USDA
Food Safety and Inspection Service
STEC Update

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Outline

- FSIS STEC Sampling
- Beef Veal Carcass Baseline Study
- Further Characterization of Strains
- Research Needs

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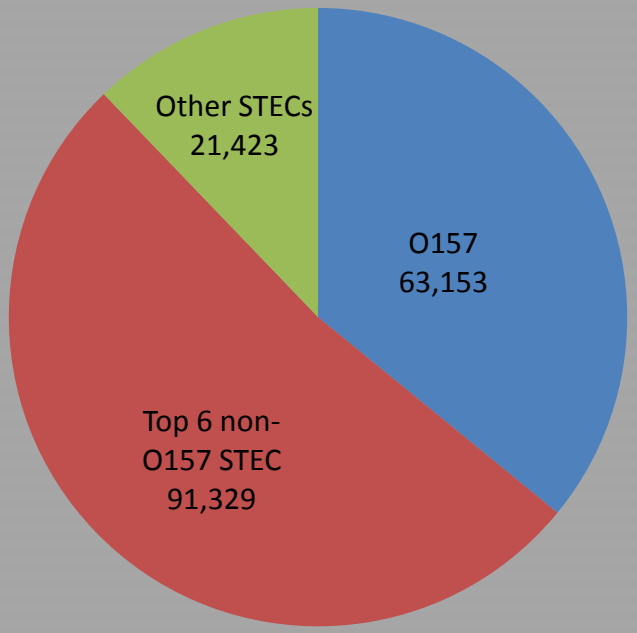
Focus on STECs

- In 1994 FSIS began testing raw beef products for *E. coli* O157:H7.
- Non-intact beef products containing *E. coli* O157:H7 were declared to be adulterated.
- In 2012 FSIS announced that the presence of additional shiga toxin-producing *E. coli* strains (STEC) in raw beef trim would render that product adulterated.
- On June 4, 2012 FSIS began sampling beef manufacturing trimmings for the 'top 6' non-O157 STECs
  - O-groups: O26, O45, O103, O111, O121, and O145
  - Strain must also include the virulence genes *eae* and *stx*

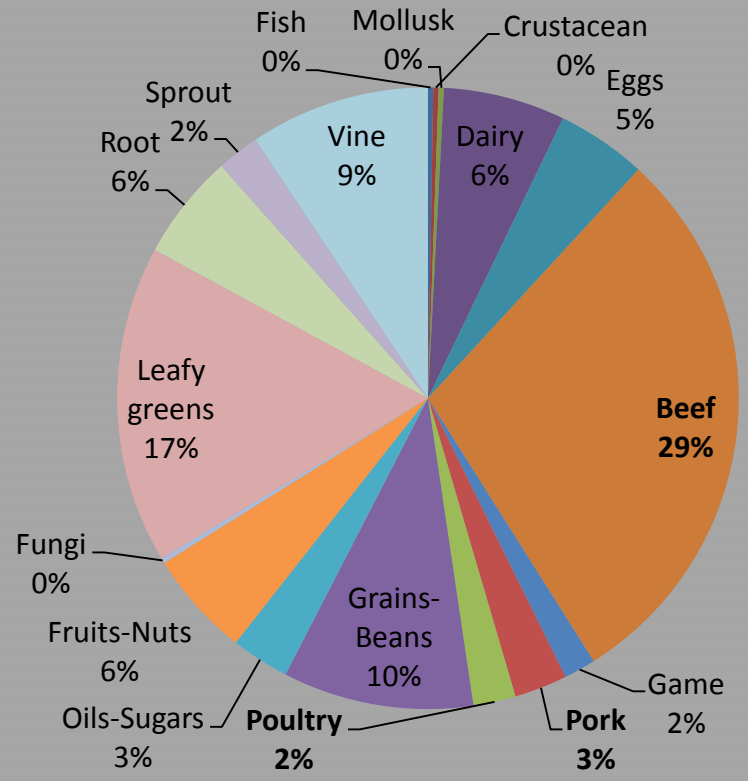
Focus on STECs

Foodborne related illnesses associated with STEC



Adapted from Scallan, *et al.,* 2011
Data collected from 2000-2008

STEC attribution to commodity



Adapted from Painter, *et al.,* 2013
Data collected from 1998-2008

STEC Test Results

(June 4, 2012 through April 27, 2014)

