

Association of BTA3 with *Mycobacterium avium paratuberculosis* Tissue Infection in Holsteins

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ABSTRACT

Johne's disease is a highly contagious fatal bacterial disease caused by *Mycobacterium avium paratuberculosis* (*Map*). Johne's disease is estimated to be present in 67% of US dairy herds and results in significant economic loss. A 70kb region on BTA3 encompassing the gene *EDN2* was found to be associated ($p < 0.01$) with tissue infection. Two variants that were associated, 671 bp and 11,161 bp from the initiation site of *EDN2*, were found to alter the transcription binding factors for *EDN2*. The objective of this study was to confirm the association found with *Map* tissue infection on BTA3 by genotyping these two variants in two independent populations of Holsteins. Tissues were collected from 270 and 205 cull Holstein cows at abattoirs in Fresno, California and Jerome, Idaho, respectively. DNA was extracted from ileo-cecal lymph nodes using the Qiagen (Valencia, CA) DNA extraction kit for genotyping and the Applied Biosystems (Carlsbad, CA) Mag-Max DNA extraction for *Map*. *Map* tissue infection was determined by quantitative PCR. Ninety-eight animals (21%) were tissue infected and defined as cases and the remaining 377 animals were used as controls. Tests for population stratification are being conducted using principal component analysis, and association tests to determine if there was an association between BTA3 and *Map* tissue infection are being conducted using χ^2 analysis. The identification of loci associated with *Map* tissue infection will allow selection for cattle that are less susceptible to Johne's disease and a subsequent reduction in the prevalence of Johne's disease.

Key Words: ileum, Johne's disease, *Mycobacterium avium paratuberculosis*

INTRODUCTION

Johne's disease (JD), also known as bovine paratuberculosis, is a prevalent threat in the cattle industry worldwide. JD is caused by *Mycobacterium avium paratuberculosis* (*Map*) bacteria. Usually infection occurs in calves, but they do not display symptoms until 2-5 years of age (Stabel, 1998). Symptoms of JD include diarrhea, emaciation, poor milk production and reproductive performance, and ultimately death (Stabel, 1998).

JD can be devastating due to the long time period between infestation and manifestation. During the years that a specimen is infected, they are spreading *Map* to other individuals within their herd. Current techniques of identifying infected individuals before symptoms occur are ineffective, which creates challenges when attempting to contain JD.

Oral-fecal transmission is the most common mode of infection. Other means of becoming infected exist, such as airborne environmental contamination (Raizman et al., 2004). It is therefore possible for a few infected animals to spread JD throughout their entire herd.

Two loci on a 70kb region of BTA3 surrounding the gene *EDN2* have previously demonstrated an association with tissue infection by *Map* (Zanella et al. 2011). These single nucleotide polymorphisms are 671bp and 11,161bp away from the *EDN2* initiation site respectively, and alter its transcription binding factors. The present study seeks confirmation by genotyping the 70kb region of BTA3 in two independent populations of Holsteins, using both cases and controls.

OBJECTIVE

Confirm previously identified associations between two single nucleotide polymorphisms in the *EDN2* promoter region with tolerance (high tissue infection and low fecal shedding of *Map*) in order to determine a favorable genotype for selective breeding and develop superior techniques for early detection of Johne's disease.



MATERIALS AND METHODS

1) DNA extraction

- DNA was extracted from the ileo-cecal nodes of 475 Holstein cows from two herds using the Qiagen DNA extraction kit (Valencia, CA).

2) Map detection

- Map* was extracted from ileo-cecal nodes using Ambion's MagMAX Total Nucleic Acid Isolation kit (Austin, TX)
- Samples were tested for *Map* using qPCR
- 96-well plates were loaded with duplicates of each sample
- Duplicates of six standard curve solutions were used

3) Genotyping

- Touch-down allele-specific PCR is ongoing to genotype the SNP 105 and SNP 208 regions using four unique primers
- Primers were designed using Primer3 and consisted of:
5'-AGGCAGTGACCTCAAAGGCTCCCAGTA-3' SNP105A
5'-GGCAGTGACCTCAAAGGCTCCCAGTG-3' SNP105G
5'-CCCTTTCCAGAAAGGTGGCTTGTGGA-3' SNP208T
5'-CCCTTTCCAGAAAGGTGGCTTGTGGC-3' SNP208G

4) Analysis

- A Chi-square analysis will be implemented to test for an association with *Map* tissue infection and these variants

DISCUSSION

The process of harvesting the ileo-cecal node from an animal to DNA extraction, *Map* detection, and genotyping was a massive undertaking that could not be completed during this summer internship. However, most of the study was completed and a tremendous number of difficulties were overcome. Cutting tissue samples provided an opportunity to learn how to identify distinct tissue types as well as practice the safe handling and disposal of bio-hazardous material. Work on extracting DNA from the samples was prohibited for undergrad students because *Map* is classified as a zoonotic pathogen to humans. There is controversy regarding if *Map* is involved in the etiology of Crohn's disease, a debilitating granulomatous enteritis in humans that has several common features with Johne's disease.

