ISSN 1466-2523

Animal Health Research Reviews **15**(2); 157–160
doi:10.1017/S1466252314000231

Results of the BRD CAP project: progress toward identifying genetic markers associated with BRD susceptibility

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Received 12 June 2014; Accepted 15 September 2014; First published online 11 November 2014

Abstract

The Bovine Respiratory Disease Coordinated Agricultural Project (BRD CAP) is a 5-year project funded by the United States Department of Agriculture (USDA), with an overriding objective to use the tools of modern genomics to identify cattle that are less susceptible to BRD. To do this, two large genome wide association studies (GWAS) were conducted using a case:control design on preweaned Holstein dairy heifers and beef feedlot cattle. A health scoring system was used to identify BRD cases and controls. Heritability estimates for BRD susceptibility ranged from 19 to 21% in dairy calves to 29.2% in beef cattle when using numerical scores as a semi-quantitative definition of BRD. A GWAS analysis conducted on the dairy calf data showed that single nucleotide polymorphism (SNP) effects explained 20% of the variation in BRD incidence and 17–20% of the variation in clinical signs. These results represent a preliminary analysis of ongoing work to identify loci associated with BRD. Future work includes validation of the chromosomal regions and SNPs that have been identified as important for BRD susceptibility, fine mapping of chromosomes to identify causal SNPs, and integration of predictive markers for BRD susceptibility into genetic tests and national cattle genetic evaluations.

Keywords: bovine respiratory disease, BRD CAP project, genetic markers, BRD susceptibility.

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Introduction

There is growing interest in the selective breeding of livestock for enhanced disease resistance. In dairy cattle, selection programs have been developed to take advantage of genetic variability in mastitis resistance, despite the fact that the heritability of clinical mastitis is low and mastitis resistance has a correlation with production traits (Rupp and Boichard, 2003). Likewise, chicken breeders have long used breeding to improve resistance to avian lymphoid leucosis complex and Marek's disease (Stear *et al.*, 2001).

The heritability of disease resistance is typically low, partly 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). Obtaining predictive markers that track disease resistance loci relies on 'linkage disequilibrium' (LD) between DNA markers and the causative loci, in this case those associated with Bovine Respiratory Disease (BRD) resistance. 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 identify and estimate the effect of all of the large and small causative loci contributing to susceptibility. As a general rule, increasing the accuracy of disease diagnosis and the size of the case:control study population results in increased power to detect loci associated with disease. Studies involving several hundred to a thousand cases and matched control animals are recommended to achieve the statistical power required to reliably detect genetic variants with small effects on relative risk of disease (Allen *et al.*, 2010).

In 2011, United States Department of Agriculture (USDA) AFRI funded a 5-year grant proposal entitled the 'Integrated Program for Reducing Bovine Respiratory Disease Complex (BRDC) in Beef and Dairy Cattle' Coordinated Agricultural Project. This effort, known as the BRD CAP, 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, USDA ARS, and the University of California, Davis. Coordinated Agricultural Projects are large-scale USDA National Research Initiative (NRI) awards intended to promote collaboration, open communication, exchange information, and coordinate activities among individuals, institutions, states, and regions. CAP participants serve as a team that conducts targeted research or a combination of research, education, and extension in response to emerging or priority area(s) of national need (Van Eenennaam, 2012).

Genome wide association studies

One of the primary aims of the BRD CAP is to reduce the prevalence of BRD in beef and dairy cattle through the identification of genetic loci associated with BRD susceptibility, and to use this information to develop DNA-based selection tools. The foundations of this research effort are two large (2000 animal) BRD case:control field studies. The first of these was carried out on a large commercial dairy calf ranch in 2011/2012 by researchers at the UC Davis School of Veterinary Medicine Teaching and Research Center in Tulare (T. W. Lehenbauer, S. S. Aly. J. H. Davis, P. V. Rossitto). Pre-weaned Holstein calves from California (CA) ($n = 2015$) were scored using the McGuirk (University of Wisconsin) scoring system which evaluates rectal temperature, cough, nasal and eye discharges, and ear position or head tilt (McGuirk, 2008). For each clinical sign, a numerical value of 0–3 was assigned based on the severity of the clinical signs. Cases were assigned as a score ≥5; controls were <5, typically 3 or less. In CA over 200,000 calves were screened to obtain 1003 cases and 1012 controls over 180 days (Neibergs *et al.*, 2013). Each day after completion of respiratory scoring and identification of cases and matched controls, a veterinarian and trial personnel then returned to those animals to collect samples. All case and control calves had nasopharyngeal and pharyngeal recess swabs collected for qPCR diagnostics (B.M. Crossley) and a second pharyngeal recess swab collected for aerobic bacteria and *Mycoplasma* respiratory pathogen culturing (P. C. Blanchard), and blood was taken for DNA extraction and genotyping using the Illumina BovineHD (~770 K) BeadChip (H. L. Neibergs). A similar protocol was followed to obtain a validation population of pre-weaned dairy calves in New Mexico (NM) ($n = 763$) (R. Hagevoort, E. Chavez).

An analogous study design was used to obtain 497 cases and 498 controls from a commercial feedlot in 2013 (H. L. Neibergs). All cases and controls were *Bos taurus* beef cattle. The mean clinical score for cases was 8.04 ± 1.23 and the mean score for controls was 2.06 ± 0.037 (Neibergs *et al.*, 2014b). Collection of additional cases and controls for beef cattle is in progress.

