

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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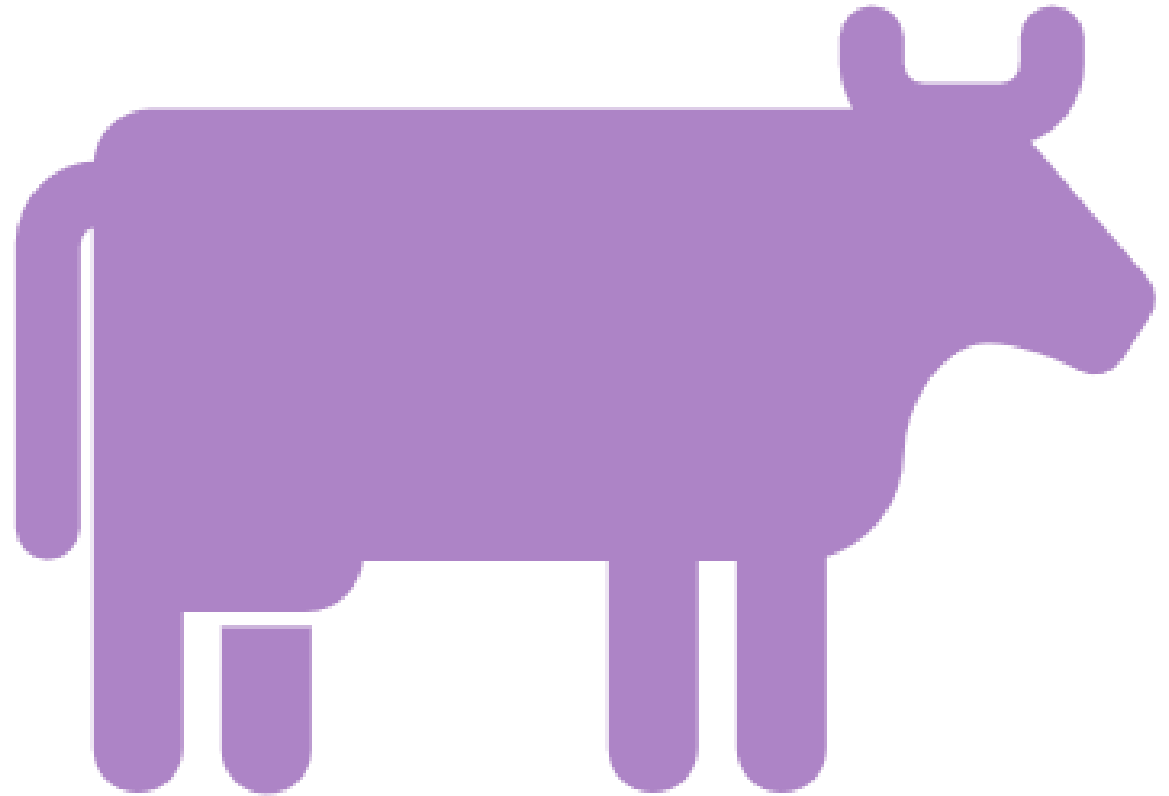


Overview

- Part 1: Master's Thesis
- Part 2: Field Experience

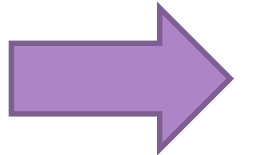
STEC Contamination in Post Harvest Cattle

