

# Putative quantitative trait loci associated with the probability of contracting infectious bovine keratoconjunctivitis<sup>1,2</sup>

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**ABSTRACT:** Infectious bovine keratoconjunctivitis, also known as pinkeye, is an economically important disease in cattle. The objective of this study was to detect QTL associated with infectious bovine keratoconjunctivitis in offspring from a Brahman × Hereford sire. The sire was mated to Hereford, Angus, and F<sub>1</sub> cows to produce 288 offspring in 1994 and mated to MARC III (¼ Hereford, ¼ Angus, ¼ Red Poll, and ¼ Pinzgauer) cows in 1996 to produce 259 offspring (547 animals total). Infectious bovine keratoconjunctivitis was diagnosed by physical examination in 36 animals of the family. Records included unilateral and bilateral frequency, but not severity. Records were binary: 0 for unaffected and 1 for affected cattle. A putative QTL for

infectious bovine keratoconjunctivitis was identified on chromosome 1, with a maximum *F*-statistic ( $F = 10.15$ ;  $P = 0.0015$ ) at centimorgan 79 of the linkage group. The support interval spanned centimorgans 66 to 110. There was also evidence suggesting the presence of a QTL for infectious bovine keratoconjunctivitis on chromosome 20, with a maximum *F*-statistic ( $F = 10.35$ ;  $P = 0.0014$ ) at centimorgan 16 of the linkage group. The support interval ranged from centimorgan 2 to 35. This report provides the initial evidence of QTL for infectious bovine keratoconjunctivitis. Although a candidate gene was identified for one of the regions of interest, further studies are needed to identify the genetic basis of resistance to the disease.

**Key words:** bovine keratoconjunctivitis, quantitative trait locus

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## INTRODUCTION

Infectious bovine keratoconjunctivitis, also known as pinkeye, is one of the most economically important diseases in cattle. The genetic effects on the incidence of infectious bovine keratoconjunctivitis have been studied, and breed differences for incidence of infectious bovine keratoconjunctivitis have been established. Animals with Hereford inheritance are more susceptible to infectious bovine keratoconjunctivitis (Webber and Selby, 1981), whereas tropically adapted breeds are less susceptible (Snowder et al., 2005). Estimates of heritability for the incidence of infectious bovine keratocon-

junctivitis range from 0.01 in Brahman to 0.28 in Hereford (Snowder et al., 2005). This indicates the existence of a genetic component that could be exploited to detect QTL for the incidence of infectious bovine keratoconjunctivitis in cattle.

As part of a larger study to detect QTL affecting economically important traits in cattle, a large half-sib family was developed from a Brahman × Hereford sire (Casas et al., 2003). This family has been used to detect QTL for growth, carcass composition, meat quality, and male reproductive traits (Keele et al., 1999; Stone et al., 1999; Casas et al., 2003; Casas et al., 2004). The use of a sire obtained from breeds divergently affected by infectious bovine keratoconjunctivitis increased the possibility of identifying genomic regions harboring genes associated with susceptibility to the infection.

The objective of the current study was to detect QTL associated with infectious bovine keratoconjunctivitis in offspring from a Brahman × Hereford sire.

## MATERIALS AND METHODS

Experimental procedures were approved and performed in accordance with US Meat Animal Research Center (USMARC) animal care guidelines and the

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Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

### Animals

A half-sib family was developed using 1 Brahman  $\times$  Hereford sire. The bull was previously used in the USDA reference population to generate the cattle linkage map (Kappes et al., 1997). The sire was mated to Hereford, Angus, and  $F_1$  cows to produce 288 offspring in 1994, and mated to MARC III ( $\frac{1}{4}$  Hereford,  $\frac{1}{4}$  Angus,  $\frac{1}{4}$  Red Poll, and  $\frac{1}{4}$  Pinzgauer) cows in 1996 to produce 259 offspring (547 animals total). Breeds of sires for the  $F_1$  cows were Hereford, Angus, Shorthorn, Charolais, Gelbvieh, Pinzgauer, Galloway, Longhorn, Nellore, Piedmontese, or Salers. Breeds of dams for the  $F_1$  cows were Hereford or Angus. Calves were weaned at an average of 187 d of age and raised from weaning to slaughter on a corn-corn silage diet. Cattle were slaughtered at a commercial packing plant at an average age of 455 d.