Raw Ground Beef Components (RGBC)			
Serotype	**Trim Verification**	**Follow-up to RGB Positive at Supplier**	**Follow-up to RGBC Positive**
***E. coli* O157:H7**	**0.42% (22/5249)**	**0.81% (5/618)**	**0.54% (8/1476)**
Total non-O157 STECs	**0.70% (34/4887)**	**1.42% (7/492)**	**2.03% (26/1279)**
Number of non-O157 STECs isolated from positive samples			
O26	9	1	11
O45	1	0	3
O103	18	2	21
O111	6	2	1
O121	0	0	0
O145	2	2	2

Beef/Veal Breakdown

Raw Beef Trim				
Source	**Serotype**	**Trim Verification**	**Follow-up to RGB Positive at Supplier**	**Follow-up to RGBC Positive**
Beef	***E. coli* O157:H7**	**0.37% (19/5162)**	**0.19% (1/537)**	**0.16% (2/1247)**
	Total non-O157 STECs	**0.58% (28/4808)**	**0.47% (2/425)**	**0.81% (9/1116)**
	Number of non-O157 STECs isolated from positive samples			
	O26	9	1	4
	O45	0	0	0
	O103	16	1	5
	O111	4	0	0
	O121	0	0	0
	O145	1	0	0
Veal	***E. coli* O157:H7**	**3.57% (3/84)**	**4.94% (4/81)**	**2.64% (6/227)**
	Total non-O157 STECs	**7.89% (6/76)**	**7.46% (5/67)**	**10.43% (17/163)**
	Number of non-O157 STECs isolated from positive samples			
	O26	0	0	7
	O45	1	0	3
	O103	2	1	16
	O111	2	2	1
	O121	0	0	0
	O145	1	2	2

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Purpose

- To gain information on the effectiveness of sanitary dressing post-hide removal and slaughter interventions pre-chill in beef/veal slaughter establishments;
- To provide process control criteria for beef/veal slaughter establishments;
- To estimate the prevalence and quantitative level of pathogenic organisms;
- To estimate the presence and quantitative levels of indicator organisms

Sample Collection Details

- Approximately, 4000 samples will be collected during both shakedown and the actual study from 133 beef and veal slaughter establishments.
- Swabs are being collected from the carcasses of all subclasses:
  - Beef (steers, heifers, cows, bulls, stag, and dairy cows)
  - Veal (heavy calves, bob veal, non-formula fed veal and formula fed veal)

Sampling Locations & Microbiological Targets

- Samples are being collected at two locations in the slaughter process:
  - "Post-hide removal": (immediately after hide removal, before evisceration, and prior to the application of any interventions)
  - "Pre-chill": at least 1 to 5 minutes after the last intervention has been applied and no more than one hour in the hotbox)
- When possible "Post-hide" and "Pre-chill" samples will be taken from leading and lagging sides of the same carcass
- Microbiological targets include:
  - Pathogens - *E. coli* O157:H7, non-O157 Shiga-toxin producing *E. coli* (STEC) and *Salmonella*
  - Indicator organisms - Generic *E. coli,* Total Aerobic Bacteria, Enterobacteriaceae and coliforms

Preliminary Shakedown Data

- Samples scheduled 798 (399 at Post-HR and 399 at Pre-Chill)
- Samples collected 664 (332 at Post-HR and 332 at Pre-Chill)
- Samples analyzed 620 (310 at Post-HR and 310 at Pre-Chill)

Distribution of Samples by Beef Subclass

Beef Subclasses	Samples by Subclass	Percent of Total Samples
Beef Carcasses		
Cow	38	12.3%
Steer	110	35.5%
Bull	9	2.9%
Dairy Cow	61	19.7%
Heifer	51	16.5%
Veal Carcasses		
Heavy Calf	9	2.9%
Non-formula fed Veal	1	0.3%
Formula-fed Veal	14	4.5%
Bob Veal	17	5.5%
Total	310	100%

Salmonella Data

Post-hide Removal

- *Salmonella* Percent Positive (60/309) 19.42%
- Serotype distribution

Serotype	Count	Percent
Montevideo	12	30.0%
Anatum	6	15.0%
Mbandaka	5	12.5%
Newport	4	10.0%
Agona	2	5.0%
Kentucky	2	5.0%
Muenchen	2	5.0%
Muenster	2	5.0%
Typhimurium	2	5.0%
Altona	1	2.5%
I 4,[5],12:i:-	1	2.5%
Infantis	1	2.5%
Total	40	100%