Performing qPCR on each of the samples was a remarkable learning experience, from preparation of the plate to understanding how to create and use a standard curve to being able to analyze results. Even using the real-time PCR machines required substantial knowledge. Designing primers for allele-specific PCR to genotype each sample proved to be the most challenging step. Four primers were needed to amplify both alleles at both loci, and it was necessary to make them highly specific. The annealing temperature for the primers was approximately 74C° because they are very long with a high GC percentage. During primer optimization, the primers proved to reliably amplify the correct product in previously genotyped control samples. The use of touch-down PCR granted further understanding of how DNA behaves during a PCR run.

Currently, 150 of the 475 samples have been genotyped. The remaining samples will be completed and analyzed this summer.



Figure 1: Johne's disease results in loss of production and chronic wasting before death.

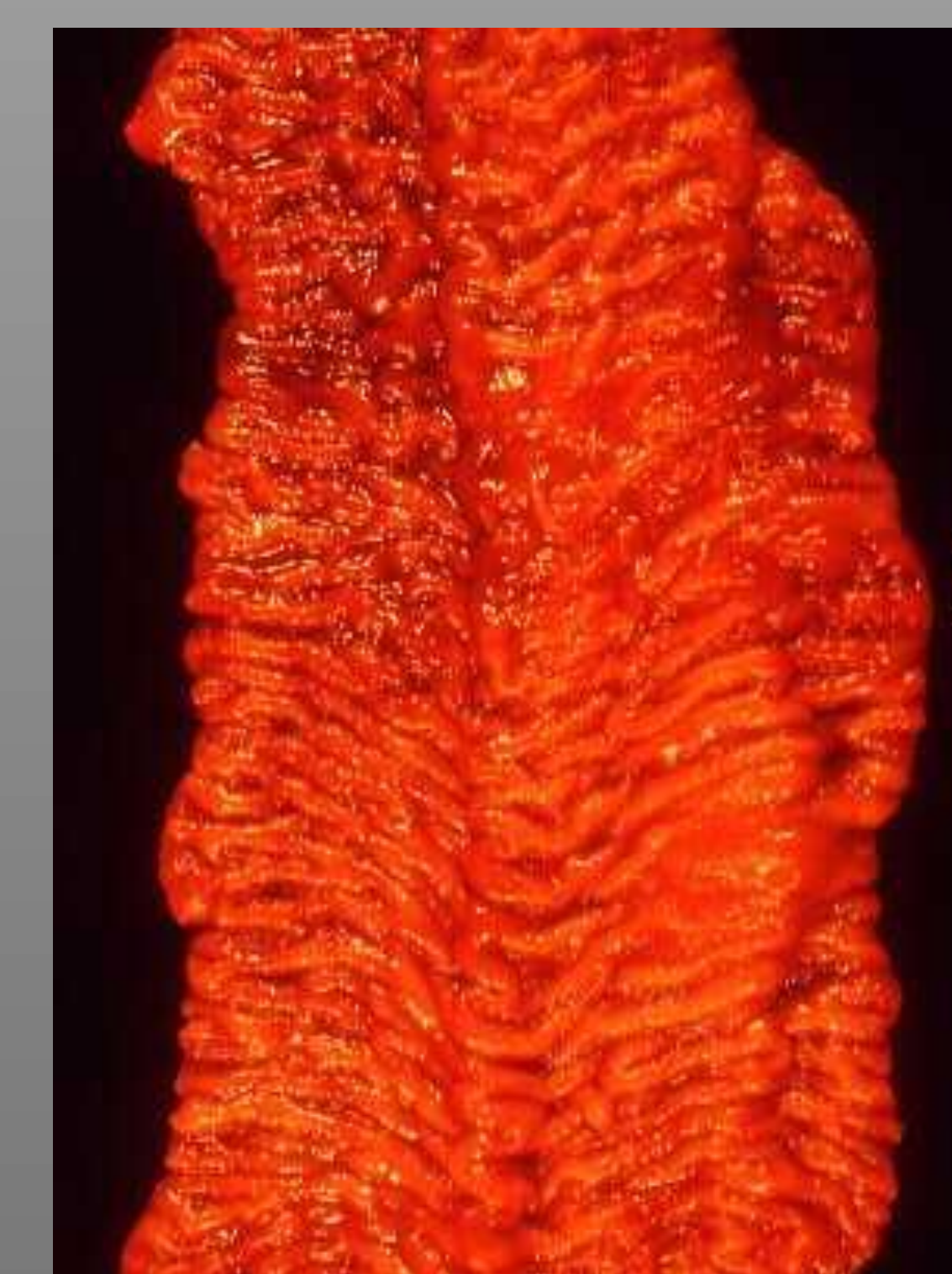


Figure 2: Bovine intestinal tract displaying symptoms of Johne's disease.

RESULTS

1) Map infection

- 19 of 270 cull Holstein cows from California (7%) were infected with *Map*.
- 70 of 205 cull Holstein cows from Idaho (34%) were infected with *Map*.

2) Genotyping

- Testing ongoing

Literature Cited

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