INTRODUCTION

Recent developments in molecular genetics and genotyping platforms offer a unique opportunity to identify a large number of the loci and genomic rearrangements associated with the spectrum of bovine respiratory disease (BRD) pathogens. This previously inaccessible wealth of genetic information will pave the way for marker-assisted selection and novel genomic selection approaches[49] to genetically-complement animal health management practices aimed at minimizing BRD occurrence. Reducing the considerable animal morbidity, mortality and economic losses associated with BRD will require the simultaneous development of genomic tools to enable the selection of resistant animals and a sustained educational effort to increase the adoption of management practices that decrease the incidence of BRD in both beef and dairy cattle**.** BRD is the leading natural cause of death in beef and dairy cattle, causing annual losses of more than one million animals and $692 million[55]. Despite extensive use of vaccines and antimicrobials, morbidity and mortality rates have actually increased in some feedlot cattle[28, 50]. Typically, cattle with BRD have clinical symptoms including fever, rapid breathing, repetitive coughing, nasal and/or eye discharge, diarrhea, dehydration, and appetite depression. Animals are more likely to be affected by BRD when stressed by sudden changes in feed, crowding, temperature, transportation, and when clean air is contaminated with ammonia, dust and pathogens. Although the environment plays a major role in BRD infection rates, there is increasing evidence that resistance to BRD is under genetic control. Differences in BRD resistance have been found between cattle breeds and sire lines[52], and a heritability estimate of 0.48 on an underlying continuous scale was reported in unweaned beef calves[74]. This suggests that selecting for BRD resistant cattle could have a substantial impact on BRD prevalence[72]. A limited number of quantitative trait loci related to bovine health, including resistance to BRD, have been reported[13, 67, 92]. Recently, we have identified additional BRD-related chromosomal loci linked with BRD in crosses of *Bos taurus* x *Bos indicus* cattle and loci associated with the incidence of pathogenic bovine viral diarrhea virus in *Bos taurus* cross-bred beef cattle[93].

Long-term goal. Our *long-term goal is to reduce the prevalence of BRD in beef and dairy cattle with resultant improvements in animal welfare and industry profitability.* Our **research hypothesis** is that genetic selection for resistance to BRD coupled with improved animal health management can provide a significant, sustainable, and profitable reduction in the prevalence of BRD. The *research objectives* of this proposal will identify genetic loci and genomic rearrangements associated with BRD and use these to develop SNP-based selection tools and diagnostic tests to identify BRD genetically resistant animals. Use of these newly-available genomic tools will enable innovative selection approaches to decrease BRD incidence. These genetic tools offer an opportunity to significantly reduce BRD losses to levels below those achieved by the prevention and control measures developed during the past 25 years[27]. Unfortunately, traditional prevention and control approaches have not resulted in a sustained decrease in the prevalence and impact of BRD[27]. This, coupled with increasing consumer reluctance to accept traditional therapeutic treatments of disease and rising public interest in animal welfare, makes implementing genomic approaches to reduce BRD a highly attractive goal[89].

Our **extension hypothesis** is that an integrated multidisciplinary approach to reducing BRD will be more successful than approaches which address only one aspect of the disease or a single sector of the cattle industry. The *extension objectives* of this CAP will develop a sustained effort to disseminate, demonstrate, evaluate and document the impact of a range of educational outreach materials and best management practices for beef and dairy cattle producers, and feedlot personnel. These efforts will outline practical methods to reduce the prevalence of BRD. Extension efforts are linked to the research projects through the use of existing extension networks of commercial operations to conduct both beef and dairy research objectives. Extension objectives are integrated with education through graduate and undergraduate students' involvement in conducting the research at the commercial cattle operations, and through the Southern Great Plains Dairy Consortium where courses are also integrated with the dairies participating in the research studies.

Our **education hypothesis** is that didactic and experiential learning approaches describing the complex issues associated with BRD in the cattle industry will be most effective in preparing students to make the management decisions needed to reduce BRD impact. The *education objectives* are to develop and offer distance and experimental multi-disciplinary learning approaches for undergraduate, graduate, and veterinary students studying BRD. Our extension and formal educational central objectives will provide educational opportunities to various stakeholder audiences (youth, undergraduates, graduate students, veterinarians, and producers) in best management practices for reducing BRD. To achieve these objectives, we have convened a unique interdisciplinary team of veterinarians experienced with BRD in dairy and beef cattle, geneticists (with specialties in animal health, disease, immunology, epigenetics, and quantitative genetics), epidemiologists, anthologists, microbiologists, livestock economists, and veterinary and livestock cooperative extension specialists (see biographical sketches). The makeup of this team aligns well with the findings of the 2009 BRD research conference, organized by the USDA CSREES Multistate Research Project NC-1027, in which a paper on future research concluded that it will be necessary to coordinate integrative teams to achieve success in reducing the incidence of BRD[48]. Our approach includes commercial collaborators from the leading beef and dairy producing states in the nation, in addition to prominent international participants. We have worked to ensure extensive stakeholder involvement in project development, implementation, and evaluation (see letters of support).

RATIONALE AND SIGNIFICANCE

This multi-institutional, multi-national CAP proposal addresses the program priority of an integrated disease management approach to reduce BRD in beef and dairy cattle. The rationale behind the proposed project is that, by fully capitalizing on recent advances in genomics, the outcomes of this project will allow for improved diagnostics of BRD and the implementation of novel genetic selection approaches resulting in a sustained decrease in the incidence of BRD in both beef and dairy cattle. Selection of BRD-resistant cattle is ultimately the most sustainable approach to reducing the incidence of disease, as resistant animals have improved animal welfare, decreased use of therapeutic antibiotics, have superior production efficiency, require less handling, and are ultimately more profitable. The research, extension and education objectives of this CAP integrate genomics, economics, animal health, and animal welfare to address a complex disease. This project is novel in that much of the research occurs at commercial operations that are part of existing extension networks with internships and extension programs flowing from these research experiences. It uses state-of-the-art genomic technologies to address disease resistance. The outcomes of the project are of great interest to industry (see letters of support), and participating states include those with the largest beef and dairy populations magnifying its potential impact. The integration of the educational material developed by this into eXtension ensures material developed will be available beyond the life of the CAP.

This proposal addresses the *Program Area Priority of integrated disease management approaches for bovine respiratory disease*, and USDA AFRI funding priority #2, Animal health and production and animal products by using an integrated approach to reduce the prevalence of BRD and reduce the substantial economic losses associated with the disease. This proposal also meets USDA Purpose and Priorities goals by proposing research, extension and education deliverables that will affect the long-range improvement and sustainability of US agriculture and food systems. This proposal advances integrated research, education and extension by confronting a disease that is of international, national, regional and multi-state importance. This proposal advances translational research stemming from the new genomic technologies that have become available to the cattle research community and extends science-based knowledge to the livestock industry, allowing them to make informed practical decisions.

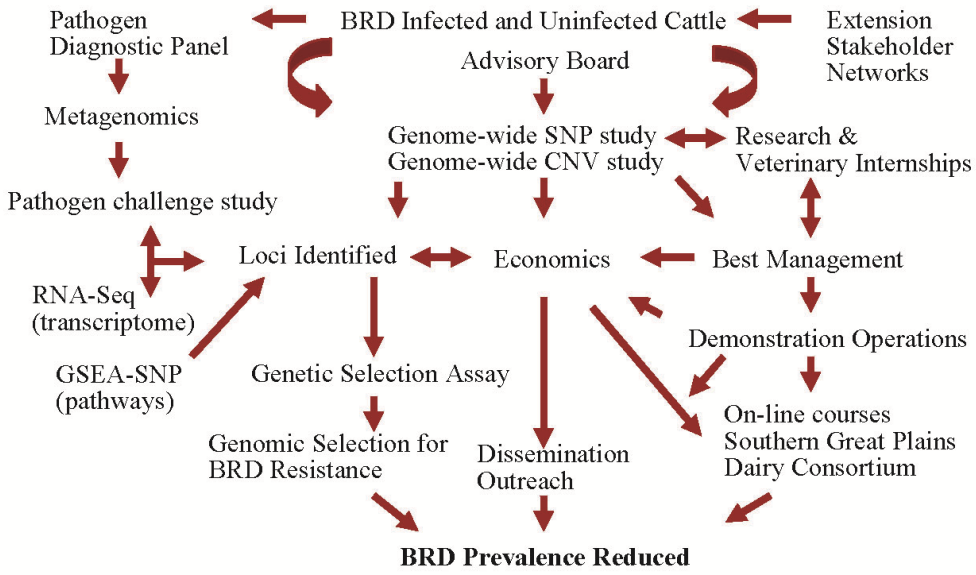


Figure 1. Overview of proposed activities.

