THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- VIBSP
  - Recruiting 400 herds or 40000 calves for testing for BVDV in Spring 2006
  - From Iowa based beef cow-calf producers
• What are the aims?
  – Evaluate pooled testing as a screening tool
  – Estimate prevalence of BVDV in Iowa
  – Estimate risk factors for BVDV in Iowa
• What it’s not?
  – Not a certification program
  – Not state sponsored
Motivation

“...it is the resolve of the Academy of the Veterinary Consultants that the beef and dairy industries adopt measures to control and target eventual eradication of BVDV from North America”
Immediate impact

- Provide beef breeders with cheap and effective methods for screening for BVDV
- Sensitivity and specificity of the pooled RT-PCR screening program
Screening tests

- Cheap
- Associated with false positive’s
- Positive’s further securitized
- PCR on Bulk milk
- Serology of sentinel heifers
Diagnostic tests

– Confirm or classify disease
– Animals are abnormal and the challenge is to identify the disease
– Individual animal: IHC, AC-ELISA
Long-range impact:
- A cost-benefit assessment of BVDV eradication program
- Prevalence of BVDV-PI positive herds
- Impact of eradication on performance
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• Enrollment phase: Oct 2005-Mar 2006
  – Recruiting veterinarians
  – Recruiting producers
Ensure veterinarians are comfortable with BVDV control programs
  – Available for veterinary meetings, extension, ICA, CVM
• Recruiting veterinarians
  – Explain the goals, enrolling, sample collection, submission, receiving results
  – Explain BVDV to vets
  – NCBA BVD and AVC BVD materials
• Vet contacts
  – 3 mailings: brochures and publications
  – Phone call for each vet
  – Follow up letters for enrolled vets
  – Producer meetings for enrolled vets
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• Recruiting producers
  – IVMA vets
  – Iowa Cattleman's meetings and magazine
  – Iowa State University Extension officers
After enrollment phase
  – Determine if there is sufficient interest to warrant continuation
  – Ensure veterinarians are comfortable with BVDV control options
• Study phase runs from Jan-July 2006
  – Sample collection
  – Testing and interpretation
  – Analysis
Enrolled producers will receive
- sample collection tubes
- sample submission boxes
- shipping labels
- sample submission record sheet
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Calving

Breeding

Window of opportunity for testing
Sample collection either at
  – Birth when calves are ID
  – Spring processing pinkeye/blackleg vaccination
• Study phase
  – Producers notch calves
  – Freeze notches in tubes
  – Submit frozen notches to our lab within 1 week of collection
• At the lab
  – Process and freeze samples
  – When collection complete then thaw and pool
  – Submit to pooled sample to VDL for PCR
• Study phase
  – Pooled RT-PCR on PBS soaked ear notches
  – Follow-up of ACE on positive pools
  – Notify veterinarians for follow up sample
Testing and interpretation

- Available through veterinarian
- **Testing paid for by grant**
- Vet and client decide next step
- Follow-up outcome
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COLLEGE OF VETERINARY MEDICINE
Pooling ear notches

Retained ear notch and individual ID for further tests if needed.

6.3 ml of PBS supernatant from 18 ear notches
<table>
<thead>
<tr>
<th>RT-PCR Pools/AC-ELISA</th>
<th>+ RT-PCR Pool</th>
<th>-RT-PCR Pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>One or more Positive AC-ELISA</td>
<td>39*</td>
<td>0</td>
</tr>
<tr>
<td>No Positive AC-ELISA</td>
<td>0</td>
<td>33</td>
</tr>
</tbody>
</table>

*Includes 36 samples spiked with a single known positive sample
### THE VOLUNTARY IOWA BVDV SCREENING PROJECT

#### POOL Samples

<table>
<thead>
<tr>
<th>POOL</th>
<th>Samples</th>
<th>BVD RT-PCR Results</th>
<th>AC-ELISA &amp; IHC results by Sample #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-100</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>2</td>
<td>101-200</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>3</td>
<td>201-300</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>4</td>
<td>301-400</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>5</td>
<td>401-500</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>6</td>
<td>501-600</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>7</td>
<td>601-700</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>8</td>
<td>701-800</td>
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</tr>
<tr>
<td>9</td>
<td>801-900</td>
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<tr>
<td>10</td>
<td>901-1000</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>11</td>
<td>1001-1100</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>12</td>
<td>1101-1200</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>13</td>
<td>1201-1300</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>14</td>
<td>1301-1400</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>15</td>
<td>1401-1500</td>
<td>Positive</td>
<td>1433 &amp; 1442 positive all others negative</td>
</tr>
<tr>
<td>16</td>
<td>1501-1600</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>17</td>
<td>1601-1700</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>18</td>
<td>1701-1800</td>
<td>Positive</td>
<td>1718 positive all others negative</td>
</tr>
</tbody>
</table>

