Loci on Bos taurus chromosome 2 and Bos taurus chromosome 26 are linked with bovine respiratory disease and associated with persistent infection of bovine viral diarrhea virus

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ABSTRACT: The objective of this study was to identify loci linked with bovine respiratory disease (BRD) and subsequently to determine if these same loci were associated with bovine viral diarrhea virus persistent infection (BVD-PI) in affected calves or their dams. A genome-wide linkage study using 312 microsatellites was conducted to identify loci linked with BRD in a Brahman × Hereford sire half-sib family. Disease incidence was recorded from birth to slaughter by daily monitoring. Linkage was suggestive for a QTL on BTA2 ($F = 7.31$, $P = 0.007$) and BTA26 ($F = 10.46$, $P = 0.001$). Six and 7 markers were added and genotyped between 110 and 126 cM on BTA2 and between 42 and 72 cM on BTA26, respectively, in the intervals where linkage was found. These markers were used to reevaluate the Brahman × Hereford family and to evaluate 3 additional crossbred half-sib families. Linkage was found with BRD on BTA2 ($F = 4.94$, $P < 0.01$), with a peak at 110 cM, and on BTA26 ($F = 4.03$, $P < 0.05$), with peaks at 42 and 52 cM. The same markers were then tested for an association with BVD-PI in 1) BVD-PI calves compared with age-matched unaffected calves from the same herd or 2) dams with BVD-PI compared with age-matched unaffected calves. Sixty commercial beef cow-calf herds were tested for BVD-PI, and 79 calves from 8 ranches had BVD-PI. Four of 6 markers were associated ($P = 4.8 \times 10^{-9}$ to $P = 0.01$) with BVD-PI on BTA2, and 4 of 7 markers were associated ($P = 0.008$ to $P = 0.04$) with BVD-PI on BTA26 when BVD-PI calves were compared with unaffected calves. The comparison of BVD-PI dams with unaffected calves detected associations with BVD-PI for all markers tested on BTA2 ($P = 3 \times 10^{-9}$ to $P = 0.005$) and for 3 of 7 markers on BTA26 ($P = 1.4 \times 10^{-6}$ to $P = 0.006$).

Key words: bovine viral diarrhea, locus, respiratory disease

INTRODUCTION

Bovine respiratory disease (BRD) contributes significantly to cattle mortality in the United States. Of all beef calves that were born alive and did not survive to weaning in 2005, 29.6% were associated with BRD (National Agricultural Statistics Service, 2006). In weaned and mature cattle, 24.3% of animals that died were associated with BRD. Combined, 28.7% of all cattle deaths were associated with respiratory disease and accounted for an annual economic loss in excess of $692 million (National Agricultural Statistics Service, 2006).

The BRD complex of diseases results from viral, mycoplasmal and bacterial pathogens. One of the pathogens associated with BRD is bovine viral diarrhea (BVD) virus (BVDV). Bovine viral diarrhea is reported to be the most costly viral disease worldwide, and exposure of healthy cattle to BVD persistently infected (BVD-PI) animals increases the risk of initial treatment for respiratory tract disease by 43% (Loneragan et al., 2005). This is consistent with the first publications describing BVDV in North American cattle as having a significant

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respiratory component (Olafson et al., 1946). A more recent study of the prevalence of BVDV infection in calves with BRD stated that 38.5% had serologic evidence of infection with BVDV type 1 and 27.9% had evidence of infection with BVDV type 2 and that these infections often occurred in conjunction with infections from other viruses associated with BRD (Fulton et al., 2000). Bovine viral diarrhea virus types 1 and 2 are detected by PCR and antigenic differences (Ridpath and Bolin, 1998, Ridpath et al., 2000). Bovine viral diarrhea virus type 1 genotypes are frequently associated with respiratory disease and fibrinous pneumonia (Fulton et al. 2000). Bovine viral diarrhea virus type 2 genotypes exhibit a range of clinical disease similar to that associated with BVDV type 1 (Fulton et al. 2002). The role of BVDV as an immunosuppressant and its synergistic effect with other BRD pathogens may be greater than its role as a primary respiratory pathogen (Campbell, 2004).

