Post Abattoir Risk-Based Meat Safety Assurance

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

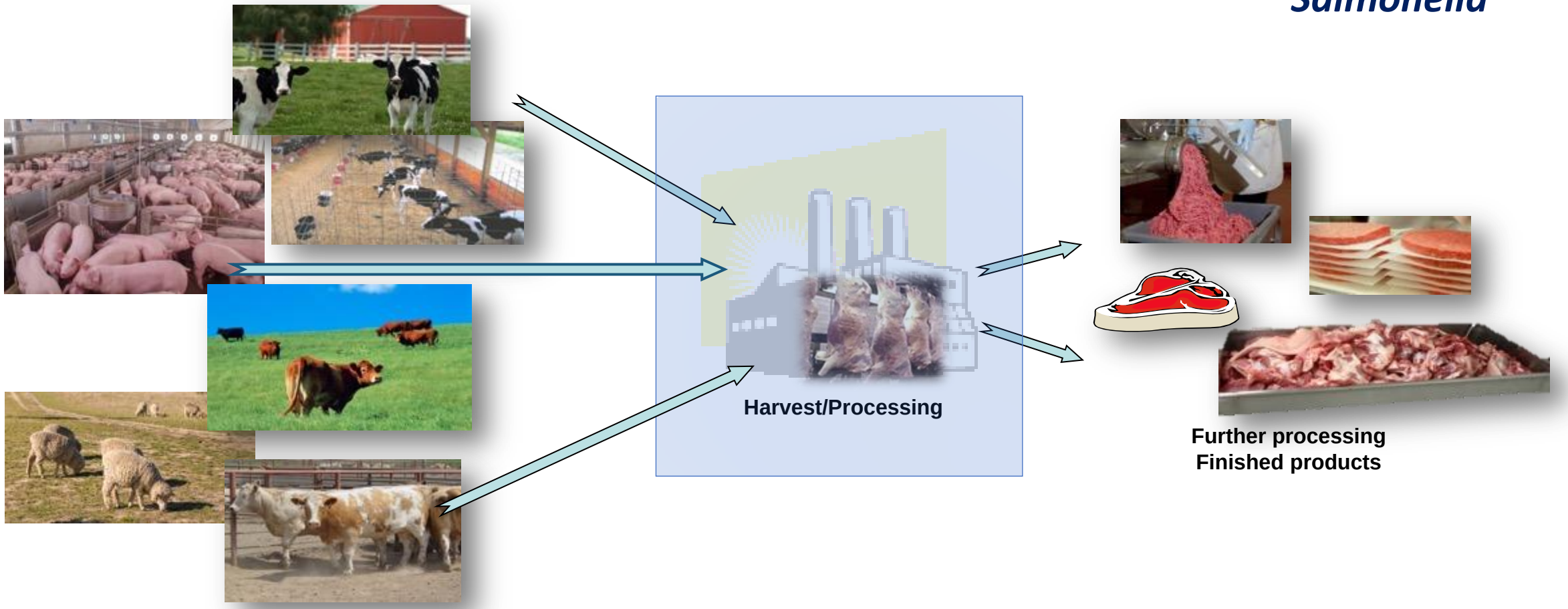
United States Department of Agriculture – Agricultural Research Service
Roman L. Hruska U.S. Meat Animal Research Center

- ~35,000 acres
- ~50 scientists
- ~150 support personnel
- Current population of:
  - 8000 cattle
  - 3000 sheep
  - 600 litters of pigs
- Abattoir annually harvests:
  - 100 beef
  - 500 swine

We can reduce the risk of pathogens across and throughout the meat chain

Shiga toxin-producing *E. coli* (STEC)

Salmonella



Pre-harvest management controls and interventions have mixed or contrary results

- Animal management practices
  - Clean feed and water
  - Scraping pens between use
- Feed additives
  - Seaweed extract, orange peel
  - Antibiotics: ionophores, neomycin sulfate, and oxytetracycline
- Probiotics - Lactobacillus-based direct-fed microbials
- Bacteriophages
- Vaccines - Siderophore Receptor and Porin (SRP) Protein Vaccines

Peri- harvest control measures

Lairage pens are routinely cleaned

Trucks are cleaned between loads of cattle

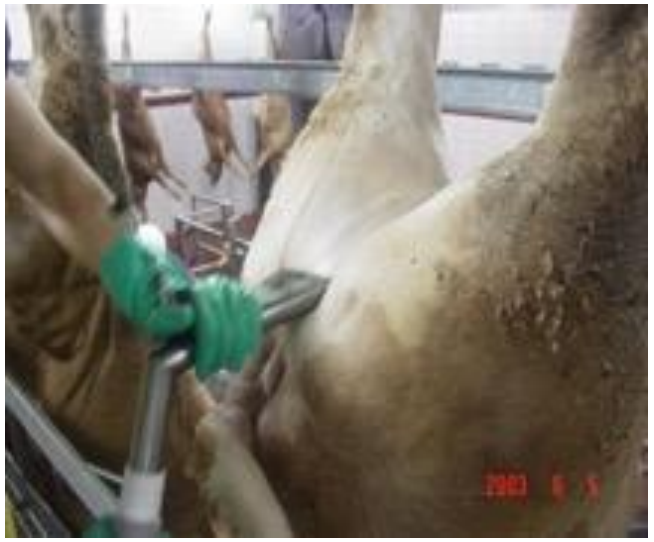
Cattle are transported in ways to avoid stress and increase shedding of pathogens

Cattle can be cleaned before slaughter

Post- harvest control measures

Hides are cleansed after stunning and exsanguination






Sanitary dressing procedures are used with careful hide removal, avoiding transferring contamination

Post- harvest control measures

Carcasses are cleansed before and after evisceration

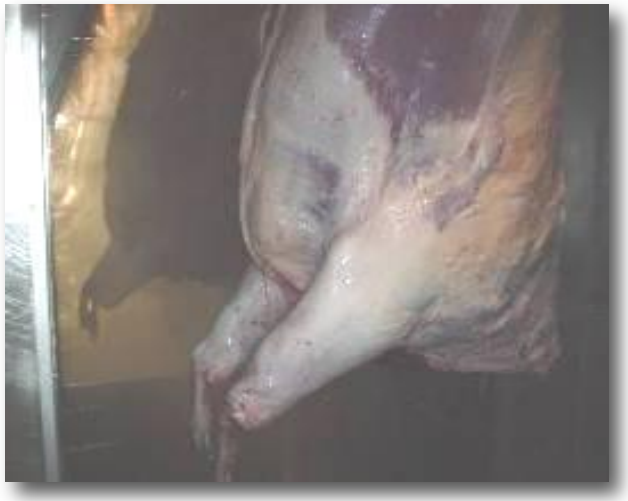


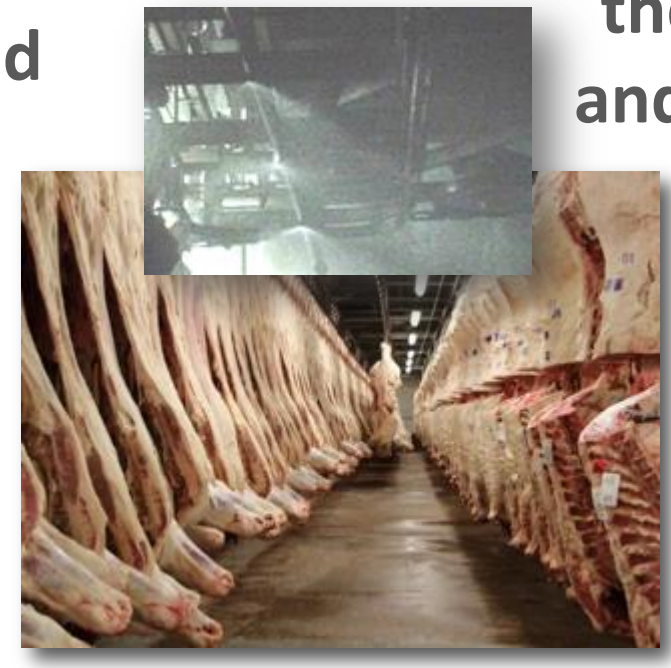

after splitting, and before chilling

Hot water, organic acids, and other antimicrobials,
as well as continuous knife trimming,

