Short communication: Refinement of genetic regions associated with *Mycobacterium avium* subspecies *paratuberculosis* tissue infection and tolerance to Johne's disease

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ABSTRACT

Johne's disease is a highly transmissible bacterial disease caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). The objective of this study was to refine the locus associated with MAP tissue infection and the locus associated with tolerance to Johne's disease. Using a genome-wide association analysis, single nucleotide polymorphisms associated with MAP tissue infection and tolerance to Johne's disease on *Bos taurus* autosome (BTA)3 and BTA15, respectively, have previously been identified. A 235-kb region on BTA3 was evaluated with 42 single nucleotide polymorphisms, and a 193-kb region on BTA15 was evaluated with 54 single nucleotide polymorphisms in a group of 209 Holstein cows. Using a single marker association analysis and haplotype tests, we refined a region of 10.6 kb on BTA3 as being associated with MAP tissue infection and a region of 6.5 kb on BTA15 as being associated with tolerance to Johne's disease.

Key words: Johne's disease, genetic association

Short Communication

Johne's disease (bovine paratuberculosis) is a chronic, incurable, bacterial disease of ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP; Buerge et al., 1978; Gonzalez et al., 2005). The fecal-oral route is the major mode of transmission; however, fetal infection has also been observed (Seitz et al., 1989; Sweeney et al., 1992a,b). In the dairy industry, Johne's disease is responsible for substantial economic losses due to premature culling and reduced production (Nordlund et al., 1996; Johnson-Ifearulundu et al., 2000; USDA, 2008). Johne's disease has increased in prevalence in the United States over the last few decades. In 1996, 21% of US dairy herds had one or more animals infected with MAP; in 2007, the number of herds with

Johne's disease had increased to 68% (NAHMS, 1997; USDA, 2008).

Animals exposed to a pathogen may defend themselves through mechanisms of resistance or tolerance. Susceptibility has been defined as the likelihood of an animal to become infected by a pathogen (Raberg et al., 2007). Susceptibility to Johne's disease has been measured by MAP tissue infection of the animal (Settles et al., 2009). Infection leads to an inflammatory response at the host level initiating an antagonistic interaction between the host and pathogen (Stecher and Hardt, 2008). Tolerance has been defined as the ability of an animal to limit the severity of the infection caused by the pathogen (Raberg et al., 2007; Zanella et al., 2011). Tolerance to Johne's disease measures the relationship between infection intensity (level of MAP in the tissues) and fitness (level of MAP fecal shedding; Zanella et al., 2011). Susceptibility to Johne's disease has been classified as a categorical trait, whereas tolerance to Johne's disease has been measured as a quantitative trait. The quantitative estimation of tolerance to Johne's disease was computed using the peak levels of MAP fecal shedding and the peak levels of MAP tissue infection as described previously (Zanella et al., 2011). The effectiveness of selecting for animals that are less susceptible or tolerant to Johne's disease is unknown. The need exists to evaluate further the potential for selection of animals that are less susceptible to MAP tissue infection or are tolerant to Johne's disease. It was the objective of this study to refine the locus on BTA3 associated with MAP tissue infection and the locus on BTA15 associated with tolerance to Johne's disease in Holstein cattle. This study was approved by the Washington State University Institutional Animal Care and Use Committee, Office of Research Assurances.

The animals used for fine mapping in this study were the same as those used for the initial genome-wide association analysis described previously (Settles et al., 2009; Neibergs et al., 2010; Zanella et al., 2011). Briefly, tissue and fecal samples from 245 Holstein cows from 4 dairy herds located in the eastern United States were harvested at slaughter and cultured for the pres-

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ence of MAP (Whitlock et al., 1996, 2000; Pradhan et al., 2009). To determine if the animals were MAP tissue infected or were shedding MAP in the feces, the presence of MAP was detected by cultures prepared from tissue taken from the ileum, ileo-cecal valve, and 2 adjacent ileo-cecal lymph nodes and from fecal samples collected at the time of slaughter using the method described by Whitlock et al. (1996). Samples with counts (cfu) of MAP >0 were classified as MAP infected. After genotyping and filtering for quality control, 90 animals were classified as MAP tissue infected (cases), and 119 animals were classified as MAP tissue negative (controls), regardless of their fecal culture status (Settles et al., 2009). None of the animals in this study were related as determined by their pedigrees or through identity-by-descent analysis. The phenotypic classification of MAP tissue infection was chosen because this phenotype had the strongest evidence ($P = 3 \times 10^{-7}$) for an association with MAP tissue infection and BTA3 (Settles et al., 2009).