Bovine viral diarrhea has an acute and a persistent form. Fetal calves become persistently infected after transplacental BVDV exposure before the development of the immune system at approximately 125 to 150 d of gestation (Brock, 2003). The ability of the virus to cross the placenta is partly due to the immunological response of the dam to the virus. Once the BVDV crosses the placenta, the stage of development of the immune system of the calf becomes paramount. The rate of the immunological development of the calf is a function of its genetic background inherited from both parents. Therefore, both the genetic and maternal components contribute to BVD-PI susceptibility or resistance of the calf. To account for the roles of the dam and the calf in BVD-PI, both the dams and calves were evaluated for an association of BVD-PI.

The objectives were 1) to identify loci linked with BRD, 2) to determine if these same loci were associated with BVD-PI, and 3) to identify whether the maternal or calf genetic effect had a greater influence on a calf becoming persistently infected by comparing the allele frequencies of the dams of the BVD-PI calves with control calves, and by comparing the allele frequencies of the BVD-PI calves and control calves.

### MATERIALS AND METHODS

All animal procedures used were exempt by the Institutional Animal Care and Use Committee of Washington State University because no live animal use was involved, except that which reused tissues obtained and submitted for routine animal health and disease diagnostic purposes. Experimental procedures were approved and performed in accordance with US Meat Animal Research Center (USMARC) Animal Care Guidelines and the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teachings (Federation of Animal Science Societies, 1999).

#### Animals for BRD Study

In the initial linkage study, a Brahman × Hereford (BH) sire half-sib family developed at the MARC was investigated (Table 1). The sire was previously included in the USDA reference population to generate the cattle linkage map (Kappes et al., 1997). The sire was mated to MARC III composite cows (one-fourth Hereford, one-fourth Angus, one-fourth Red Poll, and one-fourth Pinzgauer) in 1994 to produce 288 offspring, and in 1996 to produce 259 offspring (547 offspring total). Genotyping was conducted on 43 calves affected with BRD and 140 calves born at the same time and reared together that were unaffected. Calves were weaned at an average of 187 d and raised from weaning to castration on a corn-corn silage diet. Cattle were slaughtered at a commercial packing plant at an average age of 455 d.

A second half-sib family using a Brahman × Angus (BA) sire, also developed at USMARC, was included with the BH sire half-sib family for the interval mapping of BRD on BTA2 and BTA26 (Table 1). For this study, the BA sire was mated to Hereford, Angus, MARC III, and F1 cows from the Germlaspm Evaluation Project Cycle IV to produce 259 offspring in 1995, and was mated to MARC III cows in 1996 to produce 361 offspring (620 total). Breeds of sires for the F1 cows were Hereford, Angus, Shorthorn, Charolais, Gelbvieh, Pinzgauer, Galloway, Longhorn, Nellore, Piedmontese,

### Table 1. Characteristics of animals in the bovine respiratory disease (BRD) linkage study

<table>
<thead>
<tr>
<th>Sire breed</th>
<th>Dam breed</th>
<th>Total No. of offspring</th>
<th>Total affected by BRD (%)</th>
<th>No. affected genotyped</th>
<th>No. unaffected genotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brahman × Hereford</td>
<td>MARC III</td>
<td>547</td>
<td>49 (9%)</td>
<td>43</td>
<td>140</td>
</tr>
<tr>
<td>Brahman × Angus</td>
<td>MARC III, Hereford, Angus, F1 germplasm</td>
<td>660</td>
<td>31 (4.7%)</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>Piedmontese × Angus</td>
<td>MARC III</td>
<td>246</td>
<td>2 (0.8%)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Belgian Blue × MARC III</td>
<td>MARC III</td>
<td>209</td>
<td>2 (1%)</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

1The MARC III composite breed consists of one-fourth Hereford, one-fourth Angus, one-fourth Red Poll, and one-fourth Pinzgauer.
2F1 germplasm cows were from Hereford or Angus cows, and sire breeds consisted of Hereford, Angus, Shorthorn, Charolais, Gelbvieh, Pinzgauer, Galloway, Longhorn, Nellore, Piedmontese, or Salers.
3Total number of offspring that were diagnosed with BRD, with the prevalence indicated in parentheses.
4Total number of offspring genotyped that were diagnosed with BRD.
5Total number of offspring genotyped that were not diagnosed with BRD.
or Salers. Breeds of the F₁ cows were Hereford or Angus. Genotyping from this family was done on 31 calves diagnosed with BRD and 62 unaffected calves born at the same time and reared together with the affected calves. Two other half-sib families were produced from a Piedmontese × Angus (PA) and a Belgian Blue × MARC III (BM) sire mated to MARC III cows (Table 1). For this study, 2 animals with BRD and 2 unaffected animals were used from each of the PA and BM families. The average age of weaning for all calves was 205 d. Calves were fed a corn-corn silage diet from weaning to slaughter, and the average age at harvest at a commercial packing plant was 467 d.