PART 1: Master's Thesis



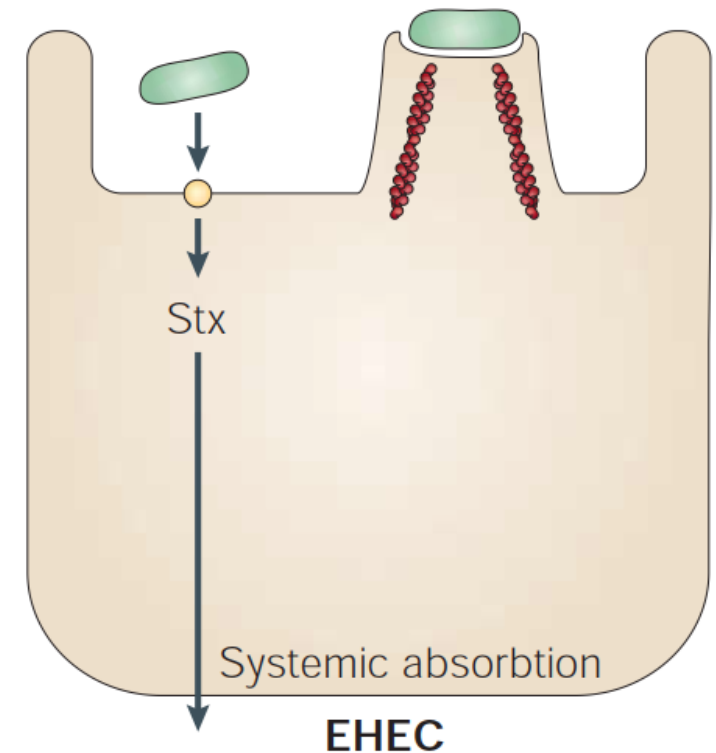
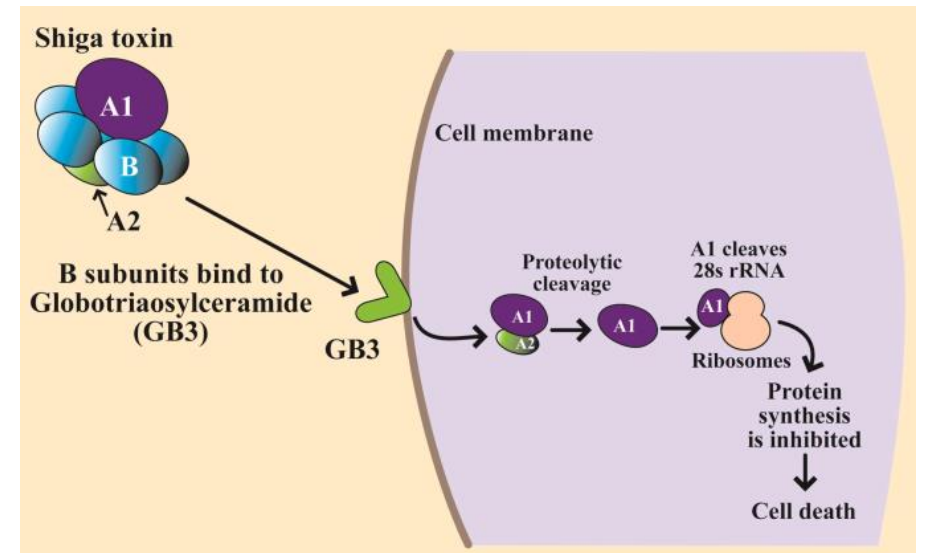
Objective

