Tackling the problems of STEC and *Salmonella* in beef

Dr. Mick Bosilevac, PhD

United States Department of Agriculture – Agricultural Research Service

US Meat Animal Research Center, State Spur 18D

Clay Center, NE 68933

mick.bosilevac@usda.gov

+1 (402) 762-4225

USDA

RIBMINS

Workshops and Working Group meetings

University of Copenhagen Frederiksberg Campus

Copenhagen, Denmark, 8 November 2019

Meat Safety and Quality Research Unit

Focus on control, prevention and detection of foodborne pathogens entering the meat chain

In Animals

During Processing

In Finished Products

and at points before, between, and after

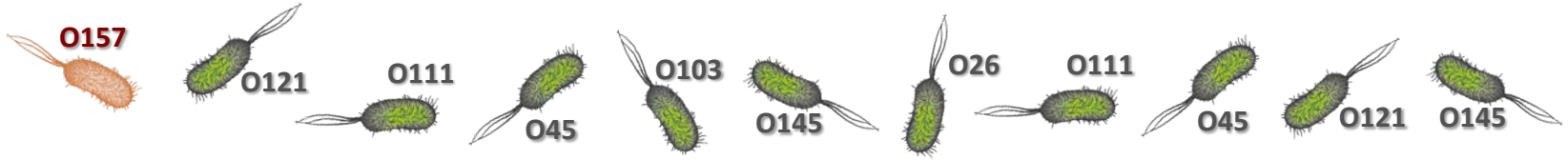
Meat Safety and Quality Research Unit

Focus on control, prevention and detection of foodborne pathogens entering the meat chain

In Animals

During Processing

Shiga Toxin-Producing *E. coli* in Red Meat Animals



Transfer rates of non-O157 STEC from meat animals during Hide/Pelt/Skin removal

	Percent of non-O157 STEC (by *stx* PCR)	
Species	Surface[a]	Pre-evis
Bovine[b]	92.0	96.6
Ovine[c]	82.6	78.6
Swine[d]	99.8	17.6

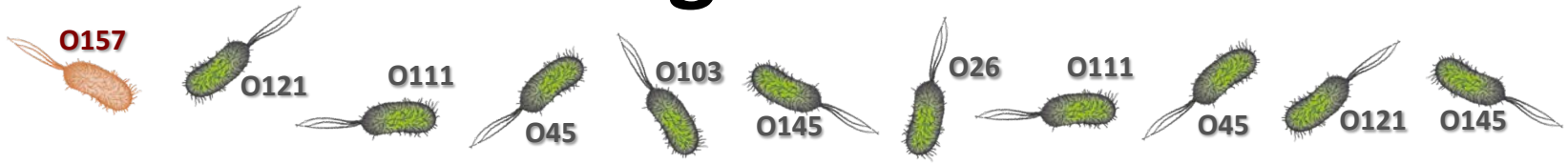
[a] Surface refers cattle hide, pelt,, and pork skin before removal, scalding, or flaming.

[b] Adapted from Barkocy-Gallagher et al ., 2003.

[c] Adapted from Kalchayanand et al., 2007.

[d] Bosilevac, 2011, personal communication.

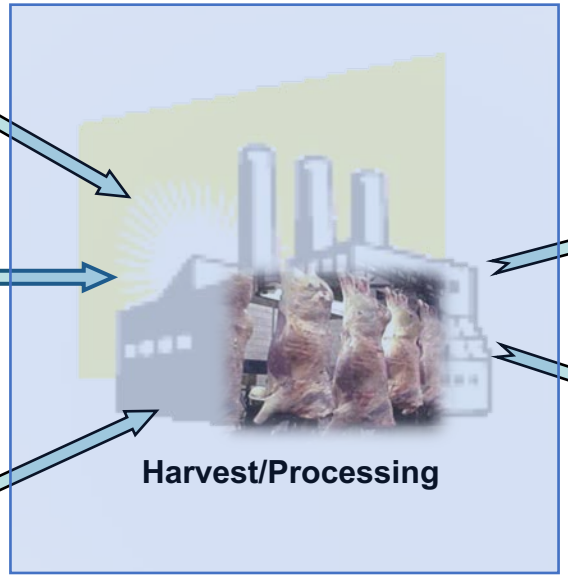
Shiga Toxin-Producing *E. coli* in the Beef Chain



Dairy
-Cull cows
-Veal calves

Production
-Cull cows

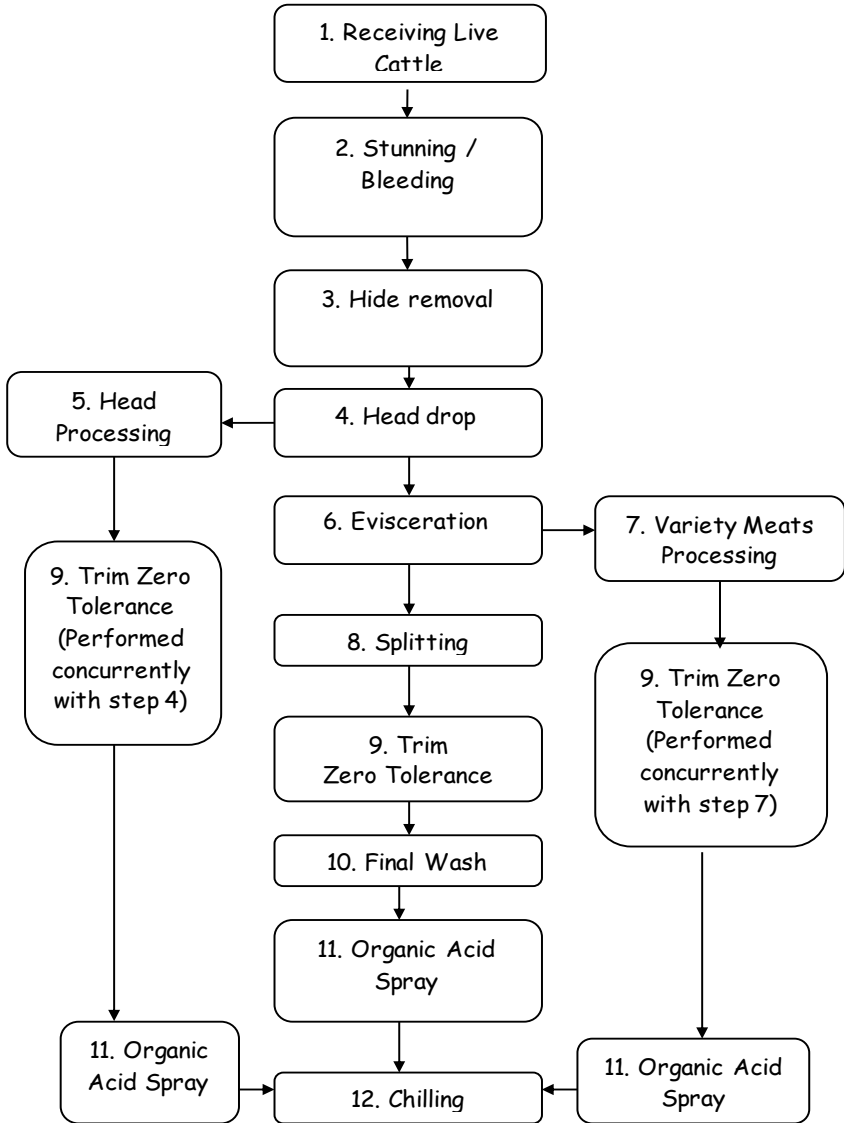
Feedlot



Harvest/Processing

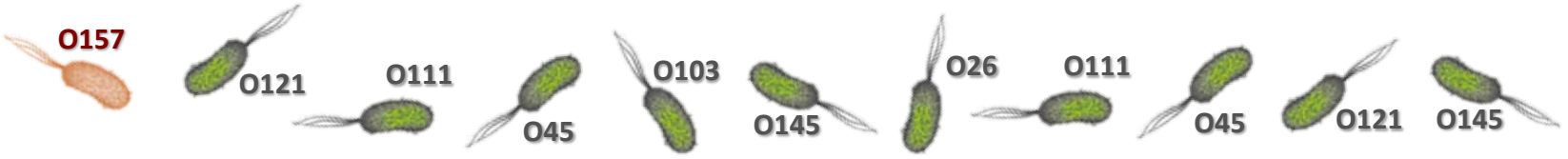
Further processing
Finished products

Beef Processing Flow Diagram



E. coli O157:H7 and non-O157 STEC are Present Throughout the Beef Chain

	Hides	Pre-evis	Final
% *stx* positive	91.7	96.5	16.2
% STEC isolate	56.6	58.0	8.9



Arthur et al., 2002; Barkocy-Gallagher et al., 2003

Prevalence of STEC on Beef Carcasses

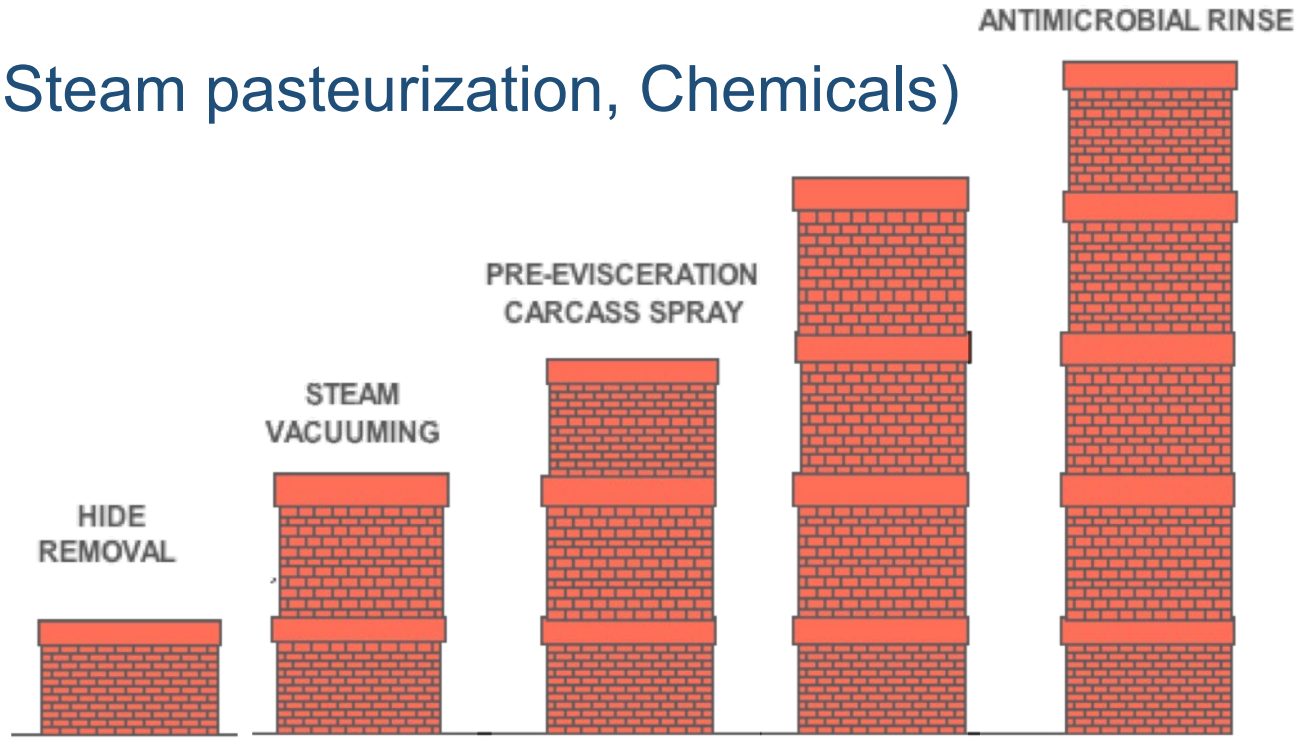
	Pre-evisceration	Post-intervention
n	334	326
stx PCR, %	76.8	13.4
STEC Isolate, %	53.9	8.3
EHEC Isolate, %	**11.7**	**1.2**

virulence factors	Pre-evis	Final
stx1	135	17
stx2	78	15
stx1, stx2	15	0
stx1, hlyA	3	5
stx2, hlyA	17	2
stx1, stx2, hlyA	23	8
stx1, eae	2	0
stx1, stx2, eae	1	0
stx1, eae, hlyA	6	2
stx2, eae, hlyA	20	0
stx1, stx2, eae, hlyA	10	2