Post- harvest control measures

Finished carcasses are chilled



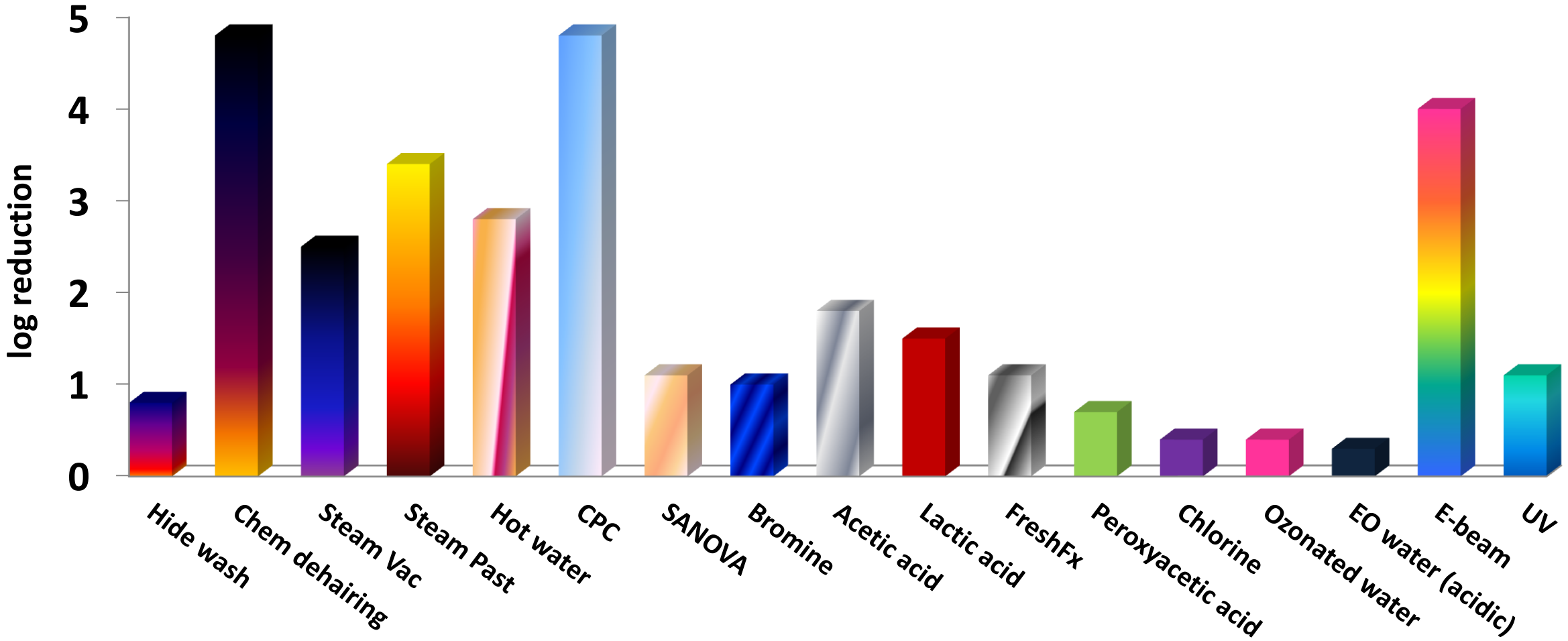


then inspected, graded, and sorted for processing

Often under a spray-chill containing an antimicrobial

Efficacy of Post-harvest Interventions

as evaluated by the Meat Safety and Quality Research Unit



Control measures work

100% STEC and *Salmonella*

0% STEC and *Salmonella*

Beef processors monitor their safety systems by routinely testing beef trimmings for *E. coli* O157:H7

Usually,
0% STEC and *Salmonella*
or very low levels (<1%).

Control measures work

0% STEC and *Salmonella*

<1% STEC and *Salmonella*

Control measures work

100% STEC and *Salmonella*

0% STEC and *Salmonella*

Beef processors monitor their safety systems by routinely testing beef trimmings for *E. coli* O157:H7

Sporadic occasional positives (<1%) show that this system of monitoring works and that safety systems are functioning.

Positive tests are referred to as "*Events*"

O157 O121 O111 O45 O103 O145 O26

E. coli O157:H7 Events

- Event: sporadic *E. coli* O157:H7 positive.
- High Event Period (HEP): multiple positive lots are clustered in a short time frame.
- FSIS definition: production interval during which slaughter establishments experience high rates of positive results for *E. coli* O157:H7 (or Shiga-toxigenic *E. coli* [STEC] or virulence markers) in trim samples.
- The cause/source for a HEP is not identified, and the contamination event will often be resolved before notable correction of the process can be performed.

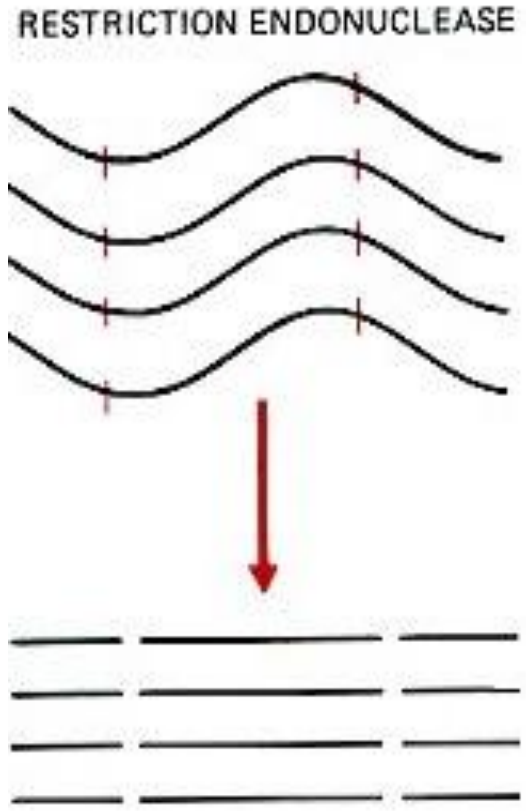
High Event Period *E. coli* O157:H7

- To understand HEP contamination events
  - *E. coli* O157:H7 isolated from beef trim during HEPs were molecular typed.
    - multiple trim lots and time points within a HEP
    - across multiple HEP at different plants
  - Is HEP contamination derived from multiple sources (harvest floor) or a single point source (processing).

  - Molecular typing *E. coli* O157:H7
    - PFGE (Pulsed-field gel electrophoresis)
    - Highly discriminative molecular typing technique that is used in epidemiological studies worldwide.
    - Based upon the variable migration of large DNA restriction fragments that allows us to compare the fingerprints of any two isolates.

Molecular typing *E. coli* O157:H7

- PFGE
- *E.coli* DNA is digested with a restriction enzyme
  - Cuts DNA at very specific locations.
- Digested DNA fragments are separated on a gel.
- Gel is stained and fingerprint is revealed for analysis.

