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The Public Health Value of Reducing *Salmonella* Levels in Raw Meat and Poultry

ABSTRACT

Non-typhoidal *Salmonella* is the most common bacterial cause of foodborne illness in the United States, causing roughly 1.2 million cases annually. *Salmonella* is commonly associated with raw meat and poultry products, and despite progress in exceeding USDA FSIS performance standards, the rates of salmonellosis in the U.S. are well above public health goals. Reducing the frequency of exposure to levels of *Salmonella* that cause illness is critical to protect public health. This paper reviews the USDA FSIS policies currently used to assess *Salmonella* and explores alternative international models focused on enumeration that are being implemented for the control of *Campylobacter*. The paper outlines how a strategy that considers enumeration of *Salmonella* may protect public health, including a review of the uncertainties and variability with regard to infectious dose and proposes that meaningful gains in public health will be achieved by reducing the levels of *Salmonella* in raw ground meat and poultry.

INTRODUCTION

Salmonellosis in the United States

CDC estimates that over one million cases of salmonellosis occur in the U.S. each year *(21)*, and data indicate that the salmonellosis incidence rate has remained unchanged over the past 15 years *(8)*. While salmonellosis is most commonly associated with gastroenteritis, infection is also associated with longer term health consequences such as reactive arthritis, aortic aneurisms and ulcerative colitis. The total economic burden of *Salmonella* illness is estimated at between $2.3B and 11.3B annually *(11, 12, 22)*.

Poultry and beef are considered to be important vectors of *Salmonella* infection *(1, 30)*. Ground turkey products have recently been implicated in outbreaks, raising concern that adherence to performance standards is insufficient to protect public health if some fraction of product, especially ground product that may not be adequately cooked, contains high levels of the pathogen *(5, 6)*.

In an analysis of investigations of outbreaks that occurred between 1998 and 2008, CDC researchers found that of the 7,724 outbreaks with a known agent and known or suspected

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food, 145 outbreaks were due to *Salmonella* associated with poultry. Of all *Salmonella* outbreaks with a known or suspected food vehicle, about 30% of them were associated with poultry and almost 10% with beef (ground products were not differentiated in the analysis) *(9)*. In an analysis of a similar data set, the number of outbreaks due to *Salmonella* and linked to poultry was 271 (out of a total of 2,469 outbreaks, of which 713 were associated with poultry), and relative to other food types, poultry was estimated to cause about 19% of the cases of salmonellosis, higher than for any other food group. Beef was the fourth most likely cause of salmonellosis, to which over 7% of cases were attributed (eggs ranked second, accounting for 14.8% of illness, and 'fruit and nuts' was third, associated with 13% of cases) *(19)*.

However, a recent analysis of 10 years of outbreak data (2001–2010) by the Center for Science in the Public Interest shows that the number of outbreaks of foodborne illness (due to all agents) has decreased for meat and poultry *(10)*, which could be due to the mandatory HACCP requirements in the industry and the resultant science-based focus on pre- and post-slaughter interventions. Still, Batz et al. estimated the public health cost of *Salmonella* associated with poultry at $693M, and that associated with beef at $229M *(2)*.

Reducing levels of *Salmonella* in food is an important public health goal, and the food safety and public health communities continue to examine how food becomes contaminated with *Salmonella,* how those exposures translate to human illness, and the factors that determine the severity of illness. Thus meat and poultry processors must remain vigilant in continuing to address *Salmonella*, as well as other pathogens. USDA FSIS recognized a number of years ago that some incidences of *Salmonella* in raw meat and poultry is probable and so have set performance standards for *Salmonella* that specify the numbers of samples within a sample set that can test positive for *Salmonella* spp. after enrichment (*Salmonella* prevalence) *(26)*. However, the performance standards do not address the actual number of *Salmonella* in a given positive sample (*Salmonella* level). In other words, a positive sample may be positive because it has one *Salmonella* organism in 25 g or because it has 10 million *Salmonella* organisms in 25 g. Both will be positive, but clearly the latter raises more concern from a public health perspective. Currently, as long as the frequency of positive samples in the sample set meet the performance standard, the product is considered "acceptable" from a regulatory standpoint, regardless of the actual load of the pathogen in the product. Questions remain as to whether improvements in public health will result from more stringent performance standards (lower tolerance for positives) or from efforts that decrease the load of *Salmonella* in ground products, or from both.