REVIEW OF RELEVANT SELECTED LITERATURE

Bovine Respiratory Disease- Beef. BRD is the most important cause of economic losses and the leading cause of illness and death for the backgrounding and feedlot cattle industries [83, 28, 73]. Of the cattle that become ill in the feedlot, 67 to 82% suffer from respiratory disease[20]. In the United States, 1.4% of all feedlot cattle perish before reaching harvest weight and of those, the majority are due to respiratory disease[81, 43]. Indeed, more feedlot cattle die from BRD than all other diseases combined and this trend is increasing[43]. The incidence of BRD has varied across a 15-year period with the annual observed incidence ranging from 5 to 44% in feedlots and from 3.3 to 23.6% in a 20-year period in unweaned calves[74, 75]. Almost all (97.6%) U.S. feedlots reported BRD prevalence, with an average prevalence of 14.4% [83]. BRD rates are seasonal with higher incidence rates in the fall and winter months[50]. More than 25% of large feedlots used a respiratory vaccine to combat the disease and almost all (99.8%) feedlot animals diagnosed with BRD were treated with an injectable antibiotic as part of the treatment regimen[83]. The costs to treat an animal for BRD, excluding labor, veterinary fees or indirect costs, ranged from $6.64 to $37.90 per animal[70, 83]. These costs were greater than for any other disease or lameness in the feedlot[83]. In addition to death-loss and treatment costs, detrimental effects of BRD include reduced carcass weights, daily gains, yield grade, carcass fat measurements, and muscle shear-force measurements[29, 64, 74, 75, 90]. Animals with BRD also experience pain which is detrimental to animal welfare. Treatment with analgesics enhances disease recovery and resulted in weight gains at slaughter[26].

Evidence that BRD prevalence is under genetic control is demonstrated by breed differences in BRD morbidity and mortality, the fact that BRD prevalence in unweaned calves and feedlot cattle is heritable, and the finding of loci identified to be linked with BRD. Prior to entry into the feedlot, the incidence of BRD in weaned calves varied by breed from a low of 10% in Angus to a high of 35% in Pinzgauer[73]. Mortality also differs by breed, ranging from 0.1% in Braunvieh cattle to 8.9% in Red Poll cattle. Susceptibility differed among various breeds, ranging from 28% in Braunvieh to 73% in Hereford[73]. Heritability estimates also reflect the genetic and environmental underpinnings of the disease. Heritability estimates for BRD prevalence in unweaned calves ranged from 0.0 to 0.26, but when estimated on an underlying continuous scale the estimate rose to 0.48[52, 74]. The heritability estimate for feedlot animals was 0.18, when adjusted to an underlying continuous scale[73]. Recently, loci on chromosome 2 and 26 were identified as being linked with BRD prevalence in crossbred beef cattle[93]. In this same study, loci in these regions were also implicated with persistent infection with bovine viral diarrhea virus, a BRD pathogen, in crossbred beef

cattle[93]. Collectively, these data provide strong support for the role of innate genetic resistance to BRD.

Bovine Respiratory Disease- Dairy. Bovine respiratory disease is also a major problem for the U.S. dairy industry. Respiratory disease accounted for 22.5% of the mortalities in unweaned dairy heifers, 46.5% in weaned heifers, and 11.3% of cow deaths[84, 85]. Losses associated with BRD in dairy replacement animals outpace those for all other diseases[58]. Dairy calves often suffer from a lack of passive transfer of colostral immunity, balanced nutrition, proper housing and vaccination, all of which put the calf at risk for BRD infection[58]. Holstein steers that enter the feedlot have higher death losses than do beef breed steers with respiratory disease causing 35.5% of all deaths[87]. Of the unweaned and weaned heifers with BRD, 93.4% were treated with an antimicrobial[85].

Breed differences in susceptibility to BRD in dairy cattle have been found to be similar to the susceptibility differences in beef breeds. Crossbreeding of Holsteins with Jerseys has improved health and survival but specific studies on BRD are lacking[44]. One study estimated the heritability of BRD in Norwegian Red calves to be in the range of 0.02 to 0.09, but heritability estimates have not been determined for Holstein or Jersey cattle[35].

Cattle genomic resources. The recent sequencing and analysis of the ~2.87 Gbp *Bos taurus* genome suggests that it contains over 22,000 genes[22]. The availability of the cattle genome sequence has allowed the development of genome-wide SNP assays which can be utilized to identify genomic regions and pathways associated with disease[45, 67]. The next generation of cattle SNP assays consists of two different 800k cattle SNP panels: one already available from Illumina and an Affymetrix assay projected to be available by the end of 2010. The 800k SNP assays will be critical for identifying and precise mapping of loci associated with BRD. In addition to revealing SNPs throughout the cattle genome, the cattle sequence has revealed more complex mutations, such as segmental duplications, which make up 3.1% of the cattle genome[22]. The majority (76%) of segmental duplications correspond to complete or partial gene duplications[22]. The duplicated genes typically encode proteins (such as immune proteins) with functions that involve interactions with the external environment. However, the majority of segmental duplications within the cattle genome are structural DNA variants known as copy number variants (CNVs).

Copy number variants. CNVs are defined as DNA fragments 1000 nucleotides or longer that vary in copy number when the tested genome is compared to a reference genome[25]. CNVs may contain DNA duplications, deletions, inversions and translocations. CNVs may be inherited or occur *de novo,* even in an inbred population[21]. It is now recognized in humans that structural genetic variation involves more DNA sequences than the sequences represented by SNP[16]. In cattle, over 850 CNV loci have been identified in 538 cattle genes, covering 22 Mb of the genome[6, 24]. Twenty percent of the CNV identified in cattle have been associated with segmental duplications and 30% with genes. Ten percent of the CNVs are found in genes that have orthologs associated with human disease. It is not known what proportion of genetic disease is caused by CNV in cattle, or if BRD is associated with CNVs, but it is known that in human disease an estimated 84% and 18% of the genetic variation in gene expression is associated with SNPs and CNVs, respectively[76]. Because there is very little overlap in the variation explained by SNP and CNV, methods to interrogate both SNP and CNV will be needed for a comprehensive evaluation of the genomic regions associated with BRD.

Genome-wide association and gene get enrichment analysis-SNP (GSEA-SNP) studies.

The availability of genome-wide SNP assays make association analysis the most powerful approach for identifying loci associated with disease[63]. This method of analysis relies upon linkage disequilibrium or the non-random association of alleles at two or more loci to detect associations between neutral markers and casual loci. Association studies do not require structured breed crosses or pedigreed populations of cattle in which the genotyped animals are separated by only a few pedigree generations, but instead use a sampling of the independent meioses present within a cattle population. Association studies require the use of high-throughput high-density SNP genotyping assays which dramatically lowers cost and

increases study power. We have found that 100,000 bp is the average distance over which linkage disequilibrium begins to deteriorate in cattle although it varies across the genome and across cattle breeds[46]. While we have successfully used a 50k SNP assay to identify loci associated with disease in cattle, using an 800k SNP assay is expected to be far more efficient by having markers in regions of the genome that were previously not well represented[67, 92]. Pathways and enriched gene sets are composed of genes that interact with one another, that act together, that are involved in similar functions or are located close to one another[36]. The correlation analysis of associated genes can have much higher statistical significance than analysis of the single genes, with the obvious limitation that the association must be functionally meaningful. GSEA-SNP is particularly useful for identifying candidate genes that produce modest effects, but it can also provide clues about the complex biological processes associated with complex diseases in cattle[68].

