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Project Title: **The Risk and Thermal Susceptibility of Non-O157 and O157:H7 shiga-toxin producing *Escherichia coli* in Non-Intact Beef Products Intended for Food Service or Retail.**

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Objectives: 1) Evaluate the internalization of shiga-toxin producing *Escherichia coli* (STECs) serogroups into non-intact beef products. 2) Determine the influence of marination and meat composition on the thermal susceptibility of internalized STECs to heat treatments previously considered lethal temperatures in meats.

Experimental Treatments: This study evaluated the internalization and cooking susceptibility of seven individual STEC serogroups (O157:H7, O26, O45, O103, O111, O121, and O145). Beef sirloin flaps (Phase 1) inoculated (10^6 log CFU/cm^2) with one of seven individual serogroups were marinated (30 or 60 min) and stored (14 d) prior to cooking (55, 60, 65, and 71°C). Pathogen presence after marination, storage, and cooking were evaluated. Beef strip loins (Phase 2) representing four USDA Quality Grade (Choice/Select) × pH (dark cutter/non-dark cutter) combinations were inoculated (10^6 log CFU/cm^2) with one of seven individual STEC serogroups prior to storage (14 d), blade tenderization, steak

portioning, and cooking (50, 60, 71, and 85°C). Pathogen presence was evaluated prior to and after storage and cooking.

Key Results: The data indicated varied internalization, translocation, and heat susceptibility patterns among the seven evaluated STEC serogroups. In Phase 1, internalization of surface pathogens was not influenced by vacuum marination length (30 or 60 min). Additionally, post-cooking analysis indicated that O26, O103, and O111 were detected in samples cooked to 55 and 60°C, while serogroup O157:H7 was detected in flap sections cooked to 60 and 65°C. Serogroup O145 was the only serogroup detected at all internal cooking temperatures (55, 60, 65, and 71°C). Results of Phase 2 indicate that the internalized populations of each STEC serovar were comparable to the populations on the subprimal and steak surfaces. Quality Grade and pH category affected ($P < 0.05$) steak surface populations for various serogroups, but no effect was noted for internalized samples. Thermal susceptibility varied among serogroups—all serogroups, except O45 and O121, were detected in steaks cooked to 50°C or 60°C. While a O26 STEC was confirmed in a USDA Select, Non-Dark Cutter samples, no STEC were detected in samples cooked to 85°C.

How can this information can be applied in the industry? The internalization and survivability of shiga-toxin producing *Escherichia coli* (STEC) serogroups in blade tenderized and marinated beef is a concern for beef processors. These data confirm the marinade and blade-mediated internalization of pathogens in non-intact meat products. As such, this supports the importance of validated subprimal intervention strategies and cooking protocols aimed to reduce the risk of non-intact beef. Overall, the results from both studies indicate the internalization, translocation, and susceptibility to cooking vary among STEC serogroups. Moreover, these results suggest STEC O157:H7 behavior in response to cooking and internalization may not accurately represent the behavior of other STEC serogroups in a meat model, and the risk of each O group must be considered individually. These results suggest that some strains are more heat tolerant than others, but the risk of those strains specifically in beef products should be considered.

Table or graph with critical information related to the results. Include proper title, units or other descriptors key to understanding the data in the table or graph.

Table 1. The percentage of cooked core samples with confirmed[1] shiga-toxin producing *Escherichia coli* (STEC) present in the cores of vacuum marinated sirloin flaps[2] and blade tenderized beef strip loins[3] inoculated with one of seven STEC serogroups prior to processing, storage, and cooking.

Phase One: Marination			Phase Two: Blade Tenderization		
Serogroup and Internal Temperature (°C)	Confirmed Samples (n = 12/temp)	% Confirmed	Serogroup and Internal Temperature (°C)	Confirmed Samples (n = 36/ temp)	% Confirmed
O26			***O26***		
55	2	16.67	50	6	16.67
60	0	0	60	0	0
65	0	0	71	1	2.78
71	0	0	85	0	0
O45			***O45***		
55	0	0	50	0	0
60	0	0	60	0	0
65	0	0	71	0	0
71	0	0	85	0	0
O103			***O103***		
55	1	8.33	50	7	19.44
60	0	0	60	2	5.56
65	0	0	71	0	0
71	0	0	85	0	0
O111			***O111***		
55	1	8.33	50	4	11.11
60	1	0	60	1	2.78
65	0	0	71	0	0
71	0	0	85	0	0
O121			***O121***		
55	0	0	50	0	0
60	0	0	60	0	0
65	0	0	71	0	0
71	0	0	85	0	0
O145			***O145***		
55	1	8.33	50	9	25.00
60	1	8.33	60	7	19.44
65	1	8.33	71	0	0
71	1	8.33	85	0	0
O157:H7			***O157:H7***		
55	0	0	50	6	16.67
60	2	16.67	60	3	8.33
65	1	8.33	71	0	0
71	0	0	85	0	0

[1] Cooked cores samples were subjected to the detection of *E. coli* cells using the rapid PCR-based BAX® system. Samples deemed as "positive" by initial detection were subjected to direct plating on selective media STEC

(Chromagar™) prior to serogroup confirmation of morphologically representative colonies using agglutination (O157:H7) or BAX® screening panels (non-O157 STEC).

[2] Beef sirloin flaps were inoculated with individual serogroups (10^6 log CFU/cm^2) , vacuum marinated for 30 or 60 min, and stored in the dark for 14 d before cooking on clam-shell style grills. Proportions represent the pooled confirmed samples for 30 and 60 min marination times.

[3] Beef strip loins from Choice dark cutter, Choice non-dark cutter, Select dark cutter, and Select non-dark cutter carcasses were inoculated with individual serogroups (10^6 log CFU/cm^2) ,stored in the dark for 14 d before blade tenderization, steak portioning, and cooking on clam-shell style grills to targeted internal temperatures. Proportions represent the pooled confirmed samples for all carcass characteristics**.**