Sources of *E. coli* O157:H7 and Interventions to Reduce/Eliminate Pathogens on Beef

Mick Bosilevac

U.S. Meat Animal Research Center

USDA-ARS

Clay Center, Nebraska

Presentation outline

- *E. coli* O157:H7 basics.
- Why focus on *E. coli* O157:H7?
- What are the sources of pathogens on beef and how do they get there?
- Interventions to combat it comtamination.

E. coli O157:H7: Basics

Shiga

Toxin producing

Escherichia

Coli

STEC

STEC virulence factors

Shiga toxins - two types: *stx*1 and *stx*2
- main virulence attribute
- ribosome inactivating proteins (cell death)
- primarily attack kidneys and brain

Intimin (*eae)* – attachment to human intestinal cells

EHEC-hemolysin – iron acquisition?

Nomenclature

E. coli O157:H7

E. coli serotyping

O157:H7

O111:H8

O26:H11

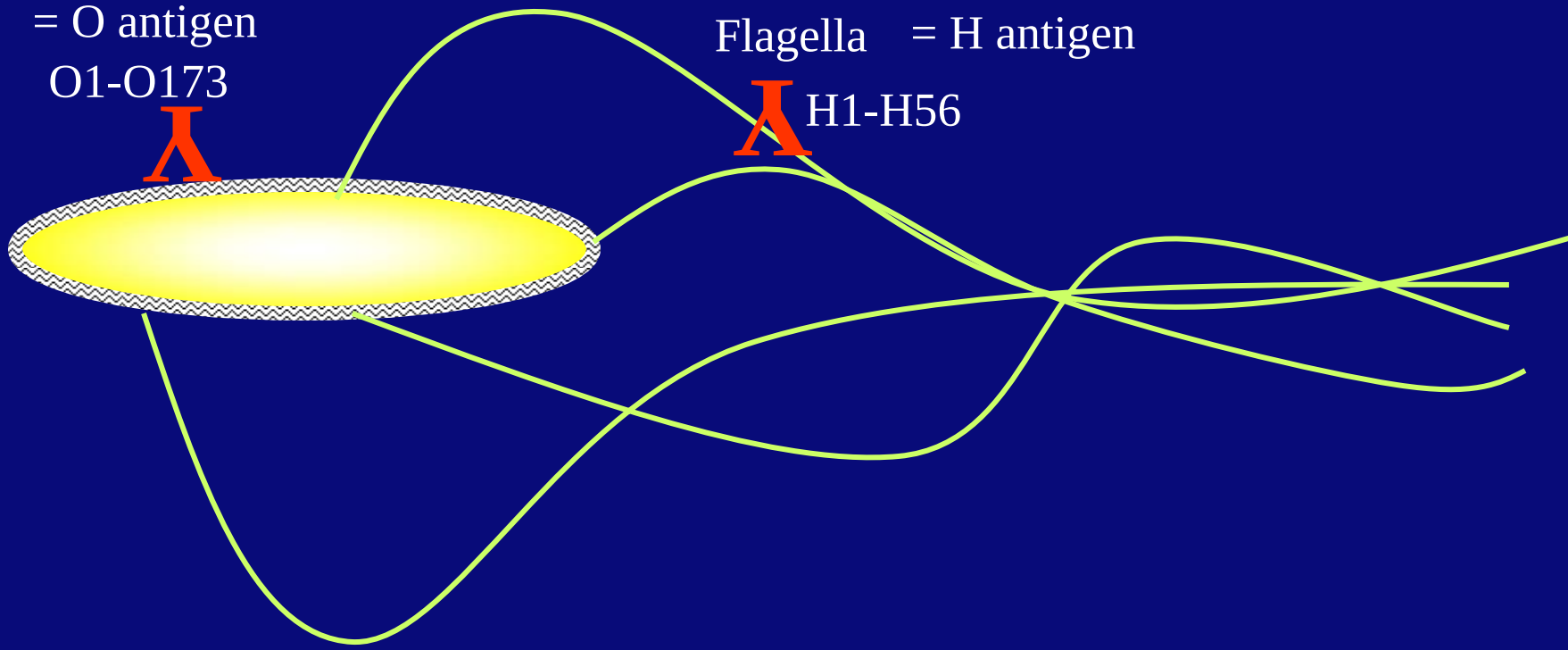
Lipopolysaccharide (LPS)

= O antigen

O1-O173

Flagella = H antigen

H1-H56



E. coli O157:H7

- Although E. coli O157 is found in the intestinal tracts of chickens, deer, sheep and pigs, cattle are considered to be the major reservoir.

- Although other foods have been implicated in O157 outbreaks, the majority of the cases have been linked to consumption of undercooked ground beef.

E. coli O157:H7

- Like other human diseases, the bacterium present the most danger to children, elderly and immuno-compromised.

Why Focus on *E. coli* O157:H7?

