BRDS 2019

Challenges and Opportunities for Reducing the Incidence of Bovine Respiratory Disease

August 7-8, 2019

Denver, CO

PROCEEDINGS

Held in conjunction with the Academy of Veterinary Consultants Summer 2019 meeting

Bovine Respiratory Disease Symposium 2019:

Challenges and Opportunities for Reducing the Incidence of Bovine Respiratory Disease

August 7-8, 2019

Renaissance Denver Hotel
Denver, CO

PROCEEDINGS

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Symposium Organizing Committee Chair

Symposium Organizing Committee:

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Dr. Grant Dewell
Dr. Robert Fulton
Dr. Terri Ollivett
Dr. Roberto Palomares
Dr. Alison Van Eenennaam
Dr. Brad White
Dr. Amelia Woolums

Welcome from the 2019 BRD Symposium Organizing Committee

Welcome to the 2019 Bovine Respiratory Disease Symposium: Challenges and Opportunities for Reducing the Incidence of Bovine Respiratory Disease. As those in attendance know well, bovine respiratory disease (BRD) exerts a major impact on the health, well-being and productivity of cattle. This year's symposium presents an important opportunity for veterinarians, scientists, educators, producers and policy makers to come together to gain additional knowledge and understanding on the state of the art in research and management related to this disease. It is our hope that the symposium presentations and panel discussions will help to close knowledge gaps and provide new ideas for making progress against the persistence of BRD as one of the most important health problems of beef and dairy cattle.

The 2019 BRD Symposium has been organized through the efforts of members of the USDA Multistate Research Project NC-1192, "An Integrated Approach to the Control of Bovine Respiratory Disease." Special thanks go to the Academy of Veterinary Consultants (AVC), whose leadership has been generous in sharing information and assistance during the planning of this symposium. We would like to specifically recognize Taylor Fix, AVC Administrative Manager, for her efforts and contributions to help plan, organize and prepare for our symposium, Staci Slaght, UC Davis Veterinary Medicine Teaching and Research Center (VMTRC) Administrative Manager, for providing administrative support and helping to prepare these proceedings, and John Leach, UC Davis VMTRC Information Technology Manager, for developing and supporting the 2019 BRD Symposium website. We would also like to acknowledge the USDA NIFA Agriculture and Food Research Initiative (AFRI) Competitive Grants Program, which provided support through a conference grant. Thanks also go to our generous sponsors who are listed on the next page.

And lastly, thanks to those of you in attendance. We are confident that this 2019 BRD Symposium will provide a forum for a variety of fruitful interactions that positively impact bovine health and productivity. These interactions wouldn't be possible without your participation.

Sincerely,
The 2019 BRD Symposium Organizing Committee

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American Association of Bovine Practitioners

Day 1: Wednesday, August 7, 2019

7:45 – 8:00 AM	Welcome from BRD Symposium Organizing Committee
BRD Stalemate	**Moderator: Dr. Grant Dewell**
8:00 - 8:35 AM	BRD Impact - Beef Cattle - Brad White, Kansas State University
8:35 - 9:10 AM	BRD Impact - Dairy Cattle - Diana Short, USDA CEAH
9:10 - 9:45 AM	Economics of BRD - Beef - Derrell Peel, Oklahoma State University
9:45 - 10:00 AM	Break
BRD Risks	**Moderator: Dr. Brian Vander Ley**
10:00 - 10:35 AM	Economics of BRD - Dairy Cattle - Mike Overton, Elanco Animal Health
10:35 - 11:10 AM	BRD Risk Factors - Beef Cattle - David Smith, Mississippi State University
11:10 - 11:45 AM	BRD Risk Factors - Dairy Cattle - Sharif Aly, University of California, Davis
11:45 - 1:00 PM	Lunch

BRD Challenges and Motivation for Making Changes to Reduce Incidence

BRD Challenges - Dairy

Panel Discussion	**Moderator: Dr. Terry Lehenbauer**
	Producer Presentations
1:00 - 1:20 PM	Dairy: Calf Raising - Paul Dunn, Dairy Farm Calf Manager, Wisconsin
1:20 - 1:40 PM	Dairy: Replacement Heifers - Bill Wavrin, DVM, Sunny Dene Ranch, Washington
1:40 - 2:00 PM	Dairy: Feeder Calves and Dairy Replacements - Mark J Thomas, DVM, Dairy Health & Management Services
2:00 - 2:50 PM	Panel Discussion - Moderator: TBA
2:50 - 3:10 PM	Break

BRD Challenges - Beef

Panel Discussion	**Moderator: Dr. Mark Hilton**
	Producer Presentations
3:10 - 3:30 PM	Beef: Cow-Calf - John Maddox, Rancher, Western Nebraska
3:30 - 3:50 PM	Beef: Stocker Cattle - Robert Smith, DVM, Stillwater, Oklahoma
3:50 - 4:10 PM	Beef: Feedlot - Tom Jones, Hy-Plains Feed Yard, Kansas
4:10 - 5:00 PM	Panel Discussion - Moderator: TBA

6:00-8:00 PM Poster Session & Reception

Day 2: Thursday, August 8, 2019

Diagnostics and Use of Clinical & Diagnostic Data

Moderator: Dr. Vickie Cooper

7:45 - 8:20 AM Overview of the Role of the Diagnostic Lab in BRD Management - R. Gayman Helman, Texas

8:20 - 8:55 AM Developments and Application of Molecular Diagnostics in BRD - John Dustin Loy, UNL, Veterinary Diagnostic Center

8:55 - 9:30 AM The Microbiome in BRD: Diagnostic Relevance? - Trevor Alexander, Agriculture & Agri-Food, Canada

9:30 - 9:45 AM Break

BRD Treatment Failures & Therapeutic Management

Moderator: Dr. Roberto Palomares

9:45 - 10:20 AM BRD Treatment Failure: Definition and Impact - Calvin Booker, Feedlot Health Management Services, Canada

10:20 - 10:55 AM BRD Treatment Failure: Clinical and Pathologic Considerations - Terri Ollivett, School of Veterinary Medicine University of Wisconsin

10:55 - 11:30 AM BRD Treatment Failure: Pharmacologic Considerations - Brian Lubbers, Kansas State Veterinary Diagnostic Laboratory

11:30 - 12:45 PM Lunch

BRD Genomics and Novel Diagnostic Approaches

Moderator: Dr. Alison Van Eenennaam

12:45 - 1:20 PM Genomics: Host Genotype, Relevance to BRD - Holly Neibergs, Animal Sciences, Washington State

1:20 - 1:55 PM Improving Cattle Against BRD Through Genomics and Novel Technologies - Kristen Parker Gaddis, Geneticist

1:55 - 2:30 PM Behavior Assessment and Algorithms for BRD Diagnosis: Dairy - M. Caitlin Cramer, Ohio State

2:30 - 3:05 PM Behavior Assessment and Algorithms for BRD Diagnosis: Beef - John Richeson, West Texas A & M

3:05 - 3:20 PM Break

Antimicrobial Stewardship and BRD Therapy

Moderator: Dr. Amelia Woolums

3:20 - 3:55 PM FDA Perspective: Antimicrobial Licensing, Adverse Events and Future Issues - Mike Murphy, FDA Surveillance Division

3:55 - 4:30 PM Antimicrobial Resistance in BRD: Prevalence and Impact - Brent Credille, University of Georgia, College of Veterinary Medicine

4:30 - 5:05 PM Responsible Use of Antimicrobials in BRD Management - Mike Apley, Kansas State, College of Veterinary Medicine

5:05 - 5:15 PM Closing remarks from Organizing Committee

Economic Considerations of Enhanced BRD Control

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Abstract

Cattle producers and animal health professionals are increasingly frustrated by the inability to reduce the impacts of bovine respiratory disease (BRD). Improved BRD control is difficult due to the complex nature of the disease; the complexity of cattle industry structure and function; and by the imbalance of economic incentives for enhanced BRD control. Success in improving BRD control will depend on an industry-wide comprehensive effort to address lifetime animal health issues as well as correcting or offsetting imbalances in economic benefits and costs for enhanced animal health management across cattle production sectors.

Bovine respiratory disease (BRD) is the most costly disease in the U.S. cattle industry, impacting all live cattle production sectors: cow-calf, stocker/backgrounding and feedlot. BRD results from a complex set of interactions between numerous pathogens, environmental factors and host factors. This paper discusses the current status of BRD; factors that make BRD such a challenge to control; and discusses considerations for improving BRD control.

BRD seems to be getting worse

Despite continued research and advances in vaccine and testing technology and much focus in the industry on respiratory disease, the impacts of BRD appear to be increasing. For example, feedlot death loss has continued to trend up with the majority of feedlot mortality attributed to respiratory disease (Figure 1). This a major source of frustration for cattle producers and animal health professionals. The question of why improvements in BRD control remain elusive is important and the frustrations of animal health professionals indicate that the reasons likely go beyond animal health science.

What has changed?

The lack of progress in BRD control raises the question of whether changes in various factors or conditions are impeding more effective BRD control. Several possible changes are discussed below.

Has the disease changed?

BRD is a disease complex that results from the interaction of numerous pathogens that combine in varied manners in all cattle production sectors. One possibility is that the disease (one or some of the specific pathogens) has changed in terms of prevalence, incidence or virulence in one or more cattle production sectors. This could be due to natural evolution of the pathogen(s) or due to some novel expression of the pathogens resulting from new combinations or changes in timing of pathogen exposure. Certainly this is a question for animal health professionals and is beyond the scope and theme of this paper but is a possibility that must be considered in this discussion.

Have cattle production practices changed?

Cattle weights continue to increase over time as a result of genetic improvement, feeding technology and management changes. Rapid change in cattle genetics in the past two decades allows cattle to grow rapidly and longer thus resulting in larger cattle. Cattle have been getting bigger for many years but the pace of increase has been faster recently. For example, steer carcass weights have increased an average of 4.3 pounds/year in the 50 years from 1969-2018. This is an increase from about 680 to 883 pounds, an increase of 30 percent from the beginning to the end of the period. In the past 25 years, average steer carcass weights have increased 5.3 pounds/year. Heifer carcasses have increased somewhat faster than steers, increasing an average of 5.2 pounds/year in the past 50 years from about 574 to 814 pounds, a 42 percent increase. Heifer carcass weights have increased an average of 5.3 pounds/year in the last 25 years.

Facilitated by genetics, much of the continued growth in cattle size has resulted from implementation of feeding technology. Aggressive use of growth promotants, ionophores and beta agonists have accounted for much of the increased cattle performance in the past 25-30 years. It has been noted anecdotally that some "high performance" cattle seem to be at higher risk of health wrecks during backgrounding and feeding but no confirming research is currently available. Is it possible that increasingly intensive cattle production systems are responsible for increased health challenges in cattle?

Management factors and environmental conditions are well known to contribute to BRD incidence and impacts. Weaning, commingling, crowding, shipping and processing are all stressors that increase the likelihood of BRD impacts. Inclement and variable weather, both heat and cold, diurnal temperature changes, wet, dusty or other weather conditions often aggravate or exaggerate disease impacts. While weather conditions are beyond control, changes in management practices could result in changes in observed BRD impacts. However, no broad-based changes in the industry use of these practices are apparent at this time.

Cattle demographics can impact the observed general cattle health situation. On average, feedlot steers have lower morbidity and mortality compared to heifers (Stehle, et. al., 2018). Lighter weight animals placed in feedlots typically have higher morbidity and mortality (Stehle et. al., 2018). Because of the nature of annual cattle production, there is a normal seasonal pattern of cattle age and gender demographics each year. Additionally, cattle numbers and changes longer term have cyclical or trend impacts on the demographics and composition of cattle flows across sectors. The beef cattle industry has experience herd growth since 2014, the first significant expansion in cattle numbers in 20-25 years. The dynamics of cattle cycles have numerous implications on cattle demographics and the average animal health situation.

Heifer retention to support herd expansion in recent years resulted in significant reductions in heifer flows through stocker and feedlot production. This resulted in the heifer percentage of total yearling slaughter in 2016 at the lowest levels since 1974. Overall cattle numbers also impacts the age demographics of cattle production flows. With increasingly limited cattle supplies prior to 2014, feedlots were often forced to utilize younger and smaller animals which

often meant increased cattle morbidity and mortality. Growing feeder cattle supplies in recent years allows feedlots to focus more on preferred cattle in terms of weight, age and management background. Normal seasonal and particularly cyclical herd dynamics impact herd demographics and the observed average health status of the industry at any point in time.

Has overall cattle health changed?

Is it possible that overall cattle health has changed over time? While there is no definitive evidence to support such a conclusion, there are indications that merit consideration. For example, research on fetal programming has shown that cow nutrition during gestation has post-natal impacts on calf health and productivity (Summers and Funston, 2013). That combined with the fact that cow weights have increased over time, raises at least the possibility of increased nutritional stress on cows during gestation in some or many situations with subsequent impacts on overall cattle health.

Why is it so difficult to reduce the prevalence, incidence and impact of BRD?

Disease characteristics

The fact that BRD is a complex involving numerous pathogens, interacting in a variety of ways, in a wide range of environmental and management conditions and across multiple production sectors significantly increase the challenges of understanding the many manifestations of the disease. There appears to be little agreement about the relative role and contributions of various pathogens and as a result, little agreement on the priority of pathogen specific control.

Bovine viral diarrhea virus (BVDv) has undoubtedly received the most individual attention and for good reason. BVDv is known to originate at the cow-calf level and has the singular characteristic of producing persistently infected (PI) animals that act as a reservoir of virus exposure across all production sectors. BVDv is usually not the ultimate cause of mortality in cattle but the immunosuppressive impact of BVDv increases the likelihood and severity of infections by other BRD organisms. However, there is little consensus regarding how much, if any, overall BRD impacts would be reduced if enhanced BVDv control could be achieved.

Complex Industry structure adds to the challenge

The inherent complexity of the beef industry, as previously noted, contributes significantly to the management needs that represent significant sources of stress for cattle. Dispersed cow-calf production and the subsequent assembly, shipping and commingling of cattle into larger management groups in other regions of the country for stocker/backgrounding and feedlot production is an inevitable consequence of the industry structure and function in the U.S.

The challenge of enhanced BRD control is significantly more difficult because of the wide range of animal health and management histories across individual operations and sectors and the frequent lack of information transfer across multiple cattle owners. The result is duplicative and worse, inevitable gaps in health management at times. The complex structure of the beef industry greatly increases the difficulty and cost of coordinated and comprehensive BRD control efforts.

Failure to recognize BRD disease/impacts

In some cases, it appears that BRD disease and impacts are not always recognized, at least for some pathogens in certain circumstances. This is arguably most true for the cow-calf sector. For instance, BVDv, which occurs with low incidence and prevalence, may be present in low levels in some cow herds without the impacts being recognized. BVDv is, on the one hand, a reproductive disease, causing lower calving percentages resulting from reduced breeding rates and abortions; and also as a calf health disease, causing increased calf mortality and further reducing weaning rates. At low levels, BVDv is often not suspected or recognized and the impacts are viewed as within the normal range of production parameters, i.e. slightly lower pregnancy/calving rates, slightly higher calf mortality/lower weaning rates. Only when BVDv reaches higher levels are the impacts obvious and attributed to the disease. Failure to recognize the disease and impacts contributes to suboptimal health management. In the cow-calf sector, respiratory vaccines are used on about one-third of calves and 25-28 percent of cows (NAHMS).

In other cases, stocker and feedlot producers may also be accepting low levels of BRD impacts which are accepted as baseline levels of morbidity and mortality. This could account, for example, for feedlots perceiving that testing for PI animals is not worth the cost. They are perhaps correct given that it is not known the extent to which PI animals contribute to observed levels of BRD in feedlot animals and that much of the impact of BVDv exposure has already happened prior to or at arrival in the feedlot.

Market failure and economic incentives for BRD control

Notwithstanding the complexities of the BRD disease and the challenges inherent in the structure of the beef industry and even in cases where BRD impacts are known, there may be a fundamental reason for less than optimal levels of BRD control: producers in the various production sectors may simply not have the proper economic incentives to invest in additional BRD control. Any economic situation where individual decisions do not result in an overall optimal outcome is known as market failure.

Market failure is, simply put, a situation where private decisions do not, collectively, lead to an aggregate (socially) optimal or efficient solution. This is usually the result of positive or negative externalities, which are positive or negative factors not accounted for in individual decisions. There many examples, large and small, of market failure. A firm that does not bear the cost of the pollution resulting from production activities will produce more pollution than it would if all costs were internalized into the firm's decision-making. Public education is subsidized because the value to society of an educated populace is greater than the amount of education individuals would invest in on their own. A weed control district is more effective and cost effective than if individuals were acting independently without a coordinated effort.

The last example is similar to the situation for BRD. The collective benefits to the industry of better BRD control very probably outweigh the total costs of enhanced control across sectors but individual producers do not capture enough benefits to warrant additional investment in BRD control. In other words, the costs and benefits of enhanced BRD control are not properly distributed across production sectors.

The BVDv example

BVDv provides a very clear example of market failure as shown in research by Hurt (Hurt, 2017). BVDv originates at the cow-calf sector but occurs with low incidence and relatively low prevalence. The majority of cow-calf producers do not have BVDv and would incur a net cost to test and verify the absence of BVDv in the herd. BVDv-infected herds, even with low levels of the disease that may not be recognized, are experiencing losses in reproductive performance and calf morbidity and mortality. These herds would ultimately benefit from diagnosing and eliminating BVDv but would incur initial losses from the testing and indemnity of infected animals. In order to positively impact overall cattle health in the industry it is imperative PI cattle be identified and depopulated from herds prior to leaving the ranch of origin.

PI-BVDv calves originate at the cow-calf level but those that survive move into stocker and feedlot production. A high percentage of PI calves either die or become chronics and thus represent direct losses to the stocker or feedlot operation. The bigger problem is that PI cattle provide a reservoir of continual exposure of all animals that have direct and indirect contact. One PI animal may expose 200-300 other animals or more during shipping; in auctions; in commingling in stocker and feedlot operations; and through fence-line contact at every step. It is not known exactly how much this exposure contributes to subsequent BRD problems. However, feedlot data clearly shows that feeder cattle sourced in auctions have increased respiratory disease morbidity and mortality compared to country-sourced cattle. The estimates used by Hurt (Hurt, 2017) suggest that there is sufficient industry value from reduced BVDv at the stocker and feedlot levels to compensate the cow-calf sector for the net additional costs of reducing BVDv and testing to verify the status of uninfected herds. There is not, however, any consistent market mechanism for such a reallocation of benefits to result in improved levels of BVDv control. For the stocker or feedlot operation, merely reducing the number of PI calves may not have much value because even a small number of PI calves remaining in the system result in continued exposure of many cattle.

Reducing BRD impacts in the cattle industry

The following sections offer some general considerations for enhanced BRD control in the cattle industry.

A comprehensive industry approach is needed

The previous discussions highlight the need for a comprehensive multi-sector approach to respiratory disease management. While the beef industry structure divides production into different sectors, animal health must be considered and managed over the lifetime of the animal. Sector specific efforts have been limited in effectiveness and will remain so.

Such a comprehensive approach takes the challenge beyond the decisions of individuals or even what markets in aggregate can coordinate. Management and coordination of comprehensive animal health programs must occur at the industry level and suggest that either the industry as a whole or perhaps government will be needed to implement and manage such a program. The situation is similar to previous efforts for Brucellosis and Tuberculosis management.

Education

Current technology for BRD control is underutilized in the industry, particularly in the cow-calf sector. Only about 25-30 percent of cows and calves are vaccinated for respiratory disease. Biosecurity management is absent or minimal in most cow-calf operations allowing introduction of outside animals and commingling without quarantine/testing, thus increasing disease threats. Continued and enhanced educational efforts can pay dividends in increased awareness and increased and more consistent use of vaccines and better animal health management.

Continued research

Despite the fact that current technology is underutilized, there is clearly a need for additional research and continued improvement/development of animal health technology. This includes continued efforts to understand the interactions of pathogens in the BRD complex and especially across production sectors. For example, just how much would enhanced BVDv control reduce BRD impacts in the industry? Better understanding of health and nutritional management on lifetime health (i.e. fetal programming) will be important, not only with respect to respiratory disease, but more broadly in the context of managing cattle generally to reduce health problems and reduce the use of antimicrobials.

Improved economic incentives

As noted previously, the imbalance of economic benefits and costs among cattle production sectors is a significant hindrance to improved BRD control. There are no consistent market mechanisms to address this imbalance. While premiums for PI-free calves have been observed, use of such incentives is sporadic, minimal and generally ineffective. However, preconditioning programs, which emphasize health management of weaned calves, are increasingly consistent in providing net benefits to cow-calf producers.

One option for improved BRD control could be a mandatory, government imposed BRD control program. Such a program could require use of vaccines; testing for BVDv; depopulation of PI animals and could be done with or without compensation. The industry would likely prefer an industry driven and managed program which would require some mechanism to ensure that beneficiaries of improved BRD control compensate the costs of improved control. Such an approach would, at least conceptually, result in improved BRD control and leave the industry as a whole better off. That said, the structure and implementation of such an industry-based program would be challenging. The costs are immediate and some benefits would accrue more slowly over time.

Summary

Cattle producers and animal health professionals are increasingly frustrated by the inability to improve control of BRD and, indeed by some indications that respiratory disease is increasing. Improved BRD control is difficult due to the complex nature of the disease; the complexity of cattle industry structure and function; and by the imbalance of economic incentives for enhanced BRD control. Success in improving BRD control will depend on an industry-wide comprehensive effort to address lifetime animal health issues as well as correcting or offsetting imbalances in economic incentives for enhanced animal health management across cattle production sectors.

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Stehle, Anna, Derrell S. Peel, and John Michael Riley. "A Profile of Cattle Feeding: Beyond the Averages" Western Economic Forum, Volume 16, No. 2, December 2018: 62-77.

Figure 1

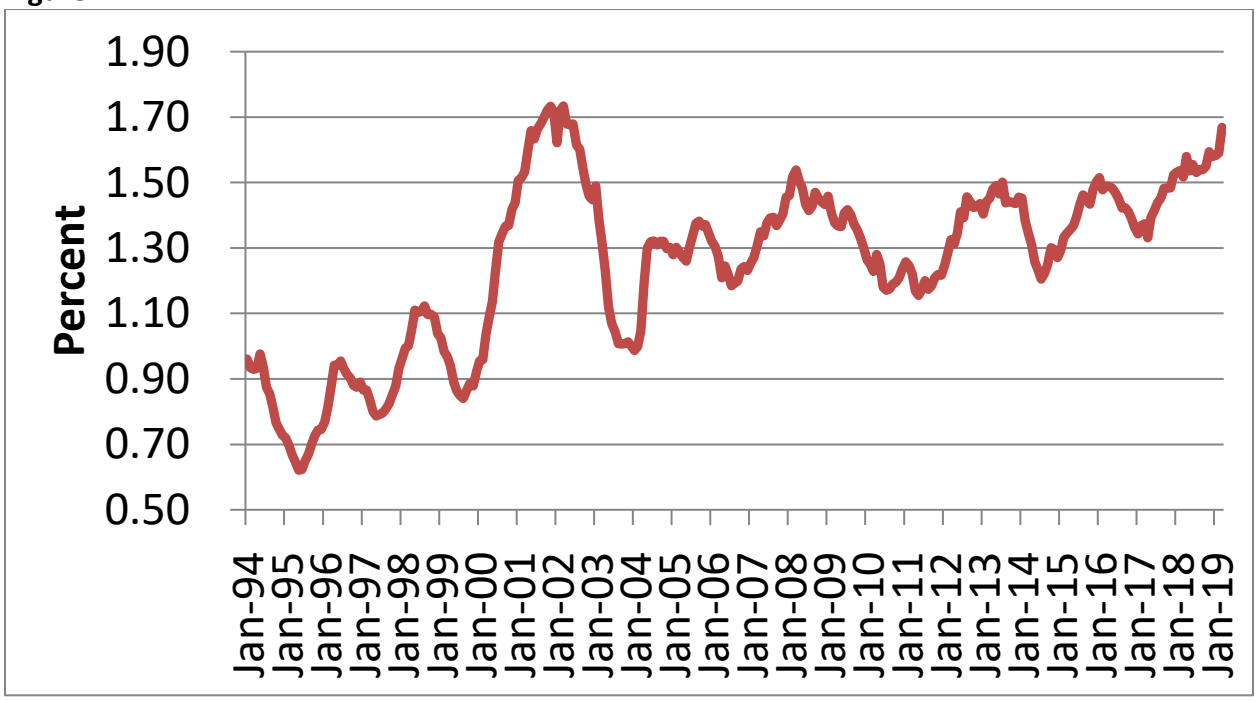

Figure 1. Feedlot Death Loss, 12 month moving average.
Source: Kansas Focus on Feedlots, data compiled by the Livestock Marketing Information Center (LMIC)

Economics of Respiratory Disease in Dairy Replacement Heifers

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Abstract

Bovine respiratory disease (BRD) is a common disease concern in dairy cattle and is most commonly diagnosed in young dairy heifers. The impact of BRD is highly variable, depending on the accuracy and completeness of detection, effectiveness of treatment, and on-farm culling practices. Consequences include decreased rate of weight gain, a higher culling risk either as heifers or as cows, delayed age at first service, delayed age at first calving, and in some cases, lower future milk production. In this data set of 104,100 dairy replacement heifers from across the U.S., 36.6% had one or more cases diagnosed within the first 120 d of age with the highest risk of new cases occurring prior to weaning. Comparison of the raising cost for heifers with BRD and those without a recorded history of BRD resulted in an estimated cost per incident case occurring in the first 120 d of age of $212 or $237, depending upon whether anticipated future milk production differences were considered or not. No additional differences in first lactation performance were evaluated. Current market conditions as reflected in this model have contributed to a cost estimate that is significantly higher than previously published estimates, driven in part by the losses associated with selective culling of a subset of heifers that experienced BRD. However, the impact on a herd's ability to selectively cull based upon genetics, resulting in even more valuable heifers at calving if BRD were eliminated or greatly reduced, was not considered. The cost of BRD in dairy replacement heifers is likely higher than many realize when all aspects of growth and performance are considered.

Introduction

Bovine respiratory disease (BRD) is a common disease concern in dairy cattle. According to the US National Animal Health Monitoring Surveys (NAHMS), BRD is the second most commonly recorded health issue in preweaned dairy calves with an incidence of 18% (second only to diarrheal disease) and the most commonly recorded health issue in weaned calves at 11.2%. In adult dairy cows, the recorded incidence is much lower at less than 3% incidence. However, it is highly likely that the true incidence is higher in all categories of dairy animals as dairies often fail to accurately detect and record clinical diseases.

The impact of BRD is highly variable, depending on the accuracy and completeness of detection, effectiveness of treatment, and on-farm culling practices. Consequences may manifest themselves immediately or later in life and include decreased rate of gain, a higher culling risk either as heifers or as cows, delayed age at first service, delayed age at first calving, and in some cases, lower future milk production (Stanton et al., 2012, Schaffer et al., 2016, Virtala et al., 1996, Closs and Dechow, 2017, Cramer and Ollivett, 2019, Bach, 2011, Ames, 1997, Waltner-Toews et al., 1986, Donovan et al., 1998, Steckler and Boermann, 2019). However, there are few published estimates of the cost of BRD in dairy animals and they likely underestimate the complete impact, in part due to the inconsistencies in disease recording that result in low, biased cost assessments and incomplete characterization of the impact on culling. Published cost estimates vary from

$20.63 to $50.64, after accounting for inflation from the time of publication until 2019 (Kaneene and Hurd, 1990, Miller and Dorn, 1990, van der Fels-Klerx et al., 2001).

Nevertheless, economic conditions in today's dairy climate are vastly different from those represented by previously published estimates. First, there is a large surplus in dairy heifer inventory relative to demand, resulting in depressed values for heifers along the entire replacement timeline that are significantly lower than the cost of raising. Second, because of the large heifer inventory, more producers are culling heifers prior to calving. While many factors combine to determine which heifers are removed from the herd to enter the beef chain, a history of BRD and/or a lower rate of gain are two important factors. Finally, since many if not most of the more severely affected heifers are removed prior to calving, measuring the carryover impact of heifer disease is much more difficult due to this culling bias. The goal of this project was to estimate the cost of BRD in heifers from birth through four months of age by evaluating the impact of BRD on treatment costs, mortality costs, rate of gain, and future value on the cost of raising heifers with and without an incident case of BRD in early life.

Commercial dairy disease data

To establish the pattern of BRD in dairy replacement heifers, a convenience sample of 23 dairy herds from across the US was created. To be included in this dataset, all herds had to record BRD in their replacement heifers on a monthly basis and use Dairy Comp 305 (Valley Agricultural Software Inc., Tulare, CA) as their on-farm record system. There was no minimum or maximum threshold to be included other than simply having monthly information recorded consistently. Dairy heifer data for 104,100 dairy replacement heifers born between January 1, 2016 and December 31, 2017 were collected in early 2019. Preliminary screening of the records for heifers born in 2016 showed that the risk of new cases of BRD past 120 days of age was less than 1%. Consequently, data from both years of birth was used allowing for follow up through at least one full year. The dates for the first, second, third and fourth recorded BRD cases was collected along with the birth dates, removal dates, and removal code (sold vs. died).

After examination of the timing of occurrence for the first BRD event using a survival plot, the first four months was chosen as the primary time at risk for study and cost estimation (Figure 1). In this data set, there was a high risk of BRD through the first four months and then the risk of new cases diminished greatly such that very few new cases were recorded between months five through twelve of age. Table 1 shows the BRD incidence stratified by age category and incident vs. total BRD risk.

Economic model construction and results

To estimate the cost of BRD occurring within the first 120 d of age in dairy replacement heifers, an existing economic model for estimating the cost of heifer raising from birth through first calving was modified and used (Overton et al., 2013). Updates were made to better account for variations in labor efficiency differences across farms of varying size and to improve the cost estimation of housing using confinement vs. extensive systems. Next, the two components of the model originally designed to compare intensively raised heifers vs. conventional raising approaches was modified so that both reflected a growing system closer to the intensive approach such that heifers calved at the appropriate height and weight at 23 months of age. The

two simultaneous models follow heifers from birth through calving using growth curves based on published research and privately collected data. The original baseline average daily gain from birth through calving is 0.86 kg/d but varies by stage. All housing, nutrition and reproductive management options are identical between the two options. Assumption within the model include a newborn calf value of $60, labor at $15 per hr, current feed ingredient prices representative of the Midwest region of the U.S and a capital cost of 6. Housing is by individual calf hutch until weaning, followed by small indoor group pens and then movement into large group pens. The nutrition program starts with a 28:18 milk replacer and 22% calf starter, followed by a 20% grower grain with hay, then proceeding on to a TMR based program designed to meet the metabolizable protein, and energy needs to support daily gain as shown in Table 2. Reproduction begins when heifers the targeted breeding size of 400 kg (based upon 57% of expected mature weight) and 130 cm in withers height.