Tolerance to Johne's disease was defined as the inverse relationship between fitness (measured by MAP fecal shedding) and infection intensity (measured by the level of MAP tissue infection). For peak fecal values, the greatest value of 4 culture tubes from 1 fecal sample was used for each animal. The peak tissue value was represented by the greatest count (cfu) for 1 of the 4 tissues evaluated. The quantitative measurement of tolerance to Johne's disease was calculated using the ratio of MAP peak counts cultured from fecal material and tissue samples as described previously (Zanella et al., 2011). This measure of tolerance was used as it provided the strongest evidence for an association ($P = 1.1 \times 10^{-7}$) with tolerance to Johne's disease and BTA15 (Zanella et al., 2011). All animals that were measured for tolerance were MAP tissue positive.

Genotyping data were assessed for quality before the association analysis. An animal's genotyping data were removed from the analysis when more than 10% of the genotypes failed. This resulted in the removal of genotyping data of 5 animals (2 cases and 3 controls) from the MAP tissue infection analysis, leaving 88 cases and 116 controls. For the fine mapping of tolerance, the removal of 2 cases resulted in a total of 87 animals evaluated.

A custom 96-SNP assay (Illumina, San Diego, CA) was designed for the genotyping of the regions on BTA3 and BTA15. Single nucleotide polymorphisms were identified through dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>) and were chosen based on a lack of repetitive regions surrounding the SNP, their nucleotide location based on the Btau_4.0 assembly, SNP with high minor allele frequency (**MAF**), whether the SNP had been validated (if they had not been validated,

SNP with confirmation of sequence variation in more than one breed were preferred), and through the selection of sequences with scores >70 utilizing the Illumina assay design tool (Liu et al., 2009; National Center for Biotechnology Information, 2010). On BTA3, 42 SNP were chosen to cover a 235-kb region (111,586,713 to 111,821,915 bp), with a median spacing of 2.3 kb. On BTA15, 54 SNP were used to cover a 194-kb region (21,155,914 to 21,349,505 bp), with a median spacing of 3.0 kb. Physical positions at each SNP were expressed relative to the forward strand of the reference genome (Table 1; Liu et al., 2009).

Single nucleotide polymorphisms were also assessed for quality before the association analysis. Single nucleotide polymorphisms were removed if MAF was less than 2%, if the SNP failed to genotype in more than 10% of the samples, or if the SNP failed the Hardy-Weinberg equilibrium test ($P < 0.001$). Eighteen SNP on BTA3 had low MAF and 2 SNP failed the Hardy-Weinberg equilibrium test and were therefore removed from the analysis (Table 1). On BTA15, 8 SNP were removed due to low MAF, 3 SNP failed the Hardy-Weinberg equilibrium test, and 5 SNP had genotyping failure in more than 10% of the samples. Four SNP failed for more than one reason, resulting in 12 SNP excluded on BTA15 (Table 1). After excluding poor quality SNP, 22 SNP with a median spacing of 3.3 kb remained for the fine mapping of MAP tissue infection on BTA3, and 42 SNP with a median spacing of 3.5 kb remained for the fine mapping of tolerance on BTA15.

Statistical analysis was conducted with PLINK (version 1.07; Purcell et al., 2007). A chi-squared test was used to test for an association of MAP tissue infection with BTA3, and the Wald test was used to test for an association with tolerance to Johne's disease and BTA15. A significance threshold for the association analysis was set at $P < 0.05$ after a chromosome-wide Bonferroni correction for multiple testing. A haplotype analysis was also performed with PLINK to identify if a haplotype was more informative than a single SNP in refining the association on BTA3 with MAP tissue infection and BTA15 with tolerance to Johne's disease. For each chromosome, an omnibus association statistic was performed to identify the overall association of the haplotype. If a significant association was identified, a haplotype-specific analysis was performed to determine which haplotype had the strongest evidence for an association with MAP tissue infection and tolerance to Johne's disease. Using the most significant haplotype blocks constructed within the region associated with the tolerance and MAP tissue infection, a multiple SNP analysis using a backward-elimination method was performed. In this method, individual SNP were first removed and the association retested, and then combi-

Table 1. Single nucleotide polymorphisms used to refine the loci associated with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) tissue infection on BTA3 and tolerance to Johne's disease on BTA15

