Testing for Non-O157:H7 Shiga Toxin-Producing Escherichia coli Serotypes in Ohio

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Background

- *E. coli* O157:H7 most common strain associated with foodborne illness in the United States
- FSIS slated to include 6 additional serotypes as adulterants in June 2012
  - Known as the “Big 6”
  - O103, O111, O26, O45, O121, O145
- The “Big 6” are not routinely tested for
  - Difficulty in identifying and confirming
  - Lack of validated laboratory methods
Background (continued)

• Ruminant animals are primary transmission source
  – Food products (beef products)
  – Manure-contaminated vegetables
  – Animal to human contact

• Ground beef is being targeted for this research
Background (continued)

- The Ohio Meat Inspection program only analyzes beef for *E. coli* O157:H7
  - Ground beef sampling program started in 2002
  - Trim sampling started in 2006
  - Over 12,000 samples analyzed since 2008
  - Seven confirmed positive findings (4 in 2009; 3 in 2011)
Problem Statement

- DMI has no baseline for the ‘Big 6’ serotypes.
- Limited test kits commercially available.
- Labs have no experience screening for non-O157:H7.
- Validation of screen kits is a concern.
Research Questions

1. Have the analytical methods been validated enough to produce accurate results?
2. Is the ODA DMI ready to regulate for these additional E. coli serotypes?
3. Is it possible to determine if non-O157:H7 STEC is present in ODA DMI-inspected beef slaughter facilities?
Methodology

- All of Ohio’s beef slaughter operations had potential for being included in project.
- 100 samples were analyzed for both *E. coli* O157:H7 and non-O157:H7.
- Inspectors responsible for random collection of samples and establishments sampled were briefed on research details.
- Samples were collected from August 22, through September 29, 2011.
Methodology (continued)

• Blind samples were used for the non-O157:H7 portion of the study.
  – Facilities were identified for the *E. coli O157:H7* portion.

• Samples were analyzed within 24 hours of collection.

• Analysis was conducted by the ODA Consumer Protection Laboratory (CPL).
Methodology (continued)

• Samples were screened using two methods
  – Bio-GX
    • Reported as potential positive if confirmed for the stx1 or stx2 gene
    • Potential positives were further analyzed using the kit’s two-plate system to identify serotype
Methodology (continued)

- **DuPont BAX**
  - 60 samples analyzed
  - Screened for either stx1 or stx 2 gene AND –eae gene
  - Further screened for wzx serogroup
  - If positive for stx1 or stx2, eae gene and wzx serogroup reported as potential positive
Methodology (continued)

- Any potential positives went through a cultural confirmation process
  - Immunomagnetic separation (IMS) beads
  - Plated on differential agars
    - 10-20 typical colonies selected
  - Rescreen on BioGX or BAX panels
  - Screen using antisera (agglutinate)
  - Biochemical identification via VITEK

- If positive through each step: reported as positive
Study Population

- Fully-inspected beef slaughter facilities in Ohio
  - 76 beef slaughter facilities in Ohio
  - 69 facilities participated in study
  - 50,500 beef slaughtered annually statewide
# Results

## Summary table of Potential Positive Findings by Method

### “BIG 6” SEROTYPES

<table>
<thead>
<tr>
<th>METHOD</th>
<th>BioGX</th>
<th>O145</th>
<th>O111</th>
<th>O26</th>
<th>O45*</th>
<th>O121*</th>
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<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
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<td></td>
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</tbody>
</table>

* NO IMS BEADS AVAILABLE
Results (continued)

• Limitations
  – Novobiocin issues (sample prep)
  – Issues with software (both)
  – Background interference (BioGX)
  – No eae gene identification (BioGX)
  – Lack of specificity (BAX)

• Difficulty in identifying ‘typical’ colonies for confirmation
Conclusions

• Due to test kit weaknesses, some questions remain unanswered.
• Regulators are not ready to implement mandatory testing.
• Too many shortcomings with screening and confirmation methods.
• More validation and real-life analyses are needed to identify and address weaknesses.
Recommendations

• More time to research and validate.
• FSIS should work with or share materials with state labs.
• Include other known serotypes of pathogenic E. coli
  – O104
• Retest Ohio beef slaughter operations
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References

Questions?