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

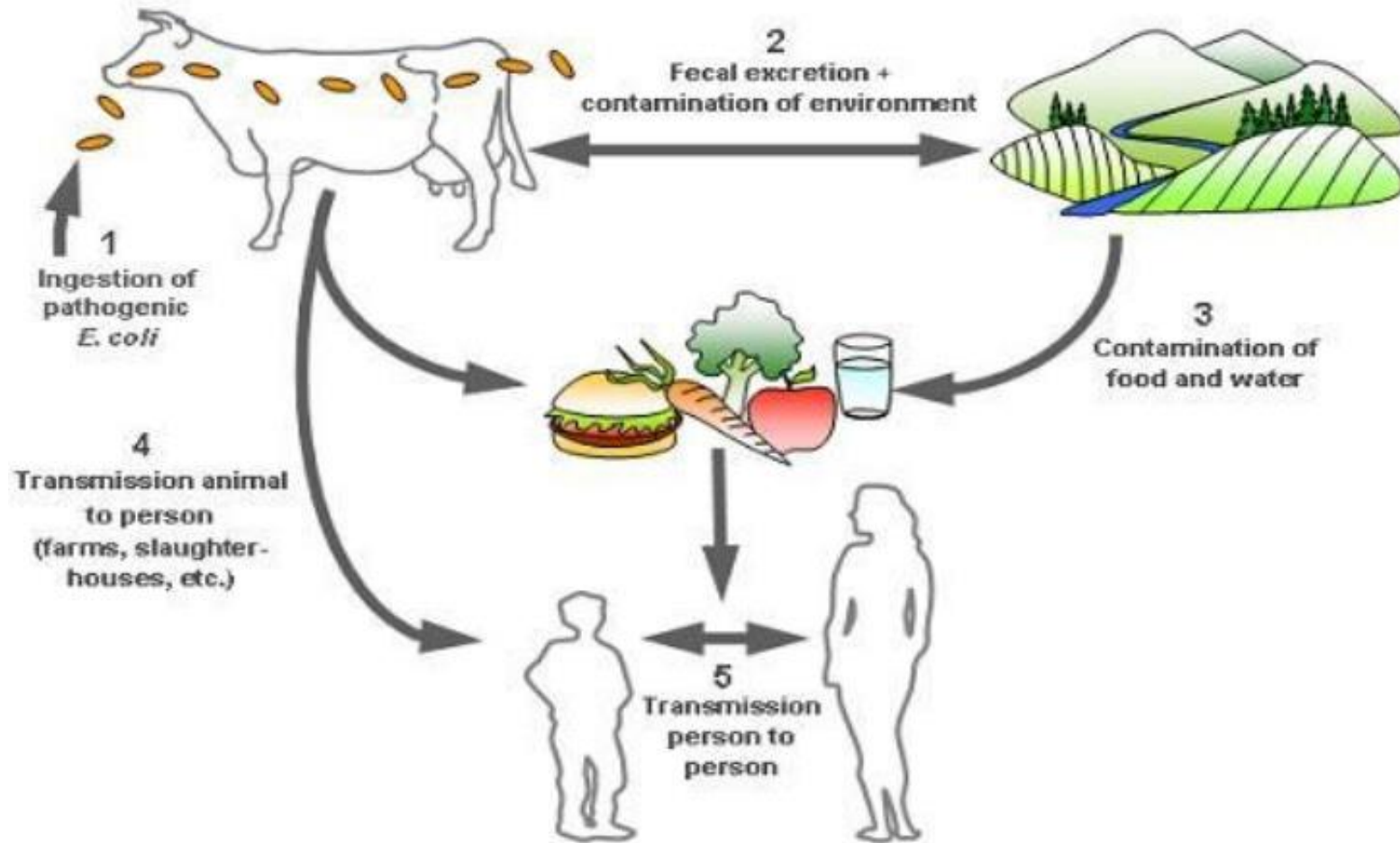


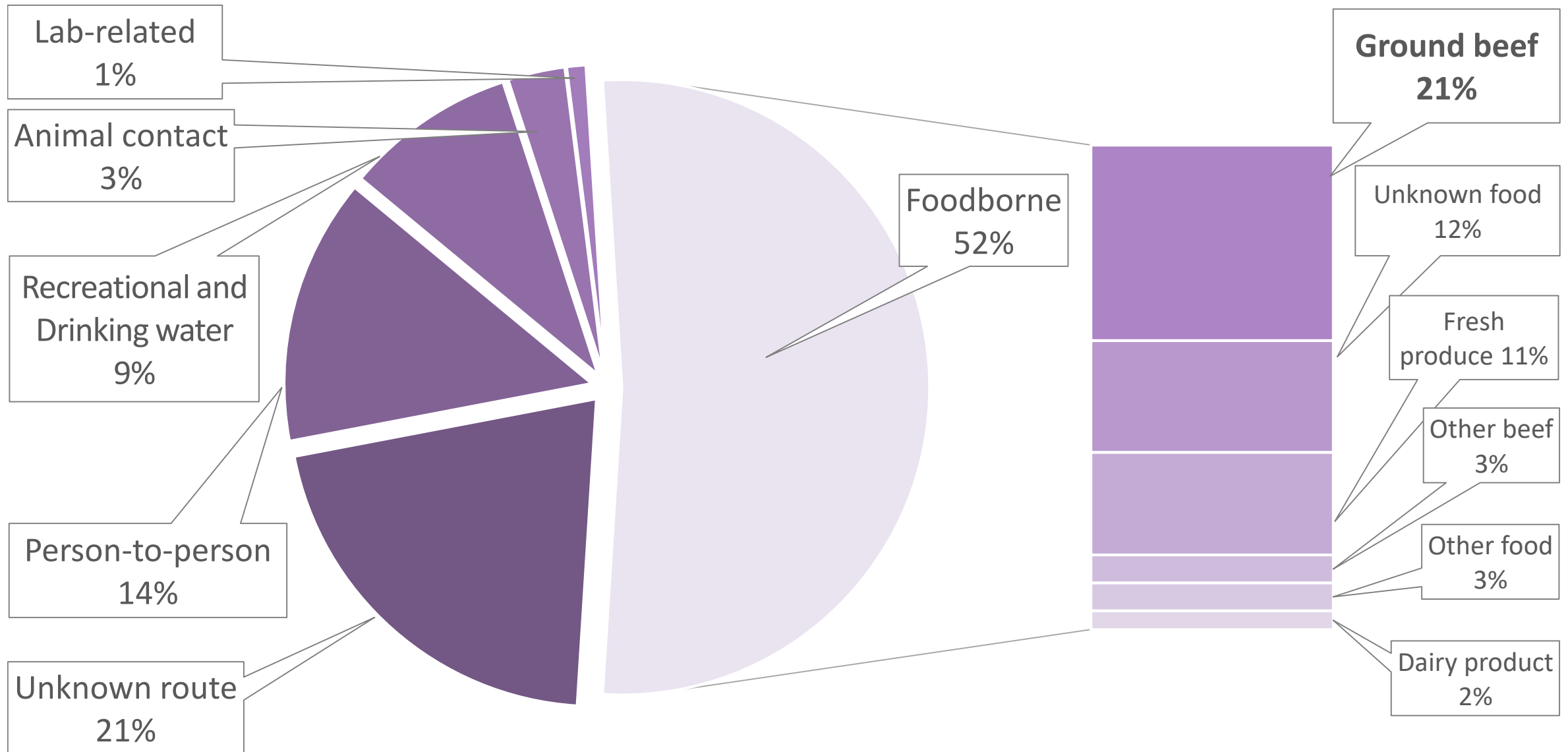
E. coli, STEC, and EHEC

- *Escherichia coli*
 - Normal inhabitants of the GI tract
 - Beneficial to their host
- STEC = *E. coli* that produce Shiga toxin (*stx*)
 - *stx1* and/or *stx2*
 - *stx2* 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





