Genome-Wide Association Study and Whole Genome Selection for Resistance to Bovine Respiratory Disease in Pre-Weaned Holstein Calves using Thoracic Ultrasonography
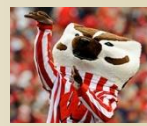
Allison Quick, Terri Ollivett, Brian Kirkpatrick, Kent Weigel

**University of Wisconsin - Madison**

Introduction

Bovine Respiratory Disease (BRD)

- **Incidence (USDA, 2012)**

  - **18.1% in pre-weaned dairy calves**

  - **11.2% in post-weaned dairy calves**

- **Pathogens**

  - **Viral: Bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), parainfluenza type 3, and bovine herpesvirus 1**

  - **Bacterial: *Pasteurella multocida*, *Manheimia haemolytica*, and *Histophilus somn***
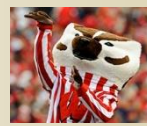
Impact of BRD in Dairy Calves

- **Symptoms**
  - **Depression**
  - **Abnormal respiration**
  - **Rumen fill**
  - **Weight-loss**
  - **Decreased appetite**
  - **Increased temperature**
  - **Nasal and ocular discharge**
  - **Drooped ears**

- **Short & Long-Term Effects**
  - **Early mortality**
  - **Premature culling**
  - **Reduced growth rate**
  - **Delayed age at first calving**
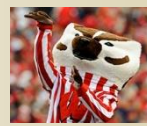 - **Decreased milk production**

Diagnostic Methods

- **Necropsy**

- **Visual Appraisal**

- **Bronchoalveolar lavage**

- **Transtracheal wash**

- **Deep pharyngeal swab**

- **Thoracic ultrasound**

Images from: Iowa State University, College of Veterinary Medicine; Wisconsin Veterinary Diagnostic Laboratory

Henderson et al., 2011

- **7,372 heifer calves at a New York calf rearing facility**

- **Health events were recorded by farm staff**

- **Opportunity period was arrival (1 to 7 d) to weaning**

- **Incidence rate for calf BRD was 38.4%**

- **Heritability estimate for calf BRD was 0.095**
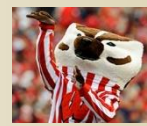
Neibergs et al., 2014

- **Holstein calves in California & New Mexico age 27 to 60 d**

- **Visual appraisal + deep pharyngeal swab**

- **Heritability estimates:** 0.20 to 0.22 within states

  0.13 combined across states

- **Regions implicated by GWAS:**

  <u>California</u>
  - **BTA15 between 30-31 Mb: *poliovirus receptor-related 1* gene**
  - **BTA14 between 63-64 Mb: *AZIN1* gene**

  <u>New Mexico</u>
  - **BTA16 between 70-71 Mb**
  - **BTA14 between 7-8Mb**

Mahmoud et al., 2017

- **31,396 heifer calves born on 43 German dairy farms**

- **Health events were recorded by farmers & veterinarians**

- **Opportunity period was birth to 2 months of age**

- **Incidence rate for calf BRD was 28.4%**

- **Heritability estimate for calf BRD was 0.07**

- **Impact of calf BRD on 1st lactation: -0.3 kg/d of milk**
  -0.015 kg/d fat
 **-0.010 kg/d protein**

Vukasinovic et al., 2018

- **Farmer-reported health events from 0 to 365 d of age**
- **300 commercial dairy farms in U.S.**

Age of Calf	No. Records	Incidence	h^2
0 to 3 d	224,112	4.6%	0.097
4 to 14 d	432,570	4.6%	0.058
15 to 50 d	633,123	9.7%	0.049
51 to 365 d	828,695	15.3%	0.039
0 to 365 d	874,170	20.2%	0.042
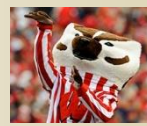
Vukasinovic et al., 2018

