likelihood of each outcome based on arrival weight and found greatest treatment success and lower risk of mortality in heavier weight classes. This finding agrees with previous research indicating that the likelihood of not finishing the feeding phase following BRD treatment tended to decrease as arrival weight increased (Theurer et. al., 2014). Similar findings were presented when evaluating the likelihood of treatment failure although weights were grouped in broad categories and the effect was modified by days on feed at first treatment (Avra et. al., 2017). The change in mortality risk following treatment was not linear in either CFRALL or CFRBRD with the two heaviest weight categories showing differences from the middle and lower weight categories. Weight is often considered a proxy for age and cattle in the top weight categories (> 900 lbs; 408.3 kg) were likely older animals and potentially more resilient to disease challenge through prior exposure or immune system development. For FTS, the likelihood of success increased in a stepwise fashion for the 5 categories starting with 600-699 lbs (272.3-317.5 kg). This impact could be due to the same reasons as changes described for CFRALL/CFRBRD. Additionally,

lighter weight cattle would potentially have longer at-risk periods during which additional treatments could be administered.

Arrival month was associated with all three outcomes with fall months (Sep, Oct, Nov) displaying some of the higher FTS and lowest CFRALL. This effect contrasts with previous research on the overall risk of BRD which indicates the fall and winter of the year as a prevalent time for BRD risk and period of higher mortality (Babcock et. al., 2013) (Cernicchiaro et. al., 2012) (Hay et. al., 2017). Previous work evaluated overall morbidity or mortality risk while the current study evaluates the post-BRD treatment outcomes which represents a different group of individuals than the population as a whole. Decreased post-treatment failure risk during this period could be related to the lack of diagnostic specificity when identifying BRD cases (Timsit et. al., 2016). Diagnostic specificity should not change based on prevalence; however, in BRD outbreaks health observers could be more aggressive in identifying cases resulting in higher level of false positive diagnoses. The potential for more false positive BRD diagnoses during this period may be supported by the fact that when a more specific mortality diagnosis was used (CFRBRD) no statistical differences among individual months were identified.

The timing of initial treatment relative to feedyard arrival was associated with all three outcomes and displayed a similar pattern with lower FTS, higher CFRALL and higher CFRBRD both early and late in the feeding phase. Initial treatment timing may be affected by the metaphylactic status of cattle which was not included in this study. This finding is similar to previous research that identified higher likelihood of treatment failure in the first 20 days on feed when all cattle treated after 40 DOF were grouped into a single category (Avra et. al., 2017). Additional research has also illustrated that in a population of cattle treated for BRD, cattle that subsequently died were treated earlier in the feeding phase (least squares mean +/- standard error

15 +/- 2.3 DOF) compared to cattle that did not die (22 +/- 0.8 DOF) (Blakebrough-Hall et. al., 2022). The early feeding phase is the most common time period for BRD occurrence (Babcock et. al., 2010) (Johnson et. al., 2023), and during this time period cattle are often transitioning to new rations, social environments, and housing conditions. This accumulation of risk factors makes disease response challenging. However, these risk factors are not necessarily at play later in the feeding period where we also saw an increase in CFRALL and CFRBRD coupled with the decrease in FTS late in the feeding phase (> 100 DOF). These findings may be related to a change in the prevalence of different pulmonary disease syndromes. While all cases were diagnosed with BRD, the clinical signs would be nearly impossible to distinguish from acute interstitial pneumonia (AIP). Later in the feeding phase is a more common time for AIP occurrence, and risk of fatality from this syndrome is higher than expected from BRD alone (Woolums et. al. 2005) (Haydock et. al., 2023). These results indicate that expectations for treatment success and mortality should be tempered with the timing of initial BRD treatment.

Rectal temperature at the time of initial BRD treatment was associated with all three outcomes, but no discernable trends were identified. Both measures of fatality (CFRALL and CFRBRD) displayed the highest mortality in the 103.0-103.9° F (39.4-40.0 °C) category which contrasts with previous research illustrating the likelihood of not finishing the feeding phase increased with higher rectal temperatures (Theurer et. al., 2014). Potential reasons for this discrepancy include differences in the method of categorization of rectal temperature and surveys of cattle from different time periods, cattle types, and employees (Theurer 2000-2009; current study 2017-2020). Interestingly, the CFRALL and CFRBRD were lower in the highest temperature category (>106°F, 41.1°C) compared to many other categories evaluated. This could occur if other syndromes beyond BRD were contributing to the fever response, or if the cattle

diagnosed in this temperature category were early in the disease process and responded well to treatment.

The day of the week was hypothesized to be associated with treatment outcomes based on changes in labor management during the week. While feedyards are in operation seven days a week, the weekends are often staffed at a different level and work hours by week-day may vary. While FTS was associated with day of the week at initial BRD treatment, few biological differences were noted. The CFRALL was also associated with day of the week, but the only two days that differed indicated that Fridays had lower CFRALL compared to Sundays.