The estimated impact of BRD on the growth curves was adapted from published work (Cramer and Ollivett, 2019, Donovan et al., 1998, Stanton et al., 2012, Steckler and Boermann, 2019). No published study described the precise differences in growth between BRD and No-BRD heifers across all stages as modeled in this project; thus, portions of each study are used to create a blended representative growth curve. Starting with the original growth curve that contained both BRD and No-BRD heifers, growth differences were estimated across each stage between the two disease classes. However, subtracting the anticipated impact of BRD from the original population curve without adjusting the No-BRD heifers upwards is incorrect. Thus, a small percent of the predicted difference in growth attributable to BRD was added to the original growth curve for the No-BRD heifers and the estimated impact of BRD was subtracted from this new curve to create the slower curve for the BRD heifers. As expected, the largest differences in growth were prior to 4 months of age, with diminishing impacts up through the prepartum period as shown in Table 2. Cumulative mortality risks, age-specific mortality risks, case-fatality risks and non-mortality culling information were collected from a number of sources and adapted into the model (Schaffer et al., 2016, Virtala et al., 1996, Sivula et al., 1996, Closs and Dechow, 2017, Stanton et al., 2012). In the baseline (no BRD) model, all predicted BRD-related treatment costs, mortality and culling were removed.

Table 2 shows the inventory, mortality risk, culling risk, and growth (weight) for both BRD and No-BRD heifers across the full raising period. The incidence of BRD in a representative commercial herd, adapted from the previously described commercial dairy data set, was assumed to be 36.6% as shown in Table 1, with 61.9% of all incident cases occurring prior to weaning, and an average of 1.7 treatments per incident case. Applying these results to the BRD-only population yields a total treatment risk of 97.5% prior to weaning (i.e., there were 975 treatments administered per 1000 BRD heifers). Age-specific weight-based treatments using an approved extended therapy macrolide (1 extended dose per treatment) and a non-steroidal (1 dose per treatment) were built into the model using current pricing from an online veterinary distributor. Based upon a case fatality risk of 3.5% of incident cases, the incremental mortality risk for the BRD group in the first and second stages was calculated and added to the baseline levels. The mortality risk in the remaining stages for the BRD group was assumed to be 10%

higher than the No-BRD group. As a result, the total mortality risk for the BRD group was 9.5% compared to 6.1% for the No-BRD group.

To estimate the higher non-mortality, non-reproductive culling risk for BRD heifers, an assumption was made that 10% of incident cases were culled into the beef market. These culls were removed 14 days after entry into the subsequent stage. Additionally, 10% of the repeat treatments that carried over into stage 3 were also removed. Because of a slower rate of gain and culling pressure applied prior to entering the reproductive program, the BRD group calves at an older age and has fewer culls attributable to reproductive failure even though the reproductive program management and performance were not different. Since breeding was based on the time at which the heifers reached 57% of mature weight, and the BRD heifers grow at a slow pace, there is a delay to first service. Second, the number of culls due to reproductive failure is lower in the BRD group as a result of fewer animals reaching the breeding period. In the No-BRD group, 6.6% of total heifers that entered the raising program were culled due to reproductive failure as compared to 5.7% of the BRD group. However, the total non-mortality culling risk was 2.6 times higher (17.3% vs. 6.6%) in the BRD group and this agrees with previously cited references. Altogether, the total removal risk was higher for the BRD heifers at 26.8% vs. 12.7%, assuming no additional selective culling based on genetics.
Culling BRD-affected heifers from the herd is costly. Under current market conditions, the cost of raising heifers is much higher than the value received from selling them for beef. The market values received for each stage represent the average values/unit of body weight obtained from online market news reports from public auction houses located in California (escalonliverstockmarket.com), Pennsylvania (ams.usda.gov), Missouri (producersliverstock.com), and Idaho (ams.usda.gov) that reported values for heifers across a range of body weights. The losses per head culled were -$223, -$289, and -$378 for stages 2, 3 and 4, respectively.

Finally, there is the issue of first lactation performance carry-over effects attributable to BRD that occurs in the young heifers. While there is no doubt that experiencing BRD is bad, the ability to measure the carryover effects is difficult for several reasons. First, as modeled in this project, there is usually additional culling of the most severely affected animals; consequently, only heifers that have recovered more completely have the opportunity to calve, thus diminishing any measurable differences. Second, disease definition, detection intensity, and accuracy of diagnostic efforts varies greatly across herds. There is likely some misclassification bias resulting in BRD-affected animals calving without the condition being identified and recorded in the records. As a result, the ability to quantify a statistically significant effect on culling risk in first lactation or on milk production is diminished. Schaffer et al. reported that heifers born on a single farm with BRD were 28% more likely to be culled in first lactation and they produced 233 kg less 305-d mature equivalent milk (Schaffer et al., 2016). Previous work by the author has resulted in very herd-specific results with some herds demonstrating similar losses, but others have no detectable effect of previous BRD. For the purposes of this project, no differences in first lactation culling risk were predicted. Meta-regression work by Soberon and Van Amburgh showed a positive relationship between preweaning ADG and first lactation milk, where milk yield = -106 kg + 1,551.4 kg * ADG (Soberon and Van Amburgh, 2013). Using this approach, BRD

heifers are predicted to produce 123 kg less milk based on the lower rate of gain preweaning, Assuming 2.33 kg of marginal milk per marginal kg of dry matter consumed, a feed price of $0.264/kg dry matter and a milk value of $0.36/kg, and 28% culling in first lactation, the net impact of this milk that is not produced is -$24.91.

Table 3 shows the overall net economic impact of BRD. As compared to the No-BRD group, the raising cost in heifers with BRD was an additional $212 per incident case. This additional expense is the estimated cost of BRD that occurs within the first 120 d of age, assuming no carry over effects into first lactation. If the economic impact of the reduction in predicted milk due to BRD-related impacts on early heifer growth is considered, the net cost per incident case of BRD rises to $237. These cost estimates for BRD are significantly higher than other previously reported values, even accounting for inflation adjustment. One big difference is in the approach taken with culling. Current market conditions reflect a very large loss when heifers are culled for the beef market. Other models have assumed a much lower additional culling risk attributable to BRD relative to the approach taken here. However, if additional non-mortality culling is eliminated from the BRD group, the cost differences due to mortality, slower rate of gain, and additional treatment costs is still $112 (no extra milk) and $137 (extra milk production in the first lactation). Nonetheless, if no heifers were selectively culled prior to first calving, the impact of BRD on productivity and culling in the first lactation would be significant with a much higher total cost per incident case than $141 or $175.

Conclusion

Heifer BRD occurring in the first 120 d of age was found to be very costly with anticipated differences in raising cost between animals with BRD and those without resulting in a cost per incident case of $212 or $237, depending upon whether anticipated future milk production differences are considered or not. Current market conditions as reflected in this model have contributed to a higher cost estimate than previously published, driven in part by the losses associated with selective culling of a subset of heifers that experienced BRD. However, the impact on a herd's ability to selectively cull based upon genetics, resulting in even more valuable heifers at calving if BRD were eliminated or greatly reduced, was not considered. Individual herd costs will vary but may be significantly higher, depending upon the initial value of the calf entering the raising program, level of BRD, the culling practices associated with BRD, the accuracy and promptness of diagnosis and treatment, and any potential carryover effects into the first lactation.

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Figure 1. Time to first BRD for all heifers in commercial dairy heifer data set, censoring at 365d of age.

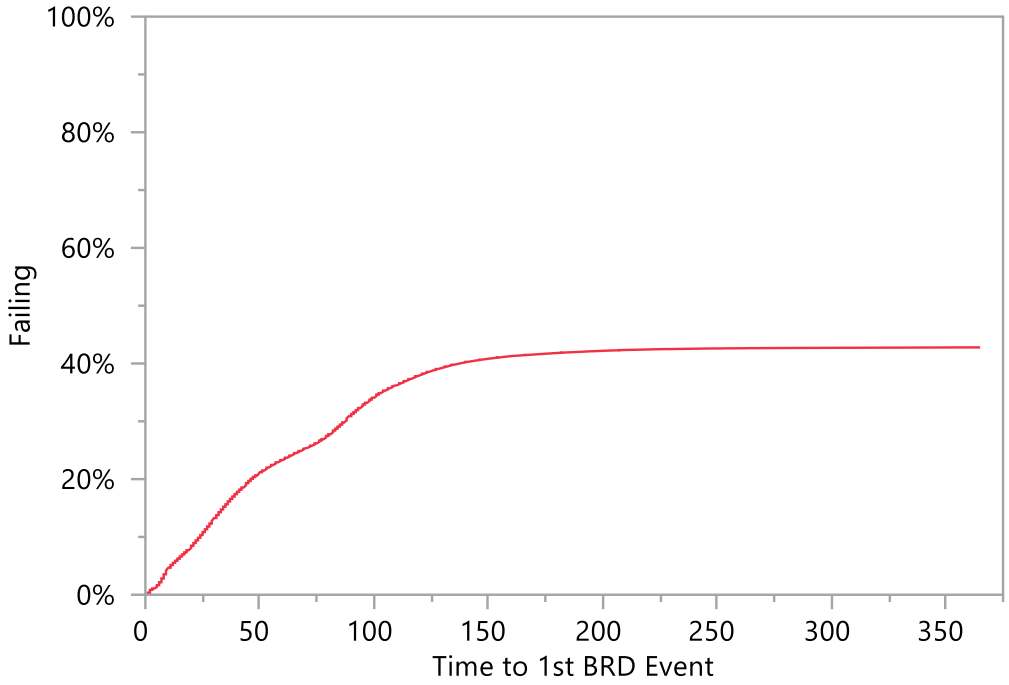

Table 1. Incidence of BRD by 30-d increments over the first 120 d of life.

	Initial Case Incidence[1]	% of Incident Cases	Total BRD Risk[2]
Incidence from birth to 30 d	12.9%	35.2%	16.1%
Incidence from 31 to 60d	9.8%	26.7%	19.6%
Incidence from 61 to 90d	7.4%	20.3%	14.1%
Incidence from 91 to 120d	6.5%	17.7%	12.4%
Total	36.6%		62.2%

[1]Initial Case Incidence = # of first time BRD cases/# of heifers born
[2]Total BRD Risk = # of new and repeat cases/# of heifers born

Table 2. Growth stages within the heifer-raising model and the corresponding daily gains, mortality risk and culls, both BRD-related and those resulting from reproductive failure.

No BRD

Stage Start (mos)	Birth	2.1	4.0	10.0	15.6	21.2	Total
Stage End (mos)	2.1	4.0	10.0	15.6	21.2	23.2	
Initial # of Heifers	1000	971	956	948	944	875	
Ending # of Heifers	971	956	948	944	875	873	873
Mortality Risk	2.9%	1.4%	0.8%	0.4%	0.2%	0.2%	6.1%
Culls (Repro)	0	0	0	0	66	0	6.6%
Initial Weight (kg)	40	91	150	323	471	609	
End Weight (kg)	91	150	323	471	609	655	655
ADG (kg/d)	0.81	1.00	0.95	0.88	0.81	0.76	0.87

BRD

Stage Start (mos)	Birth	2.1	4.0	10.0	16.4	22.0	Total
Stage End (mos)	2.1	4.0	10.0	16.4	22.0	24.0	
Initial # of Heifers	1000	949	860	816	794	734	
Ending # of Heifers	949	860	816	794	734	732	732
Mortality Risk	5.1%	2.8%	0.9%	0.5%	0.3%	0.2%	9.5%
Culls (BRD or Repro)	0	62	36	18	57	0	17.3%
Initial Weight (kg)	40	86	140	303	469	607	
End Weight (kg)	86	140	303	469	607	655	655
ADG (kg/d)	0.73	0.92	0.89	0.85	0.81	0.78	0.84

Table 3. Total Holstein heifer raising costs, from birth to calving including calf value, mortality, culling, and opportunity costs and the resulting cost of BRD.

	No BRD	**BRD**
Total Raising Costs	($1,983)	($2,196)
Cost of BRD per Incident Case (no 1st Lactation Impacts)		**($212)**
Value of Extra Milk in First Lactation	$25	
Net Raising Cost per Heifer Calving	($1,959)	($2,196)
Cost of BRD per Incident Case, Including Projected Milk Differences		**($237)**

Risk Factors for Bovine Respiratory Disease in Beef Cattle

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Abstract

Bovine respiratory disease (BRD) is the leading cause of death in beef calves three weeks of age to weaning and is the leading cause of morbidity and mortality in beef feeding and finishing systems. Part of the challenge in controlling BRD is the confusing array of factors that sometimes seem to explain the disease, but sometimes do not. Each outbreak of respiratory disease is the result of the completion of a sufficient cause, which might have also included components of viral and bacterial pathogens, a certain state of immunity, or other component causes of respiratory disease in cattle that we fail to understand. Disease is expressed when a sufficient cause is completed. Disease events we observe, such as the occurrence of BRD, usually have relationships with risk factors that are commonly the subject of epidemiologic research and the primary subject of this paper. However, it is important to understand that underlying systems produce those relationships and, ultimately, the occurrence of disease. The risk factors for bovine respiratory disease include a complex set of component causes that include bacterial and viral pathogens, level of host immunity, and environmental conditions that favor pathogen transmission and stress-induced susceptibility. During the post-weaning phase, these factors are superimposed on a system of marketing, transportation, and decisions made to support economic opportunity that further increase the risk for BRD.

Introduction

Bovine respiratory disease (BRD) is the leading cause of death in beef calves three weeks of age to weaning, costing cow-calf producers approximately $165 million annually (Wang et al., 2018). The disease is even more common and more costly after weaning and is the leading cause of morbidity and mortality in beef feeding and finishing systems (Griffin, 1998, Miles, 2009). The disease syndrome is complex and surprisingly difficult to accurately diagnose. The incidence of BRD has not waned despite widespread use of improved vaccines and antimicrobials (Miles, 2009). The anatomy and physiology of the bovine lung may make cattle inherently susceptible to BRD (Veit and Farrell, 1978). However, many other factors contribute to its occurrence.

Causal thinking

Part of the challenge in controlling BRD is the confusing array of factors that sometimes seem to explain the disease, but sometimes do not. This lack of consistent effect of putative risk factors confounds our understanding of actions that might prevent the BRD from occurring. The concepts of component and sufficient causes help explain this phenomenon. Risk factors are causal factors because they contribute to the causal pathway of disease. Risk factors may include factors related to the disease-causing agents (e.g. pathogens or toxins), the ability of the host to resist the effects of the agents, or management and other environmental factors that may affect host and agent interactions. Key determinants are those causal factors which are under management control. In disease causal theory, each factor that contributes to the development

of disease is a component cause (Rothman, 1976). Clinical signs of disease are expressed when various component causes add up to complete a sufficient cause. Each outbreak of respiratory disease is the result of the completion of a sufficient cause, which might have also included components of viral and bacterial pathogens, a certain state of immunity, or other component causes of respiratory disease in cattle that we fail to understand. Disease is expressed when a sufficient cause is completed. This is why some known component causes of BRD may be observed even though the disease is not expressed. It may appear to the livestock owner that the component cause that completed a sufficient cause was the sole reason for the disease. For example, a sudden change in weather may precede an outbreak of BRD because it completed the sufficient cause, but viral, bacterial, and immune status were unrecognized component causes. Removing one component cause (now the key determinant) means that the sufficient cause is not completed and thus disease is not observed.

Systems thinking

Thinking about the system of production may provide some additional insight into the factors that cause BRD. The science of system dynamics helps us understand how actions and decisions far removed from the immediate problem could be a cause of the problem. Disease events that we observe, such as the occurrence of BRD, usually have relationships with risk factors which are commonly the subject of epidemiologic research and the primary subject of this paper. However, it is important to understand that underlying systems produce those relationships and, ultimately, the occurrence of disease (Figure 1) (Meadows and Wright, 2008). A better understanding of how the system may lead to disease outcomes may further our understanding of why BRD occurs and what we can do to mitigate it. For example, a large regional drought might cause cow-calf producers to decide to wean calves early, seek feedlot pens to house cows, or to depopulate their herds –all of which may have effects on feedlot management and health. Small cow-calf producers may decide not to dehorn, castrate, vaccinate, or deworm calves on the farm because they lack facilities or fail to recognize an economic signal to do so. Decisions made months ago at a farm, possibly hundreds of miles away, may result in increased morbidity and mortality in the feedlot (Duff and Galyean, 2007). Those decisions don't always reflect sub-par husbandry. For example, pneumonia in calves prior to weaning is a systems problem paradoxically associated with highly managed herds (Woolums et al., 2013, Woolums et al., 2014).

The epidemiology of BRD

Traditionally, disease control programs have focused on agent-host interactions with some consideration of environmental factors. However, political, social, economic, and cultural factors also contribute to disease ecology as it affects the movement of people, animals, and animal products.

The failure of disease control programs, whether at a local, regional, or global level, frequently results from failure to consider or understand the system comprising the disease's ecology, including the decisions made by people responding to factors within the system (e.g. economic factors).

Pneumonia in calves prior to weaning

Pneumonia is a leading cause of sickness and death of calves in some cow-calf herds –especially after the first few weeks of life (USDA, 2011). This is perplexing because ranch calves typically live in conditions of little stress and relative isolation. Surveys of beef cattle producers (Woolums et al., 2013) and veterinarians (Woolums et al., 2014) from the northern plains region and southeastern US indicate that pre-weaning BRD is a problem for approximately one out of five cattle producers. Pre-weaning BRD may affect up to 10% of U.S. beef calves (Hanzlicek et al., 2013), resulting in death of 0.6% - 1.4% of all calves (USDA, 2010, Dutil et al., 1999, Snowder et al., 2005). Calves affected with pre-weaning BRD may weigh 17 - 37 pounds less at weaning, compared to calves not affected (Snowder et al., 2005, Wittum, 1994).

As with all infectious diseases, the occurrence of BRD is affected by factors of host immunity, presence of specific pathogens, and opportunity for transmission of pathogens between or within herds. Although the bacterial pathogens of pneumonia are commonly found in the upper respiratory tract of cattle, the inciting damage is often due to viral infections that may not be present in all cattle herds all the time. Commonly recognized viral BRD pathogens are bovine herpes virus 1, bovine viral diarrhea virus, and bovine respiratory syncytial virus, but many others, including bovine coronavirus (Kapil and Goyal, 1995, McNulty et al., 1984), are likely to be involved. Pathogen exposure may be necessary but it is not causally sufficient because it is difficult to replicate the clinical presentation of BRD through experimental challenge with bacteria or viruses alone.(Taylor et al., 2010a)

In confinement systems, the opportunity for pathogen transmission is high because of animal density. But, even in extensive pasture-based systems typical of cow-calf production, the opportunities for pathogen transmission may be high because cattle congregate closely around water sources and feedbunks, in shade, and when bothered by flies. Some management practices such as pasture moves and gathering for sorting also result in high animal density and greater opportunity for pathogen transmission.

Passively acquired maternal immunity is important for protecting young calves against respiratory pathogens. However, maternal antibodies wane with time. Approximately every 16 to 20 days after ingestion, the serum concentration of maternal antibodies is halved, so that by 96 to 120 days of age, a calf retains less than 2 percent of the antibodies it absorbed from colostrum. The immune system is functional but unprimed at birth, and prior to 5 to 8 months of age and the immune response of calves is weak, slow, and easy to overcome (Cortese, 2009). Therefore, even in the absence of additional stressors, calves 3 to 5 months of age may be particularly susceptible to pneumonia. This age-related susceptibility due to loss of maternal immunity may explain sudden outbreaks of pre-weaning BRD in herds with managed breeding seasons. Herd immunity is the protection afforded to susceptible individuals because most of the individuals in the population are immune. In herds with a narrow calving window, calves are similar in age and herd immunity is lost over a short span of time as the majority of calves approach 90 to 120 days of age. However, the optimum vaccination protocol to prevent BRD in calves of this age remains an important subject of investigation. Weaning, commingling groups,

and exposure to severe weather can be powerful stressors that further reduce a calf's ability to resist disease.

Other factors affecting risk for pre-weaning BRD

Health records representing over 9,900 calves from 28 cattle management groups within 7 beef cattle ranches were analyzed to test the effect of calf gender and age of the dam (Smith et al, unpublished). We concluded that the sex of calves affects their risk for BRD (males at greater risk than females). Also, of calves affected with BRD, those calves born to 2-year-old dams were more likely to become sick at an earlier age. This is consistent with the knowledge that the male sex of other species has been associated with greater risk for pneumonia (Gutierrez et al., 2006, Yamamoto et al., 1991). The age of the dam may be a correlate of colostrum absorption. Colostrum ingestion may be delayed for calves born to a young dam because of dystocia or poor mothering skills. Also, the young dam's colostrum may not contain as many antibodies, in quantity and range of protection, as older dams (Schumann et al., 1990, Odde, 1996, Odde, 1988).

Pneumonia in calves after weaning

The first several days from farm of origin to the stocker operation or feedlot can result in the accumulation of stress events that are detrimental to calf health, especially increasing the risk for BRD. Most BRD morbidity occurs in the first 21 days after arrival in the stocker or feedlot operation. By far, the most common illness of stocker calves is BRD (Miles, 2009). Other important receiving period diseases are lameness, musculoskeletal injury, diarrhea (e.g. rumen acidosis, Salmonellosis and coccidiosis), and bloat (Griffin, 1998).

Many small farm operations lack enough natural, human, or capital resources to provide an optimum health program while the calf is on the home farm. For example, the farm may lack facilities, manpower, or knowledge to dehorn, castrate, or vaccinate calves prior to weaning. Weaning often occurs the same day the cattle are marketed from the home farm, resulting in an important abrupt stress event. In addition, the common systems of marketing calves contribute additional stressors to the auction market calf. Calves may not have access to adequate feed or water or may not know how to drink from tanks or consume feed from bunks during transportation to and from the auction market. Calves are likely to be commingled with other calves, and after long distance transportation, may spend several days in an order-buyer facility as other calves are purchased to fill an order. During the phase of marketing, calves may lose rumen fill from not eating, may have shrink from dehydration, and may be exposed to a variety of enteric and respiratory pathogens. By the time calves have moved through these marketing channels and arrive at the destination feedlot or stocker facility, they may be exhausted, dehydrated, challenged by a variety of social and physical stressors, and incubating a respiratory or enteric infection.

Unfortunately, the marketing system may not reward the small cow-calf farmer for adopting practices that improve immunity and decrease stress. Calves marketed directly from the (typically larger) cow-calf farm to the stocker or feedlot operation may experience some, but often not all, of the stressors of auction market calves; however, because direct marketing is often based on

the farmer's reputation, these calves are more likely to have been preconditioned by receiving deworming treatment, vaccination at a prior to weaning, and castration and dehorning at a young age. Calves that are marketed directly, especially those undergoing a pre-conditioning program, may have less morbidity and mortality in the post-weaning phase and are, therefore, often considered calves at low risk for disease. Conversely, commingled, low body condition, freshly weaned calves, transported long distances, and marketed through an auction market are often considered high risk for disease. It is a paradox that some cattle feeders and stocker operators prefer light weight high risk calves because they can be purchased for less total dollars and, if they survive, often grow efficiently because of compensatory gains (Ives and Richeson, 2015).

Attempts to mitigate the risk for BRD in feeding or finishing systems, such as by vaccinating calves at arrival, may not reduce BRD incidence (Taylor et al., 2010b) and may sometimes increase BRD incidence, increase mortality, and lower growth performance (Griffin et al., 2018). Mass medication of calves with injectable antimicrobials at arrival has been the most consistently effective method to reduce BRD incidence (Ives and Richeson, 2015).

Conclusions

The risk factors for bovine respiratory disease include a complex set of component causes that include bacterial and viral pathogens, level of host immunity, and environmental conditions that favor pathogen transmission and stress-induced susceptibility. During the post-weaning phase, these factors are superimposed on a system of marketing, transportation, and economic opportunity that further increase the risk for BRD.

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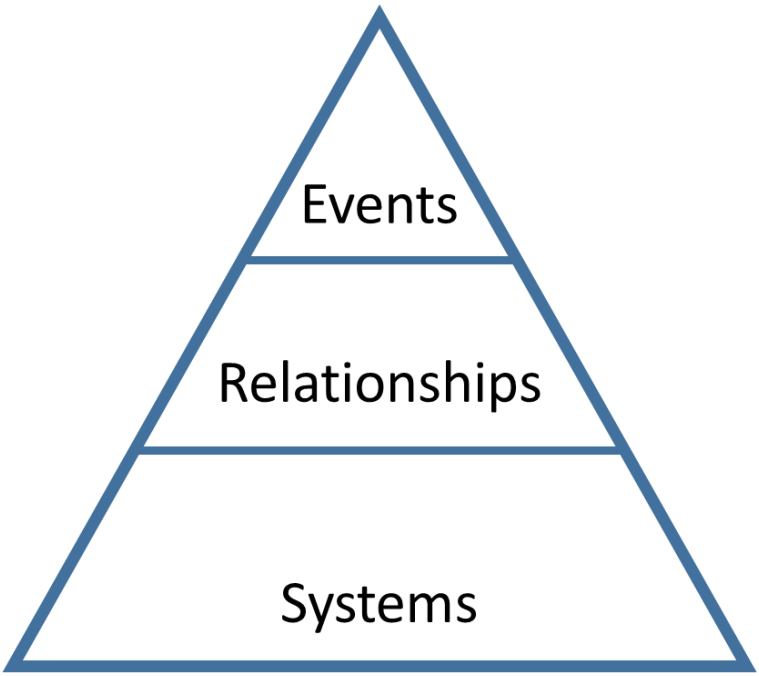
Figure legends

Figure 1. The iceberg structure of system dynamics. The disease events we observe have relationships with other causal factors that are often studied by epidemiologists and other animal health researchers, but the systems that produce those relationships and the ultimate events require further understanding.

Figure 1



The Role of the Veterinary Diagnostic Lab in the Management of BRD

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Abstract

Veterinary diagnostic labs (VDLs) are important service agencies providing essential diagnostic testing for a wide variety of domestic animal species as well as wildlife. They serve key roles in disease monitoring and diagnosis as well as surveillance for diseases of consequence. Of the many roles VDLs serve, one is being a member of the larger team of professionals dealing with the management of the bovine respiratory disease (BRD) complex. VDLs provide a number of services related to the management of BRD. These include disease outbreak investigation, abnormal morbidity characterization, routine monitoring, and biosecurity screening for a variety of infectious agents via methods such as necropsy and histopathology, bacterial culture, antimicrobial sensitivity testing, virus isolation, and serological assays. VDLs continue to look for better methods and assays as instrumentation technology also grows and improves. This is reflected in the growing proliferation of molecular-based assays that provide a high degree of sensitivity and specificity. Professional staff in VDLs work in collaboration with those in academia and private industry to conduct basic research focusing on different aspect of the BRD complex. VDLs remain a primary source of the varied field-origin infectious agents associated with BRD that are used for research purposes.

Introduction

The veterinary diagnostic lab (VDL) has for decades now been engaged in providing laboratory testing services for a diverse clientele and, for the most part covering all of the major domesticated animal species as well as exotics. Historically, the VDL has been the primary avenue for veterinarians and producers when it comes to diagnostic testing in livestock and this continues to be true today. Full service VDLs offer a broad range of options for disease investigations and more routine health and pathogen screening as well as regulatory testing. This includes a multiplicity of assays in the fields of bacteriology, serology, virology, and pathology, both anatomic and clinical. In areas of intense cattle production, beef and dairy, respiratory disease is a major disease problem and VDLs have and continue to play a significant role in the overall management of the disease complex.

Early on VDLs were involved in basic testing that included necropsy and histopathological examination of animals with pneumonia, bacterial culture/isolation of pathogen(s), antimicrobial sensitivity testing of significant isolates, and virus isolation. Necropsy and histopathology allowed for the identification of gross and microscopic patterns of pneumonia that would help veterinarians servicing the industry to be better equipped to distinguish viral from bacterial from non-infectious types of pneumonia, interstitial verses bronchial patterns, and infectious verses non-infectious and mixed causes.

These basic diagnostic disciplines enabled the veterinary medical community to better understand the complex nature of the BRD complex with regard to infectious agents and early

understandings of the pathogenesis, most of which still form the "backbone" in our understanding of BRD.
As the scientific methodology and instrumentation has advanced, it has and continues to offer increased sensitivity and specificity. Advancements in scientific methodologies have allowed for the continued growth in our understanding of the respiratory disease complex through the identification of new pathogens, interactions of previously known or suspected pathogens, and how they influence (if at all), for example, the gross and microscopic lesions of pneumonia.
Professional staff at VDLs have to one degree or another joined with academicians involved in more basic and directed research initiatives tied to various aspects of the complex. These include pathogen characteristics, pathogen metabolic byproduct(s) identification, toxins, virulence gene presence and significance, pathogen interaction with bovine immune system, pathogen interactions, environmental factors affecting pneumonia, host factors that are significant to the BRD complex management and prevention, and development of new and better testing methods and results interpretation.

One great benefit of VDLs in the management of BRD is that the diagnostic labs are a repository for organisms recovered from field cases of BRD and, thus, serve as an extremely valuable and essential source for in-house and collaborative research. For most isolates, they can be held almost indefinitely under the correct environmental conditions and be available for future study.

Looking more specifically at the various diagnostic disciplines within VDLs, it is interesting to follow the development of testing and the changes that have and are occurring over time to provide better information and service to our clients in the area of bovine respiratory disease management.

Bacteriology
Since early on in diagnostic laboratory work, traditional bacterial culture/ isolation methods have been an important component in BRD complex testing. The methodology continues to be a backbone for VDL testing for respiratory disease in cattle. However, new instrumentational technology and molecular testing methods have become more important in the area of organism isolation and identification.

One instrument that is appearing more frequently in VDLs is the MALDI-TOF (Matrix Assisted Laser Desorption/Ionization- Time of Flight). It requires routine culture methods to isolate individual bacterial colonies which are then subjected to high energy to fragment the bacteria creating a "fingerprint" of sorts that is organism specific. The instrument keeps a library which then is utilized for identification.
An important component of bacteriological testing in VDLs is antibiotic or antimicrobial susceptibility testing (AST). The process has more-or-less been fairly well standardized. Two common systems of reporting are Kirby-Bauer and MIC (Minimal Inhibitory Concentration). In each platform, results are reported as sensitive, intermediate or resistant. The goal of AST is to look at individual isolates from a diseased lung, for example, and test them against known antibiotics to hopefully find an effective compound to utilize in a treatment regimen. The other side of the coin for AST is the information it provides for detecting abnormal resistance patterns

to commonly used classes of antibiotics. This is currently a hot topic in both human and veterinary medicine. VDLs will continue to have an important role in monitoring antimicrobial resistance.

