Shiga-toxin *Escherichia coli* Contamination in Cattle Post Harvest & Educating International Students about Rabies through the Kansas State University Rabies Laboratory

Final Examination – 04/06/18

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Kansas State University
Overview

• Part 1: Master’s Thesis
• Part 2: Field Experience
STEC Contamination in Post Harvest Cattle

PART 1: Master’s Thesis
Objective

To determine the **prevalence** and **concentration** of STEC O157 and non O157 (STEC-7) in **feces** of **cull dairy cattle** at commercial processing plants
**E. coli, STEC, and EHEC**

- *Escherichia coli*
  - Normal inhabitants of the GI tract
  - Beneficial to their host

- STEC = *E. coli* that produce Shiga toxin (*stx*)
  - *stx*1 and/or *stx*2
  - *stx*2 more severe disease in humans

- EHEC (Enterohemorrhagic *E. coli*)
  - all EHEC are STEC
  - *eae* gene; encodes intimin
CYCLE OF EVENTS IN SPREAD OF STEC
RUMINANTS AND CONTAMINATION CYCLE

1. Ingestion of pathogenic *E. coli*
2. Fecal excretion and contamination of environment
3. Contamination of food and water
4. Transmission animal to person (farms, slaughterhouses, etc.)
5. Transmission person to person
STEC O157 Outbreak by Transmission routes, 1982-2002 (Rangel et al, 2005)
STEC: A Public Health Threat

Human infection
- Ingestion of small number (100 cells or less) can cause disease
- People at any age are susceptible
- Highest risk: infants, young children, older adults, and immunosuppressive persons.
- Clinical signs: non-bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS), and death

Cattle infection
- STEC do not cause disease in adult cattle
- STEC contamination in environment, fresh vegetables, beef meat
Human STEC infection in the United States

Estimation of human STEC infection per year
(Scallan et al, 2015)

<table>
<thead>
<tr>
<th></th>
<th>STEC O157</th>
<th>STEC non-O157</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cases</td>
<td>63,153</td>
<td>112,752</td>
</tr>
<tr>
<td>Hospitalizations</td>
<td>2,138</td>
<td>217</td>
</tr>
<tr>
<td>Deaths</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

- Over 100 strains of STEC (Karmali, 1989)
- “Big six” non-O157: O26, O45, O103, O111, O121, and O145 (CDC, 2015)
- **STEC-7** = STEC O157 + 6 STEC non-O157
Cull dairy cattle in U.S. beef production

- Cull dairy cattle: 9.6% of the total U.S. cattle slaughtered in 2016 (USDA, 2017).
- Cull dairy cattle contribute to 17% of total ground beef (Troutt & Osburn, 1997).
Fecal samples collection

- Commercial processing plants (California = 1), (Pennsylvania = 2)
- Summer months (August-Sept, 2017)
- Total samples = 183
- Fecal swab sample
  - recto-anal junction
  - before evisceration

Sperandio and Nguyen, 2012
Sample enrichment (40°C for 6 hrs)

IMS procedure:
- Individual: O157
- Pool 1: O26, O45, O111
- Pool 2: O103, O121, O145

Modified Posse medium & CT-SMAC media (37°C for 20-24 hrs)

Blood Agar (37°C for 20-24 hrs)

Tested for non-O157 (PCR)

Tested for O157 (agglutination, indole test, PCR)
STEC Quantification Methods (Concentration)

• Pre-enriched samples
  • Spiral plate onto 2 medias:
    • Sorbitol MacConkey (CT-SMAC) for O157
    • Modified Posse (MP) agar for non-O157
  • Incubated (37°C for 24 hrs)

• Colonies enumeration

• Blood Agar (37°C for 24 hrs)
• Tested for O157 $\rightarrow$ agglutination $\rightarrow$ spot-indole $\rightarrow$ PCR
• Tested for non-O157 $\rightarrow$ PCR
Table 1. Characteristic of the study population