Drug class was recorded at the time of treatment and was associated with all three outcomes. These findings should be interpreted cautiously as antimicrobial selection at the time of BRD treatment is often dictated by protocols which may vary by type of cattle, severity of illness, time of year, and other individual feedyard factors. The main reason for including antimicrobial drug class was not in an attempt to evaluate differences in drug classes, as these applications were biased and not randomly assigned, but rather to include this effect in the multivariable models to allow more complete evaluation of the other potential risk factors.

Limitations of this study are primarily associated with the retrospective nature of data collection and analysis. While these findings have good external validity, all potential sources of bias cannot be evaluated or controlled due to the non-random nature of cattle in each demographic group. Additionally, data were collected from multiple production operations and while this impact was controlled for in the analysis with random effects, there are likely still differences among individual operations. Results from this study form a basis for future hypotheses which may need to be further evaluated through subsequent randomized controlled clinical trials.

Another major limitation of this study is the unknown status of metaphylaxis in these cattle. Metaphylactic administration of antimicrobials at arrival may have impacted treatment responses as for cattle receiving an initial treatment for BRD that received metaphylaxis this could have been their second dose of antimicrobials after feedyard arrival. Data were unavailable to determine metaphylaxis status; therefore, the impact of this procedure could not be evaluated. Further studies would be useful to help determine if metaphylaxis administration is associated with BRD treatment outcomes.

Conclusions

This study illustrated that several arrival cattle demographics and initial BRD treatment factors were associated with the probability of post-therapeutic success and mortality. Heifers were more likely to die following treatment when measured by CFRALL and CFRBRD. Negative outcomes for all three variables were in general more likely in lighter cattle (< 900 lbs; < 408.2 kg), cattle early (< 20 DOF) and late (>90 DOF) in the feeding phase. Findings from this research can be useful in setting refined benchmarks for expectations following the initial treatment for BRD.

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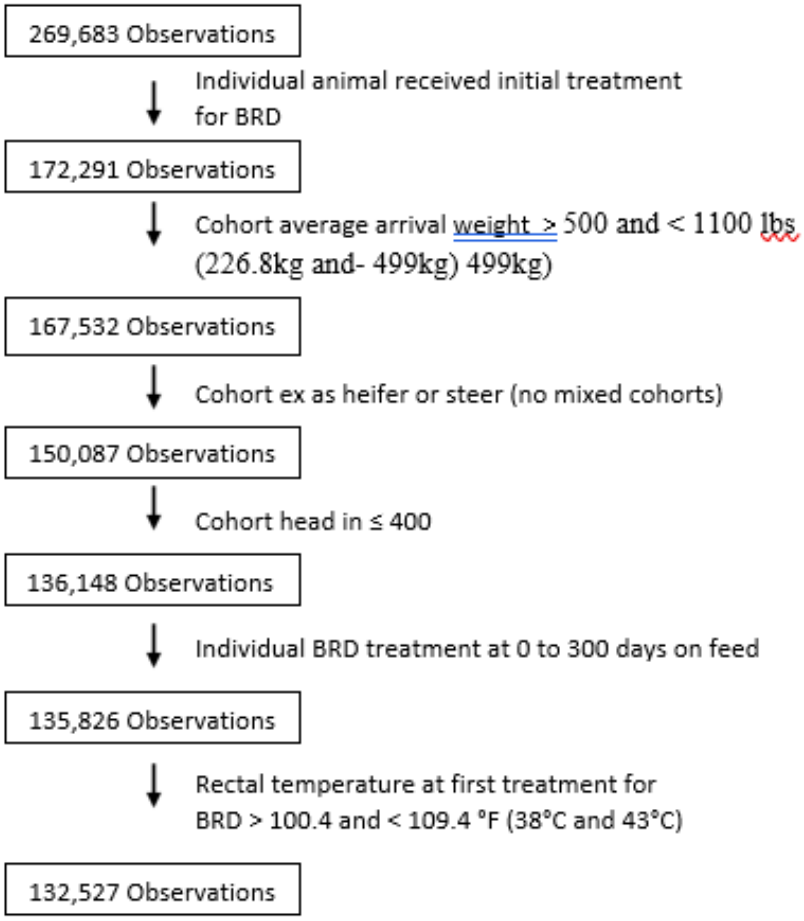

Figure 5 Data filtering on initial treatments at feedyards U.S. central high plains (n=14) to create dataset consisting of individual animal initial treatments for bovine respiratory disease (BRD) based on inclusion criteria.

Table 3 Frequency distribution of cattle initially treated for bovine respiratory disease (BRD) (n=132,521) in 14 U.S. feedyards between May 2017 and December 2020 among levels of each arrival cohort and initial BRD treatment event characteristic. Arrival information represents data collected at feedyard arrival at the cohort-level and initial event includes individual-animal data collected at the time of the first treatment for BRD.