Pre-chill

- *Salmonella* Percent Positive (10/310) 3.22%
- Serotype distribution

Serotype	Count	Percent
Newport	2	22.2%
Typhimurium	2	22.2%
Agona	1	11.1%
Heidelberg	1	11.1%
Kentucky	1	11.1%
Montevideo	1	11.1%
Not Reportable	1	11.1%
Total	9	100%

Positive Results

Table 1. BVCBS Positive Sample Results

	Number of samples (includes both Post-hide and Pre-chill	Number of Post-hide removal samples	Number of Pre-chill samples
Total Number of Analyzed Samples	620	310	310
Positive for Salmonella	60	51	9
Positive for E. coli O157:H7	2	2	0
Positive for non-O157 STEC	25	16	9
Positive for both Salmonella and E. coli O157:H7	0	0	0
Positive for both Salmonella and non-O157 STEC	9	8	1
Positive for both E. coli O157:H7 and non-O157 STEC	0	0	0
Positive for Salmonella, E. coli O157:H7, and non-O157 STEC	1	1	0

Table 2: Breakdown of BVCBS Positive STEC Serogroups

Month	Project	*E. coli* Serogroups							
		O157:H7	O26	O45	O103	O111	O121	O145	TOTAL
January	S52_PSTHR	2	1		2	1			6
	S52_PRECH					1			1
February	S52_PSTHR	1	4		4	2	1		12
	S52_PRECH		2		1	2			5
March	S52_PSTHR		7		7	1		1	16
	S52_PRECH		1		3				4
TOTALS	S52_PSTHR	3	12	0	13	4	1	1	34
	S52_PRECH	0	3	0	4	3	0	0	10
GRAND TOTALS		3	15	0	17	7	1	1	44

Beef/Veal Breakdown

Subclass	Post-Hide Removal Samples									
	Salmonella			*E. coli* O157:H7			non-O157 STECs			
	Number of Positives	Number Analyzed	Percent Positive	Number of Positives	Number Analyzed	Percent Positive	Number of Positives	Number Analyzed	Percent Positive
Beef Carcasses										
Beef Cow	10	38	26.3%	0	38	0.0%	3	38	7.9%
Bull	0	9	0.0%	0	9	0.0%	2	9	22.2%
Dairy Cow	24	61	39.3%	0	61	0.0%	3	61	4.9%
Heifer	7	51	13.7%	0	51	0.0%	3	51	5.9%
Steer	14	110	12.7%	2	110	1.8%	6	110	5.5%
Total Beef	**55**	**269**	**20.4%**	**2**	**269**	**0.7%**	**17**	**269**	**6.3%**
Veal Carcasses									
Bob Veal	4	17	23.5%	0	17	0.0%	4	17	23.5%
Formula-fed Veal	0	14	0.0%	0	14	0.0%	3	14	21.4%
Heavy Calf	1	9	11.1%	1	9	11.1%	0	9	0.0%
Non-formula fed Veal	0	1	0.0%	0	1	0.0%	1	1	100.0%
Total Veal	**5**	**41**	**12.2%**	**1**	**41**	**2.4%**	**8**	**41**	**19.5%**

Beef – Veal Breakdown

Subclass	Pre-Chill Samples									
	Salmonella			*E. coli* O157:H7			non-O157 STECs			
	Number of Positives	Number Analyzed	Percent Positive	Number of Positives	Number Analyzed	Percent Positive	Number of Positives	Number Analyzed	Percent Positive
Beef Carcasses										
Beef Cow	1	38	2.6%	0	38	0.0%	0	38	0.0%
Bull	0	9	0.0%	0	9	0.0%	0	9	0.0%
Dairy Cow	3	61	4.9%	0	61	0.0%	1	61	1.6%
Heifer	0	51	0.0%	0	51	0.0%	0	51	0.0%
Steer	2	110	1.8%	0	110	0.0%	4	110	3.6%
Total Beef	**6**	**269**	**2.2%**	**0**	**269**	**0.0%**	**5**	**269**	**1.9%**
Veal Carcasses									
Bob Veal	3	17	17.6%	0	17	0.0%	3	17	17.6%
Formula-fed Veal	1	14	7.1%	0	14	0.0%	2	14	14.3%
Heavy Calf	0	9	0.0%	0	9	0.0%	0	9	0.0%
Non-formula fed Veal	0	1	0.0%	0	1	0.0%	0	1	0.0%
Total Veal	**4**	**41**	**9.8%**	**0**	**41**	**0.0%**	**5**	**41**	**12.2%**

Timeline

- Shakedown is complete
  - Started 2nd Quarter FY2014 (January)
  - Ended April 3, 2014
- Actual Study start date
  - 4th Quarter FY2014 (July)
  - Will last for 12 months