Arthur et al., 2002

Beef Slaughter Systems Use a Multiple Hurdle Approach

- Hides
- Steam Vacuums to Treat Pattern Marks
- Pre-Evisceration Carcass Treatment
- Equipment Sanitation – Dual Knife
- Carcass Cleansing
- Interventions (Hot water, Steam pasteurization, Chemicals)



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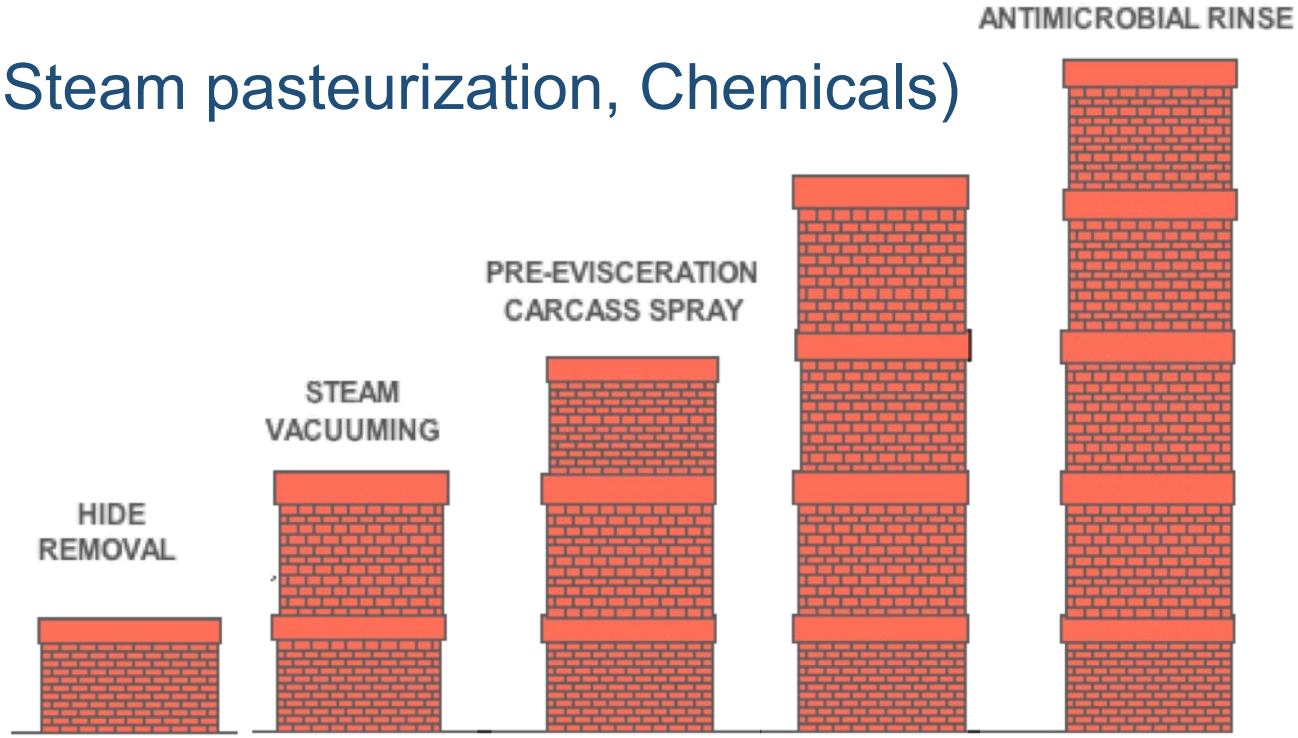
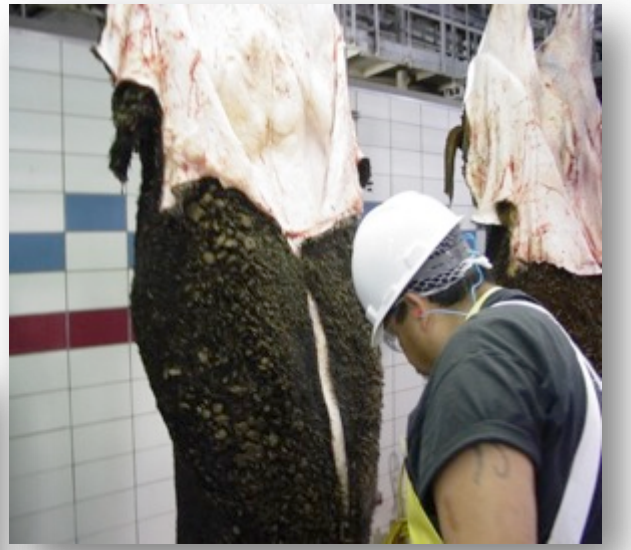


Addressing Routes of Contamination in Beef Processing

During the steps of hide removal pathogens can be transferred from the hide to the carcass



These photos present the worst case scenario, and this situation is generally quite uncommon

Industry Implemented Hide Interventions

Since the hide is the source of carcass contamination, hide directed interventions should reduce carcass contamination.

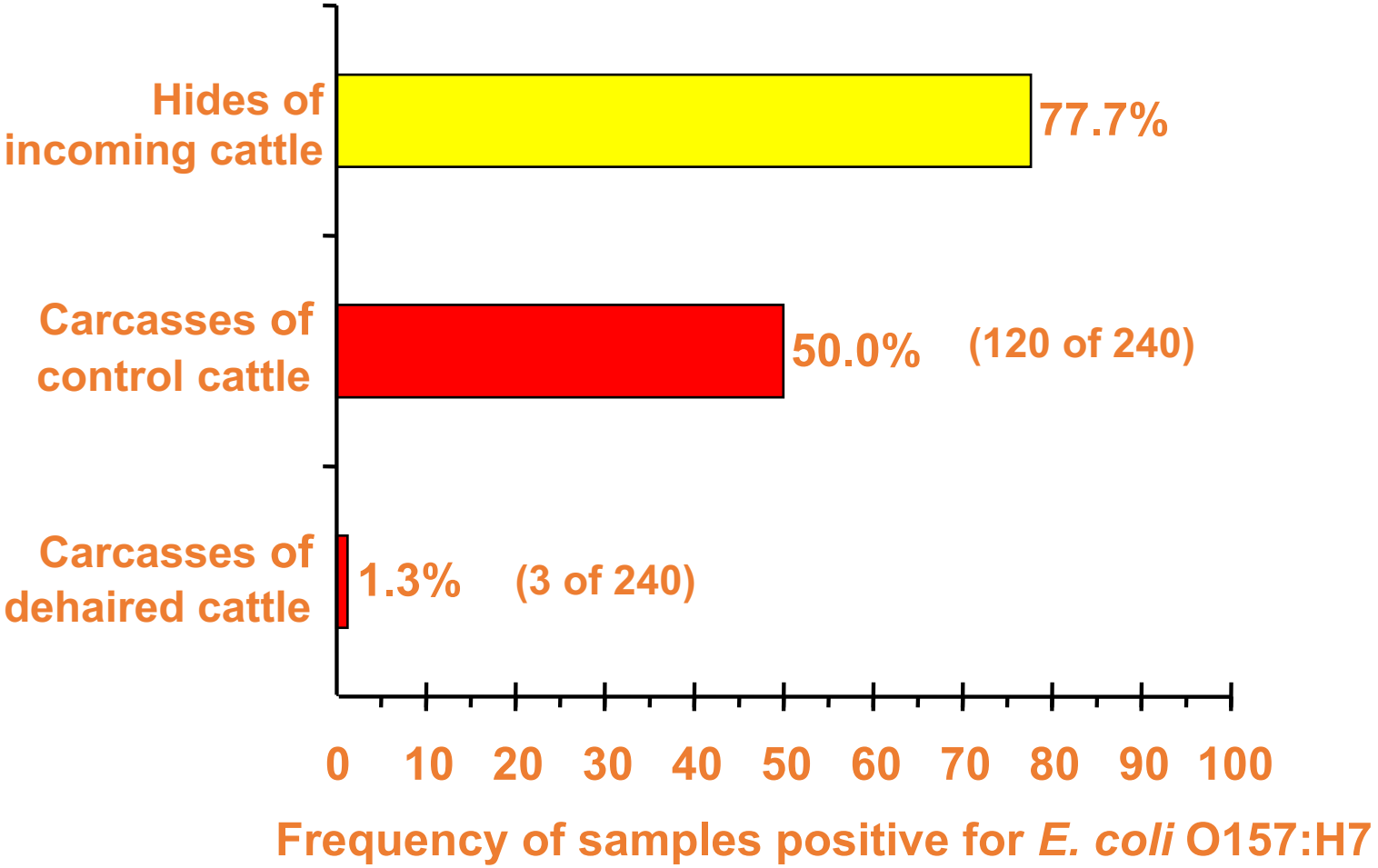
- Chemical dehairing was used to demonstrate this concept.
- The microbial status of pre-evisceration carcasses of dehaired animals was compared to controls (non-dehaired)

Chemical dehairing consists of washing hide-on carcasses with a solution of sodium sulfide, followed by hydrogen peroxide to neutralize and water to rinse.

Chemical Dehairing of Hide Correlates to Reduced Bacterial Contamination of Carcasses

	Controls	Treated
APC Aerobic Plate Count Bacteria	**5.5** $log_{10}CFU/100cm^2$	**3.5** $log_{10}CFU/100cm^2$
EBC *Enterobacteriaceae* Count	**3.2** $log_{10}CFU/100cm^2$	**1.4** $log_{10}CFU/100cm^2$

Dehairing of cattle before hide removal reduces the incidence of *E. coli* O157:H7 contamination on pre-evisceration carcasses.



Less sophisticated hide wash systems can be equally effective

- Applied right after stunning (before bleeding).
  - A very limited window of time of only 10-20 seconds.
- Thoroughly soaks hide with water.
  - 100 to 200 ppm chlorine
  - or caustic sodium hydroxide
- Excess liquid drips from hide before reaching initial point where hide opening and removal begins.

