Genetic approaches to identify genomic regions associated with decreased susceptibility to bovine respiratory disease complex

Mahesh Neupane,[1] Jennifer N Kiser,[1] Christopher M Seabury,[2] Jeremy F Taylor,[3] James E Womack,[2] The Bovine Respiratory Disease Complex Coordinated Agricultural Project Research Team, Holly L Neibergs[1]
[1]Department of Animal Sciences, Washington State University, P.O. Box 646310, Pullman, WA 99164-6310
[2]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX
[3]Division of Animal Sciences, University of Missouri, Columbia, MO

Abstract

One approach to reducing losses from BRDC is the implementation of selection of cattle that are less likely to be affected by disease by the use of dairy predicted transmitting ability (PTA) or beef expected progeny differences (EPD) for susceptibility to BRDC. The PTAs or EPDs would be based on genotypes associated with animals less susceptible to disease and incorporated into commercial genotyping platforms for industry use. The discovery of quantitative trait loci (QTL) associated with BRDC susceptibility is the first step in implementing the selection of cattle for enhanced resistance to BRDC. Feedlot crossbred *Bos taurus* (n=408) heifers from Washington were used to illustrate how genetic approaches identify BRDC susceptibility QTL that will form the basis for selection of cattle with increased resistance to the disease. The crossbred cattle consisted of 234 cases and 174 controls as defined by the McGuirk Health scoring system. Cases and controls remained in the same pens together throughout the study. The incidence of BRDC in the feedlot was 2.5% over a 15-month period. The distinction between cases and controls was based on the McGuirk health scoring system with possible integer scores ranging between 0 and 12; calves with scores ≤4 were identified as controls and those with a score ≥5 were identified as a case. Of the 174 controls, 4 (1.7%) subsequently became ill with BRDC and were reclassified as a case. The mean health score was 9.44 ± 1.19 for cases and 2.36 ± 0.61 for controls. Deep pharyngeal and mid-nasal swabs were taken for bacteriology and virology diagnostics. *Histophilus somni* had the greatest difference in prevalence between cases and controls (odds ratio = 1.65, 95% confidence interval 1.05 – 2.58, $p < 0.05$) among the detected pathogens. A genome-wide association analysis (GWAA) and genomic heritability estimate was computed using Illumina BovineHD BeadChip genotypes. Heritability of susceptibility to BRDC was estimated at 37%. After correction for population stratification using 30 principal components, the genomic inflation factor was 1.02. Single nucleotide polymorphisms (SNPs) were filtered for low call rate (<90%), Hardy-Weinberg disequilibrium ($p < 1x10^{-50}$) and low minor allele frequency (<1%) which left 669,933 SNPs for the GWAA. Animals were also filtered for low genotyping call rate (<90%) leaving 220 cases and 164 controls. The GWAA identified 43 QTL ($p < 5x10^{-5}$) on 15 chromosomes. The identification of QTL associated with BRDC susceptibility is the first step towards implementing selection of cattle that are less likely to be affected by BRDC through the use of genomic PTAs or EPDs.

Key words: cattle, genomics, EPD, BRD

Résumé

Une approche pour réduire les pertes de BRDC est la mise en oeuvre de la sélection des bovins qui sont moins susceptibles d'être affectées par une maladie par l'utilisation de capacité de transmission prédite laitiers (ATP) ou boeuf différences prévu dans la descendance (EPD) pour leur susceptibilité à BRDC. Les APT ou EPD serait basée sur les génotypes associés avec des animaux moins sensibles à la maladie et incorporés dans les plates-formes de génotypage commerciale pour utilisation par l'industrie. La découverte de loci de traits quantitatifs (QTL) associés à la susceptibilité BRDC est la première étape dans la mise en oeuvre de la sélection du bétail pour une plus grande résistance aux BRDC. Feedlot métissées Bos taurus (n=408) génisses de Washington ont été utilisées pour illustrer comment les approches génétiques identifier susceptibilité BRDC QTL qui formeront la base d'une sélection de bétail avec une résistance accrue à la maladie. Le bétail croisé se composait de 234 cas et 174 contrôles comme défini par le système de notation de Santé McGuirk. Les cas et les témoins sont demeurés dans le même enclos ensemble tout au long de l'étude. L'incidence de BRDC dans les parcs d'engraissement a été de 2,5 % sur une période de 15 mois. La distinction entre les cas et les témoins était fondée sur le système de notation de santé McGuirk avec possible integer scores variant entre 0 et 12; veaux avec des scores ≤4 ont été identifiés comme témoins et ceux avec un score ≥ 5 ont été identifiés comme un cas. Des 174 contrôles, 4 (1,7 %) est ensuite devenu malade avec BRDC et ont été reclassifiés comme un cas. Le score de santé moyenne était de 9,44 ± 1,19 pour les cas et de 2,36 ± 0,61 pour les contrôles.