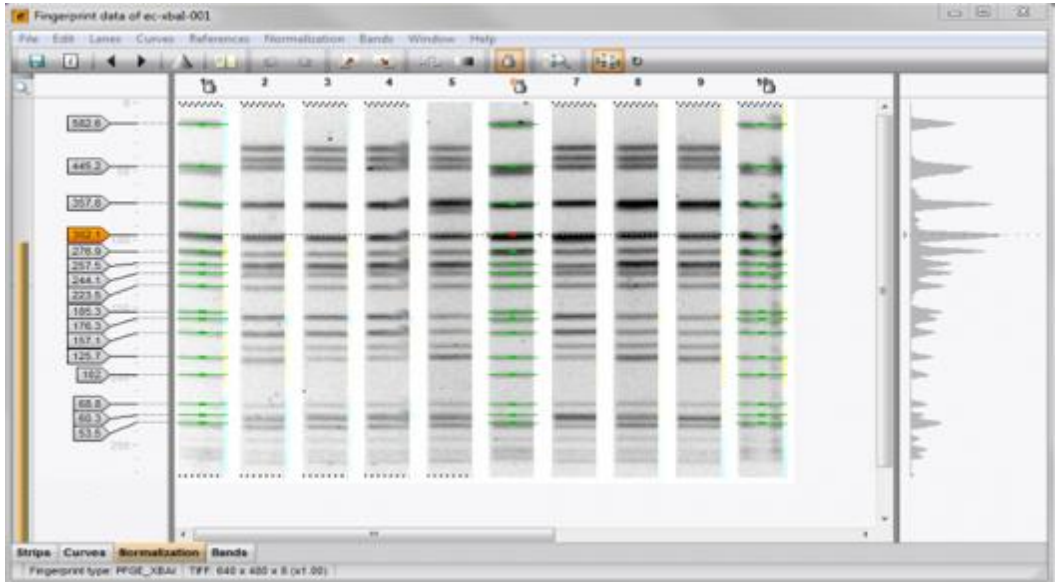


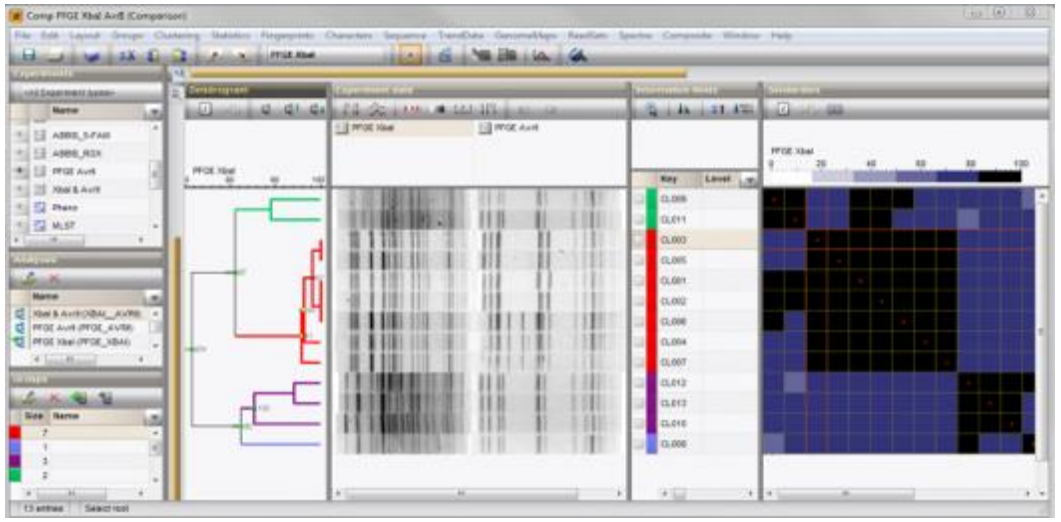
- Fingerprint – Restriction Digest Pattern (RDP)

PFGE data analysis

Each lane of digested genomic DNA is analyzed and compared to a standard run in each gel. The use of the standard normalizes the PFGE Restriction Digest Pattern (RDP) for comparison to patterns from other gels and present in data bases.

Analysis software can also create dendrograms and show apparent relatedness between RDPs of different strains.





Source: http://www.bio.davidson.edu/courses/genomics/method/pulse_field.html

High Event Period *E. coli* O157:H7

What do "Normal" *E. coli* O157:H7 PFGE types look like?

100 cattle hide samples collected each day for 3 days

Processing plant	Day	No. of isolates	No. of unique RDP
1	1	36	18
	2	76	24
	3	26	12
2	1	29	6
	2	30	12
	3	48	9
3	1	38	10
	2	22	7
	3	26	7

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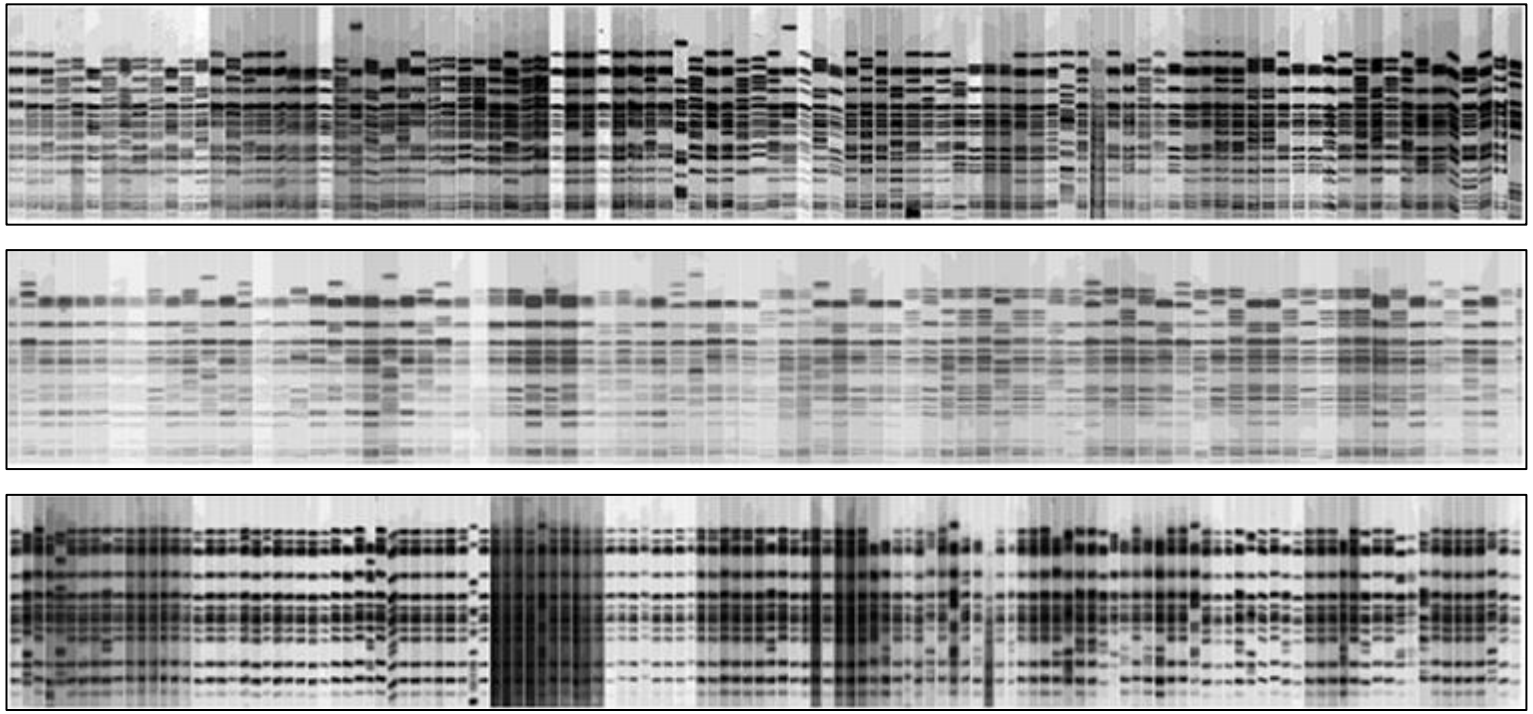
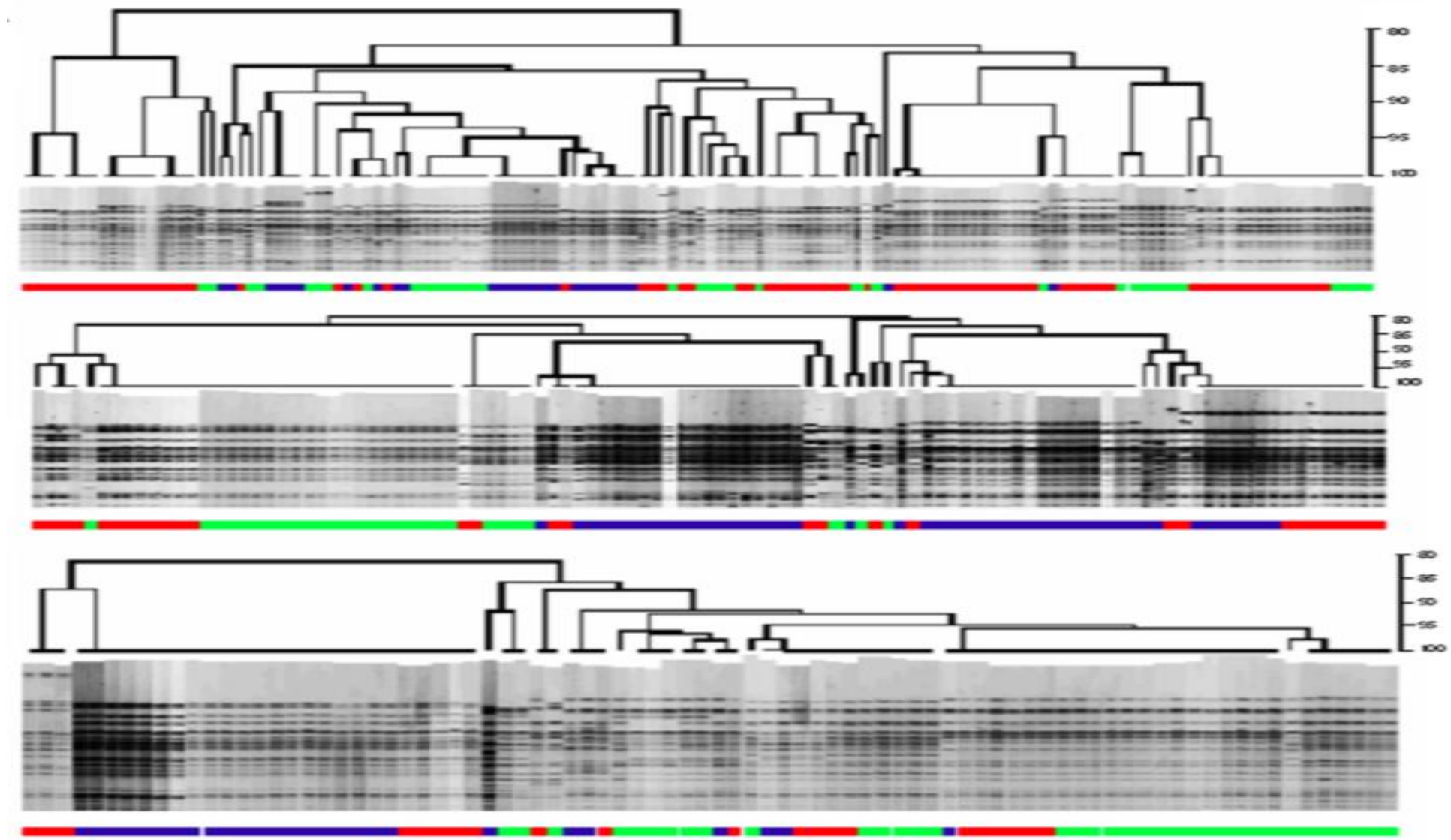