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**

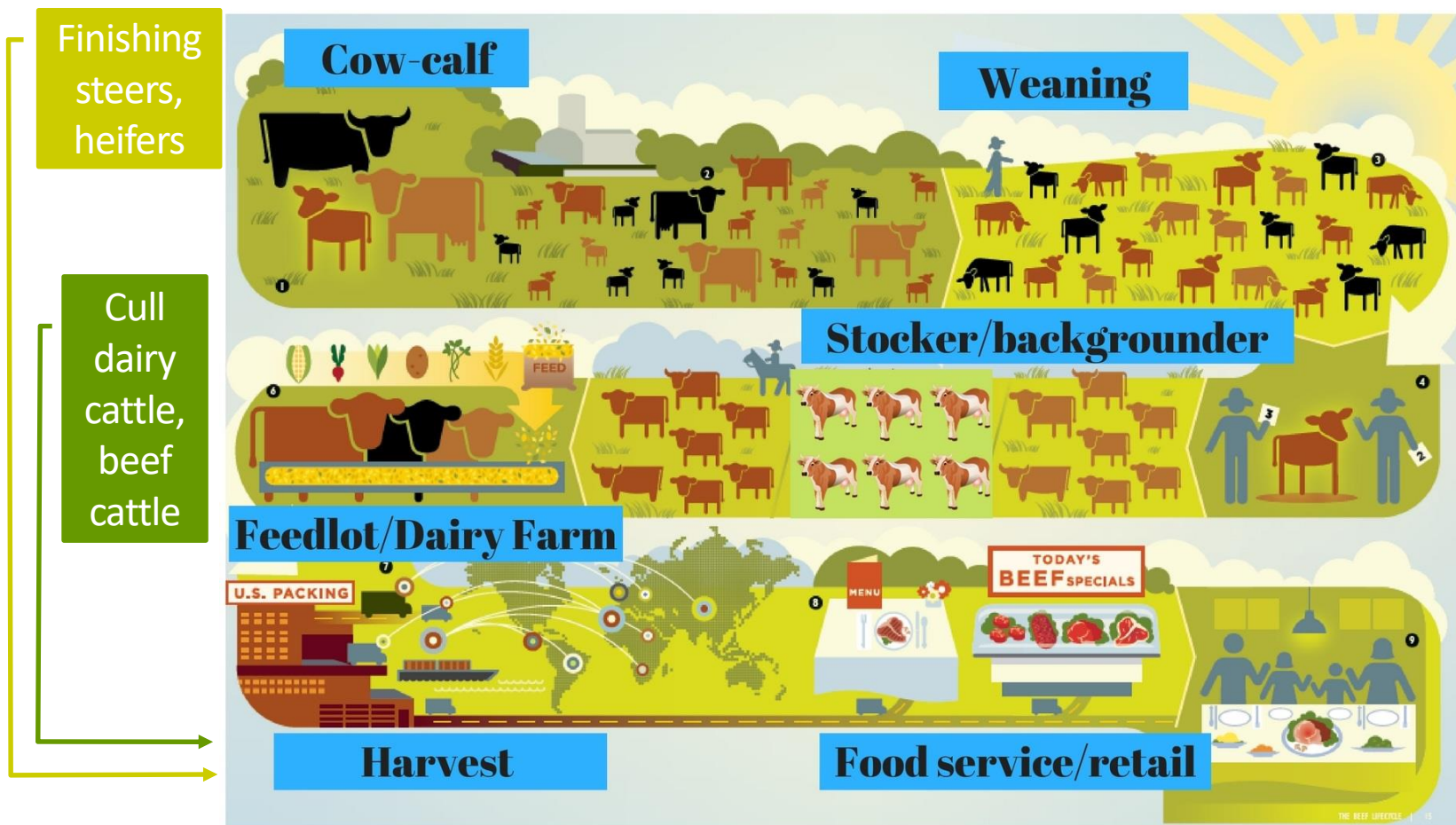
Human STEC infection in the United States

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

	STEC O157	STEC non-O157
Human cases	63,153	112,752
Hospitalizations	2,138	217
Deaths	20	0

