Identification of Genetic Regions Associated with Tolerance and Infection to Johne's Disease in Cattle Using a Fine-Mapping Approach

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ABSTRACT

Johne's disease is an incurable illness of ruminants, caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). Once an animal is exposed to *Map* it can become infected or resist infection. Susceptible animals may develop Johne's disease with different severity levels or exhibit different levels of tolerance. Selection of animals that are resistant or tolerant to Johne's disease would reduce economic losses and reduce disease prevalence. Tolerance was measured by the relationship between fitness (*Map* fecal shedding) and infection intensity (*Map* tissue infection); infection was determined by the presence of *Map* in four tissues. We previously identified associations with a region on BTA 3 and *Map* tissue infection and on BTA 15 with tolerance to Johne's disease using a whole-genome analysis. The objective of this study was to confirm the association on BTA 3 and BTA15. On BTA 15, 54 SNPs were used to interrogate a 193kb region and on BTA 3, 42 SNPs were chosen for a 235kb region. Sixteen SNPs on BTA 15 and 18 SNPs on BTA 3 were removed due to low minor allele frequencies (< 0.01) or genotyping failure (>10%). Association analyses were conducted with the Wald test (tolerance) and the Chi-square test (*Map* tissue infection). A region of 32kb was associated with tolerance (P<0.03), and an 86kb region was associated with *Map* tissue infection (P<0.05). These results support our previous findings and suggest the existence of a gene or regulatory element associated with tolerance and infection on BTA 15 and BTA 3, respectively.

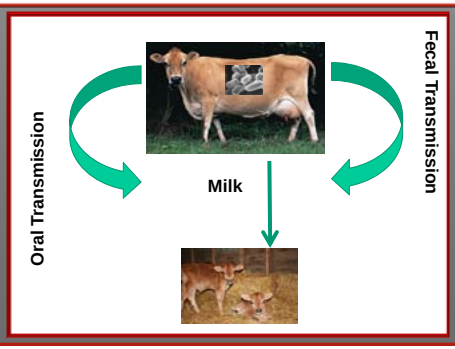
INTRODUCTION

The incidence of Johne's disease continues to increase in the United States. It is estimated to be present in 67% of US dairy herds (APHIS, 2008), resulting in annual losses exceeding $200 million US dollars (Ott et al. 1999). The disease results in lowered milk production, intermittent diarrhea, weight loss and death.
Impediments to lowering the incidence of Johne's disease are dairies with large animal numbers, low sensitivity of current diagnostic techniques and a long incubation period prior to the appearance of clinical signs (Chiodini & Merkal 1984, Collins et al. 2006). Oral-fecal transmission is the most common mode of infection between animals.
The association between Johne's disease in cattle and Crohn's disease in humans is not well known. However, *Map* was found in more than 42% of patients with Crohn's disease and was not observed in healthy individuals (Rudoler, 2004).

=> If Johne's disease is found to be zoonotic, selection of animals that will be resistant to Johne's could be used to reduce the spreading of the disease to humans.
=> If Johne's disease is **not** a zoonotic disease, selection of animals that will be tolerant to Johne's could be used to reduce the economic losses caused by the disease.

OBJECTIVE

The objective of this study was to confirm the association of *Map* **Tissue Infection** on BTA 3 and with **Tolerance** on BTA15, using a fine mapping approach.



Host can evolve to two types of defense mechanism against pathogenic infection

⇒**Resistance**
⇒**Tolerance**

MATERIALS AND METHODS

-87 Holstein cows were genotyped for the quantitative trait of tolerance
-**BTA 15**, 54 SNPs were used to interrogate a 193kb region.
- 19 SNP were excluded due to minor allele frequency (< 0.01))/genotyping failure (>10%)

- 205 Holstein cows were genotyped (89 cases and 118 controls) for the trait of *Map* tissue infection
-**BTA 3**, 42 SNPs were chosen for a 235kb region.
-10 SNPs were excluded due to low minor allele frequencies (< 0.01).

Association analyses were conducted with the Wald statistical test (tolerance) and the Chi-square test (*Map* tissue infection).

RESULTS

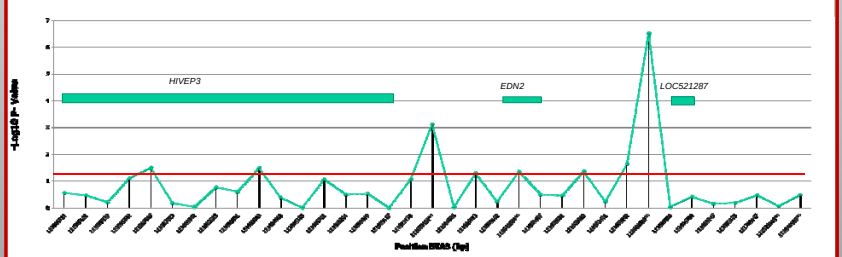


Fig. 1-Fine map. plot of -log10 (p-values) for an association of loci with tissue infection BTA 3.

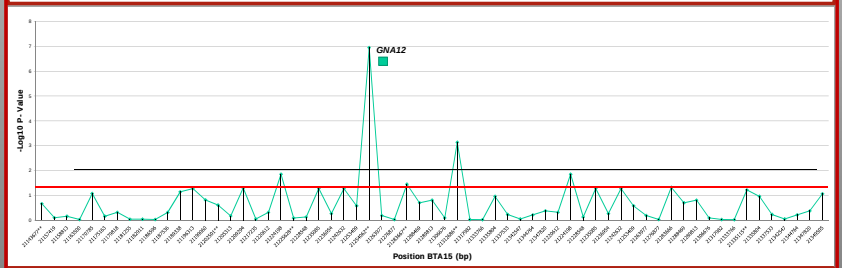


Fig. 2-Fine map. plot of -log10 (p-values) for an association of loci with tolerance to Johne's Disease BTA 15.

DISCUSSION

A candidate gene was found localized on BTA 15, Guanine nucleotide-binding proteins (*GNA12).* This gene is involved as a modulator in various trans-membrane signaling systems. On BTA 3, three candidates genes were found (*EDN2*, *HIVEP3* and *LOC521287)*. Endothelin-2 (*EDN2)* is associated with initiation of intracellular signaling events and cytokines activities as an endothelium vasoconstrictor peptide. Human immunodeficiency virus type I enhancer binding protein 3 (*HIVEP3)*, has been described in regulating patterns of gene activation in response to pro-inflammatory stimuli. *HIVEP3* is a participant in the signal transduction pathway leading from the TNF receptor to gene activation and may play a critical role in inflammatory and apoptotic responses. *LOC521287* is an important regulator of DNA replication and mitosis in a variety of cell types.

CONCLUSION

•Our findings confirmed the previous results of association between BTA 3 and *Map* tissue infection and BTA 15 and *Map* tolerance.
•The identification of the candidate genes with possible association with **tolerance** and **resistance** to Johne's disease may lead us to the discovery of the genetic component responsible for those mechanisms of defense against pathogenic infection.
•Future research will focus on sequencing *GNA12* and *EDN2*.

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