Development and Validation of a Molecular Assay for Diagnosis of Brucellosis

Brucellosis Coordination Team Meeting – Spring 2015
Pinedale, Wyoming

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Background/Problem Statement

- 1934: State-Federal Brucellosis Eradication Plan
- 80 years later: Multiple cases of spillover from wildlife → livestock
- Improved brucellosis diagnostics (quick & accurate) is the second highest priority recommended by Wyoming Brucellosis Coordination Team
- “Diagnostic tests are not ideal and need to be improved.” (Wyoming Brucellosis Coordination Team Report & Recommendations, 2005)
Background/Problem (continued)

• Each outbreak costs State of Wyoming/Producer upwards of $254,408 (Wilson et. al., 2010; Consumer Price Index USDOL)

• Seropositive animals could be:
  1. Infected and shedding
  2. Infected, not shedding but potential for future shedding
  3. Cleared of infection
  4. Infected with cross-reacting organism

• Latent Heifer Syndrome (??)
• What is Polymerase Chain Reaction (PCR)?

- Molecular method used in diagnostics
- Steps: DNA Extraction (Tissue/Blood) Reaction Mixture Run Reaction on PCR Cycler Results – *Brucella* is Present or Not
Objectives

1. Determine best DNA extraction methods on various tissues taken at necropsy for isolation of *B. abortus*.

2. Determine gene targets for *B. abortus* through a robust computer (in-silico) analysis of whole genome sequences

3. Compare the analytic and field sensitivity and specificity of PCR candidates and bacterial culture

4. Fully develop and validate a PCR assay for *B. abortus*
Methods – Objective 1 (Completed)

1. Determine best DNA extraction methods on various tissues taken at necropsy for isolation of *B. abortus*.
   a. Tissues were spike with S19 vaccine strain and extracted with commercial kits, phase-extraction, and HotNaOH.
   b. DNA concentration and purity was measured on NanoDrop to determine best extraction methods.
   c. Statistics were run on kits to determine best kits to be used for tissues and blood and its fractions.

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Brucella PCR Project – Necropsy Tissue List (Schumaker/Hull)

- **Fetus:**
  - Whole spleen
  - Medial retropharyngeal LN
  - Prescapular LN
  - Internal iliac LN
  - Mammary Gland LN
  - Pulmonary LN
  - Kidney
  - Ileum

- **Adult:**
  - Whole spleen
  - Ovaries
  - Uterus
  - Placenta
  - Medial retropharyngeal LN
  - Prescapular LN
  - Internal iliac LN
  - Mammary Gland LN
  - Pulmonary LN
  - Kidney
  - Ileum

*Listed highest to lowest priority*
Methods – Objective 2  (In Progress)

1. Determine gene targets for *Brucella abortus* through a robust in-silico analysis of whole genome sequences

   a. 95-unpublished sequences of *Brucella abortus* raw sequence files (FASTQ) from Dr. Suelee Robbe Austerman (USDA-APHIS-NVSL)
      • Every biovar known to the US

   b. Bonus: Bovine tuberculosis PCR has replaced culture
      • High-throughput system for lymph node processing
      • Trained - Fall 2014

   c. Align files for portions of genome and select gene targets
Methods – Objective 3

3. Compare the analytic and field sensitivity and specificity of PCR candidates and bacterial culture

a. RB51 vaccine trial animals challenged with S2308
   • Ames, IA (USDA-NADC): tissues harvested and DNA extracted in parallel with culture – in house WSVL

b. Collecting known + and – samples (tissues/blood) for validation of PCR assay. Working with currently quarantined herd in WY and purchasing infected animals for sampling

c. Collecting any diagnostic sample that comes to WSVL as controls (commensal organisms) \(\rightarrow\) more robust assay
3. Fully develop and validate a PCR assay for *B. abortus*

- Best extraction and gene candidates (Objective 1&2)
- Other controls
  - Negative controls of commensal organisms (Sp+)
  - Internal amplification control (Se+)
- Enrichment techniques
- Calculate test performance
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