Serology

Serological assays for bacterial and viral agents in BRD investigations continue to be popularly requested tests. It is also a discipline that has seen much growth over the decades. Tests are available for virtually all bacterial and viral agents involved with the complex. Moreover, assays are quick to appear when suspected pathogens are identified. Serological testing is relatively rapid and sensitive. Virus neutralization (VN) and ELISA have become standard testing platforms in the VDL for many serological assays with some applications utilizing the microagglutination (MAT) protocol. ELISAs may target either host antibody or the antigen. Improved technology has seen the application of more high throughput testing with instruments that can run 96 samples at a time with results available the same day of testing. VN's take a bit longer, requiring 3-days for testing results and are labor intensive. Toxin neutralization assays are helpful for bacteria like *Mannheimia hemolytica* that secretes a leukotoxin important in the pathogenesis of BRD.

The challenge with serological assays is interpretation. Does the positive result reflect exposure, active infection or vaccination? Serology is often noted to provide helpful information in terms of pathogen exposure, but can fall short in clarifying actual disease status. VDL professionals can be very helpful is helping to identify the underlying positive reaction.

Virology

Before the advent of other more rapid and sensitive assays for virus detection and identification, virus isolation was the gold standard for virus recovery and identification. The process involves pulling virus from fresh tissue specimens into cells grown in artificial media and then using a combination of visual cytopathic effects via microscopy, electron microscopy, or using fluorescein-tagged antibodies specific for the virus that allow for visual detection in the cultured sample. The culture process can be lengthy and not all viruses grow sufficiently well in artificial media for identification.

In spite of the development and expanding application of molecular diagnostic techniques to organism identification, virus isolation is still an extremely important tool in the VDL as it isolates a viable virus which can be stored and banked almost indefinitely for future study. Diagnostic labs are great resources for field isolates and provide specimens for evaluation of viral genetic changes which may influence virulence and pathogenicity, for example. Such stocks also allow for micro-evolutionary studies to look at viral changes over time and the relationship on viral strains to each other.

Molecular testing

I recall probably twenty years ago a classical bacteriologist telling me that PCR testing would never become accepted in veterinary medicine as a routine diagnostic test. It was too expensive. Basically, it was a waste of effort to develop it.

Well, that proved to be wrong. The big revolution in diagnostic laboratory testing involves a variety of molecular techniques targeting nucleic acids (DNA and RNA). Growth in this area over the past few decades has resulted in the appearance of methods that allow for organism identification in a matter of a few hours rather than days. And, the cost has come down and is affordable. Individual PCR assays can be around $25. Moreover, multiplex testing allows for the simultaneous testing for multiple organisms in one batch run. Such batch run assays can be found for $45 to $90 depending on the number of targets that are included in the multiplex panel.

For example, a multiplex bacterial assay using rtPCR may include all of the major bacterial organisms of interest in BRD complex testing – *M. hemolytica, P. multocida, H. somni, M. bovis,* and *T. pyogenes.* Or, in the case of viruses, a multiplex panel could include BRSV, PI3, BVD, IBR, BCoV, and/or Influenza D.

Another area of test development in molecular diagnostics is DNA sequencing. This is the process of determining the sequence of nucleotides in a section of DNA. This can be applied to the nucleic acids in any organism with the appropriate sample. The nucleic acid sequences are highly specific for organisms even allowing differentiation/ identification of organisms which make look identical with other testing methods.

Next generation sequencing (NGS) is one application of this technology and is being used to very specifically identify organisms; even those with very minute differences. NGS makes it possible to look for mutations, drift, and even differentiation of wild-type verses vaccine strains of organisms. It is commonly used in research applications and is making its way into VDLs.
Another aspect of molecular testing is genomics which for the purpose of this presentation broadly examines the bovine genome looking for genetic variations responsible for susceptibility/resistance to BRD. Currently, this is a research application and although it carries intriguing prospects, it remains to be seen what if any significance it will have for BRD diagnostics in the future.

Finally, the concept of microbiome has made its appearance related to the respiratory tract. What is that normal resident populations of microbes in the respiratory tract? What happens to it in disease? Does a disturbance or alteration of the microbiome allow for pathogen proliferation and, thus, aid in the pathogenesis of BRD? What are the effects of antimicrobial administration on commensal populations in the respiratory system? These are some of the questions being asked. It remains to be seen if VDLs will eventually have a “screening test” of sorts to evaluate the respiratory tract microbiome.

Epidemiology
One thing that has been relatively new for us at TVMDL is having a professional on site with an interest in and skills in dealing with data management. VDLs are storehouses of information. Years and years of data are kept in everything from paper files, microfiche, and electronically. Commercial producers of LIMS seem to come and go like restaurants. Designs vary quite a bit and it can be difficult to adjust to changes made to the programming. It just seems like there is no single, complete LIMS that does everything VDLs need. The beauty of the electronic data

storage systems is that they have made it a lot easier to access information, a lot of information, for analysis. Of course there is quite a bit of work to do on the front end to ensure the data is thorough and complete. It seems statisticians don't always think highly of the external validity of VDL data. Maybe that is why they say it has to be "cleaned up" or that it is "messy"? Since VDL's data does not represent anything like a controlled study in the classical sense, it seems too often have a lot of "holes" in it because many of the retrospective analyses do not match the original purpose of testing. The data is inherently incomplete. The VDLs have no control (or sometimes knowledge) of why a sample is submitted, what the sample represents in the population or disease process, or what testing is performed on that sample. Also, the historical and geographical information is often not included with the submission data when it was initially captured by the medical records technicians. Of course, it goes back farther to the veterinarian or clinic technician or administrator that filled out the forms for sample submission and testing.

It does seem though that the new LIMS programs are becoming more robust and have much better abilities to record, categorize, and store data that can be subjected to any variety of algorithms for drawing out conclusions. It seems that more and more VDLs are hiring an epidemiologist for staff positions. Of course, VDLs affiliated with colleges of veterinary medicine where there is one or more epidemiologists on faculty will cooperate to draw out useful information from the bank of data in the VDLs records.

The data may represent occurrence of a specific disease, a specific organism, the performance of a specific diagnostic test, or antibiotic sensitivity/resistance patterns in the case of bacteria or parasites, for example. In the case of BRD which has a complex and multifactorial causality, such data analyses will be helpful in looking at not only at the organisms and the testing associated with their presence (or absence) but if we can do a better job of capturing information on the very front end of the process where cattle are entering the pipeline for beef or dairy production.

VDL staff are reaching out and joining efforts with producers and scientists to look at the diverse nature of beef/dairy production in order to identify factors that influence the health of cattle and predisposing them to develop respiratory disease.

Telemedicine

In our region in the Panhandle of Texas, our veterinarians deal with clients widely spread over the region and even into surrounding states. It is not always possible for them to be on site when a disease outbreak occurs or maybe it is just something "uncommon" or "not routine". In those cases, it may be helpful for the veterinarian to be able to have the feedyard necropsy technician or ranch hand/ owner contact the VDL diagnostician to get "real time" consultation. Beside simple dialogue over the phone, I believe there is an interest in face-to-face contact where the producer or rancher would be able to project actual live video feed to the VDL staff member.

One example of this would be direct live video feed during the actual performance of a necropsy. We do often get photographs of gross postmortem lesions submitted by veterinarians and rarely producers.

One very important addition to our professional staff at TVMDL has been a group of veterinarians that have considerable clinical practice experience and skills. We have two in the livestock field, one in equine and wildlife, and one in small animal. In the livestock arena, these individuals because of their practice backgrounds are key to interacting and fielding questions related to diseases in cattle, like BRD, for example. Because of their clinical experience, they have the ability to dissect and identify key aspects of the clinical history from our client and provide the best diagnostic plan going forward.
In discussing services with clients, over the years, direct contact with lab professional staff whether in bacteriology, virology, or pathology remains an essential aspect of the service VDLs provide. Veterinarians and producers continue to rate this as a top service they expect from their VDL.

Client education
One of the goals of TVMDL and most VDLs has to do with outreach and client education. With the advancement of electronic media there is an ongoing revolution of sorts in clinical practice which has necessitated changes in the way VDLs interact with and provide information to our clients. Labs are finding better ways to communicate important information. VDL website's and apps are a key part in this, but also how labs package the various tests they offer for diagnostic purposes. The website also allows VDLs to attach any variety of resource documents or links to other sites to be helpful.

In our lab, Dr. Jessie Monday with assistance from other professional staff is working on a bovine diagnostic reference manual. It partially reflects recently implemented diagnostic plans for key bovine disease syndromes, but includes a significant amount of other information related to appropriate sampling, safe packaging and shipping, essential supplies, and a whole section on the necropsy procedure.

Professional staff at VDLs may also be involved with extension activities which may include demonstrations, online webinars, conferences, and publications to list a few.
In our region, TVMDL is heavily involved with the "Panhandle Livestock Professionals" (PLP) that meets monthly with a featured speaker in some field of livestock production. This group includes lab staff, veterinarians in practice, nutritionists, agricultural and environmental engineers, biopharma, animal scientists, graduate students, and university faculty. It allows for a free flow of information and discussion on important topics to the industry. Factors involved with BRD have been the source of a number of these meetings.

Client needs
In talking with bovine-oriented veterinary clients of TVMDL about their practice needs from a VDL, several themes were recurring. These include: (1) accessibility to a full-service lab with experienced professional staff in the various disciplines for in-depth consultations about testing and lab results, (2) access to a full-range of tests and testing methods, (3) rapid turnaround of results, (4) pathologists to discuss postmortem lesions, (5) serving as teachers/educators for new graduates entering into practice for both disease analysis and test selection, (6) availability to

help with “wrecks” quickly, (7) have veterinarians on staff with good clinical experience, and (8) being able to help identify specific situations which are atypical.
Areas of potential growth for VDLs include: (1) collaborative “investigations” into the role of performance parameters, feed components, and drugs and their role or interaction with animal health, (2) the use of technology to provide “distance diagnostic service”, and (3) educational programs for feedyard staff tasked with animal health.

Quality assurance
Finally, a component of the VDL that seems to get little outside recognition from its clientele, but actually plays a significant role in the overall operations of the lab to ensure uniform and consistent, validated results is the quality assurance (QA) program. In the early days of VDL work, no such organized system was present. It may or may not have existed in any form. Even into the 1990’s VDLs did not necessarily have a QA department. However, accredited VDLs under the American Association of Veterinary Laboratory Diagnosticians (AAVLD) are required to have a Quality Management System (QMS) meeting high standards which are based upon ISO 17025. In fact, some labs go beyond this standard utilizing an electronic QMS for QA fundamentals such as document control, corrective actions, training and competency of personnel.

Conclusion
Veterinary diagnostic labs have over the decades evolved to continue to keep pace with services required by veterinarians working in the area of respiratory disease management in cattle. This includes staffing the lab with highly qualified professionals directing the development of services in the various disciplines and providing necessary consultations to field veterinarians. One group that is integral to the VDL mission and working alongside with the other staff is the clinical diagnostician whose practice experience provides a highly appreciated dialogue in dissecting health issues in a population of cattle.

VDL testing services continue to expand as new organisms appear and are tied to pulmonary disease in cattle. This is driven by new methodologies, better reagents, and improved instrumentation. The explosion of highly technical procedures and the equipment to perform them is most exhibited in the field of molecular diagnostics. We will continue to see advancement in this area.

Other areas of growth for VDL’s are in the area of client services especially related to distance diagnostic medicine and identifying educational platforms that will work for remote sites. We will continue to see these develop and mature in the next decade. Moreover, if there is one important aspect of VDL services that is voiced repeatedly, it is summed up in the word “relationship”. Clients demand and highly appreciate the working, professional relationships they have with their VDL. It provides a significant and meaningful element in their practice of veterinary medicine.

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Development and Application of Molecular Diagnostics and Proteomics to Bovine Respiratory Disease (BRD)

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Abstract

Advances in molecular and proteomic technologies and methods have enabled new diagnostic tools for bovine respiratory pathogens that are high-throughput, rapid, and extremely sensitive. Classically, diagnostic testing for these pathogens required culture based approaches that required days to weeks and highly trained technical staff to conduct. However, new advances such as multiplex hydrolysis probe based real time PCR (rtPCR) technology has enabled enhanced and rapid detection of BRD pathogens in a variety of clinical specimens. These tools provide many advantages and work had demonstrated superiority over culture for co-infections/co-detections where multiple pathogens are present. Additionally, the integration of MALDI-TOF mass spectrometry into veterinary diagnostic labs has revolutionized the ability to rapidly identify bacterial pathogens associated with BRD. Recent applications of this technology include the ability to type these opportunistic pathogens to the sub-species level (specifically *Mannheimia haemolytica*) using mass spectrometry based biomarkers, to allow for identification of bacterial genotypes associated with BRD versus genotypes that are more likely to be commensal in nature.

Introduction

Bovine respiratory disease (BRD) is a multifactorial disease complex that causes tremendous economic losses to cattle industries (Griffin et al., 2010). BRD is associated with a number of viruses and bacterial pathogens, often simultaneously, making diagnosis and establishment of causal agents involved in outbreaks and clinical cases challenging (Booker et al., 2008, Fulton et al., 2009). Recent applications of molecular technologies such as polymerase chain reaction (PCR) to BRD are helping to elucidate agents involved in these cases with enhanced sensitivity (Bell et al., 2014, Tegtmeier et al., 2000). Identification of the causative agents are critical so that proper prevention and vaccination protocols can be rapidly implemented. Oftentimes, severe outbreaks occur with significant mortality, therefore, rapid diagnosis can be critical. Additionally, emergence of multi-drug resistant strains of BRD pathogens have highlighted the need to identify and further characterize these bacteria to enhance judicious and effective treatment (Lubbers and Hanzlicek, 2013, Woolums et al., 2018).

Development of molecular based diagnostics

Molecular methods can detect very small quantities of target nucleic acid in complex samples and have been used in veterinary diagnostics for decades (Lauerman, 1998). However, recent advancements in technologies and chemistries have enabled robust and cost effective assays that allow for simultaneous quantitative detection of multiple targets in a single test reaction. With these advances have come efficient nucleic acid extraction chemistries that can be utilized on high throughput extraction platforms that co-purify RNA and DNA (Berensmeier, 2006). PCR based approaches were quickly adapted to and utilized to detect viral pathogens associated with

BRD, as these methods were superior in turnaround time and interpretation compared to classical approaches like cell culture and antibody based detection, and even singleplexed conventional PCR testing had significant advantages (Vilcek et al., 1994, Schmitt et al., 1994, Masri et al., 1996). Highly multiplexed real time PCR assays (rtPCR) that include reverse transcription to detect RNA and DNA pathogens have been developed are now widely used across US diagnostic labs for virus detection (Fulton et al., 2016, Horwood and Mahony, 2011).

However, widespread implementation of these methods for detection of bacterial pathogens of BRD has lagged. There are several challenges to development and implementation of these assays. These include culture based approaches that labs have existing capacity for, the need for an isolated pathogens for downstream testing (typing, susceptibility, etc), and relative cost. In contrast to culture, PCR based testing is also inherently narrow in scope, in that you are only testing for what you are targeting with primers. However, newer technologies using 16S rRNA amplification and sequencing may hold promise for a more comprehensive and broad based molecular diagnostics tool (Timsit et al., 2018, Johnston et al., 2017). Additionally, only recently have high quality complete whole genome sequences from diverse sources become available, greatly improving the ability to identify and select robust targets for assay design (Clawson et al., 2016, Harhay et al., 2017)

Another challenge is that diagnostically, interpretation of results from molecular testing may be misleading. Many pathogens are opportunistic in nature, and are present in both normal and diseased animals, therefore, direct detection of pathogens through culture and antibody based approaches was preferred by some as more interpretable (Fulton and Confer, 2012). However, the combination of being readily able to assess the relative abundance of bacteria using real time platforms, combined with rapid cycling rotary-based real time platforms and robust enzyme mixes has enhanced the utility of molecular methods for detection of bacterial pathogens of BRD (Loy et al., 2018a, Reynisson et al., 2006).

We developed a real time based assay for the most frequently detected bacterial BRD pathogens. Newly available genome sequences were used to establish robust targets, and the assay was developed and validated on multiple instrument platforms (Loy et al., 2018a, Clawson et al., 2016). As the advantages over culture-based approaches were not immediately apparent, an extensive comparative analysis was done to evaluate PCR based detection compared to culture on samples that are routinely submitted to diagnostic labs. Both antemortem (nasal and nasopharyngeal swabs) and postmortem diagnostic samples (lungs) were included. Limits of detection for the assay are quite low (1.2-12 CFU/mL) and had near perfect agreement with culture for lung tissues with high overall levels of specificity and sensitivity. One large advantage over culture is the number of co-detections found. Co-detections were extremely under-represented when relying on culture alone, with only 25 found in the data set, with 125 co-detections using PCR based approaches, indicating a five-fold increase in detection these types of infections. (Loy et al., 2018a) Agreement between culture and rtPCR was found to be highest in lungs and lowest in nasal swabs, likely due to the limitations of culture on samples more likely to contain environmental bacteria, such as nasal swabs. The instrumentation used did not adversely affect overall method sensitivity and specificity, however, the rotary based instrument

had significantly lower Cq thresholds, indicating more efficient PCR reactions. The overall conclusions of this work indicate the multiplexed rtPCR panels are rapid, sensitive, and diagnostically useful in multiple relevant sample types and have several advantages over classical methods.

Development of proteomic based diagnostics

Another emerging technology, Matrix associated laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS) has revolutionized clinical microbiology labs and veterinary diagnostics (Seng et al., 2009, Clark et al., 2013). These instruments enable the accurate identification of a single colony of bacterial growth in minutes. Many platforms are also flexible allowing users to add locally relevant strains and species to their databases. Mass spectrum data can also be mined for biomarkers that may differentiate different phenotypes or genotypes (Mani et al., 2017, Pérez-Sancho et al., 2018). Recently we have demonstrated the utility of MALDI-TOF MS, using a bioinformatics approach for biomarker discovery, to distinguish amongst two major genotypes of *M. haemolytica* (Loy and Clawson, 2017) . This method enables near real time typing of these isolates by mining data collected during the MS identification process. This method has been validated on whole cell bacteria "direct smears" so no extraction or other processing is required and isolates can be rapidly screened and selected for downstream testing.

Applications using molecular based diagnostics

One application of rtPCR BRD panels has been epidemiological investigations to examine contributions of emerging pathogens to BRD. Workman et al has utilized rtPCR to estimate BRD pathogen shedding throughout the beef production cycle to examine the role respiratory coronaviruses play in increasing risk of BRD.(Workman et al., 2017, Workman et al., 2019) One challenge to interpreting results from these panels, is unlike viruses, where any detection of virus shedding may be clinically significant, the detection of bacteria that normally resides in the nasopharynx may not be clinically relevant. Further, work to determine Cq cutoffs or levels of relative abundance that may be clinically significant, is important to enable these tests so they be more readily interpreted from antemortem samples. In one study neither the Cq level, nor the numbers of animals classified as cases or controls which had a detected pathogen was significantly different at and following feedlot entry for bacterial pathogens. However, nasal shedding of bovine coronavirus (BCV) both in Cq values and number shedding were higher in those that were classified as cases. A follow up study using these same tools following BRD in pre-weaned beef calves was able to determine in one longitudinal study, *H. somni*, in addition to BCV, was likely contributing to clinical cases, as it was detected with higher shedding levels and in a much higher number of animals with BRD. (Workman et al., 2019) These observational studies demonstrate that molecular detection tools may be useful to examine risk factors and contributions of pathogens during BRD outbreaks.

Molecular workflows also provide for any number of culture-independent nucleic acid tests in addition to pathogen detection. One approach is to evaluate the extracted clinical samples for the presence of antimicrobial resistance genes to develop a rapid culture independent resistance detection method. Recent work has shown that detection of macrolide and tetracycline resistant

genes in BRD clinical samples have high agreement with isolation of *M. haemolytica* with increased MIC values to these antimicrobials. (Loy et al., 2018b) Such an approach could provide clinicians with information about the presence of potential resistant pathogens in samples more rapidly than culture and susceptibility testing.

Applications using proteomic based diagnostics

Another challenge with interpretation of detection results from antemortem samples is the presence of mixed intra-species populations that may not be representative of the causative organisms deeper in lung tissues. Capik et al has found using pulsed field gel electrophoresis that *M. haemolytica* populations in the nasopharyx do not always match those found in lungs of cattle with BRD (Capik et al., 2015). One potential diagnostic approach to assist microbiologists in finding those populations most likely associated with disease from NP samples is to use MALDI-TOF MS profiles to screen isolates for downstream testing. Genotype 2 *M. haemolytica* is more likely to be associated with lung invasion and contain AMR genes and ICE elements. Therefore, preferential selection of these genotypes using MALDI-TOF prior to MIC testing and other downstream assays may be useful.

Conclusions

Emerging technologies and methods developed for detection of etiologic agents associated with BRD have enabled further understanding of the role of microbes in BRD. Application of these technologies will help further elucidate the role of these opportunistic pathogens and will enable more effective disease prevention and treatment strategies.

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The Role of the Bovine Respiratory Microbiota in Health and Disease

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Abstract
Increased antimicrobial resistance in bovine respiratory pathogens poses a threat to the effective control and prevention of bovine respiratory disease (BRD). As part of continued efforts to develop antimicrobial alternatives to mitigate BRD, the microbial community residing within the respiratory tract of feedlot cattle have been increasingly studied using next generation sequencing technologies. The mucosal surfaces of upper and lower respiratory tracts of cattle are colonized by a diverse and dynamic microbiota encompassing commensal, symbiotic, and pathogenic bacteria. While a direct causal relationship between respiratory microbiota and the development of BRD in feedlot cattle has not been fully elucidated, increasing evidence suggests that the microbiota contributes to respiratory health by providing colonization resistance against pathogens and maintaining homeostasis. Certain management practices such as weaning, transportation, feed transition, and antibiotic application can disrupt the respiratory microbiota, potentially altering pathogen colonization. Microbiota-based approaches including bacterial therapeutics that target restoring the normal respiratory microbiota may provide new methods for mitigating BRD in feedlot cattle, in place of antibiotics. In addition, the distinct respiratory microbial communities observed in BRD-affected and healthy feedlot cattle may allow for future application of microbiota-based techniques used in the diagnosis of BRD.

Introduction
Multiple factors have been associated with the development of bovine respiratory disease (BRD) but bacterial species, including *Mannheimia haemolytica*, *Histophilus somni*, *Mycoplasma bovis*, and *Pasteurella multocida* are frequently implicated (Confer, 2009). The upper respiratory tract is a reservoir of these opportunistic pathogens, which can proliferate and infect the lungs when cattle immunity is compromised due to stress or primary viral infections (Hodgson et al., 2005). High risk cattle populations entering feedlots are most susceptible to BRD, and as a result, are often administered metaphylactic antibiotics to prevent infections after feedlot placement. However, there are public and scientific concerns regarding antimicrobial use in livestock production (Cameron and McAllister, 2016), and recent reports indicate high levels of resistance in important BRD pathogens from feedlot cattle, potentially limiting the antimicrobials available

for treatment (Portis et al., 2012; Anholt et al., 2017; Timsit et al., 2017). Thus novel methods to reduce antimicrobial use and mitigate BRD-related pathogenic bacteria are needed.

While pathogenic bacteria that can be cultured in the laboratory have been the main focus of research on the bovine respiratory tract, advances and affordability of next generation sequencing have led to an increased number of studies on the respiratory microbiome. Resulting from these studies is an improved understanding of the importance of the mammalian microbiome in relation to host health, and it is clear that the resident microbiota of the respiratory tract have a critical role in preventing colonization of pathogens (Bogeart et al., 2004; Cho et al., 2012). Establishment and stability of the respiratory microbiota is important for homeostasis while disruption can predispose to pathogenesis (Man et al., 2017). For example, reductions in bacterial community density and diversity following antibiotic treatment in humans have been associated with an increased risk of overgrowth of bacterial pathogens and establishment of respiratory diseases (Pettigrew et al., 2012). There is evidence showing that the cattle respiratory microbiota is also susceptible to disturbances. In beef cattle, transportation to a feedlot (Holman et al., 2017), diet composition (Hall et al., 2017), and antimicrobial administration (Holman et al., 2018; Holman et al., 2019) have previously been shown to affect the nasopharyngeal microbiota, highlighting that respiratory bacteria of cattle are perturbed by industry management practices. It has also been shown that bacterial diversity and richness were reduced in the nasopharynx of cattle that developed BRD early in the feeding period compared with cattle that remained healthy (Holman et al., 2015a) and certain bacterial communities have been associated with bovine respiratory health (Timsit et al., 2018). These data suggest that distinct microbial profiles of the respiratory tract could be used to enhance BRD diagnosis. In addition, microbiota-based interventions (e.g. bacterial therapeutics) may provide new opportunities for managing BRD.

Structure and composition of the bovine respiratory microbiota

Microbiota studies based on 16S rRNA gene sequences from beef cattle nasopharyngeal (NP) swabs, trans-tracheal aspirations (TTA) and bronchoalveolar lavages (BAL) have revealed that mucosal surfaces of the upper and lower airways are colonized by diverse microbial communities. The NP microbiota of feedlot cattle has been more extensively characterized, compared to the lower respiratory microbiota, and has been shown to be comprised of at least 29 different phyla and 300 different genera (Timsit et al., 2016a). Although the proportions of bacteria vary among individual cattle, and also the time of sampling during production, the most commonly identified phyla within the NP microbiota include *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteriodetes* and *Tenericutes,* accounting for more than 90% 16S rRNA sequences (Holman et al., 2015b; Holman et al., 2017; Zeineldin et al., 2017a; Timsit et al., 2018). Within these phyla, *Corynebacterium*, *Moraxella*, *Mycoplasma*, *Pasteurella*, *Mannheimia, Psychrobacter* and *Staphylococcus* are most relatively abundant NP genera. Overall, TTA and BAL samples from feedlot cattle have shown that the microbiota of the lungs have similar taxonomic profiles compared to the upper respiratory tract, but can have reduced diversity and geographic-specific abundances of certain bacteria (Zeineldin et al., 2017b; Timsit et al., 2018). This may be in part due to physiological differences between the upper and lower respiratory tract, including variations in pH, relative humidity, temperature, and partial pressure of oxygen and carbon dioxide (Man et al., 2017).

Management factors influencing the bovine respiratory microbiota

Several studies have shown that the NP microbiota of beef calves changes after transportation to a feedlot. In one study that sampled the nasopharynx of calves at weaning, upon feedlot arrival, and 40 days after feedlot placement, it was shown that the NP microbiota underwent a profound evolution, with the abundance of 92 operational taxonomic units (OTUs) significantly changing over time (Timsit et al., 2016a). In a subsequent study that focused on shorter time points after feedlot placement, the structure and composition of the NP microbiota was observed to change within two days of feedlot placement, increasing in both phylogenetic diversity and richness (Holman et al., 2017). In both those studies by Timsit (Timsit et al., 2016a) and Holman (Holman et al., 2017), cattle were not administered antimicrobials or implants which could have biased the results. Interestingly, transportation to and commingling at an auction market for 24 h did not significantly influence NP or tracheal bacterial communities in recently weaned beef calves (Stroebel et al., 2018) indicating that feedlot introduction has a strong influence on shaping respiratory microbiota throughout the beef continuum. It should be noted however that both farm of origin and feedlot practice can influence the pattern of respiratory microbiota evolution (McMullen et al., 2018; Stroebel et al., 2018), thus the changes observed in cattle are not necessarily common. The instability in respiratory microbiota observed after feedlot placement might explain why cattle are most likely to be affected with BRD during the first weeks after weaning and arrival at a feedlot. Factors that may lead to changes in the respiratory microbiota may include a reduction in calf immunity due to stress from weaning and transportation, and colonization by bacteria originating from the feedlot environment or new pen mates (Timsit et al., 2016b). In addition, diet transition before or after feedlot placement may influence the respiratory microbiota (Hall et al., 2017).

Administration of antimicrobials can also affect the microbiota by inhibiting growth of certain bacteria, and potentially promoting growth bacteria that have intrinsic or acquired resistance to the antimicrobial. In children, antibiotic use has been linked to an altered microbial community structure in the upper respiratory tract for up to six months after administration [10], indicating that a prolonged effect takes place. Recently, it was observed that alterations in the NP microbiota of commercial cattle were apparent 60 days after injection with either oxytetracycline or tulathromycin (Holman et al., 2018). In a controlled study analyzing the effects of these same two antimicrobials on the NP microbiota across 34 days, perturbation of the NP microbiota was greatest two and five days after administration (Holman et al., 2019). In the study by Holman (Holman et al., 2019), it took 12 days for the NP microbiota to recover after tulathromycin injection, whereas recovery was not apparent after 34 days for the oxytetracycline-treated cattle. Interestingly, shortly after administration (2-5 days), both antimicrobials reduced the abundance of *Pasteurella* spp., however one *Mycoplasma* OTU was enriched in oxytetracycline-treated cattle at the end of the study (d 34). These studies show that administration of antimicrobials to cattle can have short- and long-term impacts on the NP microbiota.

The role of the microbiota in respiratory health

An increasing number of studies suggest that the bovine respiratory microbiota plays an important role in defining respiratory health and disease in cattle (Timsit et al., 2016a; Zeineldin et al., 2019). Evidence of bacterial competition within the bovine respiratory tract was first shown

by Corbeil and colleagues, when they observed that bacteria from the nasopharynx either enhanced or limited *in vitro* growth of the BRD pathogens *M. haemolytica*, *P. multocida*, and *H. somni* (Corbeil et al., 1985). Enhancing bacteria included *Micrococcus*, *Staphylococcus*, *Corynebacterium*, *Rhodococcus*, *Moraxella*, and *Actinobacter* isolates, while isolates of *Bacillus* were the strongest inhibitors. Interestingly, it was subsequently observed that cattle sampled by deep nasal swabs at feedlot entry and later developed BRD during the feeding period, had lower proportions of NP *Bacillaceae* and *Lactobacillaceae* family members compared to cattle that remained healthy (Homan et al., 2015a). Thus members of these bacterial families may provide colonization resistance against BRD bacterial pathogens. In support of this, a case-control study compared the NP and TTA microbiota collected from feedlot calves diagnosed with BRD and healthy pen mates (Timsit et al., 2018). In that study, distinct bacterial metacommunities were observed to be associated with upper or lower respiratory tract, as well as BRD status. One metacommunity included *Lactococcus lactis* and *Lactobacillus casei*, and was mostly associated with the trachea of healthy calves. Overall, these studies highlight that differences in respiratory microbiota have been associated with BRD status in cattle. Given these observed associations, microbiota-based applications could potentially be used for both diagnosis and prevention of BRD.

Microbiota-based diagnostics for BRD

The development of microbiota-based diagnostics stems from in-depth analyses of microbial composition within a host and associations observed between specific microbial community members and a host phenotype. Microbiota-based diagnostics have been studied for gastrointestinal applications, including inflammatory bowel disease (Eck et al., 2017) and infections (Raes, 2015). Recently, Langelier (Langelier et al., 2018) developed a metagenomic sequencing-based method that simultaneously evaluated three core elements of acute airway infections, including the pathogen, airway microbiome, and host response. The method allowed for accurate diagnosis of lower respiratory tract infections in critically ill adults. Given that BRD-affected cattle have been shown to have different microbiota profiles compared to healthy pen mates (Holman et al., 2015a; Zeineldin et al. 2017a) and communities have been identified that were associated with BRD phenotype (Timsit et al., 2018), it may be possible to use microbiota data to aid in diagnosis of BRD. Further studies are needed to characterize the microbiota of beef cattle however, before realizing the full potential of microbiota for diagnosis of BRD. Complications in diagnosis may arise from the polymicrobial nature of BRD infections and the fact that cattle source and management practices can alter the microbiota.