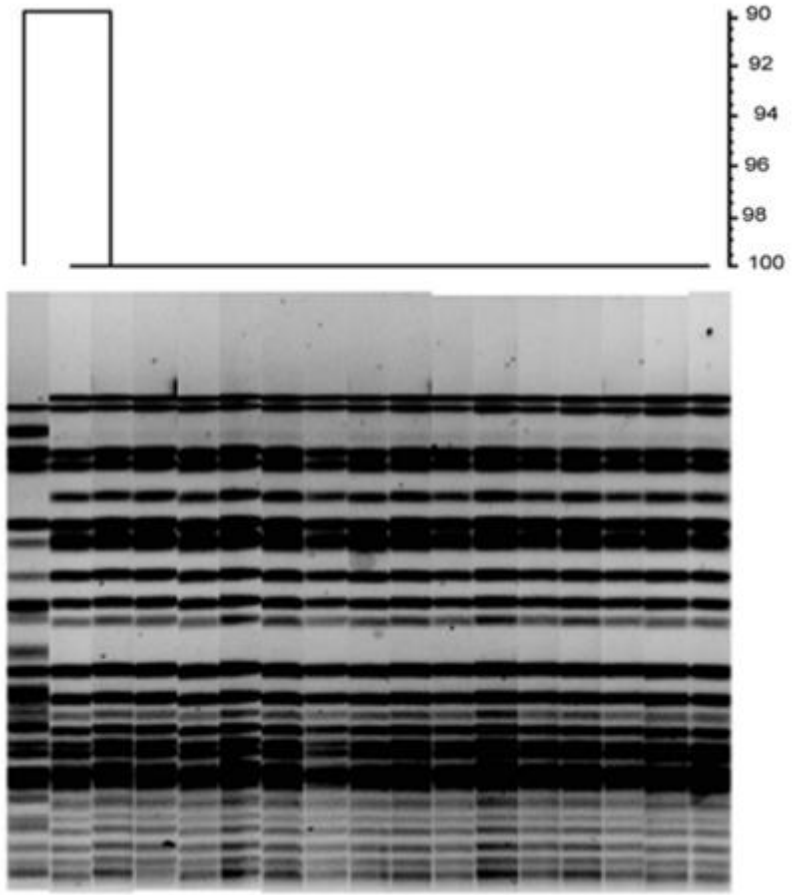
Diversity of incoming *E. coli* O157:H7 isolates on cattle hides by individual lots, *E. coli* isolates are in sequential order for each animal in a lot.

High Event Period *E. coli* O157:H7



High Event Period *E. coli* O157:H7

E. coli O157:H7 PFGE types from a typical HEP.



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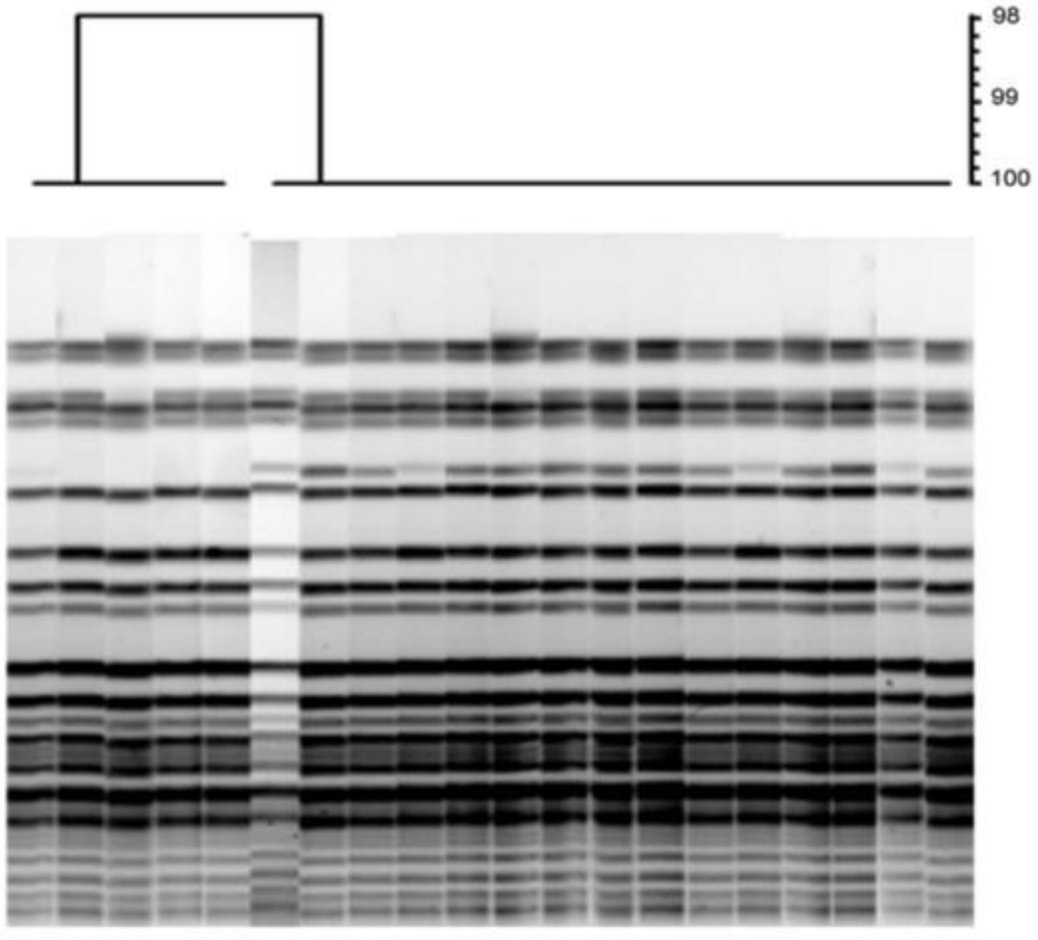

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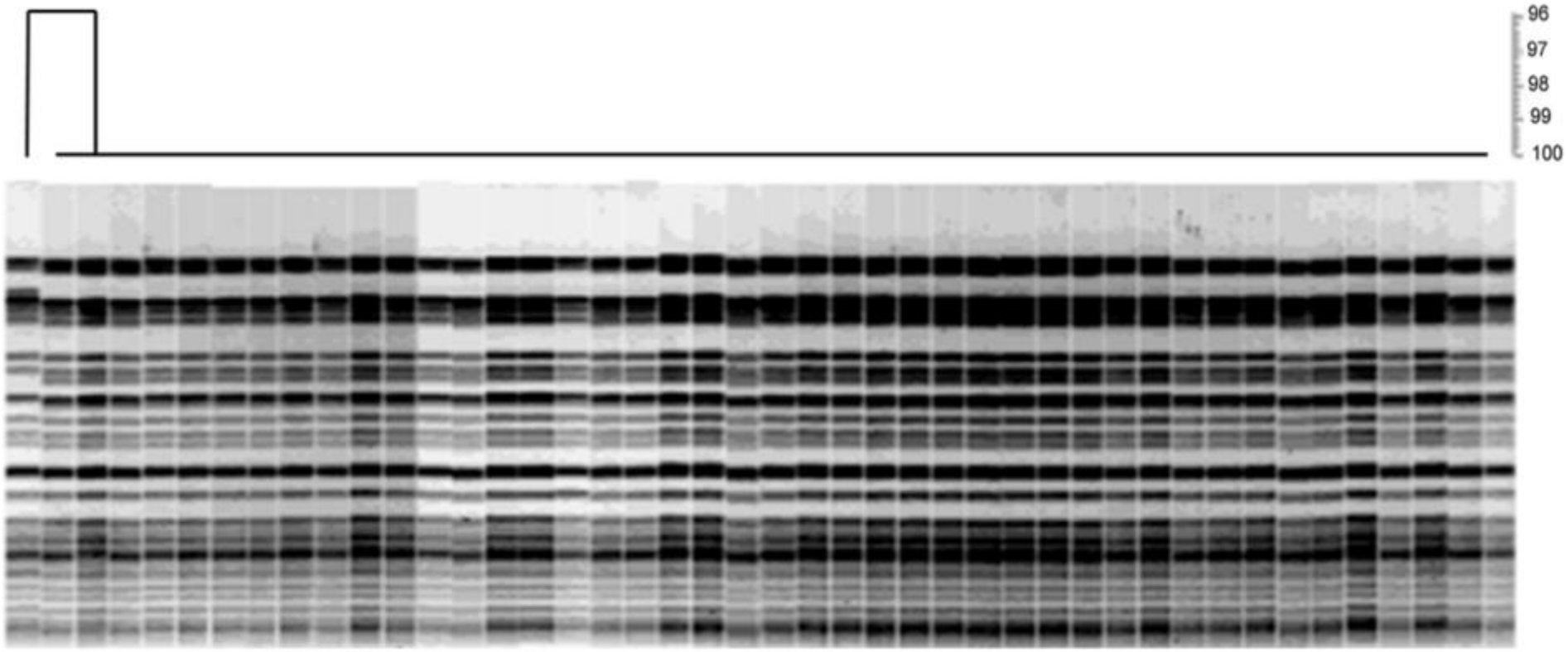