### Traits Analyzed

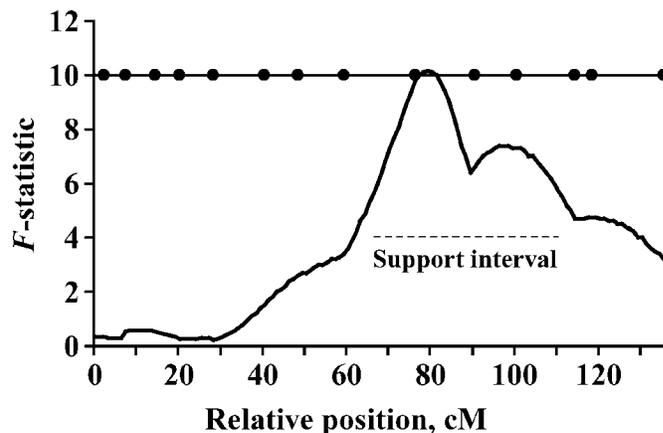
Calves were monitored daily by the staff veterinarian, the beef cattle research technicians, or both. Infectious bovine keratoconjunctivitis was detected by physical examination. Records included unilateral and bilateral frequency but not severity. Records were binary: 0 for unaffected and 1 for affected 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 infectious bovine keratoconjunctivitis or reinfection. Treatment of infected calves included injections with antibiotics (oxytetracycline and ceftiofur sodium) and topical application of cloxacillin benzathine to the eye, which was covered with an eye patch (Snowder et al., 2005). Records of coat color or eye pigmentation were not obtained.

### Genomic Screening

Casas et al. (2003) provided a detailed description of the markers used to detect QTL in this 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 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 elsewhere (Kappes et al., 1997).

### Statistical Analysis

An  $F$ -statistic profile was generated at 1 cM intervals for each chromosome by regression of 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 effects



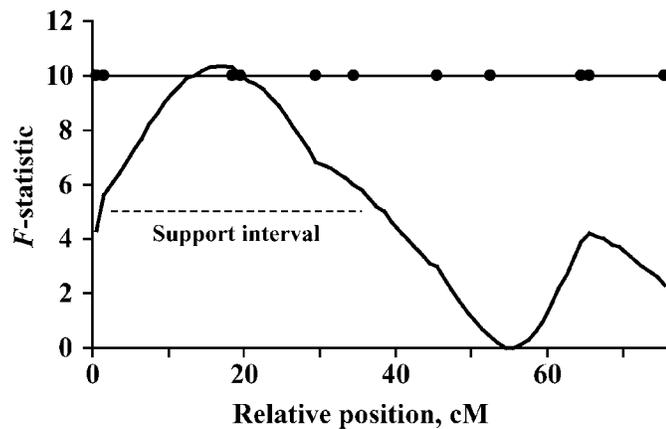
**Figure 1.**  $F$ -statistic profile and support interval for infectious bovine keratoconjunctivitis on chromosome 1. The horizontal line represents the threshold suggestive of linkage ( $F = 10.0$ ). Dots on the threshold line indicate the relative position of markers TGLA49, BMS1928, BMS2321, HEL6, ILSTS104, BMS4037, BMS948, BMS4030, BMS8246, BMS119, RM153, UWCA46, BMS918, and BMS4014.

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. The conditional probability of inheriting the Brahman allele was calculated with a FORTRAN program. Analysis for each chromosome was generated using the GLM procedure (SAS Inst. Inc., Cary, NC). The 1-LOD drop-off method was used to calculate the support interval for each putative QTL (Ott, 1992).

The experiment-wise threshold value was calculated according to Lander and Kruglyak (1995). An  $F$ -statistic was considered suggestive of linkage if it exceeded a value of  $F = 10.0$  (1 expected false positive per genomic scan). This corresponds to a nominal  $P$  value of 0.002.

## RESULTS

Thirty-six animals were diagnosed with infectious bovine keratoconjunctivitis and treated. This represents 6.58% of the total members of the family. Two putative QTL for infectious bovine keratoconjunctivitis were detected. Chromosome 1 was identified as harboring at least 1 gene associated with this condition at centimorgan 79 of the linkage group (Figure 1), with a maximum  $F$ -statistic = 10.15 ( $P = 0.0015$ ). This is, at this chromosomal region, 6.67% of the family members inherited the Hereford allele and were diagnosed with infectious bovine keratoconjunctivitis, compared with 6.49% of the family members that inherited the Brahman allele and were diagnosed with the disease. The support interval spanned centimorgans 66 to 110. Animals inheriting the Hereford allele from the sire at this