- **10-SNP sliding windows identified using ssGWAS:**
  - BTA1 between 26.7-27.1, 76.8-77.0, and 127.3-127.4 Mb
  - BTA2 between 79.3-79.8 Mb
  - BTA9 between 43.6-44.0, 94.5-95.0, 97.6-97.8, 101.0-101.3 Mb
  - BTA14 between 11.5-11.8 and 53.1-53.4 Mb
  - BTA27 between 2.0-2.3, 4.4-4.5, and 19.1-19.2 Mb
 - X between 48.6-52.7, 109.3-109.7, and 126.0-127.6 Mb
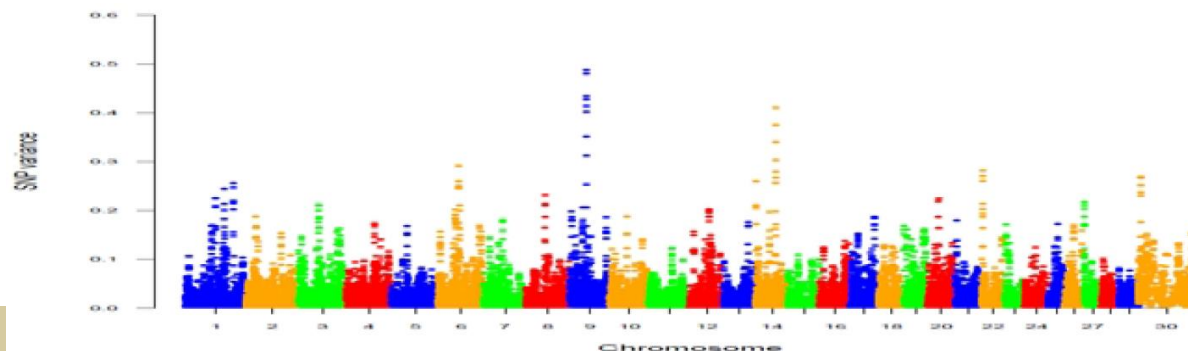
Our Study

Objectives

- **Establish a protocol for objective and efficient assessment of clinical and subclinical BRD phenotypes**

- **Build an initial genomic reference population for BRD**

- **Identify genomic regions associated with BRD**

- **Estimate genetic parameters and predict breeding values to facilitate genomic selection against BRD**
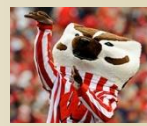
Data Collection

