An update on *Brucella ovis* serology

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Current challenges

- No standardized commercial reagents
- Inter-laboratory variation
- Indeterminate sample range

USAHA resolutions

• Develop better diagnostic tests
  • Consistent and reliable
  • Suitable for individual animal diagnosis

• Address current challenges
  • Cross-reactivity
  • Indeterminate sample range
  • Variation between laboratories

• Inconvenience and expense for producers
• Lack of confidence in labs
• Difficulty determining proper management

1 USAHA Comm on Sheep and Goats, 2005, Res 33
2 USAHA Comm on Sheep and Goats, 2007, Res 68
3 USAHA Comm on Sheep and Goats, 2009, Res 41
4 USAHA Comm on Sheep and Goats, 2014, Res 31
Comparison to NVSL assay

Sensitivity 99.1%
Specificity 100%

Negatives n=214
Positives n=214

S/P Cutoff=0.5
Indeterminates  
n=29

Negatives  
n=214

Positives  
n=243

Comparison to NVSL assay

Sensitivity  
99.1%

Specificity  
99.2%

S/P Cutoff=0.5
Evaluation by NVSL

Reagents provided to NVSL for comparison

• NVSL proficiency panel (15 samples)
  • Positive n=11
  • Negative n=4
  **100% accuracy**

• Additional evaluation ongoing
  • Timeline unknown
  • Eventual replacement of current system
Other work

- University of Wyoming
  - Evaluating multiple ELISAs and trying to find concordance
  - Comparing results on over 2000 samples
  - Difficult to define discordant samples with no gold standard

- Colorado Department of Agriculture & Rocky Mountain Regional Animal Health Laboratory
  - Investigating herd outbreaks
  - *B. ovis* semen PCR for follow-up testing
  - Timeline/course of ELISA (+) and PCR (+)
VMRD ELISA

Improved purification method for antigen extraction

- Standardized commercial product
- Very good concordance with NVSL
  - High Se/Sp using NVSL as standard
  - Proficiency panel 100% accuracy
- Improved resolution
  - Eliminate “indeterminate” range
  - Improve false positive rate
Brucella ovis management

Serology for flock surveillance
• Identifies exposed at risk for shedding
• Ewes may play role in maintenance
• Cost-effective

Sale, transport, show
• Better false positive rate on ELISA (improved extraction for more pure Ag)
• Eliminate indeterminate range

Semen PCR
• Identify active shedding
• Challenge of sample collection
SRLV cELISA: Resolution of occasional unexpected positives in freshly collected samples

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USAHA 2018 Annual Meeting
Sheep, Goat, & Camelid Committee
VMRD SRLV cELISA

USDA-licensed ELISA kit for CAEV & OPPV serology

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<thead>
<tr>
<th></th>
<th>Specificity</th>
<th>Sensitivity</th>
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<tr>
<td>Goat CAEV</td>
<td>99.6%</td>
<td>100%</td>
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<tr>
<td>Sheep OPPV</td>
<td>98.4%</td>
<td>95%</td>
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Federal Research Institute for Animal Health in Germany

- Ring trial with 13 commercial ELISA kits for SRLV diagnosis tested by 27 labs
- Historically problematic sera

VMRD demonstrated 100% accuracy
A confusing issue

• Isolated reports of unexpected positives
  • i.e. random positives from negative herds
  • Recent history of vaccination or illness?

• Investigation
  • Difficulty characterizing issue – infrequent, inconsistent
  • ONLY fresh samples, but not ALL fresh samples
  • Most samples are tested after storage
  • Revert to negative after storage

• Further assay optimization
  • Accommodation for problematic fresh samples
  • Maintain equivalent sensitivity & specificity
Initial investigation

- Collection of fresh serum samples
  - Processed & tested within 6 hours

- Storage at various conditions
  - Tendency of “false positives” to go negative over time
  - Enhanced by refrigerated storage
  - Freezing partially protective of “false positive” state

- Heat inactivation
  - 56 C for 30 minutes eliminated “false positive” effect
  - Interference by a heat-labile component in problematic samples
  - Complement and/or clotting factors?

http://www.wisegeek.com/what-is-a-renal-profile.htm#
Fresh sample set

13 identified as “false positives” (red)

%I range 35.6-50.7%
Improved kit

- “False positives” no longer positive (red)
- True positives remain positive (black)
- Better resolution
Full validation

Validation of the improved SRLV cELISA

Positive Cohort n = 49
Negative Cohort n = 220

- Positives
- Negatives
- Cutoff

Identical sensitivity
Identical specificity
Updated kit eliminates potential fresh sample issue

- Fundamental tool for CAE/OPP control
- Underlying sample issue
  - Limited to fresh samples
  - Heat-labile interfering factor
- Accommodation for problematic samples
  - Adjustment of production process
  - Modification of one kit component
  - Available since end of 2016
- Maintained high sensitivity & specificity

Photographer: Armin Kübelbeck, CC-BY-SA, Wikimedia Commons
Validation process

Fresh samples

• Sampled goat herd
• Tested within 6 hours in original SRLV cELISA kit

Positive samples

• Heat inactivated
  • True positive stay positive
  • Anomalous positives revert to negative
• Heat-inactivated samples tested alongside fresh samples

Updated SRLV cELISA kit

• Adjusted manufacturing process
• Validated with 269 field sample set (including above samples)