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Date of collection</th>
<th>Number of samples collected</th>
<th>Plant capacity (cattle/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>August 14, 2017</td>
<td>62</td>
<td>1,000</td>
</tr>
<tr>
<td>B</td>
<td>August 21, 2017</td>
<td>59</td>
<td>1,500</td>
</tr>
<tr>
<td>C</td>
<td>Sept 18, 2017</td>
<td>62</td>
<td>450-470</td>
</tr>
<tr>
<td></td>
<td>Total # of samples</td>
<td>183</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2. Cumulative prevalence of EHEC O157 and non-O157 serogroups by processing plant

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Sample size (N)</th>
<th>Prevalence % (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O157</td>
</tr>
<tr>
<td>A</td>
<td>62</td>
<td>3.2 (2/62)</td>
</tr>
<tr>
<td>B</td>
<td>59</td>
<td>1.7 (1/59)</td>
</tr>
<tr>
<td>C</td>
<td>62</td>
<td>3.2 (2/62)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>183</strong></td>
<td><strong>2.7 (5/183)</strong></td>
</tr>
</tbody>
</table>

\(^a\)Each of positive sample corresponds to have at least one non-O157 somatic antigen.
\(^b\)2 samples could not be processed by the IMS assay.
Table 3. Distribution of EHEC O157 and non-O157 by processing plant

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>O157</th>
<th>Non-O157</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>O157, stx1, stx2, eae, ehxA, fliCH7 (2)</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>O157, stx2, eae, ehxA, fliCH7 (1)</td>
<td>O103, stx1, eae (12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O111, stx1, eae (3)</td>
</tr>
<tr>
<td>C</td>
<td>O157, stx2, eae, ehxA, fliCH7 (1)</td>
<td>O26, stx1, eae (1)</td>
</tr>
<tr>
<td></td>
<td>O157, stx1, eae, ehxA, fliCH7 (1)</td>
<td>O103, stx1, eae (2)</td>
</tr>
</tbody>
</table>

Table 4. Percentage of identified EHEC by serogroup

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>O157</th>
<th>O103</th>
<th>O111</th>
<th>O26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence % (n/N)</td>
<td>2.75% (5/183)</td>
<td>7.65% (14/181)</td>
<td>1.64% (3/181)</td>
<td>0.66% (1/181)</td>
</tr>
</tbody>
</table>
Table 5. Number of quantifiable samples and concentration in CFU/g feces of non-O157* serogroups in pre-enriched samples

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Sample size</th>
<th>Number of quantifiable fecal samples</th>
<th>&lt; 500 CFU/g</th>
<th>500 &lt; n &lt; 10^4 CFU/g</th>
<th>≥ 10^4 CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>62</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>B</td>
<td>59</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>C</td>
<td>62</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*there were no enumerable samples detected for O157
N/A: Samples were unable to be processed
Conclusion

• Prevalence: EHEC Non-O157 (10.5%) was higher than STEC O157 (2.7%)
• Detectable EHEC non-O157: O103 (7.65%), O111 (1.64%), O26 (0.66%)
• Concentration EHEC non-O157: 1.6% (<10^4 CFU/g)
• Virulence factors: stx1, stx2, eae, ehxA
• Prevalence and concentration of cull dairy cattle?
Educating International Students about Rabies

PART 2: Field experience
Human rabies in the U.S.

- Human cases: 23 cases (2008 to 2017)
- Exposure occurred in the country or during overseas travel
- Not aware of the exposure
- Aware of the exposure, but not aware that PEP is needed (CDC, 2017)
Target audience: International students

- Unaware of rabies risk
  - Reservoirs are not endemic in their home countries
  - Not familiar with endemic diseases in the U.S.
- Possibility of contact with rabid animals
  - Bats enter KSU facilities
  - Raccoons in Jardine Apartment
Project Overview

Online Survey

- Knowledge, Attitude, Perception (KAP) Survey (WHO, 2008)
- Level of familiarity with the animals (Sexton & Stewart, 2007)
- Health seeking behavior