BTA	SNP ¹	Position (bp) ²	Allele 1 ³	Allele 2 ⁴	MAF ⁵
3	ss86339534	111,586,713	G	A	0.118
3	rs43364348	111,587,151	C	G	0.289
3	rs43364349	111,587,319	G	A	0.468
3	rs43364352	111,595,322	A	C	0.447
3	rs43364355	111,596,158	A	G	0.043
3	rs43364358	111,597,507	A	G	0.229
3	rs43364359	111,597,612	C	G	0.264
3	rs43364360	111,600,042	A	G	0.043
3	rs43364362 ⁶	111,602,315	A	C	0.251
3	rs43364365 ⁷	111,603,691	T	C	0.000
3	rs43364366	111,603,963	A	G	0.043
3	rs43364367	111,604,038	A	G	0.354
3	rs43364368 ⁷	111,604,478	A	C	0.000
3	rs43364369	111,605,185	A	G	0.154
3	rs43364370	111,606,780	T	C	0.446
3	rs43363223 ⁶	111,607,985	C	A	0.488
3	rs43363224 ⁷	111,608,284	G	A	0.004
3	rs43363226 ⁷	111,609,910	T	A	0.004
3	rs43363227	111,610,137	C	G	0.317
3	rs43363228	111,611,772	T	G	0.497
3	rs43364331	111,615,363	T	C	0.151
3	rs43364333 ⁷	111,619,177	T	C	0.000
3	ss86284409	111,623,092	G	A	0.451
3	rs43364335 ⁷	111,624,024	T	C	0.002
3	rs43364337 ⁷	111,626,912	T	C	0.002
3	rs43364338 ⁷	111,630,181	C	G	0.004
3	rs43364339	111,638,517	A	C	0.210
3	rs43364342	111,650,486	A	T	0.211
3	rs43364344 ⁷	111,658,290	T	C	0.000
3	rs43364345 ⁷	111,659,259	A	C	0.000
3	rs43363222	111,672,481	A	G	0.214
3	rs43363220 ⁷	111,679,242	A	C	0.002
3	ss86341066	111,682,510	A	C	0.403
3	rs43363219 ⁷	111,685,607	T	G	0.002
3	rs43363218 ⁷	111,685,833	T	C	0.004
3	rs43363217 ⁷	111,686,749	C	G	0.004
3	rs43363216 ⁷	111,690,246	T	C	0.000
3	rs43363215	111,693,185	A	G	0.129
3	rs43361040 ⁷	111,772,066	T	C	0.000
3	rs43361042 ⁷	111,780,147	A	G	0.000
3	rs43361039 ⁷	111,783,711	C	A	0.002
3	ss86321724	111,821,915	T	G	0.287
15	rs41747577	21,155,914	A	G	0.195
15	rs41747580 ^{6,7}	21,157,418	T	A	0.014
15	rs41747581	21,158,813	T	A	0.301
15	rs41747586	21,163,500	G	A	0.233
15	rs41746819 ⁸	21,170,784	A	T	0.343
15	rs41746814	21,175,163	G	A	0.372
15	rs41746792	21,179,818	G	A	0.135
15	rs41746789	21,181,254	T	C	0.033
15	rs43201145 ⁷	21,182,910	A	T	0.016
15	rs41746787	21,186,596	G	A	0.137
15	rs41746785	21,187,535	C	T	0.137
15	rs41746783	21,189,338	A	G	0.021
15	rs41746762	21,196,312	T	C	0.021
15	rs41746759	21,199,060	A	C	0.024
15	rs41746757	21,200,628	T	C	0.134
15	rs41746756	21,202,180	T	A	0.134
15	rs41746753 ^{7,8}	21,205,312	A	G	0.000
15	rs41746748 ⁸	21,209,294	A	G	0.181
15	rs41746747	21,212,440	C	T	0.031
15	rs41746743	21,214,982	G	A	0.031
15	rs41746741	21,217,235	C	G	0.021
15	rs41746739	21,220,611	C	G	0.060

Continued

Table 1 (Continued). Single nucleotide polymorphisms used to refine the loci associated with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) tissue infection on BTA3 and tolerance to Johne's disease on BTA15