Deep pharyngée et mi-nasal échantillons ont été prélevés pour diagnostics de bactériologie et de virologie. Histophilus somni avait la plus grande différence dans la prévalence entre les cas et les témoins (rapport de cotes = 1,65, intervalle de confiance à 95 % 1,05 - 2,58, p < 0,05) parmi les a détecté la présence d'agents pathogènes. A genome-wide association analyse (GWAA) et de l'héritabilité génomiques estimation a été calculée à l'aide de génotypes BeadChip BovineHD Illumina. L'héritabilité de la susceptibilité aux BRDC était estimée à 37 %. Après correction pour la stratification de la population à l'aide de 30 composantes principales, le facteur d'inflation génomiques a été de 1,02. Les polymorphismes mononucléotidiques (SNP) ont été filtrées pour de faibles taux d'appel (<90%), déséquilibre Hardy-Weinberg (p < 1x10-50) et de faible fréquence d'allèles mineurs (<1%) qui 669,933 SNPs pour l gauche GWAA. Les animaux ont également été filtrée en fonction de taux d'appel de génotypage faible (<90 %) en laissant 220 cas et 164 témoins. La GWAA identifié 43 QTL (p < 5x10-5) sur 15 chromosomes. L'identification de QTL associés à la susceptibilité BRDC est la première étape vers la mise en oeuvre de la sélection des bovins qui sont moins susceptibles d'être touchées par BRDC grâce à l'utilisation de la génomique apt ou l'utilisation d'EPD.

Introduction

The morbidity and mortality experienced in cattle from the bovine respiratory disease complex (BRDC) has not diminished over the past 2 decades despite management practices aimed at reducing stress and the utilization of vaccination and treatment programs.[8-10] A complementary approach to reducing losses from BRDC is the implementation of selection of cattle that are less likely to be affected by BRDC by the use of dairy predicted transmitting ability (PTA) or beef expected progeny differences (EPD) for BRDC. The PTAs or EPDs will be based on genotypes at loci that are associated with animals less susceptible to disease and incorporated into commercial genotyping platforms for industry use. The discovery of the genotypes and quantitative trait loci (QTL) associated with BRDC susceptibility was approached by comparing the genotypes of group-housed cattle that had BRDC to the genotypes of cattle that did not have BRDC. Feedlot crossbred *Bos taurus* (n=408) heifers from Washington were studied to illustrate how genetic approaches to BRDC susceptibility will be used to select for cattle with increased resistance to the disease.

Few studies have been conducted to identify the genomic regions (QTL) associated with bovine respiratory disease complex.[1,2,4,5] A recent study of pre-weaned Holstein calves (1379 cases and 1311 controls) identified QTL associated with BRDC susceptibility and found a moderate estimate of heritability of 21% when the analysis of the calves was separated by geographic regions (California and New Mexico).[4] However, a lower estimated heritability of 13% was found when the groups were combined and analyzed together.[4] The differences in heritability between the individual sites and the combined results may be due to differences in management, frequency of pathogens/BRDC differences, or genetic differences in the cattle. A similar study has just been completed in beef feedlot cattle (1012 cases and 1001 controls) from Colorado and Washington. The objective of this study was to use a subset of the Washington cattle (408 crossbred *Bos taurus* heifers), to illustrate the genetic approaches to the identification of genomic regions associated with decreased susceptibility to BRDC.

Materials and Methods

This study was conducted with an approved animal use protocol from the Institutional Animal Care and Use Committees at Washington State University (04110). *Bos taurus* crossbred heifers were evaluated for signs of BRDC at a single commercial Washington feedlot with a one-time capacity of approximately 12,000 cattle. Heifers were vaccinated upon arrival at the feedlot with a pentavalent MLV respiratory vaccine,[a] but did not receive prophylactic antibiotics. Samples were collected from November 2013 through January 2015 and consisted of 234 cases and 174 controls. The incidence of BRDC in the feedlot was 2.5% over this period.