**Animals for BVD Study**

Ear-notch samples (1 cm²) were collected from 8,624 calves from 44 herds in 2008 and 16 herds in 2009 from commercial beef cow-calf ranches located in 18 counties in the state of Washington. The number of animals in each herd varied: 15 herds had fewer than 50 cows, 11 herds consisted of 50 to 100 cows, 31 herds consisted of 100 to 499 cows, and 3 herds had more than 500 cows. All ear-notch samples were collected on site and sent to the Washington Animal Disease Diagnostic Laboratory at Washington State University in Pullman for BVD-PI testing. Real-time reverse-transcription PCR was performed on up to 36 pooled ear-notch samples for detection of BVDV. Each ear-notch sample from a PCR-positive pool was individually tested by commercial BVDV antigen capture ELISA (Idexx, Westbrook, ME). When fewer than 12 ear-notch samples were submitted, samples were not pooled and only the BVDV antigen capture ELISA test was performed. Calves suspected to be BVD-PI after initial testing underwent collection of a second sample (usually a 9-mL blood sample) a minimum of 21 d later to confirm its disease status by a second antigen capture ELISA test. The dam of the suspected BVD-PI calf was also sampled at that time to determine her BVD-PI status and to obtain DNA for genotyping.

Deoxyribonucleic acid extraction was performed on 10 mg of frozen ear notches from each animal. Samples were placed into a 2-mL microfuge tube containing 300 μL of Gentra PureGene (Gentra Systems, Minneapolis, MN) cell lysis solution and 10 mm stainless steel beads and homogenized with a Retsch bead homogenizer (Retsch, Haan, Germany) for 2 min at 50 cycles/s. After homogenization, extraction was performed according to the instructions of the manufacturer (Gentra Systems). The quantity and quality of DNA was assessed using 260/280 nm wavelength readings from a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). The 260/280 nm readings for all samples ranged between 1.8 and 2.0. Deoxyribonucleic acid was subsequently diluted to 10 ng/μL for use with PCR.

**BRD Diagnosis**

Disease incidence was recorded by daily monitoring from birth to slaughter by the staff veterinarian, the beef cattle research technicians, or both, as described previously (Snowder et al., 2005; Casas and Snowder, 2008). Diseases were detected by physical examination, necropsy, or laboratory analyses and were appropriately coded. Additional disease codes included for BRD were bronchitis, emphysema, pleuritis, pulmonary adenomatosis, upper respiratory infection, and pleural fibrosis. Because such diseases may be interpreted as BRD by feedlot staff, it seemed appropriate to include them with the BRD codes. The majority of the BRD classifications were for pneumonia (86%) and respiratory disease (11%; Snowder et al., 2006). Typical clinical BRD symptoms included 1 or more of the following: fever, rapid breathing, repetitive coughing, nasal or eye discharge or both, diarrhea, dehydration, and appetite depression.

Health records included frequency, but not severity, data. Records were binary: 0 for unaffected cattle and 1 for affected cattle. Animals were coded as affected if they were diagnosed with the disease at least once during their life. This was done to avoid multiple records on the same animal because of lingering BRD or reinfection. Treatment for BRD included administration of 1 or more antibiotics (oxytetracycline, ceftiofur, florfenicol, ampicillin, and sulfadimethoxine) to the calf (Snowder et al., 2005). Animals that were not detected with clinical symptoms of BRD may have been the result of an animal that resisted infection of the microbe, was not exposed to BRD-causing pathogens, or was exposed to a BRD-causing pathogen but was able to show little or no harm from the exposure (tolerant). The incidence of clinical BRD was the trait measured in this study, rather than tolerance to BRD.