Anti-Rabies Campaign
Participants’ level of familiarity with the animals

### Q10

**Please tell us how familiar you are with these animals**

<table>
<thead>
<tr>
<th>Option</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>I know that raccoon, skunk, and/or bat live in Kansas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have seen a raccoon, skunk, and/or bat in Kansas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have seen a raccoon, skunk, and/or bat in my neighborhood in Manhattan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have seen a raccoon, skunk, and/or bat inside my residence (e.g. apt, dorm, or house) in Manhattan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have seen a raccoon, skunk, and/or bat inside K-State facilities (e.g. Rec center, library, campus/laboratory buildings)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During my study in the United Study, I have bitten/scratched by a raccoon, skunk, and/or bat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Participants’ level of familiarity with skunk, racoon, and bat (N=145)
KAP Survey findings

- Response rate: 8.5% (145 responses out of 1751 international students)
- Knowledge:
  - Rabid animals, mode of transmission, biology and pathogenicity of the agent
  - 15 questions
  - 14.5% (n=21) had higher knowledge (at least 12 questions -out of 15- answered correctly)
KAP Survey Findings

• **Risk perception:** Participants’ judgment about the likelihood of rabies exposure

• 44.1% had higher perception of risk towards rabies exposure
KAP Survey findings

• **Attitude** (responses):
  • Participants’ responses toward rabies exposure and suspected rabid animals
  • Likert scale (strongly disagree-strongly agree)
  • Wounds management
  • Importance of timely medical assistance
  • Handling suspected rabid animals

• 88.3% had appropriate responses to rabies exposure
<table>
<thead>
<tr>
<th>Q11</th>
<th>Click to write the question text</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>If I see a skunk in my residence, I will catch it and then release the skunk outside the building</td>
</tr>
<tr>
<td></td>
<td>If I see a bat inside K-State facilities (e.g., Rec-center, library), I will allow it to stay if the bat doesn’t bother me</td>
</tr>
<tr>
<td></td>
<td>If a bat landed on me, I will do nothing if I don't get any serious wounds</td>
</tr>
<tr>
<td></td>
<td>If I get bitten by a skunk, I will clean up the wounds and consult a doctor/nurse as soon as possible</td>
</tr>
<tr>
<td></td>
<td>If I get bitten by a raccoon, I will call the police or animal control department to capture the raccoon and send it to the laboratory for testing</td>
</tr>
<tr>
<td></td>
<td>It’s important to get assistance from animal control department if I see a bat inside my residence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strongly agree</th>
<th>Somewhat agree</th>
<th>Neither agree nor disagree</th>
<th>Somewhat disagree</th>
<th>Strongly disagree</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
Survey findings: Health-seeking behavior

Frequency and usage of mass media and interpersonal channels (Possible range was 1 to 6; 6 being the most often used media)

<table>
<thead>
<tr>
<th>Channel</th>
<th>Variables</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Media</td>
<td>Website of official institutions</td>
<td>3.62 (1.69)</td>
</tr>
<tr>
<td></td>
<td>Other websites</td>
<td>3.23 (1.45)</td>
</tr>
<tr>
<td></td>
<td>Social media</td>
<td>3.90 (1.57)</td>
</tr>
<tr>
<td></td>
<td>Printed media</td>
<td>4.14 (1.43)</td>
</tr>
<tr>
<td>Interpersonal</td>
<td>Health workers</td>
<td>2.85 (1.46)</td>
</tr>
<tr>
<td></td>
<td>Friends and family</td>
<td>2.72 (1.33)</td>
</tr>
</tbody>
</table>
Anti-rabies campaign

- Listserv: International students listserv & Chinese Student Association listserv
- Brief talk in an ELP Class
- Facebook group: Jardine Apartment
  - Digital image
  - Web-page
  - GARC Video “Bat and rabies”

What is Rabies?
Rabies is a virus that infects the brain and spinal cord. Rabies almost always leads to death if the infected person receives no medical treatment. Rabies can be prevented if treatment is given before symptoms appear.