Arrival characteristics			**Initial BRD Treatment Event characteristics**		
Sex	n	Percent of total	Event DOF category	n	Percent of total
Steers	98,401	74.3%	0-10	15,623	11.8%
Heifers	34,120	25.7%	11-20	31,269	23.6%
Average Cohort Arrival Weight category			21-30	22,463	17.0%
226.8-272.2	2,575	1.9%	31-40	16,274	12.3%
272.3-317.5	6,858	5.2%	41-50	11,507	8.7%
317.6-362.9	28,230	21.3%	51-60	9,136	6.9%
363.0-408.2	42,570	32.1%	61-70	6,718	5.1%
408.3-453.6	37,608	28.4%	71-80	5,185	3.9%
453.7-499.0	14,680	11.1%	81-90	3,862	2.9%
Cohort arrival month			91-100	2,803	2.1%
Jan	12,997	9.8%	> 100	7,681	5.8%
Feb	9,408	7.1%	Event rectal temperature		
Mar	7,879	5.9%	< 39.4	29,223	22.1%
Apr	5,976	4.5%	39.4 – 40.0	12,003	9.1%
May	7,904	6.0%	40.1 – 40.6	59,500	44.9%
Jun	7,920	6.0%	40.7 – 41.1	26,332	19.9%

Jul	10,174	7.7%	> 41.1	5,463	4.1%
Aug	12,896	9.7%	Event day of week		
Sep	16,016	12.1%	Mon	19,448	14.7%
Oct	16,077	12.1%	Tue	18,928	14.3%
Nov	11,784	8.9%	Wed	20,013	15.1%
Dec	13,490	10.2%	Thu	19,368	14.6%
			Fri	20,012	15.1%
			Sat	17,409	13.1%
			Sun	17,343	13.1%
			Initial Event antibiotic class		
			Beta lactam	469	0.4%
			Fluoroquinolone	4,030	3.0%
			Macrolide	90,622	68.4%
			Phenicol	2,457	1.9%
			Tetracycline	34,943	26.4%

Table 4 Model-adjusted probability estimates and statistics from multivariable models for each post-initial bovine respiratory disease (BRD) treatment outcome (first treatment success, FTS; non-cause specific case fatality, CFRALL; and post-treatment deaths attributed to BRD, CFRBRD). Each model included random effects for cohort and feedyard. Data (n=132,521) included cattle initially treated for BRD in 14 U.S. feedyards between May 2017 and December 2020. Differing superscripts among levels of each covariate indicate significant ($P < 0.05$) differences among model estimated probabilities.

	FTS Main effect P-value			CFRALL Main effect P-value			CFRBRD Main effect P-value		
	Prob		SE	Prob		SE	Prob		SE
Sex	0.13			<0.01			<0.01		
Steers				0.103	*a*	0.010	0.051	*a*	0.008
Heifers				0.118	*b*	0.012	0.062	*b*	0.010
Arrival weight category	<0.01			<0.01			<0.01		
226.8-272.2	0.664	*de*	0.014	0.108	*ab*	0.014	0.054	*ab*	0.010
272.3-317.5	0.535	*a*	0.012	0.115	*ab*	0.013	0.065	*b*	0.011
317.6-362.9	0.576	*b*	0.008	0.116	*bc*	0.012	0.061	*b*	0.010
363.0-408.2	0.601	*c*	0.007	0.117	*b*	0.012	0.062	*b*	0.010
408.3-453.6	0.636	*d*	0.007	0.106	*ac*	0.011	0.052	*a*	0.009
453.7-499.0	0.660	*e*	0.008	0.100	*a*	0.011	0.048	*a*	0.008
Arrival month	<0.01			<0.01			0.04		
Jan	0.596	*ab*	0.010	0.125	*b*	0.013	0.063	*a*	0.011
Feb	0.588	*bd*	0.011	0.126	*b*	0.014	0.061	*a*	0.010
Mar	0.613	*ab e*	0.011	0.108	*ab*	0.012	0.055	*a*	0.009
Apr	0.593	*ab*	0.011	0.110	*ab*	0.012	0.054	*a*	0.009
May	0.581	*b*	0.010	0.113	*ab*	0.012	0.061	*a*	0.010
Jun	0.588	*bd*	0.011	0.110	*ab*	0.012	0.057	*a*	0.010
Jul	0.628	*ac*	0.010	0.105	*ab*	0.011	0.054	*a*	0.009