**BVD Diagnostic Testing**

All diagnostic testing was conducted at the Washington Animal Disease Diagnostic Laboratory at Washington State University in Pullman. Ear notches collected on the premises were sent to the laboratory in 13-mL polypropylene tubes. An aliquot of 2 mL of PBS was added to each tube containing the ear notch, vortexed for 7 to 10 s, and set aside for 10 min at room temperature. An aliquot of 1 mL of the supernatant was removed for total RNA extraction with the MagMax-96 Blood RNA Isolation Kit (Ambion, Austin, TX; Wilson et al., 2009). Quantitative reverse-transcription PCR was subsequently used to determine the presence of the virus by using an AgPath-ID BVDV qRT-PCR Kit (Applied Biosystems Inc., Foster City, CA). Positive extraction controls, negative extraction controls, positive amplification controls, and negative amplification controls were run with up to 36 PCR pooled samples per well on an ABI 7500 Fast Thermocycler (Applied
Reverse transcription was facilitated by incubation at 45°C for 10 min, followed by inactivation of the transcriptase by heating to 95°C for 10 min. Cycling parameters included 45 cycles of denaturing at 95°C for 15 s and annealing-extension at 60°C for 45 s. Samples were considered positive for BVDV if they were greater than threshold levels by cycle 36 according to the directions of the manufacturer (Applied Biosystems Inc.).

If a pooled sample was positive, all animals present in the pool were retested with an Idexx BVD Antigen Test kit (Idexx Laboratories, Westbrook, ME) according to the instructions of the manufacturer, to identify the individual(s) with the BVD virus. A second sample from a suspected BVD-PI calf was obtained a minimum of 21 d after the initial sample and again tested with the Idexx BVD Antigen Test kit per the instructions of the manufacturer (Edmonson et al., 2007). Thus, animals suspected of being BVD-PI were tested 3 times to determine their disease status. However, 3 calves were tested only twice because they had died, as reported by the producers. Ear-notch or 9-mL blood samples from dams of BVD-PI calves were collected when second samples were collected from suspected BVD-PI calves. Samples collected from the dams underwent the same series of diagnostic testing as those for the calves.

Genotyping

Casas et al. (2003) and Casas and Stone (2006) provided a detailed description of the markers used to detect QTL in the BH family. Briefly, a total of 312 markers were used to cover 2,850 cM of the genome. Informative markers were chosen based on their location in each chromosome and their ease of scoring. Amplification reactions for each marker were done with purified DNA extracted from blood with a saturated salt procedure (Miller et al., 1988). Amplification conditions have been described previously (Kappes et al., 1997). The interval mapping of the 16-cM region of linkage with BRD on BTA2 was initially defined by 2 microsatellite markers: BMS2519 at 110 cM and IDVGA-2 at 126 cM. To determine if an association with BVD-PI existed in this region of BTA2, 6 microsatellite markers were genotyped that had been mapped within this 9-megabase (Mb) interval (Table 2). A 9-cM region on BTA26 was linked to BRD, and 7 microsatellite markers were genotyped that had been assigned to the 11-Mb interval (Table 2). The 5′ primer of each primer set was labeled with a fluorescent tag to facilitate pooling of post-PCR samples and visualization of size differences on an ABI 3730 DNA analyzer (Applied Biosystems, Inc.). Markers BM4505, BM6041, DIK5023, and IDVGA-72 were labeled with 6-Fam (a nonproprietary blue fluorescent dye); DIK2387, BMS882, and DIK4572 were labeled with VIC (an Applied Biosystems green fluorescent dye); DIK2476, IDVGA-64 and DIK4627 were labeled with NED (an Applied Biosystems yellow fluorescent dye); and DIK2279, DIK5320 and DIK2188 were labeled with PET (an Applied Biosystems red fluorescent dye). Amplification consisted of one 2-min denaturing at 94°C, followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 55 to 62°C for 30 s, extension at 72°C for 60 s, and a final extension at 72°C for 5 min in an Applied Biosystems 9700 thermocycler. Annealing temperatures were 55°C for all primer sets except DIK4627 (53°C), DIK2476 (57°C), and DIK4572 (58°C). Each PCR was carried out in 1.0 μM 5′ and 3′ primers, 0.2 mM deoxynucleotide 5′-triphosphates, 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM MgCl2, and 1 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems) in a 10-μL reaction with 20 ng of DNA as the template.