BRDC diagnosis

Heifers were evaluated for signs of BRDC and assigned a health score for each of the following clinical signs: rectal temperature, cough, nasal discharge, eye discharge, and ear tilt. For each of these signs, a score was assessed as 0 for normal, 1 for slightly abnormal, 2 for abnormal, and 3 for severely abnormal[3] by 1 of 3 feedlot personnel. The largest of the scores assessed between the eye and ear health scores was summed with the other 3 clinical scores for temperature, cough, and nasal discharge. Health scores ranged from a low of 0 (normal clinical signs, healthy) to a high of 12 (abnormal clinical signs, unhealthy for all categories). Calves with health scores ≤4 were identified as controls and those with a score ≥5 were identified as a case. Once a case was identified and enrolled in the study, a healthy animal in the same pen was recruited as a control. Heifers that were enrolled as a control but subsequently became ill with BRDC (n = 4) were reclassified as a case and 2 healthy animals were obtained from the same pen to serve as controls; 1 for the calf newly diagnosed as a BRDC case, and 1 for the former BRDC case. Mean health scores were 2.36 ± 0.61 for controls and 9.44 ± 1.19 for BRDC cases. The time until diagnosis of cases after arrival in the feedlot was 43.8 ± 30.6 days for 452 heifers (which included all of the cases of all breeds in Washington). The dates of pen removal due to illness ranged from day 1 (on arrival in the feedlot) to 148 days after arrival in the feedlot.

Sample collection

After heifers were removed from the pens and assessed using the McGuirk system, 1 of 3 feedlot personnel collected

2 pharyngeal recess swabs with a 27-inch guarded polyester swab, a 6-inch mid-nasal polyester swab,[b] and a blood sample as previously described.[4] One of the pharyngeal recess swabs was used to identify aerobic bacteria and mycoplasma respiratory pathogens, and the other was used for qPCR diagnostics of respiratory viruses along with the mid-nasal swab. Bacterial samples were submitted daily to the Washington Animal Disease Diagnostic Laboratory at Washington State University and viral qPCR samples were stored at -122°F (-80°C) until submission to the California Animal Health and Food Safety Laboratory at the University of California at Davis. Culturing was conducted for *Mycoplasma* and for *Histophilus somni, Pasteurella multocida,* and *Mannheimia haemolytica*. Quantitative PCR for bovine corona virus, bovine respiratory syncytial virus, bovine viral diarrhea virus, and bovine herpes virus type I (the pathogen responsible for infectious bovine rhinotracheitis) were conducted from an aliquot of 3 ml of transport media that housed the virology swabs in 5 ml cryotubes as previously described.[4]

Analysis of diagnostic data

The estimated odds ratio and 95% confidence intervals for BRDC pathogens, and case status were computed. A 2-tailed Fisher's exact test was used to determine if the odds ratios differed significantly from 1, with a significance threshold of p = 0.05.

DNA isolation and genotyping

Isolation of bovine DNA was performed from 3 ml of whole blood collected in EDTA tubes using the Puregene DNA extraction kit[c] per the manufacturer's instructions. DNA samples were quantified and their purity estimated using the NanoDrop 1000 spectrophotometer.[d] Samples with 260/280 ratios of 1.8 to 2.0 were diluted to 50ng/ml and genotyped using the Illumina BovineHD Genotyping Beadchip[e] at Neogen Laboratories in Lincoln, NE.

Heritability estimation

Heritability was estimated using a genomic relationship matrix and the pseudo-heritability equation

$(h^2 = \sigma^2)$

in the efficient mixed-model association eXpedited (EMMAX) statistical test included in the SNP and Variation Suite 8.3.1 software.[f]

Genome wide association analysis

The BRDC phenotype was expressed as a binary (case-control) trait based on the McGuirk health scores taken at the time of diagnosis. To determine the QTL associated with susceptibility to BRDC, a principal component analysis approach was used within SVS8 software.[f] This method of analysis corrects for population stratification by applying principal components to genotype data to infer axes of variation which are caused either by breed composition differences or pedigree differences among the animals. Genotypes and phenotypes were adjusted using the largest 30 principal components. The modified EIGENSTRAT analyses were conducted using an additive model and consisted of single SNP tests of association.[6] As a result of principal component correction, the genomic inflation factor declined from an uncorrected value of 1.06 to a corrected value of 1.02. Data were not further normalized by the theoretical or actual standard deviation of the SNP genotype frequency.