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Research Objectives

- Identification of STECs in beef including serogroups that are not screened for during routine FSIS testing,
- Identification of co-occurrence of FSIS adulterant STECS and/or potentially pathogenic STECs in beef and
- Identification of virulence factors associated with FSIS adulterant STECS and/or potentially pathogenic STECs in beef

Procedures

- Enrichment broths of samples collected during routine FSIS sampling of beef products were blinded and sent to ARS US Meat Animal Center (USMARC) in Clay Center
  - Portions of the *E. coli* O157:H7 BAX® MP screen-positive raw beef (ground and trim) enrichment broths collected prior to the start of the non-O157 STEC testing program
  - Portions of the *stx* BAX® Real-time PCR Screening positive raw beef (ground and trim) enrichment broths collected after the start of the non-O157 STEC Testing programs
  - BAX negative raw beef samples enrichment broths were also sent to ARS.

Procedures

- ARS analyses include screening the enrichment broths for:
  - FSIS adulterant STECs,
  - Shiga toxin genes (*stx*),
  - intimin genes (*eae*)
- ARS also attempted to culture suspected STECs from enrichments that screened positive (for any of the above). Cultured isolates analyzed for:
  - O group
  - Shiga toxin genes (*stx*),
  - intimin genes (*eae*),
  - potential virulence factors

Results (To Date)

- ARS analyzed 369 broths submitted by FSIS.
  - 206 O157:H7 screen positive by FSIS
  - 163 O157:H7 screen negative by FSIS

- When ARS analyzed these broths, sixty eight of these broths (18%) screened positive for O157:H7 (BAX® MP).

Results (To Date)

- Two broths contained non-O157 adulterants STECs (one with O45 and a second with O103).
  - These adulterants possessed both the Shiga toxin gene (*stx*+) and intimin adherence gene (*eae*+).
  - The broth containing the O45 adulterant screened BAX® MP positive.
  - The broth containing the O103 adulterant screened BAX® MP negative.
  - No other adulterant STECs were culture confirmed from these broths.

Results (To Date)

- An additional non-adulterant STEC containing both the Shiga toxin gene (*stx*+) and intimin gene (*eae*+) was isolated from each of two other broths.
  - These isolates were identified as O5 and O74.
  - No adulterant *E. coli* were identified in either broth.
- Twenty broths contained non-O157 *E. coli* with Shiga toxin genes (*stx*+) but no intimin (*eae*-).
- Co-occurrence of O157:H7 and non-O157 adulterant STECs was not confirmed in any broths.
- Twenty broths contained non-O157 *E. coli* with Shiga toxin genes (*stx*+) but no intimin (*eae*-).

Preliminary Conclusions

- Potentially pathogenic STECs other that the current list of FSIS adulterant STECs are present in raw beef.
  - If these STECs are associated with severe human illness, then FSIS may consider expanding its list of adulterants STECs.
- The presence of an adulterant STEC can not be positively correlated to the presence of other pathogenic STECs.
  - The lack of consistent co-occurrence of STECs suggests that it may not be possible to identify a subset of STECs that can be used as indicator organisms for the entire suite of potentially pathogenic STECs.

Future

- Following the analysis of 1000 FSIS beef broths by ARS, the associated data will be analyzed. Possible conclusions include:
  - Additional research on this topic may be warranted
  - Publication in peer-reviewed journal
  - Changes to Agency approaches for the analysis of adulterant STECs in beef may be warranted.
  - Refinement of FSIS adulterant STEC definition