Less sophisticated hide wash systems can be equally effective

Hide sample data before and after hide wash cabinet

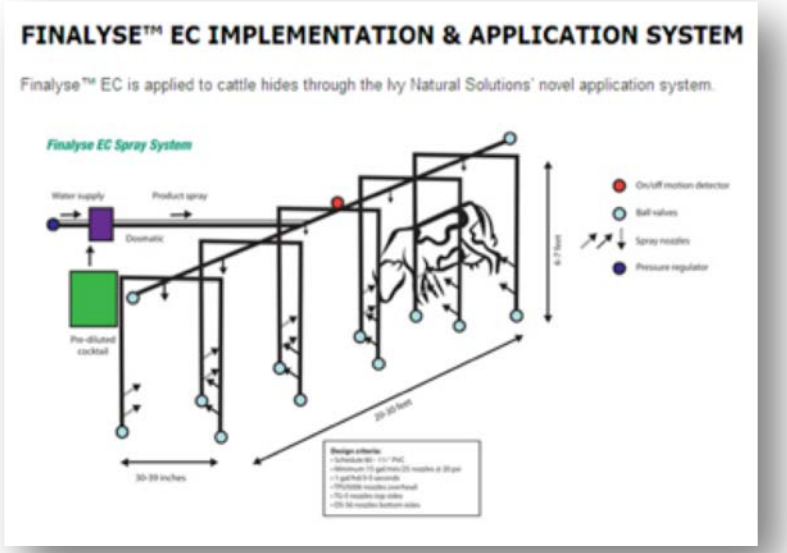
Sample	% positive
Before cabinet *E. coli* O157 prevalence	97.6
After cabinet *E. coli* O157 prevalence	89.6
Before cabinet *E. coli* O157 >20 CFU/mL	35.1
After cabinet *E. coli* O157 >20 CFU/mL	13.2
Before cabinet *Salmonella* prevalence	94.8
After cabinet *Salmonella* prevalence	68.8
Before cabinet *Salmonella* >20 CFU/mL	40.7
After cabinet *Salmonella* >20 CFU/mL	7.3

Hide enumeration = >40 CFU per 100 square cm

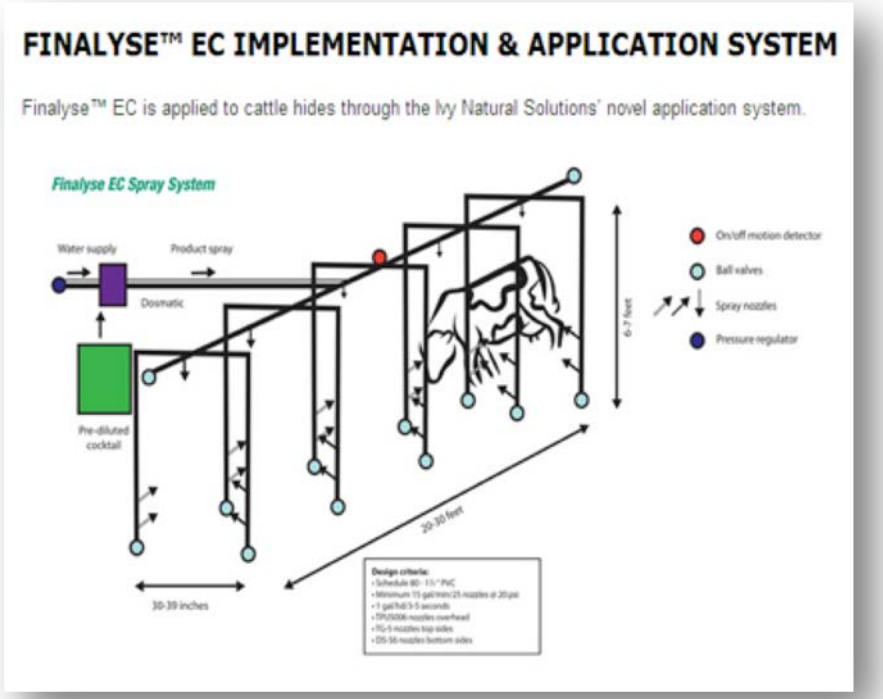
Hide interventions applied to live animals

Antimicrobial sprays

Bacteriophage



Bacteriophage Treatment Applied to Live Cattle Hides

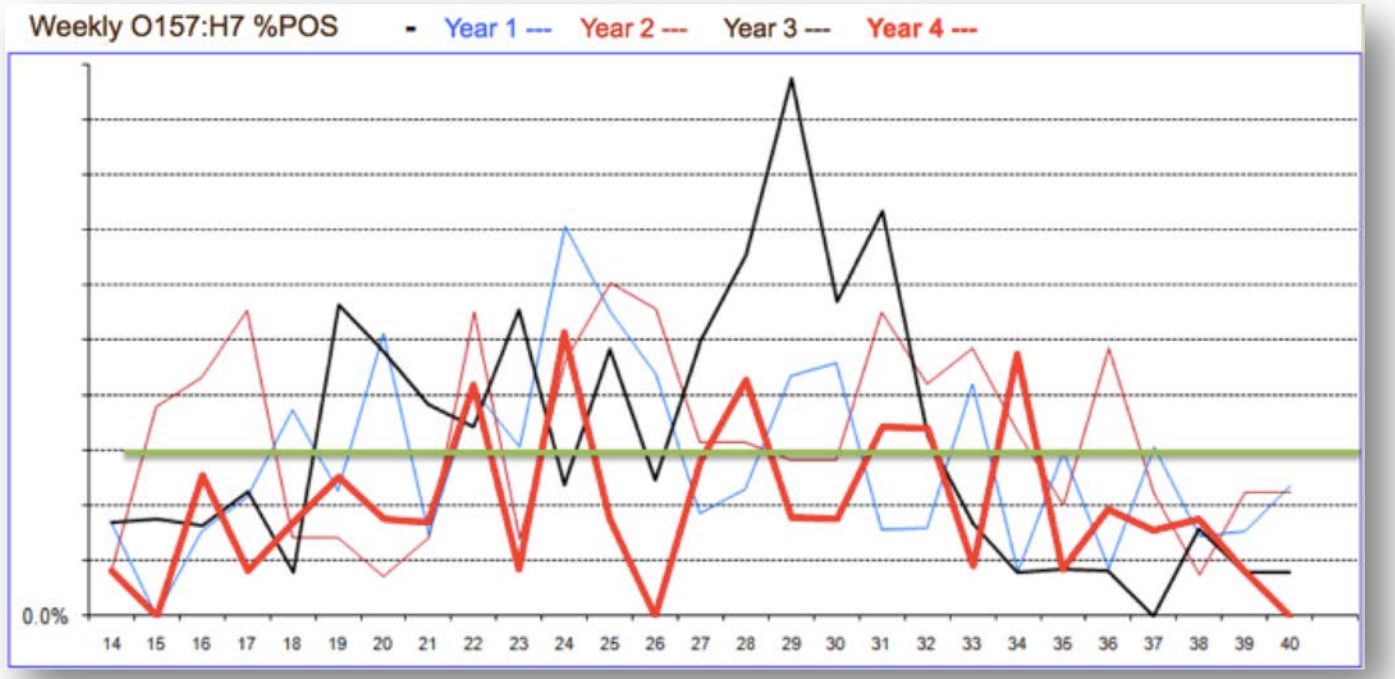


- Bacteriophage is sprayed on each animal upon arrival at processing plant.
- 1 to 4 hours prior to hide removal.
- Application is through chute with spray bars.

Effect is measured on boneless beef trim prevalence of E. coli O157:H7

Bacteriophage Treatment Applied to Live Cattle Hides

The prevalence weekly positive *E. coli* O157:H7 tests on boneless beef trim has gone down since the initiation of phage treatment.



Phage treatment began Year 4

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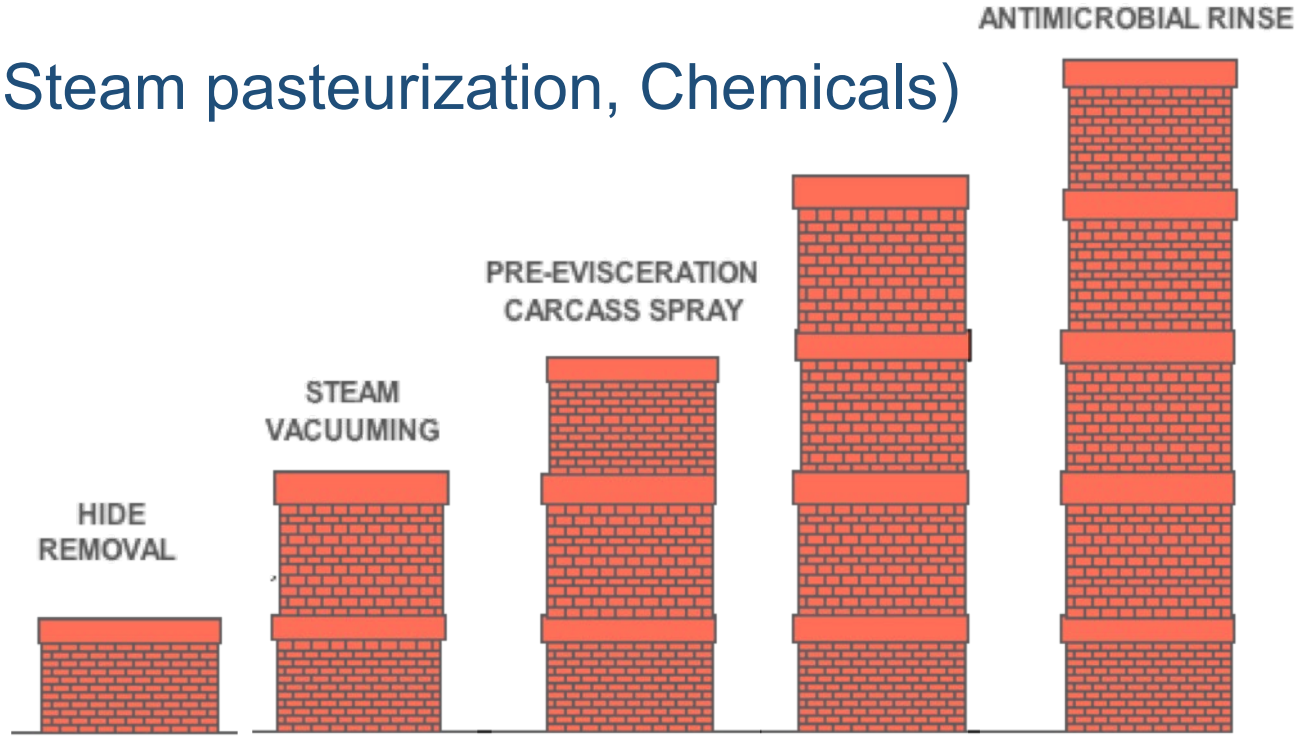
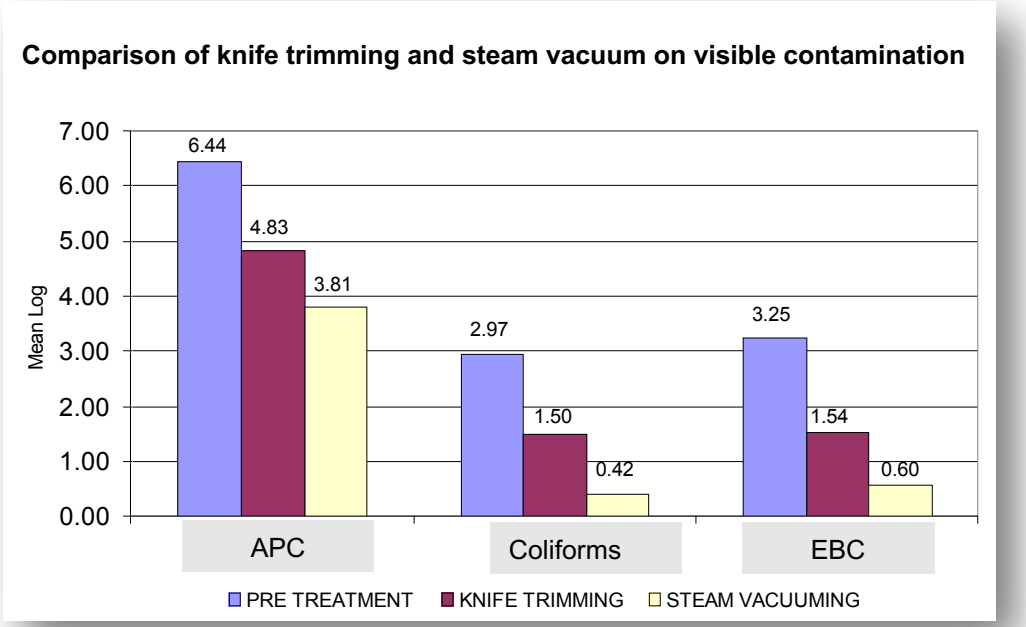


Steam Vacuum

- Early application of steam is critical, before bacterial attachment occurs.
- Only a “spot treatment” and not a whole carcass treatment.