Limited impact of current measures to affect salmonellosis

While *Salmonella* can be transmitted to humans from a number of sources, including non-food vehicles, there is a clear association between *Salmonella* and poultry products in particular.

Table 1 shows performance standards for a variety of products and shows that for ground chicken and ground turkey, the current percent positives are well below the USDA FSIS performance standards *(27)*. It also shows that prevalence is much higher in ground chicken and turkey compared to their whole counterparts. A plausible explanation is that the *Salmonella* on the occasional positive carcass with a high load becomes distributed, albeit at lower levels, when the product is ground. The use of qualitative data only allows one to determine the elimination of *Salmonella*. However, the value of reductions in pathogen load in raw meat and poultry, which can be determined only by enumeration, can be viewed as an indication of process control that should have an impact on the likelihood of illness.

The distinction between "high" and "low" levels and the establishment of a threshold level for process control is certainly debatable. As is the case for the approach used by FSIS in the initial establishment of the baseline for performance standards, a better understanding is needed of the spectrum of "positive," elucidating how often ground product contains 10^6 vs 10^1 vs 10^{-1} CFU/g and comparing these levels with the processing or other factors involved will allow the industry to better understand which interventions work and when. Resources could then be

TABLE 1. USDA FSIS *Salmonella* Performance Standards (Jan. 1 — Mar. 31, 2013)

Product	Original *Salmonella* Performance Std.	% positive:*	# Samples
Ground Chicken	44.6%	15.7	287
Broilers (carcass)	7.5%	3.5	3,786
Ground Turkey	49.9%	15.1	192
Turkeys (carcass)	1.7%	2.2	510
Ground Beef	7.5%	0.9	4,467

*(27)

prioritized according to the products (and practices) that have the highest levels of *Salmonella,* which may represent the products most likely to result in human illness. In fact, USDA FSIS has also indicated a move toward enumerating bacteria in comminuted poultry and ground beef that test positive for *Salmonella (25, 26)*.

As discussed in the section on infectious dose, the consumption of different amounts of *Salmonella* are associated with different probabilities of illness. Data suggest that the probability of illness is increased as exposure to greater numbers of *Salmonella* increases. The exact number of *Salmonella* needed to cause illness is dependent on a number of factors (including host susceptibility and serotype) and can be quite variable. However, if we operate on the premise that "more is worse," we believe that we can achieve the Healthy People 2020 goals more readily by reducing the amount of product in the marketplace that is most likely to cause illness.

Assuming that products containing low levels of *Salmonella* are just as likely to be temperature abused and misused during distribution and consumer handling as products containing high levels of the pathogen, addressing the "worst offenders" – the product that has the highest levels (CFU/g) of *Salmonella* in it – should have a public health benefit. Additionally, this insight will allow industry to investigate the factors that resulted in high loads, enabling the implementation of more effective mitigations.

As the concept of enumeration has been discussed with members of the poultry industry and USDA FSIS at meetings and conferences, a main point of discussion pertains to the availability of practical, economical methods for enumerating *Salmonella*. The detection of very high levels of *Salmonella* (not yet defined) does not necessarily require absolute quantification using expensive and time-consuming methods such as Most Probable Number (MPN). Enumerating using the MPN method requires a series of dilutions that are individually added to tubes of enrichment broth. If viable *Salmonella* are present and multiply, the tube becomes turbid. By evaluating the number of turbid and non-turbid (no growth) tubes and comparing this to a table, the most probable number of *Salmonella* in the original sample can be estimated *(28)*.

