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Genetic markers underlying resistance to Johne's disease

Key external stakeholders:
Pharmaceutical companies, DAFM, Teagasc commercial partners, veterinary surgeons, Animal Health Ireland, dairy and beef industries

Practical implications for stakeholders:

- Resistance to disease, specifically Johne's disease is heritable and selection for improved disease resistance is feasible.
- Johne's disease is spread via the faecal-oral route and we have detected proteins in the oral cavity which may improve disease resistance.
- In order to select genetically resistant cattle, the genes associated with resistance need not only to be identified but also validated, and this study used CRISPR to knock-out a critical gene associated with disease resistance (IL10RA) which validates its role in anti-mycobacterial immunity.

Main results:

- Multiple genes associated with resistance to Johne's disease have been detected, some novel and others which validate the findings of others and add weight to their potential utility as markers to select for disease resistance.
- Using a *Mycobacterium avium paratuberculosis* infection model, the effect of disease on gene expression in the oral cavity was catalogued and some important potentially protective molecules identified. This adds an important new layer of understanding of oral immunity in the cow.
- To validate the role of a specific gene identified by GWAS in the immune response, CRISPR was set up and used to knock-out a specific gene (Interleukin 10 receptor alpha – IL10RA) to validate its use in bovine epithelial cells and demonstrate the immune effects of this gene on the immune response to both mastitis and Johne's disease.

Opportunity / Benefit:

- This project will benefit initiatives to enhance oral immunity in calves and also add weight to the selection of genes to enhance disease resistance in cattle.

Collaborating Institutions:
University of Guelph

Teagasc project team: Sanjay Mallikarjunappa
Dr Kieran Meade

External collaborators: Professor Niel Karrow, University of Guelph, Canada.

1. Project background:

Selection practices can exploit genetic variation at specific genes to enhance phenotypes of interest in agriculture including fertility and milk production. However selection for disease resistance remains a challenge. Multiple genes associated with disease resistance have been identified in specific cattle populations but validation is required both at a genetic and functional level before their use can be considered. New technologies including CRISPR are now available to investigate the effects of knocking-out specific genes on the cell phenotype and immune response. The aim of this project was to combine genetic, genomic and functional immunological analyses to identify and validate genes associated with disease resistance.

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2. Questions addressed by the project:

1. What effect does MAP infection have on oral immunity in cattle?
2. What genes contribute to resistance to Johne's disease?
3. What effect will knocking out a specific gene using CRISPR have on the immune response?

3. The experimental studies:

Genome-wide association studies: Large datasets of ELISA readings from control and johne's positive cattle were used as the phenotype for animal selection. DNA from these animals was then typed using a variety of SNP typing platforms to detect an association between the disease resistance phenotype and specific genes in both Holstein-Friesian cows and AI sires. This task required significant bioinformatic analyses for SNP detection and imputation to higher density SNP typing panels.

MAP infection study: An experimental infection was conducted in conjunction with UCD/DAFM and tissues recovered from MAP+ and MAP- control cattle for gene expression analysis using RNA-seq. Again bioinformatics analysis was used to identify differentially expressed genes and pathways.

CRISPR gene knock-out: Using a bovine epithelial cell model, a gene involved in the immune response to both mastitis and Johne's disease was knock-out successfully and the edited cells were subsequently used for *in vitro* infection studies in response to MAP and LPS (bacterial stimuli).

4. Main results:

- GWAS analyses identified a number of genomic regions and specific genes associated with resistance to Johne's disease. Results validated previous findings and identified new QTL on Bos taurus autosomes 15, 16, 20, and 21. The positional candidate genes NLRP3, IFi47, TRIM41, TNFRSF18, and TNFRSF4 lying within these QTL were identified. These regions included 2 single nucleotide polymorphisms located 2 kb upstream of positional candidate genes CD86 and WNT9B, which play key roles in host immune response and tissue homeostasis. Correlations between estimated breeding values for resistance to MAP infection and other economically important traits, when significant, were favorable and of low magnitude.
- Transcriptomic changes associated with MAP exposure have been identified including reduced LF and LPO. These critical antimicrobial and immunoregulatory proteins are known to be secreted into saliva and their downregulation may contribute to disease susceptibility. The first analysis of the transcriptomic profile of salivary glands in cattle adds an important layer to our understanding of salivary gland immune function.
- CRISPR targeting of IL10RA in bovine epithelial cells was performed for the first time. Knock-out the genes has significant and widespread effects on the upregulation of inflammatory genes which identifies the mechanism by which SNPs in this gene could contribute to disease pathology. These findings support the functional role of IL10RA in both JD and mastitis.

5. Opportunity/Benefit:

This project will benefit initiatives to enhance oral immunity in calves and also add weight to the selection of specific genes to enhance disease resistance in cattle.

6. Dissemination:

Main publications:

1. Brito LF, Mallikarjunappa S, Sargolzaei M, Koeck A, Chesnais J, Schenkel FS, Meade KG, Miglior F, Karrow NA. The genetic architecture of milk ELISA scores as an indicator of Johne's disease (paratuberculosis) in dairy cattle. J Dairy Sci. 2018 Nov;101(11):10062-10075. doi: 10.3168/jds.2017-14250. Epub 2018 Sep 13.
2. Mallikarjunappa S, Sargolzaei M, Brito LF, Meade KG, Karrow NA, Pant SD. Uncovering quantitative trait loci associated with resistance to *Mycobacterium avium* ssp. *paratuberculosis* infection in Holstein cattle using a high-density single nucleotide polymorphism panel. J Dairy Sci. 2018 Aug;101(8):7280-7286. doi: 10.3168/jds.2018-14388. Epub 2018 May 10
3. Mallikarjunappa S, Adnane M, Cormican P, Karrow NA, Meade KG. Characterization of the bovine salivary gland transcriptome associated with *Mycobacterium avium* subsp. *paratuberculosis* experimental challenge. BMC Genomics. 2019 Jun 13;20(1):491. doi: 10.1186/s12864-019-5845-4.
4. Sanjay Mallikarjunappa, F.S. Schenkel, L.F. Brito, N. Bissonnette, K.G. Meade, F. Miglior, J. Chesnais, M. Lohuis and N.A. Karrow. Association of SNPs related to Johne's disease with EBVs of Holstein sires for milk ELISA test scores. (Submitted BMC Veterinary Research Dec 2019).

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5. S Mallikarjunappa, UK Shandilya, A Sharma, K Lamers, NA Karrow and KG Meade. Functional analysis of bovine interleukin-10 receptor alpha in response to *Mycobacterium avium* subsp. *paratuberculosis* lysate and Escherichia coli lipopolysaccharide stimulation using CRISPR/Cas9 (Submitted to Journal of Animal Science and Biotechnology, Dec 2019).
6. UK Shandilya, S Mallikarjunappa, A Sharma, J. Guo, K Lamers, Y Mao, KG Meade and NA Karrow Bovine Toll-like receptor 4 modulates the inflammatory response of mammary epithelial cells to *Mycobacterium avium* subsp. *paratuberculosis* cell lysate and Escherichia coli lipopolysaccharide (in prep.).

Presentations:

Research was presented at multiple national and international conferences including: World Buiatrics conference 2016, ISAG 2017, AVTRW 2017 and Plant and Animal Genome (PAG) 2018.

Popular publications:

7. Compiled by: Dr Kieran Meade

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