- **1,107 Holstein heifer calves**

- **6 dairy farms in southern Wisconsin**

- **4 trained evaluators**

- **Data collection from May to August 2017**

- **Each calf was measured at 3 and 6 weeks of age**

Clinical Disease Scoring by Visual Appraisal

- **Sensitivity = 62%**
- **Specificity = 74%**

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

Subclinical Disease Scoring by Thoracic Ultrasound

Score 0:
Normal lung

Score 2:
Lesion > 1cm

Score 3:
Consolidated Lobe
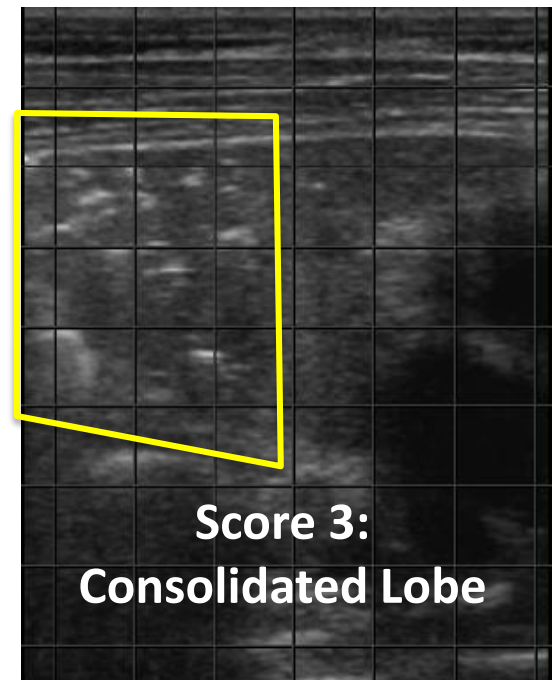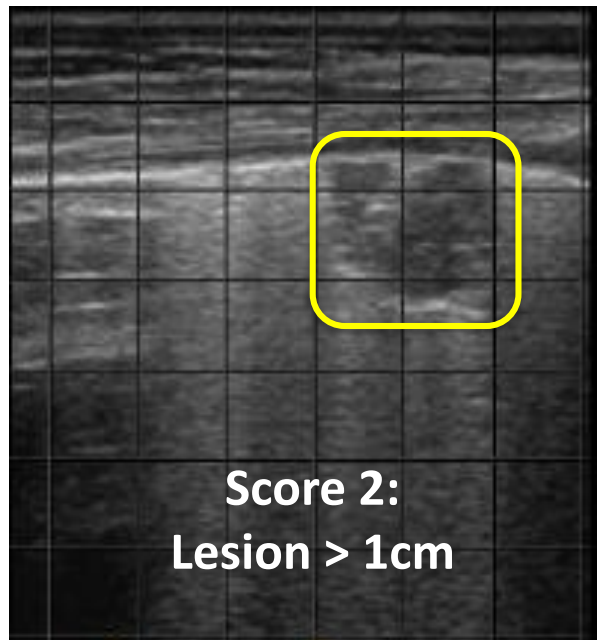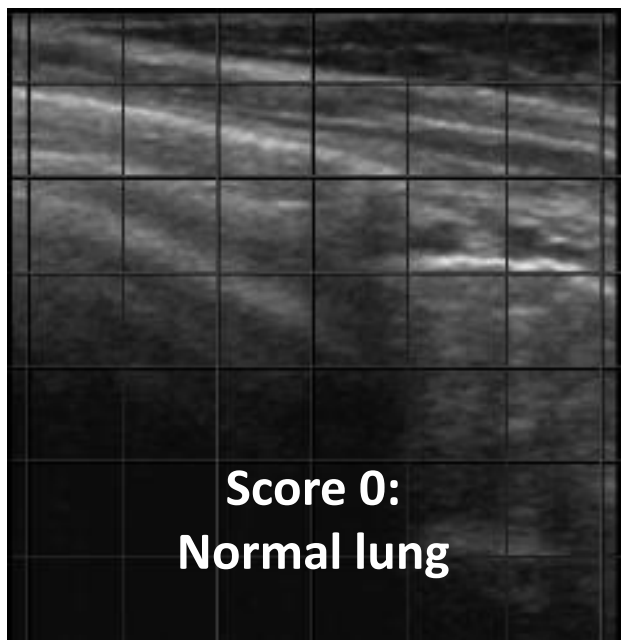
- **Sensitivity = 94%**   - **Specificity = 100%**

Detects pulmonary consolidation – regions of soft lung tissue that are swollen or hardened, and filled with liquid rather than air
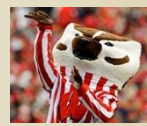
Overall Bovine Respiratory Disease (BRD) Scores

	Thoracic Ultrasound Score		
Clinical Respiratory Score	**< 2**	**2**	**≥ 3**
< 2 scores ≥ 2	Healthy 1	Subclinical Lobular Pneumonia 3	Subclinical Lobar Pneumonia 4
≥ 2 scores ≥ 2	Upper Respiratory Tract Infection 2	Clinical Lobular Pneumonia 5	Clinical Lobar Pneumonia 6

Example: Calf 19893

Clinical Scores:
    Cough = 2
    Temperature = 3

Subclinical Score:
    Ultrasound = 5

Overall BRD Score: 6

→ Clinical Lobar Pneumonia

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Overall Prevalence of BRD, by Age

	3-Week Prevalence (%)	6-Week Prevalence (%)
Healthy (1)	81.1	77.1
Upper Respiratory Tract Infection (2)	2.9	2.7
Subclinical Lobular Pneumonia (3)	11.9	12.5
Subclinical Lobar Pneumonia (4)	2.6	5.3
Clinical Lobular Pneumonia (5)	0.8	1.4
Clinical Lobar Pneumonia (6)	0.7	1.0

Overall Prevalence of BRD, by Farm

| Herd	# of Calves	Overall BRD Score (%)					
		1	2	3	4	5	6
A	57	92.8	-	6.3	-	0.9	-
B	226	72.6	3.6	18.5	4.0	0.9	0.5
C	295	90.8	3.4	5.1	-	0.5	0.2
D	257	64.8	2.8	16.5	11.2	1.6	3.1
E	245	83.1	1.9	12.1	1.3	1.5	0.2
F*	27	81.5	-	3.7	7.4	7.4	-
Total	1107	79.2	2.7	12.2	3.8	1.2	0.9

Genomic Analysis

- **1,016 animals genotyped with ZL5 chip (1,014 after edits)**

- **Imputation to 690,291 SNPs using BEAGLE 4.1**

- **Single-step GWAS with 1 Mb sliding windows (BLUPF90)**

- **Single-step GBLUP for BV prediction (BLUPF90)**

- **Phenotypes:**
  - **BRD (score 1 vs. scores 2-6) at 3 and 6 weeks of age**
  - **CON (scores 1-2 vs. scores 3-6) at 3 and 6 weeks of age**