Quality control filtering of animals was conducted to remove animals (n=24; 14 cases and 10 controls) with <90% of their genotypes called. Quality control filtering of SNPs was done to remove SNPs that provided genotyping results in <90% of animals (15,464 SNPs) and SNPs whose minor allele frequency was <1% (95,506 SNPs). A Hardy-Weinberg test of equilibrium was calculated for all 666,992 SNPs to identify SNPs whose genotypic frequencies were highly skewed ($p < 1x10^{-50}$) and that should be removed. This removed an additional 59 SNPs for a total of 666,933 SNPs remaining for the analysis.

Significance for association tests was based on the recommendation of the Wellcome Trust Case Control Consortium[11] where unadjusted p-values less than 5×10^{-7} were considered to provide strong evidence of association and unadjusted p-values between 5×10^{-5} and 5×10^{-7} were considered to provide moderate evidence of association. There was no detectable effect of pen ($P = 0.24$) on BRDC susceptibility when evaluated by a *t* test.

Results and Discussion

Diagnostic results

The bacteriology, virology and *Mycoplasma* spp diagnostic results are shown in Table 1. The vaccination of cattle with a modified-live vaccine for infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), and bovine respiratory syncytial virus (BRSV) seemed to be effective in that few cases or controls were positive for them by qPCR. Because of the small number of heifers identified as having these viruses, the 95% confidence interval for the odds ratios were extremely large, suggesting that the accuracy of assessing the odds ratio for these viruses was low. Cattle were not vaccinated against potential BRDC bacterial or *Mycoplasma* spp pathogens and a higher frequency of detection of these organisms in both cases and controls was observed. *Mycoplasma* was the most frequently detected organism as it was found in almost all cases (90%) and controls (92%). *Histophilus somni* was detected in 23% of controls and 33% of cases, and was the only BRDC pathogen tested that differed ($p < 0.05$) in frequency between the cases and controls in the crossbred heifers. These data differed from those taken from the preweaned Holstein dairy calves in that all of the organisms were higher ($p<0.05$) in frequency in cases than in controls except BVDV and IBR.[4]

Table 1. Results from aerobic bacteriology and mycoplasma culture from deep pharyngeal swabs and qPCR from virology samples taken from the mid-nasal and deep pharyngeal recess.from BRDC cases and controls.

Pathogen	Frequency in Controls & (Cases)#	Odds Ratio*	95% CI	P-Value
Arcanobacterium pyogenes	0% (0%)	0.74	0.02 to 37.26	0.88
Histophilus somni	23% (33.3%)	1.65	1.05 to 2.58	0.03
Mannheimia haemolytica	35.5% (38%)	1.12	0.74 to 1.68	0.60
Pasteurella multocida	44.8% (45.5%)	1.03	0.69 to 1.53	0.15
Mycoplasma spp	91.9% (90.2%)	0.81	0.40 to1.62	0.55
Bovine corona virus	12% (13.3%)	1.12	0.61 to 2.06	0.72
Bovine viral diarrhea virus	1.2% (0.4%)	0.37	0.03 to 4.13	0.42
Bovine herpes virus (IBR)	0.6% (2.2%)	6.76	0.36 to126.49	0.20
Bovine respiratory syncytial virus	0% (1.7%)	3.82	0.44 to 33.04	0.22

Frequency of obtaining a positive result for the pathogen in controls and cases (in parentheses), respectively, from deep pharyngeal swabs for bacteria and mycoplasma and deep pharyngeal and mid-nasal swabs for virology. Heifers classified as indeterminate for the presence of a pathogen were not included in the summary statistics.*Odds ratios were computed as the likelihood of a heifer being affected with BRDC when the pathogen was present when the animal was swabbed.

The collection of diagnostic results at 1 point during the disease process as was performed in this study does not provide a complete picture of the pathogens that may be involved in BRDC. However, it does provide a snapshot into the pathogens that tend to be more common in a group of cattle and can help inform us as to differences in the etiology of BRDC found in 1 set of cattle compared to another.