Bacterial therapeutics to mitigate BRD pathogens

Research on probiotics has significantly increased over the last 10 years, and has coincided with improved methods to culture bacteria and reduced costs of massive parallel sequencing technologies to analyze microbiota. These technologies have allowed in-depth study of host metagenomics and are providing important information on bacteria associated with certain phenotypes, such as diseases like BRD. There are ongoing efforts to identify strains of bacteria within a host's microbiota and developing them into probiotics that could potentially alter a phenotype after administration or prevent infections by pathogens. Strains of this nature however likely fall outside of the traditionally defined probiotics (e.g. *Lactobacillus*,

Bifidobacterium) and would be used for therapeutic purposes. As such, they have been coined "next-generation probiotics" (Patel and DuPont, 2015), or bacterial therapeutics. Their development employs a targeted approach combining microbiota sequencing and culturing, and is promising for tailored biotherapeutics that can be used as alternatives to antimicrobials in livestock. Recently it was shown that commercial probiotic bacterial strains were capable of colonizing bovine bronchial epithelial cells and inhibiting *M. haemolytica in vitro* (Amat et al., 2017). In a follow-up study, the same authors utilized microbiota data from cattle, and a step-wise targeted approach, to develop bacterial therapeutics against *M. haemolytica* that originated from the respiratory tract of feedlot calves (Amat et al., 2016). Intranasal application of 6 therapeutic candidate strains were capable of reducing colonization by *M. haemolytica* in challenged calves, showing that bacteria sourced from the bovine respiratory tract may have utility in reducing proliferation of BRD pathogens (Amat et al., 2018).

Conclusion

While the bovine respiratory microbiota is dynamic and can be altered by a number of management practices, it is not yet clear how perturbations directly impact BRD development. However, evidence exits to show that the respiratory microbiota contributes to cattle health potentially by providing colonization resistance against pathogens. A better understanding of how the respiratory microbiota relates to susceptibility and progression of BRD, and in maintaining respiratory health, may lead to the development of new technologies for diagnosing and mitigating BRD in feedlot cattle.

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Bovine Respiratory Disease Treatment Failure: Definition and Impact

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Abstract

Bovine respiratory disease (BRD) treatment failure occurs when animals receiving a treatment regimen for BRD fail to directly respond and recover, resulting in BRD relapse therapy, chronic illness, sale for salvage slaughter, and/or euthanasia/death. When BRD treatment failure occurs, it has direct and indirect impacts. The direct impacts include additional costs associated with BRD relapse treatment, management of chronically ill animals, reduced proceeds received for animals sent for salvage slaughter, the original purchase of the feeder animal and accumulated feed/production expenses to death, and carcass disposal. The indirect impacts include effects on infrastructure, morale of employees, animal welfare, and antimicrobial exposure.

Introduction

There are many different ways to define bovine respiratory disease (BRD) treatment failure and individual veterinarians and producers likely have different thresholds for considering something a failure. However, from a practical perspective, BRD treatment failure occurs when animals receiving a treatment regimen for BRD fail to directly respond and recover after treatment, resulting in BRD relapse therapy, chronic illness, sale for salvage slaughter, and/or euthanasia/death. When BRD treatment failure occurs, it has direct impacts (costs associated with BRD relapse treatment, management of chronically ill animals, reduced proceeds received for animals sent for salvage slaughter, the original purchase of the feeder animal and accumulated feed/production expenses to death, and carcass disposal) and indirect impacts (effects on infrastructure, morale of employees, animal welfare, and antimicrobial exposure).

Direct impacts

BRD relapse treatment

When animals receiving a treatment regimen for BRD fail to directly respond and recover after treatment, it may be necessary to provide additional treatment for BRD. In the feedlot, this generally requires identifying and separating the affected animal from pen-mates, moving the animal through an animal handling facility, making a diagnosis of BRD relapse, administering an antimicrobial drug +/- ancillary or supportive therapeutic products, recording what was done in the medical record, and returning the animal to the pen of origin directly after treatment or after spending one or more days in a hospital/convalescent pen. The direct impact is the sum of the costs associated with each of these steps.

Management of chronically ill animals

While the majority of animals treated for BRD respond directly after initial BRD treatment or after one or more additional BRD treatments, some animals develop chronic BRD that is deemed non-responsive to additional antimicrobial, ancillary, or supportive therapy. In the feedlot, animals with chronic illness, including chronic BRD, are generally housed in a designated pen(s) for

animals with chronic disease – “chronic” pens. Ideally, these pens are equipped and managed to provide a comfortable environment with easier access and less competition for feed/water than a regular production pen. In addition, animals should be evaluated on a daily and weekly basis to assess/monitor the clinical progression of each animal so that “recovered” animals can be returned to a regular production pen, chronically ill animals that are suitable for salvage slaughter and have fulfilled all pharmaceutical withdrawal times can be shipped, moribund or suffering animals can be euthanized, and animals requiring additional time in the “chronic” pen can be maintained there for a defined time period. This approach generally works well in most feedlots when the number of chronically ill animals is small and relatively constant and the personnel assigned to assess/monitor the clinical progression of each animal are well trained and have sufficient time and support to attend to their job duties. However, whenever the occurrence of BRD peaks (generally in the fall of the year in the United States and Canada), it is followed by an increase in chronically ill animals, resulting in overcrowded “chronic” pens and insufficient time/support for personnel to adequately assess/monitor the clinical progression of each animal. When this occurs, it generally requires direct intervention by a veterinarian and/or supervisor/manager to prioritize the management of chronically ill animals. The direct impact is the sum of the costs associated with managing chronically ill animals. The indirect impact of managing chronically ill animals is the effect on morale of employees and animal welfare.

Sale for salvage slaughter

Animals with chronic BRD that do not recover and have fulfilled all pharmaceutical withdrawal times may be deemed suitable for salvage slaughter. Compared to euthanasia or death, sale for salvage slaughter may seem like a substantially better economic outcome of BRD treatment failure cases. However, in many cases, especially in lighter animals, the proceeds received from salvage slaughter of animals with chronic BRD is only 25-50% (or less) of the original purchase cost of the feeder animal and does not account for the accumulated feed/production expenses from arrival at the feedlot to shipment. As a result, the direct impact of sale for salvage slaughter is a substantial loss on each affected animal.

Euthanasia/death

The most definitive form of BRD treatment failure is death. This can occur “naturally” associated with the pathologic and physiologic processes that happen in BRD or it can occur through euthanasia of animals with chronic disease or animals that are moribund or suffering. When death occurs, the direct impacts include the original purchase cost of the feeder animal, accumulated feed/production expenses to death (including processing, treatment, bedding, and yardage), and carcass removal/disposal cost. In addition, there is an “opportunity” cost to the cattle owner of not marketing the animal when cattle ownership is profitable and loss of daily margin for the feedlot until a replacement animal is able to fill the same pen space. The magnitude of these costs vary depending on the underlying value of feeder cattle and feed/production expenses.

Indirect impacts

Infrastructure

In terms of BRD treatment failure, the biggest impacts on infrastructure are pens to house animals with chronic BRD. In most cases, these are designated pens associated with hospital/treatment facilities. However, in instances where there are large numbers of animals with chronic BRD, it can be necessary to temporarily use a regular production pen as "chronic" pen. In addition to pens for chronically ill animals, it is necessary to have access to appropriate infrastructure to ship animals for salvage slaughter and carcass disposal/removal.

Morale of employees

Managing sick animals, including animals with BRD, is a serious responsibility. Trained animal personnel diagnose and treat animals in the feedlot on a daily basis. From the employee perspective, successful treatment of a sick or injured animal is a very rewarding experience. However, whenever treatment failure occurs, it usually has negative impacts on employee morale and the magnitude of the impact is usually greater the more "severe" the treatment failure. For example, the negative impact on employee morale of having a treatment failure that results in an animal with chronic BRD or death is much more severe than a treatment failure requiring one or two additional treatment regimens for BRD. Having said that, managing animals with chronic BRD seems to have a greater impact on employee morale than coping or dealing with animals that die acutely from BRD. This is not a surprising revelation, but bears significant consideration when working with animal health personnel that have to deal with large numbers of chronically ill animals on an ongoing basis to make sure that there are adequate protocols, systems, management support, and other resources in place to maintain animal welfare, as well as maintain/promote positive worker morale.

Animal welfare

Animal welfare is a priority throughout the feedlot production system, including identification and initial treatment of sick animals. When treatment failure occurs for any disease, including BRD, the probability of an adverse outcome (relapse, chronic disease, or death) goes up and, generally speaking, animals with a higher probability of an adverse outcome require closer assessment/monitoring to maintain appropriate animal welfare. As noted above, this is particularly relevant and important in animals with chronic disease to make sure that adequate protocols, systems, management support, and other resources are in place to maintain animal welfare.

Antimicrobial exposure

While we do not understand the specific impacts of each antimicrobial exposure in cattle on the development of antimicrobial resistance or the spread of resistance elements in bacteria of importance to animal or human health, it is generally believed that lower antimicrobial exposure is a good thing from an antimicrobial stewardship perspective. From a veterinary perspective, this approach makes sense as long as efforts to reduce antimicrobial exposure don't have significant negative impacts on animal health and welfare. In terms of BRD treatment failure, we have already discussed the impacts on animal health and welfare. However, when BRD treatment failure occurs, it may be necessary to provide additional treatment for BRD and this usually

includes administration of an antimicrobial. In addition, the antimicrobial used is likely to be of a different antimicrobial class than has been used previously in the same animal. As a result, an indirect impact of BRD treatment failure is increased exposure of the animal to antimicrobials to maintain animal health and welfare. While we do not know what the impact of this increased antimicrobial exposure is, if any, on the development of antimicrobial resistance or the spread of resistance elements in bacteria of importance to animal or human health, it is one more thing to keep in mind as the veterinary profession works to develop and validate more effective BRD prevention, control, and treatment strategies.

BRD Treatment Failure: Clinical and Pathologic Considerations

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In young cattle, respiratory disease is treated primarily by the administration of long acting, injectable antimicrobials intended for single dose administration. Interestingly, less than 60% of dairy producers consult their veterinarian for specific details about antibiotic usage and 85% use antibiotics in an extra label manner (USDA, 2018). High levels of disease, potential misuse, and retreatment rates contribute to the overall volume of antibiotics administered to dairy calves. This is costly and contributes to the selection pressure for antimicrobial resistance in both pathogenic and commensal bacteria. This is especially concerning because the three classes of antibiotics known for promoting antimicrobial resistance by selecting for multi-drug resistant bacteria in animals and people (3rd generation cephalosporins, fluoroquinolones, and macrolides; Guardabassi et al., 2017) are administered to nearly 50% of calves treated for respiratory disease (USDA 2018).

In the field setting, treatment response is either not measured at all, or is assessed indirectly at the herd level by looking at producer treatment records (e.g. retreatment rate, average number of treatments per calf). In the research setting, response is often gauged by clinical cure rate, clinical relapse rate, mortality rate, average daily gain, and severity of lung lesions at necropsy. Using criteria based largely on resolution of clinical signs, most reports suggest that 20 - 35% of treated calves require multiple antibiotic treatments for relapse or recurrence of their respiratory disease (van Donkersgoed et al., 1993; Windeyer et al., 2012; Heins et al., 2014).

In dairy animals less than 6 months of age, lung ultrasound can rapidly and easily detect the non-aerated or consolidated lung lesions associated with pneumonia (Ollivett et al., 2015, 2016; Buczinski et al., 2015). In addition, there is a high correlation (r = 0.92) between the amount of consolidated lung identified on lung ultrasound and gross post-mortem examination (Ollivett et al., 2013) which means we can use this tool to measure the severity of pneumonia in the live calf. Regardless of clinical picture, ultrasonographic lung lesions in dairy calves are associated with reduced preweaning ADG (Ollivett TL, 2014; Cramer et al., 2019), increased mortality (Buczinski et al., 2014), and less milk production during the first lactation (Dunn et al., 2018).

Three BRD subtypes (Ollivett and Buczinski, 2016) can be defined when a systematic clinical scoring system, such as the Wisconsin Respiratory Score (McGuirk, 2008) is incorporated alongside lung ultrasound: 1) upper respiratory tract infections, 2) clinical pneumonia, and 3) subclinical pneumonia. Although the distributions of BRD subtypes will vary from farm to farm, we have found that approximately 1/3 of new cases are subclinical and that for every case of existing clinical respiratory disease, we can expect to find 2 – 4 cases of subclinical disease (Ollivett and Buczinski, 2016; Binversie et al., 2017).

For these reasons, lung ultrasound combined with clinical respiratory scoring has become the primary way that we monitor presence of disease, competency of farm staff for detecting sick calves, and treatment response on local commercial dairies as well as research projects (Ollivett and Buczinski, 2016). In regards to measuring treatment response, once treatment has been initiated, the numbers of live bacteria within the lung are significantly reduced and the draw for new neutrophils into the airway slows down. We can expect that the neutrophils within the airways will undergo apoptosis within 1 - 2 days of arrival and that fibrin and cellular debris will be expelled from the airway through coughing and other cellular mechanisms within 7 – 10 days (Caswell, 2015). We can watch this phenomena ultrasonographically through sequential examinations and visualize lung lesion regression as the airways become aerated again (Binversie et al., 2017; Holschbach et al., 2018).

Unfortunately, data from recent studies suggest that retreatment rates can be 2 to 3 times higher than those reported in the literature (Binversie et al., 2017), ultrasonographic lung lesions associated with pneumonia initially respond to antibiotic therapy but often recur or worsen shortly after treatment (Binversie et al., 2017; Holschbach et al., 2018), and that antibiotic treatment does not always result in a bacteriological cure within the lung despite early treatment and resolution of clinical disease (Holschbach et al., 2018).

More specifically, the common definition for treatment success (rectal temperature < 104°F, normal respiratory pattern, normal attitude; as reviewed by DeDonder and Apley, 2015) used by many manufacturers when establishing efficacy of their product, would incorrectly classify 100% of the calves with severe lung disease five days after a *Mannheimia haemolytica* challenge study and 14 days after a *Pasteurella multocida* challenge (Ollivett et al., 2013; Holschbach et al., 2018).These findings indicate that despite early recognition of disease, and judicious antibiotic use, bacterial infection has not resolved at the lung level using on-label treatment regimens. We hypothesize that incomplete bacterial killing sets the stage for bacterial replication and relapse or recurrence of consolidation once the antibiotic pressure has been removed. Poor treatment response coupled with misleading clinical criteria for treatment success puts calves at risk for future clinical disease (Binversie et al., 2017) and prolonged periods of slow growth (Cramer et al., 2019).

This lecture will discuss individual and herd level factors that may be contributing to treatment failures and how ultrasound guided treatment protocols could re-shape how we measure response to treatment, how we validate dosage regimens for currently approved antimicrobial drugs as well as those drugs undergoing the approval process. Implementing ultrasound guided treatment protocols on farm should improve calf level response, result in fewer relapses, decrease duration of disease, thereby improving calf welfare and decreasing cost of disease, ameliorate effect of disease, and ensure that administered antibiotics are effective at establishing a bacteriological cure within the lungs.

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Pharmacological Considerations of Bovine Respiratory Disease Antibiotic Failures

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Abstract

Bovine Respiratory Disease (BRD) is one of the most common indications for antimicrobial therapy in beef cattle production and research trials demonstrate that antibiotic therapy greatly improves clinical outcome for BRD. These trials also show that BRD treatment success rates are less than 100% and that there are opportunities to optimize antimicrobial prescribing and improve clinical outcomes, if the underlying cause(s) of BRD treatment failures can be identified and addressed. As the etiology of BRD in an individual animal is frequently multi-factorial in nature; it is likely that BRD treatment failures also result from complex interactions between the drug, drug administrator, animal host, pathogens, and the environment. This review will focus specifically on the pharmacological aspects, specifically the interactions between the host and the drug and the drug and the drug administrator, of BRD treatment failures and the actions that veterinary practitioners can take to investigate and mitigate therapeutic failures in future cases.

Introduction

Clinical trials repeatedly demonstrate that antibiotic therapy greatly improves clinical outcome in Bovine Respiratory Disease (BRD) cases. These trials also show that treatment success rates are less than 100%; in fact, results from 30 controlled studies submitted for FDA approval of eight different antimicrobials used to treat BRD, demonstrate that average treatment success is 70% (range 51% - 92%). Treatment success rates for clinical cases of BRD in the feedyard setting are similar to those reported for drug approval [personal communication – Dr. Robert Smith]. These data suggest that veterinarians may be able to optimize antimicrobial prescribing and improve clinical outcomes, if the underlying cause(s) of BRD treatment failures can be identified and addressed.

As the etiology of BRD in an individual animal is frequently multi-factorial in nature; it is likely that BRD treatment failures also result from complex interactions between the drug, drug administrator, animal host, pathogens, and the environment. This review will focus specifically on the pharmacological aspects of BRD treatment failures; namely, the interactions that occur between the host and the drug & the drug and the drug administrator.

Host – Drug factors that contribute to clinical failure

One of the central tenets of drug therapy is that antibiotics must achieve sufficient concentrations at the site of action (bacterial receptor) to be effective. In cases of severe disease, alterations in host physiology can influence the pharmacokinetics of antibiotics. In humans, the pathologic changes associated with sepsis can lead to loss of capillary integrity, alterations in protein binding, and changes in renal clearance that potentially lower the plasma concentrations of an antibiotic and result in treatment failures; while end-stage organ dysfunction may lead to increases in plasma concentrations of antibiotics (Shah, et al., 2015). These same physiological

changes likely occur to some extent in severe cases of BRD. Although not a consideration in human medicine due to intravenous administration of antibiotics to severely ill patients, the pharmacokinetics of an antibiotic in food animals are potentially altered due to changes in absorption of drug from intramuscular or subcutaneous injection sites caused by severe dehydration (and reduced blood flow to the superficial tissues). Alterations in the absorption of an antibiotic could result in lower plasma concentrations leading to reduced efficacy and/or increased concentration of drug at the injection site leading to violative drug residues.

Factors such as end organ failure and endotoxemia may result in higher than expected plasma concentrations due to reduced clearance of drugs. These pharmacokinetic changes could result in drug toxicities, or potentially increase the likelihood of violative residues in food animals. In reality, drug concentrations in patients with severe systemic disease, such as BRD, will be increased by some of the pathophysiologic changes that occur as a result of systemic disease, while other factors may decrease concentrations of antimicrobials; thus making the sum effects on pharmacokinetics "markedly unpredictable" for these patients (Goncalves-Pereira, 2011).

Drug – Drug administrator factors that contribute to clinical failure

In human medicine, the most common drug administrator factor associated with treatment failure is selection of an antimicrobial with inadequate spectrum or improper timing of drug administration (Houck, et al., 2004; Liu, et al., 2017). While these factors also potentially contribute to BRD treatment failures, other drug-drug administrator factors should be considered when evaluating therapeutic failures in cattle treated for BRD. As antibiotics are chemical compounds subject to degradation, one of the factors that should be evaluated in cases of treatment failure is drug handling and storage. A study by Ondrak *et al.* showed that typical storage conditions (non-refrigerated truck bed box) in the summer months in Nebraska and Texas exceeded recommended manufacturer storage temperature for 32.5% and 61.8% of temperature readings, respectively (Ondrak, et al., 2015). In addition to storage and handling issues, underdosing antimicrobials can lead to therapeutic failure. Underdosing may be intentional (for economic reasons) or unintentional due to poor estimation of body weights.

Adverse event report summaries published by the US Food and Drug Administration – Center for Veterinary Medicine also show that transcription errors on prescriptions, confusion between human and veterinary brand names (for prescriptions filled by human pharmacies) and drug packaging and labeling have all contributed to cases of therapeutic failure.

Learning from treatment failures

Treatment failure ("lack of efficacy") is considered an adverse drug event by the US Food and Drug Administration (FDA) and should be reported whether the exact cause of failure can be determined or not. Clinicians or animal owners can report therapeutic failures to either the pharmaceutical company that markets the product or directly to the FDA by completing Form 1932 (https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/AnimalDrugForms/UCM048810.pdf -- Current as of 1 April 2019). Pharmaceutical companies are required by law to forward any adverse event reports to the FDA as part of their post-approval drug monitoring.

The initial step of investigating treatment failure should begin with treatment records. Treatment records can be used to determine the frequency of treatment failure, as well as, the relative timing between antibiotic administration and drug failure. Accurate treatment records are also valuable in establishing associations between treatment failures and specific antibiotics (or a specific lot of antibiotic), certain pens or groups of animals, and / or particular drug administrators.

Any potential issues with product use, handling and administration should be ruled out as part of a therapeutic failure investigation. Although seemingly obvious, medication errors are commonly documented in human medicine and also occur in veterinary medicine. Verifying that the *correct product* was dispensed and used is important to rule out these uncommon prescription errors. Confirming that the product was stored properly and used within the expiration date on the label are simple steps in ruling out product specific issues. Additionally, treatment failure investigations should include some inquiry into the specific doses and the method for determining dose (estimated weight, scale weight) used.

The underlying cause of a clinical failure may or may not be due to these pharmacological aspects of BRD therapy. Once issues with the particular antibiotic product have been investigated, the next reasonable step is to determine other factors that may present as treatment failures, such as incorrect clinical diagnosis. Finally, as a specific cause(s) for the treatment failure are determined, steps should be taken to revise treatment protocols and/or Standard Operating Procedures (SOPs) to mitigate these root causes in the future.

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Genomics: Host Genotype and Relevance to BRD

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Abstract

Genomic variation exists in cattle that affects their susceptibility to the complex of pathogens responsible for bovine respiratory disease (BRD). Heritability estimates and genome-wide association support the role of host genomic variation in BRD susceptibility. Heritability estimates for BRD susceptibility range from 0.02 to 0.29 depending on the population and the definition of the disease. Genome-wide association analysis have identified genomic regions associated with BRD in beef and dairy cattle. Commercial genotyping is now available to predict BRD susceptibility in dairy cattle and may be used for selection of replacement animals. Disease pathogen profiles vary by region and can result in genetic heterogeneity where different genomic regions are important for susceptibility to different BRD pathogens. Identification of genomic regions associated with host susceptibility to BRD provides a foundation for genomic selection to reduce disease and opens the possibilities to a better understanding of how the host defends itself.

Introduction

Bovine respiratory disease (BRD) causes significant morbidity and mortality in beef and dairy cattle. Prevention strategies to reduce BRD have included vaccination, increased biosecurity to reduce pathogen exposure, and stress reduction. Treatments for cattle affected with BRD aim to reduce death, permanent lung damage, production losses and the length of time the cattle are ill. Unfortunately, successful prevention and treatment of BRD has eluded much of the cattle industry, as 23.9% (917,090) of all cattle deaths are due to respiratory disease each year (USDA, 2015).

Evidence for a genetic role in Bovine Respiratory Disease

Not all cattle that are exposed to BRD pathogens respond the same way. Some cattle are more likely to die whereas others remain healthy when exposed to the same level and type of pathogen. When differences in susceptibility to BRD are identified in animals that are managed the same, it indicates that some of the susceptibility to disease is due to differences in cattle's innate ability to resist the disease, or its genetic predisposition to disease. Differences in morbidity and mortality found between cattle breeds and between sire or family lines support that there is a genetic component to BRD (Neibergs et al, 2014, 2011; Heringstad et al, 2008; Cusack et al, 2007; Snowder et al, 2005; Muggli-Cockett et al, 1992). By identifying the DNA regions that are associated with an enhanced ability to resist BRD, genomic selection may be used to select cattle that are more likely to stay healthy when faced with a pathogen challenge.

Heritability estimates for BRD susceptibility vary based on how the trait was measured, if the trait was binary or ordinal and if the estimates were based on pedigree information or genomic (single nucleotide polymorphism or SNP) data. Heritability estimates range from 0.02 to 0.29 for BRD susceptibility in beef and dairy cattle (Gonzalez-Pena et al, 2019; Buchanan et al, 2016; Neibergs

et al, 2014; Taylor et al, 2010; Heringstad et al, 2008; Snowder et al, 2005; Lyons et al, 1991). Higher heritability estimates tend to be identified in data sets with precise phenotypes, and phenotypes that are ordinal rather than binary (Buchanan et al, 2016).

Genomic selection uses genotypes as a tool to predict future performance of offspring to select animals that will be part of the breeding herd. Genetic selection uses performance records on an individual or its ancestors to predict future performance and select breeding animals. Genomic enhanced selection is the combination of genomic and genetic selection and is particularly powerful in traits that occur late in life, have low heritability estimates or for traits that are expensive to collect (Garcia-Ruiz et al, 2016). Bovine respiratory disease occurs throughout the life of cattle, has low to moderate heritability and recording of sick cattle and determination of the BRD pathogens present is expensive, suggesting that genomic enhanced selection is well-suited for BRD. In addition, genomic enhanced selection increases the accuracy of prediction, facilitating more rapid improvement of breeding objectives (Meuwissen et al, 2016; Hayes et al, 2009).

Genomic evaluation in dairy cattle was first released in 2008 and has grown rapidly and has resulted in the doubling of the annual genetic improvement (Council on Dairy Cattle Breeding). Most of this increase in genetic improvement is through genomic enhanced selection coupled with the use of artificial insemination (Council on Dairy Cattle Breeding). In contrast, in beef cattle, less than 10% of cattle are bred by artificial insemination so the use of genomic enhanced selection has not resulted in a doubling of genetic progress as has been experienced by the dairy industry. Health traits such as BRD are not yet included in selection indexes in beef cattle in the United States.

Health traits may be improved through selection as genomic susceptibility to viral, bacterial and parasitic diseases is well documented (Bishop et al., 2011). The identification of loci associated with enhanced resistance to trypanotolerance in N'Dama cattle and mastitis in Holstein cattle are two examples (Sahana et al, 2013; Tal-Stein et al, 2010; Hanotte et al, 2003; Klungland et al, 2001). The availability of high density genotyping assays that cover the genome at relatively inexpensive prices has paved the way for genome enhanced selection. In addition to providing information for selection, the genotyping assays provide a platform to identify causal mutations that are involved in the regulation of gene expression or gene translation. The identification of causal mutations provides an opportunity to better understand the mechanisms of host susceptibility.

Phenotypes

For genomic enhanced selection to be effective, it requires that a definition of the trait be standardized in the cattle industry. Selecting a standardized BRD phenotype that allows producers to accurately assess disease status with minimal effort would be ideal. For BRD phenotypes, the trait definition has varied depending on how the cattle were diagnosed or how many times an animal was treated for BRD. These measures have been used because they are easily applied within the industry. Currently, a standardized phenotype has not been adopted by

the cattle industry to identify BRD, but BRD assessments have been proposed that include scoring systems, ultrasonography and auscultation.

For genomic studies, scoring systems and the use of treatment records have been used to define BRD cases. These scoring systems were initially designed for dairy calves and attribute severity of symptoms to a score, so that a high score characterizes cattle with BRD clinical signs. The Wisconsin calf health scorer scores each of the BRD clinical signs with a value of zero to three and sums them together to reach a cumulative score. The clinical score is based on rectal temperature, presence of spontaneous or induced cough, nasal discharge, and the greatest of the score based on ocular discharge or head and ear position. The maximum cumulative score is 12 and the minimum score is zero and a case is defined as an animal with scores ≥ 5. A validation of this method was recently completed where the sensitivity and specificity of identifying BRD calves was 62.4% and 74.1%, respectively (Buczinski et al, 2015). The Wisconsin calf health scorer has also been applied to cattle in feedyards to identify cattle with BRD at the producer level (Neupane et al, 2018; McGuirk, 2008; McGuirk, Peek, 2014). When compared with detecting lung consolidation, the Wisconsin calf health scorer performed better than lung auscultation, but not as well as thoracic ultrasound. Thoracic ultrasonography had an increased sensitivity (79.4%) and specificity (93.9%) but its direct use in a producer setting to detect BRD cattle is limited (Buczinski et al, 2015).

Three scoring systems have been tested that use similar clinical signs as the Wisconsin calf health scorer but score the clinical signs as present or absent (with the exception of nasal discharge which has three levels in one scoring system) and have removed induced cough as a measure of clinical symptoms and added respiratory quality (Love et al, 2013). The three scoring systems proposed by Love and colleagues (2013) performed similarly in their ability to detect cases and controls and agreed (kappa > 0.8) with the Wisconsin calf health scorer.

Genome-wide association analyses

Genome-wide association analyses (GWAA) are used to identify genomic regions associated with cattle performance without limiting investigations to genes whose functions have been characterized. The analyses compare the allele and genotypic frequencies of cattle with BRD and those without BRD to identify regions that have large genetic effects on the susceptibility of the disease. Genome-wide association studies have been performed on both beef and dairy cattle to identify genomic regions associated with BRD susceptibility.

Lung lesions

Bovine respiratory disease results in damage to the lungs that may present as lesions at the time of harvest. The use of lung lesions as an indication of BRD in a case – control design for a GWAA has been performed in two studies in the United States, and one study in Israel. The first United States study consisted of pooled samples where each pool comprised 96 animals. Sixty pooled samples of cattle with lung lesions (cases) and 60 pooled samples of cattle without lung lesions (controls) were evaluated with an Illumina BovineHD BeadChip (Keele et al, 2015). Associations were identified by using a 5% genome-wide error rate threshold ($P \leq 1.49 \times 10^{-7}$) or a false discovery rate (FDR) of 5% ($P < 5.38 \times 10^{-6}$). The cattle in this study consisted of a “natural” cattle population, where cattle did not receive antibiotics, hormones or animal byproducts and were

traceable to place of birth, and a "conventional" population where cattle may have received antibiotics, hormones, animal byproducts and were not traceable to place of birth. Lung lesions were present in greater than 30% of cattle in the natural population while 9.8% of those in the conventional cattle population had lung lesions (Keele et al., 2015). Fourteen SNPs were associated with lung lesions at the 5% genome-wide error rate ($P \leq 1.49 \times 10^{-7}$) and 85 loci were associated with a FDR of 5% ($P \leq 5.38 \times 10^{-6}$; Table 1; Keele et al, 2015).

