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Using indicator organisms in performance standards for reducing pathogen occurrence on beef carcasses in the United States

Michael S. Williams*, Eric D. Ebel, Neal J. Golden

Risk Assessment and Analytics Staff, Office of Public Health Science, Food Safety and Inspection Service, United States Department of Agriculture, 2150 Centre Avenue, Building D, Fort Collins, CO 80526, USA

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ABSTRACT

Improvements in food hygienic production practices and intervention technologies have reduced the prevalence of pathogen-contaminated carcasses during slaughter. While this unequivocally reduces the risk of foodborne illness, the selection of microbiologic standards based on pathogens for application to raw meat and poultry commodities becomes more burdensome because of the large number of samples required to distinguish between good and poorly performing establishments. This study examines the feasibility of two alternative performance standards based on levels of APC contamination at different locations in the beef slaughter process. An example, based on the *Salmonella* and *E. coli* O157:H7 contamination on beef carcasses in the United States provides a case study for the potential effectiveness of the indicator organism-based performance standards. In the example, a performance standard based on the reduction in $\log_{10}$ aerobic plate counts was shown to be superior to a performance standard based on setting a maximum $\log_{10}$ aerobic plate count on finished carcasses.

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1. Introduction

In the United States, the Pathogen Reduction / Hazard Analysis and Critical Control Point (PR/HACCP) program was implemented by the Food Safety and Inspection Service (FSIS) in the mid- to late-1990s (USDA, 1996). This program intends to reduce all microbial pathogens in meat, poultry, and egg products, with most of the emphasis placed on the reduction of *Salmonella* across all products and *Escherichia coli* (*E. coli*) O157:H7 in ground beef. Since the inception of the PR/HACCP program, the apparent prevalence of *Salmonella* -contaminated carcasses at slaughter has generally decreased for all commodities, despite efforts to increase the sensitivities of the assays for some commodities by increasing the aliquot size and employing more sensitive laboratory methods (FSIS, 1996a, b, 2001 2009, 2012; Wilhelm et al., 2011; Williams et al., 2014). Reductions in pathogen occurrence have also been observed in retail surveys (Anonymous, 2010; Williams et al., 2015b), particularly in chicken. While the effectiveness of the program on overall human illness counts is arguable, most studies conclude that there were declines in human microbial foodborne illness in the years immediately following implementation of PR/HACCP (CDC, 2011; Marcus et al., 2004; Powell, 2016; Williams and Ebel, 2012), even though the program only applied to meat and poultry. Since roughly 2000, few reductions in estimated foodborne illness counts have been reported in the United States (CDC, 2011, 2013; Powell, 2016). This lack of significant improvement has prompted regulatory agencies to investigate new approaches for reducing microbial contamination on foods.

The PR/HACCP program implemented two sampling programs. The first required slaughter establishments to record and evaluate generic *E. coli* testing results using a 3-class attributes sampling plan in a moving window of 13 consecutive tests (Montgomery, 2009; USDA, 1996). Generic *E. coli* (GEC) was chosen as the indicator because it was thought to be the most appropriate indicator of fecal contamination. These testing results were maintained by each establishment and never collected or collated by FSIS for analyses of within- and between-establishment results, national status, or trends. The other sampling program described in the PR/HACCP legislation was the implementation of *Salmonella* performance standards. For a particular commodity and processing establishment, a performance standard (a.k.a., microbiological criteria, verification sampling) is based on the culture-confirmed *Salmonella* test results from a set of samples collected at the establishment. An establishment is considered to be compliant with the performance standard if the number of *Salmonella* -positive samples is less than or equal to an established threshold value. For example, the original PR/HACCP legislation stated that a compliant steer/heifer slaughter establishment could have no more than one *Salmonella* -positive carcass sponge sample collected from a set of 82 samples (USDA, 1996). The steer/heifer performance

* Corresponding author.
E-mail address: mike.williams@fsis.usda.gov (M.S. Williams).

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standard was the most stringent standard whereas establishments that produced ground turkey were allowed 29 *Salmonella* -positive samples out of a set of 53 samples. The number of allowable positive samples and the set size were determined using the mean of the prevalence of positive samples derived from surveys completed prior to the implementation of the PR/HACCP rule (USDA, 1996). Recently, more stringent standards have been proposed for poultry products to meet an objective of reducing human salmonellosis associated with these products by 25% (Ebel et al., 2012; FSIS, 2011b; HHS, 2010a).

The purpose of a *Salmonella* performance standard is to stratify slaughter establishments into subpopulations with either low or high contamination levels. Establishments with higher levels of contamination are identified as failing the performance standard. If failing establishments are motivated to reduce contamination to levels that pass the performance standard, then an overall reduction in the proportion of contaminated product reaching consumers is expected. When a commodity has a low prevalence of positive samples, the statistical power of a performance standard to correctly dichotomize establishments is poor unless very large sample sizes are employed. For example, the application of the model used to develop FSIS' new performance standards for poultry (FSIS, 2015b) to the recently collect FSIS beef carcass baseline data found that a *Salmonella* -based performance standard that could achieve a 25% reduction in contaminated carcasses would require roughly 245 samples with 1 allowable positive each year from each establishment.