Hide Opening Pattern Area Treatment

- Steam vacuums are used over hind hocks, and the hide opening patterns
- Hock blow off with steam is also used to reduce contaminants
- Continuous knife trimming of visible contamination



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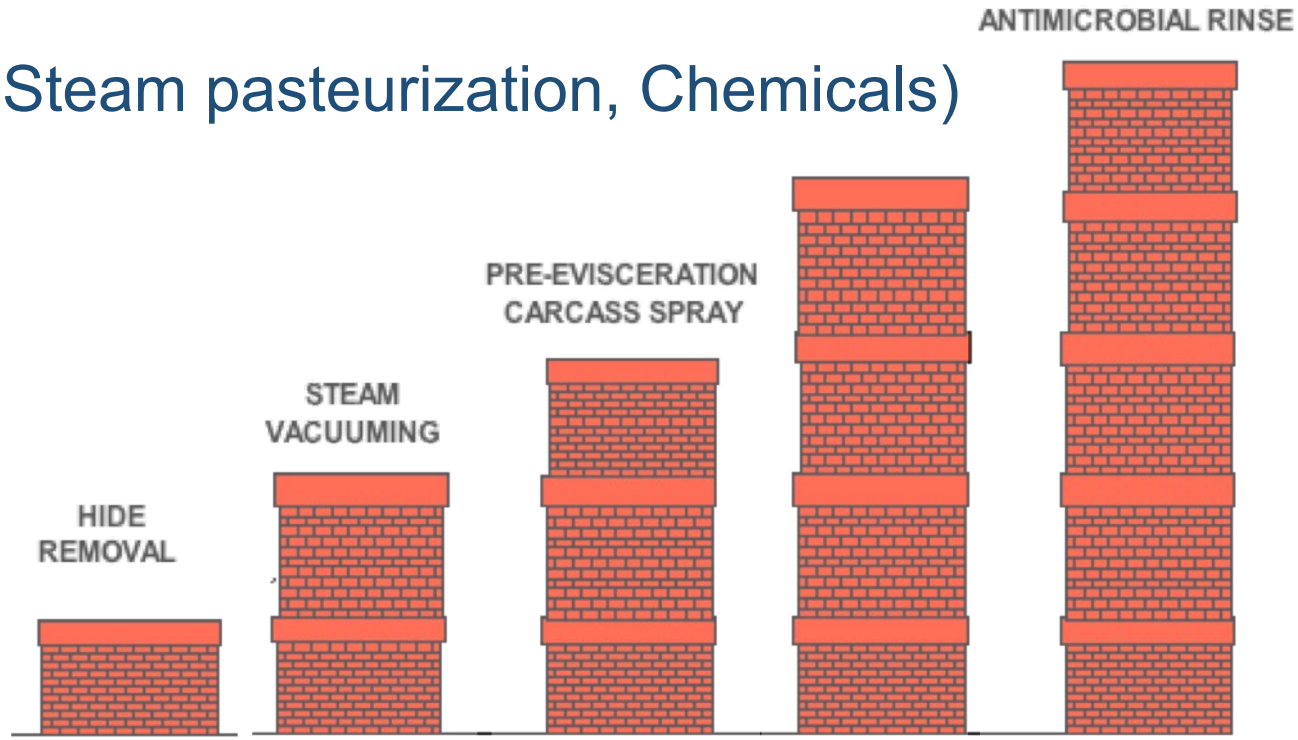

Pre-evisceration Interventions

- Pre-evisceration wash is an effective processing aid used to reduce microbiological levels.
- Focuses on exterior of intact carcass prior to evisceration and performed as soon as possible after hide removal.
- Flushes fine specs of dust from exterior using a large flow of wash ~350 L per carcass.
- Until recently most U.S. plants used lactic acid (2-4%) or hot water, but more recently, peroxyacetic acid (PAA) and Bromine are more widely used.
- One of most effective treatments has been, and always will be, hot water at 80C (175F).

Lactic acid and hot water wash treatments reduce the prevalence of *E. coli* O157:H7 on pre-evisceration carcasses

	Lactic Acid (n = 256)	Hot Water (n = 256)	Sequential (n = 256)
Before Treatment	31%	27%	19%
After Treatment	20%	5%	4%
Reduction	35%	81%	79%
P value	0.01	0.001	0.001

Effects of the interventions are not additive,

Bosilevac 2006

Lactic acid and hot water wash treatments reduce aerobic plate counts on pre-evisceration carcasses

Log_{10} APC/100cm^2	Lactic Acid (n = 256)	Hot Water (n = 256)	Sequential (n = 256)
Before Treatment	6.1	6.2	6.4
After Treatment	4.5	3.5	4.2
Reduction	1.6	2.7	2.2
P value	0.001	0.001	0.001

Lactic acid treatment followed the hot water wash and may have cooled the carcass surface, thus reducing the hot water effect.

Bosilevac 2006

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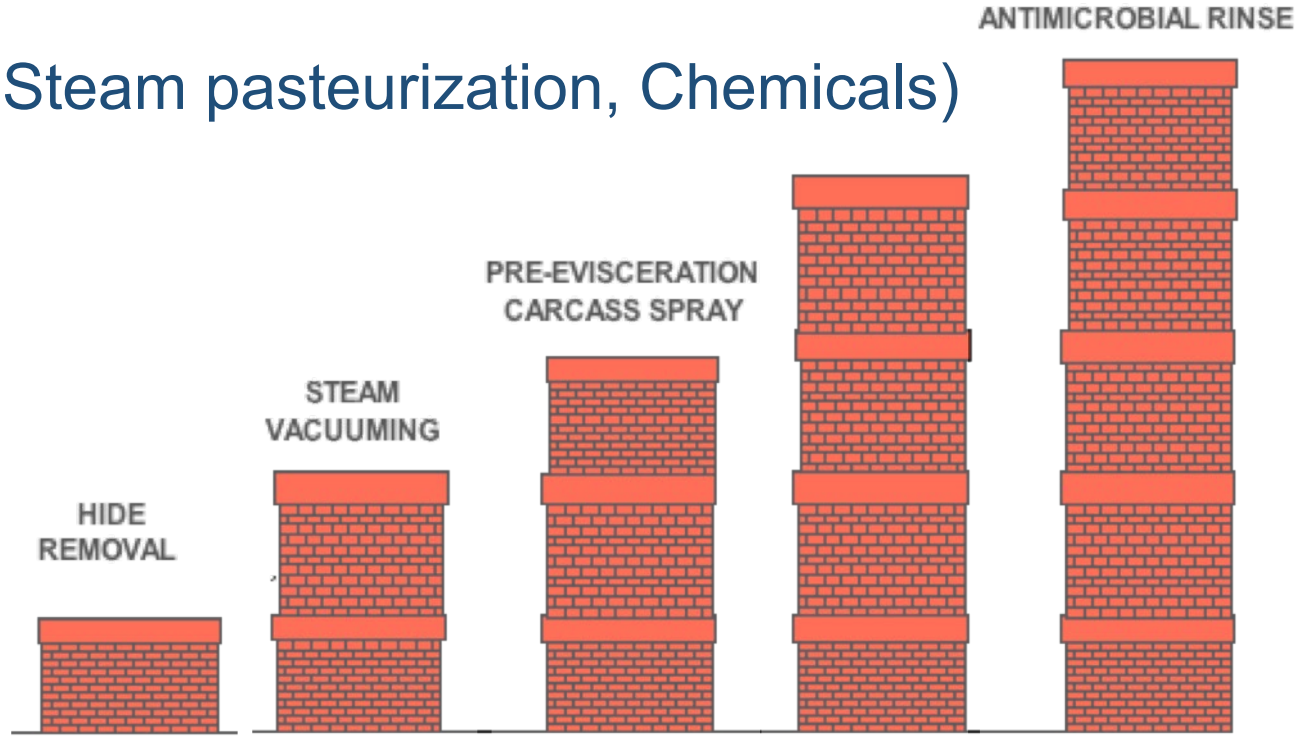
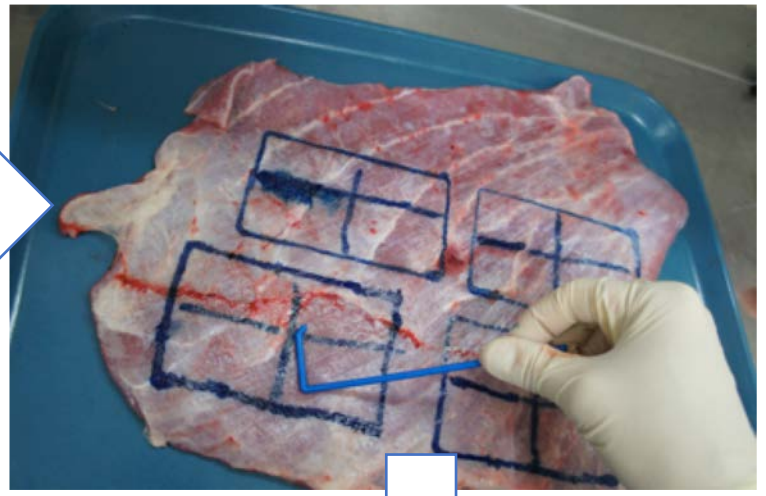


Steam Pasteurization and Hot Water Treatment of Final Carcasses

- Steam pasteurization
  - minimum of 13 seconds
  - steam minimum temperature 85ºC
- Hot Water
  - minimum of 10 seconds
  - Water temperature is >85C
- Low levels of bacteria at this point make direct testing difficult.

