Beef Sessions

Moderators: Hans Coetzee, Tye Perrett, Calvin Booker

Integrated program for reducing bovine respiratory disease complex in beef and dairy cattle coordinated agricultural project (BRD CAP)

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Abstract

There is growing interest in selective breeding of livestock for enhanced disease resistance. Bovine respiratory disease (BRD), or pneumonia, is the largest single natural cause of death in US beef and dairy cattle, and BRD resistance represents an obvious target for selective breeding programs. The heritability of disease resistance is typically low, in part as a result of suboptimal diagnosis (i.e. not all sick animals are identified, healthy animals may be incorrectly diagnosed as ill, and some susceptible animals will appear resistant when in fact they have not been exposed). Additionally, the genetic basis of BRD susceptibility is likely complex and governed by the effects of multiple genes. This suggests a large number of case:control animals will be needed in datasets for disease susceptibility marker discovery. In 2011, the Agriculture and Food Research Initiative (AFRI) funded a five-year Coordinated Agricultural Project entitled "Integrated Program for BRD in Beef and Dairy Cattle". The overarching research objective of this multi-institutional "BRD CAP" project is to use newly-available genomic tools to identify host genome regions associated with susceptibility to BRD. During 2011, 2,000 dairy calves on a single large California facility were enrolled in a BRD case:control design study using an objective scoring system, and genotyped using a high-density bovine single nucleotide polymorphism (SNP) chip. It is envisaged that analyses of these data and future studies will lead to development of DNA tests to enable the selection of animals that are less susceptible to BRD, which is much-needed given growing concern regarding both animal welfare and use of antimicrobial therapeutics.

Résumé

Nous constatons un intérêt croissant pour l'élevage sélectif de troupeaux offrant une plus grande résistance aux maladies. Le syndrome respiratoire bovin, ou pneumonie, est la plus importante cause naturelle de décès chez les bovins de boucherie et les bovins laitiers aux États-Unis, et la résistance à cette maladie constitue une cible évidente pour les programmes d'élevage sélectif. L'héritabilité de la résistance à la maladie est généralement faible, en partie en raison de diagnostics non optimaux (c.-à-d. que les animaux malades ne sont pas tous identifiés, les animaux sains peuvent faire l'objet de diagnostics erronés et certains animaux susceptibles peuvent sembler résistants alors qu'en réalité ils n'ont pas été exposés). De plus, le fondement génétique de la susceptibilité au syndrome respiratoire bovin est vraisemblablement complexe et assujetti aux effets de gènes multiples. Cela semble indiquer qu'un grand nombre d'animaux témoins seront nécessaires dans les bases de données pour découvrir le marqueur de la susceptibilité à la maladie. En 2011, l'Agriculture and Food Research Initiative (AFRI) a financé un projet quinquennal - Coordinated Agricultural Project – intitulé « Integrated Program for BRD in Beef and Dairy Cattle ». L'objectif global de la recherche dans le cadre de ce projet « BRD CAP » multi-institutionnel consiste à utiliser des outils génomiques nouvellement disponibles pour identifier les régions du génome hôte associées à la susceptibilité au syndrome respiratoire bovin. En 2011, 2 000 veaux laitiers d'une même grande exploitation californienne ont été inscrits dans une étude conceptuelle de cas-témoins sur le syndrome respiratoire bovin faisant appel à un système de notation objectif et génotypé utilisant une puce à polymorphisme nucléotidique (SNP) bovin à haute densité. On estime que l'analyse de ces données et des études à venir mèneront à la mise au point de tests d'ADN qui permettront de sélectionner des animaux moins susceptibles au syndrome respiratoire bovin, ce qui est très souhaitable, compte tenu des inquiétudes grandissantes au sujet du bien-être des animaux et de l'utilisation de produits thérapeutiques antimicrobiens.