Animal selection through genomic selection. Genomic selection is a methodology which allows the prediction of animals' breeding values using only a high-density panel of markers scored in each animal[49]. The process requires a two-stage approach in which animals with known phenotypes and genotypes are used in a training analysis to statistically establish the relationships between individual SNPs and trait variation and then the inferred equations that predict breeding value are validated in independent populations. Our experience with the Illumina BovineSNP50 assay in Holstein bulls indicates that when at least 8,000 animals are genotyped in the training population, the estimates of molecular breeding values achieved in the validation population can be as high as 80%. However, this seems to be influenced by the extent of the pedigree relationship between the animals used in the training and validation populations. All dairy breeding populations in the U.S. rely upon the use of the most superior sires within the population to produce the next generation of sons, leading to high pedigree relationships between training and target populations.

The situation within the U.S. beef population is more complex since fewer animals are often available to form the training population, necessitating the strategy of forming training populations using animals from several breeds. Our experience has been that molecular breeding value prediction equations developed for one breed perform poorly when validated in a second breed, presumably due to the fact that phase relationships are not preserved beyond about 10 kb across breeds[77] and the average resolution of markers on the BovineSNP50 assay is about 30 kb (corresponding to an average inter-marker interval of 60 kb). However, we expect that this problem will be resolved with the application of the ultrahigh-density 800k assays which will produce a resolution of ~2 kb.

RNA sequencing (RNA-Seq). RNA sequencing is a tool that is used to sequence cDNA to obtain information about genome-wide gene expression within the sampled tissue. This tool is proving to be invaluable in the study of diseases by identifying the differences in gene expression between normal and diseased states as a result of variation in transcription and post-transcriptional regulation and due to differences in genetic composition[14]. RNA-Seq is more informative than the use of expression microarrays because the approach is not limited to the genes spotted on the microarray, it is not influenced by the accuracy of the probes developed for the microarray, and it provides information on non-coding RNA. Correlation of RNA-Seq data with SNP genotypes leads to the larger discovery of expression quantitative trait loci[51]. RNA-Seq has been used to identify disease-associated genetic variants in humans[53].

RNA-Seq is possible through the use of "next-generation" sequencing or high-throughput sequencing technologies that generate millions of short reads from a library of nucleotide sequences. The library of nucleotide sequences may be developed from the tissue from a single animal or from a pooled sample of animals. Because short sequences are generated, a reference genome sequence is necessary to locate the genes or regulatory regions from which the sequence arose. The availability of the cattle genome sequence assembly, in combination with next generation sequencing, makes the use of this powerful technology possible.

Metagenomics. The term "metagenomics" is the study of organisms in their natural environment without the biases associated with the inability to characterize those organisms

which grow poorly in laboratory cultures[32]. The field of metagenomics has revealed that large groups of microorganisms have gone undetected due to their failure to culture well[57, 11, 80]. Metagenomic studies are now identifying bacterial and viral pathogens in human disease and its use in identifying bacterial and viral pathogens in cattle diseases will follow[7, 40, 53, 54].

Bovine respiratory disease pathogens. The etiologic agents associated with BRD include viruses (bovine herpesvirus 1, bovine parainfluenza virus 3, bovine viral diarrhea virus 1 and 2, bovine respiratory syncytial virus, bovine adenovirus A-D, and bovine coronavirus), bacteria (*Arcanobacterium pyogenes*, *Manheimia haemolytica, Pasteurella multocida, and Histophilus somni),* and *Mycoplasma spp.*[27]. Improving our understanding of the etiologic agents involved in the pathogenesis of BRD is critical for developing appropriate preventative, treatment, and selection strategies. Five recent studies have described the prevalence of BRD pathogens present in live and expired animals affected with BRD[4, 5, 8, 27, 28]. These studies demonstrate that *Mycoplasma spp, Pasteurella multocida, Manheimia haemolytica, Histophilus somni, Arcanobacterium pyogenes,* bovine viral diarrhea virus and bovine respiratory syncytial virus are the most prevalent known pathogens reported in cattle with BRD. It is unknown how these pathogens interact with genetically-regulated host resistance to BRD.

BRD diagnostics. Accurate diagnosis of BRD is critical. Traditional methods for detecting morbid cattle include visual appraisal once or twice daily. Animals displaying nasal or ocular discharge, depression, lethargy, emaciated body condition, labored breathing or a combination of these, should be further examined[18]. Symptomatic animals with a rectal temperature ≥ 39.7°C are usually considered morbid and given treatment. Perino and Apley[59] developed a clinical scoring system where animals with a clinical score ≥ 1 and a rectal temperature at or above 40°C should receive treatment. Scoring was based on: 0=normal animal, 1=noticeable depression without apparent weakness, 2=depression with moderate signs of weakness without an altered gait, 3=severe depression with signs of weakness and a significantly altered gait and 4= moribund and unable to rise. A second BRD scoring system, which will be used for diagnosis of BRD in this study, is based on rectal temperature, the character of nasal discharge, eye or ear appearance, and presence of a cough[41, 47]. This system provides a score based on the sum of points from the 4 categories of clinical signs, with increasing values representing progressive severity. The scoring system results in a minimum score of 0 and a maximum score of 12. Calves with scores of 6 or higher have at least 2 clinical signs of respiratory disease and are considered sick.

All of these diagnostic systems are subjective in nature. Confounding factors include the diligence and astuteness of those checking the animals, the variability and severity of the symptoms the animals experience with chronic and acute BRD, and the disposition of the animals. Animals with docile temperaments are more likely to be diagnosed than aggressive or flighty animals[12, 61]. It was postulated that aggressive cattle were less likely to show depression which may explain the higher mortality, but lower morbidity rate in these cattle. Oliphint et al.[56] suggested that disposition may also play a role in the response to vaccines which would also affect the apparent susceptibility to disease. Pulmonary lesions were examined at slaughter by Wittum et al.[90] in an attempt to confirm the live BRD diagnosis, but with mixed results. They reported that 35% of feedlot steers received treatment for BRD between birth and slaughter, whereas 72% had pulmonary lesions at slaughter. For steers treated for BRD, 78% had pulmonary lesions, whereas 68% of untreated steers also had pulmonary lesions. Thompson et al.[78] also found that many (29.7%) apparently healthy animals had subclinical disease. This study suggests that the morbidity estimates derived from clinical estimates of BRD are too low.

To culture organisms associated with BRD, pharyngeal swabs offer a less invasive, less stressful and more rapid alternative to broncheoalveolor lavage[31]. DeRosa et al.[17] reported that nasal swab cultures contained the same bacterial species as transtracheal swab cultures 96% of the time and that nasal swab cultures were genetically identical with the organism causing disease within the lung for 70% of the calves tested. The positive predictive values for nasopharyngeal swabs compared to a positive finding in the lung were 100% for *M.*

Haemolytica and *M. bovis* and the negative predictive values were 67% and 33% for *M. Haemolytica* and *M. bovis,* respectively[31]. Allen et al.[1] also reported that at a group level, nasopharyngeal swabs gave similar results to those of bronchoalveolar lavage.

BRD management. Management has a large effect on the prevalence of BRD. Preconditioning feedlot animals has been found to decrease morbidity and mortality from BRD but the practice is not widely adopted[82]. Preconditioning procedures include vaccinations for clostridial and viral diseases, treatments with anthelmintic, as well as performing castration and dehorning procedures 30 to 45 days prior to placement into the feedlot. Vaccination for IBR, PI3, BVD and BRSV are recommended 4 to 6 weeks before weaning, but if vaccination is not feasible at this time, calves are usually vaccinated at weaning and revaccinated 14 to 21 days later[18]. Exposures to animals that are persistently infected with bovine viral diarrhea affect transmission of BVD virus within groups of calves. The use of preventative medication programs with prescription antibiotics can be used as an effective means of controlling morbidity for high risk cattle[19]. Pre-shipping compared to feedlot arrival medication programs have similar efficacy. Consumer concerns about the wide-spread use of antibiotics and the potential for antibiotic resistance may limit the acceptance of these approaches in the future. The nutritional status of cattle prior to a BRD challenge is also of importance, but little is known about the specific effects of nutritional status before a BRD challenge.

Animal welfare. Diseases are a major detriment of animal welfare. Farm animals generally receive little in the way of pain relief when sick. For animal welfare to improve, a better understanding of the consequences of pain and discomfort of diseases is needed[23]. There is recognition of the pain involved in disease, particularly respiratory disease[39]. Kielland et al.[38, 39] found that when Norwegian dairy producers were surveyed, they scored respiratory disease as a 7, out of the highest possible pain score of 10. Reducing inflammation can be an important part of BRD treatment and there is considerable scope to incorporate pain relief into management of this disease. Indeed, in 1999, 40% of US feedlots used non-steroidal anti-inflammatory drugs, one type of pain relief, when treating BRD[81].

