Project Title: Do Pre-harvest Interventions Intended for *E. coli* O157:H7 Affect Fecal Shedding of Non-O157 STEC or *Salmonella* in Feedlot Cattle?

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Background

Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* are food safety issues that can have significant negative ramifications for the beef industry. Recently, the US Department of Agriculture, Food Safety and Inspection Service (FSIS) declared six non-O157 STEC serogroups as adulterants in ground meat and non-intact beef products, which is extremely concerning given the tremendous gaps in scientific knowledge. In recent years, there is also growing concern regarding *Salmonella* in beef production systems. If beef producers are to integrate pre-harvest interventions for controlling STEC and/or *Salmonella*, there is a need to determine the efficacy of interventions and whether there are any unintended consequences (positive or negative) when used in commercial production systems.

The objectives of the current research were to: 1) Determine whether commercially available pre-harvest interventions (vaccine and direct-fed microbial) that were used to reduce STEC O157 in feces of feedlot cattle had any effects on fecal shedding of non-O157 STEC (O26, O45, O103, O111, O121, O145) or *Salmonella*, and 2) Determine if the presence of STEC O serogroup-specific genes within a cattle fecal sample was associated (decrease, increase or no difference) with the presence of other STEC O serogroups or *Salmonella* within the cattle feces.

Methodology

We previously completed a field study that tested the efficacy of two interventions for controlling STEC O157 fecal shedding in large pens of commercial feedlot cattle (Cull et al., 2012). More than 17,000 cattle in 40 pens were randomized to receive one, both or neither (control) of the two interventions: a siderophore receptor and porin (SRP) proteins-based vaccine (*E. coli* SRP® vaccine, Pfizer, New York, NY) and a direct-fed microbial product (DFM; Bovamine®, Nutrition Physiology Co., Guymon, OK). Cattle in the vaccinated group received one dose (2 mL, SC) at enrollment and again three weeks later. The DFM was fed (106 CFU/steer/day) throughout the study period (mean = 87 days). Fresh fecal samples (30/pen) were collected weekly from pen floors for four consecutive weeks prior to the projected study end. The DNA extracted from samples (n = 4,800) were tested for STEC serogroup genes (O157, O26, O45, O103, O111, O121, and O145) and four major STEC virulence genes (*stx1*, *stx2*, *eae*, and *ehxA*) by an 11-gene multiplex PCR. In addition, a duplex real-time PCR assay for detecting *invA* and *pagC* genes was utilized for *Salmonella* detection. Generalized linear mixed models (GLMM) were used to analyze effects of treatments while accounting for allocation of pens within blocks and repeated measures on pens (within blocks) over time. In addition, GLMM were used to conduct within sample comparisons of presence of O serogroup and *Salmonella* genes.

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Findings

Cumulative prevalence of STEC O serogroup and virulence genes ranged from 0.2 to 45.4%. For *Salmonella*, 3.3% of samples tested positive for the *invA* gene, 0.3% to the *pagC* gene, 0.1% to both (*invA* and *pagC*) and 3.4% to at least one gene (*invA* and/or *pagC*). The SRP vaccine and/or the DFM had no significant effects on fecal prevalence of the six non-O157 STEC serogroups or *Salmonella* (all P values were > 0.1). Within sample comparisons of presence of O serogroup genes indicated that the presence of *E. coli* O157 was positively associated (all *P* values < 0.05) with the presence of STEC O26, O45, O103 and O121 genes in feces from feedlot cattle (Table 1). However, the presence of STEC O157 and *Salmonella* (*invA* and/or *pagC* genes) in a sample was not significantly associated (*P* > 0.05) (Table 1).

Implications

The beef industry has implemented intervention strategies in harvest facilities that have reduced the likelihood of carcass contamination with STEC, *Salmonella* and other potential foodborne pathogens. However, the ultimate source of these pathogens is the live animal and associated production environment. Thus, the use of pre-harvest strategies to control of pathogen loads in animals presented for harvest could have direct impact on beef safety. If beef producers are to adopt pre-harvest interventions for STEC and/or *Salmonella*, more data are needed to determine which interventions are the most efficacious and whether there are any unintended consequences when applied in a commercial production system. Our study demonstrated that two commercially available pre-harvest interventions – the SRP® O157 vaccine and low-dose Bovamine® – did not reduce fecal shedding of non-O157 STEC serogroups or *Salmonella*. This is despite the fact that the vaccine was efficacious in reducing STEC O157 shedding (Cull et al, 2012). Observed associations between the presence of different STEC serogroups within the same cattle fecal samples may suggest that common mechanisms affecting shedding of multiple STEC may be identified as potential control points. Further understanding of how animal management strategies affect beef safety risks is essential for effective food safety risk management in the industry and improved consumer confidence in beef.

Table 1. The percent (and number) of samples positive for STEC O-serogroups, STEC virulence genes (*stx*1, *stx*2 and *eae*) and *Salmonella* genes (*invA* and *pagC*) based on whether samples were positive (+) or negative (-) for STEC O157.

	Percent Positive (number of samples)											
	STEC Serogroups						Virulence genes			*Salmonella*		
	O26[T]	O45[T]	O103	O111	O121	O145	*stx*1[T]	*stx*2[T]	*eae*[T]	*invA*	*pagC*	Total
O157 (+)	43.0 (301)	4.4 (31)	20.4 (143)	1.4 (10)	10.7 (75)	0.3 (2)	34.6 (242)	57.4 (402)	83.7 (586)	3.4 (24)	0.03 (2)	14.6 (700)
O157 (-)	4.9 (201)	1.4 (57)	8.5 (350)	0.1 (6)	0.9 (35)	0.1 (6)	8.7 (355)	18.3 (749)	38.9 (1593)	3.2 (133)	0.03 (11)	85.4 (4100)
Total	10.5 (502)	1.8 (88)	10.3 (493)	0.3 (16)	2.3 (110)	0.2 (8)	12.4 (597)	24.0 (1151)	45.4 (2179)	3.3 (157)	0.3 (13)	100.0 (4800)

*Denotes statistically significant (*P* < 0.05) associations with the presence O157 within the same fecal sample

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