**Figure 2.** *F*-statistic profile and support interval for infectious bovine keratoconjunctivitis on chromosome 20. The horizontal line represents the threshold suggestive of linkage ( $F = 10.0$ ). Dots on the threshold line indicate the relative position of markers BM3517, HEL12, BMS1282, TGLA304, ILSTS068, BMS1128, BMS2361, BM4107, BM5004, BMS1719, and BMS521.

chromosomal region had a 0.9% higher prevalence of infectious bovine keratoconjunctivitis when compared with those inheriting the Brahman allele. There was also evidence supporting the presence of a QTL for infectious bovine keratoconjunctivitis on chromosome 20 (Figure 2). The maximum *F*-statistic was  $F = 10.35$ , corresponding to  $P = 0.0014$ . This value was located at centimorgan 16 of the linkage group. The support interval ranged from centimorgan 2 to 35. Animals inheriting the Brahman allele had a 0.7% higher prevalence of infectious bovine keratoconjunctivitis when compared with those inheriting the Hereford allele. At this chromosomal region, 6.61% of the family members inherited the Brahman allele and were diagnosed with the disease, compared with 6.54% of the total family members that inherited the Hereford allele and were diagnosed with the disease.

No interactions were observed in the current study. Interaction between QTL on chromosomes 1 and 20 was not significant ( $P > 0.05$ ). The interaction between the locus that produces white face in Hereford, located on chromosome 6, and chromosomes 1 and 20 were not significant ( $P > 0.05$ ).

## DISCUSSION

Several factors are involved in the expression of infectious bovine keratoconjunctivitis: the environment, season, fly concentration, the presence of the pathogen, strain of the pathogen, and genetic background of the animal play a role in the incidence, penetrance, and severity of infectious bovine keratoconjunctivitis (Brown et al., 1998). Thirty-six offspring from the family under study were diagnosed and treated for infectious bovine keratoconjunctivitis. Although the number

of diagnosed animals was low, 2 QTL associated with the disease were detected. The number and magnitude of the detected QTL for infectious bovine keratoconjunctivitis was unexpected. However, the location of the QTL identified correlates with QTL associated with other diseases in cattle (Heyen et al., 1999; Rodriguez-Zas et al., 2002; Ron et al., 2004).

The QTL for infectious bovine keratoconjunctivitis detected on chromosome 1 lies in the region where a putative QTL for somatic cell score resides. Heyen et al. (1999) detected a putative QTL for somatic cell score on chromosome 1 between markers URB039 and CSSM19, which was later verified by Rodriguez-Zas et al. (2002). The chromosomal region where Heyen et al. (1999) and Rodriguez-Zas et al. (2002) detected the QTL for somatic cell score lies within the support interval established for the QTL in the current study. These studies suggest that a gene or group of genes residing in this chromosomal region is associated with bacterial diseases in cattle.

Pentraxin 3 (PTX3) could be considered a candidate gene for the QTL on chromosome 1. This gene plays an important role in the defense against pathogens. It is released by different types of cells (mononuclear phagocytes, dendritic cells, fibroblasts, and endothelial cells) in response to primary, inflammatory signals, and toll-like receptor engagement. The common feature among the members of the pentraxin family is pathogen recognition (Bottazzi et al., 2006). Pentraxin 3 has been mapped to chromosome 1 (Snelling et al., 2005). It resides within the support interval of the QTL, in the vicinity where the maximum evidence of the presence of the QTL exists.

Quantitative trait loci for somatic cell score have been detected on bovine chromosome 20 in the region where the QTL for infectious bovine keratoconjunctivitis was detected in the current study. Rodriguez-Zas et al. (2002), using a composite interval mapping approach, identified a QTL for somatic cell score in the centromeric region of chromosome 20. Ashwell et al. (2004) identified a QTL for somatic cell score between markers RM310 and TGLA126. These markers are included in the support interval from the current study. Ron et al. (2004), studying a Holstein population in Israel, identified a putative QTL for somatic cell score on chromosome 20. Ron et al. (2004) included marker BM1225 in their study, which was associated with somatic cell score (J. I. Weller, Institute of Animal Science, Bet Dagan, Israel, personal communication). This marker resides in the support interval from the current study.