The second study performed in the United States consisted of 920 steers that were also enrolled in a "natural" cattle feeding program (Kiser et al, 2017). Half of these cattle were clinically diagnosed with BRD and removed from the natural program to receive treatment. Lung lesions were evaluated as described by Tennant et al (2014). Pseudo-heritability estimates for BRD as measured by lung consolidation was 0.25, 0.0 for the presence of fibrin tissue in the lung, 0.28 for the presence of lung consolidation and fibrin in the lung and 0.13 for hyperinflated lungs (Kiser et al, 2017). Four loci were associated ($P < 1 \times 10^{-5}$) with lung consolidation, three loci were associated with lungs that contained both consolidation and fibrin tissue and ten loci were associated with hyperinflation of the lungs (Table 1). Lung lesions were identified in greater than 60% of lungs of cattle irrespective of whether the cattle showed clinical signs of BRD (Kiser et al, 2017). None of the associations identified by Keele et al, (2015) with a 5% genome-wide error rate were identified as associated in the Kiser et al, (2017) study, but both studies identified a locus on BTA4 at 102 Mb associated with lung lesion (FDR < 0.05) and hyperinflated lungs ($P < 1 \times 10^{-5}$).

Scoring of lung lesions in Holstein male calves at harvest in Israel partitioned cattle into High (Glatt Kosher) and Low (Non-Kosher) groups (Lipkin et al, 2016). A "Kosher" animal is one in which the lung is intact after all lung lesions are removed. "Glatt Kosher" refers to animals that have no lung adhesions and make up the High group. In contrast, "Non-Kosher" (Low group) cattle have lungs with torn adhesions or that are not intact after lesions are removed. Twenty-one to thirty-one male Holstein calves formed five High and two Low groups, which were pooled for genotyping with the Illumina BovineHD BeadChip. Nineteen regions were identified as associated ($P < 2.5 \times 10^{-1}$) with Kosher status or the presence of lung lesions (Lipkin et al, 2016; Table 1). None of the loci associated with Kosher status were shared with the two lung lesion studies in beef cattle in the United States.

Clinical disease

Two GWAA have been performed using a case-control clinical BRD design to identify susceptibility loci with the Wisconsin calf health scorer system. Pre-weaned Holstein calves were sampled from California or New Mexico and a GWAA performed using the Illumina BovineHD BeadChip. Four analytical approaches were used and the most significant loci from each analysis were evaluated for concordance (Neibergs et al, 2014). Concordant results were identified for 373 SNPs in the California population consisting of 2014 calves, 370 SNPs in the 767 New Mexico calves, and 324 SNPs when the two populations were combined. The ten loci concordant across all four analysis with the strongest evidence for an association are shown in Table 2 (Neibergs et al, 2014). A second study using the same animals imputed the BovineHD BeadChip genotypes to whole genome sequence data to identify and refine the loci associated with BRD (Hoff et al, 2019). After filtering of variants, over 9 million imputed variants were evaluated with an average imputation accuracy of 84.2% (Hoff et al, 2019). The data enhanced the HD analysis by refining

the loci previously identified and increasing the proportion of variance explained by the SNP variants. Of the 100 most significant QTL in the California and New Mexico populations, 11 were shared (Table 2; Hoff et al, 2019). The differences in loci identified in the California and New Mexico populations was most likely due to genetic heterogeneity, or the host response to the different pathogens responsible for BRD identified by bacterial and viral diagnostics of the calves in the study (Hoff et al, 2019; Neibergs et al, 2014). This suggests that genomic prediction models may perform poorly in different environments where the pathogen profiles for BRD differ. Additional studies of BRD pathogen profiles in different environments need to be conducted to assist in improving the accuracy of genomic prediction for BRD susceptibility.

A third GWAA of 45,425 SNP were used to analyze 67,289 animals that had genotypic and BRD treatment data using a single-step BLUP method taken from 326 dairy producers in the United States (Gonzalez-Pena et al, 2019). In this cohort, the incidence of BRD was 21% in calves from birth to one year of age, with most cases occurring prior to 30 days of age (Gonzalez-Pena et al, 2019). The mean reliability (accuracy) for prediction for respiratory disease was 0.419 with a range of 0.189 to 0.99. Predicted transmitting ability (PTA) for respiratory disease is the prediction of the susceptibility of the offspring to respiratory disease expressed as a deviation from the mean. The PTA of bulls with a minimum of 100 offspring in the 326 dairies identified bulls with large differences in the PTA for BRD susceptibility. Bulls with the worst PTA (high values) for respiratory disease reported up to 50.4% of their progeny were affected by BRD (Gonzalez-Pena et al, 2019). Selection of bulls with low BRD PTAs would be beneficial in reducing the incidence of disease with similar levels of management. This study formed the basis for the wellness trait testing commercially provided by Zoetis and so no descriptions of the loci associated with susceptibility to BRD were provided.

Conclusions

Host susceptibility to BRD has been studied in beef and dairy cattle and it has been established that genomic variation plays a role in disease incidence. Heritability estimates suggest that selection for enhanced resistance to BRD is possible, and GWAA have identified loci and genes associated with disease. The identification of loci and genes associated with BRD provides the possibility to use genomic selection to reduce disease incidence and to better understand the host mechanisms associated with disease susceptibility.

Table 1. Genome-wide association analysis of cattle that had lung lesions as an indication of susceptibility to bovine respiratory disease.

Phenotype	**Associated Loci Chromosome (Mb)**	**Significance Threshold**	**Reference**
Lung lesion	2 (128), 3 (8, 60), 4 (31, 96), 9 (72),11(62), 14 (0), 15(67, 16),22 (59), 24 (44), 25 (26, 34)	$P \leq 1.49 \times 10^{-7}$	Keele et al, 2015
Lung lesion	85 loci	$P \leq 5.38 \times 10^{-6}$	Keele et al, 2015
Lung consolidation	11 (96), 14 (64), 18 (44), 20 (70)	$P < 1 \times 10^{-5}$	Kiser et al, 2017
Lung consolidation and fibrin	8 (81), 14 (64), 28 (0)	$P < 1 \times 10^{-5}$	Kiser et al, 2017
Hyperinflated lungs	1 (49), 2 (45), 3 (112), 4 (102), 5 (30), 6 (72), 8 (0), 11 (0), 15 (47), 23 (7)	$P < 1 \times 10^{-5}$	Kiser et al, 2017
Glatt Kosher	1 (32, 92, 136), 2 (103, 111, 112, 114), 8 (41), 9 (103), 10 (55), 12 (7), 15 (2, 36), 16 (38), 18 (58), 22 (55), 24 (27), 26 (43), 29 (30)	$P < 2.8 \times 10^{-1}$	Lipkin et al, 2016

Table 2. Genome-wide association analysis of susceptibility of cattle to clinical bovine respiratory disease.

Phenotype	**Associated Loci Chromosome (Mb)**	**Reference**
Wisconsin calf health scorer[3]	**[1]**15 (30, 14), 14 (63), 3 (119), 23 (3), 19 (9), 6 (40, 42), 4 (47), 14 (11)	Neibergs et al, 2014
Wisconsin calf health scorer[3]	**[2]**1 (147), 6 (11, 13), 13 (70), 15 (60.2, 60.7), 17 (71), 19 (9), 27 (22), 28 (11), 29 (21)	Hoff et al, 2019

[1]Top ten most significant loci identified in each study;[2]Eleven loci shared across two pre-weaned Holstein calf populations; [3]SM McGuirk and SF Peek, 2014.

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Improving Resistance of Cattle to BRD Through Genomics

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Abstract

Bovine respiratory disease (BRD) is of considerable economic importance to the dairy industry, specifically among young animals. Several studies have demonstrated that BRD has a significant genetic component, with heritabilities ranging from 0.04 up to 0.22, which could be utilized to select more resistant animals. Taking advantage of available genomic data will allow more accurate genetic predictions to be made earlier in an animal's life. Availability of genomic data does not negate the necessity of quality phenotypes, in this case, records of BRD incidence. Evidence has shown that genetic selection is possible through the use of producer-recorded health information. The national dairy cooperator database currently has minimal records on respiratory problems. There is an existing pipeline for this data to flow from events recorded by producers on the farm into the national database used for genetic evaluations. Additional data could also be collected through the expansion of currently utilized termination codes and used in conjunction with records of direct health events. Selection for animals with improved BRD resistance is possible at the national level; however, collection of additional phenotypes remains a significant hurdle.

Introduction

Up until the 1990s, selection of dairy cattle in the U.S. placed strong emphasis on improving yield traits. While selection emphasis was only on production, undesirable trends became apparent with functional traits, such as reproduction and health (Rauw et al., 1998). Since this antagonistic relationship was identified, emphasis has shifted away from solely increasing profit through increased production. Economic indices now consider decreased management costs from superior fertility and disease resistance in addition to income from production. Beginning in 1994, productive life was included into the Net Merit $ selection index (NM$), allowing producers to include considerations of overall health. Figure 1 depicts an example of the negative trend in a reproductive trait, in this case daughter pregnancy rate (DPR), occurring concurrently with improvement for production, in this case milk yield, through the early 2000s. Beginning in 2003, this trait was incorporated into the Net Merit $ selection index, with a reversal in the trend beginning shortly after that (VanRaden and Seykora, 2003). Since the early 2000s, there has been improvement in both milk yield as well as DPR. This illustrates that it is possible to improve functional traits while also continuing to improve production.

The economic considerations of dairy production are becoming increasingly important. In 2018, dairy farmers received the lowest milk payments since 2009, continuing four years of poor dairy prices (Geiger, 2019). Animals that are not healthy or have poor reproductive performance cost producers due to increased management costs (e.g., increased handling, veterinary treatments), decreased production, and possible replacement costs. Treatment for BRD can require the use of antibiotics, which results in additional cost for the producer, as well as potentially contributing

to concerns regarding antibiotic usage. Pre-weaning calf diseases, one of the most common being respiratory problems, have been associated with increased risk of morbidity prior to first calving, increased age at first calving, and decreased lifetime profitability (Henderson et al., 2011). Heifers experiencing BRD are also less likely to complete their first lactation (Bach, 2011). While societal pressures from consumers are increasing for animal handling and welfare, a reduction in the incidence of BRD will benefit multiple aspects of dairy production.

Incidence of BRD varies depending on factors including the population and how the trait is defined. The 2014 U.S. National Animal Health Monitoring Survey (NAHMS) reported that the incidence of weaned heifers reported with respiratory problems was 5.1%, however, the overall percent of cows affected by respiratory problems as reported by producers was 2.8% (USDA, 2018). Respiratory events are not a focus in adult dairy animals since they are not as common as mastitis, metabolic diseases, or lameness (Norström et al., 2001). The current incidence rate across cows of parities 1 to 5 is approximately 1% using data currently available in the cooperator database available at the Council on Dairy Cattle Breeding (CDCB; Bowie, MD). This is similar to that reported previously using similar data (Parker Gaddis et al., 2012). These data clearly indicate the need for heifer records, as the incidence rate (0.1%) and event count (n = 441) are much lower compared to cow records, while respiratory events are expected to be more common among young animals.

Genetic improvement of health

Genetic selection is one solution to improve the health of dairy animals. It is possible to select animals that have a favorable combination of alleles that are both positive for production traits and positive for health or disease resistance. The caveat to this is that disease resistance traits typically have low heritabilities. Heritability indicates the proportion of an animal's phenotype – the observable trait - that can be attributed to its genetics. An animal's phenotype can be very broadly defined as the sum of the genetic component and the environmental component. A low heritability indicates that the portion of the phenotype controlled by the animal's genetics is small compared to that controlled by non-genetic factors, such as management or the environment. Despite this, genetic improvement is an attractive solution because the gains are cumulative and permanent. A producer that selects for improved disease resistance for several generations will not immediately lose the progress made if that strategy is discontinued. The same cannot be said for changes in management or nutrition: if a herd reverts from a specific nutrition protocol, any improvement obtained will likely be lost once that protocol is discontinued.

Previous work has shown that selection for low heritability health traits is possible. The best example of this strategy is from Nordic countries, where selection for mastitis resistance has occurred since the 1980s with positive results documented (Philipsson and Lindhe, 2003). The recording of health event data is mandated, making the collection of data less of a hurdle compared to the United States. The inclusion of other lowly heritable functional traits in selection strategies has shown to slow or reverse unfavorable trends. An example of this is shown in Figure 2 with sire calving ease, which was included in the Net Merit indices beginning in 2006 (VanRaden and Multi-State Project S-1008, 2006). Selection of direct health traits that are most common in

dairy herds such as clinical mastitis and metritis has shown to be feasible in the U.S. through the use of event data recorded on farms by producers, especially since the introduction of genomic selection (Parker Gaddis et al., 2014; Vukasinovic et al., 2016). Genomic selection results in more rapid genetic improvement by decreasing the generation interval and increasing the accuracy of predictions. It does this by taking advantage of associations between a trait of interest and many genetic markers spread throughout an animal's genome. These genetic markers can be identified at birth, thus reducing the time required to estimate an animal's genetic value for the trait.

Beginning in April 2018, CDCB released genomic evaluations for six common health events that provide U.S. dairy producers with genomic evaluations for resistance to milk fever, displaced abomasum, ketosis, mastitis, metritis, and retained placenta (Parker Gaddis et al., 2018). These traits have similar heritabilities to those estimated for BRD among dairy calves. Henderson et al. (2011) estimated a heritability of 0.09 using data collected from Holstein calves in New York state. A heritability equal to 0.04 was estimated using data from Holstein heifers in Ontario (McCorquodale et al., 2013). Significant incidences of BRD are not common among Norwegian Red dairy calves, however, a heritability of 0.05 has been estimated (Heringstad et al., 2008). A case/control study conducted with Holstein calves at two locations (California and New Mexico) estimated heritabilities ranging from 0.13 to 0.21, depending on the population. A study conducted using records of heifer respiratory problems reported by producers on-farm estimated a heritability ranging from 0.04 to 0.10, depending on the time frame used after birth (Vukasinovic et al., 2018). The above selected studies all indicate that genetic improvement through selection of animals more resistant to BRD is feasible.

Phenotypes critical

Accurate genomic predictions cannot be estimated without a large number of phenotypic records. This is even more important for lowly heritable traits such as BRD. A data pipeline for reporting respiratory problems in dairy animals already exists. This is the same pipeline utilized for the previously mentioned six health traits that are currently evaluated. Data are recorded with on-farm herd management software and flow through the Dairy Herd Improvement (DHI) system. It is ultimately included in the dairy cooperator database maintained by CDCB. The data are sent to CDCB as "Format 6" records (https://redmine.uscdcb.com/projects/cdcb-customer-service/wiki/Format_6) from the dairy records processing centers (DPRC). Format 6 can be used to submit all health events (up to 20 in a single record) that a cow experiences throughout a lactation. Uniform abbreviations are currently available for twenty different events (e.g., MAST for mastitis, RESP for respiratory problems) and four management traits (e.g., BCS for body condition score). Standardization from the acronym used on farm to that accepted in a Format 6 record is performed by the DRPC. An animal with BRD can be indicated in Format 6 with the health event acronym "RESP." In reporting a respiratory problem in Format 6, there is also the availability to report specific scores for rectal temperature, cough, nasal discharge, eye discharge, and ear tilt following the McGuirk scoring system (McGuirk, 2008) if producers collect those details, however these additional details are not currently required.

Currently the CDCB cooperator database includes approximately 11,000 health events described as a respiratory problem. These events occurred from 2005 to present in heifers and cows

through ninth parity. This underlines the primary impediment to having a national genomic evaluation for BRD resistance, which is having sufficient data. This is likely partially due to the fact that there has not been a strong emphasis on calf records. Compared to milk fever, which has the fewest records of the six health traits currently evaluated, RESP has approximately 25% the amount of records. As producers begin or continue to collect respiratory event data and the dataset increases, it is very feasible to develop a genomic evaluation for BRD resistance, given the significant genetic component. Evaluations for BRD resistance would allow dairy producers to select animals genetically more resistant to developing a respiratory problem. Recording incidences of health events not only benefits genetic evaluations. Having a good recording system for health events can be used to improve management aspects. This benefits the "environment" component of phenotypic expression, which can be especially important for lowly heritable traits.

In addition to direct health event records, additional information routinely flows through the DHI system that could be exploited and/or expanded in order to help producers reduce the incidence of BRD. Termination codes are reported to indicate why a cow left the herd and included in test day milk records sent to CDCB. Currently these reasons include being sold for dairy purposes, being sold for problems such as locomotion, poor production, or mastitis, or dying on the dairy (available at https://redmine.uscdcb.com/projects/cdcb-customer-service/wiki/REFERENCES#Ref10). Termination codes to include reasons for termination in calves and heifers have been proposed as shown in Table 1. Dairy calf death losses were estimated at $327.3 billion in 2015 (Lombard et al., 2019). Common health events that are encountered in calves and heifers could be incorporated to increase available data for events such as BRD. One system was recently proposed by Lombard *et al.* (2019). Collection of this data could aid in providing benchmarks for the dairy industry, and potentially be included with data of specific health events for genetic evaluations.

Despite the limited data available, there does seem to be association between animals with recorded respiratory problems and reported termination codes compared to their contemporaries. Among animals with RESP events reported, 14% had a termination code indicating that they died on the farm, compared to 3.9% among contemporaries not recorded as having a RESP event. This may be biased by the fact that a producer is more likely to record a health event if it is more serious and an animal with a more serious health problem is less likely to survive. Further analysis is warranted as additional data become available, however, it is an initial indication that the termination codes could be useful in conjunction with the direct health records.

Conclusions

Multiple studies have indicated that BRD in dairy calves has a significant genetic component that could be exploited to select animals that have increased resistance. This strategy has the benefit of producing permanent and cumulative improvement. The challenge remains that health traits such as BRD resistance are lowly heritable and largely influenced by non-genetic factors. Thus, progress will be slow. Genomic data can aid in reducing the time required to make genetic progress compared to traditional selection. A large collection of phenotypic BRD incidence data

is still required to utilize the benefits of genomic selection. This remains a hurdle in providing national evaluations for BRD resistance to dairy producers. The pipelines are currently in place that would facilitate providing these evaluations, however, the emphasis should be placed on increasing the amount of data available.

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Table 1. Proposed termination codes for lactation data sent to the Council on Dairy Cattle Breeding. Heifer refers to a young female, i.e., newborn through one day prior to first calving. Cow refers to a female that calved at least once.

Primary (or secondary[1]) disposal coding for females		
Destination group	**Descriptive reason**	**Term. Code**
Remaining in herd	Cow lactation that ended normally without an abortion (**for cow use only)	0
	Pregnancy terminated with an abortion between 152 days after conception and: 259 days for Brown Swiss, 257 days for Guernsey, and 251 days for other breeds.	8
Remains alive for dairy purposes, but left this herd	Female transferred or sold to either a calf rearing facility, another dairy or an embryo center.	2
Sold for slaughter (include removed from herd for on-farm consumption); or Died on the dairy or had to be euthanized. If using as died along with a descriptive reason, code 6 should be put in the primary disposal position and the descriptive reason code should be placed in the secondary disposal position.	Locomotion problems (feet, legs, lameness, crampy)	1
	Low milk or component yield (not caused by other reasons) (**for cow use only)	3
	Reproductive problems (not from parturition of dam)	4
	Died on the dairy or had to be euthanized	6
	Mastitis or high somatic cell score	7
	Udder problems (udder conformation or udder injury)	9
	Unfavorable phenotype (e.g., unfavorable conformation, congenital defect) or genomic prediction	A
	Undesirable temperament (aggressive behavior)	B
	Diarrhea (scours)	D
	Other gastrointestinal problems	G
	Injury (e.g. from barn scraper), hardware	J
	Navel ill, perhaps causing joint ill (**for calf use only)	N
	Respiratory issues (e.g. pneumonia)	R
	Fresh cow transition problem (normally associated with incidents within 60 days after calving, e.g. displaced abomasum, milk fever/hypocalcemia, ketosis/fatty liver, and/or uterine infection/metritis). (** in addition, a Format 6 health event should be submitted to describe the event that contributed to this departure)	T
	Any other reason (including not specified)	5

[1]Any secondary coding provides only a termination code, not a destination-group code

Figure 1.

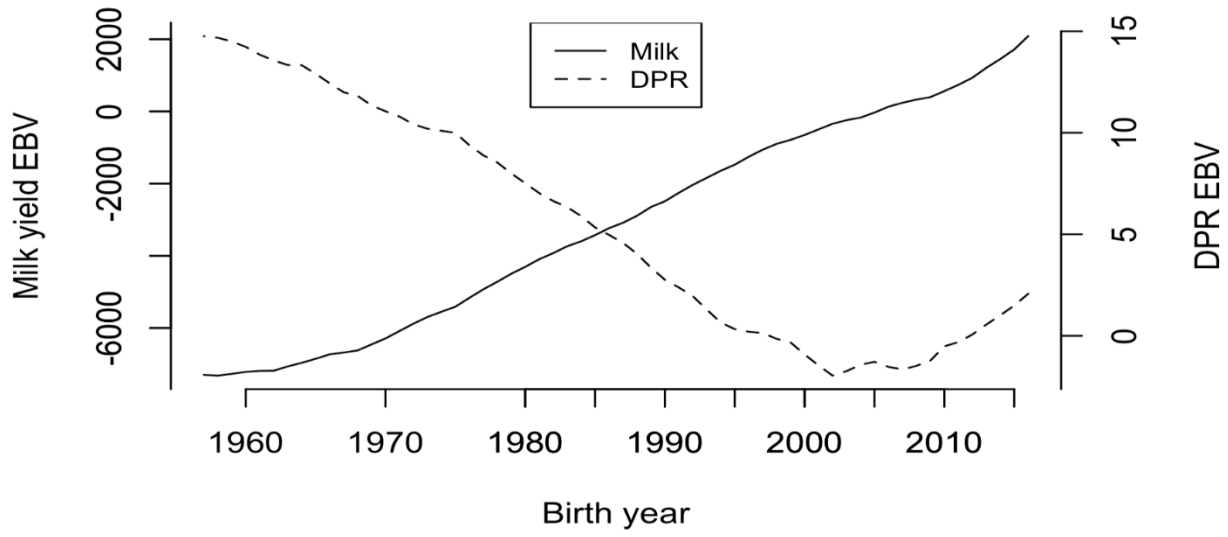

Figure 2.

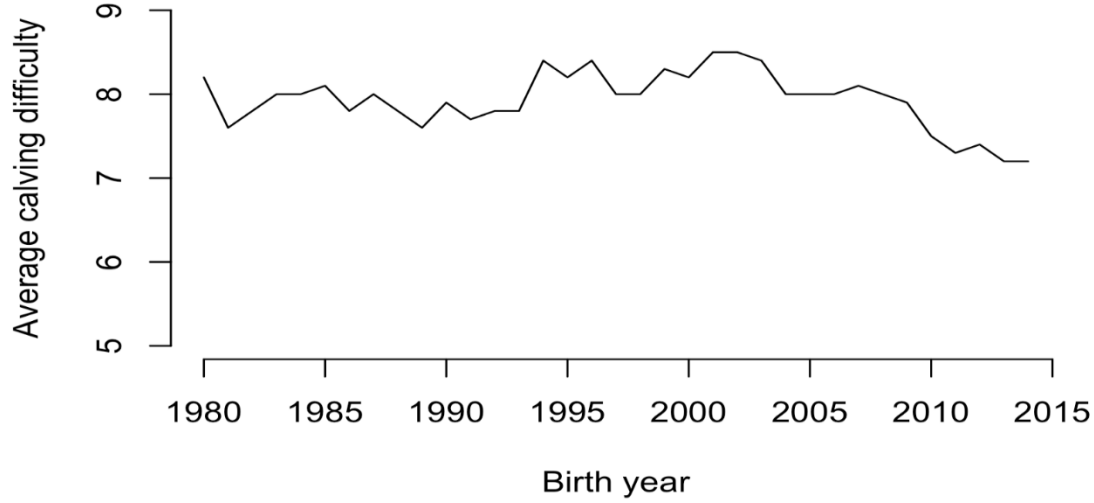

Figure 1: Comparison of trends of estimated breeding values (EBV) for milk yield and daughter pregnancy rate (DPR) from 1957 through 2016 in U.S. Holstein sires (Source: Council on Dairy Cattle Breeding, https://www.uscdcb.com).

Figure 2. Trend of sire calving ease PTA in U.S. Holsteins from 1980 through 2014 (Source: Council on Dairy Cattle Breeding, https://www.uscdcb.com).

Behavior Assessment and Applications for BRD Diagnosis: Dairy Calves

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Abstract

Bovine respiratory disease (BRD) is an important disease in dairy calves due to its long-lasting effects on the animal. Early identification results in better outcomes for the animal, but producers often struggle to identify all calves affected with BRD. Sickness behavior, which is a term used to describe behavioral changes that accompany illness, has been investigated for its usefulness as a disease detection tool. Behavioral changes associated with BRD include decreased milk intake, decreased drinking speed, depressed attitude, and less likelihood of approaching a novel object or stationary human. Behavioral measurements are useful in that they can be collected automatically (e.g. automated milk feeders) or with little financial input (e.g. behavior scores). However, one limitation of many studies that have investigated behavioral changes associated with BRD includes the sole use of either lung auscultation or clinical signs, which are imperfect systems, as the reference method. Additionally, external factors may influence the expression of sickness behavior which can affect if and when behavior can be used to identify calves with BRD. To date, behavioral measures available to detect BRD lack adequate sensitivity and specificity to be the sole means of disease detection, especially when detection tools, such as thoracic ultrasound, have better test characteristics. However, behavioral assessments, combined with other detection methods, can allow for a robust BRD detection program to improve early identification and ameliorate the consequences of BRD.

Introduction

Bovine respiratory disease (**BRD)** prevalence estimates range from 2.5% (Gulliksen et al., 2009) to 39% (Van Donkersgoed et al., 1993). Bovine respiratory disease accounts for approximately one-third of heifer deaths during the preweaning period (USDA, 2010). Immediate effects of BRD include reduced calf growth (e.g. Virtala et al., 1996) and treatment and labor costs (reviewed by Gorden and Plummer, 2010). The effects of BRD can last into adulthood; calves that get BRD are less likely to complete their first lactation (Adams and Buczinski, 2016, Teixeira et al., 2017) and will produce 1,200 pounds less milk in their first lactation (Dunn et al., 2017).

Despite years of research, the number of calves affected with BRD and deaths due to BRD have remained relatively unchanged over the last 20 to 30 years (Gorden and Plummer, 2010, USDA, 2010). On-farm personnel are primarily responsible for the diagnosis and treatment decisions regarding calves (Gordon and Plummer, 2010). Unfortunately, producers have been shown to only identify 25-56% (Sivula et al., 1996, Buczinski et al., 2014, Cramer et al., 2016) of calves that are actually sick. This presents a challenge, as late detection of illness can reduce treatment success and increase the rate of recurrence (McGuirk, 2008).

A potential avenue for improving early BRD identification includes recognizing deviations in healthy behaviors. The behavioral changes that accompany illness, and therefore result in deviations from healthy behavior, are termed sickness behaviors. Sickness behaviors include depression, anorexia, reduced water intake, decreased grooming, and decreased exploratory behavior (Hart, 1988, Haba et al., 2012). These behaviors help conserve heat and energy in order to facilitate the febrile response to infection. The expression of sickness behaviors is thought to enhance the ability of the immune system and inhibit pathogen proliferation (Hart, 1988, Johnson, 2002). In recent years, behavioral changes associated with disease have been investigated for their usefulness in BRD detection.

The objectives of this presentation are to 1) summarize research to-date on behavioral assessment for identifying BRD in dairy calves and 2) discuss limitations of using behavioral assessments.

Behavioral assessments to identify calves with BRD

Automated

The focus of disease detection in calves has primarily centered on using data collected from automated milk feeders, which automatically collect drinking speed, milk consumption, and number of visits to the feeder. In calves on a restricted milk allowance, Svensson and Jensen (2007) observed a 25% decrease in the number of unrewarded (without milk) visits in diseased calves compared to unaffected herdmates. Borderas et al. (2009) observed sick calves fed a high milk allowance drank 2.6 L/d less and had 2.4 fewer visits per day compared to unaffected herdmates. More recently, Knauer et al., (2017) observed that sick calves drank 183 mL/min slower, drank 1.2 L/d less, and had 3.1 fewer unrewarded visits compared to healthy calves. It is important to recognize that the aforementioned studies did not differentiate between disease types in sick calves.

Non-automated

A few studies have investigated behaviors other feeding behavior to determine their usefulness for BRD detection. Dairy calves with clinical BRD were less likely to approach both a novel object and stationary person on the day of BRD diagnosis (Cramer and Stanton, 2015). Additionally, a score that included five behaviors (abnormal posture when lying or standing, isolation from the group, lethargy, and two approach tests that tested the willingness of calves to approach a stationary person) was developed for use on farms who may not have automated feeders (Cramer et al., 2016). Dairy calves with clinical BRD were more likely to have an abnormal behavior score, and therefore exhibited more sickness behaviors, compared to calves without BRD (Cramer et al., 2016).

Limitations

Using clinical signs to define BRD

One limitation with the previously described studies centers on methods used to define BRD. Previous studies used clinical detection tools as the 'gold standard' reference method. The clinical detection methods included visual observations and lung auscultation (Svensson and Jensen, 2007, Borderas et al., 2009) or the Wisconsin Calf Respiratory Scoring chart (McGuirk, 2008, Cramer and Stanton, 2015, Cramer et al., 2016, Knauer et al., 2017) to define BRD. Lung auscultation and BRD scoring systems that rely solely on clinical signs lack sensitivity (Buczinski et al., 2014). Furthermore, clinical signs are transient (White et al., 2012) and have discrepancies among observers (Buczinski et al., 2016). Additionally, the prevalence of subclinical BRD (lung consolidation, but no visible signs of disease), can range from 23 to 67% (Ollivett and Buczinski, 2016), meaning a large population of calves are likely misclassified in previous studies. The limitations of clinical BRD detection methods preclude our ability to accurately identify all calves with BRD in studies, and therefore we are unable to fully grasp the behavioral changes associated with BRD.

Recently, in an effort to address the limitations of solely using clinical scoring in behavioral studies, two studies were performed (Cramer, 2018, Cramer et al., 2019) in which calves underwent twice weekly health exams. Health exams included the Wisconsin Calf Respiratory Scoring chart (McGuirk, 2008) and thoracic ultrasound (Ollivett and Buczinski, 2016). Automated changes in feeding behavior for the 3 days prior and the day of BRD diagnosis were compared among calves with clinical BRD (clinical signs of BRD with or without lung consolidation), subclinical BRD (calves with lung consolidation ≥ 1cm^2 and clinical signs of BRD), and no BRD (Cramer, 2018). Calves with subclinical BRD drank faster than calves with clinical BRD (768 vs. 664 mL/min), and calves with clinical BRD tended to drink slower than calves with no BRD (664 vs. 772 mL/min). There was no difference in drinking speed between calves with subclinical BRD and calves with no BRD (768 vs. 772 mL/min). Therefore, drinking speed may be useful to identify calves with clinical BRD. However, calves with subclinical BRD would not be identified using drinking speed. Additionally, milk intake and number of visits to the feeder failed to differentiate between calves with either type of BRD and unaffected herdmates.

In a companion study to the feeding behavior study described above, Cramer et al. (2019) compared behavioral attitude scores (normal = bright, alert, responsive; depressed = dull but responds to stimulation, slow to stand, or reluctant to lie down) among calves with clinical BRD, subclinical BRD, and no BRD (Figure 1). The attitude score did not differentiate between calves with subclinical BRD and calves with no BRD, suggesting subclinical BRD does not affect the probability of a calf having a depressed attitude.