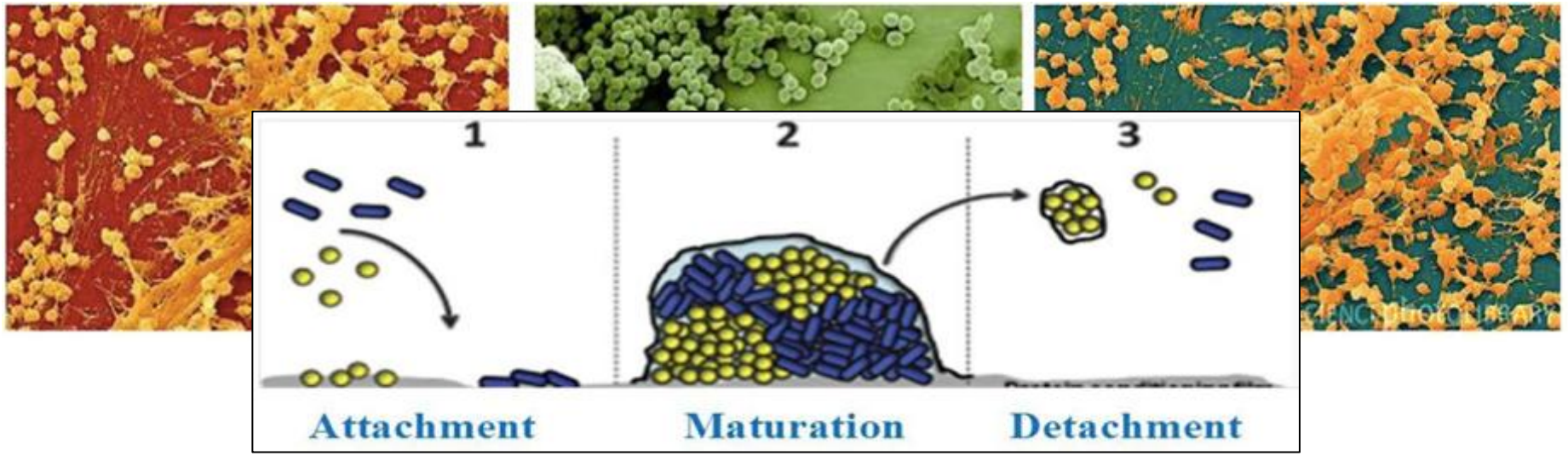
High Event Period *E. coli* O157:H7

- *E. coli* O157:H7 isolated from trim during a HEP are closely related.
  - 86% or more of the *E coli* are closely related
- Most *E. coli* O157:H7 isolated from trim during a HEP are identical.
  - Half the HEPs examined were contaminated by strains with identical patterns.
  - ¾ or more of the strains in the other HEPs were identical.
  - One exception, 50% identical, but 100% closely related.

HEP	No. of positive enrichments received	No. of enrichments from which an isolate was obtained	No. (%) of isolates identical to predominant RDP	No. (%) of isolates closely related to predominant RDP
A	8	8	8 (100)	8 (100)
B	16	9	9 (100)	9 (100)
C	11	10	9 (90)	9 (90)
D	9	9	9 (100)	9 (100)
E	7	7	6 (86)	6 (86)
F	12	8	7 (88)	8 (100)
G	7	6	6 (100)	6 (100)
H	21	18	13 (72)	18(100)
I	20	20	15 (75)	20 (100)
J	20	17	16 (94)	16 (94)
K[b]	32	10	10 (100)	10 (100)
L	9	9	9 (100)	9 (100)
M	13	12	11 (92)	11 (92)
N	18	18	9 (50)	16 (89)
O	44	44	43 (98)	44 (100)
P	65	61	61 (100)	61 (100)
Q	50	50	50 (100)	50 (100)
R	50	35	33 (94)	35 (100)
S	44	43	42 (98)	42 (98)
T	17	15	15(100)	15 (100)
U	166	157	157 (100)	157 (100)