Aug	0.632	*ce*	0.009	0.102	*a*	0.011	0.051	*a*	0.009
Sep	0.662	*f*	0.008	0.101	*a*	0.011	0.052	*a*	0.009
Oct	0.650	*cf*	0.009	0.101	*a*	0.011	0.054	*a*	0.009
Nov	0.624	*ac d*	0.010	0.111	*ab*	0.012	0.060	*a*	0.010
Dec	0.596	*ab*	0.010	0.114	*ab*	0.012	0.058	*a*	0.010
Event DOF category	<0.01			<0.01			<0.01		
0-10	0.492	*b*	0.008	0.125	*df*	0.013	0.082	*d*	0.013
11-20	0.553	*a*	0.008	0.113	*e*	0.011	0.070	*c*	0.011
21-30	0.595	*c*	0.008	0.100	*bc*	0.010	0.056	*b*	0.009
31-40	0.633	*d*	0.008	0.086	*a*	0.009	0.047	*a*	0.008
41-50	0.646	*df*	0.008	0.085	*a*	0.009	0.045	*a*	0.008
51-60	0.677	*e*	0.008	0.087	*ab*	0.009	0.045	*a*	0.008
61-70	0.666	*ef*	0.009	0.093	*ac*	0.010	0.047	*ab*	0.008
71-80	0.657	*de*	0.010	0.107	*cd e*	0.012	0.052	*ab*	0.009
81-90	0.646	*de*	0.011	0.114	*cd e*	0.013	0.055	*ab*	0.010
91-100	0.623	*cd*	0.012	0.144	*f*	0.016	0.062	*bc*	0.011
> 100	0.540	*a*	0.009	0.191	*g*	0.018	0.072	*cd*	0.012
Event rectal temperature	<0.01			<0.01			<0.01		
< 39.4	0.663	*d*	0.007	0.073	*a*	0.008	0.033	*a*	0.006
39.4 – 40.0	0.578	*b*	0.008	0.153	*d*	0.015	0.092	*d*	0.015
40.1 – 40.6	0.628	*c*	0.007	0.100	*b*	0.010	0.051	*b*	0.008
40.7 – 41.1	0.591	*ab*	0.007	0.135	*c*	0.013	0.076	*c*	0.012

> 41.1	0.603	*a*	0.010	0.106	*b*	0.012	0.048	*b*	0.008
Event day of week	<0.01			<0.01			0.12		
Mon	0.607	*ab*	0.008	0.107	*ab*	0.011			
Tue	0.610	*ab*	0.008	0.112	*ab*	0.011			
Wed	0.605	*a*	0.008	0.110	*ab*	0.011			
Thu	0.622	*b*	0.008	0.113	*ab*	0.011			
Fri	0.623	*b*	0.007	0.103	*a*	0.011			
Sat	0.609	*ab*	0.008	0.111	*ab*	0.011			
Sun	0.613	*ab*	0.008	0.117	*b*	0.012			
Initial Event antibiotic class	<0.01			<0.01			<0.01		
Beta lactam	0.535	*a*	0.025	0.100	*ab*	0.016	0.062	*bc*	0.014
Fluoroquinolone	0.495	*a*	0.010	0.157	*c*	0.015	0.073	*c*	0.012
Macrolide	0.699	*c*	0.004	0.111	*b*	0.011	0.056	*b*	0.009
Phenicol	0.629	*b*	0.014	0.120	*b*	0.015	0.063	*bc*	0.011
Tetracycline	0.691	*c*	0.004	0.077	*a*	0.008	0.035	*a*	0.006

Event DOF category	<0.01		<0.01		<0.01	
0-10	0.492 [b]	0.008	0.125 [df]	0.013	0.082 [d]	0.013
11-20	0.553 [a]	0.008	0.113 [e]	0.011	0.070 [c]	0.011
21-30	0.595 [c]	0.008	0.100 [bc]	0.010	0.056 [b]	0.009
31-40	0.633 [d]	0.008	0.086 [a]	0.009	0.047 [a]	0.008
41-50	0.646 [df]	0.008	0.085 [a]	0.009	0.045 [a]	0.008
51-60	0.677 [e]	0.008	0.087 [ab]	0.009	0.045 [a]	0.008
61-70	0.666 [ef]	0.009	0.093 [ac]	0.010	0.047 [ab]	0.008
71-80	0.657 [de]	0.010	0.107 [cde]	0.012	0.052 [ab]	0.009
81-90	0.646 [de]	0.011	0.114 [cde]	0.013	0.055 [ab]	0.010
91-100	0.623 [cd]	0.012	0.144 [f]	0.016	0.062 [bc]	0.011
> 100	0.540 [a]	0.009	0.191 [g]	0.018	0.072 [cd]	0.012
Event rectal temperature	<0.01		<0.01		<0.01	
< 39.4	0.663 [d]	0.007	0.073 [a]	0.008	0.033 [a]	0.006
39.4 - 40.0	0.578 [b]	0.008	0.153 [d]	0.015	0.092 [d]	0.015
40.1 - 40.6	0.628 [c]	0.007	0.100 [b]	0.010	0.051 [b]	0.008
40.7 - 41.1	0.591 [ab]	0.007	0.135 [c]	0.013	0.076 [c]	0.012
> 41.1	0.603 [a]	0.010	0.106 [b]	0.012	0.048 [b]	0.008
Event day of week	<0.01		<0.01		0.12	
Mon	0.607 [ab]	0.008	0.107 [ab]	0.011		
Tue	0.610 [ab]	0.008	0.112 [ab]	0.011		
Wed	0.605 [a]	0.008	0.110 [ab]	0.011		
Thu	0.622 [b]	0.008	0.113 [ab]	0.011		
Fri	0.623 [b]	0.007	0.103 [a]	0.011		
Sat	0.609 [ab]	0.008	0.111 [ab]	0.011		
Sun	0.613 [ab]	0.008	0.117 [b]	0.012		
Initial Event antibiotic class	<0.01		<0.01		<0.01	
Beta lactam	0.535 [a]	0.025	0.100 [ab]	0.016	0.062 [bc]	0.014
Fluoroquinolone	0.495 [a]	0.010	0.157 [c]	0.015	0.073 [c]	0.012
Macrolide	0.699 [c]	0.004	0.111 [b]	0.011	0.056 [b]	0.009
Phenicol	0.629 [b]	0.014	0.120 [b]	0.015	0.063 [bc]	0.011
Tetracycline	0.691 [c]	0.004	0.077 [a]	0.008	0.035 [a]	0.006

