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Prevalence, level and distribution of *Salmonella* in shipments of imported capsicum and sesame seed spice offered for entry to the United States: Observations and modeling results

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ABSTRACT

In response to increased concerns about spice safety, the United States Food and Drug Administration (FDA) initiated research to characterize the prevalence and levels of *Salmonella* in imported spices. 299 imported dried capsicum shipments and 233 imported sesame seed shipments offered for entry to the United States were sampled. Observed *Salmonella* shipment prevalence was 3.3% (1500 g examined; 95% CI 1.6–6.1%) for capsicum and 9.9% (1500 g; 95% Confidence Interval (CI) 6.3–14%) for sesame seed. Within shipment contamination was not inconsistent with a Poisson distribution. Shipment mean *Salmonella* level estimates among contaminated shipments ranged from 6×10^{-4} to 0.09 (capsicum) or 6×10^{-4} to 0.04 (sesame seed) MPN/g. A gamma-Poisson model provided the best fit to observed data for both imported shipments of capsicum and imported shipments of sesame seed sampled in this study among the six parametric models considered. Shipment mean levels of *Salmonella* vary widely between shipments; many contaminated shipments contain low levels of contamination. Examination of sampling plan efficacy for identifying contaminated spice shipments from these distributions indicates that sample size of spice examined is critical. Sampling protocols examining 25 g samples are predicted to be able to identify a small fraction of contaminated shipments of imported capsicum or sesame seeds.

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1. Introduction

The prevalence and level of pathogens in a food affect the risk of illness from consumption of that food because both influence the dose or level of exposure to the pathogen. The 2010 ILSI report *Impact of Microbial Distributions on Food Safety* (Bassett et al., 2010; Jongenburger et al., 2012a, 2012b) and other reports in the literature (Gonzales-Barron and Butler, 2011; Commeau et al., 2011; Jongenburger et al., 2011) have also demonstrated that the distributions of pathogens across food batches and within food samples influence human exposure. Bassett et al. (2010) and Jongenburger et al. (2012a) examined the impact such distributions have on the design of effective sampling plans, performance objectives and microbiological criteria. A number of studies have demonstrated that pathogen distribution can be impacted by different processes along the farm-to-table continuum (Bassett et al., 2010; Commeau et al., 2011; Jongenburger et al., 2011, 2012b). Therefore, knowledge of the distribution patterns of pathogens in products may provide insight into the source of contamination. Despite the importance of these parameters to estimate risk and evaluate risk management strategies, the data needed to characterize them is rarely collected.

The present study begins to address these data gaps for spices by determining the prevalence, level and distribution of *Salmonella* in shipments of imported dried capsicum (e.g., red hot pepper and paprika) and sesame seed spices offered for entry to the United States (U.S.). Imported spices were the focus of this study because the majority of the U.S. supply of these products (70–75%) comes from imported sources (USDA/ERS, 2012; American Sesame Growers Association, 2012). This study provides the first analysis of between- and within-shipment distributions of *Salmonella* in spice shipments. It also examines the impact of *Salmonella*

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distributions on the efficacy of sampling plans designed to identify contaminated shipments of spices. Improved surveillance sampling plans will reduce the likelihood of *Salmonella* contaminated spices entering the U.S. spice supply. This study is a part of a larger effort by the U.S. Food and Drug Administration (FDA) to conduct spice safety research to assess the salmonellosis public health risk posed by spice consumption in the U.S. and to assist the agency in identifying options to mitigate the risk (FDA, 2010).

2. Materials and methods

2.1. Sample collection

All imports of dried capsicum and sesame seeds were eligible for sampling during the study period. A total of 299 shipments of capsicums and 233 shipments of sesame seeds were sampled at the point of import into the U.S. between August and December 2010. The shipments sampled constituted approximately 10 or 20 percent of all shipments of imported capsicum or imported sesame seed shipments, respectively, offered for entry to the U.S. Sixty subsamples, each comprised of approximately 160 g, were collected randomly from each shipment. Typically, each subsample was collected from a different container or sack of spice in the shipment selected at random. Samples were sent to FDA laboratories for analysis.

2.2. Salmonella *screening and enumeration*

2.2.1. *Sample preparation,* Salmonella *screening, isolation and confirmation*

Composite samples were prepared by dividing the 60 subsamples into four groups of fifteen. Twenty-five gram analytical units of product from each of the fifteen subsamples were combined to form a 375 g composite sample (Andrews and Hammack, 2003). Each composite sample was screened for the presence of *Salmonella* using one of the following methods: AOAC's Official Methods of Analysis (OMA): 2004.03, 2001.07, 2001.08, or 2001.09 (AOAC International, 2011). All methods are validated and have similar performance criteria.