- 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

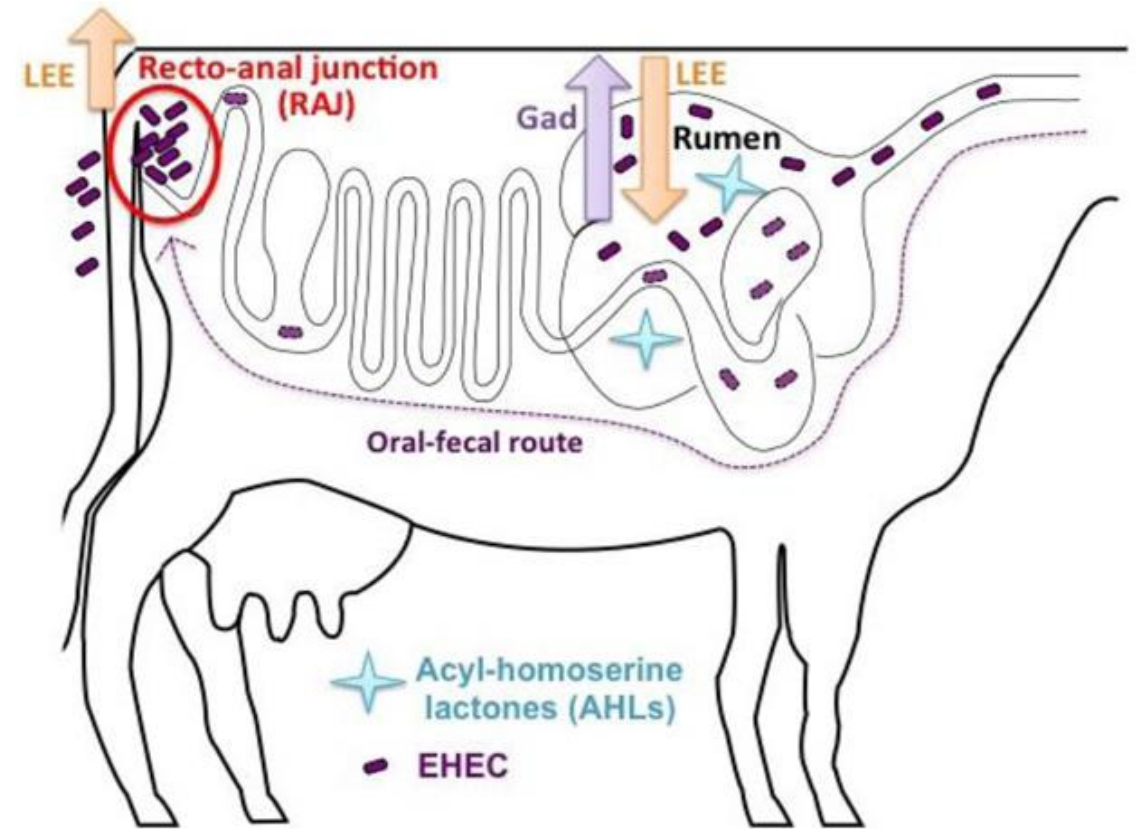
Cull dairy cattle in U.S. beef production



- 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

STEC Identification Methods (Prevalence)

- 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 → agglutination → spot-indole → PCR
- Tested for non-O157 → PCR

Table 1. Characteristic of the study population

Plant ID	Date of collection	Number of samples collected	Plant capacity (cattle/day)
A	August 14, 2017	62	1,000
B	August 21, 2017	59	1,500
C	Sept 18, 2017	62	450-470
	Total # of samples	183	

Table 2. Cumulative prevalence of EHEC O157 and non-O157 serogroups by processing plant

Plant ID	Sample size (N)	Prevalence % (n/N)	
		O157	non-O157 ^a
A	62	3.2 (2/62)	0.0 (0/60) ^b
B	59	1.7 (1/59)	25.4 (15/59)
C	62	3.2 (2/62)	6.5 (4/62)
TOTAL	183	2.7 (5/183)	10.5 (19/181)

^aEach of positive sample corresponds to have at least one non-O157 somatic antigen.

^b2 samples could not be processed by the IMS assay.