Heritability estimate

The heritability estimate for susceptibility to BRDC was 37% with the V_A = 0.09 and the V_E = 0.16. This estimate is higher than that computed for the Holstein preweaned calves, which was approximately 21% for each of the California and New Mexico groups, but only 13% for the 2 groups combined.[4] The higher heritability estimate in the feedlot crossbred heifers may have been due to a more consistent diagnosis of the BRDC phenotype, reduced variation in management practices, a more consistent response to a pathogen challenge in older animals as compared to preweaned calves, or a more limited set of pathogens causing BRDC in the feedlot heifers.

Genome-wide association analysis

After quality control filtering and population stratification correction, 43 SNPs across 15 bovine chromosomes were identified to be associated with BRDC susceptibility as shown in Figure 1 and Table 2. Positional candidate genes or genes that are located near a QTL for BRDC susceptibility are shown in Table 2. These positional candidate genes are diverse in their functions with few clear obvious functional ties to BRDC.

The QTL associated with BRDC susceptibility in feedlot heifers differed from those found in the preweaned Holstein calves.[4] These differences may be due to the maturation of the immune system in preweaned calves (≤ 70 days) compared to an older feedlot calf (6 to 15 months of age), differences in the pathogen prevalencies between the populations, or underlying genetic differences associated with crossbred beef and purebred dairy breeds. The genetic differences between breeds have been shown to limit the use or predictability of QTL across breeds.[7,12] Quantitative trait loci discovered in 1 breed may not segregate in another breed or they may have a much different allele frequency leading to a much smaller effect on the same trait.[7] Linkage disequilibrium, the non-random segregation of alleles into gametes at meiosis or into individuals which survive selection varies across breeds due to patterns of allele frequencies. Most QTL are detected based on SNPs that are in linkage disequilibrium with the underlying genetic cause of the trait variation. The lack of strong linkage disequilibrium between the causal mutation and the SNP which serves as its proxy, can result in the failure to detect or replicate detection of QTL across breeds. One way to combat this difficulty is to identify and use the causal mutation (rather than a proxy) for selection. Ideally, the causal mutation would be identified and directly incorporated into dairy predicted transmitting ability (PTA) or beef expected progeny differences (EPD) for BRDC susceptibility. This would result in a more accurate prediction of genetic merit for BRDC susceptibility across breeds and the accuracy would be maintained for a longer period of time without having to "re-train" the predictions based on new studies to account for the decay in linkage disequilibrium between the proxy SNP and causal mutations as recombination occurs between these loci. Studies to identify the causal mutations are ongoing as part of the BRDC CAP led by Dr. J.E. Womack.

To fully translate the information generated from these genetic studies to the industry, additional work must be done: uniform diagnostic criteria for BRDC have to be embraced by the beef and dairy industry, low-cost commercial genotyp-