Statistical Analysis

BRD Genome-Wide Linkage Analysis of 1 Half-Sib Family

Analysis of the BH half-sib family for the genome-wide study has been described previously (Casas et al., 2000, 2001, 2004; Casas and Stone, 2006; Casas and Snowder, 2008). The addition of the BRD phenotype to the carcass traits previously reported represented a new analysis of the preexisting genotypes of 312 microsatellites. Briefly, an F-statistic profile was generated at 1-cM intervals for each chromosome by regression of the phenotype on the conditional probability of receiving the Brahman allele. Data were analyzed using the approach suggested by Haley et al. (1994), with a model that included the effects of sex (heifers or steers), year of birth (1994 or 1996), and dam line within year of birth. The conditional probability of inheriting the Brahman allele from the sire at each centimorgan of the chromosome was incorporated as a covariate and calculated with a Fortran program (Fortran Company, Tucson, AZ). Analysis for each chromosome was generated using the GLM procedure (SAS Inst. Inc., Cary, NC). The experiment-wise significance value was calculated according to Lander and Kruglyak (1995).

BRD Interval Linkage Analysis of 4 Half-Sib Families

Before the interval linkage analysis, rare alleles with a frequency of less than 1% were combined and analyzed as 1 allele. For the interval linkage analysis of BH, BA, PA, and BM families, an F-statistic profile was generated at 1-cM intervals using QTL Express sib pair analysis (Seaton et al., 2002). The analysis used the multimarker approach for interval mapping described by Knott et al. (1996). A 2-step procedure was performed. Identity-by-descent probabilities were first calculated at specific chromosomal locations from marker data, and then the statistical model was fitted to the observations and identity-by-descent coefficients. A single-QTL or a 2-QTL model was fitted. Where evidence for a single QTL was present, a model with 2 QTL in that interval was tested. Permutation tests with 1,000 iterations were conducted to set chromosome-wide significance thresholds for the QTL given the phenotype and genotype of the animals. Specifically, 1,000 permutations of the trait data (diseased
or not) were used to identify the maximum nominal significance level to determine if the disease was linked at this position at \( P < 0.05 \) at the chromosome level. Because the analysis was limited to 2 chromosomes, genome-wide thresholds were not calculated.

**Association Analysis for BVD-PI.** Before the association analysis, rare alleles with a frequency of less than 1% were combined and analyzed as 1 allele. Fisher’s exact test was then used to compare allele frequencies between BVD-PI calves and calves matched by age and herd that were unaffected (control calves), and dams of BVD-PI calves and control calves. The test statistic was considered significant at the chromosome-wide level of \( P = 0.05 \). Polymorphism information content (PIC) values were calculated as described by Botstein et al. (1980), where PIC >0.5 is highly informative, 0.5 > PIC > 0.25 is reasonably informative, and PIC <0.25 is slightly informative.

**RESULTS AND DISCUSSION**

**Disease Prevalence**

**BRD.** The prevalence of BRD was 9% (49/547) in the BH offspring, 4.7% (31/660) in the BA offspring, 0.8% (2/246) in the BM offspring, and 0.9% (2/209) in the PA offspring (Table 1). The incidence of BRD varied by year in the feedlot, as described previously by Snowder et al. (2006).

**BVD-PI.** The presence of BVD-PI calves was identified in 8 out of the 60 herds tested (13.3%) in 2008 and 2009. Overall, a total of 79 (0.92%) calves tested positive and 2 of 79 (2.5%) dams of BVD-PI calves were positive. This is substantially greater than the prevalence reported in 2007 Montana BVD-PI study in which 106,660 animals were screened to identify 110 BVD-PI animals, for a prevalence of 0.1% (Harbac et al., 2008). A similar study done in the southern United States reported a BVD-PI prevalence of 0.5%, which is much greater than the prevalence reported in Montana but almost 50% of the prevalence we identified in the state of Washington (Fulton et al., 2009). Although 79 calves were BVD-PI, only samples from 65 BVD-PI calves and their dams were available for genotyping. Sixty unaffected contemporary calves from the same herds served as controls.

**Linkage Analysis**

**BRD Genome-Wide Linkage Analysis of 1 Half-Sib Family.** The initial genome-wide linkage analysis with the BH family identified a QTL on BTA2 and BTA26 (Figure 1). On BTA2, the maximum genome-wide \( F \)-statistic was 7.31 (\( P = 0.007 \)) and was located between \( BMS2519 \) at 110 cM and \( BM804 \) at 61 cM, with a maximum \( F \)-statistic of 10.46 (\( P = 0.0013 \)).
ers with more than 1 rare allele, rare alleles were com-

bined and analyzed as 1 allele for each marker. This was done to maximize the positional information provided by the markers. The DIK4627 marker on BTA2 had a single rare allele that was removed. Greater than 95% of all genotypes were called for all microsatellite markers and for all animals genotyped.