GWAS Results

CON3

- BTA 7, Mb region 99,919,061 to 100,911,769
  - 1.65% of variance
  - Associated with RGMB gene
    - neural development
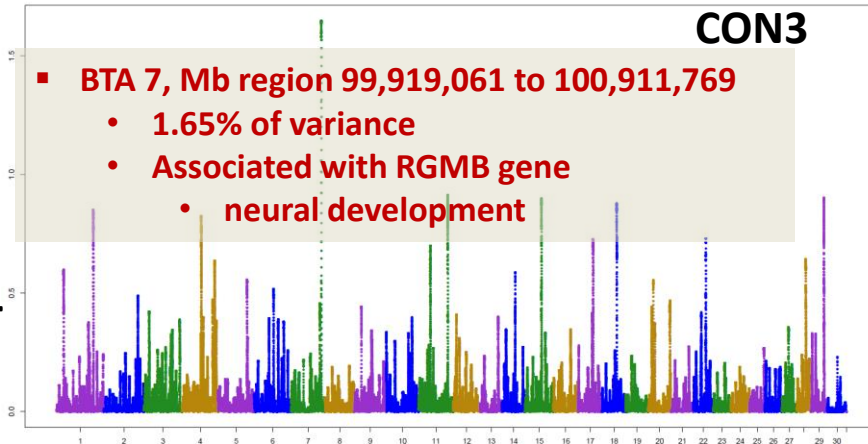
CON6

- BTA 12, Mb region 62,547,209 to 63,546,791
  - 1.23% of variance
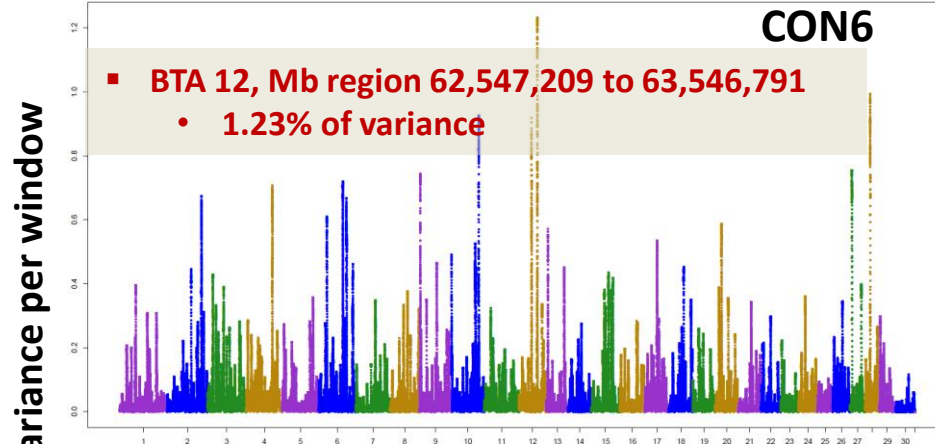
BRD3

- BTA 6, Mb region 81,293,308-82,292,618
  - 1.45% of variance
  - Associated with TECRL gene
    - lipid oxidoreductase
    - role in puberty and female fertility in cattle
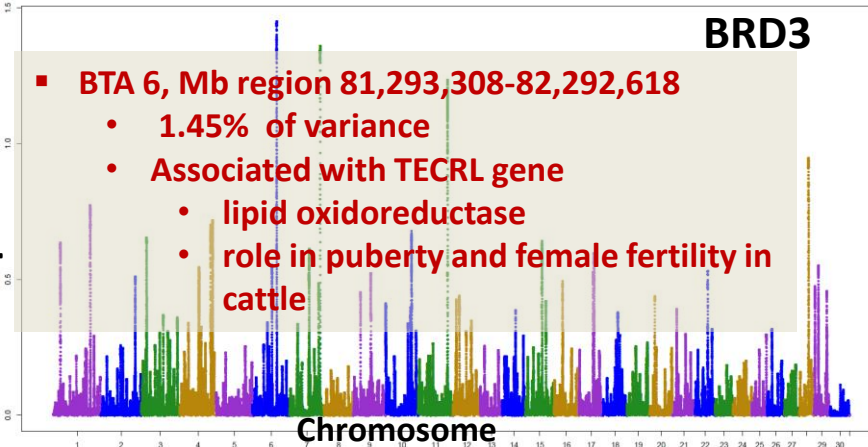
BRD6