Steam Pasteurization and Hot Water Treatment of Inoculated Carcass Surfaces



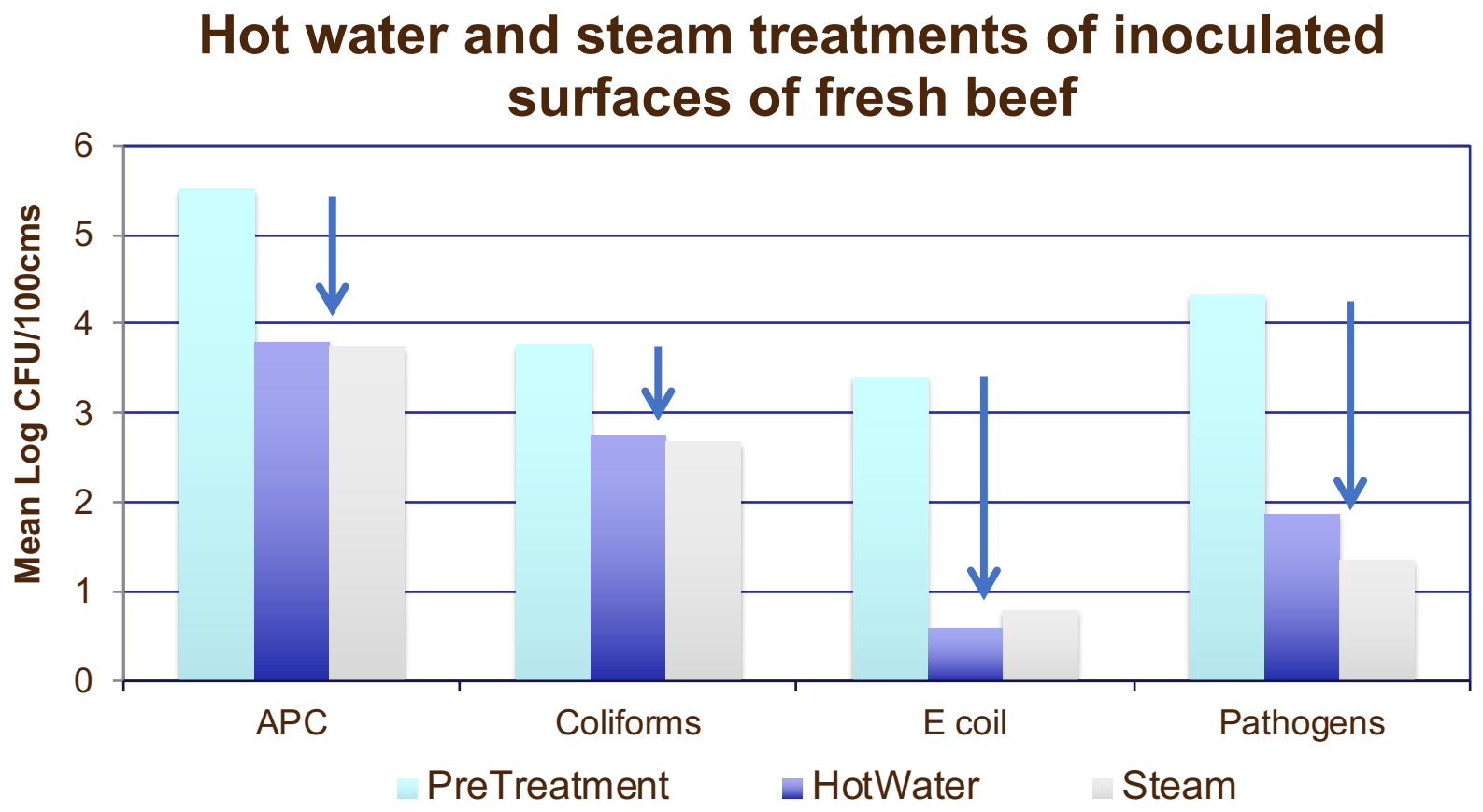
Example of steps that take place in laboratory to evaluate final carcass directed interventions when carcass has very low level of surface bacteria.

Inoculation study. Approx. 10^5 CFU per $100cm^2$ applied to carcass surface.

Inoculum is pool of pathogens and background bacterial from purge.

Inoculum allowed to adhere to surface for 20+ min before treatment applied.

Steam Pasteurization and Hot Water Treatment of Inoculated Carcass Surfaces



Pathogens = E. coli and Salmonella strains

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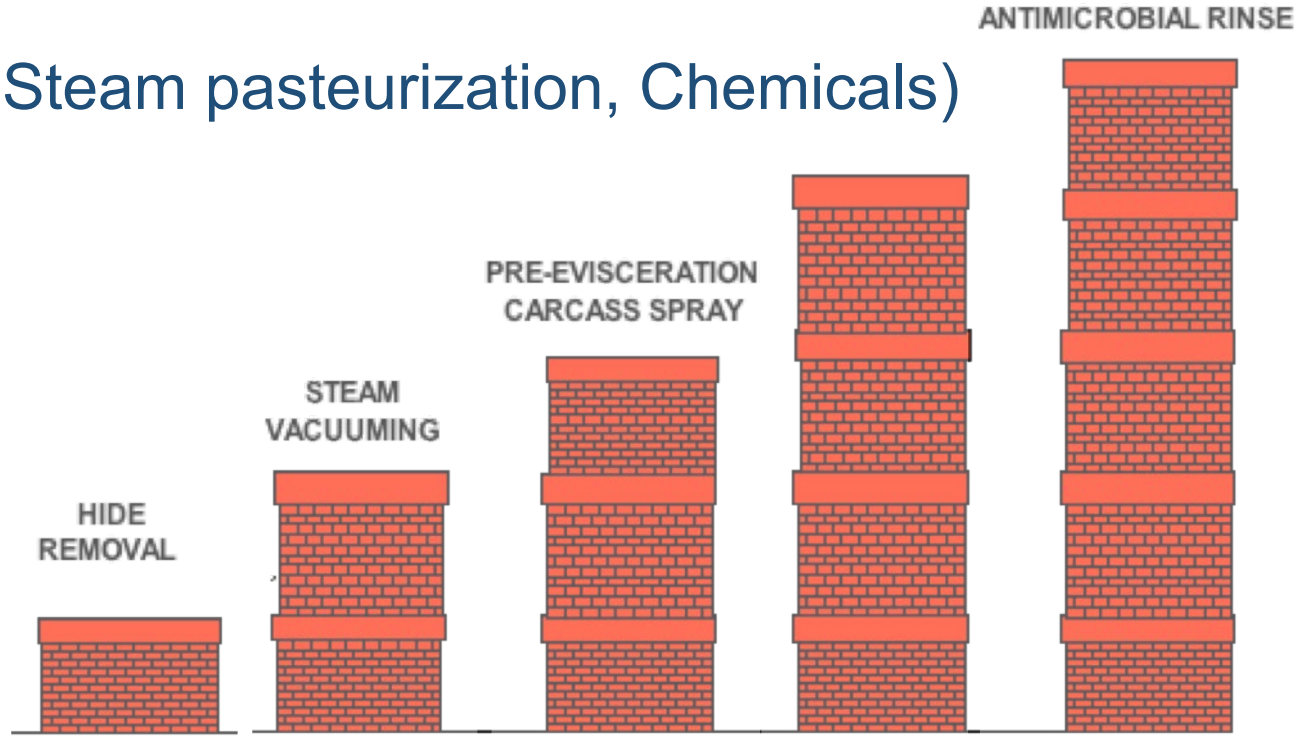
- **Points Beyond**



Cooler spray chill, and processing of subprimals & trim

Spray chill water or blast chill fogging applied to final carcass may contain an antimicrobial

Prior to vacuum packaging, primals and subprimals may be treated with an antimicrobial spray

Before grinding, trim is treated with an antimicrobial spray.

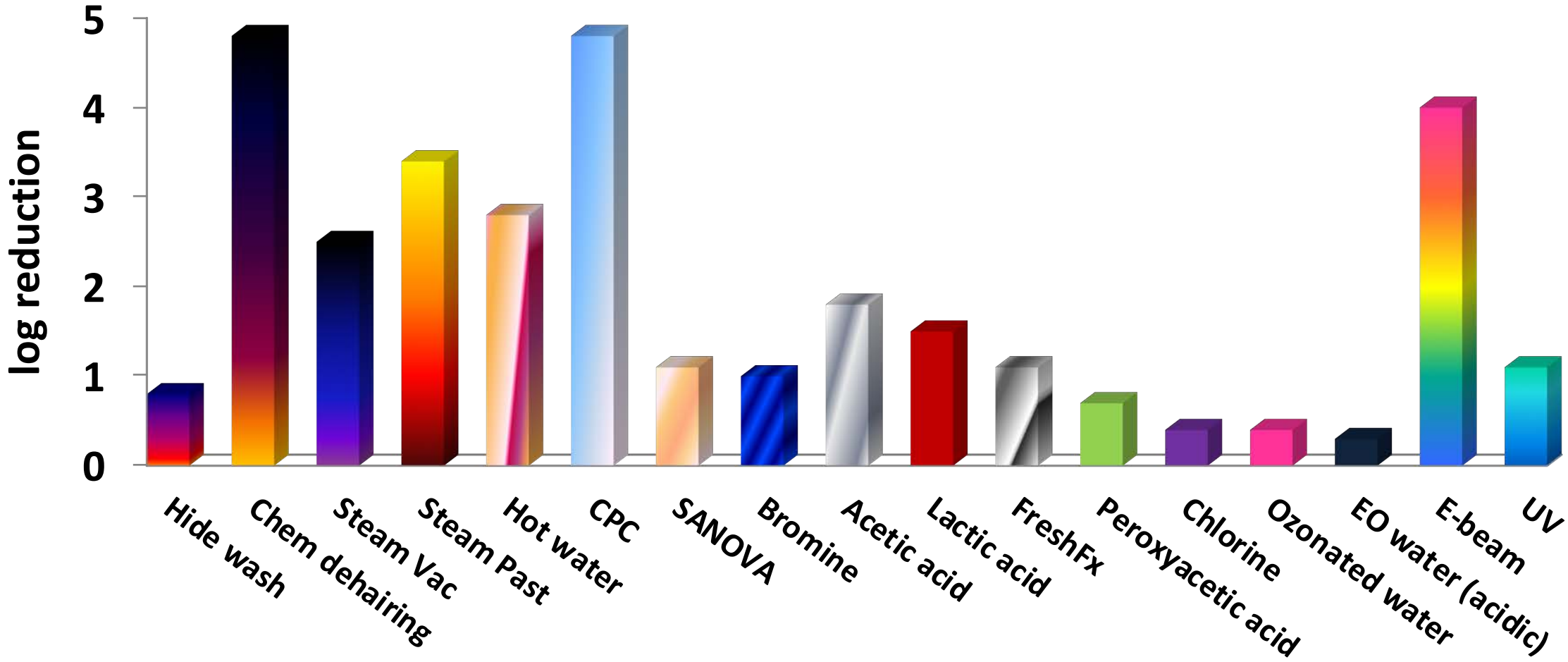
Effectiveness of Existing Antimicrobials for Reducing Pathogens

Antimicrobial Treatments and Conditions

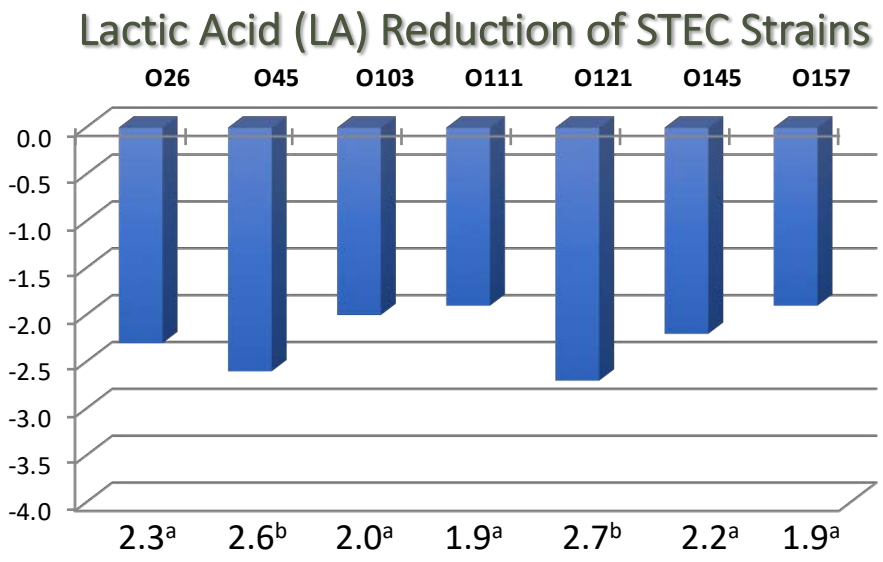
- Peroxyacetic acid; 200 ppm, pH = 2.8 (Inspexx™)
- Acidified sodium chlorite; 1000 ppm, pH =2.4 (Sanova™)
- Lactic acid; 4%, pH = 2.3
- Hot water; 85°C at nozzles
- Citric/phosphoric/hydrochloric acid blend; 2% pH = 1.7 (FreshFX™)
- Bromine compounds; 300ppm (Bromitize™, H2B™)
- 15-20 psi for 15 sec

