J. Dairy Sci. 96:1820–1833
http://dx.doi.org/10.3168/jds.2012-5528

Effects of *Bos taurus* autosome 9-located quantitative trait loci haplotypes on the disease phenotypes of dairy cows with experimentally induced *Escherichia coli* mastitis

M. Khatun,*† P. Sørensen,† H. B. H. Jørgensen,† G. Sahana,† L. P. Sørensen,† M. S. Lund,† K. L. Ingvartsen,* A. J. Buitenhuis,† J. Vilkki,‡ M. Bjerring,* J. R. Thomasen,§ and C. M. Røntved*#[1]
*Department of Animal Science, and
†Center for Quantitative Genetics, Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, PO Box 50, DK-8300 Tjele, Denmark
‡Biotechnology and Food Research, MTT Agrifood Research Finland, 31600 Jokioinen, Finland
§VikingGenetics, Elbeltoftvej 16, DK-8900 Randers, Denmark
#CMR On-Site RD, Skjernvej 4A, DK-9220 Aalborg Øst, Denmark

ABSTRACT

Several quantitative trait loci (QTL) affecting mastitis incidence and mastitis-related traits such as somatic cell score exist in dairy cows. Previously, QTL haplotypes associated with susceptibility to *Escherichia coli* mastitis in Nordic Holstein-Friesian (HF) cows were identified on *Bos taurus* autosome 9. In the present study, we induced experimental *E. coli* mastitis in Danish HF cows to investigate the effect of 2 *E. coli* mastitis-associated QTL haplotypes on the cows' disease phenotypes and recovery in early lactation. Thirty-two cows were divided in 2 groups bearing haplotypes with either low (HL) or high (HH) susceptibility to *E. coli*. In addition, biopsies (liver and udder) were collected from half of the cows (n = 16), resulting in a 2 × 2 factorial design, with haplotype being one factor (HL vs. HH) and biopsy being the other factor (biopsies vs. no biopsies). Each cow was inoculated with a low *E. coli* dose (20 to 40 cfu) in one front quarter at time 0 h. Liver biopsies were collected at −144, 12, 24, and 192 h; udder biopsies were collected at 24 h and 192 h post-*E. coli* inoculation. The clinical parameters: feed intake, milk yield, body temperature, heart rate, respiration rate, rumen motility; and the paraclinical parameters: bacterial counts, somatic cell count (SCC), and milk amyloid A levels in milk; and white blood cell count, polymorphonuclear neutrophilic leukocyte (PMNL) count, and serum amyloid A levels in blood were recorded at different time points post-*E. coli* inoculation. *Escherichia coli* inoculation changed the clinical and paraclinical parameters in all cows except one that was not infected. Clinically, the HH group tended to have higher body temperature and heart rate than the HL group did. Paraclinically, the HL group had faster PMNL recruitment and SCC recovery than the HH group did. However, we also found interactions between the effects of haplotype and biopsy for body temperature, heart rate, and PMNL. In conclusion, when challenged with *E. coli* mastitis, HF cows with the specific *Bos taurus* autosome 9-located QTL haplotypes were associated with differences in leukocyte kinetics, with low-susceptibility cows having faster blood PMNL recruitment and SCC recovery and a tendency for a milder clinical response than the high-susceptibility cows did.

Key words: *Escherichia coli*, mastitis, quantitative trait locus haplotype, phenotype

INTRODUCTION

Mastitis, an inflammatory condition of the mammary gland, is the most prevalent disease in periparturient dairy cows (Burvenich et al., 2007; Fox, 2009). Bovine mastitis causes substantial economic losses to the dairy industry because of decreased milk yield, treatment cost, prevention cost, and culling of the affected animals (Petrovski et al., 2006; Bar et al., 2008; Hertl et al., 2011). Moreover, mastitis is considered a painful condition of animal welfare concern (Rasmussen et al., 2011; Fogsgaard et al., 2012). Hence, lowering the risk and incidence, and improving recovery from mastitis through improved breeding is desirable to the dairy industry.

The gram-negative bacterium *Escherichia coli*, belonging to the *Enterobacteriaceae* family, is one of the most common causes of acute clinical mastitis in dairy cows in early lactation (Bradley et al., 2007; Ericsson Unnerstad et al., 2009; Hertl et al., 2011). *Escherichia coli* mastitis is associated with well-characterized clinical signs and subclinical and clinical changes in milk and blood components. The clinical and paraclinical