- BTA 12, Mb region 62,458,043 to 63,455,017
  - 1.42% of variance
  - Associated with SLITRK5 gene
    - left-displaced abomasum pathogenesis in Holsteins
    - other cardiovascular and synaptic functions

Proportion of SNP variance per window

Chromosome
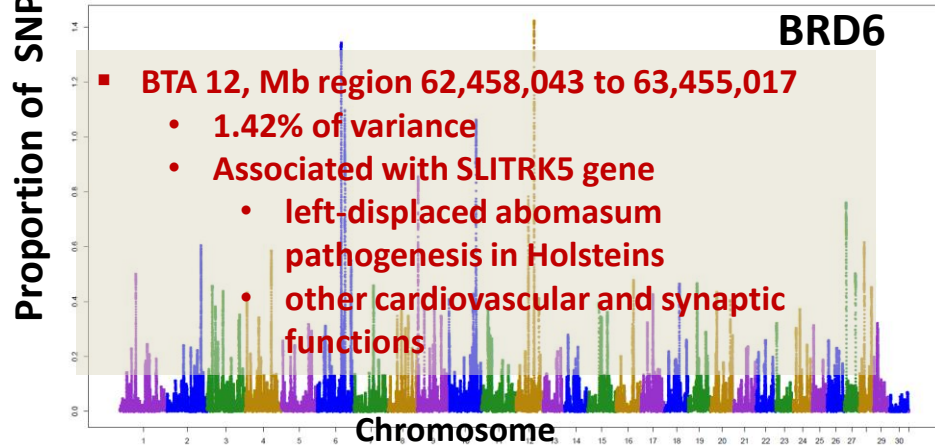
Top Genomic Regions

| BTA	Start (BP)	Phenotypes	Genes
1	129,336,133	CON3, BRD3	CLSTN2
6	81,286,240	BRD3, BRD6, CON6	TECRL
6	92,804,334	CON6, BRD6	SCARB2, FAM47E, STBD1, CCDC158, SHROOM3, SOWAHB, SEPT11, CCN1, CCNG2, LOC101901983
7	99,919,061	CON3, BRD3	RGMB
9	4,227,465	CON6, BRD6	
10	89,679,387	CON6, BRD6	NOXRED1, ISM2, VIPAS39, AP5A1, U6atac, U6, SPTCL2, ALKBH1, ADCK1, SNW1
11	91,475,811	CON3, BRD3	GGTA1, LOC525099, LOC527409
12	41,160,340	CON6, BRD6	LOC101902172
12	62,458,043	CON6, BRD6	SLITRK5
15	51,296,284	CON3	TRIM21, RRM2, STIM1, RHOG, PGAP2, NUP98, ART1, CHRNA10, TRPC2, Five genes from OR52 family, LOC407145, LOC618664, LOC407148, LOC507756
18	41,503,327	CON3	*TSHZ3*, SNORA70
27	6,593,817	CON6, BRD6	WDR17, SPATA4, ASB5, SPCS3, VEGFC, NEIL3, U6
28	19,000,877	CON6	ADO, *EGR2*, NRBF2, JMJD1C, REEP3
28	32,939,809	BRD3	KCNMA1, DLG5, POLR3A
29	40,034,122	CON3	LOC528815, LOC617765, PAG8, PGA5, VWCE, CYB561A3, DDB1, TKFC, SYT7, TMEM216, TMEM138, CPSF7, *SDHAF2*, PPP1R32, *DAGLA, FEN1*, MYRF, TMEM258

