Development of a genomic reference population for bovine respiratory disease in pre-weaning dairy calves using thoracic ultrasonography

Objectives

Establish a protocol for objective and efficient assessment of bovine respiratory disease (BRD) phenotypes in dairy calves to build a large reference population and enable genomic selection for reduced incidence and severity

Specific Aim 1: Identify markers associated with BRD in a genome wide association study using the established reference population

Specific Aim 2: Estimate genomic breeding values of reference population animals and relatives to facilitate whole genome selection against BRD
Establishing a Reference Population

- 1107 Holstein calves
- 6 dairy farms in southern Wisconsin
- 4 trained evaluators
- Data collection from May to August 2017
- Each calf measured at 3-weeks and 6-weeks of age
- Clinical Disease Scoring
  - UW-Madison Vet Med Calf Health Scorer iPad tool
- Subclinical Disease Scoring
  - Thoracic Ultrasound (TUS) Evaluation
Clinical Scoring

- Sensitivity = 62%
- Specificity = 74%
- Compared with gold standard post-mortem evaluation (Buczinski et al., 2015)

https://www.vetmed.wisc.edu/dms/fapm/fapmtools/calves.htm
Subclinical Scoring: TUS

- Sensitivity = 94%
- Specificity = 100%
- Compared with gold standard post-mortem evaluation (Ollivett et al, 2015)
- 0 to 5 Scale (4 and 5 are multiple consolidated lobes)

Score 0: Normal lung
Score 2: Lesion > 1cm
Score 3: Consolidated lobe
## Overall BRD Scores

### Example:
**Calf 19893**

**Clinical Scores:**
- Cough = 2
- Temperature = 3

**Subclinical Score:**
- Ultrasound = 5

**Overall BRD Score:** 6

**Clinical Lobar Pneumonia**

### Table: Thoracic Ultrasound Score

<table>
<thead>
<tr>
<th>Clinical Respiratory Score</th>
<th>Thoracic Ultrasound Score</th>
<th>≤ 2JB</th>
<th>2</th>
<th>≥ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 scores ≥ 2</td>
<td>Healthy</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subclinical Lobular Pneumonia</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2 scores ≥ 2</td>
<td>Subclinical Lobar Pneumonia</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Upper Respiratory Tract Infection</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical Lobular Pneumonia</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical Lobar Pneumonia</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Frequency using Overall BRD Scores

<table>
<thead>
<tr>
<th>Condition</th>
<th>3-Week Incidence Rate (%)</th>
<th>6-Week Incidence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (1)</td>
<td>81.1</td>
<td>77.1</td>
</tr>
<tr>
<td>Upper Respiratory Tract Infection (2)</td>
<td>2.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Subclinical Lobular Pneumonia (3)</td>
<td>11.9</td>
<td>12.5</td>
</tr>
<tr>
<td>Subclinical Lobar Pneumonia (4)</td>
<td>2.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Clinical Lobular Pneumonia (5)</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Clinical Lobar Pneumonia (6)</td>
<td>0.7</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Preliminary Genetic Analysis

- **1016 animals genotyped on ZL5 chip** (Zoetis Genetics, Kalamazoo, MI)

- **Quality Control:**
  - PLINK 1.90b5.2 (Purcell and Chang; [www.cog-genomics.org/plink/1.9/](http://www.cog-genomics.org/plink/1.9/))
  - Call rate 95%, MAF < 0.05, HWE 1e-6
  - Before: 35334 SNPs       After: 28696 SNPs

- **Imputation:**
  - BEAGLE 4.1 (Browning and Browning, 2016)

- **GWAS:**
  - R Packages: gaston, GeneticsPed, qqman
Genome Wide Association Study

- Phenotype: 3-week and 6-week scores
  - Overall BRD Score - 2 Levels
    • Healthy (1), Affected (2 to 6)
  - Overall BRD Score - 6 Levels
    • Categories from 1 to 6 assuming increasing severity

- Wald Test
  - Linear Mixed Model
  - Threshold: $5 \times 10^{-5}$
Statistical Model

\[ y = m_j b_j + Zu + e \]

- \( y \): Vector of disease scores (2 - logistic, 6 - normal approximation)
- \( m_j \): Vector with genotypes (0, 1, 2) for marker j
- \( b_j \): Allele substitution effect for marker j
- \( Z \): Incidence matrix relating \( y \) to \( u \)
- \( u \): Polygenic random term, \( u \sim N(0, \sigma_g^2) \)
- \( e \): Residual effects, \( e \sim N(0, \sigma_e^2) \)
GWAS using 3-Week BRD Scores

2 Levels

6 Levels

-log_{10}(p)
GWAS using 6-Week BRD Scores

2 Levels

6 Levels

-\log_{10}(p)

Chromosome

MT
## Genes Associated with Significant SNPs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>3-Week 2 Levels</th>
<th>3-Week 6 Levels</th>
<th>6-Week 2 Levels</th>
<th>6-Week 6 Levels</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEKR1</td>
<td>1</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
<td>Metabolic nature</td>
</tr>
<tr>
<td>SLC6A17</td>
<td>3</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Transporter for presynaptic uptake of neurotransmitters</td>
</tr>
<tr>
<td>MSMO1</td>
<td>17</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Cholesterol Synthesis</td>
</tr>
<tr>
<td>MND1</td>
<td>17</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
<td>Meiotic Recombination</td>
</tr>
<tr>
<td>PLEKHH3</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Protein Coding</td>
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</tbody>
</table>
Conclusions

- Scoring system allows objective and efficient assessment of BRD for building a reference population that can be used for genomic selection against BRD in dairy calves.

- Preliminary analysis shows significant markers associated with BRD incidence and severity at 3 and 6 weeks of age.
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Thank you!