### Pool Samples

<table>
<thead>
<tr>
<th>POOL</th>
<th>Samples</th>
<th>BVD RT-PCR Results</th>
<th>AC-ELISA &amp; IHC results by Sample #</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>1801-1900</td>
<td>Positive</td>
<td>1899 positive all others negative</td>
</tr>
<tr>
<td>20</td>
<td>1901-2000</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>21</td>
<td>2001-2100</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>22</td>
<td>2101-2200</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>23</td>
<td>2201-2300</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>24</td>
<td>2301-2400</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>25</td>
<td>2401-2500</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>26</td>
<td>2501-2600</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>27</td>
<td>2601-2700</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>28</td>
<td>2701-2800</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>29</td>
<td>2801-2900</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>30</td>
<td>2901-3000</td>
<td>not detected</td>
<td>No Positives</td>
</tr>
<tr>
<td>31</td>
<td>3001-3100</td>
<td>not detected</td>
<td>No Positives*</td>
</tr>
<tr>
<td>32</td>
<td>3101-3200</td>
<td>not detected</td>
<td>No Positives*</td>
</tr>
<tr>
<td>33</td>
<td>3201-3300</td>
<td>not detected</td>
<td>No Positives*</td>
</tr>
<tr>
<td>34</td>
<td>3301-3400</td>
<td>not detected</td>
<td>No Positives*</td>
</tr>
<tr>
<td>35</td>
<td>3401-3500</td>
<td>not detected</td>
<td>No Positives*</td>
</tr>
<tr>
<td>36</td>
<td>3501-3599</td>
<td>not detected</td>
<td>No Positives*</td>
</tr>
</tbody>
</table>

*AC-ELISA correlated with IHC on first 3016 samples afterward IHC was discontinued*
<table>
<thead>
<tr>
<th>RT-PCR pools/ AC-ELISA</th>
<th>RT-PCR BVD +</th>
<th>RT-PCR BVD -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool contained positive AC-ELISA (1 or more)</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Pool did not contain positive AC-ELISA</td>
<td>2 (one pool contained 50 samples* the other contained 38 samples**)</td>
<td>38</td>
</tr>
</tbody>
</table>

*Samples from herd where a PI was found: **testing not completed for herd
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• 400 + herds
• 40 PI-BVDV positive herds
• Identifying PI’s early allows them to be removed from the herd before the next breeding season;
• Otherwise, PI suckling calves may infect cows carrying early-gestation fetuses and create more PIs in next year’s calf crop.
• Only need to test dams of + calves (dams are rarely positive, but must test).
Finding BVDV PI Calves

• Options for identifying BVDV PI neonates:
  – BVDV isolation (VI) from serum or buffy coat:
    • False negatives due to interference by maternal Ab.
  – BVDV antigen detection in serum with Ag-capture ELISA:
    • False negatives due to interference by maternal Ab.
Options for identifying BVDV PI neonates:

- RT-PCR on serum (individual or pools):
  - Need blood samples, $$ unless pool.
- Ear notch tests
Three tests for BVDV can be performed on ear notches:

1. Immunohistochemistry (IHC):
   Fix notch in formalin, embed in paraffin, apply antibody to section to detect antigen.
2. Ag-capture ELISA:
   – Soak notch in saline, (freeze), run ELISA on fluid to detect antigen.

3. PCR:
   – Soak notch in saline, run RT-PCR on fluid to detect RNA. Can pool fluid of 100 notches.
1. Immunohistochemistry (IHC):
   Sensitivity = \(~100\%\), but a small % of acutely infected calves are positive.

2. Ag-capture ELISA:
   Sensitivity = \(~100\%\), but a small % of acutely infected calves are positive

3. PCR:
   Sensitivity = \(~100\%\) on individuals and pools.
   May also detect acute infections
• Producers notch calves at ear-tagging.
• Place notches in numbered tubes with saline and freeze.
• After all calves have been notched, send frozen notches to VDL.
• At VDL, ear notch fluids for each herd pooled into 1 or more pools of \( \leq 100 \).
• RT-PCR for BVDV RNA on each pool.
VIBSP Testing Strategy

• If pool is PCR-negative, no PIs present.
• If pool is PCR-positive
  – Ag-capture ELISA will be done on fluid from each calf in pool.
  – Ag-capture ELISA positive calves should be confirmed PI by re-testing (buffy coat PCR) >30 days after notches obtained.
• Aim:
  – Estimate the prevalence of BVDV PI in Iowa beef breeding herds
  – Evaluate the efficacy of pooled testing to cheaply screen herds for BVDV
Enrollment phase: Winter 2005