- **TSHZ3:**
  - up-regulating miRNAs during metritis events
- **EGR2:**
  - required for the development and maintenance of myelin structure and function
  - smooth muscle formation
  - abnormalities of the gene in humans lead to restrictive pulmonary disease, recurrent pneumonia, and respiratory failure
- **SDHAF2:**
  - assembly of succinate dehydrogenase, an enzyme complex also referred to as respiratory complex II
- **DAGLA and FEN1:**
  - nervous, immune, respiratory, metabolic systems
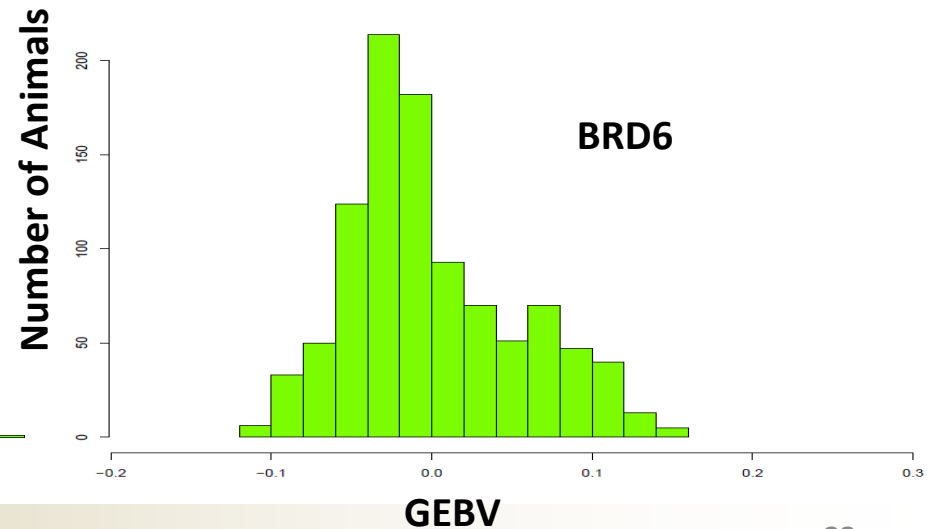 - FEN1: poor survival of stage-I lung adenocarcinoma in humans
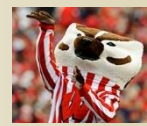
Genetic Parameter Estimates

Phenotype	σ_g^2 (SE)	σ_e^2 (SE)	h^2
CON3	0.027 (0.010)	0.100 (0.010)	0.214
BRD3	0.036 (0.013)	0.112 (0.012)	0.241
CON6	0.012 (0.010)	0.136 (0.011)	0.084
BRD6	0.018 (0.018)	0.144 (0.012)	0.111