Received March 12, 2012.
Accepted November 28, 2012.
[1]Corresponding author: cmr@cmr-on-site.dk

changes, termed "evident phenotype" (Detilleux, 2009), are considered the disease phenotypes during mastitis. The common clinical disease phenotypes during *E. coli* mastitis are high fever and anorexia with lowered milk production (Burvenich et al., 2003; Bannerman et al., 2004; Kornalijnslijper et al., 2004). The disease phenotypes expressed in milk are a brief excretion of high numbers of *E. coli*, followed by a dramatic increase in SCC and inflammatory mediators and altered milk appearance (Kornalijnslijper et al., 2004; Vangroenweghe et al., 2004; Vangroenweghe et al., 2005), depending on the severity of the infection (Bannerman et al., 2004; Suojala et al., 2008). Similarly, the important disease phenotypes expressed in blood are leukocyte mobilization with short-term leukopenia, followed by leukocytosis (Vels et al., 2009; Rasmussen et al., 2011) and secretion of large quantities of inflammatory mediators including cytokines (Bannerman et al., 2004) and acute-phase proteins (Jacobsen et al., 2005; Suojala et al., 2008). Among the commonly investigated acute-phase proteins are milk amyloid A (**MAA**) and serum amyloid A (**SAA**), which have been suggested to be associated with the degree of infection (Jacobsen et al., 2005; Suojala et al., 2008) and the related tissue trauma (inflammation) (Cray et al., 2009).

Host defense against mastitis is controlled by multiple genes (Pighetti and Elliott, 2011), and the outcome of clinical mastitis may be affected by several host factors, including QTL or QTL haplotype (Lund et al., 2008; Sodeland et al., 2011). In Nordic Holstein-Friesian (**HF**) cows, the SCS is affected by a QTL located on BTA9 that is partly associated with *E. coli* mastitis, with one haplotype being more susceptible and another being more resistant (Sørensen et al., 2008). Indeed, increasing numbers of studies are investigating the underlying molecular mechanism of *E. coli* mastitis from an immunological point of view (Buitenhuis et al., 2011). Furthermore, numerous studies investigating the effect of SNP in the bovine immunological genes on mastitis susceptibility are emerging (Leyva-Baca et al., 2008; He et al., 2011; Pighetti and Elliott, 2011). Although several QTL affecting mastitis incidence and mastitis-related traits such as SCS exist in dairy cows, only few studies have examined the effect of specific QTL or QTL haplotypes in an experimental setup. Recently, primary bovine mammary gland epithelial cells originating from cows with specific SCS-associated QTL on BTA18 were infected with *E. coli* and investigated for multiple gene expression using quantitative real-time PCR and microarray expression chip technology (Griesbeck-Zilch et al., 2009; Brand et al., 2011). However, so far no one has investigated the direct effect of the BTA9 QTL haplotypes on common clinical, milk, and blood disease phenotypes during experimental *E. coli* mastitis. Studying the effect of QTL haplotypes on clinical outcome and disease recovery may provide novel insights into the genetic basis of the pathophysiology of bovine *E. coli* mastitis.

The aim of the present study was to test the hypothesis that the BTA9 QTL haplotypes are partly associated with *E. coli* mastitis, with one cow haplotype being more susceptible and another being more resistant to *E. coli* mastitis. The effect of QTL haplotypes on the clinical and paraclinical disease phenotypes associated with *E. coli* mastitis was tested in an experimental in vivo model using a low bacterium dose. We also collected liver and udder biopsies from half of the cows for gene expression analyses. The results from these studies were presented in Buitenhuis et al. (2011) and Jørgensen et al. (2012). As this combined biopsy procedure may have caused additional stress and inflammation, the biopsy procedure was included as a statistical factor in our study. The main effect of the factor biopsy is presented elsewhere (M. Khatun, P. Sørensen, K. L. Ingvartsen, M. Bjerring, and C. M. Røntved, unpublished data), whereas the interaction effect between biopsy and QTL haplotype on the investigated parameters are presented here.

MATERIALS AND METHODS

Selection of Animals

The experiment was performed at Aarhus University, Department of Animal Science (Foulum, Denmark). All procedures involving animals were approved by the Danish Animal Experiments Inspectorate in accordance with the Danish Ministry of Justice Law no. 726 (September 9, 1993) and acts 739 (December 6, 1988) and 687 (July 25, 2003) concerning animal experimentation and the care of experimental animals. Inspection was carried out by members of the Danish Animal Experiments Inspectorate committee during the acute stage of the disease.

The Danish HF breed was used in this study. Potential carriers (bull daughters) of the high-or low-mastitis-resistance QTL haplotype were identified in the Danish National Cattle Database (https://www.landbrugsinfo.dk/Kvaeg/RYK/Sider/Registreringsblok_web.pdf; http://www.glr-chr.dk/pls/glrchr/chrmenu$.menu) by assessing the haplotype status of their sire and grandsire. Daughters were also matched with regard to age, expected calving date (1.5 to 2 mo before expected calving date), and health status record (i.e., free of *Salmonella dublin*, bovine viral diarrhea virus, group B *Streptococcus*, infectious bovine rhinotracheitis virus, and low antibody titers against *Para tuberculosis* in milk and serum). Forty-two pregnant heifers (20 in