Estimated illness, hospitalization and death caused by foodborne pathogens

Agent	Illness	Hospitalization	Deaths
Bacterial	30.2%	59.9%	71.7%
parasitic	2.6%	5.3%	21.2%
Viral	67.2%	34.8%	7.1%

Incidence (per 100,000 population) of cases of bacterial infection – Foodborne Disease Active Surveillance Network, United States, 2005

Bacteria	Incidence
Campylobacter	12.72
Listeria	0.30
Salmonella	14.55
Shigella	4.67
STEC – O157	1.06
STEC, Non-O157	0.33

Estimated illness, hospitalization and death caused by foodborne pathogens

Bacteria	Deaths
L. mono.	27.6%
Salmonella	30.6%
O157:H7	2.9%

Why Focus on *E. coli* O157:H7?

Why Focus on *E. coli* O157:H7?

Adulterant

In October 1994, in response to an outbreak of foodborne illness that resulted in several deaths from the consumption of undercooked ground beef contaminated with *Escherichia coli* (*E.coli*) O157:H7, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) declared *E. coli* O157:H7 an adulterant in raw ground beef and began a sampling program to test for *E. coli* O157:H7 in raw ground beef prepared in federally inspected plants and in retail stores.

FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food

Year	Population	Confirmed cases	Campylobacter	*Salmonella*	*E. coli* O157	Non-O157
1996	13.2	7,223	23.5	14.5	2.7	-
1997	16.11	8,576	25.3	13.6	2.3	-
1998	20.72	9,787	21.4	12.3	2.8	-
1999	25.85	10,697	17.5	13.6	2.1	-
2000	30.54	12,631	15.4	14.2	2.0	0.19
2001	34.33	13,705	13.8	15.3	1.6	0.18
2002	37.96	16,580	13.3	16.2	1.7	0.09
2003	41.50	15,600	12.6	14.4	1.06	0.11
2004	44.1	15,806	12. 8	14.6	0.90	0.25
2005	44.5	16,614	12.72	14.55	1.06	0.33

U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) Microbiological Results of Raw Ground Beef Products Analyzed for *Escherichia coli* O157:H7

Year	# of positives	# Tested	% Positive
1994	0	891	0.0
1995	3	5407	0.05
1996	4	5703	0.07
1997	4	6065	0.07
1998	14[a]	8080	0.17
1999	32[b]	7785	0.4
2000	55	6375	0.86
2001	59	6770	0.87
2002	55	6708	0.82
2003	20	6392	0.31
2004	14	7603	0.18
2005	19	10975	0.17
2006	20	10510	0.19

[a] During October 1997, the amount analyzed was increased from a 25 g sample to a 325 g sample to provide increased detection sensitivity.

[b] On September 3, 1999, a new selection and detection method was introduced to further increase test sensitivity.

What are the sources of pathogens on beef carcasses and how do they get there?

Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides and carcasses of beef cattle during processing.

U.S. Meat Animal Research Center

Clay Center, Nebraska

Agricultural Research Service
United States Department of Agriculture

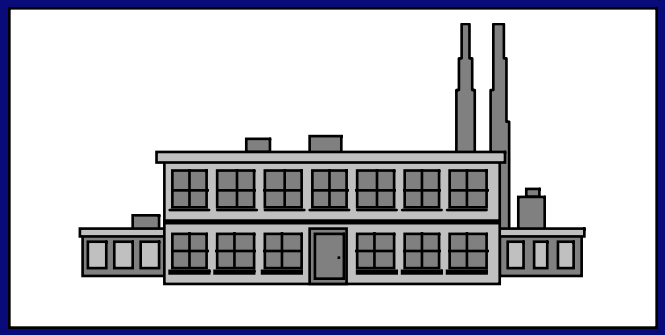
Problem:

Does prevalence of *E. coli* O157 infection of beef cattle influence contamination of carcasses?

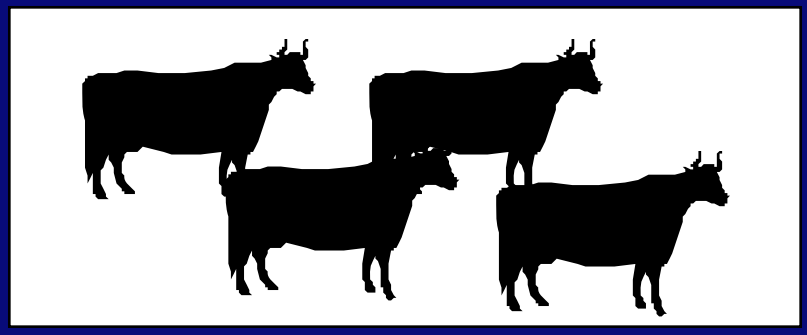
Alternative possibilities:

- airborne contamination
- plant personnel
- other?

