A multi-pronged approach to understanding bovine respiratory disease at USMARC

Larry A. Kuehn, Carol G. Chitko-McKown, Tara G. McDaneld, Amanda K. Lindholm-Perry

USDA, ARS, U.S. Meat Animal Research Center

BEEF CATTLE GENETIC RESOURCES

Bovine respiratory disease complex (BRDC)

- Most costly disease to the cattle industry
  - Cattle treated for BRDC expected to return at least $40 less than untreated calves Fulton et al., 2002
- Relative economic value is 37.7 times that of yearling weight (Van Eenennaam and MacNeil, 2011)
- Antibiotics, vaccination, and management all can effectively decrease incidence
  - Concerns over antibiotic resistance
  - Hard to control all beef cattle sectors

Often hard to diagnose…

BRDC pathogens

- Bacterial infections
  - *Mannheimia Haemolytica*
    - Most implicated – shipping fever
    - Leukotoxin, lipopolysaccharide
    - Most defined/studied
  - *Pasteurella multocida*
  - *Mycoplasma bovis*
  - *Histophilus somni*

BRDC pathogens

Several vaccines available

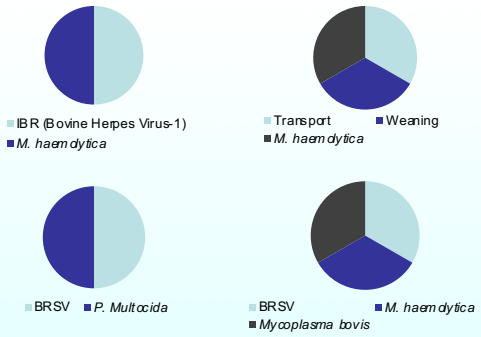
- At least five primary viral agents
  - Parainfluenza-3 (PI_3)
  - Infectious Bovine Rhinotracheitis (IBR)
  - Bovine Viral Diarrhea (BVD; 2 strains)
  - Bovine Respiratory Syncytial Virus (BRSV)
  - Bovine Coronavirus (BCV)
    - Implication relatively recent
- Gateway to bacterial infection, likely due to damage to respiratory clearance

Taylor et al., 2010

Environmental factors

- Feedlot
  - Entry weight, gender, transport distance, commingling, receiving ration, prophylactics, social dominance/disposition
- Prior treatment
  - Vaccines, passive colostrum transfer, persistent infection (BVDV), weaning management
- STRESS
  - Immune system dynamics

Plausible BRDC models



Difficulties of BRDC treatment records as phenotypes

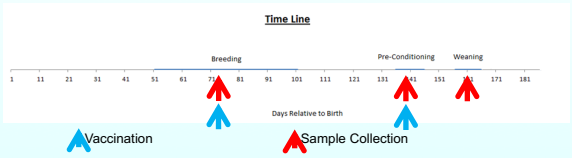
- Disease incidence measured as 0 or 1
- Subclinicals/shedders analyzed as healthy
- Symptoms not precise indicators
- Multiple pathogens may cause the same disease/set of symptoms
- Prior exposure often unknown
- Can't guarantee level of exposure

Overcoming difficulties

- Large population size
  - Subset of USMARC Germplasm Evaluation Program
  - All spring-born natural service calves
- Data collection
  - Improve quality of diagnoses/necropsy
  - Increased number of measures
  - Identify subclinical animals
  - Identify susceptible animals in years with low exposure

USMARC disease resistance population (700-800 hd/yr)

- Vaccinated with BRSV/IBR/PI3/BVDV vaccine
- Nasal samples taken at each point(8 yr) Blood phenotypes measured (10 yr; vaccine response)
- Lung Scores recorded at harvest (~ 420d)
- Resulted in several related projects



Genetic correlations (delta)

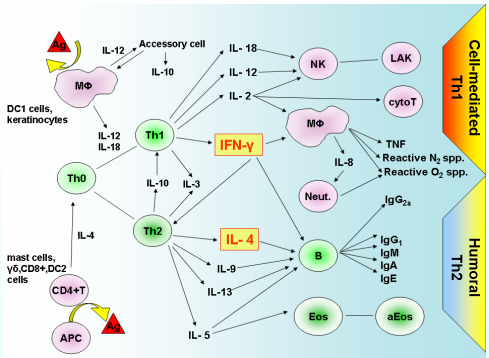
Trait	Diagnosis		Lung Lesions	
	Corr	SE	Corr	SE
Neutrophils	-0.16	0.33	-0.28	0.39
Lymphocytes	-0.67	0.21	0.22	0.30
Monocytes	0.12	0.32	0.29	0.37
Eosinophils	0.16	0.27	-0.39	0.34
Basophils	-0.23	0.27	-0.52	0.37