BTA	SNP ¹	Position (bp) ²	Allele 1 ³	Allele 2 ⁴	MAF ⁵
15	<i>rs41746737</i>	21,224,197	C	G	0.156
15	<i>rs41745722</i>	21,228,547	T	C	0.375
15	<i>rs41745716</i>	21,235,084	T	C	0.149
15	<i>rs41745713</i> ⁷	21,236,954	C	A	0.004
15	<i>rs41745710</i> ⁷	21,242,632	A	G	0.007
15	<i>rs41748392</i>	21,251,461	G	A	0.454
15	<i>rs41748407</i>	21,253,408	T	C	0.101
15	<i>rs41748401</i>	21,257,974	T	C	0.147
15	<i>rs41748400</i>	21,260,261	C	G	0.152
15	<i>rs41748395</i>	21,262,642	T	C	0.283
15	<i>rs41748410</i>	21,263,976	T	C	0.318
15	<i>rs41748412</i>	21,270,896	A	G	0.320
15	<i>rs41748414</i> ⁶	21,275,277	A	G	0.370
15	<i>rs41749247</i>	21,276,876	T	C	0.146
15	<i>rs41749241</i>	21,280,720	G	C	0.139
15	<i>rs41749235</i>	21,283,665	T	C	0.151
15	<i>rs41748422</i>	21,288,468	T	G	0.342
15	<i>rs41748420</i>	21,289,813	G	A	0.156
15	<i>rs41748415</i>	21,295,290	C	T	0.322
15	<i>rs41749248</i>	21,306,676	A	G	0.079
15	<i>rs41749249</i>	21,311,151	T	C	0.077
15	<i>rs41749250</i> ^{6,8}	21,313,152	G	A	0.026
15	<i>rs41749253</i>	21,316,588	G	A	0.290
15	<i>rs41749254</i> ^{7,8}	21,317,982	A	C	0.016
15	<i>rs41749267</i>	21,332,723	G	C	0.000
15	<i>rs41749265</i>	21,333,766	A	T	0.131
15	<i>rs41749264</i>	21,335,803	T	C	0.266
15	<i>rs41749261</i>	21,337,533	A	G	0.135
15	<i>rs41749270</i> ⁷	21,342,547	C	A	0.000
15	<i>rs41749273</i>	21,344,763	C	T	0.454
15	<i>rs41749274</i>	21,347,820	A	G	0.449
15	<i>rs41749275</i>	21,349,505	G	A	0.197

¹GenBank accession number for the SNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>).²Location of the SNP on the chromosome based on Btau_4.0 (Liu et al., 2009).³Allele with lowest frequency observed when cases and controls were combined.⁴Allele with greatest frequency observed when cases and controls were combined.⁵Minor allele frequency: frequency of the allele with the lowest frequency observed when cases and controls were combined.⁶SNP that were excluded due to lack of Hardy Weinberg equilibrium ($P < 0.001$).⁷SNP that were excluded due to minor allele frequency < 0.01 .⁸SNP that were excluded because more than 10% of the genotypes were missing.

nations of SNP were included and excluded manually from each haplotype block using PLINK to define the region associated with each phenotype.

Within the 235-kb region tested on BTA3, 2 loci located 59.4 kb apart (*ss86284409*:G > A at 111,623,092 bp and *ss86341066*:A > C at 111,682,510 bp) were associated with MAP tissue infection ($P < 0.05$; Table 2). These 2 SNP were in strong linkage disequilibrium (LD) with each other ($r^2 = 0.784$, $D' = 0.99$). The locus *ss86341066* provided the strongest evidence for association ($P = 0.0001$) with almost a 2-fold difference in the allele frequency between the cases (0.26) and the controls (0.5). This was also the locus previously reported as being associated with MAP tissue infection (Settles et al., 2009; Neibergs et al., 2010). Of the 22

SNP evaluated on BTA3, 6 loci (*ss86284409*:G > A, *rs43364339*:A > C, *rs43364342*:A > T, *rs4336322*:A > G, *ss86341066*:A > C, and *rs43363215*:A > G) comprised a single haplotype block that spanned a 70-kb region. This haplotype (ACTGCG) was associated ($P = 0.0003$) with MAP tissue infection because it was found at higher frequency (0.64) in animals that were MAP tissue positive than in animals that were MAP tissue negative (0.46; Table 2). This haplotype provided supporting evidence that a causative mutation for MAP tissue infection resides in this region of BTA3, but it did not provide stronger evidence than the association detected with *ss86341066*:A > C alone ($P = 0.0001$). The systematic elimination of SNP from the most significant haplotype block demonstrated that the

Table 2. Loci associated with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) tissue infection on BTA3