E. coli O157:H7/NM in-plant study

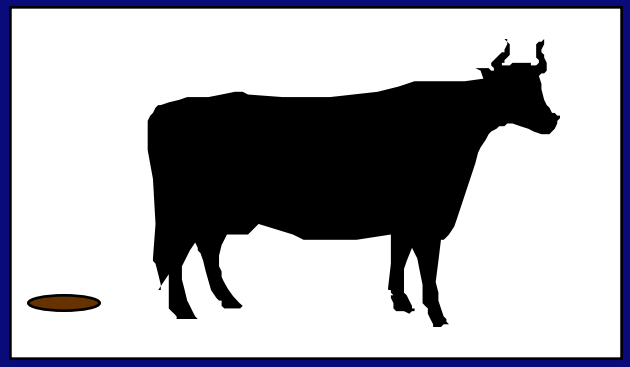


4 large packing plants, two trips each



3-4 lots of 35-85 animals each trip

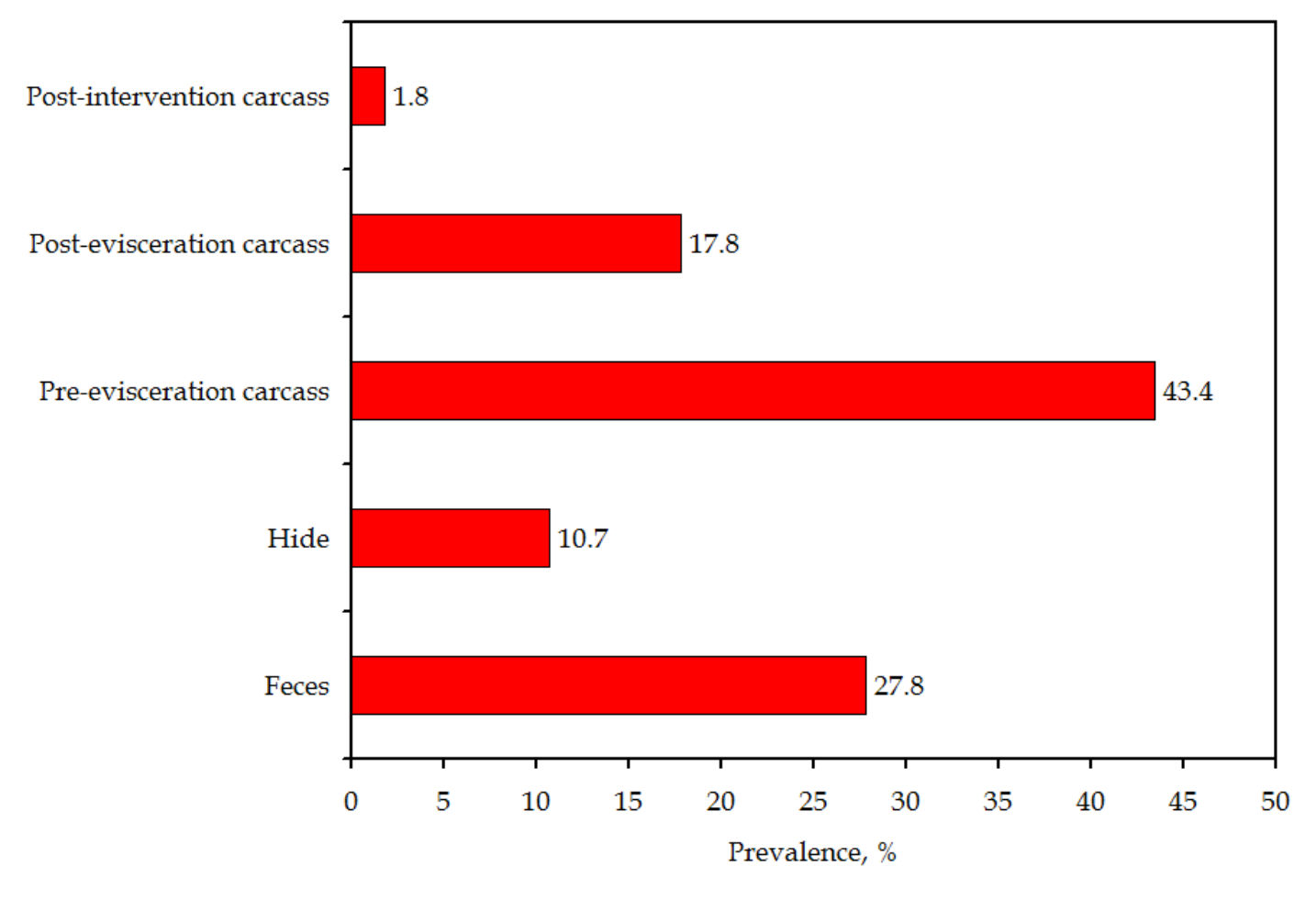
Sample 20% of each lot:



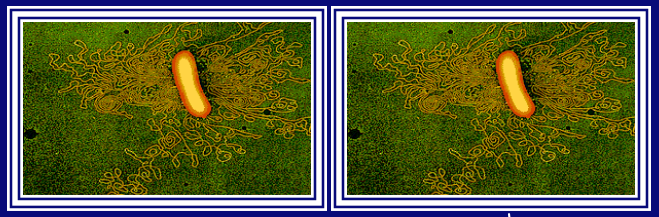
Preharvest: hides, feces

Postharvest (tracked carcasses): preevisceration, postevisceration, and after final interventions (in the cooler)

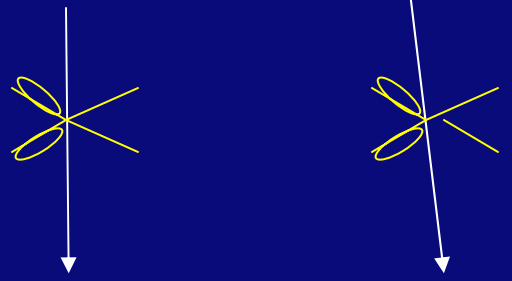
Prevalence of *E. coli* O157 in four large beef processing plants