- Some indication of cell-mediated response to vaccine

  » Leach et al., 2012
- Less luck with humoral response

Simplified pathway

O'Neill, thesis 2005



Improving BRDC diagnosis

- Additional measures – subclinicals
  - Chute scoring system (Love et al., 2014)
  - Severe lung lesions
  - Look at combination of heath records and other diagnostic phenotypes
- Need to be cautious of traditional multi-trait analysis (likely mixture distribution; Bishop and Woolliams, 2010)

Assessing BRDC risk

- Vaccine/antigen response
  - Titers vs. neutralizing antibodies
  - Cell-mediated vs. humoral responses
- Cell counts (T, B, CD, Neut, Macro, etc.)
- Acute phase/response proteins
- Cytokine pathways
- Measures of stress and stress response
  - Interaction with all of the above
- Would prefer all in response to a stimulus

Resource populations

- Academic institutions
  - Easier to collect blood/tissues for immunological measures
    - Hopefully more heritable
    - Limited numbers of animals
      - 4,500 spring calves/yr at USMARC, 200-300 cases
  - Commercial Partners (DNA pooling)
    - Collections at commercial abattoirs
    - Commercial feedlots

Methods

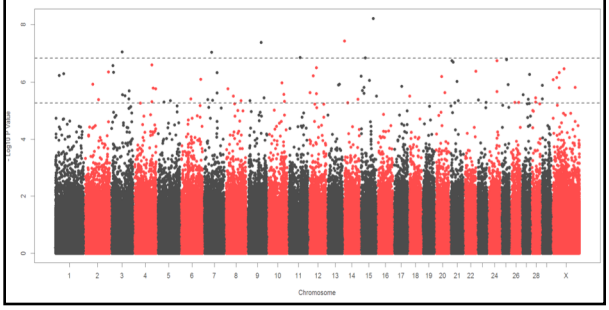
- 11,520 lungs were sampled from a central Nebraska beef processing plant with a throughput of 2,500 cattle per 8 h shift.
- On average 900 lungs were sampled per day.
- The majority of the lungs came from cattle raised without antibiotics.
- Case – Control Definitions
  - Half (5,760) had severe lung lesions (Case)
  - Half (5,760) had mild or no lesions (Control).

Methods

- Lungs were scored as severe if they had greater than 50 % of lung tissue affected with lesions associated with BRDC including pleural adhesion to the thoracic cavity.
- Sampling variation in lung lesions– Lesion (L), Normal (N); green for sample and red for don't sample.
  - LLLLLLLLLNNNNNNNNNLLLLLLLLLLNNNNN
  - LLNLNNLLNLNNNLLLNLNNLLLLNNLLNLLLNLN
  - NNNNNNNNNNLNNNNNNNNNNNNNNNLNN

7 SNP achieved genome wide significance at the 5 % level.

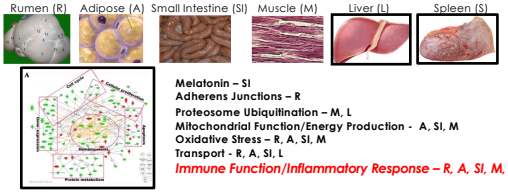
84 SNP achieved a false discovery rate of 5 % or less.



Cytokine profiles and BRD

Inflammatory Response and Feed Efficiency

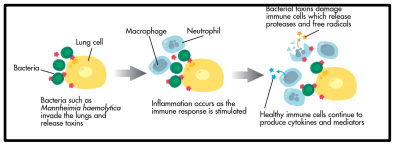
Rumen (R) Adipose (A) Small Intestine (SI) Muscle (M) Liver (L) Spleen (S)



Melatonin – SI
Adherens Junctions – R
Proteosome Ubiquitination – M, L
Mitochondrial Function/Energy Production - A, SI, M
Oxidative Stress – R, A, SI, M
Transport - R, A, SI, L
Immune Function/Inflammatory Response – R, A, SI, M, L, S