End notes

[a]R Studio Version 2023.12.1.402. RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/

Chapter 3 - Systematic review to identify knowledge gaps in bovine respiratory disease antimicrobial therapy research

Abstract

Much research has been done to find the best antimicrobial treatments for bovine respiratory disease (BRD). This systematic review sought to identify information relative to antimicrobial use in BRD control and metaphylaxis. Specific research areas included case fatality risks, first treatment success, switching drug classes between treatments, switching drug classes between metaphylaxis and first treatment, post-metaphylactic or post-treatment intervals, as well as concomitant therapies. Databases searched included PubMed, CAB Direct, and Agricola, resulting in 418 publications meeting the search criteria. Manuscripts were then sorted into topic categories for review. Case fatality risk decreases with treatments of tulathromycin when compared to other antimicrobials. Similar trends were seen with first treatment success and included other macrolides as well resulting in higher success rates. Studies showed longer post-treatment intervals had better morbidity resolution than shorter post-treatment intervals. No prospective manuscripts were found regarding switching drug classes between metaphylaxis and first treatment or first and subsequent treatments. The research that evaluated concomitant therapy for BRD treatment did not show an advantage compared to just single antimicrobial treatment. This literature review identified several knowledge gaps related to specific application methods of treatment and more research in these areas could be conducted to better understand the best methods for treating BRD in cattle.

Introduction

Bovine respiratory disease (BRD) continues to be a major health issue in the cattle industry. BRD has been estimated to cost the cow calf industry alone over $165 million per year (Wang et al, 2018) and the US feedlot industry over $800 million per year (Blakebrough-Hall et al., 2020). Although considerable research has been conducted on BRD, especially antimicrobial therapeutic research, knowledge gaps still exist. The mainstay treatment for BRD in cattle continues to be antimicrobial therapy. There remains much debate on which treatment or regimen is best however. The objective of this research was to identify information relative to antimicrobial use in BRD control and therapeutic interventions. Specific areas included case fatality risks, first treatment success, switching drug classes between treatments, switching drug classes between metaphylaxis and first treatment, post metaphylactic or post treatment intervals, as well as concomitant therapies. By identifying significant knowledge gaps or areas which have not examined closely we can guide future research to address these issues.

Materials and Methods

Three literature databases (PubMed, CAB Direct, and Agricola) were searched in June 2022. No restrictions were placed on the study country of origin or the publication date for the initial search. PubMed searches were completed using the preferred MeSH terms as follows: {(cow) OR (cows) OR (cattle) OR (steer) OR (heifer) OR (calf) OR (calves)} AND {(bovine respiratory disease) OR (BRDC) OR (BRD) OR (bovine respiratory disease complex)} AND {(anti-infective agents) OR (antibiotics).

CAB Direct terms were determined using the thesaurus function. Search terms included: (Cattle) AND (Bovine respiratory syncytial virus OR Bovine respiratory disease) AND (Antiinfective agents OR Antibiotics). Agricola terms used were the same as the CAB Direct

terms; (Cattle) AND (Bovine respiratory syncytial virus OR Bovine respiratory disease) AND (Antiinfective agents OR Antibiotics).

After the initial searches of each database was conducted using the chosen search terms, publications from each database were evaluated for content based on title and abstract review. Criteria for retaining a publication in the review included: using cattle (dairy or beef breed), induced or natural BRD infection models as well as any in vivo study. Any clinical definitions of BRD in the publications were included such as pneumonia, fever, and respiratory disease; and studies of the following BRD pathogens; *Mannheimia haemolytica*, *Pasteurella multocida, Histoplasma somni*, *Trueperella pyogenes*, or *Mycoplasma bovis*. Abstracts only and case reports were excluded, as well as studies published in any language of print other than English.