Preliminary results

Calves identified as BRD cases in the California dairy study using the criterion of a McGuirk score ≥5 were significantly associated with positive cultures of BRSV, *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, and *Mycoplasma* spp. All 2030 samples tested for BVDV were negative (Lehenbauer *et al.*, 2013).

Heritability estimates for BRDC susceptibility in dairy calves were 19–21% for NM and CA as individual populations and 13% when combined (Neibergs *et al.*, 2014a). In beef cattle, the heritability estimates were 17.7% for the binary case-control phenotype, and 29.2% when using numerical values of the McGuirk system (that ranged from 0 to 12) as a semi-quantitative definition of BRDC (Neibergs *et al.*, 2014b). These estimates are higher than a prior estimate of 0.18 that was obtained for beef feedlot animals, when adjusted to an underlying continuous scale (Snowder *et al.*, 2005). The higher heritability estimate may be attributable to the use of the objective calf health scoring system to more precisely identify BRD cases and controls. Heritability is known to increase when the accuracy of the measured phenotype (i.e. correct assignment

https://doi.org/10.1017/S1466252314000231 Published online by Cambridge University Press

of calves to BRDC case or control group) improves (Neibergs *et al.*, 2014b).

A case-control genome wide association study (GWAS) was conducted on the dairy calf data in the laboratories of H. L. Neibergs at Washington State University, C. M. Seabury at Texas A&M University, and J. F. Taylor at the University of Missouri, using four different analytical approaches (GBLUP, EMMAX, SNP and Variation Suite 7 and PLINK). The SNP effects explained 20% of the variation in BRD incidence (Neibergs *et al.*, 2013), and 17–20% of the variation in clinical signs (Seabury *et al.*, 2014). All analytical approaches identified concordant single SNP associations on bovine chromosomes 3, 15 and 23. Twelve additional chromosomes provided evidence for association with two or more approaches. When chromosomal regions (rather than single SNPs) were compared, 29 regions on 13 chromosomes were associated with BRD including those identified in the single SNP association comparison (Neibergs *et al.*, 2013). These results represent a preliminary analysis of ongoing work to identify loci associated with BRD. Subsequent work will include fine mapping in regions associated with BRD (C. M. Seabury, TX) to attempt to identify the causal mutations that actually result in BRDC susceptibility rather than predictive markers that are located in linkage disequilibrium with these mutations. Additional analyses will look at the interaction between host genotype and the results from the diagnostic laboratory.

It should be noted that the GWAS studies detailed in this paper are just one component of the BRD CAP research being conducted to identify genomic regions associated with BRDC susceptibility. Other approaches include the identification of DNA structural variants (differences in the number of copies of a DNA region that alters gene expression) that have major effects on BRDC susceptibility (L. Skow, and S. Dindot, TX); gene expression (RNA-sequencing) studies from challenged animals to identify genes differentially expressed in cattle in response to a pathogen (L. Gershwin, CA and J. F. Taylor, MO); and pathway analysis to identify genes whose individual effects on BRDC susceptibility are small but whose effects on BRDC susceptibility are great when taken together with other genes present in a biological pathway (H. L. Neibergs, WA). It is envisaged that jointly these studies will result in the information required to enable the much-needed development of DNA tests for the selection of animals that are less susceptible to BRDC.

A validation of the chromosomal regions and SNPs that have been identified as important for BRD susceptibility will be conducted by estimating the predicted transmitting abilities (PTA) of the sires of the CA and NM Holstein calves and evaluating whether their predicted PTA is confirmed by the prevalence of disease in their offspring. The development of genomic breeding values for Holstein sires that are less susceptible to BRDC is underway (C. Van Tassell, MD). Ultimately, the trait of BRD susceptibility will need to be included in the dairy industry economic selection index ($NetMerit). The appropriate selection emphasis for this trait will need to be weighted by its effect on profitability relative to other economically important traits.

The translation of results to the beef industry presents a more challenging situation. It has been found that the accuracy of prediction equations developed in one breed of beef cattle have not proven to be useful in other breeds when using the more sparsely spaced 50 K SNP chip (Van Eenennaam *et al.*, 2014). The BRD CAP envisioned that by performing the GWAS with the Illumina BovineHD BeadChip using ~770 K SNPs, predictive markers would be associated with many loci associated with BRD susceptibility and that such markers would be predictive across beef breeds. It is not yet known if this will in fact be the case. There are also developing efforts to whole genome sequence numerous prominent beef bulls throughout the world as a result of the ever decreasing cost of sequencing, and these efforts may further accelerate the identification of causal SNPs that directly affect susceptibility to BRDC.

To date the US beef industry has made little use of economic selection indexes (Garrick and Golden, 2009). Although there is undoubtedly huge value associated with selection against BRD susceptibility (Van Eenennaam and MacNeil, 2012; Neibergs *et al.*, 2014b), to incentivize the inclusion of genomic BRD susceptibility criteria in breeding decisions, and to offset the concomitant decreased selection pressure on growth traits that return value directly to producers who do not retain ownership (i.e. sell on a weight basis prior to feeding), there would need to be some mechanism analogous to a backgrounding premium to transfer the value derived from delivering animals that are less susceptible to BRD to the feedlot and back up the supply chain to the producers and breeders involved in making the selection and management decisions that ultimately impact the prevalence of BRD in the beef cattle industry.

Acknowledgments

The authors acknowledge the Bovine Respiratory Disease Consortium Coordinated Agricultural Project team members, and USDA Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30367 from the USDA National Institute of Food and Agriculture.

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