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Salmonella spp. in lymph nodes of fed and cull cattle: Relative assessment of risk to ground beef

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ABSTRACT

Ground beef products have been implicated as the vehicle for the transmission of *Salmonella* in a number of outbreaks. Although carcass surface interventions have proven effective, *Salmonella* contamination in ground beef still occurs. Recent studies indicate that deep tissue lymph nodes (DTLNs) may be an important source of *Salmonella* contamination in ground beef products. We developed a stochastic simulation model covering the pre-to post-harvest stages to assess the relative contribution of DTLN as compared with carcass surface, to *Salmonella* in ground beef, and the impact of various pre- and post-harvest interventions. The model addressed fed and cull cattle, and in high and low prevalence seasons. Contamination from carcass surfaces and DTLNs was simulated separately. Linear relationships were used to describe the changes of *Salmonella* surface concentration and prevalence at different processing stages. The baseline results indicate that on average over 90% of the *Salmonella* CFU load in a 2000 lb (907 kg) production lot originates from DTLN contamination as compared with carcass surface contamination. The relative contribution of DTLN contamination was fairly robust to changes in model parameters for ground beef from fed cattle, while it was comparatively more sensitive to changes in model parameters for cull cattle. The predicted mean *Salmonella* CFU load from DTLN contamination was considerably greater in ground beef production lots from fed cattle compared with cull cattle. Correspondingly, our scenario analysis suggested that generic pre-harvest interventions which can reduce *Salmonella* contamination in DTLNs would cause a greater total CFU load reduction in ground beef production lots from fed cattle compared with cull cattle. The study provides some valuable information for prioritizing control measures targeted at *Salmonella* contamination from the beef carcass surface or DTLNs based on the current knowledge.

1. Introduction

Ground beef products have been implicated in a number of salmonellosis outbreaks (Centers for Disease Control and Prevention [CDC], 2011, 2012, 2013). Cattle hide contamination is primarily responsible for the contamination of carcass surfaces, which may result in contaminated ground beef if the pathogens survive carcass interventions during processing (Arthur, Bosilevac, et al., 2008; Barkocy-Gallagher et al., 2003; Brichta-Harhay et al., 2008). Previous investigations indicate a fairly low level of *Salmonella* prevalence (on average less than 1%) on post-intervention carcasses due to the application of various carcass interventions in commercial beef processing plants (Brichta-Harhay et al., 2008; Rivera-Betancourt et al., 2004). Despite the success of carcass interventions, however, testing results from the United States Department of Agriculture, Food Safety Inspection Service (USDA/FSIS) indicate little reduction of *Salmonella* contamination in ground beef during the past decade, with generally over 2.0% prevalence in ground beef samples (25 g) during the past years

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(USDA/FSIS, 2012). These discrepancies imply other potential sources for ground beef contamination, among which lymph nodes have received increasing attentions in recent years.

A growing number of studies indicate that *Salmonella* may be harbored in bovine deep tissue lymph nodes (DTLNs), such as subiliac, iliofemoral, and superficial cervical LNs, which are embedded in adipose tissues that are frequently included in ground beef and thus could potentially contaminate the final product (Arthur, Brichta-Harhay, et al., 2008; Brichta-Harhay et al., 2012; Gragg, Loneragan, Brashears, et al., 2013, Gragg, Loneragan, Nightingale, 2013; Haneklaus et al., 2012; Koohmaraie et al., 2012). A two-year survey of *Salmonella* contamination in subiliac lymph nodes ($n = 3327$) of cull and fed cattle across the nation indicated an average prevalence of 7.5%, and *Salmonella* harborage appeared to be affected by season, region, and animal type (Gragg, Loneragan, Brashears, et al., 2013). Since *Salmonella* harbored in DTLNs are likely to evade the antimicrobial carcass surface interventions currently implemented in the processing plants, other potential pre- and post-harvest interventions, such as vaccination, probiotics, lymph node removal, and final product treatment, could be explored to reduce or eliminate *Salmonella* originating from DTLNs.

To harness limited resources to reduce the *Salmonella* burden, it is critical for the beef industry to employ a science-based, risk-informed approach to choose the most effective intervention strategies among a variety of control options. Therefore, the objective of this study was to develop a stochastic simulation model to assess the relative contribution of DTLNs to *Salmonella* contamination of ground beef products, and exercise the model with best available data to evaluate the relative impact of various pre- and post-harvest intervention strategies. The following outcomes were evaluated: (i) relative contribution of DTLN contamination to the *Salmonella* CFU load in a 2000 lb (907 kg) production lot; (ii) total *Salmonella* CFU load in a production lot. The model could aid producers and beef packers in making informed decisions regarding the choice of effective control measures, and direct future research needs, especially for gathering appropriate data to improve the predictive ability of the model.