The 6 markers used within 110 to 126 cM of BTA2 in the 4 half-sib families resulted in a maximum chromosome-wide F-statistic of 4.94 (P < 0.01) at 110 cM (IDVG-A-64) with a 1-QTL model (Figure 2). The F-statistic was less (F = 4.05) with a 2-QTL model (with one QTL located between IDVG-A-64 and DIK5023 near 111 cM and the other QTL located between DIK4572 and IDVG-A-72 near 116 cM), indicating that the 1-QTL model was a better fit. Linkage was demonstrated with a 2-QTL model on BTA26 (F = 4.03, P < 0.05), with the 2 regions of linkage at 42 cM near BM4505 and 52 cM between BM6041 and BMS882 on BTA26. The F-statistic was less with a 1-QTL model (F = 3.92), indicating that the 2-QTL model was a better fit. These results confirmed the initial linkage identified in the BH family and further refined the regions of interest.

**Association Analysis for BVD-PI**

Rare alleles were observed for BMS882 (5 alleles), DIK2387 (2 alleles), IDVG-A-64 (2 alleles), DIK2476 (5 alleles), DIK3320 (3 alleles), DIK2188 (3 alleles), DIK4572 (5 alleles), and BM4505 (3 alleles; see additional details in the supplemental information: http://jas.fass.org/content/vol89/issue4). Rare alleles were combined into a single allele group for each marker for Fisher’s exact analysis. All markers were highly informative (PIC > 0.5) using the method of Botstein et al. (1980), with the exception of DIK2188 (PIC = 0.36), which was moderately informative.

**BRD Interval Linkage Analysis of 4 Half-Sib Families.** The 13 microsatellite markers used for interval mapping were highly informative, with PIC values exceeding 0.5 by using the method of Knott et al. (1998) within the QTL Express program. Allele frequencies were assessed to determine if an allele had a frequency of less than 1% (Supplemental Tables 1 to 13; http://jas.fass.org/content/vol89/issue4). Rare alleles (frequency of <1%) were observed for markers DIK4627 (1 allele; Supplemental Table 5), IDVG-A-64 (3 alleles; Supplemental Table 5), DIK4572 (4 alleles; Supplemental Table 2), BMS882 (9 alleles; Supplemental Table 8), BM4505 (5 alleles; Supplemental Table 9), DIK3320 (2 alleles; Supplemental Table 13), and DIK2476 (2 alleles; Supplemental Table 12). For markers with more than 1 rare allele, rare alleles were com-

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**Figure 1.** A) Represents the linkage of bovine respiratory disease on BTA2. Each closed circle represents the location of a polymorphic marker used in the linkage study. Markers consisted of BMC9007 (0 cM), BY312 (1.5 cM), ILSTS026 (7.5 cM), TEXAN-2 (22.5 cM), URB042 (33.9 cM), BMS803 (41 cM), RM556 (51.9 cM), BM2808 (59.9 cM), BMS353 (63 cM), TGLA226 (80 cM), BMS889 (91.5 cM), BM356 (100.3 cM), BMS2519 (101.5 cM), IDVG-A-2 (117.8 cM), and FCB11 (120.4 cM), with the centromere near 0 cM. B) Represents the linkage of bovine respiratory disease on BTA26. Each closed circle represents the location of a polymorphic marker used in the linkage study. Markers consisted of BM4505 (39.7 cM), HELI11 (20.7 cM), BM6041 (26 cM), BM6041 (49.2 cM), BM805 (59.6 cM), ILSTS091 (69.9 cM), and MAF36 (72.6 cM), with the centromere near 0 cM. The computed F-statistic is shown on the y-axis and the relative position in centimorgans is shown on the x-axis, based on the US Meat Animal Research Center cattle linkage map (Ihara et al., 2004).

