Genetic Analysis of ELISA Scores for Paratuberculosis in Beef Cattle

University of Florida

Important Diseases
- Foot and Mouth Disease
- Bovine leukemia
- Paratuberculosis
  - Tuberculosis
  - Mastitis
  - Brucellosis
  - Salmonellosis
- Trypanosomiasis
  - Theileria
  - Babesia
- Helminthosis
- Ticks

Motivation for Improving Disease Resistance
- Economic
  - Improve Productivity and Decrease Costs
- Changes in Resistance of Pathogens and Parasites
  - Bacteria Resistant to Antibiotics
  - Ticks Resistant to Acaricides
- Human Health Concerns
  - Transmission of Cattle ParaTBC to Humans

Paratuberculosis (Johne’s Disease)
- Chronic Progressive Disease of the Small Intestine of Ruminants (Cattle, Sheep, Goats)
- Mycobacterium avium subsp paratuberculosis (MAP)
- MAP may be related to Chron’s Disease in Humans

Transmission
- Intrauterine infection
- Ingestion of contaminated feces on teats & hair
- Milk from infected dams
- Newborn calves are the MOST susceptible
  - Susceptibility decreases with age
Subclinical Signs

- Lower milk production
- Lower growth
- Lower feed efficiency
- Higher susceptibility to other diseases

Subclinical stage can last for many years

Infected animals shed bacteria through feces

Clinical Symptoms

- Frequently appear under stressful conditions
- Diarrhea
- Poor body condition
- Lower milk production
- Unresponsive to treatment
- Weight loss even with normal appetite

Emaciation and Death

Economic Losses Due to Paratuberculosis

US Livestock Industry
US$1,500 million per year

Premature culling
Reduced availability of replacements
Decreased milk production
Reduced growth and feed efficiency

Increased susceptibility to other diseases
Increased veterinary costs
Loss of market value

Stages of Paratuberculosis

- Stage IV
  - Final Phase (1)
- Stage III
  - First Clinical Signs (2)
- Stage II
  - Silent Carrier (3)
- Stage I
  - Initial Infection (4)

Diagnosis

- Agar Gel Immunodiffusion (AGID; 1 day)
- Enzyme-Linked Immunosorbent Assay (ELISA; 1 day)
- Fecal Culture (12-16 weeks)
- Polymerase Chain Reaction - DNA Test (1 day)

UF Beef Cattle Research

- ELISA
- ELISA + Fecal Culture
- Most Common Diagnostic Procedures

- Angus-Brahman Multibreed Herd
  - ELISA
  - Low ability to detect infected animals (50%)
  - High ability to detect non-infected animals (99%)
**Goal and Objective**

- Improve effectiveness of ELISA as a tool in prevention and control programs of paratuberculosis in beef cattle
- Identify and Evaluate Genetic and Environmental Factors Related to MAP ELISA Scores

**ELISA S/P Ratios**

- Blood Sample → Serum
- IDEXX Antibody Test kit → Immunoglobulins
- Spectrophotometer → Color (Optical Density)

Optical Density (OD) is proportional to amount of antibodies in a serum sample

- \( S = OD \text{ Sample} - OD \text{ Negative Control} \)
- \( P = OD \text{ Positive Control} - OD \text{ Negative Control} \)

**ELISA Scores**

- 0 = s/p ratios (0.0 to 0.09) → negative
- 1 = s/p ratios (0.10 to 0.24) → suspect
- 2 = s/p ratios (0.25 to 0.39) → weak positive
- 3 = s/p ratios (0.40 to 0.99) → positive
- 4 = s/p ratios (0.99 to 10.0) → strong positive

**Data**

- UF Angus-Brahman Multibreed Herd
  - 2003 to 2004
  - 238 Cows
  - 352 ELISA Scores (1.5 per cow)
    - (late May blood samples)
  - Cow Condition Score (late May)
  - Cow Days Pregnant (mid August)
  - Cow Days in Lactation (late May)
  - Cow weights (late November and late May)
  - Calf weights (birth and late May)

**Number of Cows**

<table>
<thead>
<tr>
<th>Breed Group of Sire</th>
<th>A</th>
<th>75 A</th>
<th>Br</th>
<th>50A</th>
<th>25A</th>
<th>B</th>
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**Cow ELISA Scores**

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<td>61</td>
<td>38</td>
<td>46</td>
<td>74</td>
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Mixed Model – Part 1

Cow ELISA Score

Year (2003, 2004)
Age of dam (3, 4, 5 and older)
Brahman breed [Regression on prob(B)]
Heterosis [Regression on prob(A/B)]

Mixed Model – Part 2

Cow weight change from November to May
Cow days in lactation until May
Cow condition score in May
Cow days pregnant in August
Calf birth weight
Calf preweaning gain until May

Mixed Model – Part 3

Cow random effect
Cows Unrelated
Mean Zero; Common Variance

Residual
Uncorrelated
Mean Zero; Common Variance

Results

Breed Group Combination Means
Estimates of Fixed Effects
Repeatability Estimate
Graphs of Mean Predicted ELISA Scores