Salmonella was isolated from each of the composite samples testing positive using the procedures described in Chapter 5 of FDA's *Bacteriological Analytical Manual* (BAM) (Andrews et al., 2011). Presumptive-positive *Salmonella* isolates were confirmed with OMA methods 978.24 or 991.13.

Salmonella isolates recovered from the spices were serotyped (Ewing, 1986). Further details of the methods used and data collected are provided to the interested reader in the Supplementary material.

2.2.2. Salmonella *enumeration*

A dilution assay was undertaken for composite samples of spice that were found to contain *Salmonella* by the screening test. The serial dilution protocol involved a three tube analysis on each of four different dilutions of spice. Spice sampled for the dilution assay analysis were drawn from a composite (thoroughly mixed) sample created from equal proportions of the same set of 15 subsamples used in the corresponding *Salmonella*-positive screening test. Separate composite spice product portions of 100, 10, 1, and 0.1 g were each rehydrated at a 1:9 ratio with a tryptic soy broth pre-enrichment medium by swirling or soaking as instructed in the BAM method (Andrews et al., 2011). This procedure was repeated three times (for three tubes) for each of the four different dilutions (100, 10, 1, and 0.1 g). The enrichment tubes were kept at room temperature for 60 ± 5 min, pH adjusted to 6.8 ± 0.2, if necessary, and then incubated for 24 ± 2 h at 35 ± 2 °C. Once incubation was complete, the BAM *Salmonella* culture method was followed (Andrews et al., 2011). The relative likelihood of each reported dilution assay pattern for a thoroughly mixed sample was evaluated on the basis of the rarity index (Blodgett, 2010a; Jarvis et al., 2010).

Most Probable Number (MPN) values and 95% confidence intervals for each of the four composite samples were determined from the five results, screening test plus the four dilutions using the excel spreadsheet provided in the BAM (Blodgett, 2010b), where the screening test was treated as another "dilution" for the MPN analysis. For these analyses, we assumed the screening test has a perfect sensitivity and specificity to detect one *Salmonella*, if present, in the sample. For a few shipments, the procedure described above was not followed. For samples in one shipment of capsicum and six shipments of sesame seeds, the dilution assay result patterns were not reported by the field labs but rather the presence/absence of *Salmonella* was reported for the enumeration experiment as a whole. In these cases, we interpret the experiments as providing a second screening test with total spice mass of 333.3 g. For samples from two other shipments of sesame seeds with confirmed positive *Salmonella* samples, enumeration experiments were not performed. MPN estimates and confidence intervals for the mean *Salmonella* level in a shipment were calculated taking into account the full set of screening test and dilution assay results.

The assumption of Poisson-distributed contamination within shipments, which was used to estimate the shipment mean level of contamination and is part of some of the parametric models developed, was examined. We evaluated for each of the 4 composite sample results, a rarity index (Blodgett, 2010a). Specifically we evaluated the probability to obtain the pattern of results observed for one composite sample given the estimated mean shipment level divided by the probability to observe the most probable pattern of results in the composite sample given the estimated mean shipment level. This statistic has the advantage of being quantifiable for all of the outcomes obtained in this study including missing dilution assay results and binary dilution assay result outcomes. The second advantage is that this rarity index evaluates in one statistic the adequacy of the assumption of within shipment Poisson distribution and within dilution assay Poisson distribution. There is no *a priori* expectation that contamination levels in different composites from the same shipment should be the same/similar because the spice contained in different composites are from different locations in the shipment. If the local concentration in a given composite is far higher or lower than the value estimate at the shipment level, the probability to observe the given pattern will be low with regards to the most probable one, leading to a low rarity index. We use the recommendations of Jarvis et al. (2010) for thresholds of probability: the pattern of results is likely to occur if its rarity index is ≥ 0.05; is expected to occur only rarely if the rarity index falls within the range $0.01 <$ rarity index < 0.05; and is expected to occur extremely rarely if the rarity index is ≤ 0.01.

2.3. Probabilistic models

Probabilistic models of imported spice shipment contamination were examined for their ability to describe the *Salmonella* sampling data. Features included in the models were selected for their ability to describe between- and within-shipment distributions of *Salmonella* in spices. Each model was fit to the capsicum and sesame seed data separately, and evaluated for its quality of fit. Mathematical descriptions of the models, development of the likelihood functions, and derivation of the maximum likelihood solutions, where applicable, are presented in the Supplementary Material.