Freedom from pain, injury and disease are crucially important aspects of domestic animal husbandry and welfare[10]. However, pain assessment in farm animals is often difficult. For example, cows may show no obvious signs when in chronic pain other than a reduction in milk yield[69]. Our previous work demonstrated that pain associated with lameness in cows causes a reduction in social behaviour, higher Cortisol/DHEA ratio[2] and high gene expression in immune cells of IL-2, MMP13, IL-6 e IL10[3]. Similarly, other illnesses, such as metritis, result in decreased feeding time two to three weeks before clinical diagnosis of the disease in dairy cows[37]. A number of other behavior changes are also associated with illness, such as reduced grooming and lying inactive[9]. There is growing evidence that treatment with non-steroidal anti-inflammatory drugs improves the welfare of sick animals (e.g. with diarrhea in calves)[79].

APPROACH

Research aims

1. ***Identify genomic loci associated with BRD resistance/susceptibility in beef and dairy cattle.***

We will identify loci associated with BRD using a multi-tiered approach. The first approach will be a genome-wide association study that uses a single nucleotide polymorphism (SNP)-by-SNP comparison with disease to identify genes with major effects. To identify genes of modest effects, GSEA-SNP will be used to evaluate pathways and functional groups of genes for an association with BRD. Copy number variants will be investigated using array comparative genomic hybridization and multiplex ligation-dependent probe amplification to determine if they are associated with BRD. Gene expression differences will be determined to further understand the biological processes involved in the etiology of BRD and to identify candidate genes in these pathways that, if perturbed, would enhance BRD resistance. The *expected outcomes* are that loci will be identified that are

associated with BRD and the biologic pathways and processes will be identified that contribute to the host response to BRD pathogens. The *importance* of these expected outcomes is that they will provide tools for selecting animals that carry gene associated with BRD resistance and testing whether these animals are resistant to BRD.

a. ***Genome-wide association analysis***. Our first approach will be to conduct genome-wide association studies using SNPs to identify loci associated with BRD resistance. Genome-wide association studies will be conducted with 800k SNP assay data in dairy (dairy calves and replacement dairy heifers) and in beef (crossbred feedlot steers and purebred bulls). A 70% power to detect loci associated with resistance to BRD (based on our reported heritability estimates) will require 2000 animals. To maximize power to detect BRD loci by minimizing variation in calf history, the first study will take place with 2000 one-day-old calves in a highly controlled calf rearing facility. Due to the strict management of this facility, heritability, and therefore power to detect loci, is expected to actually exceed our previously published estimates. This study will be followed by a validation study using 1000 dairy replacement heifers. To determine if different loci are associated with resistance to BRD in beef, 2000 crossbred feedlot steers will be studied and a follow-up validation study will be conducted in 1000 purebred beef bulls. Blood samples will be taken from all animals for DNA extraction. These 6000 animals will be genotyped with the 800k SNP chip.

- POPULATION A**:** Two thousand Holstein dairy calves at Grimmius Cattle in California will be monitored for clinical BRD symptoms through their first three months of life. Each BRD case will be defined as a calf with two or more clinical signs of BRD diagnosis that has been confirmed by pharyngeal swabs. Matched controls will consist of a contemporary calf observed to be healthy and housed in the same or adjacent hutch. Controls will also be examined for pathogens by a pharyngeal swab. These samples will serve as the locus discovery training cohort for dairy.
- POPULATION B**:** One thousand female Holstein dairy calves (500 each from DoRene and Highland Dairies in New Mexico) will be followed through their first lactation. Cases and controls will be determined as described in POPULATION A. These samples will serve as a locus validation cohort for dairy.
- POPULATION C: Two thousand cross-bred beef calves in the Graham Feedlot in Texas will be monitored for BRD from the time of entry into the feedlot through harvest. Cases and controls will be defined in the same way as the dairy calves. These samples will serve as a locus discovery training cohort for beef.
- POPULATION D: One thousand purebred bulls from the Angus, Hereford, Charolais, Red Angus, and Wagyu breeds will be monitored for BRD during a 100 days feeding trial at Snyder Livestock in Nevada. Cases and controls will be defined as previously stated. These samples will serve as a locus validation cohort for beef.

b. ***Copy Number Variants.*** Our second approach will be to identify regions of the genome that are associated with resistance to BRD due to copy number variation.

- *Identifying genomic rearrangements by array comparative genomic hybridization (aCGH).* We developed a 400k oligonucleotide array encompassing 20,344 genes in the bovine genome. The array includes protein coding genes, predicted genes, microRNAs, rRNAs, snRNAs, snoRNAs and miscellaneous RNAs. Each gene is represented by approximately 20 oligonucleotides and is focused towards the 5′ and 3′ untranslated regions and exons. Scanned images will be processed and normalized using Agilent's Feature Extraction Software. Analysis of data will be performed using the Agilent's Genomic Workbench aCGH software. CNV will be identified using the ADM-2 aberration algorithm with high aberration filters (i.e., > 0.5 log2 ratio averaged over 5 probes). Output files of genomic aberrations will be generated with genomic coordinates, gene names, gene type (i.e., protein coding, microRNA, etc.), and GO identification. Eighty feedlot animals from POPULATION C will be used for aCGH.
- *Multiplex ligation-dependent probe amplification (MLPA) genotyping assay.* Ligation half probes will be designed for each CNV using the two-color MLPA design[88]. Each ligation pair of half probes will be selected to differ in length from other ligated probes by at least two

bases and probe sequences will be checked for uniqueness by BLAST to insure specific hybridization. Simultaneous amplification of the multiplexed ligated probes will be allowed by synthesis of 3' and 5' tails on each probe pair that are complementary to a common PCR primer pair such that the lengths of the amplified ligation products will fall within the size range of 80-125 bp. To increase the number of MLPA targets, we will use two sets of amplification primers, labeled with FAM or HEX dyes, respectively. These primer pairs are designed to specifically and reliably amplify in multiplexed reactions[88]. MLPA reactions will be performed as per published protocols[66]. Following amplification, product separation and identification will be by capillary electrophoresis on an ABI 3730 (Applied Biosystems). For each probe set, probes for two unlinked reference loci will be included and calculation performed only within a probe set as described by[88]. DNA samples from the remaining feedlot steers (n=1900 of POPULATION C) will be used for the MLPA assay. A subset of animals will be tested twice to determine the coefficient of variation for the assay.

c. ***RNA-Seq***. Our third approach to identify genomic regions associated with resistance to BRD will utilize RNA sequencing (RNA-Seq) to determine the transcription profiles of all genes being expressed in the lung, and pharynx derived from animals with specific pathogen challenges.

- POPULATION E: Eighty-four 9 month-old steers from the UC Davis commercial cow-calf herd will remain unvaccinated for BRD and will be sorted into seven challenged and seven unchallenged pens at the UC Davis feedlot. Seven pens will contain unvaccinated and pathogen challenged (n=6) steers, and control pens will contain unvaccinated and unchallenged (n=42) steers. Animals in the pathogen-challenged group will be experimentally challenged with one of the seven major pathogens (*Mycoplasma spp., Mannheimia haemolytica, Arcanobacterium pyogenes, Pasteurella multocida, Histophilus somni,* bovine viral diarrhea virus and bovine respiratory syncytial virus) that comprise the BRD complex of disease. Animal numbers and experimental approach were based on the study by Gershwin et al.[30]. Clinical signs will be evaluated using a method adapted from Collie et al.[15]. This system of evaluation uses a check sheet containing weighted parameters for cough, temperature, nasal and ocular exudates, character of respiratory effort, abnormal lung sounds, depression, and anorexia. Samples for RNA sequencing will be collected from all animals from each pen *post mortem*. Challenged steers will be euthanized eight days after the onset of clinical signs and lung and pharynx tissue will be immediately collected and frozen for RNA extraction (See 2. below for a description of the experimental and analytical approaches).

d. ***GSEA-SNP***. Our fourth approach to identify genomic variation associated with resistance to BRD will be to utilize SNP data collected from the genome-wide association study (Aim 1a) to find genes within biological pathways that are altered in animals resistant to BRD. Gene set enrichment analysis utilizing SNPs (GSEA-SNP) will be conducted to determine the genes and biological pathways associated with BRD resistance in the 2000 Holstein calves (POPULATION A), and 2000 feedlot steers (POPULATION C) (Aim 1a).