SNP ¹	Position (bp) ²	MAF ³		Unadjusted <i>P</i> -value ⁴	Bonferroni-adjusted <i>P</i> -value ⁵
		Cases	Controls		
<i>rs43363227</i>	111,610,137	0.36 (C)	0.28 (C)	0.0800	1.0000
<i>rs43364331</i>	111,615,363	0.19 (T)	0.12 (T)	0.0400	1.0000
<i>ss86284409⁶</i>	111,623,092	0.35 (G)	0.53 (G)	0.0003	0.0100
<i>rs43364339⁶</i>	111,638,517	0.15 (A)	0.25 (A)	0.0100	0.4200
<i>rs43364342⁶</i>	111,650,486	0.15 (A)	0.25 (A)	0.0100	0.4200
<i>rs43363222⁶</i>	111,672,481	0.16 (A)	0.25 (A)	0.0200	0.9700
<i>ss86341066⁶</i>	111,682,510	0.26 (A)	0.50 (A)	1.5×10^{-6}	0.0001
<i>rs43363215⁶</i>	111,693,185	0.07 (A)	0.17 (A)	0.0020	0.0800

¹GenBank accession number for the SNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>).

²Location of the SNP on chromosome 3 based on Btau_4.0 (Liu et al., 2009).

³Case: variant nucleotide (listed in parentheses) that represents the allele with the lowest frequency (minor allele frequency or MAF) in animals that are MAP tissue positive, regardless of whether they were shedding MAP in the feces; Control: variant nucleotide (listed in parentheses) that represents the allele with the lowest frequency (minor allele frequency or MAF) in animals that are MAP tissue negative, regardless of whether they were shedding MAP in the feces.

⁴Chi-squared asymptotic *P*-value before Bonferroni adjustment for multiple testing.

⁵Chi-squared asymptotic *P*-value after Bonferroni adjustment for multiple testing.

⁶SNP that comprise the haplotype block associated with MAP tissue infection ($P = 0.0003$).

elimination of *ss86284409* from the haplotype analysis improved the significance of the association (3×10^{-4} to 7.8×10^{-6}). Additional removal of *rs43364339* did not alter the significance of the haplotype ($P = 8 \times 10^{-6}$). Additional removal of *rs43364342* and *rs43363222* improved the significance ($P = 1.55 \times 10^{-6}$); moreover, the use of only *ss86341066* and *rs43363215* provided the strongest evidence ($P = 1.55 \times 10^{-6}$) for an association with tissue infection. These 2 SNP redefine the region of 235 kb to a region of 10.6 kb that most

likely harbors the functional mutation responsible for the association of MAP tissue infection on BTA3.

Within the 194-kb region tested on BTA15, tolerance to Johne's disease was associated ($P < 0.05$) with 2 loci that were located 22.9 kb apart (*rs41745722:T > C* at 21,228,547 bp and *rs41748392:G > A* at 21,251,461 bp; Table 3). Two SNP, *rs41745722* ($P = 0.001$) and *rs41748392* ($P = 3.4 \times 10^{-5}$), were associated with tolerance to Johne's disease in the same region that previously provided strong evidence for an association

Table 3. Loci associated with tolerance to Johne's disease on BTA15

SNP ¹	Position (bp) ²	MAF ³	Unadjusted <i>P</i> -value ⁴	Bonferroni-adjusted <i>P</i> -value ⁵
<i>rs41746737</i>	21,224,197	0.15 (C)	0.030	1.00
<i>rs41745722⁶</i>	21,228,547	0.37 (T)	3.7×10^{-5}	1.0×10^{-3}
<i>rs41745716⁶</i>	21,235,084	0.15 (T)	0.030	1.00
<i>rs41748392⁶</i>	21,251,461	0.44 (G)	6.38×10^{-7}	3.4×10^{-5}
<i>rs41748407⁶</i>	21,253,408	0.09 (T)	0.490	1.00
<i>rs41748401⁶</i>	21,257,974	0.15 (T)	0.030	1.00
<i>rs41748400⁶</i>	21,260,261	0.15 (C)	0.030	1.00
<i>rs41748395⁶</i>	21,262,642	0.28 (T)	0.020	1.00
<i>rs41748410⁶</i>	21,263,976	0.32 (T)	0.014	0.75
<i>rs41748412⁶</i>	21,270,896	0.32 (A)	0.018	0.97
<i>rs41749247</i>	21,276,876	0.14 (T)	0.030	1.00
<i>rs41749241</i>	21,280,720	0.14 (G)	0.020	1.00
<i>rs41749235</i>	21,283,665	0.15 (T)	0.050	1.00
<i>rs41748422</i>	21,288,468	0.34 (T)	0.070	1.00
<i>rs41748420</i>	21,289,813	0.15 (G)	0.050	1.00
<i>rs41748415</i>	21,295,290	0.32 (C)	0.030	1.00

¹GenBank accession number for each SNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>).