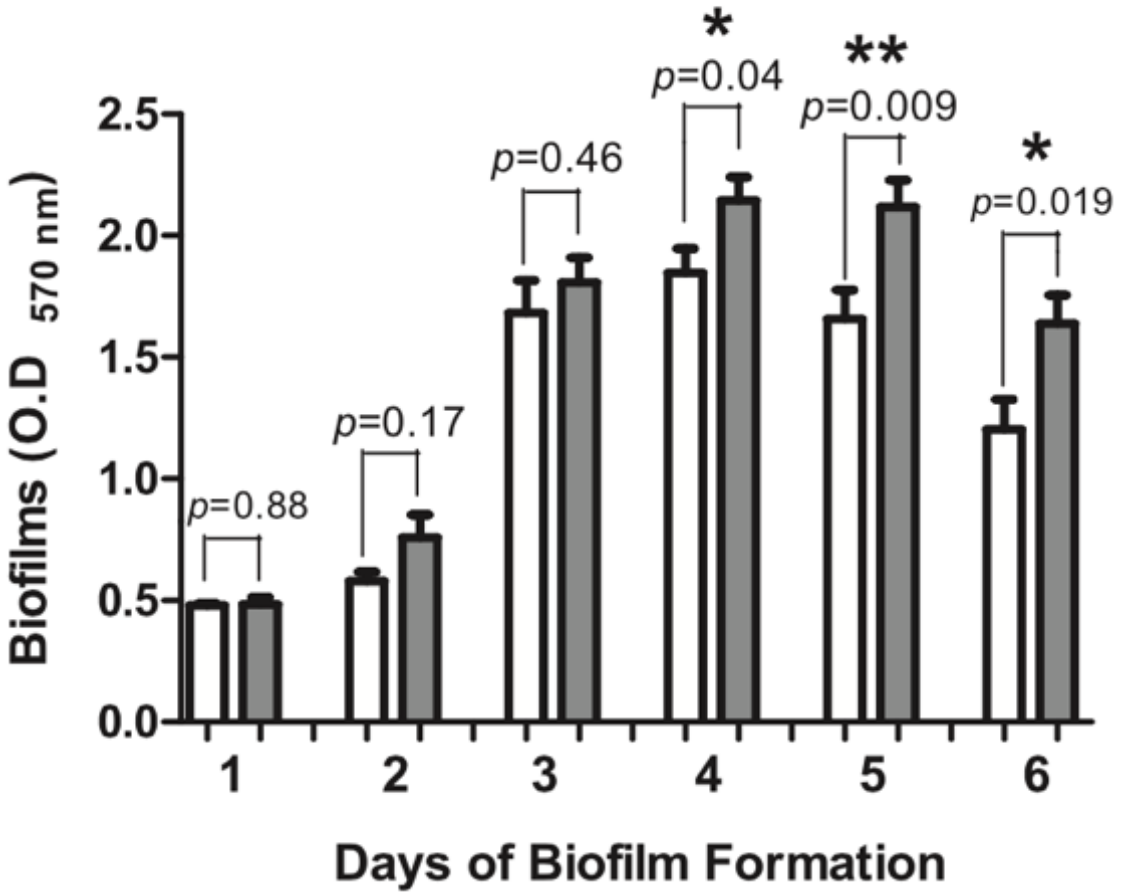
High Event Period *E. coli* O157:H7

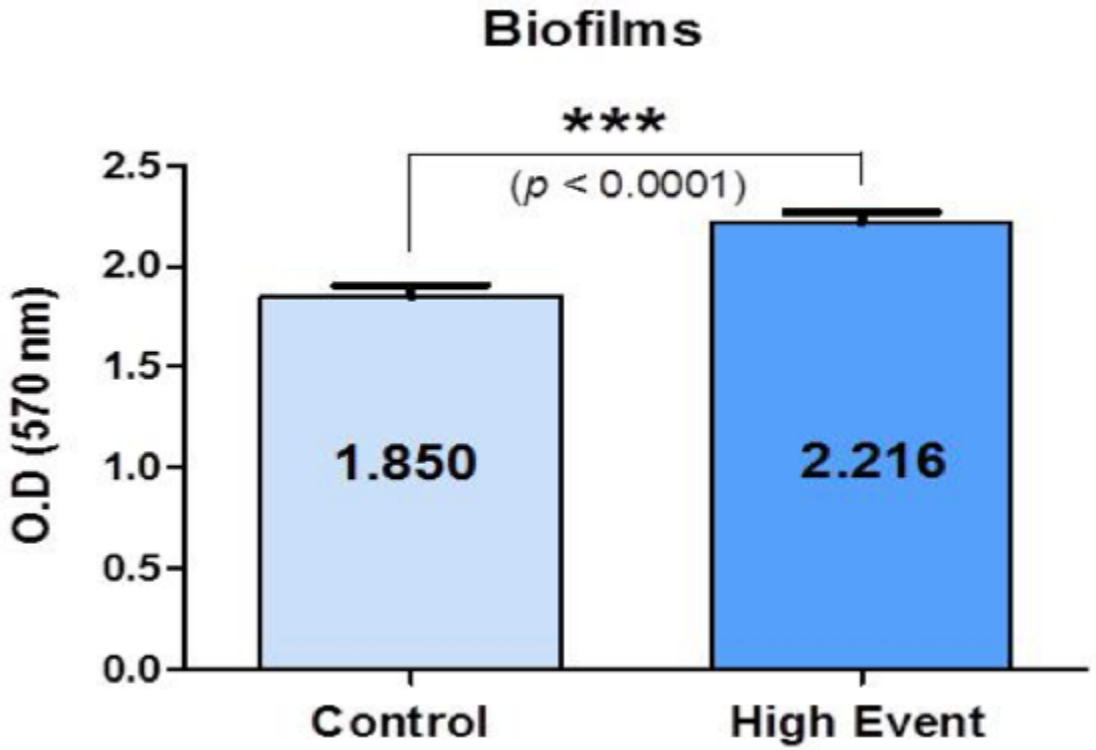
- So what's going on?
  - Could certain HEP *E. coli* be able to adhere to carcasses and/or resist interventions and make it off the harvest floor and into processing areas?
  - Could HEP *E. coli* be present in processing areas persisting in biofilms?
    - Evidence?
    - Biofilms: bacteria colonized on solid surfaces in a 3-D structure.



HEP *E. coli* O157:H7 biofilms

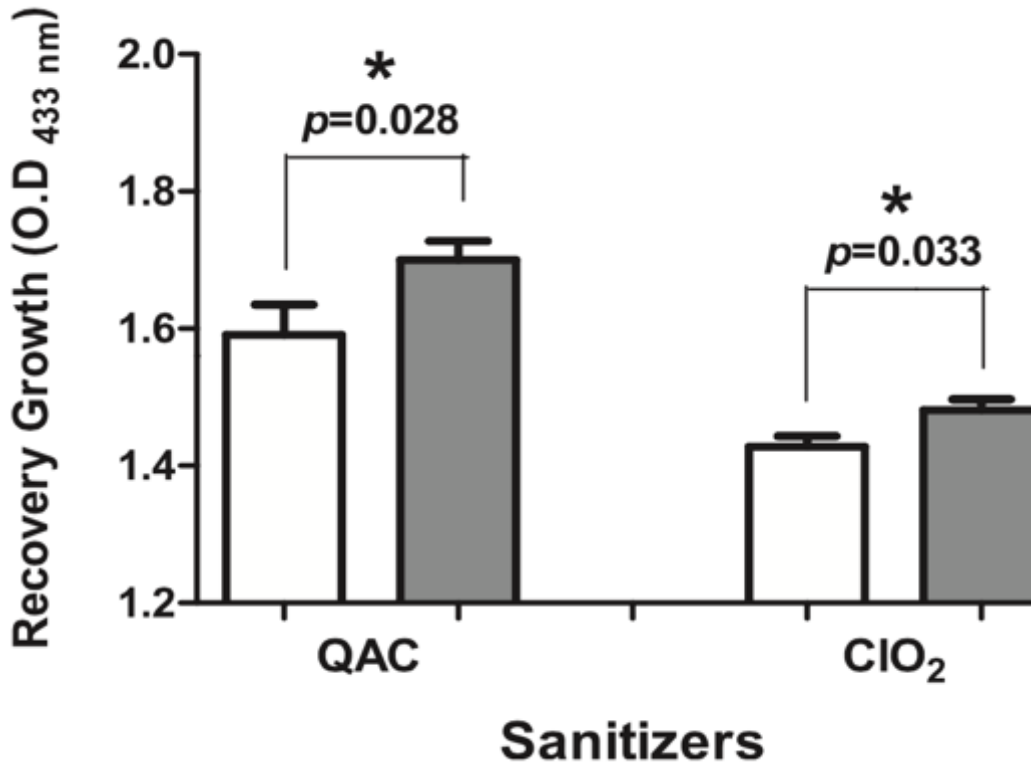
- HEP *E. coli* O157:H7 are stronger biofilm formers than non-HEP *E. coli* O157:H7





HEP *E. coli* O157:H7 biofilms

- HEP *E. coli* O157:H7 in biofilms are more resistant to sanitizers than non-HEP *E. coli* O157:H7



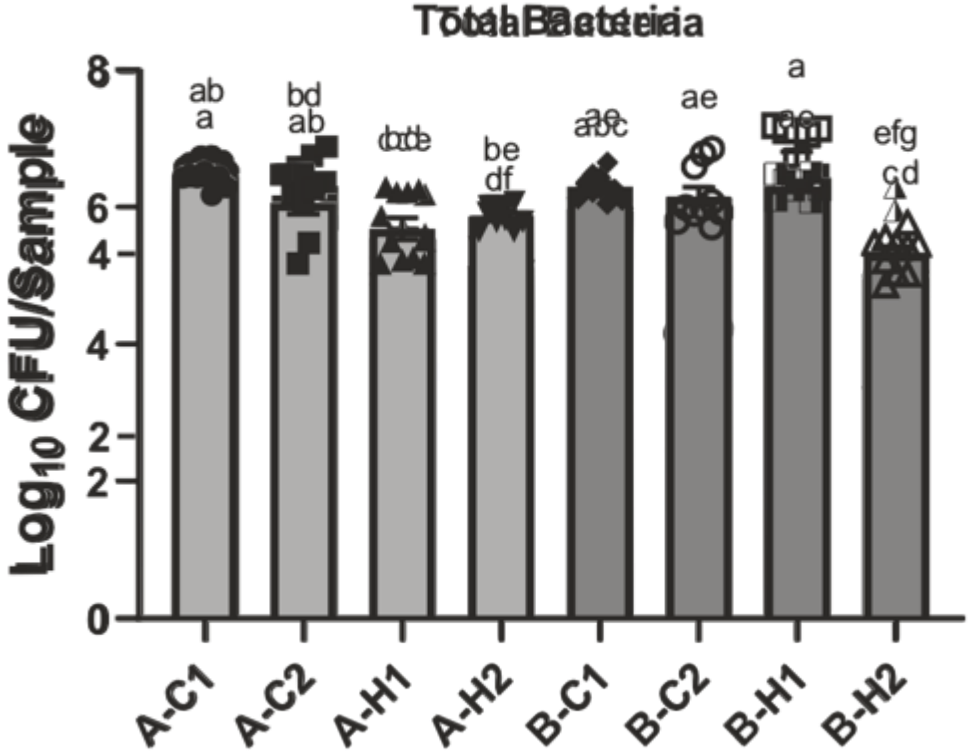
However, single strain biofilms of bacteria do not typically exist naturally, instead a biofilm is made up of multiple species, genera, families, and phyla.