Papers were exported from each database and input into a spreadsheet where duplicate papers were removed. Individual study characteristics were extracted from each published manuscript including: Country of study, BRD risk level of cattle (as defined by the manuscript author), cattle age, cattle type (beef, dairy, veal, bulls, heifers, etc.), sector (feedlot, dairy, stocker, etc.), infection type, BRD classification type, BRD pathogen, morbidity risk, mortality risk, case fatality risk (CFR), first treatment success (FTS), ancillary treatments, switching drug classes between treatments, switching drug classes following metaphylaxis, post metaphylactic interval, post treatment interval, experimental unit, total number of study units, positive or negative control used.

Results and Discussion

Database Results

The database search of PubMed resulted in 1,169 relevant publications between the years of 1948 and 2023. The search of CAB Direct yielded 1,006 results from to 1951-2021. The

search of Agricola results in 114 publications from the database between 1991 and 2021. Upon applying the refinement criteria, 181 papers were included from PubMed, 211 from CAB Direct, and 26 from Agricola. Duplicates were removed in the spreadsheet leaving a total of 418 manuscripts related to BRD therapeutic strategies were evaluated in this review.

Case Fatality Risk (CFR)

Four publications were identified describing case fatality risk for BRD treatments in the article title or abstract.

Van Donkersgoed and others evaluated the efficacy of tilmicosin and tulathromycin as metaphylaxis in feedlot calves in 2008. Tulathromycin was shown to have significantly lower treatments risk for undifferentiated fever, those with no fever (rectal temperature <104°F), and BRD. Although those cattle had lower treatment risk, no differences were detected between the two treatment groups in regards to BRD relapse risk, mortality risk, BRD mortalities, or railers (Van Donkersgoed et. al., 2008). This study also confirms in order to reduce treatments metaphylaxis should be done with tulathromycin. Another study assessing florfenicol-flunixin compared with tulathromycin for BRD treatment showed no difference among the groups in the crude case fatality risk. BRD case fatality risk and Histophilus case fatality risk was significantly different between groups with the florfenicol treated calves having a lower risk for each (Van Donkersgoed et. al., 2008). In this instance tulathromycin did not come out on top and was shown to have no difference when evaluated against florfenicol-flunixin combination.

A 2012 study comparing enrofloxacin and ceftiofur at first treatment for BRD determined a lower-case fatality risk in the group of cattle which received enrofloxacin treatment. While a difference was detected in case fatality risk, no statistical differences were seen in chronicity of the disease (how long the duration of clinical signs were) between the two groups. There were

also fewer BRD relapses in the enrofloxacin treatment group (Abutarbush et. al, 2012). If a lower relapse rate is desired in low risk cattle enrofloxacin has greater success over ceftiofur.

Another study evaluated treatment of BRD with three antimicrobials; oxytetracycline, penicillin, and trimethoprim-sulfadoxine. Comparison of these three antimicrobials showed no significant differences in case fatality rate. The mortality risk was numerically higher in the penicillin and oxytetracycline groups as compared to the trimethoprim-sulfa group, but this did not prove to be statistically significant. Treatment with trimethoprim-sulfadoxine also had the most favorable economic returns when cost of treatment and the mortality cost of the calf was considered compared to either the penicillin or oxytetracycline treated cattle (Mechor, et. al, 1988). If treatments options for BRD in feedlot cattle are between oxytetracycline, penicillin, or trimethoprim-sulfadoxine feedlot management can choose whichever they like, or the cheapest drug, without a concern of significantly different case fatality rates. Several studies have evaluated CFR as outcome and relatively few show significant differences; while this is an important outcome it may be how the studies are powered and more work may be needed to truly evaluate CFR differences

First Treatment Success (FTS)

Twenty-one papers were found regarding first treatment success for BRD in cattle meeting our search criteria. There were several treatment administration studies in the reviewed literature. In a BRD study in dairy calves, first treatment success was shown to be statistically better in the tulathromycin treatment group compared to negative controls (Binversie et. al, 2020). Meta-analysis work of BRD studies in beef cattle has shown there is an advantage in BRD retreatment morbidity odds for tulathromycin (Abell et. al., 2017). Although the authors found this association, they also recommended more research be done in the area to determine

best retreatment options. Based on this research though tulathromycin should be considered a very effective drug for BRD treatment, leading to a high first treatment success.

High-risk calves have been shown to have a higher probability of initial treatment failure (PTF) compared to low risk cattle. The PTF in this study was affected by quarter of arrival, arrival weight, days on feed, and the rectal temperature at first BRD treatment of these cattle (Avra et. al., 2017). In other BRD treatment research, for a 7-day success risk comparing marbofloxacin and florfenicol there was not found to be an advantage in using one treatment over the other (Grandemange, et. al., 2012). As long as we are tracking cattle out to 7 days it does not make a difference between these two antimicrobials in this study.