BRD and Cytokines

- **Evaluate potential relationships between cytokine gene expression, complete blood counts (CBC) and disease or disease susceptibility in animals at various time points**
  - **Pre-weaning, Weaning and at Diagnosis of BRD**



Whole Blood for CBC

N=791

2 Times:
Preweaning (~120d)
Weaning (~150d)

3rd Time point: At Diagnosis (N=13 BRD, 75 Case Control)

CBC Data from BRD and CC Animals

- Higher levels of neutrophils and lower levels of basophils were detected in the sick animals compared to the asymptomatic controls at the time of diagnosis ($P_{corrected}<0.05$).
- Lower levels of monocytes were identified at weaning ($P<0.05$) in those calves that went on to develop BRD in the feedlot compared to calves that remained asymptomatic in the first 42 days after weaning.

	Asymptomatic Control Animals (n=75)			Animals with BRD (n=13)		
Parameter[1]	Time 1 Ave(SD)[2]	Time 2	Time 3	Time 1	Time 2	Time 3
WBC (10^9/L)	11.3 (0.29)	11.2 (0.32)	10.2 (0.29)	11.5 (0.59)	11.4 (0.65)	11.5 (0.58)
NEU (10^9/L)	3.7 (0.14)	3.6 (0.17)	***2.8 (0.17)***	3.4 (0.29)	3.7 (0.35)	***4.9 (0.35)***
LYM (10^9/L)	6.8 (0.21)	6.8 (0.25)	6.7 (0.21)	7.2 (0.42)	7 (0.5)	5.9 (0.43)
MONO (10^9/L)	0.57 (0.02)	***0.61 (0.022)***	***0.42 (0.019)***	0.57 (0.04)	***0.47 (0.044)***	***0.46 (0.038)***
EOS (10^9/L)	0.19 (0.013)	0.16 (0.007)	0.18 (0.018)	0.16 (0.027)	0.14 (0.015)	0.14 (0.036)
BAS (10^9/L)	0.08 (0.005)	0.1 (0.004)	***0.1 (0.005)***	0.09 (0.01)	0.09 (0.009)	***0.07 (0.011)***

Conclusions

- Need a multi-pronged approach of large animal volumes and intensive, more highly heritable phenotypes
  - Much of this work is underway by multiple groups
  - Cost saving measures such as pooling are effective
  - Just the first stage of examining candidate phenotypes

METAGENOMICS AND BOVINE RESPIRATORY DISEASE

T. G. McDaneld

USDA Meat Animal Research Center
Clay Center, NE, 68933, USA

USDA

BOVINE RESPIRATORY DISEASE (BRD) COMPLEX

- BRD one of the most studied livestock diseases (Fulton, 2009)
- Despite decades of research, effective immunization or antimicrobial therapies have not been developed that substantially reduce the prevalence or severity of BRD

USDA

METAGENOMICS

- Identify genomics of bacterial and viral communities (microbiome) in environmental samples