E. coli O157:H7/NM genomic fingerprinting by PFGE

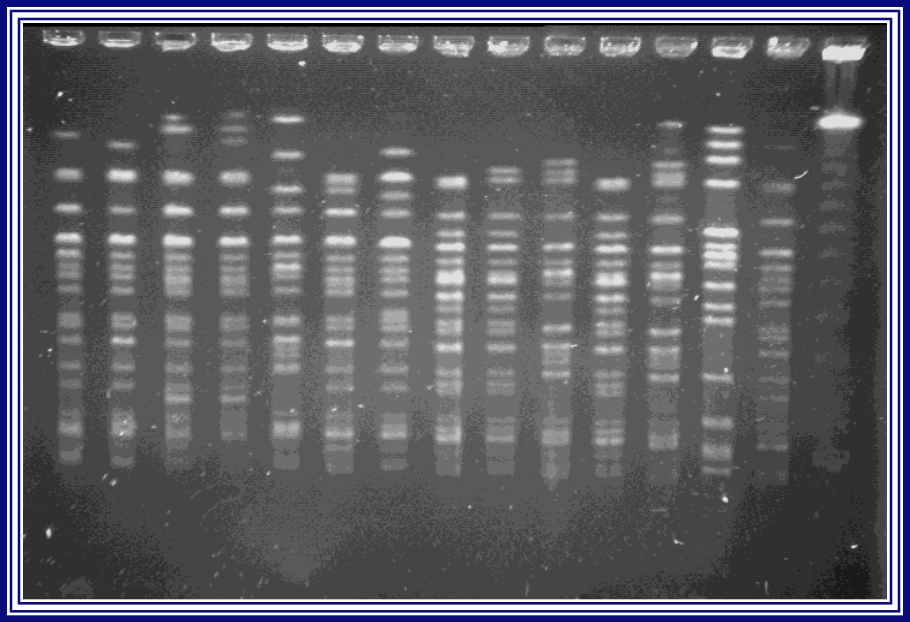


E. coli isolates are treated to release their DNA

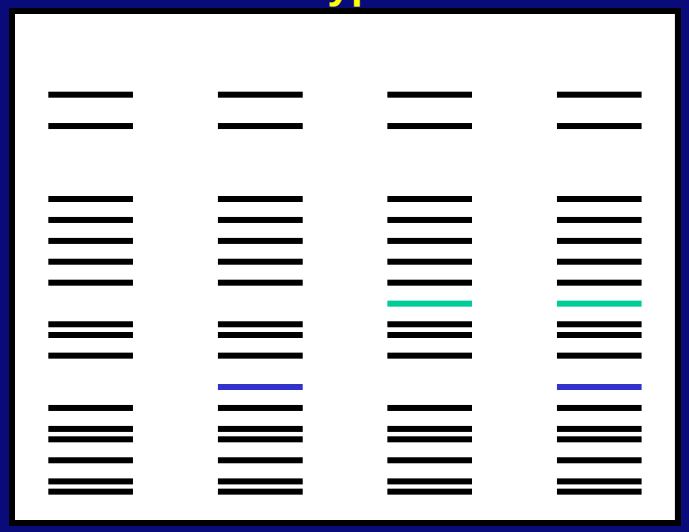


The DNA is cut into large pieces

The pieces are size- separated into “fingerprints”



Type 1



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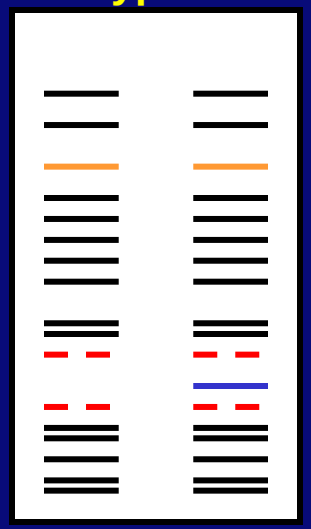

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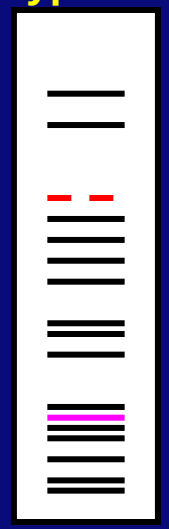


PFGE Results

- 343 isolates
- 77 fingerprint patterns (grouped into 47 types).

Conclusions

- O157 more common than previous estimates.
- The genetic fingerprints of O157 on the carcasses were the same as the genetic fingerprints associated with the cattle in that lot.
- Carcasses are contaminated before evisceration… i.e., the contamination occurs during hide removal.