**Figure 2.** The computed F-statistic is shown on the y-axis and the relative position in centimorgans is shown on the x-axis, based on the US Meat Animal Research Center (USMARC) cattle linkage map (Ihara et al., 2004). Each closed circle represents the location of a polymorphic marker used in the linkage study. Markers consist of IDVG-A-64 (110 cM), BM4505 (112 cM), DIK4572 (114 cM), IDVG-A-72 (119 cM), DIK4627 (125 cM), and DIK2188 (126 cM). The centromere is located near 0 cM.
To determine if the QTL linked with BRD were associated with BVD-PI, an association analysis was conducted on BVD-PI calves, their dams, and control calves from the same herd. Table 2 summarizes the significance of the association with BVD-PI (calves and dams) and the number of alleles observed for each of the genetic markers. Stronger associations were found with the dams of the BVD-PI calves than the calves themselves with all the genetic markers on BTA2 and with most of the markers on BTA26. These strong associations with the dams of the BVD-PI calves also extended for a longer distance on both chromosomes than was observed with the BVD-PI calves.

On BTA26, none of the 4 loci associated with BVD-PI in calves (BM4505, DIK2287, BMS882, DIK2279) were concordant with the 3 loci associated with BVD-PI in the dams (BM6041, DIK2476, DIK5320). This indicates that although this large 11-Mb region harbors loci associated with BVD-PI, disease susceptibility resulting from the maternal genetic effects occurs through different mechanisms than the genetic effects of the calves. For these regions, on BTA26, selection of loci associated with the incidence of BVD-PI in calves may not have positive effects on the genetic contribution of the dams. This is in contrast to the 4 loci concordantly associated with BVD-PI calves and dams of BVD-PI calves on BTA2. These loci include DIK5023 (112 cM), IDVGA-72 (119 cM), DIK427 (125 cM), and DIK2188 (126 cM). These loci span 7 Mb and most likely represent several genes or regulatory regions that are affecting the resistance or susceptibility to BVD-PI. Once these regions are refined by fine mapping, selection for these loci could benefit disease resistance from the maternal side through the ability of the dam to limit placental transfer of the BVD virus to her calf as well as by enhancing the development of the immune system of the calf. Two additional loci (IDVGA-64, P = 0.004, and DIK5372, P = 0.005) on BTA2 were associated with BVD-PI through the dams of BVD-PI calves.

The peak F-statistic (4.8) for the linkage analysis on BTA2 with BRD was located near IDVGA-64, where an association exits with BVD-PI and the dams of BVD-PI calves (P = 0.005). This suggests that the linkage of BRD with BTA2 may be due in part to a locus associated with the BVD virus within the BRD complex. The identification of loci associated with resistance or susceptibility to specific pathogens would be particularly helpful in understanding the pathogenesis of the BRD complex of diseases. Alternatively, the linkage and association of the same loci for BRD and BVD-PI may reflect loci that are involved in a broad spectrum of disease.

The 2 linkage peaks on BTA26 with BRD are near BM4505 and between BM6041 and BMS882. No association with BVD-PI calves or their dams was identified at BM4505. However, an association with BM6041 and the dam of BVD-PI calves (P = 1.4 × 10⁻⁶), and BMS882 and BVD-PI calves (P = 0.01) is supportive of this region containing a locus that is important in the resistance or susceptibility to BVD-PI and BRD. Like the loci associated with BVD-PI and linked with BRD on BTA2, the region defined by BM6041 and BMS882 may represent a locus that is associated primarily with BVDV within the BRD complex or that implies resistance or susceptibility to a host of pathogens. Interestingly, the associations found in this 2-Mb region of linkage with BRD are associated with dams of BVD-PI calves as well as the calves, demonstrating that the genetic and immunological contributions of both the dams and the calves are important in fighting the BRD complex of diseases.

Evidence exists that some animals are innately more resistant to BRD than others because heritability estimates for resistance range from 0.10 to 0.48, depending on the age and breed of animals and the scale used for the study (Muggli-Cockett et al., 1992; Snowder et al., 2005, 2006; Heringstad et al., 2008). Substantial breed differences have been reported in the susceptibility of calves to BRD, and crossbreds are generally less susceptible than purebred cattle (Muggli-Cockett et al., 1992; Snowder et al., 2005, 2006). Heritability estimates specifically for BVD-PI cattle have not been reported. However, because BVD is one of a complex of diseases that are categorized under BRD, the estimates for heritability for BRD also account, in part, for the estimate of heritability of BVD. The heritability estimates taken together with the differences in incidence of disease among cattle breeds suggest that if resistance to BRD or BVD were selected for, gains could be made in reducing the incidence of the disease.