Looking at the risk of retreatment in the first 7-10 days after first treatment, tulathromycin had a higher first treatment success than did enrofloxacin. This trend was also seen with risk of a third treatment between 7 and 10 days using enrofloxacin (Heins et. al., 2014). This means tulathromycin in this study used over enrofloxacin increased the first treatment success of BRD cattle. In another study tulathromycin was shown to have a better first treatment success than tilmicosin, as well as a negative control of saline administration (Kilgore et. al., 2005). Showing another drug that tulathromycin should be chosen over in treatment successes for this population of cattle. In a high-risk cattle study for BRD the efficacy of tulathromycin was tested against both florfenicol and tilmicosin. The first treatment success of tulathromycin was significantly higher than the other two treatments. Those cattle treated with tulathromycin also had less likelihood of developing clinical signs of BRD throughout the rest of the feeding phase following treatment (Nautrup et. al., 2013). In summary, first treatment success in this study varied by drug choice.

Stocker cattle were also evaluated for association between BRD treatments with tilmicosin, tulathromycin, or florfenicol and treatment success. In stocker cattle, tulathromycin also seems to be the best choice to increase first treatment success rates for BRD. This was also seen when florfenicol was added in as a possible treatment and showed tulathromycin had a higher cure rate than both tilmicosin and florfenicol (Nutsch et. al., 2005).

In 2 study locations comparing tulathromycin to enrofloxacin for treatment of BRD, tulathromycin demonstrated an improved FTS over the first 60 days of the feeding period (Robb et. al., 2007). Not only does tulathromycin prove to be a good treatment, it also continues to be a better choice than enrofloxacin throughout the period after treatment. Comparing the efficacy of tulathromycin to tilmicosin and florfenicol in cattle in Colorado, Idaho, and Texas feedlots that were at high-risk for development of BRD, first treatment success over the first 28 days as well as the entire feeding period was significantly higher for tulathromycin compared to both tilmicosin and florfenicol (Rooney et. al., 2005). In these areas and for high risk cattle tulathromycin would be the best choice of these drugs. Metaphylaxis using tulathromycin in feedlot calves at moderate risk for BRD proved to have a lower first treatment risk of BRD compared to those receiving tilmicosin metaphylaxis. While there was an economical advantage at the timepoint of this study in the tilmicosin treated group, there was a trade-off between cost of metaphylaxis and efficacy. There was also no difference in the overall BRD relapse risk between the different treatment regimens (Van Donkersgoed et. al., 2008). While tulathromycin usually has a better first treatment success, this shows that when treating moderate risk BRD calves metaphylaxis with tilmicosin has its advantages.

Gamithromycin was evaluated for the treatment of BRD in feedlot cattle compared to a negative-control saline injection in a 2011 study. The overall treatment success was significantly

higher for animals given gamithromycin compared to the saline control, showing it is effective against BRD (Sifferman et. al., 2011). When evaluating gamithromycin for use as metaphylaxis there was a significantly higher first treatment success at 86% than saline at 61% (Baggott et. al., 2011). Torres and others compared the efficacy of gamithromycin to that of tulathromycin for treatment of BRD in feedlot calves. Overall, a greater number of gamithromycin treated calves required retreatment within the following 120 days (125 calves versus 71 calves). This was a statistical significance as well that calves in this group had a higher retreatment risk, meaning a lower first treatment success (Torres et. al., 2013). Although gamithromycin is better than the saline negative control for a treatment, tulathromycin was shown to be the better option. Another study, similar to the previous, evaluated calves at high risk for development of BRD showed again a higher retreatment risk for gamithromycin-treated cattle compared to tulathromycin-treated cattle (Torres et. al., 2013).

When comparing enrofloxacin with ceftiofur for a BRD treatment, enrofloxacin was found to have the advantage when looking at the first treatment success of undifferentiated fever in cattle. This was also true with the number of cattle needing a third treatment and case fatality risk. Enrofloxacin had the economic advantage too when comparing the two treatments (Abutarbush et. al., 2012).

A study investigating the PTI for BRD treatment of feedlot cattle using ceftiofur crystalline free acid administered subcutaneously in the caudal aspect of the ear found that a 7-day retreatment interval had improved cure rates, defined as not requiring retreatment on the first day eligible, compared to a 3- or 5-day interval. While the cure rate was improved this was not associated with a better overall treatment success ((McClary et al., 2011) Hibbard et. al., 2003). When ceftiofur crystalline free acid administered subcutaneously was evaluated it produced a

better 14-day treatment success rate than the negative controls. This route and formulation were shown to have a lower success risk at 3.0mg CE/kg BW and 4.4mg CE/kg BW compared to an injection of tilmicosin. However, in a separate part of the study, day-14 treatment successes were significantly higher for ceftiofur groups administered 4.4-8.8mg CE/kg BW compared to tilmicosin (Hibbard et. al., 2002). This shows that in using 14-day post treatment success evaluations for subcutaneously administered ceftiofur a higher dosage was better than treatment with tilmicosin. A lower dosage of ceftiofur was not as effective as tilmicosin.

Metaphylaxis using oxytetracycline or tilmicosin administered to cattle in Western Canada feedlots were compared. The overall treatment risk was found to be higher in the oxytetracycline group. Relapse risk between first, second, or third treatments of the groups were not different however (Schunicht et al., 2002).

