Evaluation of Diagnostic Tests Used for Detection of Bovine Viral Diarrhea Virus and Prevalence of BVDV Subtypes 1a, 1b, and 2a in Persistently Infected Cattle Entering a Feedlot

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Oklahoma State University
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- Grant from The Noble Foundation, Ardmore, Oklahoma (NF)
  - Drs. A. Confer and R. Fulton, Project Directors

- Collaboration
  - Agricultural Division (NF)
  - Dr. Billy Cook (NF)
Publication of Study Results

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“Evaluation of Diagnostic Tests used for the Detection of Bovine Viral Diarrhea Virus and Prevalence of BVDV subtypes 1a, 1b, and 2a in Persistently Infected Cattle Entering a Feedlot”
Investigators

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- Julia Ridpath, PhD, USDA NADC
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- Lurinda Burge, MS, OSU VPB
- Mark Payton, PhD, OSU Dept Statistics
- Rebecca Funk, OSU VPB
Materials and Methods

- Feedlot collections: July-December 2004
- 21,743 cattle entering feedlot
- Initial collection: Fresh ear notch in PBS
- Initial test: Antigen capture ELISA (ACE) at veterinary clinic
- Subsequent collections for confirmation at OSU CVHS
Subsequent collection on ACE positives on initial test

- All initial collections of ACE + were repeated: >90% within 48 hours after initial test
- Second fresh ear notch in PBS for ACE
- Formalin notch for IHC
- Serums and nasal swabs for PCR and virus isolation and titration
- Subtyping for BVDV 1a,1b,2a,and 2b
Results of Second (Confirmatory Testing)

- 86/88 were ACE positive and 2 were ACE negative, IHC neg, VI neg
- 86/86 ACE positives also IHC positive
- 86/86 ACE+ and IHC+ = Viral isolation+ and PCR + on serum
- The two ACE+ on initial test and ACE/IHC/VI negative on second test were potential false positive for PI

- 86 of 21,743 = PI (0.4%)
Positive IHC BVDV ear notch from PI calf: OADDL
Dr. Bill Johnson: Feedyard Calf
Predictive value of positive ACE test on ear notch at processing

- 86 of 88 initial ACE positive = PI
- 97.7% considered as PI using additional tests
BVDV Subgenotypes

BVDV1b
- BVDV1b-NY-1
- BVDV1b-Hast
- BVDV1b-TGAN

BVDV1a
- BVDV1a-NADL
- BVDV1a-Singer
- BVDV1a-C24V

BVDV2b
- BVDV2b-VS-260
- BVDV2b-VS-63
- BVDV2b-Soldan

BVDV2a
- BVDV2a-890
- BVDV2a-296nc
- BVDV2a-125nc
BVDV subtypes in US vaccines

- BVDV1a CP
- BVDV1 NCP
- BVDV2a CP
- BVDV1a NCP
- BVDV 1 not specified
Distribution of BVDV subtypes in diagnostic lab cases

- BVDV1a 37/131 (28.2%)
- BVDV2a 34/131 (26.0%)
- BVDV1b 60/131 (45.8%)

*Veterinary Microbiology* (2005), 111, pp 35-40
Distribution of BVDV subtypes in PI cattle entering the feedlot: 2004 current study

- BVDV 1a: 10/86 (11.6%)
- BVDV 2a: 9/86 (10.5%)
- BVDV 1b: 67/86 (77.9%)
Distribution of BVDV subtypes in PI cattle entering the feedlot: 2005-2006 follow up to current study

- Kansas feedlot
  - BVDV 2a: 10/78 (12.8%)
  - BVDV 1a: 8/78 (10.3%)
  - BVDV 1b: 60/78 (76.9%)