The difficulties of developing and implementing performance standards when the prevalence of pathogen-positive samples is low have led to suggestions that alternative performance standard methods should be explored. One alternative approach is to sample carcasses for more easily detected microorganisms with an expectation that the occurrence or levels of these indicator organisms co-vary with the pathogen of interest (e.g., *Salmonella*). Previously, it has been demonstrated that stratifying establishments based on their average $\log_{10}$ reduction in aerobic plate counts generated substantial differences in the resulting lognormal distributions of *Salmonella* and *Campylobacter* concentrations among post-chill swine and broiler chicken carcasses (Williams et al., 2015a). The conclusion of that study suggests that carcasses produced in establishments with larger than average reductions in the levels of an indicator organism are less likely to be *Salmonella* -positive than carcasses produced by establishments with smaller reductions in the levels of the indicator. Furthermore, monitoring carcasses with the purpose of reducing the indicator organisms should produce a corresponding reduction in *Salmonella* occurrence on the carcasses (Altekruse et al., 2009; Duffy et al., 2014; Ghafir et al., 2008). A performance standard designed to dichotomize food processing establishments into passing and failing categories on the basis of indicator organisms might succeed in reducing overall pathogen exposures to consumers if the indicator organisms and pathogen co-vary sufficiently.

In this study, we examine the feasibility of two performance standards for beef carcasses that are based on testing for indicator organisms. The first standard sets an upper limit on the mean of $\log_{10}$-transformed aerobic bacteria counts (aerobic plate counts, APC), derived by plating, prior to chilling of a finished carcass. The second standard sets a minimum $\log_{10}$ reduction in APC based on sampling results just after removal of the hide from the carcass and just prior to chilling of the finished carcass. We use methods previously developed for pathogen-based performance standards to determine indicator-based performance standards that are expected to achieve a targeted reduction in human exposures and illnesses. To assist in the selection of thresholds for determining passing and failing categories, we examine the operating characteristics of the performance standards.

2. Data description

This study relies on data from a national survey of cattle slaughter establishments conducted by FSIS. The data report the occurrence of indicator organisms and pathogens at two locations in the slaughter process. The data for each establishment are summarized for an assessment of the effects of indicator organism-based performance standards on overall *Salmonella* occurrence across the beef industry. Given its food safety importance to beef, we also examine the potential effects of the performance standards on *E. coli* O157:H7 occurrence on finished cattle carcasses. The results for *E. coli* O157:H7 are only included as an illustrative example of the effects of an indicator organism-based performance standard on multiple pathogenic species. We consider these results to be illustrative because the occurrence of *E. coli* O157:H7 in this dataset is too infrequent to make definitive statements regarding the effectiveness of the performance standards for this pathogen.

The sampling frame of the FSIS survey comprised establishments that slaughter the majority of all cattle in the United States. FSIS personnel collected sponge samples at 138 slaughter establishments from August 2014 through July 2015. For each sampling event at an establishment, the inspector randomly selected one carcass for sampling immediately following the removal of the hide from the carcass (post de-hiding). The same finished carcass was sampled again just prior to entry into the chiller (pre-chill) and after the application of any antimicrobial treatments. There were a total of 956 samples collected at post de-hiding and 962 at pre-chill. The mismatch in the number of samples at each location was due to laboratory issues with a small percentage of samples. A total of 953 samples had matching results for both sampling locations and pathogens (i.e., an average of 6.9 matched samples per establishment with a range of 1 to 25).

At post de-hiding, two sterile sponges were moistened with 10 ml of buffered peptone water (BPW) and used to swab one side of the carcass. One sponge was used for the upper half of the carcass and the other for the lower half. Each sponge was used to collect approximately 4000 cm^2 of surface area. Whenever possible, the same carcass was located at pre-chill and the other side of the carcass was sampled with two additional sponges using the same procedure.

All four sponges were sealed in individual bags and the samples were shipped to an accredited lab where one sponge from each sampling location was analyzed using a screening test to determine the presence *Salmonella* and *E. coli* O157:H7 . In this assay, 50 ml of BPW was added to the sponge to achieve a total sample volume of 60 ml. The sample was then enriched and incubated for 24 h, after which it was analyzed using a BAX PCR (Dupont Qualicon, Wilmington, DE) assay to identify presumptive positive samples. Each presumptive positive sample was subjected to extensive confirmatory testing prior to a sample being classified as positive (FSIS, 1998, 2011a, 2014, 2015a).

The contents of the other sponges were used for enumeration of four indicator organism classes. In this assay, BPW was added to the sponge for a total diluent volume of 25 ml. For enumeration, 1 ml of diluent was further diluted using 9.0 ml of fresh BPW (10^{-1}dilution) and vortexed. Serial dilutions from 10^{-1} to 10^{-4} were plated onto the appropriate Petrifilm^{TM} to enumerate APC, *Enterobacteriaceae*, coliforms, and GEC. The proportion of samples where the concentration of indicator organisms was sufficiently high for non-zero enumeration values is listed in Table 1 for each class of indicator organisms.

In addition to the microbial testing data, the annual number of cattle slaughtered by each of the sampled establishments (denoted by V_i) was included in the data. These values were used to weight