Sensitivity and specificity of behavioral assessments

Detection methods for BRD should have high sensitivity in order to identify a large proportion of sick calves, as well as a moderate specificity to avoid false-positives. When comparing sensitivity and specificity among studies, it is important to consider the study population and the 'gold-standard' or reference method. Test characteristics are often not reported for behavioral studies, in part because no research has addressed cutoffs for feeding alarms to indicate BRD+ or BRD-. Four behavioral studies reported sensitivities and specificities (Table 1). Overall, behavioral assessments examined thus far for BRD identification have less than desirable sensitivity and specificity.

Sickness behavior is motivational and non-specific

Using behavior to identify calves with BRD is challenging due to the nature of sickness behavior, which is both motivational (Aubert, 1999) and non-specific (Hart, 1988). An animal may or may not be motivated to express sickness behavior, depending on internal or external factors (Aubert, 1999). Factors that affect the expression of sickness behaviors have been investigated in other species and include reproductive status (Owen-Ashley and Wingfield, 2006), environmental conditions (Aubert et al., 1997), social conditions (Lopes et al., 2012), and gender (Avitsur et al., 1997). In calves specifically, milk allowance affected the expression of sickness behavior whereby calves fed 4 L per day displayed fewer sickness behaviors compared to calves fed 12 or more L per day (Borderas et al., 2009), suggesting calves fed less milk were more motivated to drink milk than to express sickness behavior.

Sickness behaviors are also common across species, non-specific, and often associated with fever (Hart, 1988, Haba et al., 2012). Therefore, it is difficult to find behaviors associated solely with BRD, as the behaviors often associated with BRD (e.g. depression, anorexia) can also be indicative of other calf diseases as well.

Conclusion and future directions

The changes that accompany illness represent a potential avenue that can be utilized to improve BRD detection in dairy calves. Currently, behavioral measures available to detect BRD lack adequate sensitivity and specificity to be the sole means of disease detection, especially when detection tools, such as thoracic ultrasound, have better test characteristics. However, behavioral assessments may improve the ability of farm staff to identify disease if no systematic scoring occurs on-farm (Cramer et al., 2016) and may be combined with other detection methods to form a robust BRD detection program which results in earlier detection and mitigation of negative consequences of BRD.

Future research regarding behavioral changes associated with BRD should consider the following: 1) use accurate BRD detection tools as the reference methods, 2) investigate both internal and external factors that change the expression of sickness behaviors in dairy calves, and 4) seek to better understand if and when calves with subclinical BRD exhibit behavioral changes.

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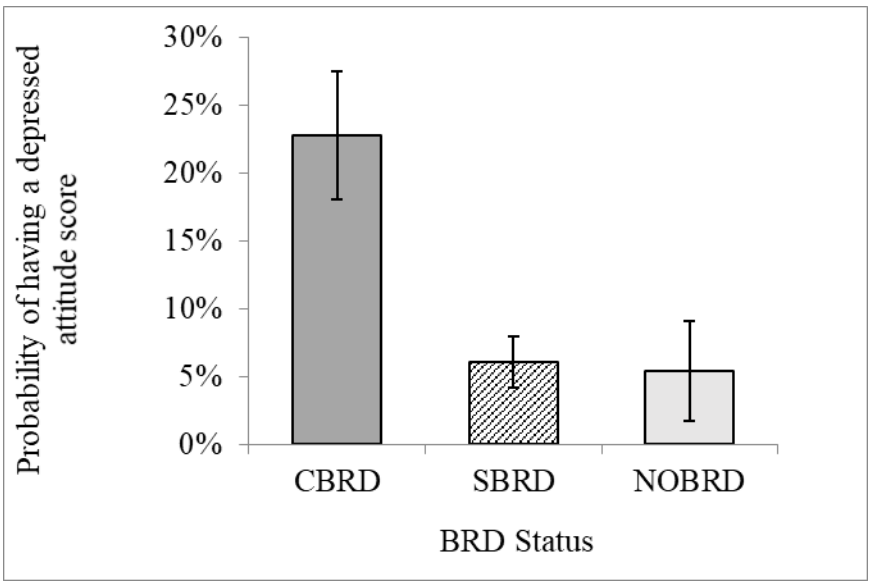
Table 1. Summary of test characteristics for studies that investigated behavioral changes associated with BRD

Study	Behavioral measure	Reference method	Sensitivity	Specificity
Cramer and Stanton, 2015	Novel object approach test	Wisconsin Calf Respiratory Scoring Chart	64%	43%
Cramer and Stanton, 2015	Stationary human approach test	Wisconsin Calf Respiratory Scoring Chart	68%	43%
Cramer et al., 2016	Behavior score, which included abnormal lying or standing posture, isolation from the group, approach tests, and lethargy	Wisconsin Calf Respiratory Scoring Chart	48%	79%
Cramer et al., 2019	Behavioral attitude scores (normal = bright, alert, responsive; depressed = dull but responds to stimulation, slow to stand, or reluctant to lie down)	Thoracic ultrasonography combined with Wisconsin Calf Respiratory Scoring Chart	23%	95%

Figure 1.



Cramer Figure 1 caption

The predicted probabilities (LSM ± SEM) of having a depressed attitude score, by bovine respiratory disease status (BRD status; *P* = 0.0008). Proc Glimmix in SAS (version 9.3; SAS Institute Inc., Cary, NC) was used. Attitude scores were obtained from the Wisconsin Calf Health Scoring App; Normal= Bright, alert responsive; Depressed: dull but responds to stimulation; depressed, slow to stand or reluctant to lie down. We determined the mean age of all calves with subclinical BRD (SBRD) and clinical BRD (CBRD; 31 ± 8 d old) and for the NOBRD calves, selected the attitude score from a health examination day that most closely corresponded with this mean age. BRD status determined from twice weekly health exams, which included a clinical respiratory score (CRS; - or +; calculated with nasal, eye, ear, cough, and rectal temperature scores; McGuirk, 2008) and a lung ultrasound score (0 to 5, based on severity of lung consolidation; Ollivett et al., 2016). BRD status for each calf was defined as CBRD (n = 79; clinical BRD; CRS+ with or without lung consolidation) or SRBD (n = 64; subclinical BRD; calves with any lung consolidation ≥ 1cm^2 and CRS-;), based on the first BRD event. Calves with NOBRD (n = 37) never had lung consolidation ≥ 1cm^2 or CRS+.

Behavior Assessment and Applications for BRD Diagnosis: Beef

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Abstract

Assessment of individual animal behavior is a longstanding strategy to aid in the diagnosis of clinical bovine respiratory disease (BRD) in beef cattle. Cattle with systemic inflammation caused by infectious pathogen(s) and/or physiological stress display predictable behavioral adaptations compared to healthy cohorts. Behavioral alterations in cattle with respiratory infection may include lethargy, social isolation, and anorexia. However, behavior assessment to support BRD case definition in the production setting is challenging for several reasons: 1) other infectious or metabolic bovine diseases cause behavior alterations similar to respiratory infection; 2) cattle have inherent prey instinct to disguise behavioral signs of clinical disease during human evaluation; 3) time and labor constraints in commercial settings dictate very brief observation of individual animal behavior; and 4) traditional behavior assessment is subjective and agreement between observers (pen riders) is often poor. Some of these challenges may be overcome with the use of advanced technologies that allow continuous, non-invasive, and objective behavior assessment of individual cattle. Automated methodologies for behavior assessment in real-time include three-axis accelerometers that quantify physical behaviors, systems that document feeding and watering behavior, and triangulation systems that continuously document spatial behavior within a pen or pasture. Each of these behavior-monitoring approaches generate unique information and have shown promise for early detection of BRD compared to human evaluation. Nevertheless, successful adoption of behavior assessment technologies/systems for BRD diagnosis in beef operations hinges upon improved sensitivity and specificity, positive economic return on investment, and integration within existing BRD management practices.

Introduction

Bovine respiratory disease (BRD) is arguably the most complicated mammalian disease that exists. The pathogenesis of BRD in newly received beef calves is influenced by the segmented infrastructure of the beef production system, social and cultural factors that influence management decisions (or lack thereof) by beef producers, marketing strategies, genetics, environment, stress-induced immunosuppression, and multiple viral and bacterial agents (Taylor et al., 2010). It is also difficult to accurately predict and diagnose BRD in individual beef cattle within a population; it was determined that the sensitivity and specificity of traditional BRD detection methods was 61.8 and 62.8%, respectively (White and Renter, 2009). Diagnostic difficulty in the commercial setting is further demonstrated by previous research that retrospectively correlated lung lesions presented at slaughter with clinical BRD treatment during the feeding period (Wittum et al., 1996; Thompson et al., 2006; Tennant et al., 2014). These studies indicate cattle with gross lung lesions present at slaughter that are never treated for BRD during the feeding period; conversely, some cattle that are treated for BRD during the feeding period have no evidence of lung lesion at slaughter. In the feedlot, where BRD is most prevalent, disease diagnosis is dependent upon human evaluation and the basic strategy of pulling individuals for further evaluation of BRD has not changed in decades. Pen riders are professional field diagnosticians and a critical component of BRD management,

but pen rider availability and expertise are limited. Furthermore, an individual pen rider may be responsible for the daily evaluation of up to 10,000 cattle in a feedlot so observation time of individual cattle or pens must be brief. Advanced technologies such as accelerometers, radio frequency identification, and global positioning systems have potential to enhance BRD field diagnosis, but their practicality in the commercial setting requires positive cost benefit, reliable function in harsh environments, and integration into current health management systems.

Traditional behavior assessment for BRD diagnosis

We have used visual evaluation of cattle behavior for purposes of disease diagnosis for many decades, with questionable success. In the feedlot, the pen rider is responsible for daily monitoring and clinical determination of individual cattle health and the methods for BRD detection have remained relatively unchanged since the advent of commercial cattle feeding. Advantages and disadvantages of traditional visual monitoring for disease diagnosis exist. Perhaps the greatest advantage a pen rider has over any technology is human intuition and the ability to think. Experienced pen riders often comment that a steer or heifer "just doesn't look right" as justification for BRD diagnosis. Although rudimentary, indeed this is often an effective tactic with an experienced eye, but it illustrates the disadvantage of subjectivity and potential disagreement between pen riders. Perhaps depression is the foremost behavior alteration that a pen rider uses in supporting clinical BRD diagnosis. Depression in cattle can be characterized by droopy ears, compact or extended posture, dull eyes, and general lethargy. Biological reasons for depression in BRD affected cattle include energy conservation for immunological processes and indirect effects of the febrile and inflammatory response against infectious agents (Hart, 1988). However, it is known that other diseases including metabolic acidosis result in similar behavioral alterations so it is likely that an unknown proportion of acidotic animals are incorrectly diagnosed as BRD cases in the feedlot (Richeson et al., 2019). The lack of pathognomonic behavior associated with BRD is problematic for both traditional and novel behavior assessment.

It is doubtful that feedlot pen riders will be replaced by technology. If novel behavior assessment provides early and superior sensitivity and specificity for BRD detection, the need for removal of BRD cases from their home pen to a hospital will always require pen riders unless robotic technology for animal handling advances tremendously. Understandably, pen riders may view implementation of novel behavior assessment systems for BRD detection in the feedlot as a threat. Therefore, feedlot managers and consulting veterinarians will be required to inform pen riders of their continued importance in feedlot health management for novel behavior assessment to be successfully implemented in the production setting.

Novel behavior assessment for BRD diagnosis

Currently, there are three primary technological options to assess behavior in beef cattle and each method has potential to detect BRD early. The available systems include: 1) three-axis accelerometers that quantify physical behaviors such as steps, standing/lying time, rumination time, or overall activity index, 2) systems that document feeding and watering behavior via RFID detection at the feed bunk and water trough, and 3) triangulation systems that continuously document spatial behavior within a pen, including the ability to locate important features in the pen such as feed or water source and monitor those specific activities. Previous reviews (Theurer et al., 2013; Wolfger et al., 2015; Richeson et al., 2018) outlined the specific functions of each behavior assessment system and highlighted some of

the research conducted. The focus of this review was to provide general discussion on the potential benefits and challenges associated with implementation of novel behavior assessment systems to assist in BRD detection in the commercial feedlot. The novel behavior monitoring systems previously identified offer the benefit of being non-invasive, continuous, and objective, but they will require significant investment and adaptation to use effectively.

Non-invasive monitoring

Monitoring behavior of individual cattle for BRD detection via advanced technology systems offers several advantages over human observation. First, these systems are non-invasive compared to traditional visual observation by a pen rider. Most of the technologies use an ear-tag accelerometer or transponder that communicates activity or location, respectively to a central computer system for further processing of data. Although pedometer devices can better detect differential physical behaviors, they are typically attached as an ankle bracelet and may transiently alter behavior in some cattle until they adapt to wearing the pedometer device. Poor retention and adverse scenarios such as misplacement are more likely for pedometers vs. ear-tags, as many of the commercial pedometers designed for livestock are intended in dairy applications and do not appropriately fit smaller beef calves. Nevertheless, the non-invasive advantage of behavior monitoring technologies is clear. Cattle are more likely to disguise sickness behavior in the presence of a human evaluator because of evolutionary-driven prey instincts, making early or timely BRD diagnosis more difficult for visual diagnostic approaches.

Continuous monitoring

Continuous monitoring afforded by novel behavior monitoring technologies is another advantage over visual observation and has potential to save time and labor in the commercial feedlot if data are managed effectively through an accurate and precise algorithm and complimentary software system. Daily visual evaluation of individual animals in a large feedlot requires extensive time and labor. In production settings, it is typical to house 50 to >250 cattle in a feedlot pen. Large pen populations may overwhelm less experienced pen riders causing false negative or false positive BRD outcomes, but technology can overcome this challenge because each animal is monitored constantly.

The constant logging of behavior data allows comparison of individual animals to the pen average, transient changes in behavior within a given animal, and circadian behavior patterns. Pillen et al. (2013) reported changes in activity index of naturally occurring BRD cases several days prior to clinical diagnosis by pen riders in a commercial feedlot (Figure 1). Tomczak et al. (2019) evaluated average daily active minutes logged by an accelerometer device in high-risk, newly received feedlot calves and reported differences between BRD cases and control calves never diagnosed with BRD (Figure 2). Similarly, circadian differences existed between BRD cases and controls (Figure 2; Tomczak et al., 2019). It is important to note that the activity differences between BRD cases and controls reported by Tomczak et al. (2019) only existed between the hours of 0800 (morning feeding time) and 2000 (cessation of evening activity). This suggests that future behavior monitoring applications for early BRD detection may only require data logging between these hours of increased overall activity, which could reduce the amount of data generated and simplify application of novel behavior assessment systems.

Objective monitoring

Fluctuations in work schedules, social dynamics, weather conditions, and the experience of horse and pen rider affect the efficiency of visual monitoring of behavioral exceptions for BRD

diagnosis across feedlot pens. Technology may offer the advantage of consistent and objective monitoring that is not affected by emotion, environmental conditions, or experience. The value of novel behavior assessment for BRD diagnosis is influenced by the true sensitivity and specificity of BRD diagnosis by individual pen riders and other complicated dynamics that vary from one feedlot to another. It is possible that some of the better pen riders could perform BRD diagnoses at a similar level of sensitivity and specificity as novel behavior assessment systems. In this case, it is difficult to justify the investment cost for novel behavior assessment. However, where I see repeatable benefit with continuous, objective behavior monitoring is in scenarios where multiple lots of high-risk calves are being received over a period of several weeks, such as during the "fall run" in feedlots that typically procure a large number of high-risk cattle. It is human nature and often feedlot SOP to more closely or frequently monitor pens with new arrivals (i.e., <60 days on feed). If novel behavior monitoring is used in a group of cattle through re-implantation or beyond, technology is perhaps more likely to identify late BRD cases within a lot group after primary BRD outbreak has passed because pen rider focus may shift to more recently received groups of cattle.

Implementation challenges

Cost benefit is probably the primary challenge with implementation of novel behavior assessment systems in the commercial feedlot. If BRD diagnostic sensitivity is improved with technology, the BRD morbidity rate (and thus antimicrobial treatment cost) could actually increase for novel vs. traditional BRD detection methods. Therefore, the widespread adoption of novel behavior assessment systems for early BRD detection in the feedlot may hinge on cost savings from improved treatment success (i.e., reduced respiratory relapse rate), better growth performance, and less death loss. Conversely, it is possible that antimicrobial treatment cost savings could be realized for novel systems if specificity is improved. Table 1 displays a theoretical example of economically important health outcomes between novel and traditional BRD detection.

Another implementation challenge is the requirement for individual identification of cattle and behavior data management. To evaluate behavior of individual animals electronically, they must be uniquely identified to effectively use the data for individual treatment decisions. Obviously, this would require feedlots using lot-level identification to convert to more intensive individual animal identification, which requires additional cost investment and management. However, an accelerometer ear-tag may be equipped with radio-frequency identification built-in as part of the cost structure.

Let us briefly visualize how invested use of novel technology for BRD diagnosis in the feedlot might affect pen rider duties. The software system generates a "suspect" list of animals each morning based on behavior deviation and algorithmic (or decision tree) cutoff. The novel behavior system could be complimentary to the pen riders traditional duties because it can be used to enhance their diagnostic decision-making. Health management SOP for a feedlot may or may not dictate that pen riders pull every animal on the daily suspect list, or pull animals that are not identified by the novel behavior assessment system. My opinion would be to require a pen rider to pull all behavior "suspect" animals for further evaluation, but allow pen riders the flexibility to make pulling decisions on their own if a particular animal appears clinically ill and eligible for BRD treatment but was not identified according to the behavior assessment technology. The "suspect" list brings about another challenge; as a former cattle manager, I can imagine some of the responses I might get from a pen rider if I gave them a printed list of 200 individually identified cattle scattered across ten or twenty

different feedlot pens that they must find and sort out! Therefore, accelerometer or other devices used for behavior assessment and early BRD detection in the commercial feedlot will require a self-contained signaling mechanism such as a flashing light to facilitate timely pulling.

Conclusions

Cattle behavior is an important component of clinical BRD diagnosis, and emerging technology may greatly enhance our understanding and application of cattle behavior for animal health management in the production setting. Novel behavior assessment systems provide the benefit of being non-invasive, continuous, and objective but further research is needed to understand their integration into existing health protocols for use as a tool for early BRD detection. The various behavior assessment systems will need to demonstrate positive cost benefit, enhance existing health management protocols, and provide satisfactory retention and reliability in harsh conditions to gain widespread adoption in the commercial setting. Preliminary efforts suggest that continuous cattle behavior monitoring using various technology systems may allow earlier detection of BRD.

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Table 1. Theoretical health and economic outcomes of traditional vs. novel BRD diagnostic systems[1].

	Traditional	Novel	Cost Difference for Novel
BRD morbidity, %	40	55	$375.00
Relapse rate, %	40	30	-$250.00
Respiratory mortality, %	5	3	-$1,600.00
Novel system cost/animal, $	-	10.00	$1,000.00
Theoretical ROI, $[2]	-	4.25	$475.00

[1]Assumes sensitivity of 61.8% for traditional (White and Renter, 2009) and 75% for novel in 100 head lot size with average treatment cost of $25.00/animal and average death loss cost of $800.00/animal.

[2]Based on 100 animal lot size and assumes $10.00/animal total investment cost for novel implementation. Example does not consider performance or closeout differences between systems.

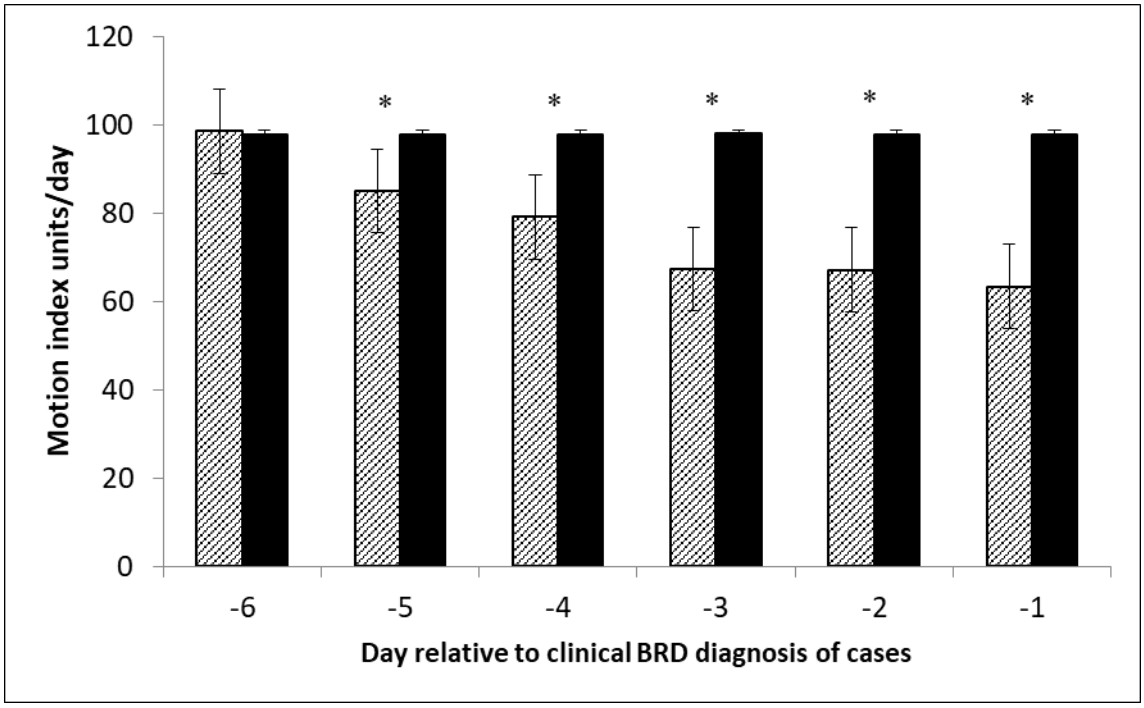

Figure 1. Average motion index units/day for clinical bovine respiratory disease cases (shaded bars) and controls (solid bars) on the day relative to BRD diagnosis in a commercial feedlot. Effect of BRD ($P < 0.001$), day relative to BRD ($P = 0.99$), and their interaction ($P < 0.001$). Adapted from Pillen et al. (2016); Bov. Pract. 50(1):1-8.

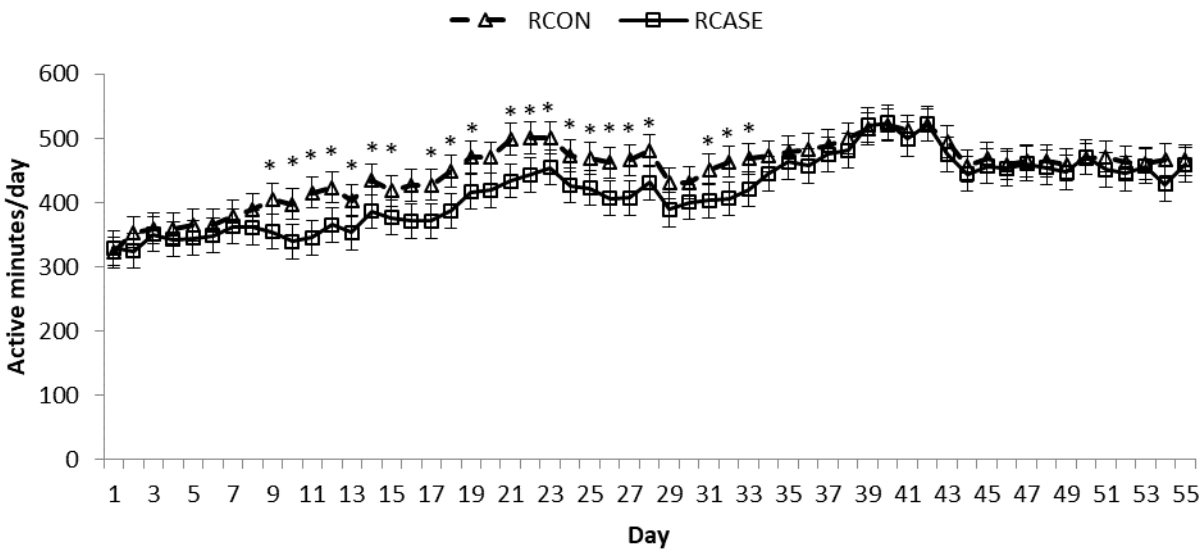

Figure 2. Daily activity differs between calves diagnosed and treated at least once for BRD (RCASE) and those never treated (RCON). Active minutes was generated from an accelerometer collar (Allflex Livestock Intelligence, Madison, WI) with daily means averaged by BRD status from 2 h data logging periods. Effect of BRD, $P = 0.02$; day, $P < 0.01$; and BRD x day, $P < 0.01$. *RCASE differs from RCON within day ($P < 0.05$). Adapted from Tomczak et al. (2019); J. Anim. Sci. 97:2015-2024.

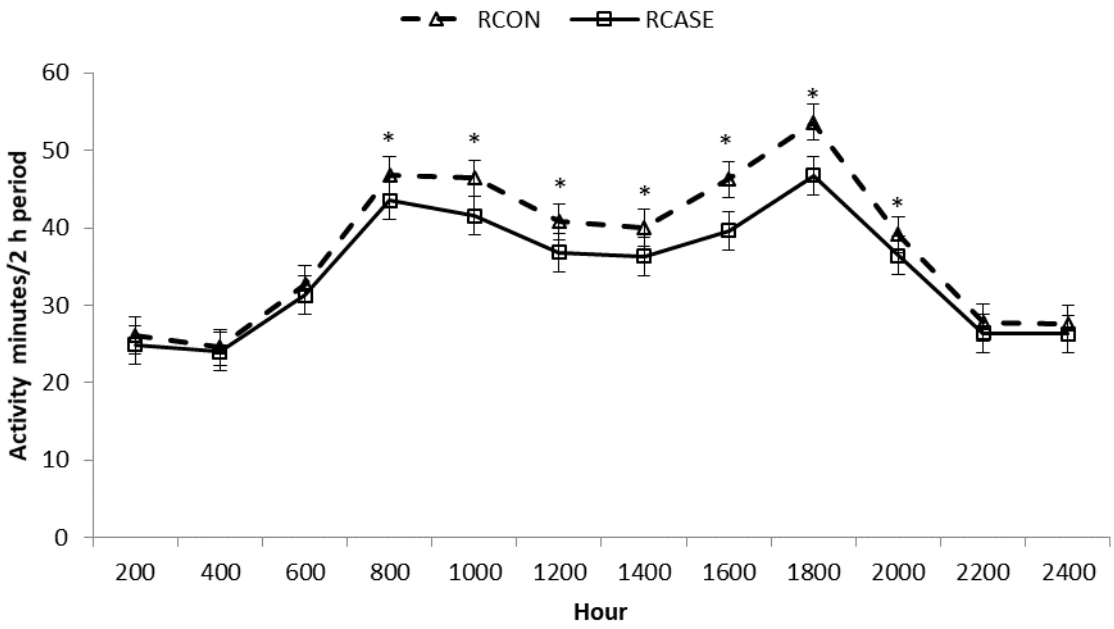

Figure 3. Circadian activity differs between calves diagnosed and treated at least once for BRD (RCASE) and those never treated (RCON). Active minutes was generated from an accelerometer collar (Allflex Livestock Intelligence, Madison, WI) with 2 h data logging intervals averaged by BRD status across the 56-d following feedlot arrival. Effect of BRD, P = 0.71; hour, $P < 0.01$; and BRD x hour, P = 0.04. *RCASE differs from RCON within hour ($P <$ 0.05). Adapted from Tomczak et al. (2019); J. Anim. Sci. 97:2015-2024.

Antimicrobial Resistance in Bovine Respiratory Disease Pathogens: Prevalence and Impact

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Abstract

Bovine respiratory disease (BRD) is the most common cause of morbidity and mortality in North American beef cattle. In recent years, isolation of strains of *Mannheimia haemolytica* that are resistant to multiple different classes of antimicrobials has become commonplace. New research would suggest that the routine use of antimicrobials by some cattle operations might be driving emerging resistance patterns, with the majority of the spread observed due to propagation of specific clones of *M. haemolytica* that harbor integrative conjugative elements (ICE). To date, there is little information evaluating the impact of antimicrobial resistance on clinical outcome in cattle with BRD.

Introduction

Bovine respiratory disease (BRD) is the most common and costly disease affecting beef cattle in North America (Magstadt et al., 2018). Within feedlots, BRD is responsible for approximately 75% of all morbidity and 50% of all mortality. In stocker calves, BRD occurs at a much greater frequency than what is commonly seen in feedlot cattle and is estimated to be responsible for 90% of all morbidity and mortality in these operations. While multiple factors play a role in the development of BRD, bacteria, particularly *Mannheimia haemolytica*, are ultimately responsible for the clinical signs observed in affected cattle. For this reason, antimicrobials are a mainstay of BRD therapy. Unfortunately, antimicrobial resistance is an emerging issue in BRD pathogens and isolation of multi-drug resistant (MDR) strains of *M. haemolytica* has become a more frequent occurrence. The purpose of this manuscript is to review the literature as it pertains to antimicrobial resistance in common BRD pathogens, particularly *M. haemolytica*, and how resistance might impact the outcome of therapy in cattle diagnosed with BRD.

Resistance defined

Veterinarians are most concerned about clinical resistance, in other words, what is the probability that a specific antimicrobial will effectively treat an animal infected by a specific pathogen causing a particular disease (Apley, 2003). The concept of clinical resistance is based on clinically derived breakpoints developed by the Clinical and Laboratory Standards Institute Veterinary Antimicrobial Susceptibility Testing Committee (CLSI VAST) using the following criteria (Apley, 2003):

1. Range of *in vitro* MICs of an antimicrobial for a representative population of a specific bacterial pathogen.

2. PK/PD parameters established on the basis of the relationship between drug concentration and microbial susceptibility.

3. Results of clinical trials in the target species.

For BRD, the CLSI has approved bovine respiratory disease specific breakpoints for penicillin (broth dilution only), ceftiofur, danofloxacin, enrofloxacin, florfenicol, spectinomycin sulfate, tulathromycin, gamithromycin, tildipirosin, tetracycline (broth dilution only), and tilmicosin (Table 1). With these antimicrobial agents, a susceptible result indicates that the likelihood of treatment success is significantly greater than if the result indicated resistance. It is important to remember, however, that the relationship between antimicrobial susceptibility testing and clinical outcome is not perfect and these breakpoints apply only when the antimicrobial is used according to label directions and the susceptibility testing is performed using CLSI approved methods and interpretive criteria. It is also important to realize that antimicrobial susceptibility testing does not guarantee a specific clinical result in an individual animal. Susceptibility breakpoints attempt to take an *in vitro* test result and extrapolate it to an *in vivo* response and, often times, disease outcome is influenced by factors such as host immune status, variations in individual pharmacokinetic parameters, or increased disease severity/prolonged disease duration. For antimicrobials without CLSI approved breakpoints, the interpretations have been adapted from interpretive criteria extrapolated from plasma and interstitial fluid in other species. Examples of this approach include penicillin G (disk diffusion), tetracycline (disk diffusion), potentiated sulfonamides, aminoglycosides, and erythromycin. For these antimicrobial agents, a susceptible result is certainly better than a resistant one. However, there are no data available to correlate the results of susceptibility testing and expected outcome in cattle with BRD.

