BEEF INDUSTRY SAFETY SUMMIT
March 13-15, 2013
Dallas, TX

Project Title: **Analysis of growth of the Shiga toxin-producing *Escherichia coli* (STEC) in culture medium and impacts of subprimal chilling status, storage temperature and time on STEC attachment**

Presenter: **K.R. Kirsch**[1], D. Griffin[2], A. Castillo[3], T.M. Taylor[3], D. B. Marx[4], L. Smith[4]

[1]Department of Nutrition and Food Science, College of Agriculture and Life Sciences, Texas A&M University, College Station, TX; [2]Department of Animal Science, Texas A&M AgriLife Extension, College Station, TX; [3]Department of Animal Science, Texas A&M AgriLife Research, College Station, TX; [4]Department of Statistics, College of Arts and Sciences, University of Nebraska, Lincoln, NE

Email: krkirsch@neo.tamu.edu

Mailing address: 303 Kleberg Center, College Station, TX 77843-2471

Category: Methodology Improvement

Published: No

Objective: The objectives of this study were to investigate the growth of eight Shiga toxin-producing *Escherichia coli* (STEC; serotypes O26:H11, O45:H2, O103:H2, O104:H4, O111:H-, O121:H19, O145:NM, and O157:H7) in culture medium and evaluate STEC attachment to pre- and post-chilled beef as a function of post-inoculation storage temperature and elapsed incubation period.

Experimental Treatments: Growth of rifampicin-resistant STEC was determined by culturing and inoculating isolates individually into test tubes containing 9.9 mL sterile tryptic soy broth (TSB) to 2.0 $\pm$ 0.1 $\log_{10}$ CFU/mL, followed by incubation at 35 °C. At 0, 1, 2, 4, 6, 8, 12 and 24 h post-inoculation, STEC were serially diluted in 0.1% (w/v) peptone diluent and pour plated using sterilized, tempered molten tryptic soy agar (TSA). Colony counts were recorded after incubation for 24 h at 35 °C. Mean generation times from four distinct replications (n=4) were determined using the portion of the growth

curve representing the exponential phase for each serotype. For the attachment phase of this study, paired beef briskets (n=24) from split pre-chill carcasses were obtained from an FSIS-inspected facility. One brisket from each pair was kept warm and the second chilled to ≤ 5 °C prior to inoculation of the lean side with a prepared cocktail of the eight STEC to 6.3 ± 0.1 $\log_{10}$ CFU/cm^2. Inoculated briskets were stored at 5 or 25 °C. After 0, 30, 60, 90 and 120 min post-inoculation, 30 cm^2 of tissue was aseptically excised, and STEC were enumerated by spreading onto mPosse medium (40 g/L MacConkey Agar Base, 6 g/L sucrose, 6 g/L sorbose, 1.5 g/L bile salts #3, 0.05 g/L X-gal, 0.05 g/L IPTG, 100 mg/L rifampicin). Inoculated plates were then incubated for 48 h at 35 °C prior to enumeration. Data were analyzed using Analysis of Variance (ANOVA) and the Generalized Linear Model (GLM) in SAS.

Key Results: The STEC did not grow at identical rates, though all reached 9.0 ± 0.3 $\log_{10}$ CFU/mL within 24 h at 35 °C. Briskets inoculated post-chilling were found to have significantly greater recovery of attached cells versus those inoculated pre-chilling, with recoveries of 4.0 and 3.7 $\log_{10}$ CFU/cm^2, respectively ($p < 0.05$). Recovery of attached cells peaked at 0 min (4.1 $\log_{10}$ CFU/cm^2), with STEC recovery at this time point being significantly different ($p < 0.05$) from all others except at 30 min. Storage temperature (5, 25 °C) did not significantly influence cell recovery and no significant interactions were found ($p \geq 0.05$) between chilling status, incubation period, or time.

How can this information be applied in the industry: An understanding of STEC growth characteristics and the influence of beef temperature, storage temperature and time on STEC adherence to tissue could aid in the assessment of when to inoculate meat for subsequent research based on whether or not the product will likely be chilled. These data indicate that differences in the attachment of STEC as a function of meat chilling status should be considered by those designing experiments seeking to validate process interventions for preservation of beef safety.

Table 1. ANOVA for attachment of STEC8 on beef brisket

Effect	DF	SS	Mean Square	F Value	Pr > F
Brisket Temperature[1]	1	3.40	3.40	21.63	<.0001
Storage Temperature[2]	1	0.41	0.41	2.59	0.1105
Time[3]	4	3.83	0.96	6.10	0.0002
Brisket Temperature[1] * Storage Temperature[2]	1	0.59	0.59	3.79	0.0545
Brisket Temperature[1] * Time[3]	4	1.32	0.33	2.10	0.0862
Storage Temperature[2] * Time[3]	4	0.43	0.11	0.68	0.6087
Brisket Temperature[1] * Storage Temperature[2] * Time[3]	4	0.23	0.06	0.37	0.8305

[a] Values presented represent statistical differences in numbers of both unattached and attached STEC8 as a function of treatment-specific effects identified by analysis of variance (ANOVA). Sources consisting of multiple treatment variables linked with an asterisk (*) represent the combined interaction effects of the identified variables. [1] Pre-inoculation beef temperature (pre- vs. post-chilled), [2] Storage temperature (5 vs. 25 °C), [3] Elapsed time between inoculation and tissue excision (0, 30, 60, 90, or 120 min.).