- One SNP per bovine annotated gene will be assigned as a gene proxy from the 800k SNP genome-wide association study. The Kyoto encyclopedia of genes and genomes (KEGG) pathways and Gene Ontologies (GO) gene sets will be tested for enrichment to identify genes within critical pathways and gene sets that are key for BRD resistance in dairy and beef.

2. Identify the interaction of the cattle genome with the pathogens responsible for BRD.

Multiple pathogens are involved in BRD. We will determine whether the host's genome affects the BRD pathogen(s) with which they are infected. Pathogens attack the host using different mechanisms so that it is difficult for the host to defend itself. Because of this complexity, loci associated with resistance to one BRD pathogen may not necessarily be of critical importance in defense against another BRD pathogen. To define these differences, the pathogens causing BRD must be diagnosed for each affected animal. This will provide an opportunity to characterize the loci that are specifically associated with specific pathogens. The *expected outcomes* are that the pathogen(s) responsible for BRD in each animal will be identified, and the loci associated with resistance to each pathogen will be identified. The

importance of these expected outcomes are that they provide tools for selection of animals resistant to specific pathogens that comprise BRD.

a. ***Diagnosis of BRD***. Diagnosis will be based on the BRD scoring system[41, 47] and be confirmed from pharyngeal swabs. To determine the proportion of animals with subclinical disease, lungs from the animals in POPULATION C will also be examined for pulmonary disease. Although this will identify control animals with subclinical bacterial respiratory infections that escaped detection, it will not identify subclinical viral infections. Animals with pulmonary infections will be treated as subclinical cases. Currently, several different tests are required to identify the pathogens associated with BRD. Developing a diagnostic panel for BRD pathogens will significantly improve BRD diagnosis (see below).

b. ***Host x pathogen interaction***. Animals diagnosed with BRD (POPULATIONS A and C) and their controls will be sampled for BRD complex pathogens and genotyped with the 800k SNP assay. Genotypes of the cattle diagnosed with the same BRD pathogens will be compared to genotypes of cattle without the presence of that specific pathogen using the R statistical environment and PLINK[60].

3. Identify novel pathogens present in animals with BRD, and development of diagnostic tests.

Metagenomics will be used to identify the novel and emerging pathogens present in cattle exhibiting signs of BRD. The *expected outcome* is that a BRD diagnostic panel will be developed based on available sequence of already known BRD pathogens, and emerging BRD pathogens identified in the metagenomic study. The *importance* of this expected outcome is that an inexpensive diagnostic test will enable the rapid diagnosis and appropriate management response to the presence of specific disease agents.

a. Metagenomic analyses. Metagenomic analyses will be conducted on pharyngeal and lung tissues of 16 animals (a minimum of two animals infected with one of the seven major pathogens) terminally ill with BRD immediately *post mortem*. These analyses will be used to identify the BRD pathogens present and their relative abundance. This will provide information for the development of the BRD diagnostic panel and will constitute the most important known and recently discovered pathogens in the BRD complex of pathogens.

- DNA and RNA samples will be prepared based on standard procedures and either RNA-Seq or genomic library preps will be made by the DNA Core Facility at University of Missouri at Columbia (UMC). All sequence data will be quality filtered and barcode-sorted based on standard procedures implemented at UMC. The entire RNA-Seq data set for each tissue (91.8 Gb) will be used to generate a *de novo* tissue-specific transcriptome using SoftGene's NextGene software running on a 48-node server with 256 Gb of shared memory at UMC. The produced transcriptomes will contain all identifiable transcripts present within the 16 animals which will be annotated by searching public databases for sequences based on similarity. Each transcriptome will serve as a reference against which to align the reads from individual animal/tissue samples to obtain animal/tissue specific gene expression profiles. Gene expression data will be derived as counts within 100 bp bins across each transcript which may then be normalized for total reads per library and transcript length. Normalized reads per transcript will then be logarithmically transformed, as necessary to establish linearity, and a mixed linear model fit to examine transcript difference among challenged and unchallenged animals. The metagenomic samples will be similarly analyzed. First, all produced reads will be aligned to a bovine repeat library to filter bovine repetitive elements and then to the bovine sequence assembly to filter bovine genomic sequences. The remaining reads will be used to create a *de novo* "genome" of the microflora present in the lungs of cattle. This will serve as a reference to align reads from individual animals that will yield count data as to the species present within each animal. To establish a baseline for bacterial species, polymorphisms present within the sequence pile-up for the ribosomal 16S gene and counts of identical reads will be used to establish the number and relative numbers of each bacterial species sampled from each tissue. All laboratory and sequence analysis procedures follow standard operating procedures used by the Animal Genomics group at UMC which has previously analyzed over 388 Gb of genomic and 12 Gb of RNA-Seq data (unpublished data).

b. ***Develop a BRD diagnostic SNP-chip assay based on the sequence of all known BRD pathogens***. There is a need for the development of rapid and sensitive methods to enhance the clinical diagnosis of BRD[48]. In collaboration with the veterinary diagnostic laboratories associated with this study, we will develop a chip-based BRD pathogen diagnostic panel based on known pathogen (viral and bacterial) sequences, and novel pathogens from the metagenomics study, to quickly and effectively identify the specific pathogens present in an animal. This pathogen panel will be based on the pathogens observed in the samples collected in POPULATIONS B (replacement heifers) and C (feedlot steers) and validated using a subset of the diagnosed samples from POPULATIONS A (dairy calves) and D (bulls). The effective diagnosis of the pathogen infecting each animal will facilitate the study of the interaction of the host's genome with the pathogen's genome.

4. *BRD genetic selection panel.*

Tools to accurately predict the health risk of calves for BRD are not currently available (Smith 2009). Following the identification of loci that are associated with BRD, a panel of SNPs and CNVs will be developed to rapidly asses the genotype of the tested animals. The *expected outcome* is that an inexpensive genetic test (tool) will be developed that will enable the identification and selection of animals resistant to BRD. The *importance* of this expected outcome is that it will enable the reduction of BRD through genetic selection.

a. ***Assay development.*** Using Sequenom MassARRAY MALDI-TOF instrumentation and related chemistries, a final deliverable in the form of a genotyping assay will be developed based on loci strongly associated with BRD in genome-wide association, GSEA-SNP, and RNA-Seq studies to rapidly assess the genomic breeding value for resistance to BRD in beef and dairy cattle. At present, we anticipate that ≤ 384 BRD predictive SNPs will be queried via 9-10 highly cost-effective multiplex reactions, with genotypes resolved on the Sequenom MassARRAY instrument.

- This assay will be trained on samples from POPULATION A (dairy calves), C (feedlot steers) and E (challenged calves).
- This assay will be validated with POPULATION B (dairy replacement heifers) and D (bulls). We will also have the opportunity to validate the assay in population of 3000 feedlot animals that were evaluated in a Pfizer BRD study (see letter of collaboration). This validation will leverage existing DNA samples and phenotypes from a BRD study conducted by Pfizer. In return, a sample of similar size will be available for validation of Pfizer genetic markers. This exchange of resources will hasten the availability of genetic markers to the industry so that they may be used for genetic selection of animals resistant to BRD.

5. *Develop genomic estimates of breeding values for resistance to BRD.*

We propose to genotype 6000 animals (3000 Holsteins, 2000 crossbred beef and 1000 purebred bulls of Angus, Hereford, Charolais and Wagyu breeds) with an 800k SNP assay and utilize this process to develop molecular breeding values that will enable selection to the US beef and dairy industries to reduce animal susceptibility to BRD. To facilitate the incorporation of BRD resistance into beef cattle selection decisions, selection indexes for breeding objectives that include phenotypes indicative of the health of feedlot cattle will be developed for the beef cattle industry (see MacNeil letter of collaboration).We will develop estimates of breeding value for animals resistant to BRD to use in selecting breeding animals. Genetic selection for BRD resistance can be targeted through the identification of cattle with genotypes that confer resistance to BRD through estimates of breeding values. The use of Genomic Selection will enhance the frequency of resistance loci associated with BRD. The *expected outcome* will be that these estimated breeding values will provide a more comprehensive means to select for cattle resistant to BRD. The *importance* of this expected outcome is that animals resistant to BRD will be healthier, more productive and more profitable.