The second type of resistance is defined based on data surveilling changes in profiles of susceptibility distributions in wild-type populations of bacteria (Lubbers and Turnidge, 2015). Rather than providing data correlated to clinical outcome, these epidemiologic cut-offs represent deviations of the MIC from the original bacterial population and can be used to indicate the appearance of resistance genes. As a result, epidemiologic cut-offs might declare resistance at an MIC that is different (often lower) than a clinical breakpoint (Lubbers and Turnidge, 2015). For the purposes of this discussion, we will be most concerned about clinical resistance and this definition of resistance will be used throughout the rest of this manuscript.

Antimicrobial resistance in BRD pathogens

The earliest published MIC distributions for *M. haemolytica* established using modern diagnostic laboratory methodology and CLSI approved breakpoints were derived from a survey of animals that died of BRD over a several year period from 1988 to 2002 (Watts et al., 1994). In this study, 461 *M. haemolytica* isolates were submitted by numerous veterinary diagnostic laboratories across the U.S. and Canada (Pennsylvania, Wyoming, Iowa, Washington, California, Missouri, Nebraska, Oregon, Kansas, Arizona, Texas, South Dakota, Montana, Minnesota, Oklahoma, Colorado, Utah, Saskatchewan, Alberta, and Quebec) to an Upjohn laboratory for MIC determination. The results of this study are reported below in Table 2. It is important to note that the interpretive criteria for tilmicosin has changed since this study was published and the prevalence of resistance to this drug would be dramatically decreased (>90% susceptible) using currently accepted criteria.

In another study, the susceptibility of 390 *M. haemolytica* isolates obtained from the lungs of beef cattle that died from BRD and submitted to the Oklahoma Animal Disease Diagnostic Laboratory between 1994 and 2002, was investigated (Welsh et al., 2004). This study found

that the susceptibility to tetracycline and spectinomycin varied over the course of the study period but was consistently low for each drug. In contrast, the susceptibility to ceftiofur and enrofloxacin remained high and relatively stable throughout the duration of the study (Table 3).

A landmark study from Kansas State University evaluated the prevalence of multi-drug antimicrobial resistance and antimicrobial co-resistance patterns in *M. haemolytica* isolated from the lungs of cattle with BRD over a 3-year period (Lubbers and Hanzlicek, 2013). This work showed that, between 2009 and 2011, the proportion of isolates resistant to 5 or more antimicrobials increased from 5% to 35%. In addition, isolates resistant to either oxytetracycline or tilmicosin were significantly more likely to be resistant to at least one other antimicrobial class.

Several studies have investigated the prevalence of antimicrobial resistance in feedlot cattle between feedlot arrival and exit. In one study, samples obtained from 10% of animals from 30% of feedlot pens in two feedlots in southern Alberta were submitted for isolation and susceptibility testing of M. *haemolytica* (Klima et al., 2011). Swabs were collected from cattle at the time of feedlot entry and again within 30 days of feedlot exit. Over the course of the study, 409 *M. haemolytica* isolates were obtained and resistance to all antimicrobials tested was low, ranging from 0.2% to 3.9% with resistance to oxytetraycline being most common (Klima et al., 2011). It should be noted that many of the antimicrobials evaluated in this study do not have CLSI established breakpoints for *M. haemolytica* and BRD, making some of the conclusions from this study difficult to interpret. In another study that sampled nearly 5,500 cattle from 4 feedlots in Canada, deep nasopharyngeal swabs (DNP) were collected from enrolled animals at the time of arrival and again at a time point prior to feedlot exit (Noyes et al., 2015). In this study, susceptibility to 21 different antimicrobials was evaluated for 2,989 individual *M. haemolytica* isolates. Overall, resistance was rare, with 87% of isolates susceptible to all antimicrobials tested (Noyes et al., 2015). As with the aforementioned work, it should be noted that many of the antimicrobials evaluated in this study also did not have CLSI established breakpoints for *M. haemolytica* and BRD, making some of the conclusions from this study difficult to interpret.

In a study from Canada evaluating resistance patterns in *M. haemolytica* isolated from healthy cattle and cattle with BRD, a resistant phenotype was found in 18% of *M. haemolytica* isolates tested (Klima et al., 2014). Overall, resistance was more common in isolates collected from cattle with BRD (32%) than isolates collected from healthy cattle (2%). Resistance to tetracycline was the most common phenotype observed and, generally speaking, if an isolate was resistant to one drug it was also resistant to at least one other antimicrobial class (Klima et al., 2014).

Work from our lab evaluating the prevalence of resistance in *M. haemolytica* after metaphylaxis with the long-acting macrolide tulathromycin has yielded surprising results (Snyder et al., 2017). In this study, lightweight, unweaned calves entering a stocker facility in Northeast Georgia were given tulathromycin at the time of arrival to the facility to prevent BRD. DNP were collected from each animal at the time of arrival and again 10–14 days later. For all antimicrobials except ceftiofur, there was a significant increase in the proportion of isolates classified as intermediate or resistant at the time of second sampling compared to samples collected at arrival (Snyder et al., 2017). Of the 123 calves with *M. haemolytica*

cultured at the time of second sampling, 1 (0.8%) had only pan-susceptible isolates, 30 (24.4%) had at least one isolate classified as intermediate or resistant to two antimicrobial classes (fluoroquinolones and macrolides), and 92 (74.8%) had at least one isolate classified as intermediate or resistant to three antimicrobial classes (fluoroquinolones and macrolides in addition to either phenicols or cephalosporins) (Snyder et al., 2017). Additional work by our group evaluating efficacy and resistance in both enrofloxacin and tulathromycin in high-beef calves produced similar results (Crosby et al., 2018).

Similar work by researchers at Mississippi State University produced comparable results. In this study, DNP were collected from calves at day 0 and then 7, 14, and 21 days after arrival processing and mass medication with tildipirosin (Woolums et al., 2018). In these calves, nearly 100% of *M. haemolytica* isolates collected from calves at 7, 14, and 21 days after arrival processing and exposure to tildipirosin were classified as MDR and were resistant to all drugs tested except ceftiofur (Woolums et al., 2018).

Mechanisms of multi-drug antimicrobial resistance

It is clear that resistance in BRD pathogens, particularly *M. haemolytica*, is on the rise. It is also clear that MDR is becoming more common place as well. The question is then, how does resistance to multiple antimicrobials arise after exposure to only one drug? In *M. haemolytica* the primary driver for the increase in MDR strains is the integrative conjugative element (ICE) (Clawson et al., 2016). Integrative conjugative elements are mobile genetic elements that integrate into the bacterial chromosome and, under the right conditions, transfer to neighboring bacterial cells (Wozniak and Waldor, 2010). These ICEs can carry multiple genes associated with antimicrobial resistance and, with exposure to one antimicrobial drug, the ICE and the rest of the resistance genes carried can be transferred to other bacterial cells (Snyder et al, 2019). Currently, there are two ICEs commonly found in *M. haemolytica* that confer resistance to all of the major classes of antimicrobials commonly used in cattle with BRD (Snyder et al, 2019).

Antimicrobial resistance: impact on therapeutic outcome

First treatment success, defined as the proportion of animals successfully responding to antimicrobial therapy at the time of first pull, has historically been high in most populations in cattle on feed. Generally, a first treatment success risk of > 80% is considered acceptable. However, a recent retrospective study evaluating risk factors for treatment failure found that over 30% of cattle failed to respond to first treatment (Avra et al., 2017). In this study, high-risk calves demonstrated a greater risk of treatment failure than low-risk calves (Avra et al., 2017). Unfortunately, little work has been done to evaluate the impact of antimicrobial resistance on clinical outcome in cattle with BRD. In the one published study that the author is aware of, 62% of cattle infected with susceptible *M. haemolytica* isolates (n=688) responded to treatment with tilmicosin compared to 38% of animals (n=6) with resistant isolates (McClary et al., 2011).

Conclusions

Despite the importance of BRD to the North American cattle industry, there are few well-designed studies that evaluate antimicrobial resistance in bacterial pathogens important to this disease syndrome. The majority of published literature includes diagnostic laboratory submissions obtained from dead cattle that have been treated multiple times with multiple different antimicrobials. Nevertheless, general trends would suggest that a decrease in the susceptibility of *M. haemolytica* has occurred over time. Recent work suggests that antimicrobial use practices that are common within certain cattle operations might be the primary factor driving selection of resistant clones. As a result, it is critical that clinicians working with cattle recognize the importance of antimicrobial resistance in BRD pathogens and how this might affect the treatment efficacy in animals with clinical disease.

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Table 1. Antimicrobial-pathogen combinations with CLSI-approved breakpoints for bovine respiratory disease

Antimicrobial	Pathogens
Ceftiofur	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Danofloxacin	*Mannheimia haemolytica* *Pasteurella multocida*
Enrofloxacin	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Florfenicol	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Gamithromycin	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Penicillin[a,b]	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Spectinomycin	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Tetracycline[b]	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Tildipirosin	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*
Tilmicosin	*Mannheimia haemolytica*
Tulathromycin	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*

[a]Only applies to the procaine penicillin G formulation used at 22,000 IU/kg IM q 24 h
[b]Approved breakpoints only valid for broth dilution

Table 2. Antimicrobial Susceptibility of *M. haemolytica* Isolates Collected from the Lungs of Cattle that Died of BRD.

Organism	# of Isolates	Antimicrobial	% Susceptible
M. haemolytica	461	Tilmicosin	69.1
		Ceftiofur	100
		Tetracycline	57
		Spectinomycin	83.5

Table 3. Susceptibility of M. haemolytica Obtained from the Lungs of Feedlot Cattle from 1994-2002

	Year								
Antimicrobial	**1994**	**1995**	**1996**	**1996**	**1998**	**1999**	**2000**	**2001**	**2002**
Ceftiofur	97	98	100	100	98	100	98	96	97
Enrofloxacin	-	-	-	-	-	96	98	89	98
Florfenicol	-	-	100	96	98	97	96	87	90
Spectinomycin	65	49	71	53	55	63	45	29	51
Tilmicosin	90	78	93	83	80	74	85	71	79
Tetracycline	23	46	74	58	42	63	44	34	54

Responsible Use of Antimicrobials in Bovine Respiratory Disease Management

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Abstract

Responsible, judicious, and stewardship are terms commonly thrown around in discussing antimicrobial use. This presentation doesn't define what I think is responsible use. This presentation clarifies who defines these terms. The first two terms involve evaluation of our decisions and judgements by both our clients and the consumers of the products our clients produce. Stewardship of antimicrobials also involves efforts to avoid the use of antimicrobials by finding other ways to prevent, control, and even treat disease. We have extensive data on the good antimicrobials can do for us in addressing BRD. However, our societal license to produce food products is based on their real or perceived threats to human and animal safety based on our practices. I think "Responsible" is usually predominantly judged by veterinarians on the basis of making the best antimicrobial use decision for our clients when we focus on the veterinary-client-patient relationship. However, the advent of social media, combined with marketing campaigns based on differentiating food products for various attributes has widened the population which judges how responsible we are being with our antimicrobial decisions. While potential selection for resistance remains a nebulous criterion for antimicrobial use decisions, our future decisions will increasingly need to take into account market access for our clients based on precautionary and opportunistic market chain decisions. This abstract almost sounds like resistance is being discussed as just a mythical threat dreamed up to impede our ability to produce food animals; that isn't true, resistance is a real issue for both humans and animals.

Introduction

Words matter. Their definitions matter. An example is the discussion of sustainability. We must define for whom sustainability is desired and by what measures success or failure will be judged. Sustainability next week or next century? Then, who is responsible for assuring sustainability? It seems this R word (responsible) constantly reappears in the context of discussing multiple societal issues.

When antimicrobial use in humans and animals is discussed, common terms include responsible, judicious, and stewardship. Just like discussing sustainability, discussion of these terms needs to start with definitions, move to who is responsible, and end with some type of metric-driven evaluation of how we are doing. Simple, right?

Definitions

Today we can instantly access definitions on-line, just like most any bit of information. For words like responsible and judicious, that is fine. However, stewardship in relation to antimicrobials needs to be defined by those with special knowledge and appreciation for the context in which the definition will be used.

Responsible is defined on macmillandictionary.com with several meanings. One is "someone who is responsible for someone or something is in charge of them and must make sure that what

they do or what happens to them is right or satisfactory". In the case of antimicrobial use for BRD, this still begs the definition of by what standards "right or satisfactory" is judged. Perhaps the second meaning of responsible adds more clarity, "deserving to be blamed for something that has happened". And yet a third meaning, "sensible, reliable, and able to be trusted to do the right thing". Do the right thing by who?

Judicious will be more simply defined, this time on merriam-webster.com. "Having, exercising, or characterized by sound judgement" is their standard for this term. As in all definitions, we need to then define key words used in the definition, this time in determining the appropriate components of the decision process involved in "judgement".

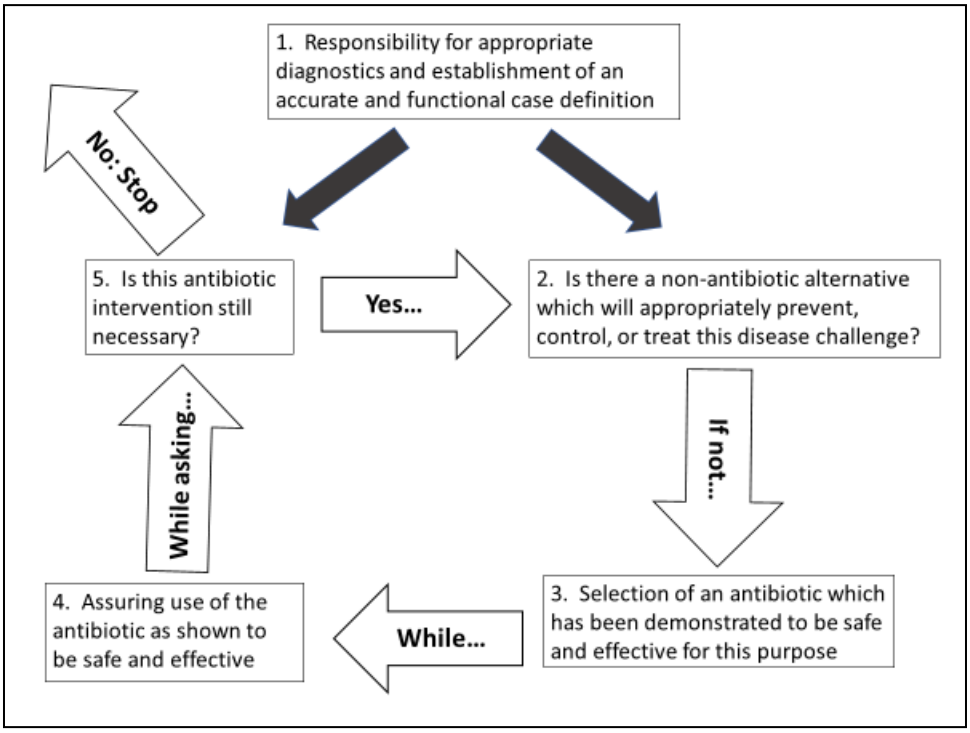
Antimicrobial Stewardship as defined by the American Veterinary Medical Association (AVMA) "refers to the actions veterinarians take individually and as a profession to preserve the effectiveness and availability of antimicrobial drugs through conscientious oversight and responsible medical decision-making while safeguarding animal, public, and environmental health."

The AVMA goes on to define the Core Principles of Antimicrobial Stewardship in Veterinary Medicine. **"**Antimicrobial stewardship involves maintaining animal health and welfare by implementing a variety of preventive and management strategies to prevent common diseases; using an evidence-based approach in making decisions to use antimicrobial drugs; and then using antimicrobials judiciously, sparingly, and with continual evaluation of the outcomes of therapy, respecting the client's available resources."

The AVMA stewardship definition and core principles emphasize the broad nature of antimicrobial stewardship and that the judicious (responsible?) use of antimicrobials is but one part of the overall cycle. Simply put, the process of making sure an effective antimicrobial is used in the most appropriate manner does not, by itself, meet our overall obligation to antimicrobial stewardship.

Figure 1 illustrates the breadth of our responsibilities (see all 3 definitions above) in antimicrobial stewardship for any food animal infectious disease process. The diagnostic and case definition responsibility #1 applies both at initiation of addressing a disease challenge in food animals and consistently throughout the lifecycle of antimicrobial use for a disease challenge. In responsibility #2, we are being asked to evaluate different methods for preventing, controlling, or even treating infectious disease. Responsibilities #3 and #4 constitute the judicious use of an antimicrobial when it is indicated while assuring that the use is as directed. Responsibility #5 is informed by continuous attention to #1. None of these responsibilities exist in a vacuum, and all affect (and inform) the others.

Figure 1: The Antimicrobial Stewardship Cycle in Food Animal Medicine



Social License

I found a definition of social license on Investopedia.com. "The social license to operate (SLO), or simply social license, refers to the ongoing acceptance of a company or industry's standard business practices and operating procedures by its employees, stakeholders and the general public."

Wait, how did we get from a discussion of applying the proper evidence-based medicine approach in support of antimicrobial stewardship in food animal therapeutics to an esoteric discussion of social license? Because, the definitions of responsible and judicious are not necessarily ours to create. Remember that the definition of judicious involves the term "sound judgement".

Sound judgment in the immediate best interest of our clients, and therefore ourselves, involves pulling out the stops to prevent, control, and treat infectious disease by all means possible which make economic sense. We can cover up doubters with efficacy data for using antimicrobials to improve case resolution and decrease mortality in acute BRD cases, and even controlling BRD by administering injectable antimicrobials to all cattle in a group.[1] That meets responsibility #3 in the stewardship cycle, and we can extend it to responsibility #4 with client cooperation. I believe most veterinarians would soundly rebuff a far-away consumer questioning the disease/pathogen/food animal matchup with the appropriate antimicrobial. However, a tiered system of antimicrobial priority and diagnostic criteria for moving to antimicrobials of higher human medical importance are the norm in some countries.

Where the social license is the most evident is in responsibility #2. This responsibility is illustrated by the question "why do you have to use antimicrobials to address this disease"? Or, more clearly, "what is wrong with your production system that this illness is so prevalent"? "What else could you be doing besides using antimicrobials?"

These questions might be discounted as attributed to societal outliers trying to advance nefarious agendas upon food animal producers. Discounted, that is, until the social license starts to be interpreted and enforced by restaurants and food retailers through buying practices coupled with marketing initiatives. If you think that this is a far-fetched, over-reaching application of the social license for antimicrobial stewardship, replace "antimicrobial stewardship" with "animal welfare" then let's circle back through the concept again.

The take-home concept is that antimicrobials are viewed as a societal resource and it is well recognized that by using antimicrobials we can select for resistance in both target pathogens and commensal populations. The nuances of antimicrobial regimen magnitude and duration on antimicrobial selection pressure are varied and often ill-defined. Equally ill-defined is the relationship between bacterial populations in animals, humans, and the environment. However, in this author's opinion, to think that these populations are not related is to deny the basic tenets of bacterial population dynamics and mechanisms of genetic transfer.

The frustration with identifying presence, extent, and effects of links between food animal use and human antimicrobial resistance has resulted in bypassing the need for such evidence by invoking the precautionary principle. The precautionary principle may be defined in multiple ways, but a quite useful definition was published by Ricci and Sheng.[2]

"Precautionary principles are the foundations for policy when it has to deal with weakly understood causes of potential catastrophic or irreversible events, and where protective decisions require certain and costly policy interventions that may not solve the problem that they are designed to correct. These principles provide – when developed by statutes that reflect the intent of the principles – a legal justification for acting, even though scientific causation is either incomplete or perhaps unavailable. The dilemma that those principles create is that the ethical choice underpinning precautionary principles, better safe than sorry, can be costly because an action designed to avoid potential damage can be counterproductive for society by creating other hazards that are incorrectly analyzed."

The reader should be clear that while invoking the precautionary principle through regulatory actions may require statutory support, no such preparation need be made for consumers or food suppliers to utilize this principle in purchasing and marketing. Legislators may also take this approach in their thought processes for introducing and voting on legislation.

Dilemmas

When there appears to be multiple right answers in a problem, you are in the middle of a dilemma. Rushworth Kidder defined the 4 dilemmas, for which any decision encompasses at

least one.[3] When making tough decisions, you are contemplating a mixture of these dilemmas whether you recognize it or not. They are…

Justice vs. Mercy
Truth vs. Loyalty
Short Term vs. Long Term
Individual vs. Community

All of our definitions of "responsible" entail a unique mix of these decisions, especially when we are judging someone else's application of them in decisions which affect or are perceived to affect us. The last two dilemmas in the list directly apply to the subject of this paper. As a profession, we can espouse our commitment to public health (the community) and long-term sustainability of the antimicrobial resources we have, but in reality our immediate duty, and the one also most aligned with our immediate best interests, is to use antimicrobial resources in the best economic interest of our clients and to also address the reality of morbidity, mortality, and the associated animal welfare issues within the production system as it stands that day. To us this seems quite responsible, a reasonable judgment in light of the circumstances.

The external judgment of how responsible our antimicrobial use in food animals is comes from the community, and they are most likely to abandon any scientific assessments in favor of the precautionary principle. When the community feels that the individual is gaining at their expense, then they revoke the social license to operate through laws, regulations, and buying preferences. Addressing the actual science takes too much effort and who can you trust anyway? Raising the animal welfare issue quickly cycles us back to the argument of whether the way we raise the animals makes the use necessary to address welfare and production losses.

Conclusion

Responsible to who? The decision to use an antimicrobial for BRD primarily involves the best interests of the client, the patient, and the veterinarian. The thought of not using an antimicrobial to address BRD based on possibly selecting for resistant bacterial populations which may cause treatment failures in some other setting in some unspecified future time would be unthinkable to many veterinarians.

Perhaps the potential to interfere with future therapy in this specific production setting might have more impact on the definition of "responsible"? Would a veterinarian compromise real or perceived immediate efficacy for the potential to have improved efficacy 10 – 20 years hence? I think the evidence would have to be overwhelming that the immediate use is harmful to future efficacy for this thought process to dominate, and the nature of projecting the resistome of bacterial populations makes a future surety of this relationship very doubtful.

However, a consumer may not think that these are such unreasonable compromises. And if these thoughts are present in consumers, marketers would reasonably be expected to capitalize on them in order to provide the solution; the most simple marketing message is the removal of antimicrobial use rather than trying to communicate the nuances of "responsible", "judicious",

and “stewardship”. Legislators and regulators might also think the potential for harmful effects to be more immediate, and in the best interest of the public and themselves to act.

Our benefit/harm considerations in controlling and treating BRD may in the future be heavily influenced by including potential restrictions on marketing options for our clients, coupled with pricing differentials as well as regulatory environments which are currently state-specific in some cases. Marketing and/or regulatory restrictions might be based on tiered antimicrobial formularies keyed to importance in human medicine (already present in many European Union countries) as well as intense diagnostic requirements before certain classes of antimicrobials may be used (also present in some countries).

“Right and satisfactory” now becomes a balance between immediately addressing animal disease and the need to preserve market access due to documented or precautionary concerns. Responsible use is being defined by an ever-widening population which sits in judgement (remember the judicious definition?) of our actions. The best way to avoid all of these complicated relationships is to avoid the need to use the antimicrobials in the first place. There is nothing I can tell you that will make you alter antimicrobial use practices in the short-term based on potential long-term benefits. Consumers and marketers can. Our best immediate strategy as veterinarians is to minimize ineffective and unnecessary use today, and prepare our clients for a future with less use of antimicrobials for at least prevention and control of disease.

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Abstract Session

1. Effect of an Aerosolized Innate Immune Stimulant on Development of Bovine Respiratory Disease

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We evaluated the effect of innate immune stimulation on development of bovine respiratory disease. A bacterial lysate of killed *Staphylococcus aureus* and *E. coli* was administered by aerosol to calves, and induced an innate immune response within 24 hours that was characterized by transient fever, elevated serum haptoglobin and fibrinogen, increased neutrophils and inflammatory cytokines in bronchoalveolar lavage fluid, and neutrophil infiltration in bronchioles and alveoli. The bacterial lysate or saline control was administered by aerosol to 60 auction-derived high-risk beef calves on the morning after arrival to a feedlot. The calves did not receive metaphylaxis, and were monitored over the subsequent 28 days. Clinical illness requiring treatment developed in 72% of lysate-treated animals and 53% of control animals. Similarly, compared to saline-treated controls, the lysate-treated animals had earlier time to first treatment, reduced weight gains, mildly increased serum haptoglobin and fibrinogen, and higher nasal loads of *Mycoplasma bovis*. Six of the lysate-treated calves died of *Mycoplasma bovis* pneumonia, compared to 1 of the control calves. Thus, the innate immune stimulus given on arrival was associated with increased prevalence and severity of respiratory disease. The effects were further investigated by administration of the bacterial lysate to colostrum-deprived calves 24 hours prior to challenge with aerosolized *Mannheimia haemolytica*. In this model, the bacterial lysate had a partial protective effect, with less severe clinical signs, lower blood leukocyte counts, a lower proportion of consolidated lung at postmortem, but similar numbers of *Mannheimia* isolated from lung. Together, these studies indicate that an inflammatory response in the respiratory tract when stressed beef calves arrive to the feedlot exacerbates BRD, but this adverse outcome was not seen in non-stressed calves challenged with *Mannheimia haemolytica*. These findings challenge the notion that immunosuppression is the mechanism by which stress and viral infection lead to BRD. Instead, the results suggest that increased inflammatory responses (or dysregulated inflammatory responses) are a basis for development of this disease, with implications for disease prevention.

2. The Predictive Value of Using Antemortem Bacterial Culture and Susceptibility Testing to Determine Clinical Outcome of Bovine Respiratory Disease in Feedlot Cattle

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Bacterial culture and antimicrobial susceptibility testing are commonly used diagnostic tests to help determine the bacterial pathogens involved in causing BRD and to select antimicrobial agents for the treatment or control of BRD. The main objective of this study was to determine how closely the results of antemortem bacterial culture and susceptibility testing correlated with clinical outcome in high risk, newly received cattle. Over one thousand heifers were procured from sources with a perceived history of tulathromycin resistance and transported to a research facility in Texas, USA. Within 36 hours of arrival at the research center, all cattle enrolled in the study were processed using standard arrival protocols. A Deep Nasopharyngeal Swab (DNS) was used to collect a sample for bacterial culture. All animals were given an injection of tulathromycin per label (2.5 mg tulathromycin/kg BW) to control signs respiratory disease. After a 7-day post-metaphylactic interval (PMI), animals were eligible for first pull treatment of BRD if they were identified with clinical signs of BRD based on having a Clinical Attitude Score (CAS) of 1 and rectal temperature >39.7°C or a CAS of >2 regardless of rectal temperature. Prior to treatment, all animals were again sampled for bacterial culture and susceptibility testing using the DNS procedure. As well, a nasal swab (NS) was taken for viral PCR testing. Animals were not eligible for additional treatment of BRD for another period of 7 days after administration of tulathromycin. All DNS samples were submitted to a commercial microbiological laboratory and were cultured. Tulathromycin susceptibility was determined using one representative colony from each sample for all *Mannheimia haemolytica* or *Pasteurella multocida* isolates. Results from this study suggest that only using bacterial culture and tulathromycin susceptibility testing to predict clinical outcome or evaluate efficacy of tulathromycin for treatment or control of respiratory disease in newly received cattle, without consideration of other factors, may be clinically misleading.

3. Comparative Efficacy of Metaphylaxis with Tulathromycin and Pentavalent Modified-Live Virus Vaccination in High-Risk, Newly Received Feedlot Cattle

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Metaphylactic administration of antimicrobial (META) and vaccination with modified-live virus respiratory vaccine (MLV) upon feedlot arrival in high-risk beef calves are typically implemented with the goal of controlling and preventing bovine respiratory disease (BRD), respectively. Research-based evidence to support the efficacy of META is abundant; however, there are few published studies that demonstrate MLV to improve health outcomes in high-risk feedlot cattle. Our objective was to compare the efficacy of META with tulathromycin and vaccination with a pentavalent MLV respiratory vaccine in high-risk beef calves during a 56-day feedlot receiving period. Crossbreed beef bull (n=372) and steer (n=106) calves were stratified by arrival body weight (BW; 515 ± 2.9 lb), sex and health status, and randomly assigned to 1 of 4 treatments arranged in a 2 × 2 factorial: 1) no META or MLV administration (CON), 2) META administration on day 0, 3) MLV administration on day 0 with revaccination on day 14, and 4) META and MLV administration on day 0 with revaccination on day 14. Interactions and main effects were analyzed as a generalized complete block design with 10 pen replicates/treatment (n=20/main effect) and 12 animals/pen. Body weight and feed refusal was recorded on days 0, 14, 28, 42 and 56 to determine interim and overall (day 0 to 56) gain performance, dry matter intake (DMI), and gain:feed (G:F). A 7-day post-metaphylactic interval was implemented for META treatments and BRD cases were determined by blinded investigators according to clinical presentation and rectal temperature ≥104° F. The META groups had greater ADG from days 0 to 14, 14 to 28, and overall ($P \leq 0.02$) and BW on day 56 was increased ($P < 0.01$) 29.8 lb for META. Conversely, MLV did not affect ADG or BW ($P \geq 0.12$). For each interim period and overall, DMI was increased ($P < 0.01$) for META, but MLV did not affect DMI ($P \geq 0.11$). There was an improvement in G:F from day 0 to 14 and overall for META ($P < 0.01$); whereas, the main effect of MLV improved ($P = 0.02$) G:F from day 28 to 56. The BRD morbidity rate was less (18.5 vs. 51.2%; $P < 0.01$) and days to first BRD treatment was more (19.8 vs. 8.5; $P < 0.01$) for META; however, BRD morbidity was not improved for MLV ($P = 0.37$). The percentage of calves deemed chronically ill was reduced ($P < 0.01$) for META (1.7 vs. 8.4%), but not MLV (4.6 vs. 5.5%; $P = 0.67$). Calves that arrived with a pre-existing ranch tag were treated for BRD less often ($P = 0.03$) compared to calves that did not arrive with a ranch tag. These data indicate that on-arrival META with tulathromycin improves health and performance of high-risk feedlot cattle, but on-arrival MLV with revaccination on day 14 does not.