As an alternative to traditional MPN enumeration, methods being used by industry currently allow for the differentiation of samples that exceed a specific level, and improvements in testing methodologies are expected to facilitate enumeration or semi-quantification. The use of methods that rely on thresholds have the advantage of providing actionable results more quickly than the traditional MPN method. The first step in implementing a threshold approach is to determine the threshold that triggers action. For example, if a firm determines that a level of *Salmonella* greater than 1 CFU/g warrants additional action, then studies are undertaken to determine the length of time samples with 1 CFU/g need to incubate in an enrichment broth to become detectable by a DNA- or RNA-based test. Once the incubation time is determined, the method is validated to ensure sensitivity and specificity that meet or exceed USDA test method validation guidelines. This technique is currently being used to identify when a finished ground beef or poultry product contains levels of *Salmonella* that could present an increased risk to consumers (Cargill, unpublished data). Like other applications of PCR, the replication of DNA from dead as well as live cells could impact the result, albeit in a way that is conservative and protective of public health.

It is also recognized that without strict maintenance of the cold chain, *Salmonella* can grow. A study modeling the growth of *Salmonella* Typhimurium in chicken skin showed that samples inoculated at 0.9 log CFU did not show growth after 10 days of storage if the temperature was below 8°C *(18)*. At higher temperatures, growth occurred as indicated in *Table 2*.

In a separate study, the same researcher explored the dependence of serotype on growth rate. There are over 2,500 serotypes of *Salmonella*. Some serotypes, such as Kentucky, are commonly isolated from poultry products, but rarely cause outbreaks in the United States *(7, 29)*. In contrast, the serotypes Heidelberg and Hadar have been associated with multiple outbreaks with poultry as the suspected vehicle, while the serotype Typhimurium causes a high number of human illnesses when all vehicles of infection are considered. For this reason, researchers often examine the impact of serotype in microbiological studies. When growth rates were evaluated, serotypes Typhimurium and Hadar exhibited similar growth patterns in inoculated chicken skin stored between 5 and 50°C for up to 8 hours; serotype Kentucky grew more slowly *(17)*. Ingham et al. *(14)* conducted similar studies identifying lag time and growth rates for four serotypes of *Salmonella* (Typhimurium, Heidelberg, Infantis and Enteritidis) in the temperature range of 10–43.3°C in multiple matrices, including ground pork and ground turkey. In ground turkey, at the lowest temperature tested, 50°F/10°C, the lag phase was roughly 22 hours, with a growth rate of 0.024 log/h (0.576 log/day). In ground beef under the same conditions, the lag phase was 46 hours, with a growth rate of 0.012 log/h (0.288 log/day).

While growth does occur during temperature abuse, the type of abuse needed to achieve a log growth in these raw products is extreme and would likely cause organoleptic spoilage as well.

Comparable approach: Process hygiene criterion for *Campylobacter* in broiler meat

Evaluating the pathogen load, as opposed to relying strictly on presence/absence data, has been increasingly examined and used to improve food safety relative to other pathogens in other countries. Although *Campylobacter* is quite different from *Salmonella*, both in terms of levels commonly associated with raw poultry as well as the hardiness of the organisms, the applicability of enumeration of *Campylobacter* to achieve a public health benefit is worthy of review. Recently, the Dutch government issued

TABLE 2. Log increase in *Salmonella* after 10-day storage (as reported in *(18)*)

Temperature (°C)	log increase
9	0.7
10	1.1
11	1.8
12	2.9

its analysis of how different process hygiene criteria for *Campylobacter* (10,000; 1,000; or 100 CFU/g) on broiler meat, as measured after chilling, reduces campylobacteriosis *(23)*. The analysis was undertaken as a result of studies cited that demonstrate that "the main consumer risks are associated with the most highly contaminated products and that risk management strategies aimed at preventing such highly contaminated products from reaching the consumer are both effective and efficient" *(23)*.