In a 1988 study, trimethoprim-sulfadoxine did not have a significant advantage in treatment success over penicillin or oxytetracycline. The number of relapses between the three groups was also not significantly different than any other (Mechor et. al., 1988). Research evaluating literature on ancillary drugs in BRD treatment it was shown that an antimicrobial in combination with an NSAID could decrease treatment failures, increasing first treatment success. However, this fact was only significant in 1 of the 6 studies (Francoz et. al., 2012). This means it has not been proven to be advantageous for feedlots to be adding in NSAIDs when treating for BRD due to efficacy and increased costs of the NSAID.

Post Treatment Interval and Post Metaphylactic Interval

Two factors that can be evaluated in BRD treatments are post-treatment intervals and post-metaphylaxis intervals. Post treatment interval can be defined as the amount of days between two treatments for BRD of an individual animal, while post metaphylactic treatment is

the amount of days between metaphylaxis and the first treatment for BRD of an individual animal.

While there were a few papers relating to post treatment intervals based on drug treatments for BRD, only one involved prospective research. The paper found a decrease in the morbidity of cattle with metaphylaxis treatment with tilmicosin, and furthermore those with a 10-day PMI had the lowest BRD morbidity compared to 3, 5, and 7-day PMIs (McClary et. al., 2008). This suggests that when using tilmicosin for metaphylaxis a 10-day interval was better as opposed to one of a shorter duration.

Changing antimicrobial drug class following treatment

This literature search resulted in no prospective studies that evaluated outcomes in comparing switching or using the same antimicrobial drug class following first treatment for BRD.

Concomitant Antibiotic Treatments

The first study examined used cattle in feedlots in the western US who needed treatment for undifferentiated bovine respiratory disease. Researchers found similar results in regards to using combination treatments and evaluated ceftiofur compared to combinations of oxytetracycline with sulfadimethoxine, ampicillin with sulfadimethoxine, or penicillin with sulfadimethoxine. Ceftiofur alone was shown to significantly lower average temperature by day-two, while the sulfadimethoxine concomitant treatments took longer to show the same effect. Cattle treated with ceftiofur were also shown to return to feed or stay on feed better than those treated with any of the other combination of drugs. Along with ceftiofur having a reduced cost, no benefits were found by using combination antimicrobial treatment (Hansen, et al, 1993). Again, no advantages were seen when using multiple antimicrobials therapies together, even

some antagonistic effects were shown by combining some with others. In this study, drug choices and availability were different than what is available today which also must be taken into consideration. While it has been shown that in Chlamydia-infected calves, rifampin combination treatment reduced the number of viable pathogens, no advantage was found using the combination compared to the sole therapy in the severity of disease or the course of the disease (Prohl et al, 2015). Even in Chlamydia infected calves no advantages were seen using multiple antimicrobials and single therapy should continue to be used.

Conclusion

The objective of this literature review was to identify information regarding antimicrobial use in BRD control and interventions including; case fatality risks, first treatment success, switching drug classes between treatments, switching drug classes between metaphylaxis and first treatment, post metaphylactic or post treatment intervals, as well as concomitant therapies. If we can better understand areas for further research we can ensure we are using the best practices in order to treat cattle with BRD.

Six publications were found in this literature search evaluating case fatality risk in BRD. In most cases, the best-case fatality risks (lowest CFR) were associated with using tilmicosin as either a treatment or metaphylaxis. The only time this was not the case was in Holstein cattle which showed gamithromycin was a better option.

From the review twenty-one papers assessed first treatment success rates in BRD treatment. Overall, tulathromycin seems to be the clear choice in order to increase FTS in BRD cattle. In studies where tulathromycin was not evaluated, other drugs belonging to the macrolide class were good options.

Three papers looked at post treatment intervals of different antimicrobials for BRD, as well as one paper looking into the interval between metaphylaxis and first treatment for BRD. For both the post metaphylactic treatment interval as well as the post treatment interval, the studies showed more favorable outcomes when a longer interval was used compared to the shorter ones in these trials. A common interval which had good success was 7 days.

This literature review resulted in no papers being found explicitly investigating switching drug classes between metaphylaxis and the first treatment of an animal for BRD which would be a good area for future research.

Data from this systematic literature review does not support using concomitant therapy for the treatment of bovine respiratory disease. While only three papers evaluating this were found the results all stated it was not advantageous to use concomitant therapy, which may be the reason more research has not been done.

Although antimicrobials have been used for decades for control and treatment of BRD in cattle, there are clinical use practices that are unsubstantiated by the current scientific literature. With limited prospects for the development of new antimicrobials for use in cattle, it would be advantageous to optimize the use of currently approved medications. This literature review highlights several knowledge "gaps" regarding optimal clinical use practices for BRD therapy. Given the significant animal welfare and economic considerations attributed to BRD and the potential for selection for antimicrobial-resistant bacteria, future work in these areas should be considered a critical priority for researchers and funding agencies.

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