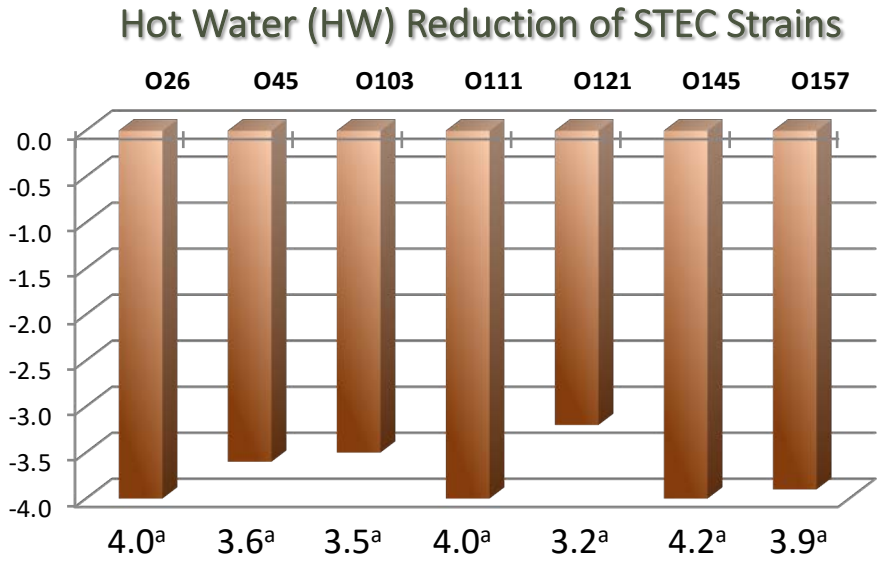
Efficacy of Post-harvest Interventions

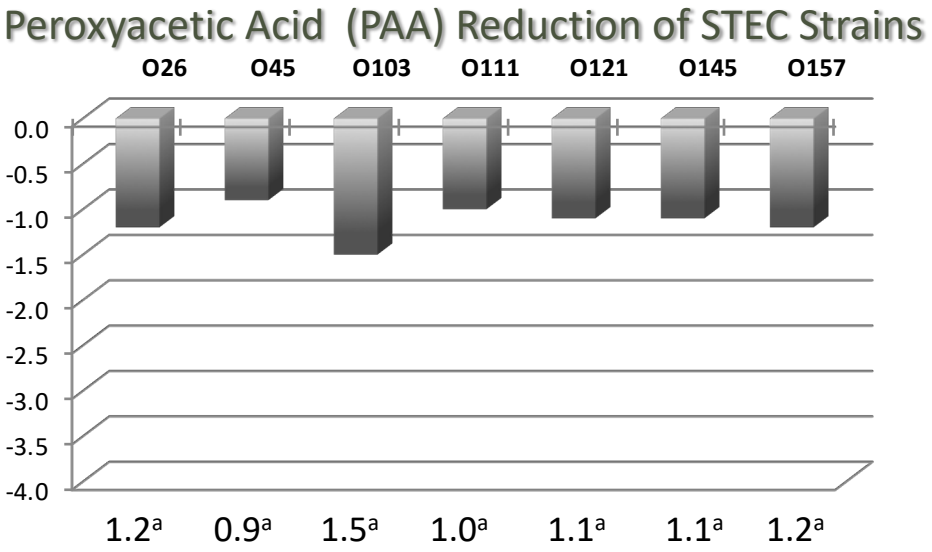
as evaluated by the Meat Safety and Quality Research Unit

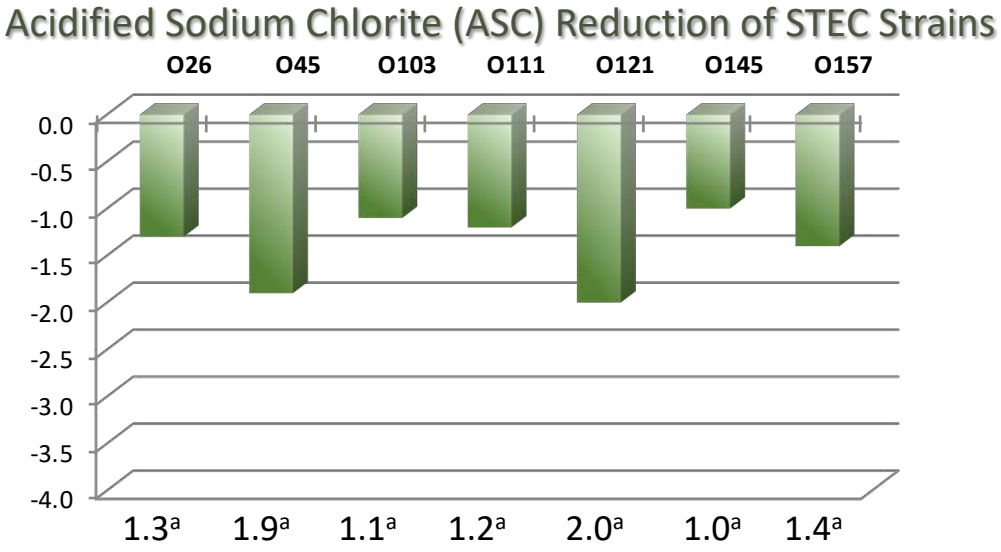


Efficacy of interventions is measured on pooled pathogens to obtain direct comparisons between individual non-O157 STEC and *E. coli* O157:H7 (*Salmonella* serovars often included too).









Tackling the problems of STEC and *Salmonella* in beef

- STEC and *Salmonella* are completely different beasts when it comes to their control and elimination from beef products.
- While both are surface contaminants, *Salmonella* can be an internal contaminate as well.

- Colonizes bovine lymph nodes
  - Not exposed to in-plant interventions
  - Released in grinding of beef trim

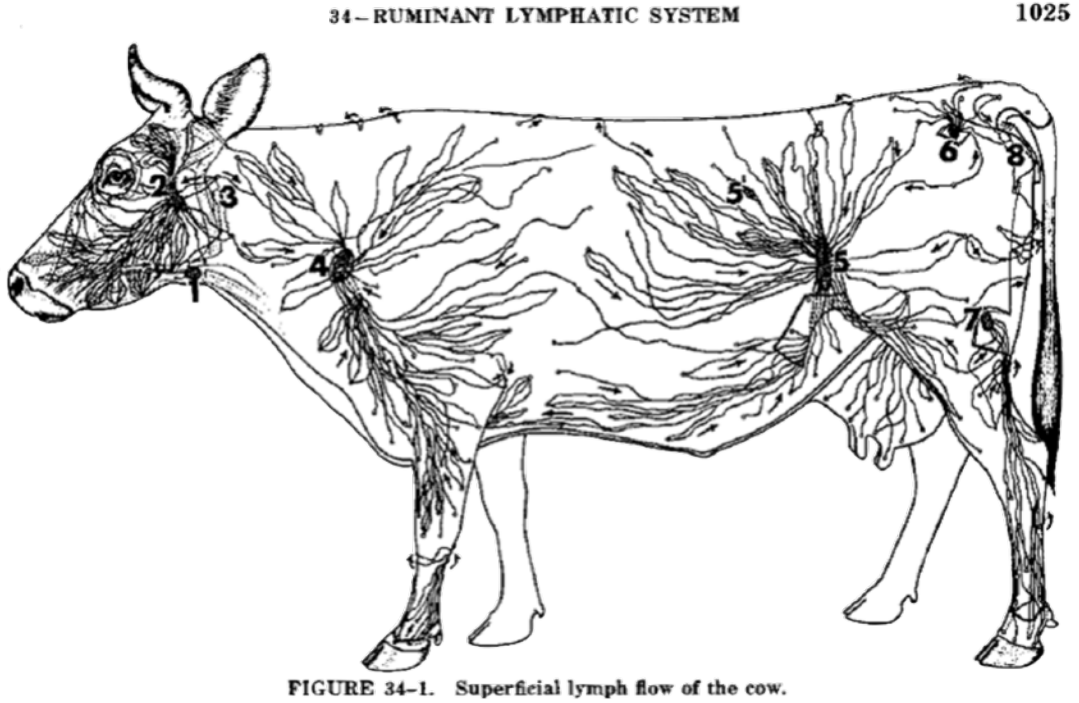


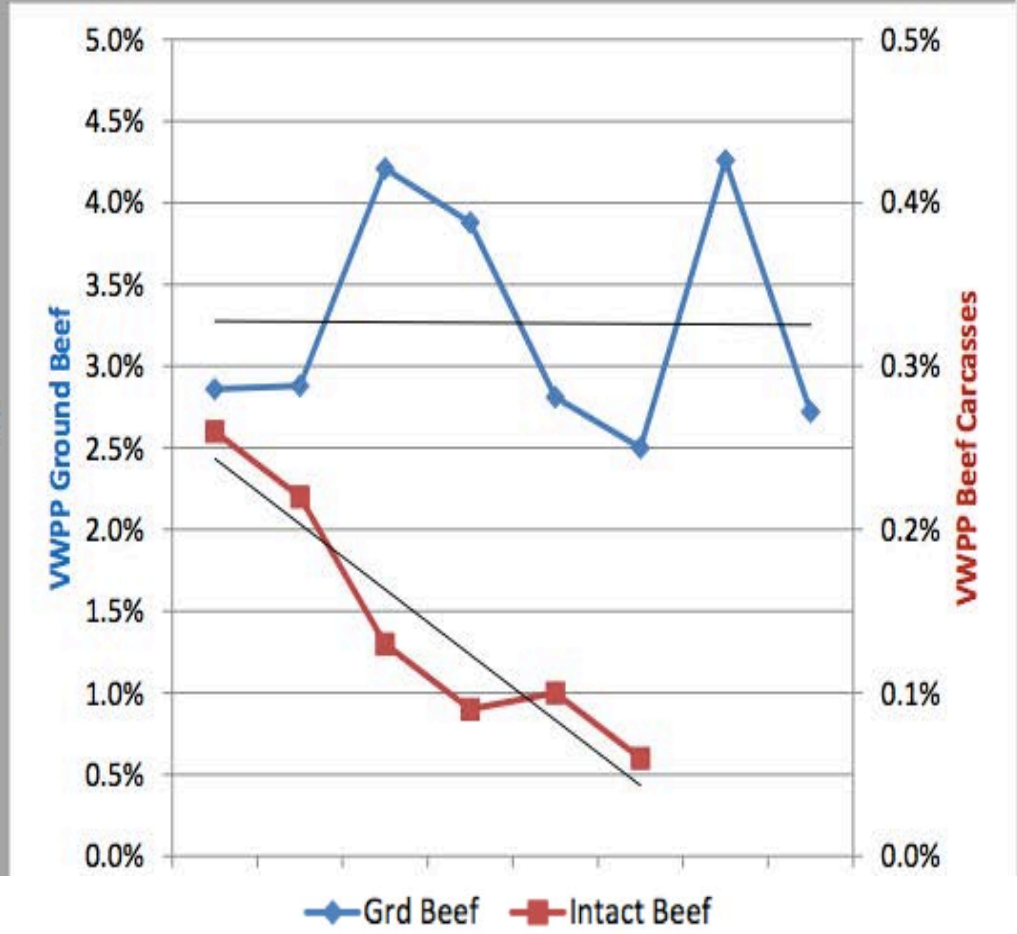
FIGURE 34–1. Superficial lymph flow of the cow.