HMP

USDA

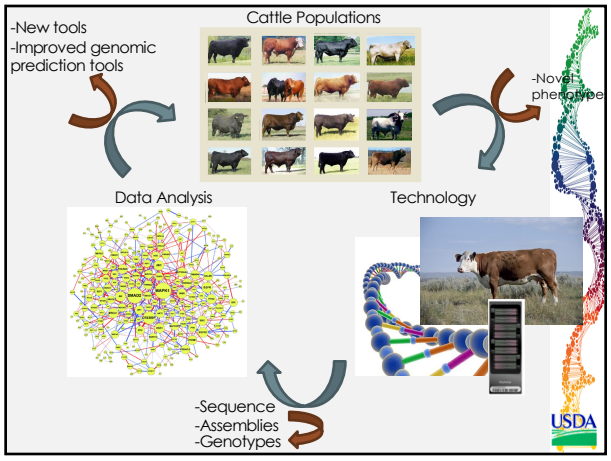
METAGENOMICS

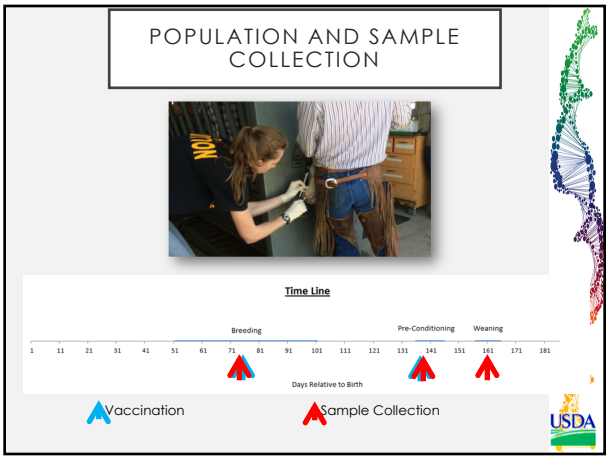
- Not a single isolate
- Identify all contributers in the community

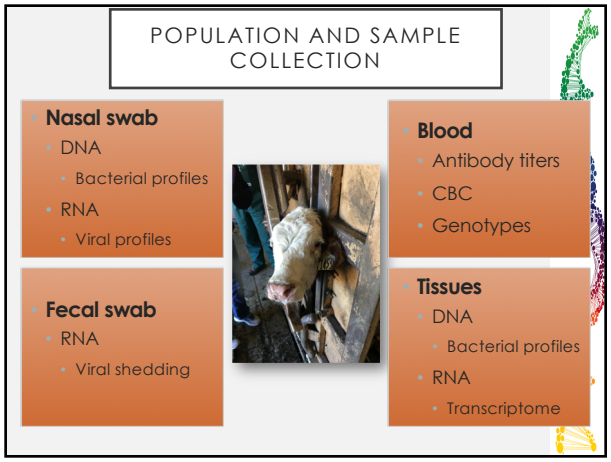
USDA

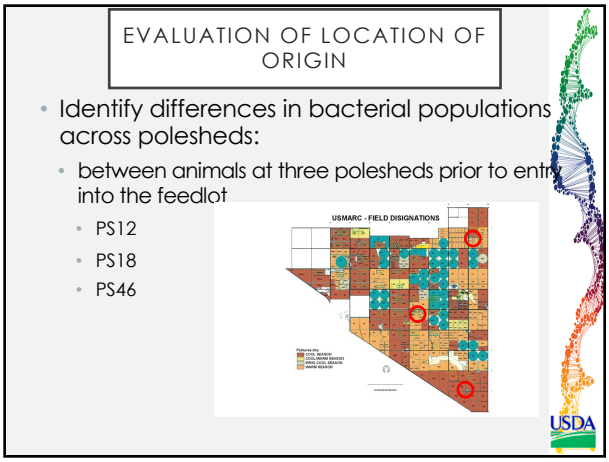
- What distinguishes the bacterial communities of sick and healthy animals?

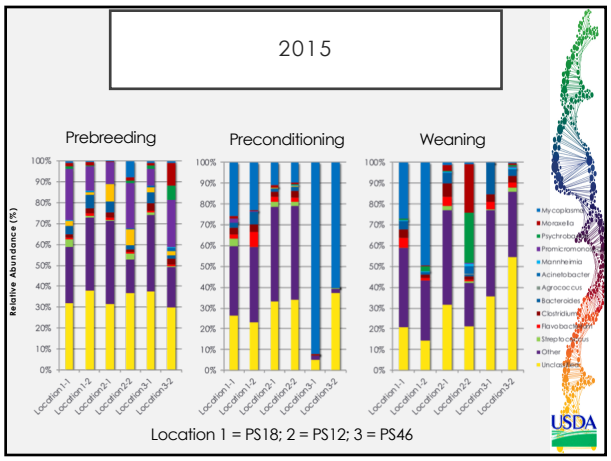
USDA

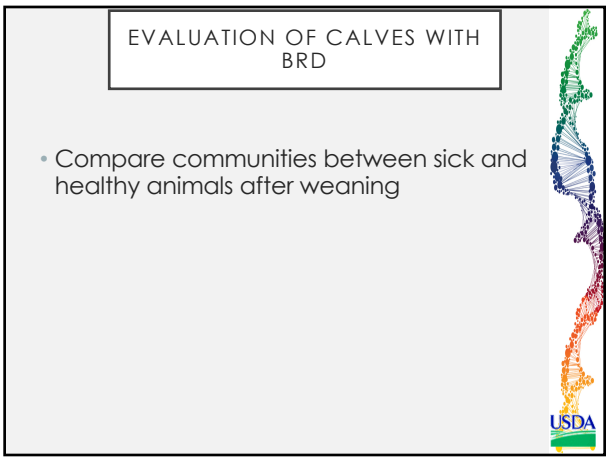

-New tools
-Improved genomic prediction tools
Cattle Populations
-Novel phenotype
Data Analysis
Technology
-Sequence
-Assemblies
-Genotypes
USDA