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Introduction

Bovine respiratory disease (BRD), also known as shipping fever or pneumonia, is the leading cause of illness and death for the backgrounding and feedlot cattle industries and is the most important cause of disease-related economic losses. Of the cattle that become ill in the feedlot, 67 to 82% suffer from respiratory disease. In the United States, 1.4% of all feedlot cattle perish before reaching harvest weight, and the majority of this loss is the result of respiratory disease. Indeed, more feedlot cattle die from BRD than all other diseases combined, and this trend is increasing. This, despite the fact that more than 90% of large feedlots use a respiratory vaccine to combat the disease, and almost all (99.8%) feedlot animals diagnosed with BRD were treated with an antibiotic as part of the treatment regimen. In addition to death loss and treatment costs, detrimental effects of BRD include reduced carcass weight, daily gain, yield grade, carcass fat measurements, and muscle shear-force measurements.[6]

It is well known that stressors like weaning, transportation, commingling at the sale yard, and dust can all increase the incidence of BRD. There is a reason that buyers offer a backgrounding premium for cattle that have been weaned prior to sale, are enrolled in a known vaccination program, and are shipped as an entire load that can be fed as a pen, thereby avoiding the stress of commingling necessitated by smaller lots. These management factors help to minimize the likelihood that an animal will get BRD.

Evidence that BRD susceptibility also has a host genetic component is demonstrated by breed differences in BRD morbidity and mortality, and the fact that BRD prevalence in unweaned calves and feedlot cattle has been reported to be heritable.[6,7] One study reported that the heritability of BRD incidence in feedlot animals was 0.18.[6] Recently, loci on chromosomes 2 and 26 have been identified as being linked with BRD prevalence, and loci in these regions were also implicated with persistent infection with bovine viral diarrhea virus (BVDV).[8] Collectively, these data provide strong support for the role of innate genetic resistance to BRD.

Heritability (h^2) is defined as the proportion of observed variation in a particular trait that can be attributed to inherited genetic factors, in contrast to environmental ones. It is the relative importance of genetics versus environment in determining phenotype. A trait that has high heritability (e.g. height) is largely determined by genetics, whereas a trait that has a low heritability is greatly influenced by the environment. With a trait such as height, phenotype definition is straightforward and relatively easily measured. The same cannot be said for host susceptibility to infectious diseases.

There are some technical reasons why BRD heritabilities tend to be low under field conditions. These include suboptimal diagnosis (e.g. not all sick animals are identified and healthy animals may be incorrectly diagnosed as ill), and some susceptible animals will appear resistant to a disease when in fact they have not been exposed to the disease agent (viruses and/or bacteria in the case of BRD). These confounding factors add environmental noise to field data, which decreases heritability. Field studies therefore likely underestimate the true importance of genetics in BRD incidence, and thus also undervalue the potential gains that could be made by breeding for disease resistance. It has been observed that while traits describing immune responses to infections are often highly heritable, the disease outcomes that these traits influence tend to be lowly heritable. This is due in part to the fact that the estimable genetic variation between animals will be influenced by variable exposure and the sensitivity of diagnosis.[1]

What is the BRDC Coordinated Agricultural Project?

Newly available genomic tools offer an opportunity to employ novel genetic approaches to select for more disease resistant cattle. It was with this overarching goal in mind that a interdisciplinary team of veterinarians with BRD complex experience in both dairy and beef cattle, geneticists (with specialties in animal health, disease, immunology, epigenetics, and quantitative genetics), epidemiologists, animal behaviorists, microbiologists, livestock economists, and veterinary and livestock cooperative extension specialists developed a successful five-year USDA grant proposal entitled "Integrated Program for Reducing Bovine Respiratory Disease Complex (BRDC) in Beef and Dairy Cattle." The goal of this program is to reduce the incidence of BRD in beef and dairy cattle by capitalizing on recent advances in genomics to enable novel genetic approaches to select for cattle that are less susceptible to disease. This effort, known as the BRD CAP (Coordinated Agricultural Project), involves a multi-institutional team led by Dr. James Womack at Texas A&M University, and involves research groups from Washington State University, University of Missouri, Colorado State University, New Mexico State University, and University of California, Davis. Coordinated Agricultural Projects are large-scale USDA National Research Initiative (NRI) awards intended to promote collaboration, open communication and exchange of information and to coordinate activities among individuals, institutions, states, and regions. CAP participants serve as a team that conducts targeted research, education, and extension in response to emerging or priority area(s) of national need. For more information on the BRD CAP see www.BRDComplex.org.