Study phase: Spring and Summer 2006
Enrollments to date

- Calves - 15000
- Producers - 102 average herd size ~ 150
- Vets - 51
- Clinic - 40
• Issues to date
  – What to do with fall and spring calving herds
  – What to do with spread out calving seasons
  – Concern about ears appearance after notching
• Do you produce seedstock? 36/71
• Do you background or feedlot calves? 49/71
• Do you purchase replacements, bulls or cows? 70/71
• Do you exhibit etc? 38/71*
• Do you have nose to nose contact? 53/71
• Do other animals have contact with cattle feed etc? 71/71
• Conception > 90%? 60/71
• Weaning rate > 90%? 62/71
• BVDV vaccination yearly? 64/71
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- 81% of non BVDV herds vaccinate?
- 90% of herds diagnosed with BVDV vaccinate?
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- Project issues of the outcomes
  - Sensitivity of Pooled PCR
  - Risk factor analysis
  - Prevalence estimates
• Sensitivity
  – The PCR test on the calf subset
  – The calf subset as a herd test
Causes of imperfect sensitivity in the calf subset

- Excessive dilution
- Inhibition
• 100 of 400 samples
• Prevalence of false-ve pools - 1%
• ACE = 95% sensitive & 100% specific
• If all samples test negative, there is 95% confidence that the true prevalence is false negative is < 2.76%
  – 1 - false negative prevalence = sensitivity
  – 95% confident the sensitivity of pooled PCR is greater than or equal to 97.24%
• 100 of 400 samples; prevalence of false -ve pools- 1%
  – 100% sensitivity & 100% specificity of ACE
  – If all samples test negative, there is 95% confidence that the true prevalence is < 2.1%
## The Voluntary Iowa BVDV Screening Project

<table>
<thead>
<tr>
<th>Test result</th>
<th>BVDV+</th>
<th>BVDV-</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>39</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>356</td>
</tr>
</tbody>
</table>

- **PVP**: 
  \[ \frac{39}{43} = 91\% \]

- **PVN**: 
  \[ \frac{356}{357} = 97\% \]

- **40** (sen=97%)
- **360** (spec=99%)
Test result

<table>
<thead>
<tr>
<th></th>
<th>BVDV+</th>
<th>BVDV-</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>360</td>
</tr>
</tbody>
</table>

40 (sen=97%) 360 (spec-100%)

PVP = 39/39 = 100%
PVN = 360/361 = 99.7%
•99% sensitivity & 100% specificity of ACE
  –From an infinite population testing 300
  –If all samples test negative, there is 95% confidence that the true prevalence is < 0.01.

Sensitivity of the calf subset as a herd level diagnosis

- Follow-up of herds that decide to herd test after finding a calf PI
- Next funding phase
• Project issues of the outcomes
  – Risk factor analysis
  – Prevalence estimates
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<table>
<thead>
<tr>
<th>Exposure factor</th>
<th>BVDV+</th>
<th>BVDV-</th>
<th>RR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>200</td>
<td>1600</td>
<td>1.1</td>
<td>1.01-1.47</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>44%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>200</td>
<td>2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 (10%)</td>
<td>3600 (90%)</td>
<td></td>
<td></td>
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<tr>
<td>Exposure factor</td>
<td>BVDV+</td>
<td>BVDV-</td>
<td></td>
<td></td>
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<tr>
<td>----------------</td>
<td>-------</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>20</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>44%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>20</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 (10%)</td>
<td>360 (90%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RR-1.1
95%CI - 0.6 - 2.1
<table>
<thead>
<tr>
<th>Exposure factor</th>
<th>BVDV+</th>
<th>BVDV-</th>
<th>RR-1.4</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>+</td>
<td>28</td>
<td>180</td>
<td>1.4</td>
<td>1.1 - 1.8</td>
</tr>
<tr>
<td></td>
<td>70%</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>12</td>
<td>180</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 (10%)</td>
<td>360 (90%)</td>
<td></td>
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Exposure factor

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>60</td>
<td>122</td>
</tr>
<tr>
<td>-</td>
<td>60</td>
<td>158</td>
</tr>
</tbody>
</table>

RR - 1.2
95%CI - 0.8-1.6

120 (30%) 280 (70%)
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Exposure factor

<table>
<thead>
<tr>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (50%)</td>
<td>80 (40%)</td>
</tr>
<tr>
<td>200 (50%)</td>
<td>120</td>
</tr>
</tbody>
</table>

RR-1.2
95%CI – 1.02-1.48
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Exposure factor

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>80 (66%)</td>
<td>140 (50%)</td>
</tr>
<tr>
<td>-</td>
<td>40</td>
<td>140</td>
</tr>
</tbody>
</table>

RR-1.6
95% CI – 1.1-2.2

120 (30%)
• Where next?
  – Funding for longitudinal animal health and enterprise analysis
  – Collaboration and sample supply
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• http://www.census.gov/population/www/socdemo/school.html