

Genetic Resistance to Johne's Disease in Four Cattle Breeds: A Candidate Gene Case Control Study, Preliminary Results

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Introduction

Paratuberculosis is a chronic debilitating, infectious disease of ruminants, caused by *Mycobacterium avium* subsp. paratuberculosis, and characterized by progressive weight loss and profuse diarrhea. The disease has been suspected to have a genetic component and estimations of heritability of 0.15 have been reported. Crohn's disease (CD) is an inflammatory bowel disease in humans resembling many aspects of paratuberculosis. Susceptibility to CD has a strong genetic component and one gene involved has been identified as CARD15 (Caspase recruitment domain 15), with a role in the intracellular recognition of bacterial cell walls as a part of the immune response. The association between mutations in this gene and increased risk of CD has been extensively described. Since Johne's disease has been demonstrated to have a genetic component and genetic variation for infection resistance exists in cattle, CARD15 could also determine differences in resistance to paratuberculosis in the bovine. This candidate gene study focused on determining the alleles present in the CARD15 gene in a population of infected and of non-infected cows. Our objective was characterizing the distribution of two polymorphisms in this gene and testing their association with paratuberculosis infection in Florida cattle with the aim of finding resistance alleles useful in livestock selection.

Materials and Methods

The study population included 397 Holstein, Jersey and Brahman-Angus adult cows in five operations. The infection status for cases and controls was determined using five diagnostic tests (serum ELISA, milk/blood/fecal PCR, fecal culture). Parallel interpretation of results was used to account for the limitation in sensitivity of available diagnostic tools, with a final ratio of cases to controls of 1:3. Two previously reported single nucleotide polymorphisms (SNP1: C733R, and SNP2: Q1007L) in the bovine CARD15 gene, responsible for two amino acid substitutions, were examined for this study population by the TaqMan genotyping assay. The statistical analysis consisted OF Chi square and Fisher

exact test, to test significant differences in allele frequencies between cases and control populations. Logistic regression models were proposed to estimate odds of infection related to the presence of singular alleles. Our hypothesis was that infected and non-infected cows will present different variants for our candidate gene that could represent resistance alleles.

Results

Frequencies for the major allele in SNP1 and SNP2 were 0.957 and 0.527, respectively, and the population was in Hardy-Weinberg equilibrium only for SNP2. Most of the individuals carrying the variant allele for SNP1 were Brahman cattle and the SNP2 variant allele was evenly distributed in the breeds. The statistical analysis resulted in significant differences in allelic frequencies between cases and controls for SNP1 ($p < 0.001$) indicating a association between infection and variant allele. A significant association was also found between infection and SNP1 diplotypes ($p < 0.0001$) and infection and combination of both SNPs ($p < 0.01$). Six models were tested for the logistic regression analysis, considering infection as outcome variable. The analysis resulted in significant parameters for SNP1 genotype (alone and with SNP2) and for breed of animal (alone and combined with SNP1). The low representation of the variant allele for SNP1 in Holstein and Jersey breeds raises the concern of a potential role of breed as a confounding for its association with infection. A new subset of Brahman animals will be added to the analysis to clarify this point.

Significance

Preliminary results suggest a role for CARD15 gene in the susceptibility to paratuberculosis infection. Amino acid substitution C733R (SNP1) appears to be associated to paratuberculosis infection in Florida cattle. These results could be the basis for further research to create a rapid method to select for more resistant individuals genetically contributing to the control of Johne's disease.