4. Whole Blood RNA-Seq Analysis of Stocker Calves at Arrival Provides Immunological and Metabolic Distinction Related to BRD

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Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in post-weaned beef cattle. The complex interactions between infectious agents, immune response, and environmental risk factors responsible for BRD are poorly understood. We hypothesize that altered transcriptional profiles in the blood of stocker calves represent regulatory pathways responsible for protection from and resistance to BRD. Whole blood was collected from the jugular vein of bull and steer calves (n=80, mean=206 kg) into Tempus tubes at arrival. Animals were monitored for clinical signs of BRD based on an adapted version of the DART system. Cattle diagnosed with BRD within 14 days following arrival (n=6), and cattle without signs of BRD over the 84-day study (n=5) were selected for blood RNA sequencing (Illumina HiSeq 3000). Sequencing reads (80M paired-end/sample) were quality filtered and aligned to the bovine reference genome assembly ARS-UCD1.2. False discovery rate (FDR) adjusted p-values of 0.10 were applied to identify differentially expressed genes (DEGs), utilizing edgeR. WebGestalt and String v11.0 were used to identify biological functions, pathways, networks, and interactions represented by DEGs. Fifty-two DEGs were identified between healthy and diseased groups at arrival; thirty-six downregulated and sixteen upregulated in diseased calves. We identified biological processes related to inflammatory mediation, metabolic processes, and stress regulation. Pathway analysis revealed upregulation of inflammatory resolution pathways in healthy calves and dysfunctional metabolic pathways in diseased calves. The identification of DEGs in whole blood at arrival revealed a clear distinction between calves diagnosed with BRD and those that remained healthy. Our analysis shows an indication in the use of these genes as biomarkers for disease prediction and risk association. With the use whole blood transcriptomic analysis, this study provides a novel insight to host immunity in response to BRD at time of stocker facility arrival.

5. Health Status and Endoscopic Evaluation of the Upper Respiratory Tract of Dairy Bull Calves Inoculated with BVDV2 and BHV1 After Vaccination and Trace Minerals Injection

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Bovine respiratory disease (BRD) is a major illness that affects cattle industry worldwide. *Bovine viral diarrhea virus* (BVDV), and *Bovine herpes virus 1* (BHV1) are pathogens commonly involved in BRD. These viruses cause immunosuppression and damage of the upper respiratory tract (URT), respectively, increasing host susceptibility to opportunistic bacteria which results in mild to fatal illness. Strategies to prevent BRD include adequate management and biosecurity, along with multivalent vaccination. The use of injectable trace minerals (ITM) has demonstrated beneficial effects on the immune response and protection elicited by vaccination against respiratory pathogens. In addition, efforts to reduce the impact of BRD involve early diagnosis of affected animals. Clinical examination and health score for BRD diagnosis may fail to identify cases of subclinical pneumonia. Endoscopy is a tool that permits prompt evaluation of the URT of affected animals, before bronchial and lung lesions appear. The objective of this study was to determine if administration of ITM at the time of vaccination enhances protection against BVDV2 and BHV1 infection in dairy calves by decreasing clinical signs of disease and level of inflammation and damage of the URT, evaluated by endoscopy. Twenty-four dairy calves (1 mo) were administered a modified-live virus (MLV) IN vaccine containing BHV1, BRSV, PI3V (Inforce 3®), and randomly assigned to subcutaneous (SC) administration of ITM (Multimin®90 containing Se, Cu, Zn & Mn; ITM, n=12) or saline (Sal, n=12). Ten weeks later, the calves received a booster of the same IN vaccine, and a second dose of ITM, or saline, according to the treatment groups. Moreover, calves in both groups were administered a dose of a SC MLV vaccine containing BVDV1 & 2 (Bovi-shield gold BVDV®). Additionally, 12 calves did not receive vaccine or treatment and served as a control group (Unvac, n=12). Forty-nine days after booster vaccination, all calves were intranasally inoculated with BVDV2 (5 $x10^5$ $CCID_{50}$); and seven days later with BHV1 (8 $x10^6$ $CCID_{50}$). Health status was evaluated every day using the University of Wisconsin's health scoring system. Five days after BHV1 inoculation, a random subset of calves (ITM= 5; Sal= 5 & Unvac= 3) were selected for evaluation of the URT (nasal cavity, pharynx, larynx, trachea, and bronchi) using endoscopy (Tele Pack Vet X led-Karl Storz®). Each segment of the URT was visually assessed for vascularization, integrity of the mucosa, and secretions by three evaluators that were blinded of group assignment. An endoscopic score (ES) from 0 to 3 (0: normal; 1: mild; 2: moderate; 3: severe) was assigned for each characteristic. An overall ES was calculated for each calf and the means were compared among groups using ANOVA of SAS®. All groups had comparable rectal temperature, health scores, body weight and ES before virus challenge. Rectal temperature significantly increased in the Unvac group after BVDV2 & BHV1 challenge. Health score was higher ($P < 0.05$) in Unvac compared with the vaccinated groups on days 6, 10 and 12 after BVDV2 inoculation. There was no significant difference regarding health score between vaccinated

groups. Unvaccinated calves had the highest mean URT ES (27.5 ± 4.47) after BVDV2 and BHV1 challenge, showing marked mucosal congestion, severe lymphoid hyperplasia and ulcers at the pharynx and larynx with abundant purulent secretions along the URT, compatible with severe pharyngitis, laryngitis, tracheitis, and bronchitis. Calves treated with ITM showed significantly lesser URT ES (18.2 ± 1.25) compared with Sal (24.9 ± 1.0) and Unvac groups ($P < 0.01$), presenting milder inflammation and damage of the URT mucosa. Calves in the Sal group also had lesser URT ES than unvaccinated calves, but with no statistical difference. In conclusion, administration of ITM concurrent with IN vaccination resulted in lesser inflammation and tissue damage after BVDV2 + BHV1 inoculation compared with Unvac and Sal groups. To the knowledge of the authors, this is the first report on endoscopic evaluation of the URT in cattle infected with BVDV2 and BHV1.

6. Efficacy of NASYM Against a Heterologous Challenge with a Virulent BRSV Strain in the Presence and/or Absence of MDA.

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The aim of the present study was to evaluate efficacy of NASYM (HIPRA, Spain) under the influence of maternally derived antibodies (MDA) against an experimental heterologous challenge of bovine respiratory syncytial virus (BRSV) conducted 8 weeks after the intranasal application in young calves. Thirty six newborn Holstein calves were fed 1.5L of pooled dairy colostrum either negative to BRSV antibodies (MDA-; n=18) or BRSV antibody-positive (MDA+; n=18). These animals were randomly assigned to NASYM or phosphate buffered saline (PBS). Hence, animals were divided into 4 groups of 9 animals: group 1 (MDA-NASYM), group 2 (MDA+NASYM), group 3 (MDA-PBS) and group 4 (MDA+PBS). NASYM or PBS was administered at two weeks of age approx. (7 to 17 days of age), by intranasal route (2ml) using a cannula coupled to a syringe. During the vaccination phase, animals were daily monitored for clinical signs, including signs of depression, and respiratory clinical signs were recorded and scored. Blood was collected to study the decay of MDAs in serum. Eight weeks after vaccination, all animals were challenged as a single group by aerosol exposure to a virulent BRSV strain (Asquith). Clinical assessment was conducted daily during the eight days after challenge. Calves were euthanized on day 8 or earlier if those animals presented severe respiratory clinical signs caused by the BRSV infection. These criteria were consistent with the Canadian Council of Animal Care guidelines that were approved by the Committee on Animal Care and Supply at the University of Saskatchewan. On necropsy, the percentage of pneumonic tissue of all animals was evaluated. Nasal swabs were collected 8 days after challenge to assess virus shedding. Before challenge no calf showed respiratory clinical signs or alterations in behavior. After challenge, calves in all groups developed variable signs of respiratory disease characteristic of BRSV infection. Five out of nine MDA-PBS and four out of nine MDA+PBS required euthanasia for ethical reasons, whereas only one MDA+NASYM animal was euthanized among all vaccinated animals. No significant differences were observed between MDA+ and MDA- vaccinated animals, or between control groups (MDA+ and MDA-). Thus, the percentage of control animals (50%; 9 out of 18) that required euthanasia for ethical reasons was significantly greater ($p<0.05$) compared to vaccinated animals (5.6%; 1 out of 18), indicating an 88.9% reduction of mortality expressed as of euthanized animals. In terms of shedding, 12 out of the 18 vaccinated animals had negative samples 8 days after challenge. On the other hand, only 2 out of the 18 control animals had negative samples. The percentage of negative samples in vaccinated animals (MDA-NASYM and MDA+NASYM) was 66.7%, whereas only 11.1% of control animals (MDA-PBS and MDA+PBS) had negative samples. These results suggest that vaccinated animals, independently of the presence or absence of MDAs, reduced virus shedding faster than PBS groups. The lung lesions of vaccinated groups were similar between them, no significant differences were observed between MDA+ and MDA-

vaccinated animals. Similarly, no significant differences were observed between control groups, comparing MDA+ and MDA- control animals. However, lung lesions of vaccinated groups (average of 16.1%) were significantly lower than those observed in the control groups (average of 29.3%) . Overall, the results demonstrate that intranasal vaccination with NASYM during the first two weeks of life, significantly reduced mortality, lung lesions and shedding of BRSV caused by an experimental infection with a virulent heterologous strain of BRSV.

7. Efficacy of NASYM Against a BRSV Challenge in Young Calves (from 10 weeks of age) with Maternally Derived Antibodies

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The aim of the present study was to evaluate efficacy of the live attenuated vaccine NASYM (HIPRA, Spain) under the influence of maternally derived antibodies (MDA) against bovine respiratory syncytial virus (BRSV). This BRSV experimental heterologous challenge was conducted 21 days after the intramuscular vaccination in young calves from 10 weeks of age. One group of 7 calves without BRSV antibodies (MDA-) was included in the study and vaccinated with NASYM. Fourteen calves with BRSV antibodies (MDA+) were also included and randomly assigned to a vaccinated group with NASYM or a mock-vaccinated group with phosphate buffered saline (PBS). Hence, animals were distributed in 3 groups: control MDA+, vaccinated MDA+ and vaccinated MDA-. A first dose of NASYM or PBS was administered at 10 weeks of age (10 to 15 weeks of age), by intramuscular route (2ml), and a second dose was administered 4 weeks later. During vaccination phase, animals were monitored on a daily base for clinical signs, including signs of depression and respiratory clinical signs. Blood was collected to study the decay of MDAs in serum. Twenty-one days after vaccination, once MDA levels of the unvaccinated group had dropped, all animals were challenged as a single group by aerosol exposure to virulent BRSV. Clinical assessment was conducted daily during 14 days after challenge. Calves were euthanized on day 14 in order to assess lung lesions induced by the BRSV infection. Nasal swabs were collected daily after challenge to assess virus shedding. Before challenge, no calf had respiratory clinical signs or alterations in behavior. After challenge, calves in all groups developed variable signs of respiratory disease characteristic of a BRSV infection. Both MDA+ and MDA- vaccinated animals showed significant ($p<0.05$) reduction in respiratory rate, respiratory distress, cough and ocular discharge at several time-points compared to control animals. The total score of respiratory clinical signs was also significantly lower at days 7, 8, 10, 11, 12 and 13 in vaccinated groups compared to the control. Consequently, the average of total respiratory signs score in the MDA+ and MDA- vaccinated animals during the post-challenge period (from challenge to 14 days later) was significantly ($p<0.05$) lower than in the control group. A reduction in respiratory signs of 28% (MDA+) and 34% (MDA-) was achieved in vaccinated animals. In terms of shedding, 11 out of the 14 vaccinated animals had negative samples 1 week after challenge. On the other hand, only 1 out of the 7 control animals had negative samples at day 7. Hence, at this time-point the mean virus titer was significantly ($p<0.05$) lower in both vaccinated groups, than in control group. These results suggest that vaccinated animals, independently of the presence or absence of MDAs, controlled virus excretion better than the control group. The lung lesions of vaccinated groups were similar between them, no significant differences were observed between MDA+ and MDA- vaccinated animals. Both vaccinated groups showed a notable reduction in lung lesions respect the control group. A reduction of 52% of lung lesions was achieved in MDA+ vaccinated animals. Overall, these results demonstrate that vaccination with NASYM protected the vaccinated calves, independently of the presence of MDA, in terms of clinical signs, virus shedding and lung lesions.

8. Systematic Review and Meta-Analysis Comparing Arrival Versus Delayed Vaccination of High-Risk Beef Cattle with 5-Way Modified-Live Viral Vaccines Against BHV-1, BRSV, PI3, and BVD Types 1 and 2

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Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in North American beef cattle, and thus a major economic and welfare concern of individuals involved in the beef cattle industry. The most common viruses implicated in BRD are Bovine Herpesvirus 1 (BHV-1), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 (PI3), and Bovine Viral Diarrhea Viruses types 1 and 2 (BVD 1 and 2), and it is common practice to administer vaccines against these viruses to cattle entering feedlots and stocker operations within the United States and Canada at arrival. However, there have long been concerns that cattle may not mount an optimal immune response when vaccinated at this time. A number of studies have been conducted to evaluate the effects of vaccination timing on morbidity and mortality, but often include other interventions that might confound interpretation of the results. This can make an objective evaluation of the effects of only vaccination timing difficult. It is therefore our goal to provide a systematic review of these studies, and extract relevant data to perform a meta-analysis of vaccine timing on BRD morbidity, retreatment rate, and mortality. A literature search of PubMed, CAB, and Bovine Practitioner was done with the following inclusion/exclusion criteria to find studies that compared the efficacy of arrival vaccination to delayed vaccination of high-risk beef cattle with a MLV pentavalent vaccine against viral BRDC pathogens. The vaccines needed to be labeled for the prevention of or the aid in prevention of disease caused by BHV-1, PI3, BRSV, and BVD types 1 and 2. Time points for interventions needed to include vaccination at arrival, or vaccination at a time point greater than 7 days following arrival. Studies needed to be conducted on high-risk beef cattle, and report clinically relevant outcomes; that is, morbidity, mortality, and retreatments, either as total case numbers or percentage of a population, and disease had to be naturally occurring. Means of diagnosis of respiratory disease had to be clearly described, and had to have a case definition that included clinical signs of BRD such as depression, diminished appetite, increased respiratory rate, cough, nasal or ocular discharge, and increased rectal temperature. After identification of all suitable studies, a Mantel-Haenszel risk ratio was calculated using a random effects model, along with a 95% CI and presented in a Forest plot, for BRD morbidity, retreatment risk, and mortality. The Cochran Q statistic was also calculated. A $P \geq 0.10$ for this statistic, and an $I^2 > 50\%$ were used to indicate potential heterogeneity of the studies. A confidence interval that crosses 1 was considered indicative of no significant difference between the compared variables. A total of eight studies were identified that met all search and inclusion/exclusion criteria. In regards to morbidity, when all studies are evaluated, the overall risk ratio is 0.99 (95% CI 0.93, 1.06), indicating that there is no difference in regards to morbidity between vaccination at either time point. The overall risk ratio for retreatment risk is 1.01 (95% CI 0.91-1.13), indicating that retreatment risk is not impacted by vaccine timing. Regarding mortality risk, there does appear to be a trend toward delayed vaccination being advantageous;

the risk ratio is 0.78. However, the 95% CI still crosses 1 (0.57, 1.10), again indicating no difference in mortality risk between calves vaccinated at arrival or delayed. Based on the data from the studies analyzed, it would appear that there is not an advantage or disadvantage in terms of morbidity, retreatment rate, or mortality in delaying vaccination. In light of the results of this meta-analysis, and when we consider the high morbidity rates seen in many of these studies, it may be that vaccination of high-risk beef cattle in the feedlot or stocker setting is equally ineffective regardless of when it is performed. More research is needed in larger groups of cattle with fewer confounding variables to evaluate the timing of vaccination as a factor in the control of bovine respiratory disease.

9. Development of a Novel Biosensor for Early Detection of Respiratory Disease in Stocker Cattle

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Beef production in the US is a dynamic, multi-stage system including cow-calf, stocker, and feedlot. The stocker stage involves the purchase of weaned calves and feeding of a high pasture diet to achieve increased body weight and uniformity among their cohort prior to entering a feedlot. Due to a variety of factors, weaned calves entering the stocker system are at high risk for bovine respiratory disease (BRD), an important economic and animal welfare concern and the leading cause of sickness and death. Infected calves exhibit a variety of clinical signs including high fever, nasal discharge, coughing, and difficult breathing. Visual observation of these clinical signs is the primary method of diagnosis, which lacks sensitivity and specificity, and often occurs late in pathogenesis. Currently, there are several commercially available biosensors that have been primarily used in dairy cattle for detection of health and performance changes. However, none of these available biosensors have been validated for use in stocker-aged beef calves, and the algorithms developed to classify behaviors are based upon adult dairy or feedlot cattle. Therefore, there is a critical need to develop a technology that is capable of monitoring and recording changes in movement patterns, social networks, and intensity of activity specific to stocker cattle. The short-term objective of this study is to determine proof of concept for a novel biosensor and utilize various machine learning methods to develop algorithms to report specific activities in recently weaned beef calves. Our long-term objective is to utilize this technology for early detection of respiratory disease in commercial beef stocker cattle production. Toward this short-term objective, we have begun a 4-week data collection, observational study. Sixteen Angus steer calves (400-500 lbs. body weight) were received from the East Tennessee Research and Education Center (ETREC). Steers were housed in two adjacent pens, with 8 steers per pen. Upon arrival at the facility, steers were fitted with collars containing tri-axial accelerometers and global positioning systems. Video surveillance data will be paired with biosensor data to characterize different behavioral movements. All behavioral data will be collected for 4 weeks and entered into a database management system. Both neural networks and random forests machine learning approach will be used to classify raw data into various activities. K-Nearest Neighbors (k-NN) will be used for cluster algorithms development. Data will be analyzed using R version of 3.6.0. Results from the development of this biosensor will be presented. The algorithms developed from this study will be used to classify specific behaviors, intensity of activity, social interactions, and time spent in focal areas within the pen. In conclusion, this study will provide a basis for early BRD detection based upon behavioral evaluation through the development and testing in healthy, recently weaned steers.

10. Whole Genome Sequencing of Upper Respiratory Tract Microbiome of Beef Calves

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Much attention has been focused on the microbiome of the respiratory tract, and more specifically changes in the microbiome, as possibly a significant factor that may predispose to bovine respiratory disease (BRD). For example, Timsit, Workenstine, et al., (Vet Micro, 2018) found that the microbiomes of the upper and lower respiratory tracts (URT and LRT) of cattle with BRD has distinctly different bacterial metacommunities, compared to those of healthy feedlot cattle. Other work has shown dynamic changes in the microbiome over time (Zeineldin, et al.; Vet Micro, 2017). They also found differences in the microbiome of calves treated for BRD vs. those not treated, including differing shifts in population from feedlot entry to time of diagnosis. However, they only examined from time of entry to 30 days on feed. Thus, it seems apparent that not only the microbiome composition, but also changes in that composition over time, is critical to disease development. It remains to be determined what factors serve to stabilize or perturb that microbiome, and what degree of variation is likely in the absence of external perturbation. One project took a longer timeframe assessment of dynamics of the URT microbiome of calves from different ranches (McCullen, et al.; Vet Micro 2018). They found notable dynamism over time, but absence of a common pattern of evolution. As such, additional work is warranted to characterize alterations over time, and what may influence those changes. Moreover, all reported work to date has utilized 16s sequencing methodology. While this approach provides a summary overview, it has notable deficiencies compared to whole genome sequencing. The objective of our project was to use whole genome sequencing to assess dynamics of the URT microbiome of calves from prior to weaning, through the weaning process, and to 21 days post weaning. Six commercial calves, approximately 7 months of age were randomly selected from a group of 29 for sampling. Calves were previously vaccinated with an intranasal modified live bovine herpesvirus (BHV-1) (approximately 2 months prior to study). Calves were also vaccinated with a modified live viral vaccine containing BHV-1, bovine viral diarrhea virus (BVDV) types 1 and 2, bovine respiratory syncytial virus (BRSV) and bovine parainfluenza 3 virus (PI-3) immediately following first sample collection. Deep pharyngeal samples were collected using sterile cotton swabs, and immediately placed in storage media (DNA/RNA Shield, Zymo Research). Samples were collected D-21, D0 and D21 relative to fence line weaning. Samples were stored at 4°C in storage media until processed for DNA extraction. Extraction was followed by depletion of methylated DNA by use of commercial kits (Zymo and New England Biolabs). Shotgun metagenomics library preparation was done using published methods, followed by Illumina MySeq sequencing, and composition assessment via Centrifuge, using available datasets for alignment. Statistical calculations were performed using the python Scikit-Learn and Statsmodels libraries. Sequencing work and analysis has been completed. Interpretation of results is ongoing, but will be completed by August 1st.

11. Economic Evaluation of Metaphylactic Antimicrobial Administration in High-Risk Calves at Time of Arrival.

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The use of metaphylactic antimicrobial treatment upon arrival of high-risk calves at backgrounder and feedlot operations is proven to be beneficial to decrease the incidence of bovine respiratory disease. The objective of this study is to analyze the economic impact of arrival metaphylactic antimicrobial treatment with Tulathromycin or Tilmicosin in high-risk calves compared to no metaphylaxis. Salebarn-sourced, crossbreed beef bulls (n=211), steers (n=128) and heifers (n=1,014) with average body weight of 386.59 ± 70.17 pounds were enrolled. At arrival, calves within a pen were randomly assigned to receive one of two treatments: META (calves receiving metaphylactic treatment at the time of arrival) or NO-META (calves did not receive antimicrobial treatment at the time of arrival). Calves were kept in one of 17 pens across two different farms. Arrival drugs and pulls were consistent across the farms and did not differ between META and NO-META within the same farm. The total cost was calculated using the cost of enrollment (the cost of antimicrobial at arrival) + hospital cost (cost of antimicrobial used at hospital + $5 chute fee for hospital costs) + death cost (cost of animal purchase + cost of feed consumed until the day that animal died). The average cost of metaphylaxis was $13.58 ± $1.06 per calf. Analysis of variance was performed using the MIXED procedure of SAS (SAS v9.4, SAS Inst. Inc., Cary, NC). The statistical model included the main effect of treatment group (META vs NO-META) and random variables were farm and pen. Odds ratio for BRD event was calculated using the LOGISTIC procedure of SAS. Calves in the META group had lower morbidity (46.33% vs. 63.27%; P<0.0001) and mortality rate (9.03% vs 14.41%; P-0.002) than calves in the NO-META group. Furthermore, calves not treated with metaphylaxis were at 2 times the odds to be pulled and treated due to a respiratory event than calves receiving metaphylaxis at arrival. Although the death cost per head that died ($599.74 ± $20.51 vs. $597.30 ± $19.70) did not differ (P=0.79), the hospital cost/head ($13.74 vs. $19.97; P<0.0001) and total cost per head ($76.03 vs. $99.76; P=0.02) was lower for META than NO-META. In conclusion, the use of metaphylaxis reduced losses associated with hospital treatments and mortality resulting in less total cost/calf enrolled in the study.

12. Between-Location Heterogeneity of Bilateral Nasopharyngeal Swabs and Bronchoalveolar Lavages Based on MALDI-TOF Identification of Bovine Respiratory Disease Pathogens

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Bovine respiratory disease (BRD) pathogens such as Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni have been cultured from different locations in the respiratory tracts of sick and healthy cattle for decades. However, uncertainty remains regarding which samples are the most appropriate for the detection of these opportunistic bacteria at different time points relative to BRD incidence. The objective of this research was to evaluate the agreement of bilateral nasopharyngeal swabs (NPS) and bronchoalveolar lavages (BAL) collected from feeder calves on arrival at the feedlot (ARR), from clinically healthy animals (CON), and cattle diagnosed with BRD (SICK). A total of 117 auction-derived beef calves (186 kg – average weight) were transported to a feedlot in Texas and did not receive metaphylaxis on arrival. Thirty randomly selected calves were sampled via bilateral NPS and BAL on arrival to the feedlot. Over the next 28 days, calves exhibiting clinical signs of BRD plus a rectal temperature of ³40°C were classified as BRD cases and sampled immediately before first treatment (n=72). Additionally, 16 clinically healthy calves were sampled during the first 28 days on feed. Samples were cultured for M. haemolytica, P. multocida, and H. somni with up to 15 phenotypically identified colonies of each bacteria saved for MALDI-TOF species confirmation. Agreement beyond chance between tests was evaluated via the Kappa statistic. Kappa values of <0.00 = Poor, 0.00-0.20 = Slight, 0.21-0.40 = Fair, and 0.41 – 0.60 = Moderate. For ARR calves, agreements were Fair, Fair, and Slight; in CON calves, agreements were Poor, Slight, and Slight; and for SICK calves, agreements were Slight, Slight and Slight for left NPS vs BAL, right NPS vs BAL, and URT vs BAL, respectively. Agreements between left NPS and right NPS were Fair, Moderate, and Moderate for the CON, ARR, and SICK calves, respectively. These results indicate that agreement between these techniques is variable depending on the group being sampled. These data also suggest that left or right NPS differ in the ability to identify the bacteria that will be recovered from a BAL sample and that pooling URT samples results in minimal improvement in agreement with BALs.

13. Determination of Ethyl Carbamate in Bovine Plasma by GC-MS

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Ethyl carbamate (EC), also known as urethane, is a compound commonly found in fermented foods and beverages. In its metabolism, it can produce vinyl carbamate epoxide forming DNA adducts and is classified as a Group 2A probable human carcinogen by the International Agency for Research on Cancer. Literature on analyzing EC in bovine plasma is limited. A previous report on EC measurement in mouse whole blood uses a lengthy procedure and had a limit of detection (LOD) around 50ng/mL. A sensitive analytical method for EC in bovine plasma is needed to further understand EC metabolism and its physiological effects in cattle. An improved quantification method presented here used methyl tert-buyl ether (MTBE) to extract EC and the extract was directly injected to gas chromatography coupled to mass spectrometry (GC-MS). The ratio of extraction solvent to sample volume was optimized. A calibration curve was created using ethyl-d5 carbamate as internal standard, with varying concentrations of EC (1- 500 ng/mL). Method validation including linearity, inter-day and within-day repeatability, and detection limit were conducted. The LOD of current method is 10 ng/mL. Acceptable repeatability with coefficients of variation at three representative concentrations were achieved and averaged below 8%. The modified method and lowering of the LOD greatly enhances the ability to quantify EC in bovine plasma.

14. A Description of Bovine Respiratory Disease from an Antimicrobial Use Perspective in Canadian Feedlot Cattle

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This project (funded by the Canadian Beef Cattle Check-Off through the Beef Cattle Research Council, Alberta Beef Producers and Agriculture and Agri-Food Canada) was designed to provide robust Canadian feedlot cattle antimicrobial use (AMU) estimates, interpret AMU data and relate it to Canadian feedlot production practices, validate Population Corrected Unit estimates for Canadian feedlot cattle, and assess how a standardized collection method/system might be applied nationally. As part of this, AMU indications were identified, including treatment and control of bovine respiratory disease (BRD), which allowed summary of use data related to this important disease complex, and in particular summary of medically important antimicrobials related to BRD. Detailed AMU data (2,615,082 cattle from 36 feedlots in western Canada) were collected over 4 years of animal placements (November, 2008-October, 2012). The majority of cattle entering feedlots were male (63%; 1,643,528), considered low risk for developing bovine respiratory disease (BRD; 61%; 1,593,443), identified as yearlings at feedlot entry (55%; 1,434,583), and arrived during the fall/winter (62%; 1,616,686). Descriptive AMU data (parenteral and in- feed) included trends over time, use based on Health Canada categories of importance to human medicine, and use by antimicrobial class (e.g., quinolones, macrolides, tetracyclines). AMU comparisons were made between sexes and seasons. To allow detailed insight into AMU practices in Canadian beef production and antimicrobial exposure in Canadian feedlot cattle, use indications were stratified by animal classification at feedlot arrival for risk of developing BRD (high or low), and by antimicrobial class. A total of 92.9% of all parenteral AMU (based on the number of animal use daily doses [nADD]) was targeted against BRD treatment and control. The majority (>90%) of this BRD use was macrolide (Health Canada Category II) and tetracycline (Health Canada Category III) use. Of the AMU associated with BRD, 65% of nADD were administered to calves and 62% of nADD were administered to cattle classified as high-risk for developing BRD. Antimicrobial drugs (AMDs) are used in Canadian feedlot cattle production systems to support and maintain health and as therapy for illness. This project provides unprecedented AMU data for western Canadian feedlot cattle linked to indications for use. Historically, comprehensive published data on AMD types used, extent of use, common use indications, and demographics of cattle populations receiving antimicrobials have been limited. Antimicrobial stewardship is a priority for the Canadian Beef Industry, and a thorough understanding of baseline AMU is important for meaningful assessment of associated public

health risk and to inform stewardship activities. This project highlights the importance of continued research efforts to identify effective non-antimicrobial BRD mitigation strategies to maintain animal health and welfare and to support reduced AMU for BRD treatment and control.

15. Atypical Bovine Respiratory Disease Associated with *Chlamydia pecorum* in Adult Lactating Dairy Cows

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Recently, a local dairy farm in Maricopa County, Arizona has experienced an abnormally high incidence of pneumonia in their adult dairy cows despite routine and preventative care. Such care includes vaccination of cows and heifers with the same modified live multivalent respiratory vaccines. Sixty-five (65) lactating Jersey cows were submitted to the Midwestern University Diagnostic Pathology Center for necropsy following death or euthanasia between August 2018 and April 2019. Forty-seven (47) of those cows had active pneumonia (72.3%), however, other comobidities such as mastitis, enteritis, and encephalitis were discovered. The pathogens isolated from lung tissues included *Mannheimia hemolytica*, *Pastuerella multocida*, *Mycoplasma bovis*, *Trueperella pyogenes*, Streptococcus suis, and Pseudomonas aeruginosa. Incidentally, the same farm also experienced higher incidences pneumonia and diarrhea in calves as well as late term abortions with encephalitis and fibrinous polyserositis. Abortions were extensively worked up by multiple collaborating diagnostic laboratories. Diagnostics performed included PCR and bacteriology on fetal tissues (no placenta available) for common causes of bovine abortions. Pathogens that were ruled out included BVD, IBR, *Leptospira*, *Brucella*, *Neospora*, *Listeria*, and *Campylobacter*. Chlamydial PCR was positive in all tested fetal tissues (brain, heart epicardium) and IHC demonstrated intralesional organisms. Sequencing of the 16S rRNA resulted in a 100% match with *Chlamydia pecorum*, though with only 1 base pair mismatch from C. psittaci and C. abortus. Because some cows (5) and calves (4) had similar lesions of encephalitis and vasculitis, IHC and PCR was pursued on some and tests detected chlamydiosis, also sequenced as C. pecorum in one calf. Additional molecular testing for *Chlamydia pecorum* in cows and calves is ongoing. Investigations are underway to determine the extent, if any, of *Chlamydia pecorum* involvement in the high incidence of bovine respiratory disease in this herd. Concurrently, epidemiological investigations are being carried out to determine factors that might have contributed to the unusually higher bovine respiratory disease incidence in this herd.

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