Effect of season on the incidence of *E. coli* 0157:H7 at various stages during the processing of beef carcasses in three Nebraska beef processing plants (n = 1,200)

	Hide	Feces	Pre-evisceration carcass	Post-wash carcass
Spring	73.8[a]	3.9[bc]	38.9[a]	3.1[a]
Summer	73.5[a]	12.9[a]	40.8[a]	1.0[b]
Fall	67.2[a]	6.8[b]	27.3[b]	1.0[b]
Winter	29.4[b]	0.3[c]	1.2[c]	0.0[b]

Conclusion

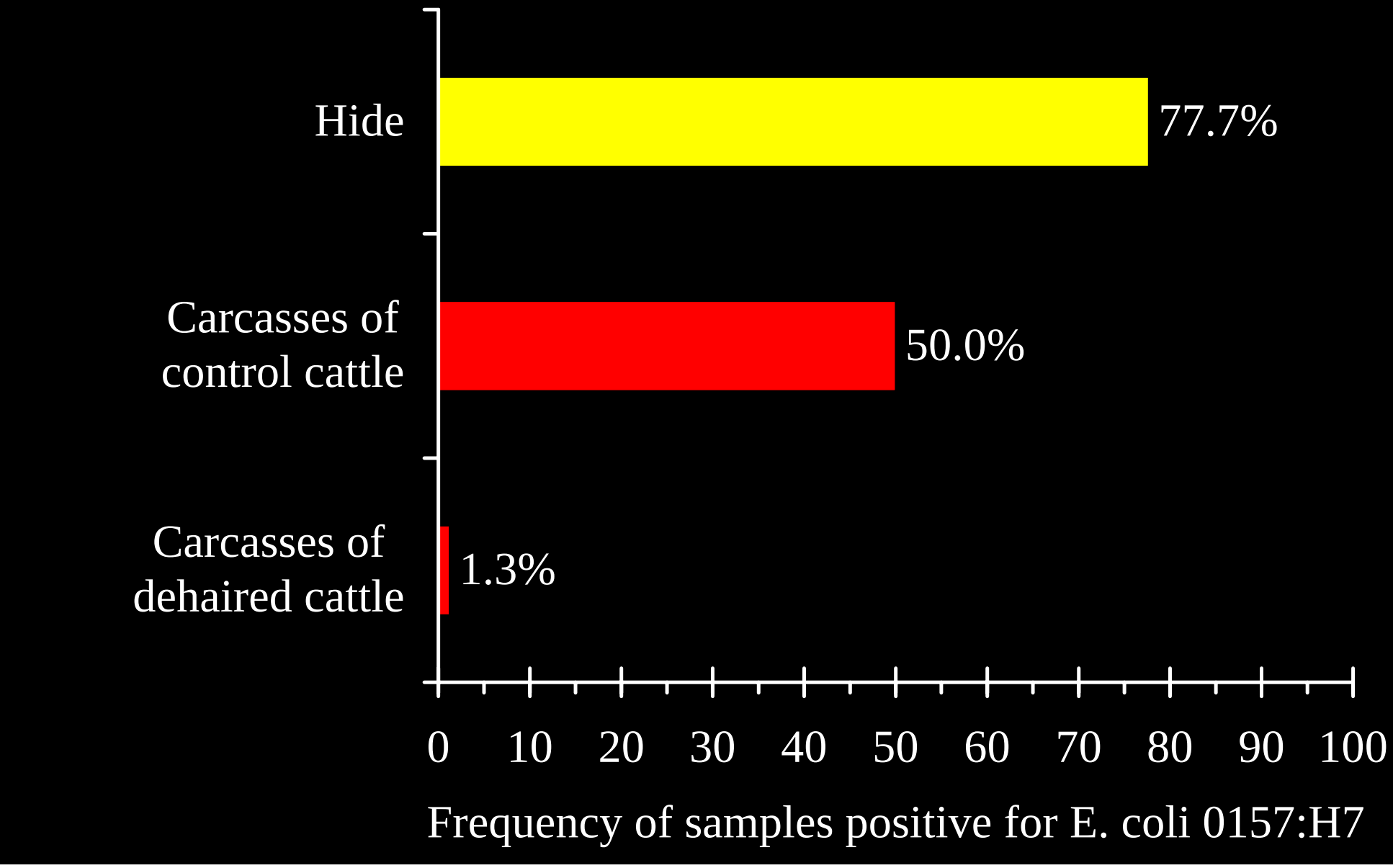
- In Winter the *E. coli* O157:H7 load coming into the plants on the hides and in the feces of cattle is lower and this results in lower levels being transferred to the carcasses during the hide removal process.
- Hide prevalence in Spring through Fall was approximately 70%.

Hypothesis:

If we reduce the load on the hide, the level of *E. coli* O157:H7 contamination on the carcass will be reduced

Tested hypothesis with chemical dehairing.