If host BRD resistance is a quantitative trait governed by the action of many genes, as might be expected for this complex trait, then a large dataset of case and control animals will be needed to detect all of the large and small effect loci. As a general rule, increasing the size of the case-control study population results in increased power. Studies including several hundred to a thousand case and control studies are recommended to achieve the statistical power to detect genetic variants with small effects on relative risk of disease.[1] The Bovine HapMap consortium data further suggests that case-control studies undertaken in cattle should endeavor to use animals of the same breed and genetic heritage to maximize the probability that any differences observed in SNP allele frequency are due to differences in disease susceptibility to BRD, and not to population substructure (i.e. breed-related differences in allelic frequencies).[1]

Obtaining markers that track disease resistance loci relies on "linkage disequilibrium" (LD) between DNA markers and the causative loci, in this case those associated with disease resistance. With the widely-used Bovine 50K SNP marker panel (Illumina) which contains 50,000 SNP genetic markers evenly distributed across the 3 billion base pairs that make up the bovine genome, a marker associated with a trait in one breed is often found to not be associated in the same way in another breed (Figure 1A). The reason for this is that the relatively sparse spacing of SNP markers means that they may be located a "long way" from the gene, and so the relationship (phase) between the SNP and the variant of the gene causing a given phenotype may vary between breeds.

In cattle it has been estimated that SNP need to be spaced less than 10 kb apart to show consistent LD phase across breeds.[3] It is anticipated that by increasing the number of SNP markers to > 700,000, as has been undertaken in the new generation of high density SNP arrays (e.g. Affymetrix Bovine 650K, Illumina Bovine HD SNP Array), it will be possible to track loci that are associated with disease susceptibility.[1] More densely spaced markers will increase the likelihood of finding SNPs that are situated close to the gene (Figure 1B, red SNP), and it is hoped that such markers will be predictive in more than one breed (Figure 2, red SNP). These high density bovine marker panels may also allow for the pooling of valuable datasets from multiple *Bos taurus* breeds to increase the size of the "discovery" dataset and enable the development of DNA tests that are predictive of host disease resistance in multiple breeds. There is also the tantalizing prospect that the whole genome sequencing of individual bulls, currently ongoing on hundreds of bulls throughout the world due to the ever decreasing cost of sequencing, may further accelerate the identification of SNP that are directly affecting BRD resistance.

Preliminary data

The BRD CAP research plan includes experiments designed to identify loci associated with BRD resistance in beef and dairy cattle. Because BRD susceptibility is most likely a complex genetic trait governed by the effects of many genes, a large number of cases and controls will be needed to detect all of these variants, so that datasets for disease resistance marker discovery will
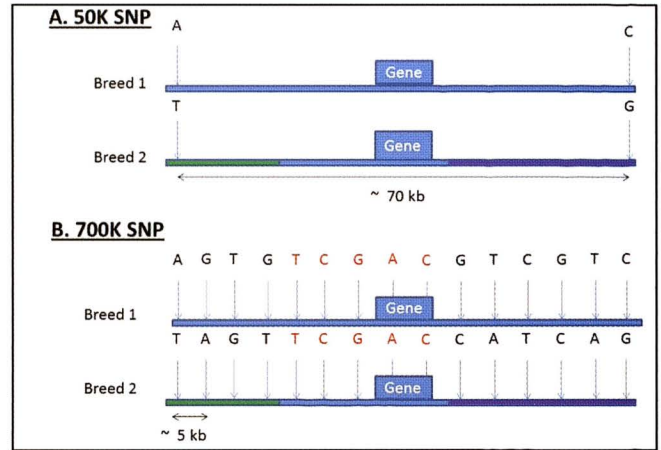