Means Cow ELISA Scores

<table>
<thead>
<tr>
<th>Breed Group of Sire</th>
<th>BGDam</th>
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<tr>
<td>75 A</td>
<td>0.50 0.89 1.00 0.36 0.55 1.20 0.74</td>
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<tr>
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<tr>
<td>50A</td>
<td>0.91 0.86 0.77 0.22 1.26 1.08 0.89</td>
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<tr>
<td>25A</td>
<td>0.67 1.00 0.86 0.80 1.82 1.29</td>
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<tr>
<td>B</td>
<td>0.71 1.20 1.00 - 1.00 1.29 1.24</td>
</tr>
<tr>
<td>All</td>
<td>0.67 1.03 0.97 0.50 1.00 1.30 0.91</td>
</tr>
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</table>

Year Effects

| Effect | Estimate | SE | P > |t| |
|--------|----------|----|-----|---|
| Year 2003 | 1.26 | 0.56 | 0.006 |
| Year 2004 | 1.34 | 0.68 | 0.045 |

Significant ...
No surprise here ...
Cow Age Effects

Effect | Estimate | SE | P > |t| |
--- | --- | --- | --- | --- |
(3–5) yr old Cows | 0.13 | 0.14 | 0.358 |
(4–5) yr old Cows | 0.13 | 0.14 | 0.246 |

Cows infected mostly later in life
Speed of progress too variable within ages
Sensitivity of ELISA too low to separate ages
Dataset too small to differentiate ages

Genetic Group Effects

Effect | Estimate | SE | P > |t| |
--- | --- | --- | --- | --- |
(B - A) Breed Cow | 0.846 | 0.28 | 0.017 |
Maternal Heterosis | 0.28 | 0.696 |

High % Brahman Cows → more susceptible
High % Brahman Cows → more resistant
Eating Behavior High %B ≠ Low %B Cows
Antibody Response High %B ≠ Low %B Cows

Cow Regression Effects 1

Effect | Estimate | SE | P > |t| |
--- | --- | --- | --- | --- |
Cow WT Change | 0.006 | 0.009 | 0.002 |
Days in Lactation | 0.006 | 0.003 | 0.021 |

Higher ELISA Scores
⇒ Less Gain if Positive WT Change
⇒ More Loss if Negative WT Change

Increased Antibody Response as Resources Allocated to Milk Production Decreased
Cow Regression Effects

| Effect       | Estimate | SE  | P > |t| |
|--------------|----------|-----|-----|---|
| Condition Score | 0.028    | 0.077 | 0.622 |
| Days Pregnant  | 0.000    | 0.001 | 0.982 |

Cond Score => Low Sensitivity to Subclinical MAP
Small Decreasing Trend for Predicted ELISA Scores

ELISA During First Third of Pregnancy
=> Expected Small Effect on ELISA Scores
Decreasing Trend for Predicted ELISA Scores

Calf Regression Effects

| Effect       | Estimate | SE  | P > |t| |
|--------------|----------|-----|-----|---|
| Birth Weight | -0.022   | 0.010 | 0.355 |
| Preweaning Gain | -0.000   | 0.003 | 0.001 |

Cows with Higher ELISA Scores
=> Lower Calf Birth Weights
=> Lower Calf Preweaning Gains
=> Lower Milk Production?
Random Effects

Cow Variance = 0.34 ± 0.11
Residual = 0.65 ± 0.09
Repeatability Ratio = 0.34 ± 0.01
Sizable Variation Among Cow ELISA Scores
ELISA Scores Repeatable Within Cows Across Years

Conclusions

Measurable Breed Effect
High % Brahman ≠ High % Angus

High ELISA Scores
Lower Cow Weights
Lower Calf Birth Weights
Lower Calf Preweaning Gains
Lower Milk Production?

Implications

Although ELISA has
Low Sensitivity (50%; Infected Animals)
High Specificity (99%; Non-infected Animals)
There appears to be significant negative impact of subclinical paratuberculosis on production traits of dams and calves in beef cattle

Applications

Improve Control and Eradication Measures
Help Diagnosis During Subclinical Stages
Decrease Prevalence of MAP in Infected Herds

Current Control Measures
Angus-Brahman Herd

Reduction of Exposure to Fecal Infection
Use of hay rings and special water containers
Separation of pre-partum cows by age and paratuberculosis status
ELISA and DNA testing of Cows with Clinical Symptoms
More Specific Cow Groups

Use breed group of dam, dam weight changes, calf WT and calf gains to create cow groups

| Prepartum Cows | Age × Breed Group × ELISA Score |

| Postweaning Cows | Age × Breed Group × ELISA Score × Preweaning Calf Growth |

Next Steps

- Analyze Angus-Brahman data from a production trait perspective
  - Gestation Length
  - Cow Weight Maintenance
  - Calf Birth WT, Prewean Gain, Postwean Gain
  - Carcass Traits

- Reevaluate Angus-Brahman herd with more data (serological, bacteriological, DNA) from future years