Dehairing of cattle before hide removal reduces the incidence of *E. coli* 0157:H7 contamination on pre-evisceration carcasses



Hide interventions

- The dehairing data, clearly demonstrate that hide intervention should be a priority as a part of comprehensive program to reduce/eliminate pathogens

- Alternative to chemical dehairing

Hide decontamination protocols

Washes:

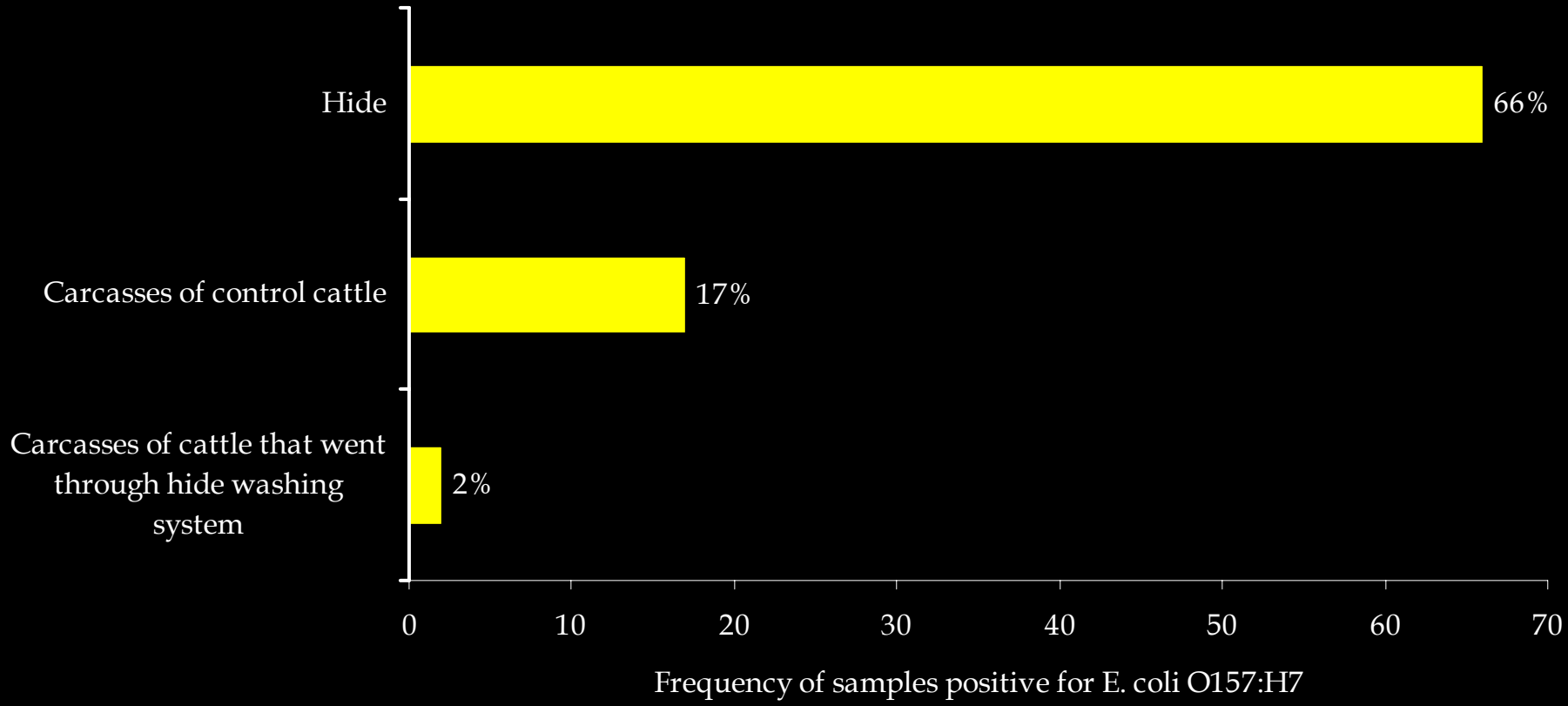
- CPC
- Sodium Hydroxide
- Trisodium Phosphate
- Phosphoric Acid
- Chlorofoam

Rinses:

- Water
- Acidified chlorine

Vacuum step

Effect of hide washing system on incidence of *E. coli* 0157:H7 contamination on pre-evisceration carcasses



Less sophisticated, but equally effective hide wash system

- Applied right after stunning (before bleeding).
- Thoroughly soaks hide with water.
- 100 to 200 ppm chlorine
- Has stainless steel enclosure.
- Drips partially dry before hide is opened.

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 enumeration	35.1
After cabinet *E. coli* O157 enumeration	13.2
Before cabinet *Salmonella* prevalence	94.8
After cabinet *Salmonella* prevalence	68.8
Before cabinet *Salmonella* enumeration	40.7
After cabinet *Salmonella* enumeration	7.3