1, Mandibular ln.; 2, parotid ln.; 3, lateral retropharyngeal ln.; 4, superficial cervical ln.; 5, subiliac ln.; 5′, lnn. of paralumbar fossa; 6, gluteal ln.; 7, popliteal ln.; 8, tuberal ln. (After Baum, 1912.)

Lymph node borne *Salmonella* contamination of beef products to be ground

Salmonella verification program in beef

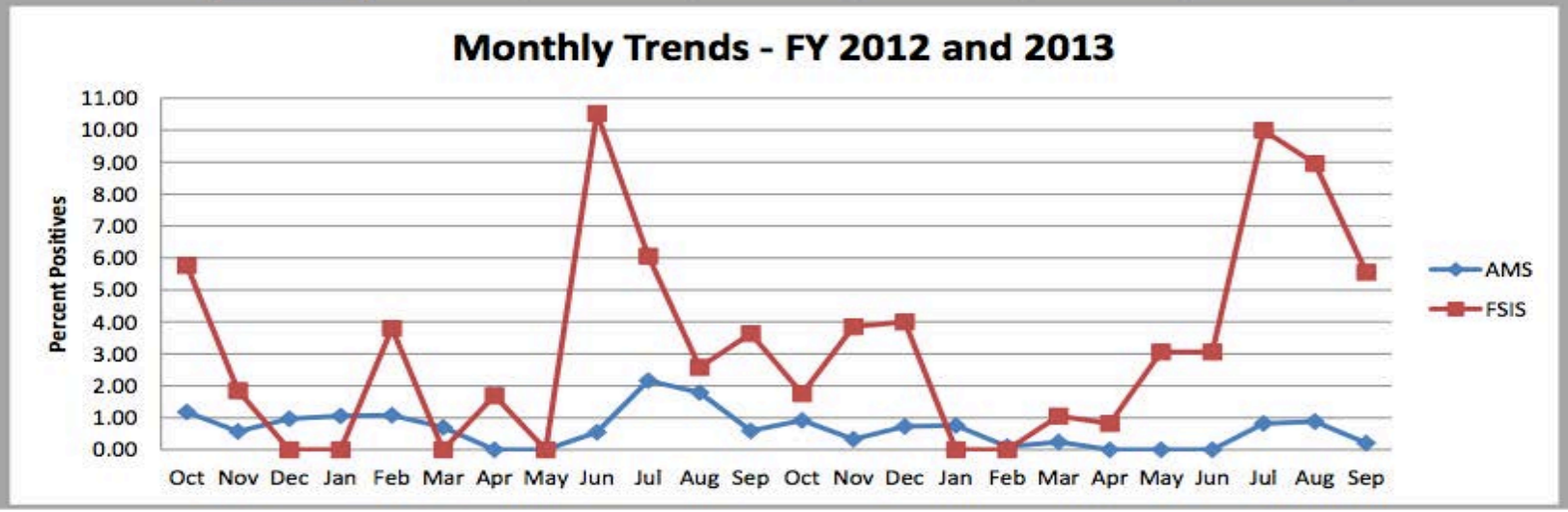
- *Salmonella* VWPP in ground beef has remained unchanged while beef carcass have improved
- This disconnect suggests *Salmonella* on carcasses might not be the primary source of *Salmonella* in ground beef
- Contamination from trim/ fabricated parts or internal contamination, such as lymph nodes, could be greater contributors



Lymph node borne *Salmonella* contamination of beef products to be ground

Preliminary Assessment of *Salmonella* in Ground Beef Between AMS And FSIS Samples

Percent *Salmonella* Positive For FY 2012 and 2013						
Agency	Percent Positive			VWPP		
	2012	2013	Overall	2012	2013	Overall
AMS	1.04	0.54	**0.79**	1.02	0.54	**0.89**
FSIS	2.9	3.27	3.09	2.85	2.05	2.40



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AMS began requiring the largest peripheral lymph nodes be removed from beef destined for grinding to supply the school lunch program.

FSIS monitors all ground beef produced, much of which does not require lymph node removal.

Lymph node borne *Salmonella* contamination of beef products to be ground

Journal of Food Protection, Vol. 71, No. 8, 2008, Pages 1685–1688

Research Note

Prevalence and Characterization of *Salmonella* in Bovine Lymph Nodes Potentially Destined for Use in Ground Beef†

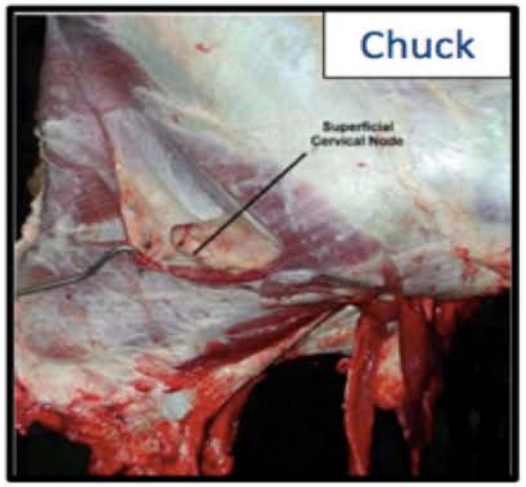
TERRANCE M. ARTHUR,* DAYNA M. BRICHTA-HARHAY, JOSEPH M. BOSILEVAC, MICHAEL N. GUERINI, NORASAK KALCHAYANAND, JAMES E. WELLS, STEVEN D. SHACKELFORD, TOMMY L. WHEELER, AND MOHAMMAD KOOHMARAIE

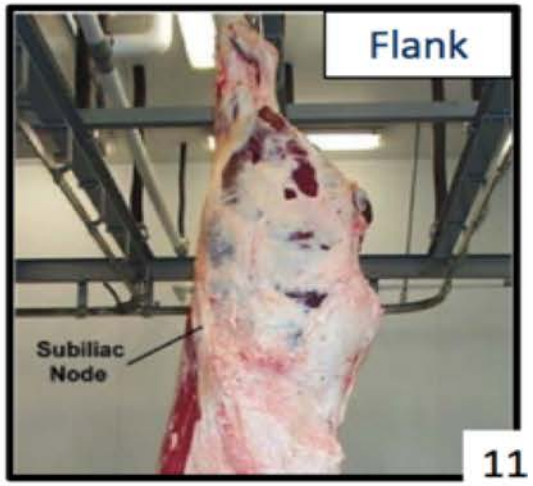
Percent *Salmonella* Prevalence

Cattle type	Chuck	Flank	Overall
Cull Cow	1.0	3.9	2.5
Fed Beef	0.3	1.0	0.7
n=1,140			1.6

9 Serotypes observed from 18 positive nodes:

- Cerro (6)
- Typhimurium (3)
- Montevideo (2)
- Newport (1)
- Anatum (1)





Lymph node borne *Salmonella* contamination of beef products to be ground

TABLE 1 | *Salmonella* percent prevalence in subiliac peripheral lymph nodes (PLN) of feedlot-fattened (FF) and cull cattle at harvest by region and season.

Season	Cull cattle PLN			All Cull	FF cattle PLN			All FF	Overall by season
	Region A	Region B	Region C		Region A	Region B	Region C		
COOLER SEASON									
Sample sets	1	9	3	13	12	9	5	26	39
Number of PLNs	76	561	245	882	892	551	379	1,822	2,704
Positive (*n* =)	0	12	3	15	2	36	11	49	64
Mean %	0	2.14	1.22	1.70	0.22	6.53	2.90	2.69	2.37
WARMER SEASON									
Sample sets	2	9	3	14	10	9	6	25	39
Number of PLNs	145	567	246	958	754	570	464	1,788	2,746
Positive (*n* =)	7	6	5	18	8	177	22	207	225
Mean %	4.83	1.06	2.03	1.88	1.06	31.05	4.74	11.52	8.19
OVERALL BY REGION									
Sample sets	3	18	6	27	22	18	11	51	78
Number of PLNs	221	1,128	491	1,840	1,646	1,121	843	3,610	5,450
Positive (*n* =)	7	18	8	33	10	213	33	256	289
Mean %	3.17	1.60	1.63	1.79	0.61	19.0	3.91	7.09	5.3

Webb et al, 2017. Frontiers in Microbiol fmicb.2017.02214

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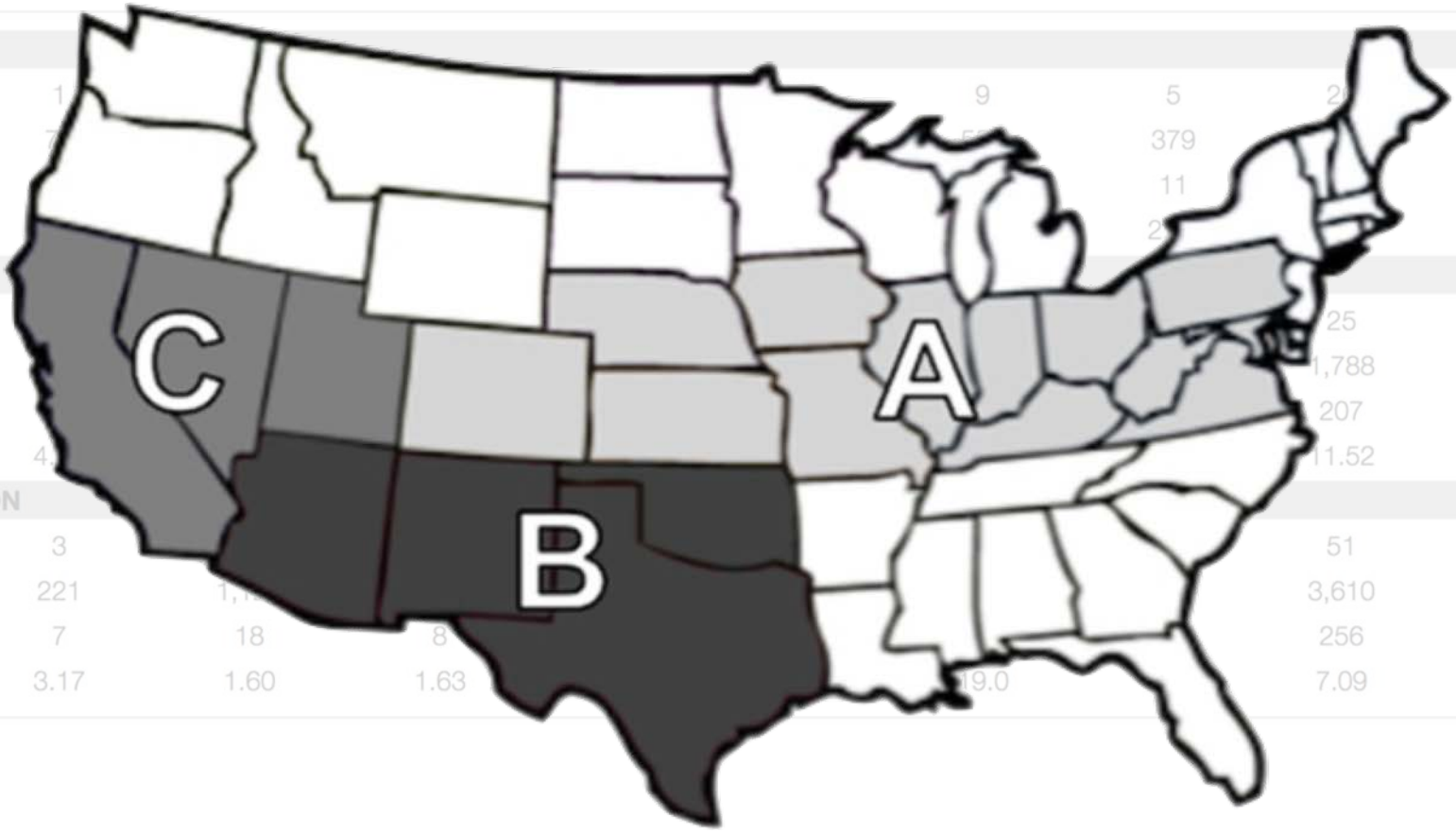
	Region A	Region B	Region C		Region A	Region B	Region C		
COOLER SEASON									
Sample sets	1					9	5	2	39
Number of PLNs	7						379		2,704
Positive (*n* =)							11		64
Mean %							2		2.37
WARMER SEASON									
Sample sets								25	39
Number of PLNs								1,788	2,746
Positive (*n* =)								207	225
Mean %	4.							11.52	8.19
OVERALL BY REGION									
Sample sets	3							51	78
Number of PLNs	221	1,1						3,610	5,450
Positive (*n* =)	7	18	8					256	289
Mean %	3.17	1.60	1.63			19.0		7.09	5.3