- Several approaches have been described for producing prediction equations for the estimation of molecular breeding values[86]. However, the most straightforward approach is a simple modification of the mixed linear model approach of Henderson[34] for the best linear unbiased prediction (BLUP) of breeding values using pedigree information and observed data

in an animal model. With this approach, we first use the genotypes produced for animals in the training population to estimate a genomic relationship matrix G[86] which is incorporated into the mixed model equations:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + G^{-1}\lambda \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} X'Y \\ Z'Y \end{bmatrix}$$

where X and Z are design matrices, Y is a data vector, *b* is a vector of fixed effects and *u* are breeding values which are estimated as molecular breeding values under this model via the use of the genomic relationship matrix. In the equation below, *i* is a scale parameter of variance components which may be estimated or assumed known *a priori*. Finally we produce BLUP estimate SNP allele substitution effects as:

$$\hat{\alpha} = (2\sum_i p_i q_i)^{-1} M'G^{-1}\hat{u}$$

Where p_i and $q_i = (1 - p_i)$ are SNP allele frequencies and M is a matrix determined by animal's genotypes[86]. From these estimates of allele substitution effects, the molecular breeding value of any newly genotyped animal is obtained as:

$$\hat{u} = M\hat{\alpha}$$

6. ***Assess how animal welfare is affected by BRD in cattle.***

Diseases, particularly the ones that cause pain, are the most serious but least studied challenges to animal welfare. We predict that effective pain alleviation will improve disease recovery and will decrease animal suffering in animals infected with BRD. We will assess the behavioral and physiological changes associated with BRD, with emphasis on changes that could serve as sub-clinical indicators of BRD, such as reduced feed intake. We will also study the effects of effective pain alleviation for animals diagnosed with BRD. The *expected outcome* is that early detection of BRD and reducing BRD prevalence will enhance dairy and feedlot animal welfare. The *importance* of this expected outcome is that improved animal welfare results in higher production, improved consumer perception of animal agriculture, and improved profitability.

a. **Clinical and sub-clinical changes in feed intake associated with BRD.** Feed intake will be measured in POPULATION D (bulls) animals in a GrowSafe system over 70 days to determine if animals administered pain medication will have a shorter period of feed intake depression than animals without pain medication.

- Subsequent to a diagnosis of BRD, half of the beef bulls will be administered injections of meloxicam at 26-h intervals (half life of this drug in bovine serum) for 7 days. Meloxicam has been selected because it is a non-steroidal anti-inflamatory drug that attenuates clinical signs of diarrhea in calves[79] and the 7-d administration period captures the duration of clinical symptoms in calves challenged with BRSV[62]. Each BRD animal treated with meloxicam will be paired with a healthy control that will also receive meloxicam, resulting in four treatments in total: BRD+meloxicam, healthy+meloxicam, BRD only and healthy only. Animals will be implanted with a DSI PhysioTel® transmitter prior to the trial to allow the telemetric recording of body temperature, respiration and cardiac activity throughout the study.

b. **Characterization of behavioral and physiological changes associated with BRD.** Behavioral and physiological changes associated with BRD will be determined along with how pain control affects these responses in animals in POPULATION E (challenged calves).We predict that animals affected with BRD without pain relief will spend less time feeding and grooming and more time lying inactive than BRD animals with pain control. We also predict that these animals with have higher respiration rates, will be more sensitive to pain (as measured via pain threshold), and have elevated immune responses compared to control and NSAID-treated animals.

- Eighty-four nine month-old steers from the UC Davis commercial cow-calf herd will remain unvaccinated for BRD and will be sorted into 14 pens at the UC Davis feedlot as described in Research Aim 1c. Within each pen, half of the animals would receive injections

of meloxicam every 26 h for 7 days, beginning on the day of the challenge. Behavioral and physiological responses will be measured for 3 days before and 8 days after the challenge. Behavior will be measured with direct, structured observation and through automated devices, namely a logger for recording lying behavior (Onset Pendant G, HOBO). Data on pain threshold will be obtained using an electronic algometer (Commander Algometer, JTECH Medical Industries, Salt Lake City, Utah, USA) before and six days post challenge to identify potential long-term consequences of BRD-related discomfort on the organization of the pain system. Biomarkers of inflammatory pain[3] will be measured before challenge and six days post-challenge in peripheral blood mononuclear cells (PBMC) using quantitative real-time RT-PCR to assess interleukin (IL)-2, IL-10, matrix metalloproteinase-13, and chemokine C-C motif receptor-5.

Extension Aims

1. *Organize an annual advisory board face-to-face meeting and BRD research conference.*

a. **Advisory Board.** An advisory board consisting of veterinarians, researchers, stakeholders and industry personnel will serve to guide the CAP research, extension and education efforts. The advisory board will meet at least quarterly by teleconference in addition to the annual BRD conferences (see Management Plan). The *expected outcome* of the advisory board is to provide guidance, oversight and maintain the relevance of the project to the industry. The *importance* of this outcome is to enhance the project's relevance, credibility, ability to meet expectations and produce project deliverables.

b. **Annual BRD Conference.** We will hold an annual BRD conference modeled after the 2009 BRD research conference organized by the USDA CSREES Multistate Research Project NC-1027. Ideally in collaboration with that group, the BRD conference will be located in the participating states with a focus that will alternate between feedlot and dairy BRD. This design will facilitate exchange of information between researchers, veterinarians, students, stakeholders and industry. The *expected outcome* of the conference is to provide an exchange of information and progress reports to industry stakeholders and project participants. The *importance* of this outcome is the conference will provide a means of dissemination of project results that will be critical to the success of the project and further links the research, extension and education efforts.

2. Utilize existing extension networks to integrate producers, industry, veterinarians, researchers, graduate and veterinary students into the conducting and interpreting of the research trials.

Existing extension networks in California, Nevada, New Mexico, Texas and Washington will be used to bring producers, researchers, veterinarians, students and industry together to effectively conduct the proposed research and extension activities. Research POPULATIONS A-D are all based on commercial operations and these research sites were facilitated through extension networks. This approach leverages current relationships and operations to reduce the cost of the research while providing daily interactions with the stakeholders and industry. The *expected outcome* of this aim is that the integration of the research trials with students, stakeholders, veterinarians, researchers and educators will facilitate the research and provide experiential learning opportunities for students, stakeholders and the industry. The *importance* of this outcome is that the research will be relevant and responsive to the needs of the stakeholders, industry and students.

3. Enhance the eXtension Beef Cattle Community of Practice (CoP) by contributing new content, increasing membership in the CoP, and creating a cross-disciplinary partnership with DAIReXNET in the area of BRD research and outreach.

Dr. Van Eenennaam and a full-time extension associate will write, produce and fully integrate the research, extension and educational outcomes of this CAP into eXtension (see letters of support). The *expected outcome* of this aim is to extend information on means to reduce BRD through extension outreach. The *importance* of this outcome is that research, extension and educational materials will reach a larger audience to effect greater reduction of BRD prevalence.

- Materials will be developed for eXtension that include fact sheets documenting best-management practices to minimize BRD incidence based on current literature and CAP research findings. Where appropriate, these materials will be translated into Spanish for Spanish-speaking agricultural workers.
- Slide sets from presentations, webinars, film vignettes, Moodle (a software course management system) learning modules from the distance learning courses, and 4-H curriculum will be placed into eXtension.

4. Develop community-based programming and work with county-based local extension agents (farm advisors) to identify farms/ranches/managers (beef, dairy, and feedlot) interested in participating in an evaluation of their BRD management practices.

