Preliminary data on laboratory diagnostics for Bovine Respiratory Disease Complex in the U.S.

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US Bovine Respiratory Disease
Coordinated Agricultural Project
http://www.brdcomplex.org

The “Integrated Program for Reducing Bovine Respiratory Disease Complex (BRDC) in Beef and Dairy Cattle” Coordinated Agricultural Project is supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30367 from the USDA National Institute of Food and Agriculture.
USDA Awards Grants to Improve Cattle Production and Health

COLUMBIA, Mo., April 15, 2011 – Roger Beachy, director of the U.S. Department of Agriculture’s National Institute of Food and Agriculture (NIFA), today announced two grant awards to the University of Missouri and Texas A&M University to support research, education and outreach on cattle production to increase global food security.

“The United States is the world’s largest producer of beef and milk and has the largest fed-cattle industry in the world,” Beachy said. “As the demand for food rises due to a growing global population, it will be critically important to develop methods to produce more food with greater efficiency, while lowering the prevalence of bovine respiratory disease that inflicts significant losses each year.”

NIFA also awarded a **$9.75 million grant** to Texas A&M University to support research led by Dr. James Womack to reduce the prevalence of bovine respiratory disease (BRD) in beef and dairy cattle. BRD is the leading natural cause of death in beef and dairy cattle, causing annual losses of more than 1 million animals valued at nearly $700 million.

Womack and colleagues will use a DNA-based approach to identify cattle that are resistant to disease-causing pathogens. In addition to studying known pathogens, they will identify novel pathogens responsible for BRD. The data will be used to develop BRD diagnostic tests and genetic selection tools to identify BRD-resistant animals, while also assessing the welfare of cattle with BRD. The researchers intend to share their results with producers and develop undergraduate courses and related educational materials and instruction for 4-H youth.

Womack’s team includes scientists from the University of California-Davis, Colorado State University, the University of Missouri, New Mexico State University, Washington State University and USDA’s Agricultural Research Service.
Long-term goal is to reduce the incidence of BRD in beef and dairy cattle by capitalizing on recent advances in genomics to enable novel genetic approaches to select for cattle that are less susceptible to disease.
Funding for this project is provided by the National Institute of Food and Agriculture.

- Jim Womack, PD
- Alan Dabney
- Scott Dindot
- Noah Cohen
- Laurel Gershwin
- Terry Lehenbauer
- Cassandra Tucker
- Alison Van Eenennaam
- Jerry Taylor
- Chris Seabury
- Lawrence Falconer
- Lauren Skow
- Gary Snowder
- Milt Thomas
- Mark Enns
- Mike MacNeil
- Curt Van Tassell
- Holly Neibergs
- Shannon Neibergs
- Robert Hagevoort
- Tim Ross
- Daniel Pomp (NC)
- Shiela McGuirk (WI)
- Adroaldo Zanella (Norway)

OTHER COLLABORATORS

Bovine Respiratory Disease Consortium
Location of US collaborators

Bovine Respiratory Disease Complex
Coordinated Agriculture Project

http://BRDComplex.org
Bovine Respiratory Disease Complex
Coordinated Agriculture Project

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Our goal is to integrate research, education, and extension activities to develop cost-effective genomic and management approaches to reduce the incidence of BRD in beef and dairy cattle

Dr. Jim Womack, Texas A&M University, College Station, TX

The objective of this multi-institutional project is to reduce the incidence of bovine respiratory disease by:

• Capitalizing on recent advances in genomics to enable novel genetic approaches to select for disease-resistant cattle

• Developing improved DNA-based tests for disease diagnosis

• Providing educational opportunities for undergraduate, graduate and veterinary students to generate a future human resource for the continued reduction in bovine respiratory disease incidence

• Producing and delivering a variety of educational materials for beef and dairy cattle producers, and feedlot personnel on best management practices to reduce disease incidence
**Goal of relevance to this group**

*Develop a BRD diagnostic assay using genomic technologies.* There is a need for the development of rapid and sensitive methods to enhance the *clinical diagnosis* of BRD*. In collaboration with the veterinary diagnostic laboratories associated with this study, we will develop a diagnostic panel based on known pathogen (viral and bacterial) sequences, and novel pathogens from the metagenomics study, to quickly and effectively identify the specific pathogens present in an animal.


Developed a Diagnostic lab survey to evaluate “market”
Survey diagnostic laboratories about BRD diagnostics using prototype survey located at [https://www.surveymonkey.com/s/H5T3M8Z](https://www.surveymonkey.com/s/H5T3M8Z)
OLIGO ARRAY: Schematic view of a microarray, showing single-stranded DNA oligo probes attached to substrate, with fluorescently labeled (green) target DNA strands bound to selected oligos.
Diagnostic Lab survey

- States responding to Survey (n=9)
  CA, IL, KY (x2), MS, NE (x2), NJ, SD, TX, WI

- What percentage of your BRDC testing is for the following industries:

![Bar chart showing percentages for different states and industries]

- Cow calf
- Calf raiser/Back grounder
- Feedlot
- Dairy
Approximately how many tests do you perform per year from respiratory samples that are suspected to contain BRDC causing pathogens?

<table>
<thead>
<tr>
<th>Number of Serological tests</th>
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<th>100-500</th>
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<th>5001-10000</th>
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</table>
Do you perform multiplex BRDC assays, and if so on what platforms, for what organisms and using what sample types?