2. Materials and methods

2.1. Model development

A stochastic spreadsheet based simulation model covering the pre-harvest to post-harvest stages was utilized to predict the relative contribution of *Salmonella* from DTLNs. The model scope and key assumptions are summarized in Tables 1 and 2. The model is comprised of three modules as shown in Figs. 1–3: (1) *Salmonella* prevalence on carcass surfaces; (2) *Salmonella* concentration on carcass surfaces; and (3) *Salmonella* prevalence and concentration in DTLNs. The parameters and calculations for the three modules are listed in Table 3.

2.1.1. Modeling Salmonella prevalence on carcass surfaces

The carcass surface prevalence module starts with post-stun hide prevalence (Fig. 1). The transfer of *Salmonella* from live animals' feces to hide surfaces was not attempted because previous studies have showed that the transport and lairage stages after harvest and before slaughtering have a commingling effect which can increase the magnitude of contamination and mess up the genetic association between pre- and post-harvest bacterial isolates (Arthur et al., 2007, Arthur, Bosilevac, et al., 2008). Hide prevalence (P_1) for fed and cull cattle, in high and low prevalence seasons, was estimated from multiple studies across different geographical regions (Table 3). During dehiding, *Salmonella* can be transferred to the carcass surface through direct contact with the hide or airborne particulates created during the process. A linear transfer coefficient (P_2) was assumed to describe this process and relate the *Salmonella* prevalence from 1000 cm^2 hides to the prevalence on 8000 cm^2 pre-evisceration carcasses (P_3) (hide and carcass sampling areas in most data were 1000 and 8000 cm^2, respectively). Similar linear regression models have been used previously to simulate the transfer of *Escherichia coli* O157:H7 prevalence from feces to pre-evisceration carcasses (Hurd & Malladi, 2012; USDA/FSIS, 2001). Studies that used tagged and matched hide and carcass samples were chosen to estimate the linear transfer coefficient (Arthur, Bosilevac, et al., 2008; Barkocy-Gallagher et al., 2003; Brichta-Harhay et al., 2007, 2008; Rivera-Betancourt et al., 2004). Uncertainty associated with this parameter was estimated by linear regression parameter bootstrapping. To be more specific, the hide and carcass prevalence (denoted as independent and dependent variables, respectively) from

Table 1
Scope of the risk assessment model.

1. The risk assessment model is designed for a typical high volume (16,000 production lots per year) beef processing plant that slaughters cattle, produces meat trims, and finally grinds the trims to ground beef.
2. It addresses fed cattle (steers and heifers) and cull cattle (cows and bulls) separately, based on their respective contamination data on hides and in DTLNs.
3. Generic, non-specific serovars of *Salmonella enterica* are considered.
4. Seasonal variations are modeled based on "high" (summer and fall) and "low" (winter and spring) prevalence times.
5. Plant data across different geographical locations are aggregated based on sample sizes and expressed as average values.
6. The surface contamination model starts from hide contamination at abattoir and ends at contamination in ground beef after grinding.
7. Internal model parameter estimates are based on averages from multiple published studies.
8. Data (publicly available) were collected from studies conducted for U.S. beef production and processing.
9. The prevalence and concentration (of positive samples) of *Salmonella* on carcasses and ground beef are estimated on a production lot basis, which is defined as a 2000 lb (907 kg) load of ground beef.
10. The risk reduction effect of post-grind interventions is not evaluated in the model.
11. No exported trims or trims from other packing plants are considered.

Table 2
Key assumptions of the risk assessment model.

1. Contamination of a production lot is independent of previous lots processed, i.e., lot-to-lot contamination is negligible.
2. Pre-evisceration carcass prevalence is a linear function of in-plant hide prevalence.
3. No mixing of ground beef from cows and bulls with that from steers and heifers is assumed.
4. Simple linear fits were assumed to be appropriate for relating *Salmonella* prevalence and concentration at various production and processing stages.
5. *Salmonella* is assumed to be randomly distributed in combo bins of ground beef (although clusters of bacteria may exist) and modeled with Poisson distribution.
6. All contamination is assumed to be on the external surface of the carcass and in DTLNs.
7. The *Salmonella* prevalence and concentration on all 8000 cm^2 segments of carcass surface is assumed to be similar to the values estimated from the 8000 cm^2 sampling area in the Brichta-Harhay et al. (2007, 2008) studies.
8. The *Salmonella* prevalence and concentration on all 1000 cm^2 segments of hide surface is assumed to be similar to the values estimated from 1000 cm^2 sampling area in the Brichta-Harhay et al. (2007, 2008) studies.
9. External carcass contamination is assumed to come mostly from direct contact with contaminated hide during dehiding.
10. Internal carcass contamination from GI (gastro-intestinal) content was not considered in the model.
11. During fabricating, trimming, and grinding, cross-contamination from hands, equipment, and contaminated carcasses or trims is assumed not to differentially add new bacteria into the grinding load.