n = 288

Hide enumeration = 40 CFU per 100 square cm

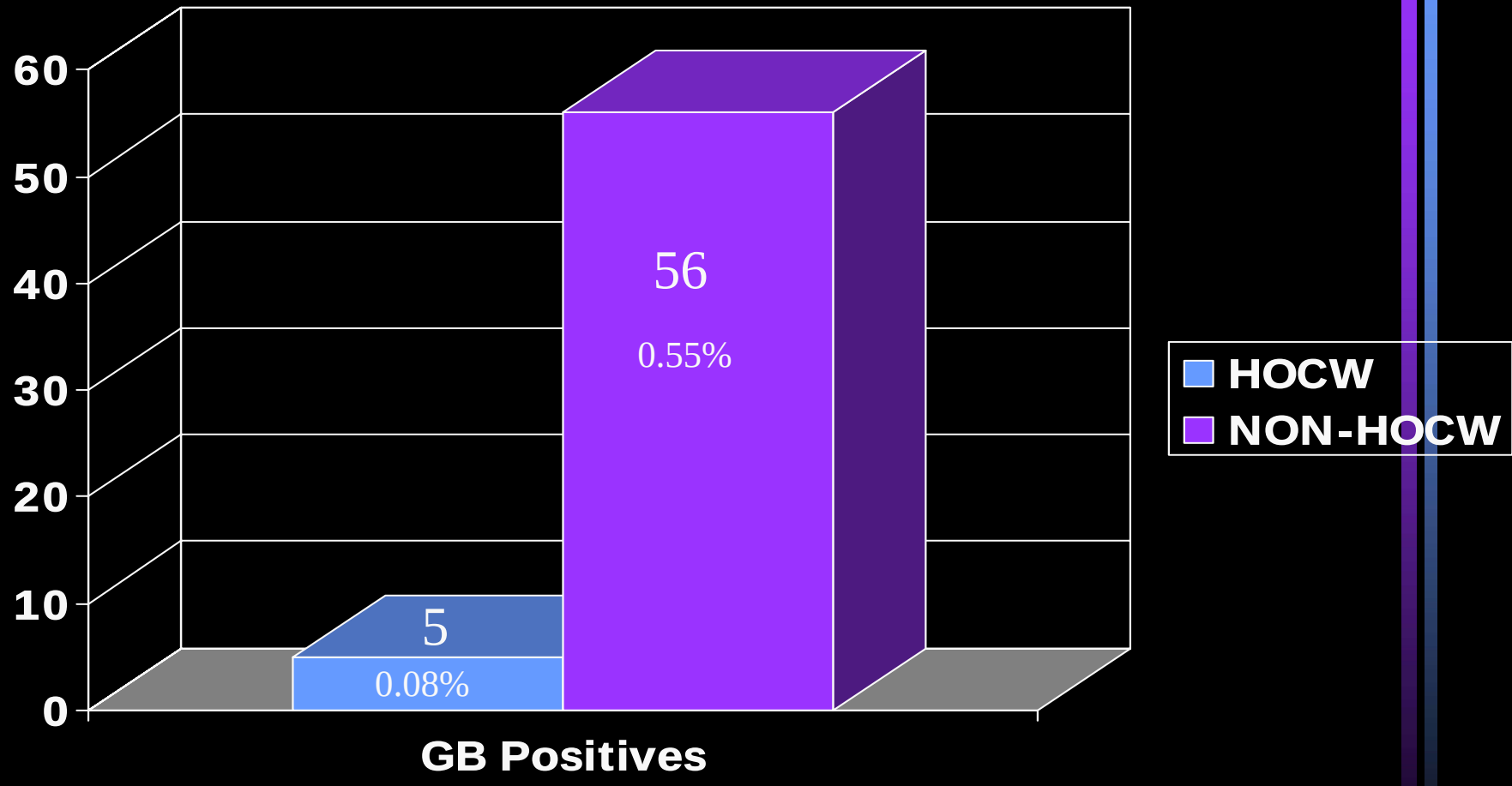
Distribution of enumeration data

CFU/100 cm^2	Before	After
<LOD	187	250
40-99	51	24
100-999	42	12
1,000-9,999	7	2
10,000-99,999	1	0

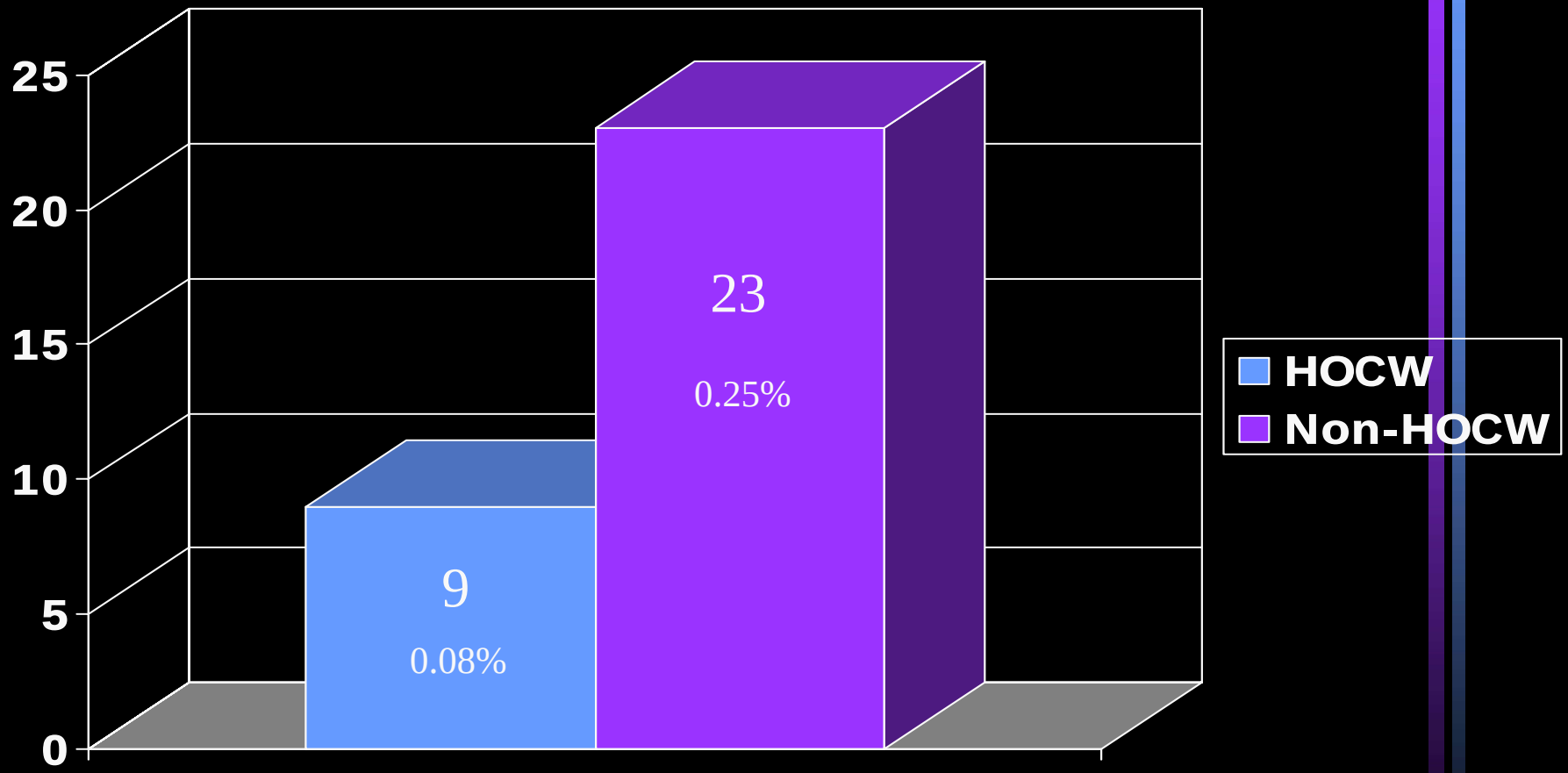
LOD, limit of detection = 40 CFU/100cm^2.