The study relied on industry-collected enumeration data over a two-year period. This information was combined with dose-response data and modeled. The results provided an indication of the public health benefit by limiting the levels of *Campylobacter* to the specific levels, balanced against the percent of non-compliant product that would need to be further processed. Intuitively, broiler meat with the lowest levels (100 CFU/g) *Campylobacter* would be expected to have the greatest impact on illness, with the risk to consumers estimated to be reduced by 98% in this product, compared with the illness rates at that time. Models also examined the impact of establishing a limit of 1,000 CFU/g and 10,000 CFU/g, which corresponded with increased numbers of expected cases of illness *(23)*. Thus the researchers did not strive to determine a "safe" level of *Campylobacter* in broiler meat but rather explored the impact of several options.

The study also showed that a linear relationship did not exist between reduction of levels of *Campylobacter* in the products and reduction of illness. Decreasing the load from 10^5 CFU/g to 10^4 CFU/g did not have as great a public health impact as decreasing the levels from 10^3 to 10^2 CFU/g *(23)*, suggesting that 10^4 CFU/g is still often an infectious dose and only below that does an impact on the likelihood of illness occur. Similarly, the fraction of illness that is preventable remains close to 100% illness prevented when levels of *Camplylobacter* on broiler chicken post chiller are between 0 and 100 CFU/g. Thus, the number of *Camplyobacter* present on the product has an impact on the likelihood of illness, with the likelihood being lower with lower levels of *Campylobacter* on the product. Although a "safe" level of *Campylobacter* was not established, the results directly speak to the relationship between levels of pathogens in products and the likelihood of illness.

New Zealand undertook a *Campylobacter* risk management strategy beginning in 2008. Before implementation, the mean per-carcass count was determined to be 4.16 log CFU/carcass *Campylobacter (15)*. The government established a *Campylobacter* Performance Target (CPT) of 3.78 log CFU/carcass in order to reduce the load of the pathogen on positive carcasses. The program has had two notable effects. First, a dramatic and immediate decrease in cases of poultry-associated camplylobacteriosis was observed. Compared to a baseline level of illness in 2005–2006, campylobacteriosis associated with poultry dropped by 74% in 2008, without a corresponding drop in other zoonotic foodborne disease *(16)*.

Modeling studies exploring the factors that impact campylobacteriosis have also been undertaken. An analysis of "what if" scenarios demonstrated that in one particular instance a 30-fold reduction in the number of cases of campylobacteriosis could be achieved either by reducing the load of *Campylobacter* on poultry carcasses by 2 logs or reducing flock prevalence 30 fold *(20)*.

Infectious dose

The infectious dose is presumed to be higher for *Camplyobacter* than for *Salmonella*, but the range of factors that determine infectious dose makes it difficult to pinpoint an exact number. However, it is important not to confuse exploring the value of enumeration and reducing levels of *Salmonella* on raw meat and poultry with trying to answer the question, "what is the acceptable level of measurable *Salmonella*?"

Infectious dose can be defined as the minimum number of live *Salmonella* bacteria that it will take to cause illness. This is dependent on a number of factors, including host susceptibility and medications being taken by the host, the food matrix, and virulence factors of the pathogen (which may be serotype dependent). As with most foodborne pathogens, it is extremely difficult to determine with certainty the minimum infectious dose of *Salmonella*.

It has been assumed conventionally that consumption on the order of 10^6 salmonellae is required to cause illnesss *(13)*. The early studies on infectious dose were undertaken using volunteers, with strains of *Salmonella* that had been passed multiple times in the laboratory, and an analysis of these studies shows that the experimental designs were inadequate to assess the likelihood of infection at lower doses *(4)*. Other studies have measured the actual number of *Salmonella* in specific food items linked to illness and are thus a much better indicator of infectious dose. Food affords protection to pathogens as they pass through the stomach, increasing the likelihood that they are still viable as they reach the lower intestines, where they act *(3)*. Recent studies modeling outbreak data where the pathogen load was known suggest that as few as 36 colony forming units can cause illness *(24)*, which supports earlier hypotheses and outbreak investigations. A review of 11 outbreaks in which the number of organisms ingested could be estimated shows that the dose ranged from tens of organisms to millions *(4)*. In some cases, the attack rate could also be estimated and was found to be related to the dose ingested. For example, in an outbreak related to water, when a liter contained 17 *Salmonella* cells, the attack rate was roughly 12%; when outbreaks were associated with the ingestion of 10^5 CFU *Salmonella*, 100% of the exposed population became sick *(4)*. Blaser and Newman *(4)* reviewed additional outbreaks, which show a direct relationship between the number of *Salmonella* ingested and the likelihood of illness.