The Hereford breed has been recognized as being more susceptible to infectious bovine keratoconjunctivitis than other breeds (Snowder et al., 2005). In the large half-sib family used in the current study, 36 calves were diagnosed and treated for infectious bovine keratoconjunctivitis. The sire of this family was obtained by crossing a Brahman sire to a Hereford cow. A second family developed at the US Meat Animal Research Center was developed from a sire derived from crossing a Brahman

sire with an Angus cow (data not shown). In this family, only 1 of 620 calves was diagnosed and treated for infectious bovine keratoconjunctivitis. Thus, the family was excluded from the study. Diagnosis and treatment of infectious bovine keratoconjunctivitis in these families confirm the findings by Snowden et al. (2005) that some breeds are more susceptible than others to infectious bovine keratoconjunctivitis.

Defense mechanisms of the tear film are poorly researched in cattle. Tear proteins identified in humans include immunoglobulins, lysozyme, lactoferrin, and transferrin (Brown et al., 1998). The lysozyme gene resides on bovine chromosome 5 (Gallagher et al., 1993). The gene responsible for the expression of lactoferrin lies on bovine chromosome 22 (Martin-Burriel et al., 1997). Transferrin is produced by a gene located on bovine chromosome 1 (Kappes et al., 1997); however, the location of this gene is outside the limit of the support interval of the QTL on this chromosome. The major histocompatibility complex, which contains genes important in the effectiveness of the immune response, has been mapped to bovine chromosome 23 (Kappes et al., 1997). Genes associated with defense mechanisms of the tear need to be better understood before they can be excluded as responsible for the QTL associated with infectious bovine keratoconjunctivitis.

The gene that produces white face in Hereford can be excluded as responsible for the incidence of infectious bovine keratoconjunctivitis in the breed. The gene responsible for white face is located on bovine chromosome 6 (Grosz and MacNeil, 1999). There was no evidence of interaction between chromosome 6 and chromosomes 1 and 20 in the current study (data not shown).

Snowden et al. (2005) indicate that Hereford cattle are more susceptible to infectious bovine keratoconjunctivitis than Brahman cattle. The QTL detected on chromosome 20 has the opposite effect. This is, animals inheriting the Brahman allele had a higher prevalence of infectious bovine keratoconjunctivitis than those inheriting the Hereford allele. Quantitative trait loci exerting a similar effect on a trait have been previously reported in other species (De Vicente and Tanksley, 1993; Zidek et al., 1998; Rohrer et al., 2001). Zidek et al. (1998), using a population derived from the DBA/2J and the C57BL/6J strains of mice that differ in their testicular weight (0.22 and 0.16 g, respectively), detected 3 QTL for this trait on different chromosomes. On mouse chromosome 3 and 13, those animals inheriting the C57BL/6J exhibited heavier testicular weights (0.047 and 0.06 g, respectively) as compared with those inheriting the DBA/2J. In pigs, Rohrer et al. (2001) identified several QTL associated with FSH plasma concentration. They used a backcross and an F<sub>3</sub> population derived from Meishan (Chinese) and White Composite (commercial) lines. Meishan pigs have high concentrations of plasma FSH compared with the White Composite. Four QTL were detected in the study. For 2 of those QTL (pig chromosomes 3 and 8), animals

inheriting the White Composite allele had a higher concentration of plasma FSH than those inheriting the Meishan allele. The explanation of why these QTL have the opposite effect than what it is observed in the breed is missing in both studies. De Vicente and Tanksley (1993) define transgressive segregation, or transgression, as the appearance of individuals in segregating populations that fall beyond their parental phenotypes. De Vicente and Tanksley (1993) used an F<sub>2</sub> cross of cultivated and wild type tomato to identify QTL for 11 production traits. Thirty-six percent of 74 QTL detected showed transgressive segregation. This is, individuals inheriting the wild type tomato allele at the QTL showed higher productivity than those inheriting the cultivated tomato allele. A similar pattern could be influencing the results obtained for the infectious bovine keratoconjunctivitis on chromosome 20.

Data reported herein provide the initial evidence of QTL for infectious bovine keratoconjunctivitis. Putative QTL were detected on chromosomes 1 and 20. They reside in similar regions where QTL for other conditions in cattle have been detected. These findings should motivate future studies with the objective of identifying the genetic base of infectious bovine keratoconjunctivitis resistance or susceptibility.

## IMPLICATIONS

This is the first report of putative quantitative trait loci associated with infectious bovine keratoconjunctivitis. The number and magnitude of the quantitative trait loci suggest that one could be real. Pentraxin 3 could be considered a candidate gene for the quantitative trait loci detected on chromosome 1. Further studies are needed to assess these quantitative trait loci in other populations to determine the extent of their usefulness.

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