What is the impact of the work?

Number of Ground Beef Positives – Hide Wash Plants vs. Non- Hide Wash Plants



Number of Trim Positives – Hide Wash Plants vs. Non- Hide Wash Plants



Hide removal best practices

- One can also arrive at the same results by employing good practices during the hide removal process to minimize transfer of pathogens from hide to carcass

During the steps of hide removal *E. coli* O157:H7 is transferred from the hide to the carcass

Data for a single plant before and after best practices training

Results for 6 previous sampling trips

Sampling trip	Hide Prevalence	Carcass Prevalence
1	84%	74%
2	100 %	69%
3	60 %	36%
4	100 %	58%
5	47 %	28%
6	36 %	31%
Mean	71%	50%

Results

Day	Hide Prevalence	Carcass Prevalence
1	72%	8%
2	67%	9%
3	84%	10%
Mean	74%	9%

What should processors do to minimize the probability of ground beef contamination with *E. coli* O157:H7 and other foodborne pathogens?

What to do?

- Remember that kill floor is where all the problems begin. If you have any problem, the solution can be found on the kill floor.

- FSIS policy – looking back upstream for source.

Slaughter Process

- Hide-on side of the plant
- Hide-off side of the plant
- Keep them completely separate (physical barrier, workers, airflow etc)

Hide Removal

- The most important piece of information is that hide is the source of all pathogens at slaughter
- If at all possible, use a hide-on carcass wash with antimicrobials
- Train employees (and monitor their performance) for sanitary hide removal
- Use some sort of intervention after every hide opening step.

Hide on side

- Spot cleaning
  - Knife trimming of visible contaminations
  - Steam Vacuum
  - Hind leg steam boot
- Sterilizer dips for utensils between each carcass

STEAM VACCUUM

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

Pre-evisceration Interventions

- Focuses on exterior of intact carcass prior to evisceration.
- Performed soon after hide removal.
- Flushes fine specs of dust from exterior.
- Until recently most plants were using 2% lactic acid.
- Most effective is hot water – 180 F

Effects of lactic acid, hot water wash or combined treatment on the prevalence of *E. coli* O157:H7 on pre-evisceration carcasses

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

Pre-evisceration cabinet

Hide Off Side

- Final Carcass Rinse
  - Single or two hot water cabinet
  - The concept is to increase the surface temperature to >160 F and maintain it at this temperature for at least 10 s
  - The most effective intervention.

Process monitoring

Test-and-Hold

- The process:
  - Sample ground beef or ground beef raw material (trim) and test for the presence of *E. coli* O157:H7
  - If sample is positive for *E. coli* O157:H7, then lot is discarded (rendered) or diverted to cooked product (reduced value)
  - A lot of trim is 10,000 pounds
  - A lot of ground beef is one hour of production which could be as high as 30,000 pounds in large fed-beef plant

An effective test-and-hold strategy

- Proper sampling
  - N = 60 for raw material (trim)
    - Carcass surface material
  - Batching for ground beef and minimum of 4 subsamples per batch (lot)
  - Rapid test (in-house or commercial labs)

Test and hold

- Lessons of the last year have reinforced the need for monitoring at all levels.
- Must have effective testing system.

Microbial Sampling to Verify Slaughter Process and Recent Development in Hide interventions

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