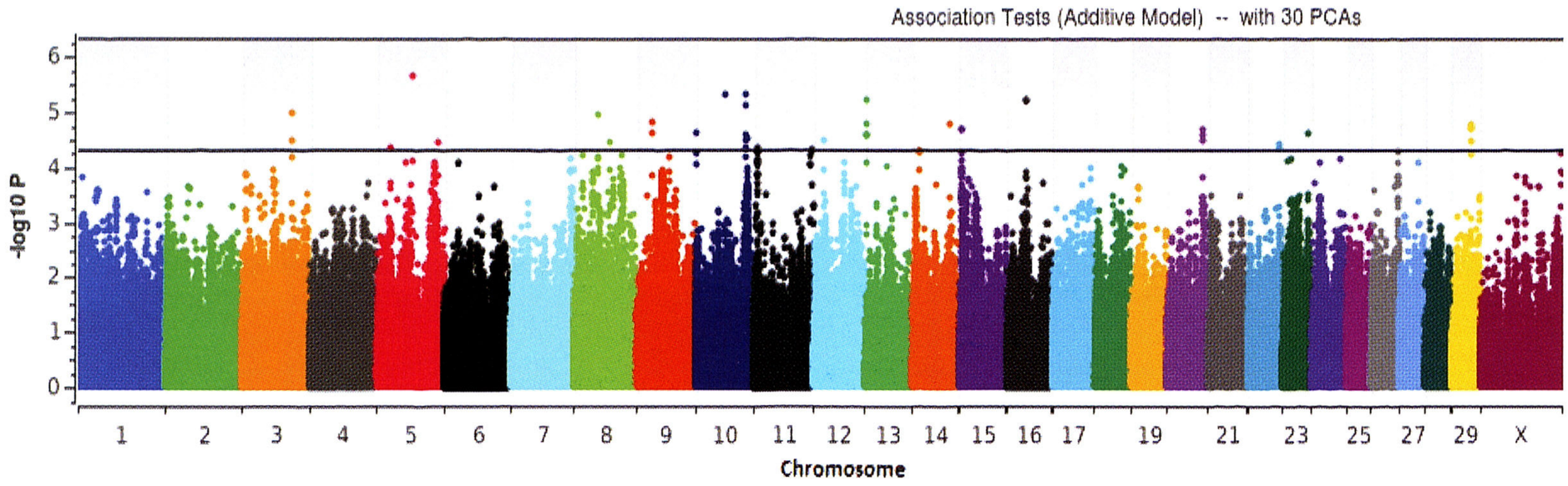

Figure 1. Manhattan plot of QTL associated with BRDC susceptibility in crossbred heifers using a modified EIGENSTRAT method.

Case-control phenotypes were corrected for breed or pedigree stratification by using 30 principal components. The –log10 P-values are indicated on the Y axis, and the bovine chromosomes are listed on the X axis. The black line indicates the Wellcome Trust threshold[11] for SNPs with modest evidence for association with BRDC susceptibility.

Table 2. Genome wide association analyses for the Washington crossbred heifers with the QTL that are associated with BRDC susceptibility with an additive model.

Bovine chromosome	Location (Mb)	Lead SNP (No. SNPs)#	P value of lead SNP	Positional candidate genes*
5	63-64	*rs133139217* (1)	2.26 x 10^{-6}	*IKBIP*(intron), *TMPO, SLC25A3, APAF1, snoRA53*
10	89-90	*rs133099391* (5)	4.94 x10^{-6}	*POMT2* (intron), *TMEM63C, NGB, GSTZ1*
10	51-52	*rs132639112* (1)	5.01 x 10^{-6}	*MYO1E* (intron), *CCNB2*
13	2-3	*rs29018207* (5)	6.14 x 10^{-6}	*MRPL33*
16	34-35	*rs41798809* (2)	6.19 x 10^{-6}	*AKT3* (intron)
3	89-90	*rs133281954* (2)	1.08 x 10^{-5}	*DAB1* (intron)
8	43-44	*rs42504347* (1)	1.15 x 10^{-5}	*DMRT2*
9	26-27	*rs136007637* (2)	1.59 x 10^{-5}	none
29	34-35	*rs135-38561* (4)	1.72 x 10^{-5}	none
14	66-67	*rs110130542* (1)	1.76 x 10^{-5}	*SPAG1* (intron), *RNF19A*
20	64-65	*rs137118255* (3)	2.08 x 10^{-5}	*SEMA5A* (intron)
15	4-5	*rs134143723* (2)	2.10 x 10^{-5}	none
10	0-1	*rs43711688* (1)	2.45 x 10^{-5}	*MCC* (intron)
23	46-47	*rs42032717* (1)	2.58 x 10^{-5}	none
10	91-92	*rs135001438* (1)	3.1 x 10^{-5}	none
12	16-17	*rs110205276* (1)	3.36 x 10^{-5}	*LRCH1* (intron)
5	108-109	*rs110637038* (1)	3.65 x 10^{-5}	*ERC1* (intron)
8	62-63	*rs42591069* (1)	3.69 x 10^{-5}	none
22	56-57	*rs133019015* (2)	3.93 x 10^{-5}	*VGLL4* (intron)
5	23-24	*rs135884310* (1)	4.51 x 10^{-5}	none
11	4-3	*rs110593550* (3)	4.65 x 10^{-5}	*TSGA10* (intron), *C2ORF15*
11	105-106	*rs110156380 (1)*	5.18 x 10^{-5}	*ENTPD8* (intron, synonymous codon, 5' UTR)
26	49-50	*rs109550921* (1)	5.49 x 10^{-5}	*MGMT* (intron)

rs SNP identification number of the lead SNP is the SNP with the smallest p value of the SNPs associated with BRDC in that genomic region; Number of SNPs with a p value < 5.5 x10^{-5} in the same genomic region. * Genes within 50kb 5' or 3' of the genomic region defined by the significant SNPs are listed. If one of the associated SNPs falls within a gene, it is noted.

