Bovine Tuberculosis: Antemortem Diagnosis and the IDEXX *M. bovis* Ab Test

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USDA United States Department of Agriculture
ars Agricultural Research Service
NADC National Animal Disease Center

Outline

1. Background and Context of Antibody-Based TB Tests

- Antemortem tests: In Use and Recent Development
- Antigens
- Cross Reactivity
- Confounding Variables
- Antibody-based Tests for Tuberculosis?

2. IDEXX *M. bovis* Antibody Test

- Highlights of the Assay
- Development and Validation
- Accuracy
- Recent Developments
- Applications

Antemortem Tests: In Use and Recent Development

1. Cell-mediated immune (CMI) responses

a. Delayed type hypersensitivity (skin test)
b. Interferon-(IFN) γ responses (Bovigam)
c. others (lymphocyte blastogenesis, IP-10, granulysin, etc.)

2. Specific antibody (Humoral Immunity)

a. Multi-antigen ELISA (CSU)
b. Fluorescence polarization assay (FPA)
c. Western blot (ARS research tool)
d. Multi-Antigen Print ImmunoAssay (MAPIA, Chembio)
e. Vet TB Stat Pak (Chembio)
f. Dual Path Platform (Chembio)
g. Seralyte Mbv (magnetic beads)
h. Enferplex TB immunoassay (Enfer Scientific)
i. IDEXX *M. bovis* Ab Test

3. Detection of the organism by culture, PCR, etc

a. Trunk wash culture for elephants

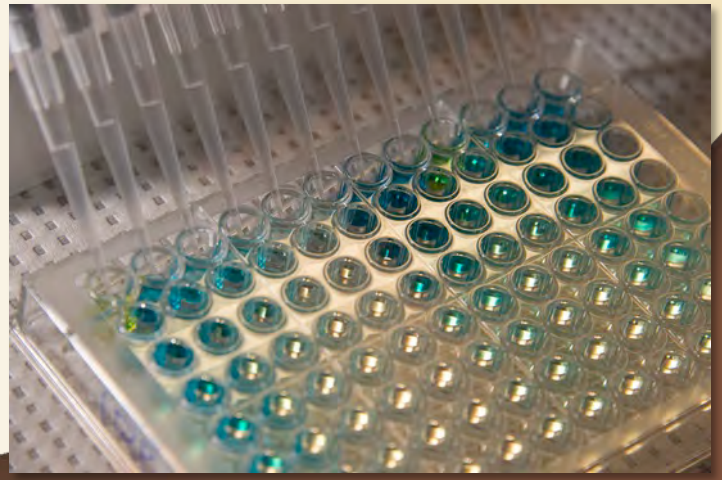
Antigens for Immune Based Assays

1. Complex

a. Purified protein derivatives (PPD) or Tuberculin
b. Whole cell sonicates (WCS)
c. Culture filtrates (CF)
d. Lipoarabinomannan-enriched (LAM)
e. others

2. Specific

a. Early secretory antigenic target 6 (ESAT-6)
b. Culture filtrate protein 10 (CFP-10)
c. MPB70 (secreted, late response)
d. MPB83 (surface, early response)
e. MPB64, MPB59, and many many others



Cross Reactivity

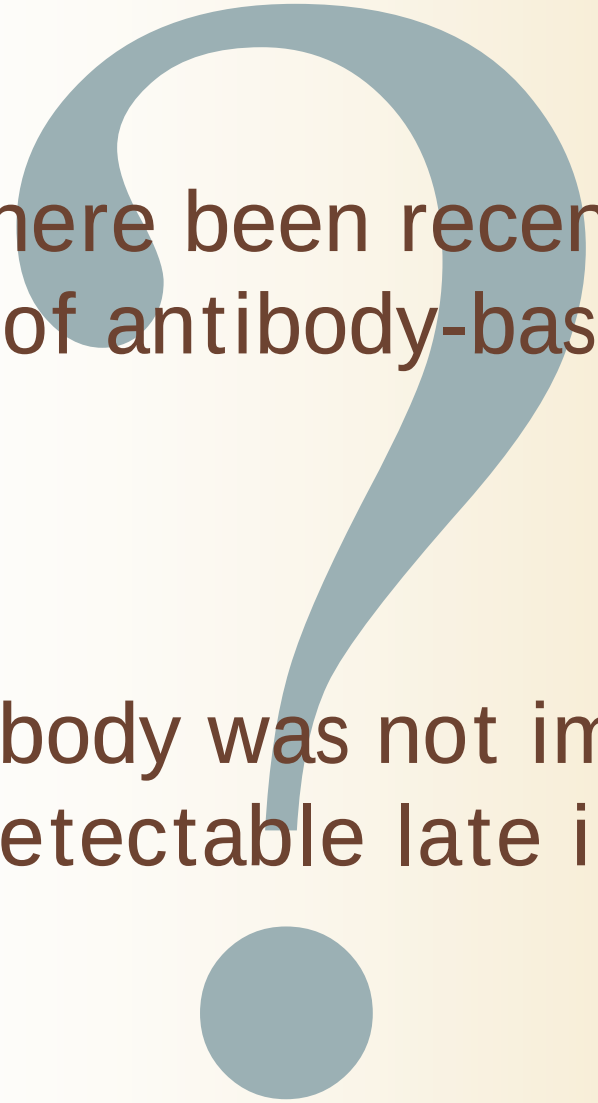
- **Shared antigens between *M. bovis* and other mycobacteria**
- ***M. avium* and *paratuberculosis* common**
- **Differential reactivity to PPDa and PPDb for CCT (scattergram) and Bovigam (PPDb - PPDa > 0.1 OD)**
- **Use of specific antigens (e.g., ESAT-6, CFP-10, and MPB83)** [*M. kansasii* is particularly problematic, ESAT-6, CFP-10, and MPB83 (others include *M. szulgai*, *M. marinum*)]
- **Johne's vaccination (Gudair, Mycopar, Silirum)**