Table 3. Distribution of EHEC O157 and non-O157 by processing plant

Plant ID	O157	Non-O157
A	O157, stx1, stx2, eae, ehxA, fliCH7 (2)	-
B	O157, stx2, eae, ehxA, fliCH7 (1)	O103, stx1, eae (12) O111, stx1, eae (3)
C	O157, stx2, eae, ehxA, fliCH7 (1) O157, stx1, eae, ehxA, fliCH7 (1)	O26, stx1, eae (1) O103, stx1, eae (2)

Table 4. Percentage of identified EHEC by serogroup

Serogroup	O157	O103	O111	O26
Prevalence % (n/N)	2.75% (5/ 183)	7.65 % (14/181)	1.64% (3/181)	0.66% (1/181)

Table 5. Number of quantifiable samples and concentration in CFU/g feces of non-O157* serogroups in pre-enriched samples

Plant ID	Sample size	Number of quantifiable fecal samples		
		< 500 CFU/g	500 < n < 10 ⁴ CFU/g	≥ 10 ⁴ CFU/g
A	62	N/A	N/A	N/A
B	59	N/A	N/A	N/A
C	62	2	1	0

*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)



A bat caught in Utah - Utah Division of Wildlife Resources

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



I know that raccoon, skunk, and/or bat live in Kansas



I have seen a raccoon, skunk, and/or bat in Kansas



I have seen a raccoon, skunk, and/or bat in my neighborhood in Manhattan

I have seen a raccoon, skunk, and/or bat inside my residence (e.g. apt, dorm, or house) in Manhattan

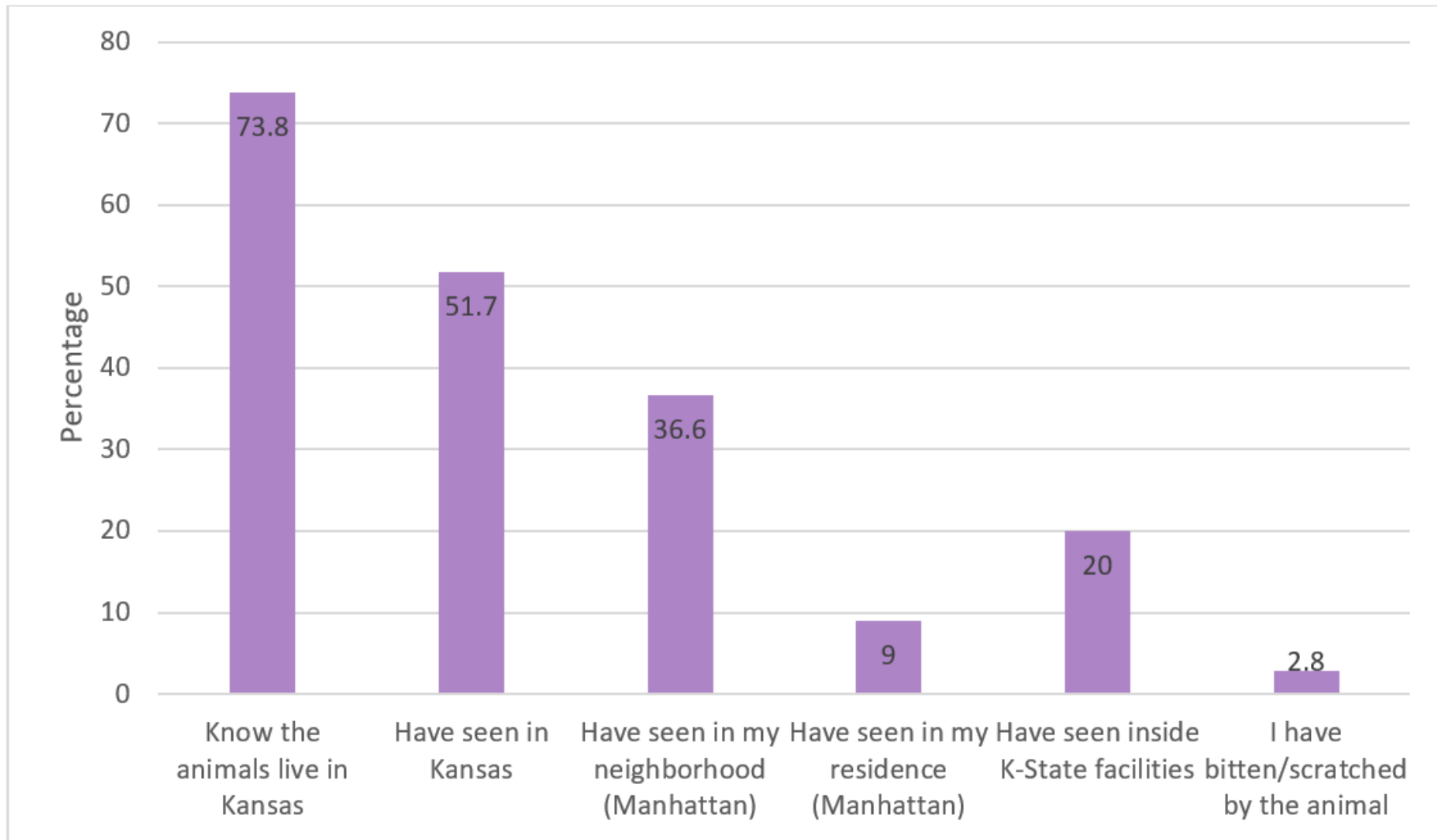
I have seen a raccoon, skunk, and/or bat inside K-State facilities (e.g. Rec center, library, campus/laboratory buildings)