The etiology of BRD is complex, involving viral, bacterial, and mycoplasmal pathogens; however, it is the immune response of the animal to these agents that is responsible for the clinical disease. The relationship between BVDV and BRD has been studied extensively (Fulton et al., 2000, 2002, 2009). Animals infected with BVDV become immunosuppressed, increasing the risk for opportunistic infections from other pathogens associated with the BRD complex. Potgieter (1997) concluded that BVDV played a pivotal role in potentiating the virulence of other BRD pathogens.

Bovine viral diarrhea is the result of an infection from a virus in the Pestivirus genus and is distributed worldwide. Bovine viral diarrhea virus may cause acute or persistent infection. Acute BVD may be mild or severe and is characterized by an increase in body temperature, immunosuppression, loss of appetite, diarrhea, hemorrhaging, dehydration, a decrease in milk production, an increased incidence of reproductive failure, and even death (Baker, 1995; Grooms et al., 2002). The magnitude of the incidence of BVD is often unappreciated because 70 to 90% of BVDV infection results in a subclinical presentation (Ames, 1986). It is estimated that 85% of cattle become infected during their first year of life.

Persistently infected BVD animals may appear healthy but will continue to shed the virus and initiate
further infection among the herd. Bovine viral diarrhea virus has also been reported to be present in semen and in the ovaries of individuals that are no longer viremic but that have been acutely infected with the virus (Grooms et al., 1998; Voges et al., 1998). Acutely infected animals may also significantly contribute to the introduction of the virus to uninfected animals in BVDV-free herds. The ability of BVDV to induce immunosuppression or increase susceptibility of the animals to other pathogens may be rooted in the innate immune response of the individual animal. Animals that are more likely to fail to seroconvert, or to mount a successful resistance to co-infecting organisms, may be innately genetically predisposed to BVDV.

This study consisted of field data collected at the USMARC in Nebraska and at cow-calf commercial operations across the state of Washington. Field studies provide the collection of disease data in situations that are pertinent to the livestock industry that are not replicated in research settings. Research settings differ from commercial cattle settings in 2 ways: field data allow a much greater number of herds and animals to be evaluated than if the animals were taken from a research herd or a small sampling of herds, and they also provide additional variation in the environmental conditions under which the animals are raised and exposed to disease. For example, across the state of Washington, the climate and terrain varies from desert regions to rain forests and from valleys to mountains. The variety in environment and management that is reflected by field data across the state cannot easily be replicated in a research study. By sampling cattle exposed to a variety of conditions, rather than a very limited set of conditions, the research results become more relevant and reflective of the diverse conditions that face cattle across the United States.

However, field studies suffer from the uncontrolled exposure (or lack of exposure) of each animal to the pathogens associated with the BRD complex. Exposure to pathogens generally varies within a herd or pasture. Differences in pathogen exposure may have significant effects on the ability of genetic studies to identify loci associated with disease. Lack of pathogen exposure may portray animals that are susceptible to disease as resistant to disease. The misclassification of disease-resistant and disease-susceptible animals reduces the power of the study to detect loci associated or linked with disease. To minimize the differences in pathogen exposure, animals that were diagnosed with BRD were not only half-sibs of those that were unaffected, but were also reared together throughout their lives. For BVD-PI, the time of exposure of the dams to BVDV is critical. Dams of the BVD-PI calves and dams of the unaffected calves were all housed together during gestation and the BVD-PI calves and unaffected calves were the same age so that the dams would have been exposed at the same point of fetal development of their calves. Although the possibility remains that some animals were classified as resistant because they were not exposed to the pathogen, efforts were taken to minimize this possibility.

Fine-mapping studies of the regions on BTA2 and BTA26 identified in this study are ongoing and will more thoroughly characterize the loci associated and linked with the pathogens of the BRD complex. High-density genome-wide association studies may also be helpful in identifying additional loci involved in the pathogenesis of BRD. The identification of loci associated with BRD will be useful in selecting animals that are more disease resistant, enhancing the sustainability of cattle production.

LITERATURE CITED


Loci for bovine respiratory disease and bovine viral diarrhea


Supplementary Material

Supplementary material can be found at:
http://www.journalofanimalscience.org/content/suppl/2011/03/18/jas.2010-3330.DC1.html

References

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