²Location of the SNP on chromosome 15 based on Btau_4.0 (Liu et al., 2009).

³Variant nucleotide (listed in parentheses) that represents the allele with the lowest frequency (minor allele frequency, MAF) in animals evaluated for tolerance.

⁴Wald test asymptotic *P*-value before Bonferroni adjustment for multiple testing.

⁵Wald test asymptotic *P*-value after Bonferroni adjustment for multiple testing.

⁶SNP that comprise the haplotype block associated with tolerance ($P = 8.09 \times 10^{-6}$).

(Zanella et al., 2011). Both of these SNP are in strong LD ($r^2 = 0.417$ and $D' = 0.914$).

A 42-kb haplotype block (TCACCGCCG), composed of 9 SNP including *rs41745722* and *rs41748392*, was associated ($P = 8.09 \times 10^{-6}$) with tolerance to Johne's disease on BTA15 (Table 3). The haplotype analysis on BTA15 did provide stronger evidence of an association than the individual loci identified as being associated with tolerance to Johne's disease. This reduced the region of interest from 194 to 42 kb. Methodically eliminating combinations of these 9 SNP by backward elimination provided an additional narrowing of the region associated with tolerance to Johne's disease. The SNP providing the most evidence for an association using this approach were *rs4174522*, *rs41745716*, and *rs41748392* ($P = 5.5 \times 10^{-6}$) that span a 22.9-kb region of BTA15. As *rs4174522* and *rs41748392* were in high linkage disequilibrium ($D' = 0.914$), these results suggest that the 6.5-kb region between *rs4174522* and *rs41745716* is the most likely to harbor a mutation associated with tolerance to Johne's disease.

Loci previously identified as associated with MAP tissue infection and tolerance to Johne's disease in a whole-genome association analysis were further characterized in the current study (Settles et al., 2009; Zanella et al., 2011). The objective of this study was to refine these loci to narrow the region to explore for a mutation associated with MAP tissue infection and tolerance to Johne's disease. The locus for MAP tissue infection was refined to 2 SNP 10.6 kb apart on BTA3 and the locus for tolerance to Johne's disease to 3 SNP, 2 of which are in high LD, further refining the region to 6.5 kb on BTA15.

It is possible that the 2 narrow regions identified in this study do not host the functional mutations for these associations. This could occur if the 2 SNP that define these regions were in LD with the functional mutation but were positioned outside the regions targeted in this study. If the initial genome-wide association studies (Settles et al., 2009; Zanella et al., 2011) had had perfect placement of SNP in relationship to the changes in LD throughout the genome, then the identification of LD with *ss86341066* and *rs433623215* on BTA3 and *rs4174522* and *rs41745716* on BTA15 and other genomic regions would have been detected. However, it is known that the SNP placement in the Illumina BovineSNP50 BeadChip is imperfect and so the possibility exists that the SNP that bound the 10.6-kb and 6.5-kb regions may not represent the boundaries of the location of the functional mutations but merely represent a region in LD with the functional mutation. Resequencing of the DNA in this region along with functional studies will be needed to determine if these

regions contain the functional mutations responsible for the associations with Johne's disease.

Positional candidate genes were not identified immediately within the refined regions associated with MAP tissue infection on BTA3 or tolerance to Johne's disease on BTA15. However, the region associated with MAP tissue infection on BTA3 is highly conserved between human, chimpanzee, dog, and cow, harbors several different regulatory elements, and is adjacent (30 kb) to endothelin-2 (*EDN2*) (National Center for Biotechnology Information, 2010). The region associated with tolerance to Johne's disease on BTA15 is partially conserved among human, dog, and cow, has not been shown to contain regulatory elements, but is 19 kb from guanine nucleotide binding protein (G protein) α 12 (*GNA12*) (National Center for Biotechnology Information, 2010).

In conclusion, this study has refined a locus for MAP tissue infection and a locus for tolerance to Johne's disease. Further investigation of these loci for the mutations responsible for these associations will contribute to our understanding of the mechanisms underlying MAP tissue infection and tolerance to Johne's disease and provide resources for the selection of cattle less susceptible to Johne's disease.

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