Several surveys have addressed the current state of BRD in the feedlot industry[43, 91, 65]. Likewise many studies have documented animal health management practices that decrease BRD incidence. However, the clinical impact of BRD continues, and many operations do not implement best-management practices for reducing the incidence of BRD. Dairy producers and feedlot operations may be willing to take steps to prevent BRD through management changes, but this will require new guidelines and explanations for the changes to be made. The *expected outcome* of the development of community-based programming is that implementation of BRD-focused herd health management will reduce the risk and prevalence of BRD. The *importance* of this expected outcome is that the reduction of the prevalence of BRD in these demonstration operations will improve profitability, production, animal welfare and incentive for other operations to follow similar practices. This information will be applied to the course curriculum for the courses on-line and through the Southern Great Plains Dairy Consortium.

a. **Risk assessment**. A site-specific evaluation tool to assess BRD prevalence, concerns, management, herd health practices and risk factors for individual operations will be developed.

b. **Demonstration operations**. Dairy and feedlot operations will be invited to participate in a BRD evaluation of their operations by local extension agents. Agents will use the evaluation tool to develop baseline data for participating operations, and advise cooperators of BRD best management practices to deal with the risk factors that are found at each location. Subsequent assessments will be taken to determine if BRD has been reduced through these changes. Agents will visit each participant annually to track metrics, document changes in behavior or management, and inform cooperators of new developments and relevant research findings resulting from the CAP. Data from all participants will be compiled to determine the impact of the CAP at changing behavior and reducing the incidence of BRD on the participating operations.

5. ***Determine the economic cost of BRD to dairies and feedlots and develop stochastic bio-economic models for the net cost-benefit of implementing strategies for reducing the prevalence and severity of BRD.***

Improving the net economic return of prevention and treatment of BRD is a driving motivation to reduce the prevalence of BRD. Feedlot operators and dairyman cannot use tools to reduce the prevalence of BRD if they do not pass a cost-benefit analysis. The direct costs of treatment, prevention and death, as well as the long-term costs associated with loss of production, health and growth of cattle will be measured. There are few studies available on the economic aspects of BRD and there remains a knowledge gap with regard to the economics of the disease and the economic net return of the disease management protocols relative to BRD epidemiologic characteristics. Integration of the research, extension and education missions of the proposal ideally involves all three entities in the research process. These studies will have the *expected outcome* of identifying economically viable genetic and management approaches to reduce the prevalence of BRD. These will then be implemented in community-based programming (demonstration) feedlots and dairies to test the recommended procedures and provide demonstration examples. The *importance* of this outcome is that only economically viable approaches to reduce BRD are sustainable. These results will be used in recommendations for improving profitability to the demonstration operations. In addition,

economic research results will be presented in national, regional and local extension programs and be integrated into the on-line and Southern Great Plains Dairy Consortium courses. The economic results will also guide research toward sustainable approaches to reduce the prevalence of BRD.

Comparative budget models will be developed to evaluate the economic cost of BRD prevalence for beef and dairy production systems. Loss in production will be valued at current market values, and disease treatment and herd health management costs will be valued at current cost. The profit difference between BRD and healthy animals will estimate disease economic losses. A spreadsheet modeling approach will be used to develop the comparative budgets.

Bio-economic models will be developed to examine the cost effectiveness of BRD management strategies within a beef feedlot and a dairy herd over time. The biological component of the models will simulate BRD exposure and infection in a feedlot or dairy using a Reed-Frost epidemic model of BRD[42]. The model will estimate the resulting BRD prevalence rate and production losses relative to disease management control strategies such as genetic selection of disease resistant animals, early identification and treatment of infected animals, and the effectiveness of alternative herd health management practices. The economic model will account for the disease control and treatment management costs and will value production losses and profitability of the feedlot and dairy herd over time. The GAMS (General Algebraic Modeling System) will be used to program the models. GAMS is a widely applied modeling tool that can efficiently manage biological production functions, time dynamics and determine net economic return. It can also easily be programmed to account for stochastic parameters such as disease exposure, prevalence and production losses.

a. **Feedlot performance.**

- Animals in POPULATION C (feedlot steers) will be compared for average daily gain, live weights in and out and carcass data for both healthy and BRD diagnosed cattle to determine the cost of BRD infection.
- Animals in POPULATION D (bulls) will be pre-conditioned and fed in a GrowSafe system while monitored for the presence of BRD. Feed intake, residual feed intake, average daily gain, feed consumption, live weights, and ultrasound measurements of subcutaneous fat and rib eye area will be recorded on an individual basis to determine the cost of BRD infection. This population will also be evaluated with pain control for BRD and a cost-benefit analysis of this treatment will be determined.

b. **Dairy replacement heifers.**

- Animals in POPULATION B (replacement heifers) will be followed from birth to three years of age. Differences in growth, production, conception rates and dystocia of the healthy and BRD diagnosed heifers will be measured and the cost identified.

6. ***Develop and deliver educational programs on best management practices for integrated and economically sustainable animal health management, genomic, and animal breeding approaches to reduce BRD.***

These activities will have the expected outcomes of integrating and facilitating the research trials through extension while providing experiential learning opportunities for students, stakeholders and the industry. This will provide an exchange of information and linkages enhanced through stakeholder and industry meetings, BRD conferences programs, eXtension materials distributed via a range of media, and local meetings. Empirical data on changes in adoption of best management practices and economic approaches to reduce BRD prevalence will be documented and disseminated through extension programs and meetings, and linked to education through on-line courses and the Southern Great Plains Dairy Consortium.

a. **National programs.**

- Six ten minute video vignettes on the diagnosis and management approaches to decrease the incidence of BRD will be developed for viewing and distribution via various outlets including YouTube, eXtension, and the National Cattlemen's Beef Association (NCBA) TV show "Cattlemen to Cattlemen".

- Best management practices developed for eXtension will be incorporated into the NCBA Cattlemen's college curriculum and Beef Quality Assurance manuals and materials (see letters from Tom Field, NCBA Executive Director of Producer Education and John Maas, UC Veterinary Medicine Extension)

b. **Regional and local programs.**

- Project team members will participate in local educational events in the participating states in collaboration with local extension agents (e.g. Annual UC Dairy Herdsman Short Course, State Cattlemen's and Cattle feeder's meeting, Western Dairy Management Conference, Southern Great Plains Dairy Consortium meetings, Beef Improvement Federation Annual Meeting, etc.)

Education Aims

1. Develop a nationally accessible distance-learning course on the integration of animal health management with genomic and animal breeding approaches to reduce livestock disease.

Distance learning courses will be developed in Moodle and made available on eXtension. Graduate students enrolled in universities in the U.S. will be eligible to register for the distance learning course. These students will learn integrated approaches to reduce BRD. The *expected outcome* of this education aim is that students, who would not otherwise have such a course at their university, will learn multidisciplinary approaches to reduce BRD. The *importance* of this outcome is that multidisciplinary approaches are needed to solve complex problems, such as BRD, and this course will provide resources for students to learn these approaches.

- An on-line course will be developed on "Genetic improvement of BRD resistance." Credit and registration for the course will be granted through a special topics course listing at the institution the student attends.
- An on-line course will be developed on "Animal health and genetic approaches to disease." Credit and registration for the course will be granted through a special topics course listing at the institution the student attends.

2. *Develop an undergraduate summer research internship program, with an emphasis on students from minority-serving institutions, to expose and train them in multidisciplinary integrated research to reduce BRD prevalence.*

Undergraduate research will be completed in research labs that are identifying ways to reduce the prevalence of BRD under the mentorship of a Project Director. Student participation in research projects not only benefits the research projects but benefits the students. These experiences often lead to an increased interest in the field of study or other advanced areas of study. New Mexico State University, a minority-serving institution, will lead the effort to promote undergraduate summer internships in BRD research. The *expected outcome* of undergraduate students summer internships is that students will have an enhanced knowledge of disease prevention and will present their research findings at the annual BRD conferences or the Southern Great Plains Dairy Consortium. The *importance* of this outcome is that the work of the students advances the research aims, exposes the students to the research process and educates the livestock community.

- Four undergraduate summer research internships will be available in a choice of laboratories of the BRD research investigators. Interns will be mentored, provided a project that they will complete during the summer, and will present the completed project at the annual BRD conference.

3. Develop a veterinary feedlot and dairy internship program.

Veterinary internships will be completed at commercial dairy and feedlot operations, offering real-life experiences under the guidance and mentoring of an established veterinarian. The opportunity to work in a feedlot or dairy will often aid future veterinarians in determining whether this is an area that they would like to continue to work in and expose them to a field that they might otherwise not have considered. Veterinarians from minority-serving institutions will be encouraged to participate. The *expected outcome* of providing veterinary internships to four veterinarians is that they will gain experience critical for

diagnosing, treating and preventing BRD and will learn of the daily challenges and rewards of working with cattle in a feedlot or dairy. The *importance* of this outcome is that advanced knowledge and experience in identifying BRD and reducing its prevalence utilizing a multidisciplinary approach will be a skill these individuals will carry with them the rest of their careers.