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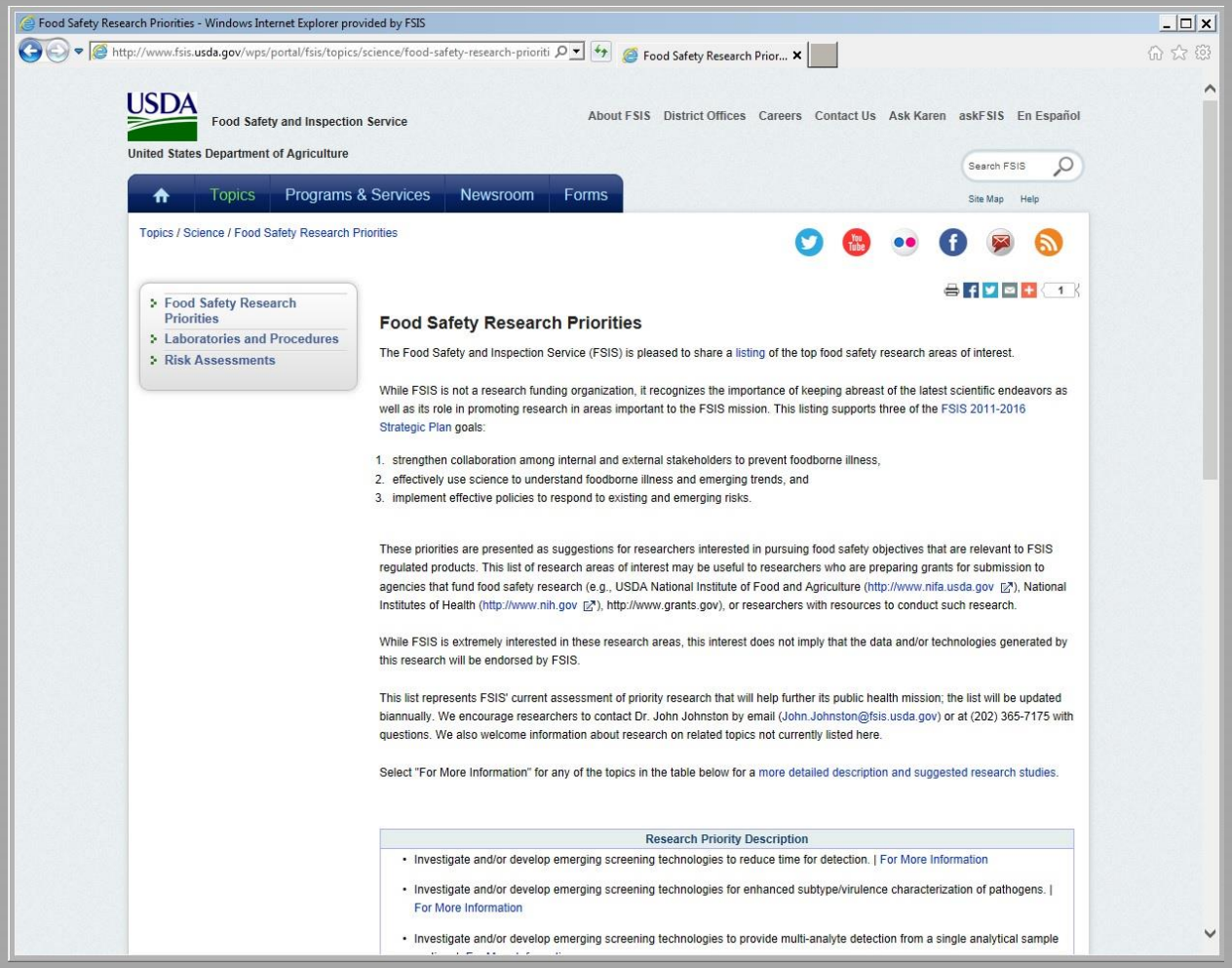
FSIS Research Priorities



http://www.fsis.usda.gov/wps/portal/fsis/topics/science/food-safety-research-priorities

Relevant FSIS Research Priorities

- Development of pathogen detection methodologies: Screening, enumeration, and characterization
  - Investigate and/or develop emerging screening technologies to reduce time for detection.
  - Investigate and/or develop emerging screening technologies for enhanced subtype/virulence characterization of pathogens.
  - Investigate and/or develop emerging screening technologies which are applicable to FSIS regulated products (meat, poultry, egg products and foods containing these products).
  - Develop or refine testing methods for *quantifying* target pathogens in meat, poultry and egg products.
  - Identify and evaluate alternative approaches to N60 sampling.
  - Identify and/or develop emerging technologies for real-time testing for higher levels of contamination prior to slaughter.
- Develop and evaluate interventions: Pre-harvest and at slaughter
  - Evaluate the potential effectiveness of pre-harvest interventions on finished products.
  - Determine the effectiveness of parallel and/or simultaneous application of more than one pre-harvest and/or post-harvest intervention as a control strategy.
  - Determine (validate) the effectiveness (log-reduction) of interventions used by industry to reduce levels of pathogens on FSIS regulated products.
  - Identify and/or develop pre- and post-harvest interventions to reduce levels of pathogens and chemical hazards for each class of veal (bob veal, non-formula-fed, formula-fed, and heavy calves).
- Conduct *ex post* evaluation of regulatory initiatives. Determine the presence and contributing factors for antimicrobial resistant strains in poultry and cattle.

Questions?