ing platforms with BRDC SNPs must be available, selection indexes must be developed that incorporate the BRDC PTA/EPD information, and a validation of the SNPs as a predictor of BRDC susceptibility must be completed. A consensus must be reached on the diagnostic criteria to be used for identifying an animal with BRDC in the beef and dairy industries. Diagnostic criteria must be chosen with a consideration of how it affects the heritability of BRDC susceptibility as the objective is to use it for selection of resistant cattle. Diagnostic criteria that are too general will lower the heritability and will reduce the ability for genetic selection to make gains in reducing BRDC. Criteria that are too time-consuming, expensive or cumbersome to complete in a commercial setting will not be adopted or used. Beef and dairy committees are working on this important task. As the BRDC-CAP consortium identifies SNPs associated with BRDC susceptibility, the QTL information is being published and provided to commercial vendors for incorporation into their genotyping panels so that they can be used within the industry. Dairy PTA predictions are underway and EPDs will follow when the beef data are analyzed in their entirety. Validation studies are underway in independent beef and dairy cattle populations to confirm the QTL with the greatest effect on BRDC susceptibility have been identified.

Conclusions

Bovine respiratory disease complex continues to sicken and kill millions of cattle in the United States each year. Susceptibility to BRDC has a genetic basis that can be used to lower the impact of this disease on the cattle industry. This study has shown that heritability estimates for BRDC susceptibility in commercial crossbred beef cattle are moderate (37%) and that QTL can and have been identified that can predict an animal's susceptibility to BRDC. These predictions will be incorporated into selection indexes to be used by producers as another tool to reduce the prevalence of BRDC.

Endnotes

[a]Bovishield Gold 5, Zoetis, Floram Park, NJ
[b]Kalajian Industries, Signal Hill, CA
[c]Gentra, Minneapolis, MN
[d]Nanodrop 1000 Spectrophotometer, Wilmington, DE
[e]Illumina BovineHD Genotyping Beadchip, San Diego, CA
[f]SVS8; Golden Helix, Bozeman, MT

References

1. Casas E, Garcia MD, Wells JE, Smith TP: Association of single nucleotide polymorphisms in the *ANKRA2* and *CD180* genes with bovine respiratory disease and presence of Mycobacterium aviu subsp. paratuberculosis. *Anim Genet* 42:571-577, 2011.
2. Leach RJ, O'Neill RG, Fitzpatrick JL, Williams JL, Glass EJ: Quantitative trait loci associated with the immune response to a bovine respiratory syncytial virus vaccine. *PLoS One* 7:e33526, 2012.
3. McGuirk SM: Disease management of dairy calves and heifers. *Vet Clin North Am Food Anim Pract* 24:139–153, 2008.
4. Neibergs HL, Seabury CM, Wojtowicz AJ, Wang Z, Scraggs E, Kiser J, Neupane M, Womack JE, Van Eenennaam AE, Hagevoort GR, Lehenbauer TW, Aly S, Davis J,. Taylor JF: Susceptibility loci revealed for bovine respiratory disease complex in pre-weaned Holstein calves. *BMC Genomics* 15:1164, 2014.
5. Neibergs HL, Zanella RJ, Casas E, Snowder GD, Wenz J, Neibergs JS, Moore D: Loci on BTA2 and BTA26 are linked with bovine respiratory disease and associated with persistent infection of bovine viral diarrhea virus. *J Anim Sci* 89:907–915, 2011.
6. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D: Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 38:904–909, 2006
7. Raven LA, Cocks BG, Hayes BJ: Multibreed genome wide association can improve precision of mapping causative variants underlying milk production in dairy cattle. *BMC Genomics* 15:62, 2014
8. Snowder GD, Van Vleck LD, Cundiff LV, Bennett GL: Influence of breed, heterozygosity, and disease prevalence on estimates of variance components of respiratory disease in preweaned beef calves. *J Anim Sci* 83:1247–1261, 2005.
9. USDA: Cattle and calves nonpredator death loss in the United States, 2005. Fort Collins: USDA-APHIS-VS, CEAH; 2005. CO #N462.0507.
10. USDA: Dairy 2007, Part V: Changes in dairy cattle health and management practices in the United States, 1996–2007. Fort Collins, CO: USDA-APHIS-VS, CEAH; 2007. #519.0709.
11. Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447:661–678, 2007.
12. Wiener P, Edriss MA, Williams JL, Waddington D, Law A, Wooliams JA, Gutiérrez-Gill B: Information content in genome-wide scans: concordance between patterns of genetic differentiation and linkage mapping associations. *BMC Genomics* 12:65, 2011.