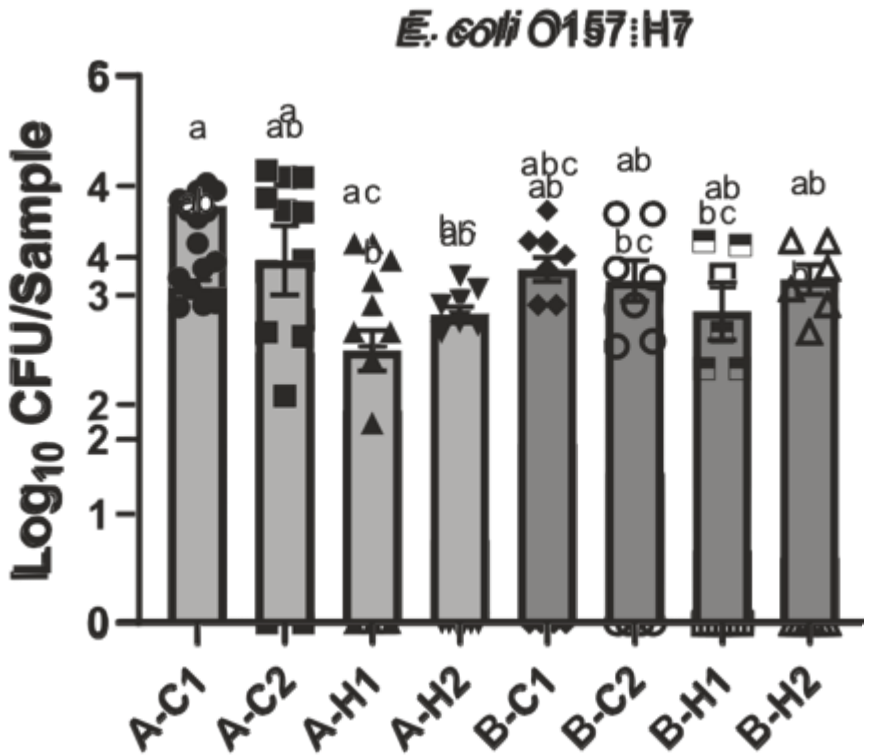
Certain biofilms protect *E. coli* O157:H7

- Biofilms present in processing plant drains represent the various microbial species present in the surrounding environment.
- Some of these biofilms can protect *E. coli* O157:H7 from sanitizers.

Certain biofilms protect *E. coli* O157:H7

- Visited a plant experiencing HEPs and a control plant and collected biofilm samples from floor drains Hot Boxes and Coolers.
  - Co-cultivated *E. coli* O157:H7 in each biofilm.
  - Treated the biofilms with a quaternary ammonium compound (QAC) sanitizer.





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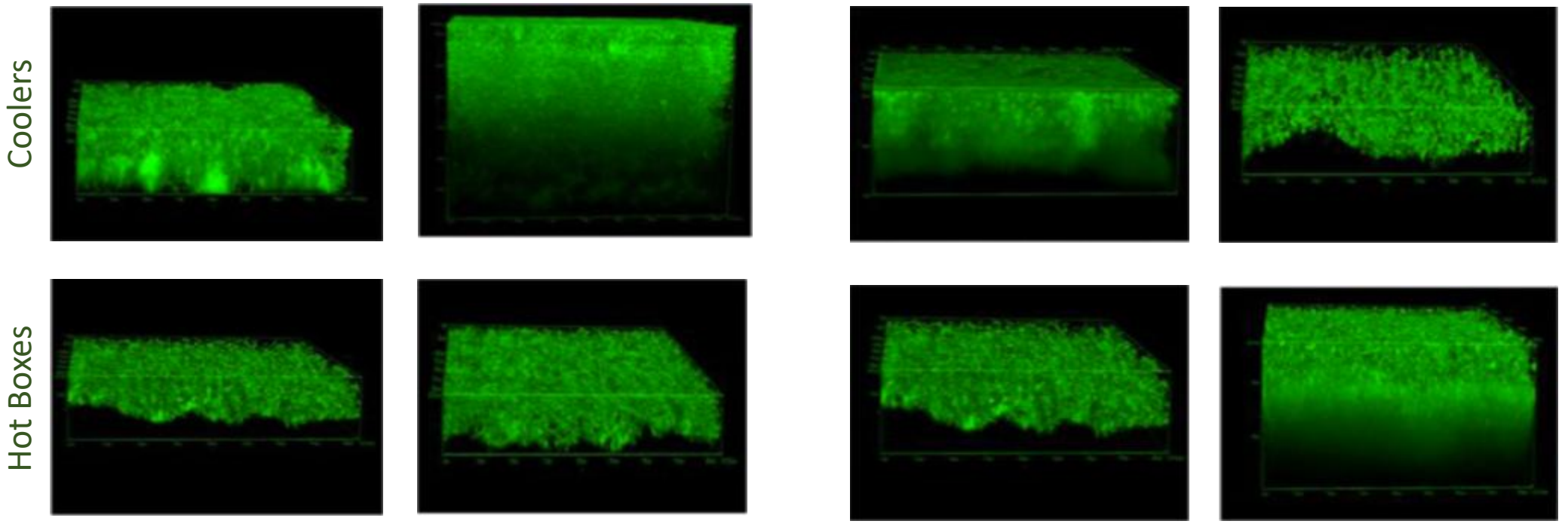
- Visited a plant experiencing HEPs and a control plant and collected biofilm samples from floor drains Hot Boxes and Coolers.
  - Co-cultivated *E. coli* O157:H7 in each biofilm.
  - Examined 3D structures with confocal laser scanning microscopy



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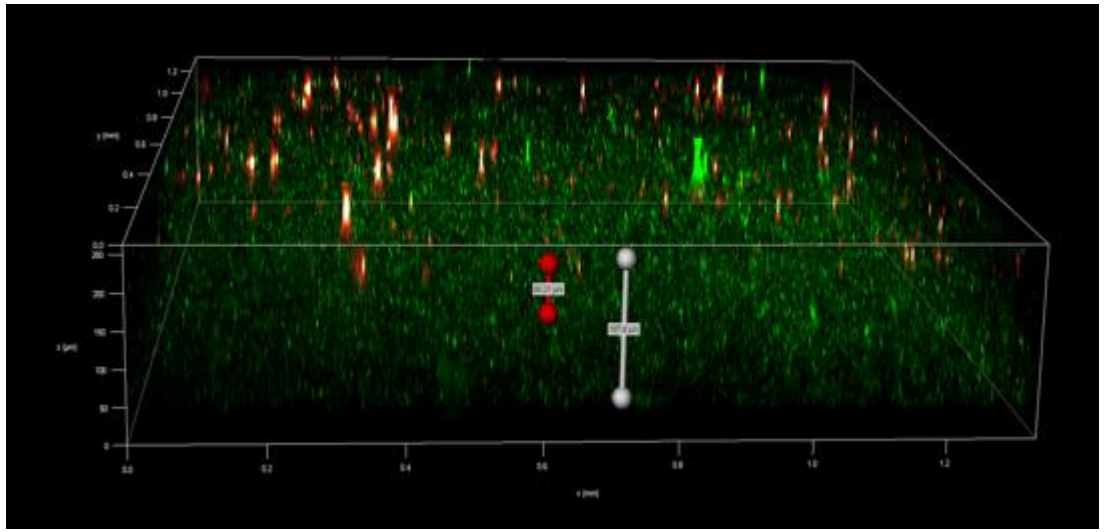
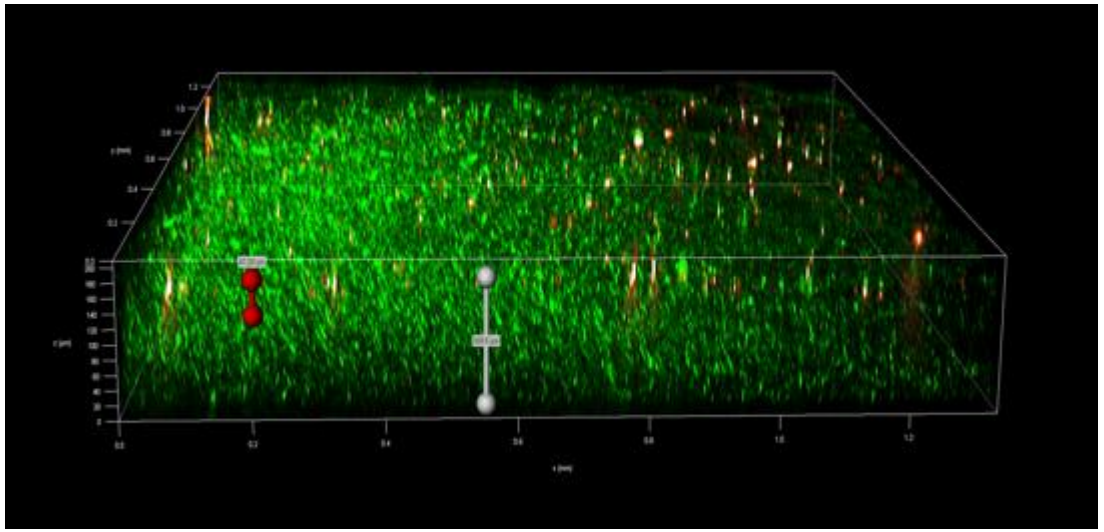
  - Co-cultivated *E. coli* O157:H7 in each biofilm.
  - Examined 3D structures with confocal laser scanning microscopy
  - Included counter fluorescing *E. coli* O157:H7s