Figure 1. Marker location relative to the gene of interest in two breeds when using (**A**) a 50K SNP marker panel (markers spaced at 70 thousand base pair (70 kb) intervals), or (**B**) a high-density 700K SNP marker panel (markers spaced at approximately 5 thousand base pair (5 kb) intervals).
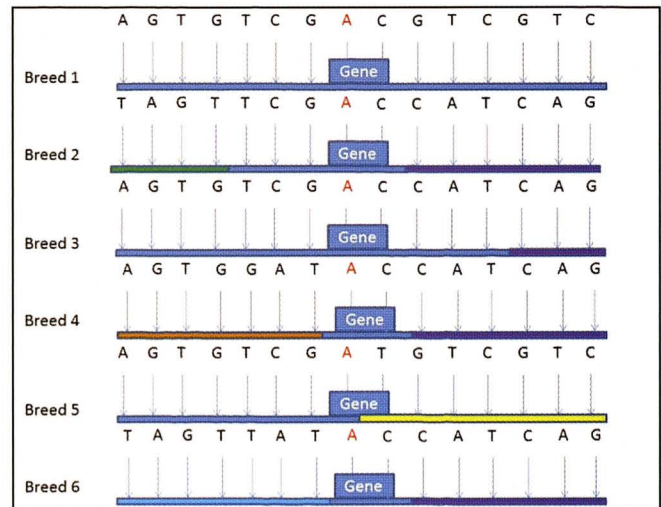

Figure 2. High density SNP marker panels may enable the discovery of markers that are predictive of disease susceptibility in more than one breed (i.e. red "A" SNP located in the gene).

need to be comprised of observations on several thousands of individuals.[2] To address this need, a large field trial was carried out in 2011 on a calf-raising ranch in the central valley of California. This trial was led by Dr. Terry Lehenbauer, DVM, Associate Professor and Director, Veterinary Medicine Teaching and Research Center in Tulare. The objective of the study was to identify host loci associated with susceptibility to BRD in Holstein calves between day 10 and weaning (day 63).

One-day-old Holstein calves transported to a calf ranch in Hanford, California were monitored daily for signs of BRD using established calf-health scoring criteria.[4,5] BRD-affected calves scored ≥ 6, and controls scored < 6. Controls were selected from hutches adjacent to cases. Over 200,000 calves were screened to obtain 1,003 cases and 969 controls over 180 days. Blood samples and diagnostic swabs (mid-nasal and two deep pharyngeal) were obtained for each calf. DNA was extracted from blood using the Qiagen DNA extraction kit, and genotyped using the Illumina Bovine HD SNP Array. Seventy-six calves were discarded from the analysis due to a single nucleotide polymorphism (SNP) call rate of < 95%, and 28 were removed as outliers following principal component analysis, leaving 1865 calves for the genome wide association study (GWAS). SNP were discarded if their minor allele frequency was <1% (143,357), more than 10% failed (16,603), there was a difference in the proportion that failed between cases and controls (29), or they failed the Hardy-Weinberg Equilibrium test (490 SNPs <1x10^{-90}), thereby leaving 617,483 SNPs for analysis.

A genome wide association study (GWAS) was conducted with PLINK in the R environment. Multiple testing corrections were based on modified Welcome Trust recommendations that accounted for array size. Strong evidence for an association was found on BTA27 (p=1.5x10^{-7}) and moderate evidence (p<3x10^{-5}) for associations involving 155 SNPs were found across 33 genomic regions on bovine chromosomes. Bovine chromosomes 4, 11, 12, 17 and 18 possessed the vast majority of moderately associated SNPs, with 23 SNPs (two regions), 25 SNPs (four regions), 24 SNPs (three regions), 15 SNPs (two regions), and 29 SNPs (two regions), respectively (H. L. Neibergs, Washington State University, personal communication). These results represent a preliminary analysis of ongoing work to identify loci associated with BRD. Further analyses will examine the interaction between host genotype and the results from the virology and bacteriology analyses reported by the diagnostic laboratory. In 2012 an analogous experiment is planned with 1000 case and 1000 control *Bos taurus* cattle in a feedlot setting.

Conclusion

The advent of high-density SNP chips concurrently with the infusion of USDA research funding focused on the development of large "training" populations of genotyped animals with phenotypes on hard to measure traits (e.g. feed efficiency and BRD resistance), offers the tantalizing prospect that DNA-based selection criteria to enable genetic improvement in hard to measure traits of high value may soon be available for the cattle industry. Developing large multi-breed training data sets for disease phenotypes may allow for the development of DNA tests that are predictive of host genetic merit for disease resistance in multiple breeds. The development of DNA tests to enable the selection of animals that are less susceptible to BRD is an important component of the BRD CAP's approach to reduce the considerable animal morbidity, mortality, and economic losses associated with BRD in US cattle production systems.

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