How Does It Spread?
Rabies spreads when an infected animal bites or scratches a human or another animal. You can also get it from contact with the saliva of an infected animal.

The known sources of rabies in the U.S. are:
- Dogs
- Cats
- Ferrets
- Raccoons
- Skunks
- Foxes
- Coyotes
- Bats

If you see the animal with these unusual behaviors, they are more likely to have rabies:
- Unable to fly, flying during day time
- Making a lot of noise or otherwise acting sick

However, an infected animal may transfer the virus before they show the symptoms.

Public Health Services:
The Lafene Health Center
785-532-4544

The Riley County Health Department
785-776-4779

Kansas Department of Health and Environment
785-296-1059
during normal business hours or epidemiology hotline
1-877-427-7317 for immediate assistance.

You Can’t Always Tell If You’ve Been Bitten
Unlike other animals, bats have very small teeth. So, it’s possible to be bitten and not know it, and a bite may not leave a clear mark. Treatment to prevent rabies may be needed if a bat is found in the room with anyone who is:

- Sleeping
- An unattended child
- Mentally or physically challenged
- Intoxicated
Program Evaluation

• Online-based campaign (challenging to get feedback)
  • Learned something new
  • Easy to understand (picture and written text)

• In class presentation (anonymous close-ended questionnaire)
  • Learned something new
  • Information important
  • Sharing the information could be beneficial to their friends and families
Conclusion and limitation

• Anti-rabies communication strategies:
  • Knowledge and Risk perception

• Health seeking behavior:
  • Social media and printed media
MPH Core Area Competencies

✓ Biostatistics
  • Data interpretation and analysis

✓ Environmental Health
  • Environmental factors that determine disease

✓ Epidemiology
  • Distribution of disease in populations and associated risk factors

✓ Administration of Health Care Organizations
  • Management of health care system (government, private, NGOs)

✓ Social and Behavior Science
  • Social determinants and human behavior that contribute to population health, health communication
MPH Courses

- MPH 754  Introduction to Epidemiology  3  F2016
- MPH 802  Environmental Health  3  F2016
- MPH 701  Fund Methods of Biostatistics  3  F2016
- MPH 818  Social/Behavioral Bases of Public Health  3  S2017
- MPH 720  Admin of Health Care Organization  3  S2017
- DMP 815  Multidisciplinary Thought/Presentation  3  S2017
- DMP 854  Intermediate Epidemiology  3  S2017
- DMP 770  Emerging Disease  3  Su2017
- DMP 880  Problem in Pathobiology  3  F2017
- MC 750  Strategic Health Communication  3  F2017
- DMP 899  Master’s Research  6  F2017&S2018
- DMP 705  Principal of Vet Immunology  3  S2018
- MPH 840  Field Experience  3  S2018

Total MPH Program Credits : 42
Thank you!!

• **STEC CAP Internship**
  Dr. Natalia Cernicchiaro
  Leigh Feuerbacher, Neil Wallace, Joaquin Baruch

• **Rabies Field Experience**
  Dr. Susan Moore (KSU Rabies Laboratory)
  KSU International Students

• **Committee members**
  Dr. Robert Larson
  Dr. Justin Kastner
  Dr. Michael Sanderson

• **MPH Office**
  Dr. Ellyn Mulcahy
  Barta Stevenson
“Thank you”
References


References


Questions?
Contamination at feedlot

• Cattle have high tolerance to STEC infection (Gb3 receptor)
• Present in intestine
• STEC persistence in environment (low moisture, lower temperature)
• Houseflies, wild animals, water troughs, feed, super shedder
• Picture speaks better
Slaughter plant

• Cattle arrival
• Hide removal
• Decontamination after hide removal
• Evisceration
• Second decontamination
• Chilling
• Carcass fabrication (trim as a byproduct vs trim as the major product)