- Two, twelve-month veterinary internships will be conducted at the Gonzalez, Texas feedlot with Dr. Charles Graham.
- Two, three-month veterinary dairy internships will be conducted at the University of California at Davis, Tulare Veterinary Center with Dr. Terry Lehenbauer.

4. Sponsor graduate and undergraduate students to attend the Southern Great Plains Dairy Consortium where extension and research activities will be ongoing.

The Southern Great Plains Dairy Consortium in conjunction with New Mexico State University, a minority-serving institution, will provide an intensive dairy management course for sponsored undergraduate, graduate and veterinary students. The Southern Great Plains Dairy Consortium's goals are to establish multi-disciplinary faculty teams to address the industry's identified problems and issues through collaborative research, education, extension and service programs and to provide coordinated and enhanced undergraduate and graduate training programs. The *expected outcome* of sponsoring 10 undergraduate and 5 graduate students to attend the Southern Great Plains Dairy Consortium is that these students will have advanced management skills and have learned multi-disciplinary approaches to reduce BRD. The *importance* of this outcome is, like the veterinary internships, these students will gain valuable knowledge, experiences and contacts within the industry that will aid them in future careers in animal agriculture.

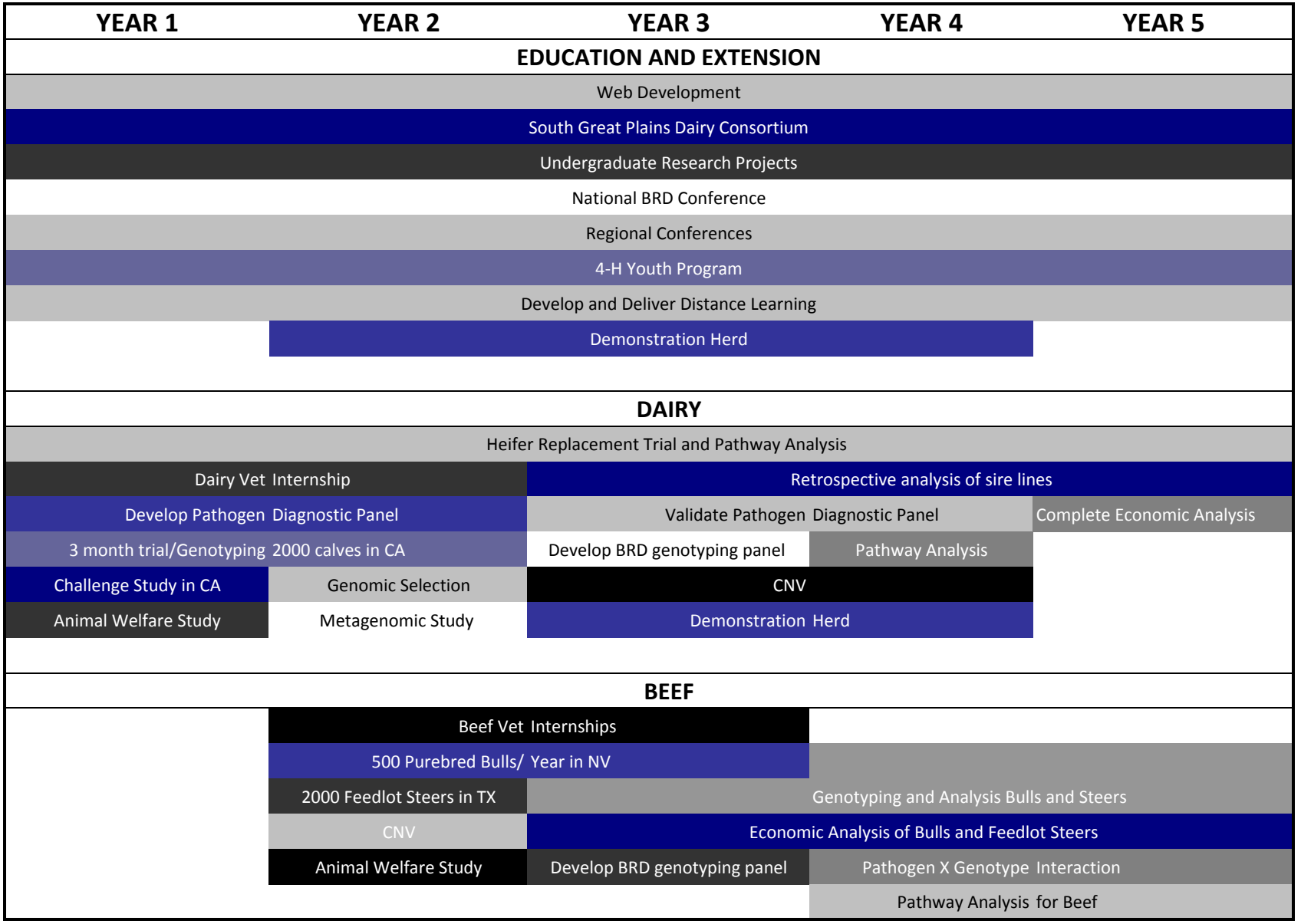
- Two undergraduate students and one graduate student per year will be funded to attend and learn about dairy management and BRD prevention. Special effort will be made to encourage participation from groups that are under-represented in science, including Native Americans, African-Americans and Hispanics.

5. A 4-H curriculum will be developed to expose and train youth on animal disease using BRD as an example application.

4-H youth livestock programs involve thousands of youth nationwide. These programs provide youth with the opportunity to use live animals to develop livestock production knowledge and life skills. Animal health management is an important component of their livestock project as they apply quality assurance practices to vaccination procedures and animal handling. Beef shows where participants bring their steers to a show grounds and then bring them home before they sell their project at their fair or sale show, are increasing in popularity. This represents a particular example where BRD is likely to impact their project. For shows involving beef and dairy heifers, these animals will likely return to production herds after exposure to BRD at the show. The objective of this education aim is to build on this project's research discoveries and Extension outputs to develop 4-H education products. The *expected outcome* of this aim is that 4-H youth will be able to identify approaches to minimize the risk of BRD in the 4-H cattle projects. The *importance* of this outcome is that the 4-H youth, many of whom will be future cattle producers, will have heightened awareness of the risk of BRD thereby reducing the prevalence of the disease.

a. **Develop interactive education games on animal health and BRD**. Interactive games will be developed for educating 4-H youth about BRD. These games will be developed for use in group 4-H meetings, at livestock shows and exhibits, and for individual use. Interactive games have been found to be effective for 4-H audiences as a learning tool.

b. **Develop an animal health management curriculum**. Animal health curriculum (leaders guide, youth workbook and interactive games) will be developed and available for use by 4-H leaders for animal health and BRD education and activities. These materials will be incorporated into eXtension and 4-H websites. A proposal will be submitted to use animal disease as the 4-H National Youth Science Day experiment topic. If the proposal is accepted, we will develop an animal disease 4-H National Youth Science experiment.


YEAR 1
YEAR 2
YEAR 3
YEAR 4
YEAR 5
EDUCATION AND EXTENSION
Web Development
South Great Plains Dairy Consortium
Undergraduate Research Projects
National BRD Conference
Regional Conferences
4-H Youth Program
Develop and Deliver Distance Learning
Demonstration Herd
DAIRY
Heifer Replacement Trial and Pathway Analysis
Dairy Vet Internship
Retrospective analysis of sire lines
Develop Pathogen Diagnostic Panel
Validate Pathogen Diagnostic Panel
Complete Economic Analysis
3 month trial/Genotyping 2000 calves in CA
Develop BRD genotyping panel
Pathway Analysis
Challenge Study in CA
Genomic Selection
CNV
Animal Welfare Study
Metagenomic Study
Demonstration Herd
BEEF
Beef Vet Internships
500 Purebred Bulls/ Year in NV
2000 Feedlot Steers in TX
Genotyping and Analysis Bulls and Steers
CNV
Economic Analysis of Bulls and Feedlot Steers
Animal Welfare Study
Develop BRD genotyping panel
Pathogen X Genotype Interaction
Pathway Analysis for Beef