

Genome-wide analysis of metritis status and time-to-metritis

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Summary

Metritis is an important uterine disease that negatively impacts cow health and profitability. Single nucleotide polymorphisms (SNPs) associated with metritis status and time-to-metritis detection were detected using information on approximately 600,000 SNPs from approximately 2100 dairy cows in 16 US herds. The SNPs were evaluated using logistic and survival analysis procedures. On average, metritis was detected on 30.4% of the cows at 6.8 days postpartum. Metritis status and days-to-metritis were associated (P-value < 0.0001) with 199 and 7 SNPs, respectively. Most of these SNPs were mapped to intergenic regions and within known genes. Information from these genetic variants can be applied in genomic selection programs to reduce the incidence of metritis and accelerate detection with the goal of improving the overall health of the herd and minimize costs.

Keywords: logistic, survival analysis, SNP

Introduction

Metritis is a uterine disease prevalent during early lactation. The incidence of metritis rapidly increases after calving reaching 40% between 4 and 12 days postpartum (Sheldon *et al.* 2009). This disease can be potentially costly to the dairy industry impacting the herd's fertility and incidence of subclinical endometritis after day 21 postpartum (Sheldon *et al.* 2009).

The characterization of the genetic variation of two complementary metritis indicators, metritis status and time-to-metritis detection, is essential for developing genetic selection schemes that reduce the incidence of metritis. Metritis status was studied as a binary phenotype with two outcomes: metritis or healthy status within the first weeks post-calving. Time-to-metritis detection is the time from parturition up to 2 to 3 weeks postpartum when cows can be diagnosed with metritis. Different approaches must be used to describe these metritis indicators due to the differences in assumptions and interpretations involved (Southey *et al.*, 2001; Southey *et al.* 2003).

Material and Methods

Metritis status and number of days postpartum to metritis detection were measured up to 15 days postpartum between November 2012 and October 2014 on approximately 2100 Holstein cows from 16 herds situated in 6 US states. Cows negative for metritis were considered censored at 15 days postpartum. Vaginal discharge score (1: clear to 5: foul) was recorded 5 days postpartum (LeBlanc *et al.*, 2002). Genomic variant information was collected using the BovineHD Genotyping BeadChip platform (Illumina Inc., San Diego, CA) that contains 777,962 single nucleotide polymorphisms (SNPs) (Zimin *et al.* 2009). Approximately 600,000 SNPs were analysed after quality control removal.

Identification of significant explanatory variables used a logistic regression for metritis status and a proportional hazards regression for days-to-metritis detection implemented in SAS (SAS Systems, Cary, NC, USA). The explanatory variables included: herd, parity, and vaginal discharge score at 5 days postpartum. Critical variables to include in the final models were identified using backwards elimination and a P-value < 0.05 threshold.

A progressive variable selection approach was used to identify SNPs associated with metritis and time-to-metritis (Rodriguez Zas & Southey, 2001). The SNP genotypes were analysed within chromosome. Stepwise variable selection using P-value < 0.05 threshold for SNP entry and removal. The groups of selected SNPs within chromosome were fitted together and backwards elimination using a P-value < 0.05 threshold was used to identify the significant SNPs within each chromosome.

Results and discussion

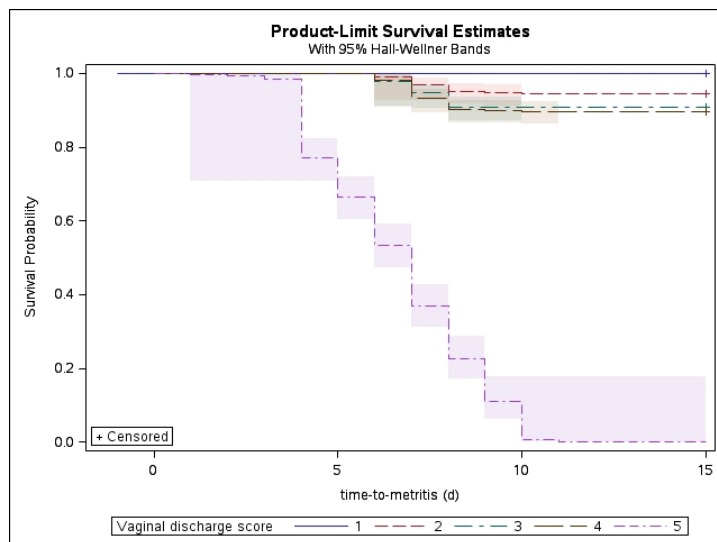


Figure 1. Survival curves for time-to-metritis detection by vaginal discharge scores at 5 days postpartum.

Metritis was detected in 30.4% of the cows studied and ranged from 11.5% to 51.5% of the cows within herd. The significant ($P < 0.001$) effect of herd on metritis status reflects substantial variation in conditions and health management practices across the herds studied. Parity was marginally significant ($p < 0.1$) with multiparous cows having slightly lower

probability of metritis and a longer time-to-metritis than primiparous cows. The significant ($P < 0.001$) odds ratio of vaginal discharge score was 31.5 (23.1-43.0 95% confidence interval) indicating that the odds of metritis detection increased with increasing vaginal discharge score. This result reflects the established usage of vaginal discharge score as a valuable indicator of the risk to develop metritis (LeBanc *et al.*, 2003, Sheldon *et al.* 2009).