Confounding Variables

- Stage of disease [typically, early cell-mediated immune (CMI) response and later antibody response]. CMI anergy may occur late
- Coinfection: (e.g., helminths may skew the immune response, BLV may cause an immunosuppressive effect, other *Mycobacteria sp.* elicit cross reactive responses, etc)
- Stress (e.g., shipping, production demands, malnutrition, etc.)
- **Effects of PPD administration for skin test (boosting and desensitization)**
- Sample (particularly with Bovigam)
- Immunosuppressive drugs (Dexamethasone)
- Age (with Bovigam must be > 6 months)

So, why has there been recent development of antibody-based tests for bovine TB?

I thought antibody was not important for TB and only detectable late in the course of disease?



1. Various Hosts Respond Differently!

- **Humans:** Poor antibody response, robust Delayed Type Hypersensitivity (DTH, Skin Test) response
- **Elephants**: Robust antibody response, poor DTH (difficult to apply)
- **South American Camelids:** Moderate antibody response, poor DTH
- **Eurasian Badgers**: Poor DTH response, antibody to MPB83 correlates w/bacterial load & ability to transmit as well as level of pathology
- **Cervids:** DTH (? Accuracy), moderate antibody response, immune response varies between the various cervid species
- **Cattle:** Robust DTH response, antibody to MPB83 correlates w/ level of pathology

2. Recent advancement of antibody-based tests

- Improved Platforms (testing technology)
- Improved Antigens (MPB83, MPB70, ESAT-6, CFP10)

3. Injection of PPD for skin test boosts low-level antibody response to detectable levels

IDEXX *M. bovis* Ab Test

- Utilizes standard ELISA methodology – easily adapted for use in diagnostic laboratories
- Assay validated with worldwide sample sets, including a dozen independent worldwide evaluations
- Fast three-hour protocol that delivers objective, quantitative results compatible with typical laboratory data management systems
- May use serum or plasma (ease of shipping and storage)
- Antigens: MPB83 and MPB70

IDEXX *M. bovis* Ab Test: Development and Validation

- Specific antibody detected ~90 to 100 days after experimental infection [aerosol (USDA, ARS), intratracheal (AgResearch, NZ) inoculation]

- No cross reactivity to experimental *M. avium* subsp. *avium (n = 8)* or *M. avium* subsp. *paratuberculosis (n = 8)* sensitization / infection. No false positives with sera (n = 19) collected from cattle naturally infected with *M. avium* subsp. *paratuberculosis*

- False positive responses to *M. kansasii* sensitization with 1 of 2 sera sets (n = 4 and 8)

- Injection of PPD's for skin test boosts the antibody response from 7 to at least 60 days post PPD injection.

IDEXX *M. bovis* Ab Test: Accuracy (Published)

- Sensitivity ~63% (range, 30 – 97%), n = 478, >89 herds
- Specificity ~98% (range, 88 – 100%), n = 1,473, >58 herds
- Sensitivity lower w/ samples from NZ and US, maybe due to early infection associated with active and relatively successful control programs
- **Timing of PPD injection may also explain the variable Se between sample sets**

IDEXX *M. bovis* Ab Test: Updated Sensitivity

- Diagnostic Sensitivity (relative to TB culture positive status) is approximately 65%
- For US origin cases, diagnostic sensitivity ranges from 30% – 75% for various subsets tested
- For comparison, the diagnostic sensitivity of CFT is estimated to be ~ 85% for US cattle
- However, the IDEXX test detects a subpopulation of TB-infected cattle not identified by either skin test or Bovigam®