POPULATION AND SAMPLE COLLECTION
Time Line
Breeding
Pre-Conditioning
Weaning
Days Relative to Birth
Vaccination
Sample Collection
USDA

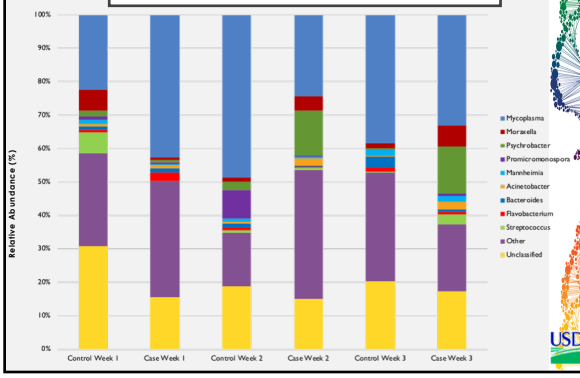

POPULATION AND SAMPLE COLLECTION
Nasal swab
DNA
Bacterial profiles
RNA
Viral profiles
Fecal swab
RNA
Viral shedding
Blood
Antibody titers
CBC
Genotypes
Tissues
DNA
Bacterial profiles
RNA
Transcriptome
USDA


EVALUATION OF LOCATION OF ORIGIN
Identify differences in bacterial populations across polesheds:
between animals at three polesheds prior to entry into the feedlot
PS12
PS18
PS46
USMARC - FIELD DISIGNATIONS
USDA


2015
Prebreeding
Preconditioning
Weaning
Relative Abundance (%)
Location 1 = PS18; 2 = PS12; 3 = PS46
USDA


EVALUATION OF CALVES WITH BRD
Compare communities between sick and healthy animals after weaning
USDA

2015

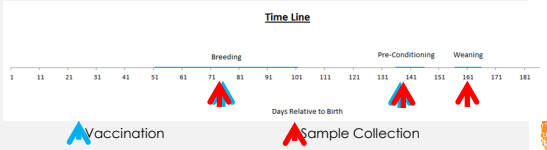


CONCLUSIONS

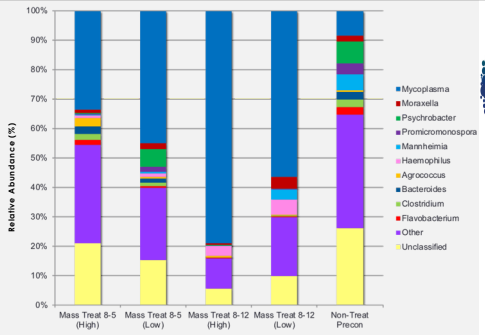
- Identified differences in bacterial populations:
  - between time points prior to weaning
  - between animals diagnosed with BRD and control cohorts

MASS TREATMENT 2016

- 2 groups of calves mass treated for BRDC at PS18 2-3 weeks before preconditioning
  - August 5 (n=93, 15% showing signs) and August 12 (n=141, 30% showing signs)
  - Third group at PS18 not treated



MASS TREATMENT 2016



CONCLUSIONS

- Mycoplasma predominant genus at mass treatment (33-79%)
- Haemophilus present at mass treatment (1-5%)

- MARC cattle operations
- Larry Kuehn
- John Keele
- Aspen Workman
- Carol Chitko-McKown
- Tammy Sorensen
- Sam Nejezchleb
- Stacy Bierman
- Amy Abrams (SDSU)
- Tasha Womack (University of Houston)
- Maci Lienemann (UC Davis)
- Kaitlin Dickson (CSU)
- Martha McCoy (USC)
- Amber Martinez (TAMU Kingsville)
- Amber Johnson (TU)
- Kylie Butterfield (NMSU)
- Megan Kaiser (HC)
- Core Lab (USMARC)
- Amanda Lindholm-Perry
- Linda Flathman


THANK YOU
USDA