In 2002, the World Health Organization (WHO) and Food and Agricultural Organization (FAO) of the United Nations published a risk assessment of *Salmonella* in broiler chickens and eggs to understand the relationship between microorganisms, food, and human illness *(30)*. The report states "Clearly, the risk per serving is variable when we consider individual egg servings (e.g., a serving containing 100 organisms is much more likely to result in illness than a serving containing just 1 organism)," and this is accounted for in the estimates for probability of illness that are used: the report states that the probability of illness given an average dose of 1, 10 or 100 organisms is 0.2%, 2.2% or 13%, respectively.

Teunis et al. *(24)* evaluated non-typhoidal *Salmonella* outbreaks to determine a dose-response model that could be utilized when the *Salmonella* dose or the number of exposed was unknown. This study also found that as the dose increased, the probability of illness increased. Doses above 10^2 CFU had probabilities of illness ranging from 0.05 to 1.0, where doses less than 10^2 CFU had probabilities of illness ranging from 0.01 to 0.56 *(24, 30)*.

While none of the studies are attempting to define a precise infectious dose, few would dispute that higher levels of *Salmonella* are more likely to cause illness. Thus we propose that strategies be focused on enumerating *Salmonella* so that products containing higher levels of the pathogen are identified, enabling industry to investigate the root cause of the high level of contamination. In turn, this will allow the development and evaluation of mitigation methods to reduce both levels and prevalence of *Salmonella*, with a positive public health impact.

SUMMARY

Qualitative performance standards versus enumeration and thresholds

Qualitative performance standards initially had a positive impact on public health and provided USDA FSIS, the industry, and public health officials with useful information. However, progress appears to have stalled and alternative approaches should be considered.

If strategies that consider enumeration are deliberated, the potential for growth of the pathogen also needs to be considered in order to determine if an enumeration strategy affords an appropriate level of public health protection as compared with the current performance standard approach. The development of a model to better illustrate the public health impact of shifting the focus from prevalence of *Salmonella* (the percent of product with detectable levels of *Salmonella*) to reducing the numbers of *Salmonella* within the product will help inform risk management decisions.

A prerequisite to model development is the collection of data, beginning with an understanding of the levels of *Salmonella* that currently exist in ground products. The relationship between levels of *Salmonella* in products that leave a processing facility and levels ingested by consumers can be modeled using published growth rates as a function of temperature if the times and temperatures at supply chain points are known.

The following factors support the exploration of transiting from solely qualitative performance standards to having data that provide both qualitative and quantitative or semi-quantitative measures (threshold) of *Salmonella*:

- Inadequate progress in meeting healthy People 2020 Goals for *Salmonella*;
- Continued outbreaks of salmonellosis associated with ground meat and poultry products despite decreased prevalence of *Salmonella*;
- Inadequate data on infectious dose and growing evidence that the likelihood of illness increases with higher doses;
- Lack of data regarding levels of *Salmonella* in ground product.
  Collection of quantitative or semi-quantitative data on current levels of *Salmonella* in ground products, as well as modeling efforts, should explore whether reducing exposure to high levels of *Salmonella* will have a public health impact comparable to qualitative performance standards, or if there is the potential to reduce the risk of *Salmonella* illnesses attributable to meat and poultry products, thus improving public health and helping reach the U.S. Healthy People 2020 goals.

As stakeholders continue to explore the concept of enumeration, the best method(s) to obtain results will need to be determined and the cost of changing methodology will need to be considered. There will also need to be discussion as to whether enumeration is used by industry as a tool to inform risk management, or a regulatory approach. Ultimately, the objective is to reduce the public health burden of salmonellosis.

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