- Clayton NMSU study
  - BVDV 1a: 3/17 (17.6%)
  - BVDV 1b: 14/17 (82.4%)
<table>
<thead>
<tr>
<th>State</th>
<th>No.</th>
<th>PI</th>
<th>% PI</th>
<th>PI pens</th>
<th>PI pen rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>403</td>
<td>8</td>
<td>2.0%</td>
<td>3/ 4</td>
<td>75.0%</td>
</tr>
<tr>
<td>N.Carolina</td>
<td>851</td>
<td>4</td>
<td>0.47%</td>
<td>4/ 11</td>
<td>36.4%</td>
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<tr>
<td>Florida</td>
<td>1930</td>
<td>3</td>
<td>0.16%</td>
<td>3/ 18</td>
<td>16.7%</td>
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<tr>
<td>Kentucky</td>
<td>415</td>
<td>1</td>
<td>0.24%</td>
<td>1/ 4</td>
<td>25.0%</td>
</tr>
<tr>
<td>Missouri</td>
<td>1323</td>
<td>2</td>
<td>0.15%</td>
<td>2/ 15</td>
<td>13.3%</td>
</tr>
<tr>
<td>Mississippi</td>
<td>756</td>
<td>1</td>
<td>0.13%</td>
<td>1/ 8</td>
<td>12.5%</td>
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<tr>
<td>Oklahoma</td>
<td>8184</td>
<td>42</td>
<td>0.51%</td>
<td>36/ 88</td>
<td>40.9%</td>
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<tr>
<td>Tennessee</td>
<td>1227</td>
<td>7</td>
<td>0.57%</td>
<td>7/ 14</td>
<td>50.0%</td>
</tr>
<tr>
<td>Texas</td>
<td>5691</td>
<td>15</td>
<td>0.26%</td>
<td>15/ 67</td>
<td>22.4%</td>
</tr>
<tr>
<td>Virginia</td>
<td>963</td>
<td>3</td>
<td>0.31%</td>
<td>2/ 11</td>
<td>18.2%</td>
</tr>
<tr>
<td>Total</td>
<td>21,743</td>
<td>86</td>
<td>0.40%</td>
<td>74/ 240</td>
<td>30.8%</td>
</tr>
<tr>
<td>Buyer</td>
<td>State</td>
<td>No.</td>
<td>Pl</td>
<td>% PI</td>
<td>PI pens</td>
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<tr>
<td>-------</td>
<td>-------</td>
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<td>----</td>
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<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>FL</td>
<td>1604</td>
<td>2</td>
<td>0.12%</td>
<td>2/15</td>
</tr>
<tr>
<td>2</td>
<td>FL</td>
<td>326</td>
<td>1</td>
<td>0.31%</td>
<td>1/3</td>
</tr>
<tr>
<td>3</td>
<td>TN</td>
<td>1227</td>
<td>7</td>
<td>0.57%</td>
<td>7/14</td>
</tr>
<tr>
<td>4</td>
<td>NC</td>
<td>850</td>
<td>4</td>
<td>0.47%</td>
<td>4/11</td>
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<tr>
<td>5</td>
<td>VA</td>
<td>277</td>
<td>2</td>
<td>0.72%</td>
<td>1/3</td>
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<tr>
<td>6</td>
<td>VA</td>
<td>686</td>
<td>1</td>
<td>0.15%</td>
<td>1/8</td>
</tr>
<tr>
<td>7</td>
<td>KY</td>
<td>314</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
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<tr>
<td>8</td>
<td>KY</td>
<td>102</td>
<td>1</td>
<td>0.98%</td>
<td>1/1</td>
</tr>
<tr>
<td>9</td>
<td>MS</td>
<td>756</td>
<td>1</td>
<td>0.13%</td>
<td>1/8</td>
</tr>
</tbody>
</table>
# Individual order buyer: PI