During my study in the United Study, I have bitten/scratched by a raccoon, skunk, and/or bat

Yes

No





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)

Rabies is a bacteria that is transferred by animals to humans

- ☐ True
- ☐ False
- ☐ Don't know

If it is not treated, rabies almost always causes death to humans

- ☐ True
- ☐ False
- ☐ Don't know

Humans can get rabies through the bite of an infected animal

- ☐ True
- ☐ False
- ☐ Don't know

If a rabies infected animal licks or scratches a person, the person can be infected with rabies

- ☐ True
- ☐ False
- ☐ Don't know

KAP Survey Findings

- **Risk perception:**
Participants' judgment about the likelihood of rabies exposure
- 44.1% had higher perception of risk towards rabies exposure

If you wake up with a bat in the same room you were sleeping in, how likely are you to be exposed to rabies?

- ☐ Very unlikely
- ☐ Unlikely
- ☐ Likely
- ☐ Very likely

If you find a bat in a room with an unattended child, a disabled or intoxicated person, how likely is she/he to be exposed to rabies?

- ☐ Very unlikely
- ☐ Unlikely
- ☐ Likely
- ☐ Very likely

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

Q11

Click to write the question text



If I see a skunk in my residence, I will catch it and then release the skunk outside the building

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

If a bat landed on me, I will do nothing if I don't get any serious wounds

If I get bitten by a skunk, I will clean up the wounds and consult a doctor/nurse as soon as possible

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

It's important to get assistance from animal control department if I see a bat inside my residence

Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree
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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)

Channel	Variables	Mean (SD)
Mass Media	Website of official institutions	3.62 (1.69)
	Other websites	3.23 (1.45)
	Social media	3.90 (1.57)
	Printed media	4.14 (1.43)
Interpersonal	Health workers	2.85 (1.46)
	Friends and family	2.72 (1.33)

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”](#)

Rabies in the United States

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.

Anxiety.
Aggressiveness.
Seizures, stumbling, and vocalizing.



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

What To Do If You Are Exposed to Rabies?

Anyone who had contact with an infected animal or its saliva is at risk of getting rabies. This includes bites, scratches, or any other bare skin contact with the animal or its saliva.



1. Wash the bite, scratch, or contact area immediately with soap and water for at least 10 minutes.
2. Seek medical attention immediately. Contact your health care provider or Public Health service for guidance about the treatment.
3. If you think your pet was exposed to rabies, call your veterinarian.

Public Health Services:

The Lafene Health Center
• **785-532-6544**

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”



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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)