The analysis of time-to-metritis revealed that metritis was detected, on average, at 6.8 days postpartum and ranged from 1 to 15 days postpartum across herds. The significant ($P < 0.001$) hazard ratio of vaginal discharge score was 10.6 (8.9 - 12.6 95% confidence interval) indicating increasing risk of metritis with increase increasing vaginal discharge score. The distinct time-to-metritis profiles among cows presenting different vaginal discharge scores are evident in survival curves depicted in Figure 1. This finding demonstrates that the vaginal discharge score can also be a reliable indicator when metritis is expected to become symptomatic.

Table 1. Location and result of significant SNP genotypes associated with known genes for Metritis status and days-to-metritis detection.

T ¹	SNP	C ²	Position	Gene ³	Loc ⁴	GenR ⁵	PV ⁶	Ratio ⁷
M	rs132845222	2	6734250	SLC40A1	intron	TC_CC	3.7E-10	0.01
						TT_CC	NA	NA
M	rs109974048	3	104311603	PPCS	3' UTR	AC_AA	4.4E-04	0.02
						CC_AA	9.7E-08	0.00
M	rs43370606	3	116167167	AGAP1	intron	TG_GG	3.1E-09	1.93
						TT_GG	NA	NA
M	rs110762359	8	90207700	SEMA4D	intron	AG_AA	5.8E-08	1.12
						GG_AA	4.1E-05	11.37
M	rs110470183	9	68575065	LAMA2	intron	TG_GG	7.1E-08	12.86
						TT_GG	1.4E-01	0.71
M	rs42402217	15	63281076	ELP4	intron	TC_CC	8.7E-09	0.49
						TT_CC	2.4E-08	0.41
M	rs135462315	15	81949123	PRG3	intron	TC_CC	1.3E-08	0.76
						TT_CC	5.5E-05	1.80
M	rs43718992	22	60225844	SEC61A1	intron	TC_CC	4.3E-08	1.20
						TT_CC	1.7E-04	0.68
D	rs136262145	5	49264290	TBC1D30	intron	AG_AA	4.9E-04	0.53
						GG_AA	3.3E-05	0.46
D	rs110650712	16	58775379	RFWD2	intron	TC_CC	5.0E-05	0.21
						TT_CC	1.0E-04	0.23
D	rs109983747	27	35574279	ZMAT4	intron	TG_GG	2.3E-01	1.14
						TT_GG	7.1E-05	4.88

¹ Trait analysed: M= Metritis status and D: days postpartum when metritis was detected

² Chromosome or BTA and location on the chromosome (bp units)

³ Gene symbol: SLC40A1=Solute Carrier Family 40 Member 1; PPCS=Phosphopantothenoylecysteine Synthetase; AGAP1= ArfGAP With GTPase Domain, Ankyrin Repeat And PH Domain 1; SEMA4D= Semaphorin 4D; LAMA2= Laminin Subunit Alpha 2; ELP4= Elongator Acetyltransferase Complex Subunit 4; PRG3= Proteoglycan 3, Pro Eosinophil Major Basic Protein 2; SEC61A1= Sec61 Translocon Alpha 1 Subunit; TBC1D30= TBC1 Domain Family Member 30; RFWD2= Ring Finger And WD Repeat Domain 2; ZMAT4=Zinc Finger Matrin-Type 4

⁴ Location of SNP variant within gene

⁵ Genotypes compared (first genotype versus second genotype)

⁶ P-value of the difference between genotypes

⁷ Odds (for M) or Hazard (for D) ratio between the compared genotypes: NA: Not available due to data structure.

The genome-wide analysis detected 23 and 1 SNPs (P -value $< 1.0 \times 10^{-7}$) for metritis status and days-to-metritis detection, respectively. In addition, 89 and 2 SNPs were detected ($1.0 \times 10^{-7} < P$ -value $< 1.0 \times 10^{-5}$) for metritis status and days-to-metritis detection, respectively. Lastly, 87 and 4 SNPs were detected ($1.0 \times 10^{-5} < P$ -value $< 1.0 \times 10^{-4}$) for metritis status and days-to-metritis detection, respectively. The majority of the SNPs exhibiting the most significant associations that were located within known genes are provided in Table 1. An unexpected finding is that some genotypes are associated with opposite trends for odds of metritis and hazard ratio of time-to-metritis. This finding suggests differences in the molecular mechanisms influencing the development of metritis symptoms and the timing postpartum.

Among the SNPs associated with metritis, rs109974048 is mapped to the 3' UTR region of Phosphopantothencysteine Synthetase (PPCS). This gene codes for an enzyme in the pathway for the biosynthesis of coenzyme A (CoA) from pantothenic acid (vitamin B5). The odds ratio for rs109974048 indicated that the AC and CC genotypes have substantially lower odds of metritis than the AA genotype. Other SNPs associated with metritis are located in the intron from genes known to be involved in immune system including: Proteoglycan 3, Pro Eosinophil Major Basic Protein 2 (PRG3), Sec61 Translocon Alpha 1 Subunit (SEC61A1), Semaphorin 4D (SEMA4D), and Solute Carrier Family 40 Member 1 (SLC40A1). Subsequent next-generation sequencing studies of the regions proximal to the detected SNPs may provide a more comprehensive evaluation of genetic variants associated with metritis (Southey *et al.*, 2016). The identified SNPs provide genetic variants underlying metritis susceptibility that can be used for the genetic improvement of cow welfare and profitability. The support of USDA NIFA PROJ NO: ILLU-538-909 is appreciated. These findings contribute to a long-term multistate project database USDA-NIFA-AFRI-003542.

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