<table>
<thead>
<tr>
<th>Buyer</th>
<th>State</th>
<th>No.</th>
<th>PI</th>
<th>% PI</th>
<th>PI pens</th>
<th>PI pen rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>MO</td>
<td>1323</td>
<td>2</td>
<td>0.15%</td>
<td>2/15</td>
<td>13.3%</td>
</tr>
<tr>
<td>11</td>
<td>Ark</td>
<td>403</td>
<td>8</td>
<td>2.0%</td>
<td>3/4</td>
<td>75.0%</td>
</tr>
<tr>
<td>12</td>
<td>TEX</td>
<td>3698</td>
<td>12</td>
<td>0.33%</td>
<td>12/45</td>
<td>26.7%</td>
</tr>
<tr>
<td>13</td>
<td>TEX</td>
<td>1575</td>
<td>1</td>
<td>0.06%</td>
<td>1/17</td>
<td>5.9%</td>
</tr>
<tr>
<td>14</td>
<td>TEX</td>
<td>427</td>
<td>2</td>
<td>0.47%</td>
<td>2/5</td>
<td>40.0%</td>
</tr>
<tr>
<td>15</td>
<td>OK</td>
<td>7480</td>
<td>38</td>
<td>0.51%</td>
<td>32/80</td>
<td>40.0%</td>
</tr>
<tr>
<td>16</td>
<td>OK</td>
<td>221</td>
<td>1</td>
<td>0.45%</td>
<td>1/2</td>
<td>50.0%</td>
</tr>
<tr>
<td>17</td>
<td>OK</td>
<td>100</td>
<td>1</td>
<td>1.0%</td>
<td>1/1</td>
<td>100.0%</td>
</tr>
<tr>
<td>18</td>
<td>OK</td>
<td>383</td>
<td>2</td>
<td>0.52%</td>
<td>2/5</td>
<td>40.0%</td>
</tr>
</tbody>
</table>
## Viral Titers in Serums and Nasal Swabs from PI Calves

<table>
<thead>
<tr>
<th>Calf no.</th>
<th>Sample</th>
<th>Viral titer/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>4276</td>
<td>serum</td>
<td>$4 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>nasal swab</td>
<td>$1.4 \times 10^4$</td>
</tr>
<tr>
<td>4061</td>
<td>serum</td>
<td>$2.2 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>nasal swab</td>
<td>$4.5 \times 10^3$</td>
</tr>
<tr>
<td>13984</td>
<td>serum</td>
<td>$2.2 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>nasal swab</td>
<td>$4.5 \times 10^4$</td>
</tr>
</tbody>
</table>
BVDV IHC in PI calf Respiratory Tract
Diagnostic Tests for BVDV

- IHC on ear notches: formalin notch
- Antigen capture ELISA (ACE): PBS fresh notch
- ACE on serum
- PCR on serum
- PCR on fresh notch in PBS
- PCR on whole blood
- Viral isolation (VI) on serum/whole blood
Diagnostic Tests for BVDV

- IHC on ear notches: formalin
- Antigen capture ELISA (ACE): PBS notch

These two tests less likely to get positive result with acute infection or MLV vaccination
Diagnostic Tests for BVDV detection for feedlot, stocker, and breeding herd

- Antigen capture ELISA (ACE): PBS notch
  - Isolate and Retest calf
  - Potential false positive for PI status
    - OD readings positive but very close to positive should be retested
Diagnostic Tests for BVDV detection

- ACE on serum
- PCR on serum
- PCR on whole blood
- Viral isolation (VI) on serum/whole blood

These tests could/will give positive result for some acute infections or MLV vaccinates
OSU Experiences with Tests

- ACE on PBS notches
  - PI positives (with confirming tests; IHC and VI) have stayed positive on repeated tests
  - Have not found ACE + notch in acute infections
- Occasional ACE PBS notch + will be IHC negative: False positive, thus repeat
- PCR on Ear notches
  - Some PI calves: PCR negative in fresh notches
  - Acutely infected BVDV calves can be PCR positive in serum
  - Dilutions of 1:2, 1:5, and 1:10 of one positive in PBS
Current and future projects for BVDV testing

- Evaluation of management options for BVDV test positive cattle: stockers and feedlots
- Examination of additional tests
- Evaluation of BVDV vaccine responses to cattle exposed to PI cattle
- Focusing on breeding herd testing as goal
- Stressing animal ID to assist breeding herd decisions/biosecurity/vaccinations
Which animal(s) is/are persistently infected with Bovine Viral Diarrhea Virus?