Distributions of Predicted Breeding Values
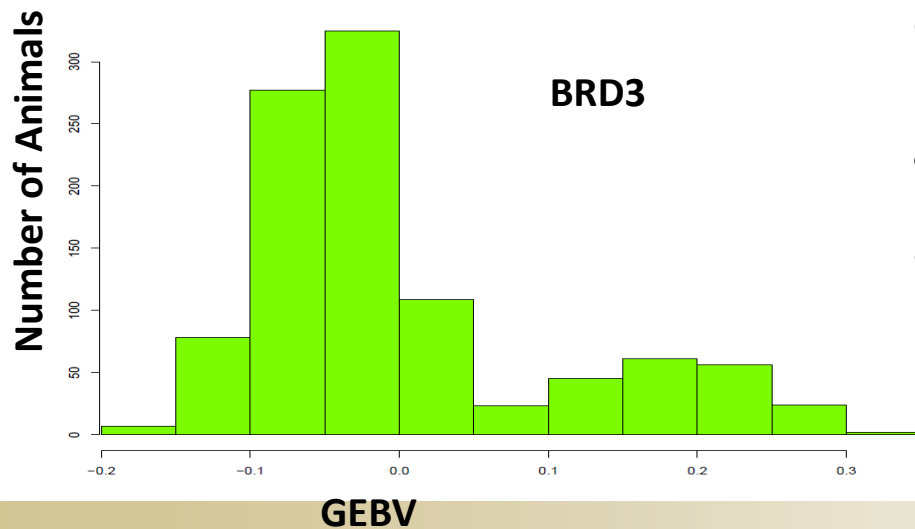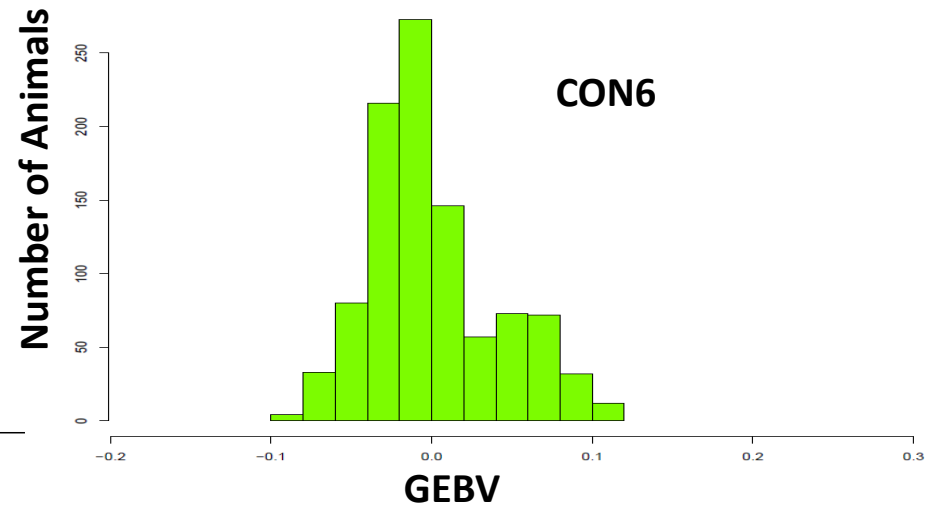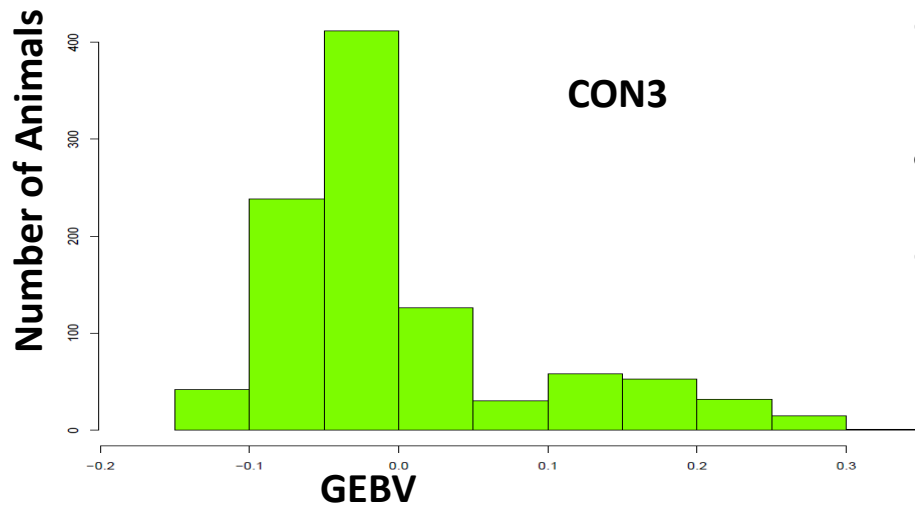
Summary of Our Project

- **Scoring protocol allowed objective, efficient assessment of BRD phenotypes to build a genomic reference population**

- **GWAS identified regions containing putative genes with functions related to respiration and immunity**

- **Moderate heritability estimates suggest potential for achieving genetic progress in reducing the incidence of BRD**

- **3-week phenotypes gave higher heritabilities due to smaller influence of management and chance pathogen exposure**

Practical Implementation
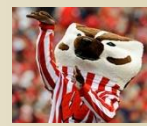
Vukasinovic et al., 2018

- **Calf health GPTAs now available from Zoetis**

- **Respiratory disease at various ages**

Age of Calf	# Genotyped Calves	SD of GPTAs (%)	Mean REL (%)
0 to 3 d	220,571	2.51	50.1
4 to 14 d	202,946	1.65	41.7
15 to 50 d	198,900	2.23	42.3
51 to 365 d	212,895	2.50	38.1
0 to 365 d	212,201	3.01	40.1

Vukasinovic et al., 2018

- **Calf Wellness (CW$) index is now available from Zoetis**
- **8% relative weight in Dairy Wellness Profit Index (DWP$)**

Trait	Relative Weight in Calf Wellness (CW$) Index
Respiratory Disease	**29%**
Livability	**43%**
Scours	**28%**

Overall Conclusions

- **Selection tools to improve dairy calf health are overdue**

- **Zoetis offers genomic predictions for calf scours, livability, and respiratory disease via farmer-reported health events**

- **Thoracic ultrasound offers an objective assessment of lung consolidation that can be implemented very efficiently**

- **Future research should focus on expanding the genomic reference population and developing systems to assess elite young bull and heifer calves in genetic nucleus herds**

Mooving Forward.

DAIRY SCIENCE AT WISCONSIN

Questions?