Webb et al, 2017. Frontiers in Microbiol fmicb.2017.02214

Additional points to consider when examining ways to tackle the problems of STEC and *Salmonella* in Beef

- Sample collection during processing from hides and carcasses
  - Hides: 500 cm^2
  - Carcasses: 4,000 or 8,000 cm^2, FSIS has started to adopt this for research studies, but still maintains the 3 x 100 cm^2 regulatory sample areas.
- Bench top study results do not often relate to pilot-scale results or on-line use in processing plants.
- The impacts of antimicrobial interventions on meat quality and consumer preferences should also be considered.

Sample Collection

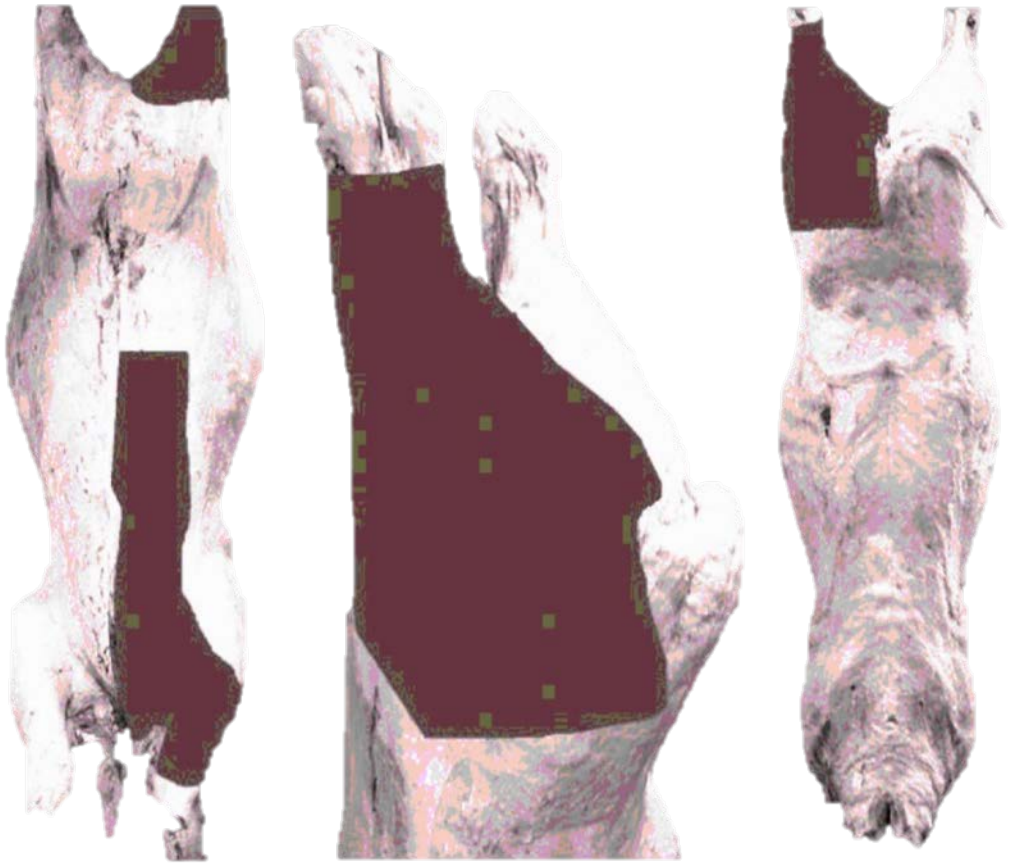
Boxed area represent location where hide sponge samples are collected.

- Samples collected from hide where accessible. Usually near along hide opening pattern lines.
- Hide sample covers 500 cm^2, about the area of a sheet of paper (8 x 10 inch)
- One sponge used, turned over half way through collection, using vertical and horizontal motions.

Arthur 2004; Kalchayanand 2009.

Sample Collection

Carcass sampling areas.



Shaded areas represent locations where carcass sponge samples were collected

- Samples collected from “hot spots” along hide opening pattern lines.
- Carcass samples cover 4,000 or 8,000 cm^2.
- One sponge used on top and round, and a second sponge used on brisket and fore shank, then combined.

Arthur 2004; Wang 2014.

Bench top studies often do not relate to on-line use

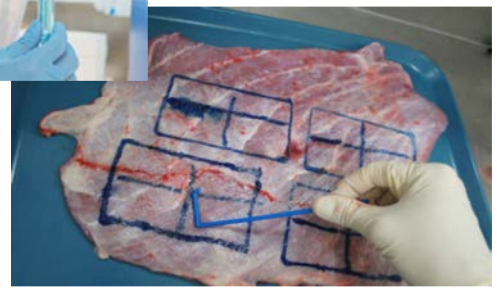
EO water, LA-SDS, UV and other studies performed at USMARC were effective on the bench top and to some extent in the pilot-scale system. However for commercial adoption significant effects must be observed.

Hot Water:	**5.1-6.3 log**	**3.5-4.0 log**	**1.9-2.5 log**
Lactic Acid:	**4.4-5.3 log**	**1.5-2.6 log**	**0.9-1.6 log**
Poultry Fresh:	**6.2-7.0 log**	**0.4-0.8 log**	**--**

For example, here are the reductions observed for commonly used (hot water and lactic acid) interventions and a new product under evaluation (Poultry Fresh).

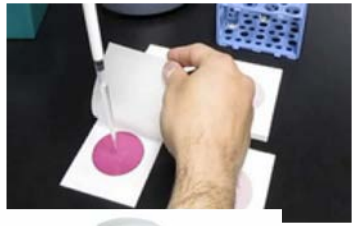
Carcass surface inoculation studies allow best estimate of on-line efficacy of an intervention

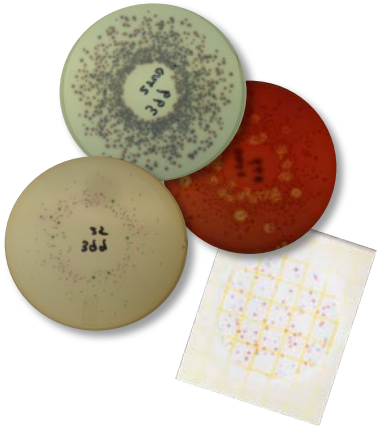
Inoculate



Treat

Collect samples and plate

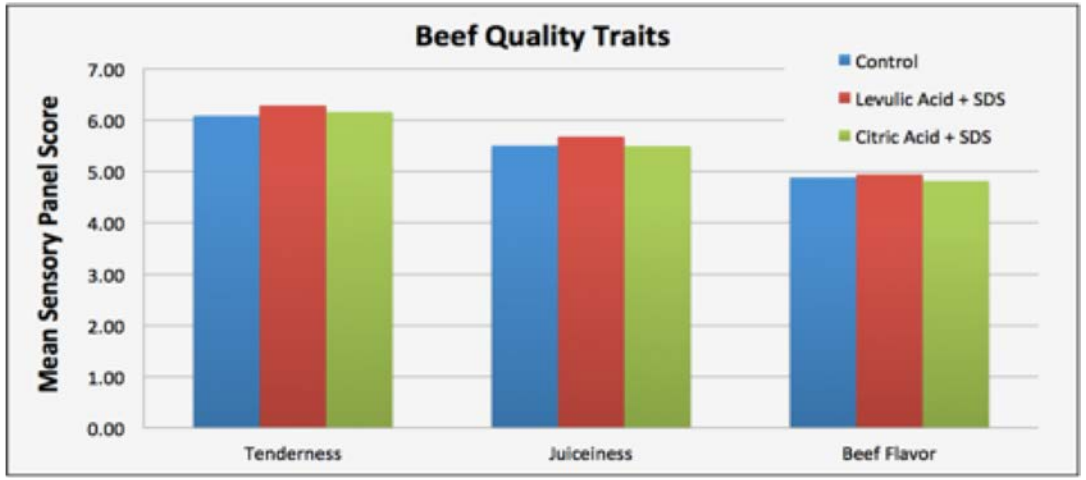


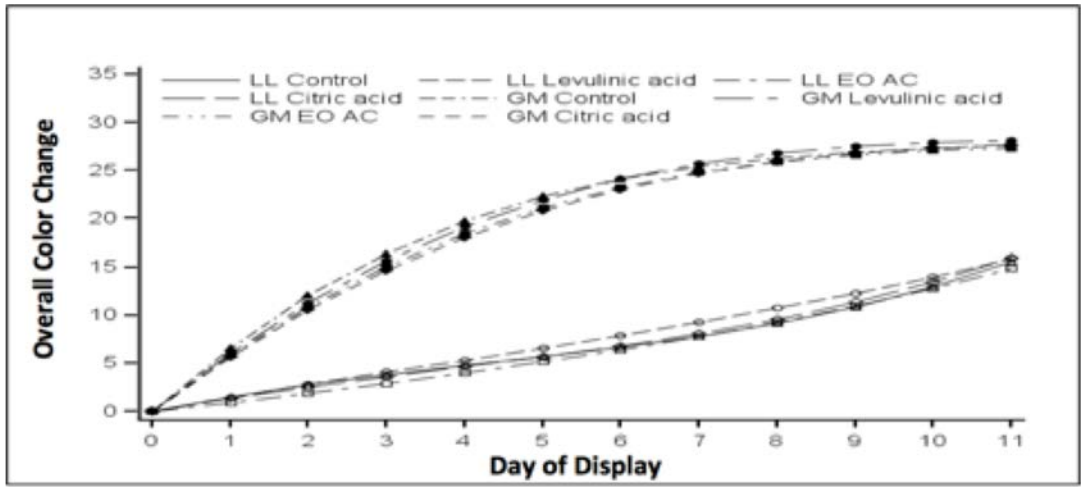


Record results

Pooled strains diluted in beef purge provide simultaneous measurement of each STEC group, Salmonella serovar*, and indicator organisms.*

Do novel antimicrobials that reduce contamination effect meat quality?





- Neither treatment had a significant impact on sensory panel scores for beef tenderness or juiciness.
- Differences in flavor profiles were found but were very small in magnitude and not likely to be of practical importance.
- Effects of both treatments were the same as controls on color change during retail display of strip loin and sirloin steaks.