In both cases, *E. coli* O157:H7 locates to the upper region of the biofilm structure

Certain biofilms protect *E. coli* O157:H7

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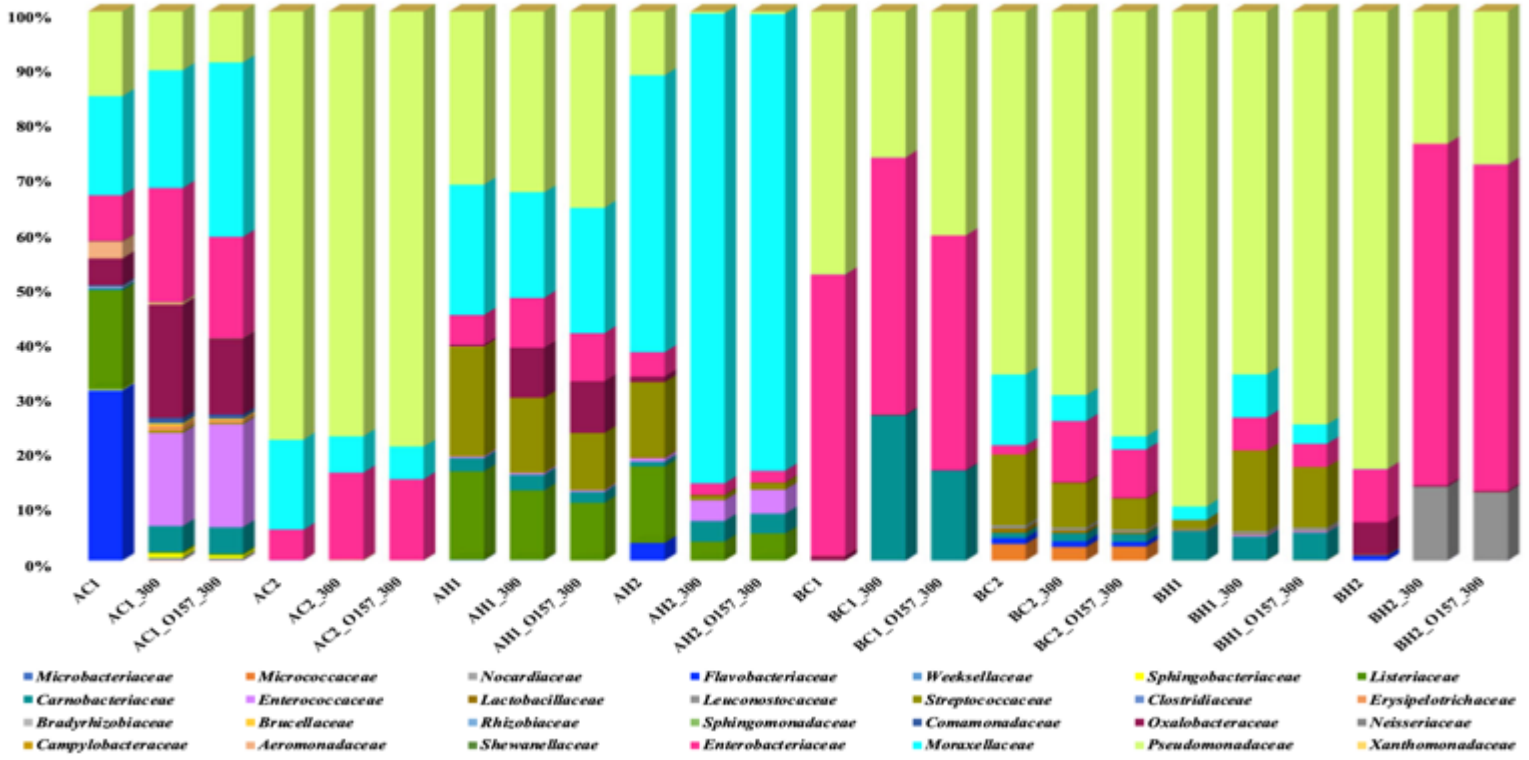
  - Co-cultivated *E. coli* O157:H7 in each biofilm.
  - Examined the metagenomic community composition (16S rRNA sequencing)



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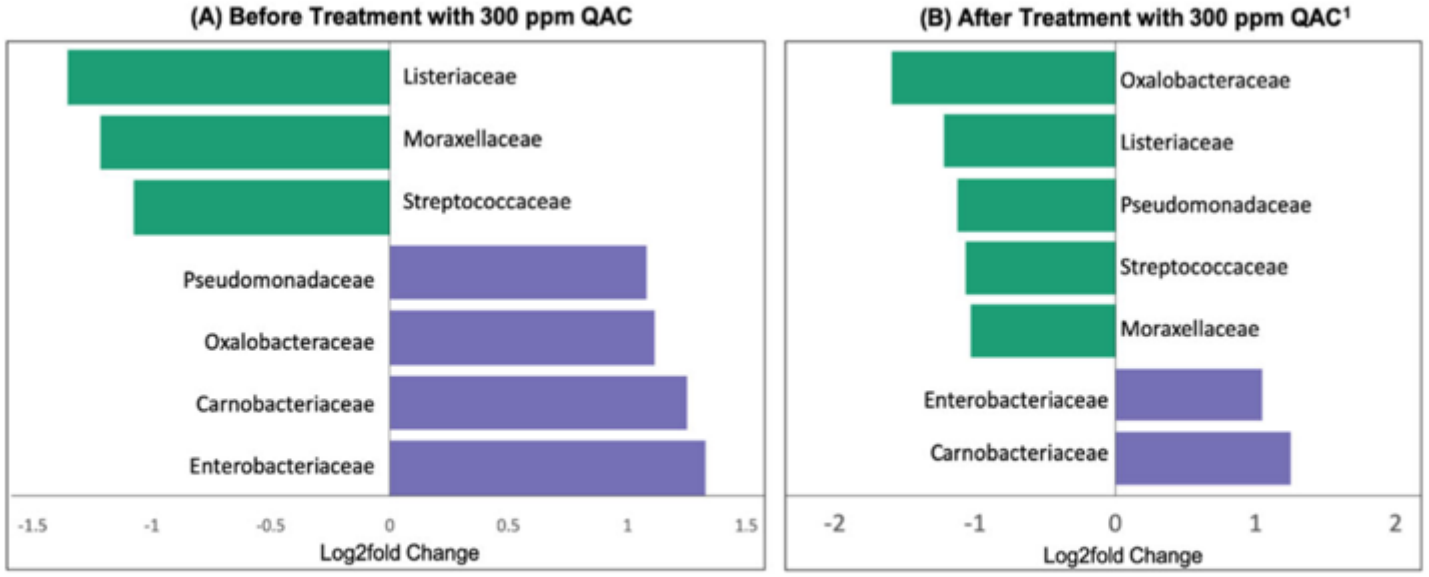
  - Co-cultivated *E. coli* O157:H7 in each biofilm.
  - Examined the metagenomic community composition (16S rRNA sequencing)
  - Analyzed the community structures comparing the non-protector to the protector biofilms.



[1] While the Leuconostocaceae family was found in more than 1% of the microbiome, the sparseness of it in the protector (0.35 CSS average) verses the non-protector (7993.26 CSS average) group made the log2fold change comparison not meaningful.

Conclusions

- Despite the use of pre-, peri-, and post- harvest control measures contamination events can still be identified after processing carcasses that were low risk of being contaminated.
- When HEPs of *E. coli* O157:H7 were investigated, the strains were found to be closely related within the HEP, and HEP O157:H7 strains formed stronger biofilms than control O157:H7 strains.
- Certain bacteria making up microbial communities in different zones meat processing environments (coolers, boning/fabrication lines) formed stronger biofilms, tolerated routine sanitization steps, and protected pathogens.
- These results suggest that HEPs and other contamination events mat be a result of pathogens harbored in the boning/fabrication environment.
- Even within a safety system of successful interventions, contamination can and does occur at sites after harvest and these sites should be considered and included in a risk-based meat safety assurance system.

Questions?

Beef Trim Sampling

Beef Trim Sampling Device

- Continuous sampling devise (CSD), collects sample from throughout combo as it fills.
- Provides an organism recovery greater than or equal to, N60 or N60+ methods for detection pathogen detection.
- Surface samples collection of organisms that eliminates the loss of product through N60/N60+.




New Trim Sampling Device

- Manual method most popular.
  - Scrub trim for 90 seconds.
  - Firmly with constant pressure.
  - Reaching in and around pieces of trim.