- **ASSAY 1 Platform**: Realtime PCR, ABI 7500 fast in standard mode (n=5).
- **Sample type**: nasal sab, lung tissue, nasal swab, semen, whole blood, buffy coat, various, deep nasopharyngeal swab, lung, nasal swab, lung homogenate
- **Cost per assay**: $21.50; $40 in-state, $50 out-of-state; $44/panel; $29/pair
- **Which organisms**: BVDv, BRSV, coronavirus, IBR (BoHV-1); BHV-1, BHV-5, BVDV; BVD, BHV-1, BRCoV, BRSV; BVD, BRSV, IBR; BVDV/IBR and BRCV/BRSV
- **Additional organisms**: BLV, can do PI3 and BAV-3 if requested

What percentage of samples are diagnosed to NOT contain a known BRD-associated pathogen (n=7)?

- Difficult to determine
- Cannot determine
- Virology ~ 40%
- Bacteriology ~ 78.2%; these estimates from the lab doing the most testing
- 27%, 30%, 50%; 70%
What are the biggest challenges to conducting BRDC assays?

- Serum only or formalin fixed tissue only so limited to serology and IHC. Biggest challenge is chronic infections post treatment so hard to grow bacteria and viruses have come and gone.
- Antimicrobial therapy affects the success rate of isolation and identification of bacteria.
- Viral etiologies such as BRSV are difficult to isolate, however, PCR alternative provides greater success and shorter turn-around-time.
- As many of these agents are vaccinated for prevention of BRD, identification and differentiation of vaccination and wild type strains is challenging.
- Client asked for testing for only 1-2 of the potential agents and not willing to pay for full work up.
- The current technology for viral BRDC requires that an acute and convalescent sample be submitted. This is difficult for both owners and veterinarians to accomplish.
- Clients must use a deep nasopharyngeal swab in order to adequately assess BRDC.
If an assay were developed to identify the presence of multiple BRDC associated-viruses and bacteria in a single sample, please rank what you would consider to be the most important considerations in developing such an assay.

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Not a concern</th>
<th>Least important</th>
<th>Moderately important</th>
<th>Most important</th>
<th>Rating Average</th>
<th>Response Count</th>
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</thead>
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<td>Cost - equipment</td>
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<td>0</td>
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<td>1</td>
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<td>4</td>
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<td>3</td>
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<td>4</td>
<td>3</td>
<td>4.30</td>
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<td>Ability to differentiate vaccine vs wildtype strains</td>
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<td>1</td>
<td>2</td>
<td>4</td>
<td>4.50</td>
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<td>Can use multiple sample types</td>
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<td>5</td>
<td>2</td>
<td>4.50</td>
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<td>2</td>
<td>5</td>
<td>2</td>
<td>4.20</td>
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</tbody>
</table>

Other suggestions and comments for choosing a single assay platform for BRD diagnosis:

- **Open platforms are suggested such that labs can customize ordering of reagents from multiple sources**
- **Panels that could be tested on Multiple platforms such as Life Technologies, Biorad or others.**
- **A BRDC has been developed that works very well and is used in many AAVLD labs. Resources would be better directed to fill other gaps. These questions are not relevant for our lab.**
A miniature qualitative microarray chip technology which could cover all the viral and bacterial respiratory agents as well as antimicrobial resistance genes, virulence factors, toxins, serotyping, key strain typing, and other attributes will be revolutionary if it could be offered in a cost effective manner, and require minimal hands on training for data analysis. Till date, no such single miniature microarray is commercially available, but it will be a wonderful resource for BRDC diagnosis.

Ion Torrent sequencing of infected animal samples vs noninfected
Comparison of different sequencing platforms

<table>
<thead>
<tr>
<th>Platform</th>
<th>Sequencer</th>
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<th>Read length</th>
<th>Accuracy (%)</th>
<th>Run time</th>
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<td>Roche/454 sequencing</td>
<td>GS Junior System</td>
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<td>~400 bp</td>
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<td>Up to 1000 bp</td>
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<td>Up to 600 Gb</td>
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<td>99.99 consensus accuracy; 99.5 raw accuracy</td>
<td>&lt;2 h</td>
</tr>
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Moore's Law is the observation that over the history of computing hardware, the number of transistors on integrated circuits doubles approximately every two years.
We are entering a new frontier of “Diagnostic technology for the genomics era”

Diagnosis by sequencing as an example:
Pathogenica’s sequencing analysis architecture generates a complete picture of the pathogens present in a sample in under 30 minutes and enables analysis of antibiotic resistance dissemination, infection spread between sites and individuals, or discrimination of hospital acquired from community acquired infections (i.e. DIVA)

http://www.pathogenica.com/

Figure 1: Three Hour Workflow

- Extract Nucleic Acid
  Total nucleic acid extraction with standard commercial kit

- Interrogate
  Thousands of pathogens assayed simultaneously

- Amplify
  Individually barcoded patient samples

Reactions performed by serial addition to a single tube, minimizing workflow, enabling automation and reducing the risk of cross-contamination.
Is there a place for this technology in vet diagnostic labs?

The assay screens a sample for 12 pathogens and 18 antibiotic resistance markers in a single tube, combining the workflow and cost of a dozen assays into a comprehensive low cost solution.

Highly multiplexed assay technology provides the only effective means to:

• screen up to 48 samples for a dozen pathogens and drug resistance markers in one assay
• distinguish closely related pathogens in order to track transmission of infections in a hospital setting
• achieve single base resolution for the cost of a traditional PCR assay
• No need to culture – aerobic/anaerobic bacteria and viruses

Power of sequencing – multiple pieces of information from single test

• Rapid identification of multiple pathogens
• Track outbreak transmission between patients
• Distinguish antibiotic resistance genotypes
• Confirmation of complex multi-species infections
• Improve clinical infection control and management
• Simple work flow allows fast, single shift turnaround
Discussion

The “Integrated Program for Reducing Bovine Respiratory Disease Complex (BRDC) in Beef and Dairy Cattle” Coordinated Agricultural Project is supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30367 from the USDA National Institute of Food and Agriculture.