IDEXX *M. bovis* Ab Test: Accuracy

- In Ireland: SICCT or Bovigam +, without Visible lesions, Se 46%, n = 50
- In Ireland: SICCT or Bovigam +, with Visible lesions, Se 70%, n = 50
- In Ireland: SICCT+ AND Bovigam +, with Visible lesions, Se 90%, n = 30
- **Thus, antibody levels may increase with the progression of infection and Se is greatest when lesions are visible**

- In Ireland: SICCT- and Bovigam -, with Visible lesions, Se 20%, n = 10
- **Thus, potential to detect a low percent of animals negative on CMI-based tests (anergic?)**

IDEXX Test: Recent Developments

- Johnes Vaccination (Gudair) elicits a false positive response only after injection of PPDs for skin test.

- Cursory review of *M. avium* subsp. *paratuberculosis* genome reveals no obvious homologue to the antigens used in the IDEXX test (MPB83 and MPB70)?

- *M. avium* sensitization followed by injection of PPD's for skin test elicits false positive responses (Jeff Nelson studies)

- Evidence from *M. bovis* sensitized animals suggest that blood may be shipped on the clot for identity verification without loss of reactivity and skin test boost lasts greater than 60 days (Jeff Nelson studies)

IDEXX *M. bovis* Ab Test: Applications

- CVB-licensed and USAHA TB-SAS, USAHA TB committee, and APHIS/VS recommended as a parallel test for use in TB-affected herds. Use is limited to no sooner than 7 days after tuberculin injection to gain benefit in sensitivity afforded by skin test boosting affect.

- Although its specificity is greater than that of CFT, the IDEXX test is not suitable as a slaughter surveillance tool at this time. Given a specificity of ~97.9%, for every million head of cattle tested, ~ 21,000 would be seropositive.

- Another possible application for the test would be for use with the CFT for import / export purposes; however, additional data are needed to evaluate this application. Study options are being considered.

- Not a Primary Test to replace CFT

Veterinary Service Webinar

Proposed TB Program Use of the IDEXX *M. bovis* Ab Test

February 2013

APHIS

Safeguarding Animal Health

VS Veterinary Services

Approved use:

- In TB-affected cattle herds during the removal phase of test-and-remove herd management plans.
- Other uses considered on a case-by-case basis.
  - Clause included to allow for ongoing evaluation of test for other uses in the program.
- At the discretion of the Designated Tuberculosis Epidemiologist (DTE) with approval required by the Regional Tuberculosis Epidemiologist (RTE).

Sample Collection:

- Only regulatory veterinarians may collect blood samples for the IDEXX Ab test.
- Blood samples must be collected no sooner than 7 days after the CFT is **injected** and no more than 60 days after the CFT is injected.
  - Recommend collection 7 and 14 days after the CFT is injected to ensure rapid identification and removal of potentially infected animals.

Laboratory Testing:

- Initially, testing will be limited to the National Veterinary Services Laboratories (NVSL).
- In the future, VS may approve additional laboratories to conduct the test.

USDA, James Fosse

IDEXX Ab Test Results:

- Reported as positive or negative based on a sample-to-positive (S/P) ratio.
- S/P ratio = $\frac{\text{Sample A(450)} - \text{Mean of Negative Controls}}{\text{Mean of Positive Controls} - \text{Mean of Negative Controls}}$
- Manufacturer's recommended cut-off is an S/P ratio of 0.3.
- S/P ratio ≥ 0.3 is considered test positive.

Interpretation of Test Results:

- Interpret the CFT and IDEXX Ab test results in **parallel** to increase diagnostic sensitivity.
- A non-negative result to either the CFT, the IDEXX Ab test or both the CFT and IDEXX AB test should be interpreted as a “positive” result for the animal.

CFT Result	IDEXX Result	Parallel Interpretation
+	-	+
-	+	+
+	+	+
-	-	-

Classification of Animals:

- The AEO or DTE will classify animals.
- Animals negative on the CFT and IDEXX Ab test, S/P ratio < 0.3, should be classified as negative.
- Animals non-negative on the CFT or the IDEXX Ab test, S/P ratio ≥ 0.3, must be examined postmortem for evidence of TB.

Ongoing Evaluation of Test Performance

VS will continue to evaluate the performance of the IDEXX AB test to determine if additional program uses are appropriate.

We Want your Feedback!!

- **Please submit written comments no later than March 1, 2013.**
- Draft guidance document was distributed for review and comment in early February.
- Comments may be submitted directly to Charles.W.Hench@aphis.usda.gov.

Next Steps:

Action	Proposed Timeline
Draft Guidance Document into VS Clearance	Week of March 11
Implement as an Official TB Test in TB-Affected Herds	As soon as possible upon finalization of Guidance Document

Questions?