Minimization of *Salmonella* Contamination on Raw Poultry*

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Abstract

Many reviews have discussed *Salmonella* in poultry and suggested best practices to minimize this organism on raw poultry meat. Despite years of research and conscientious control efforts by industry and regulatory agencies, human salmonellosis rates have declined only modestly and *Salmonella* is still found on raw poultry. Expert committees have repeatedly emphasized the importance of controlling risk, but information about *Salmonella* in poultry is often limited to prevalence, with inadequate information about testing methods or strains of *Salmonella* that are detected by these methods and no information about any impact on the degree of risk. This review examines some assumptions behind the discussion of *Salmonella* in poultry: the relationships between sampling and cultural methodology, prevalence and numbers of cells, and the implications of serotype and subtype issues. Minimizing *Salmonella* contamination of poultry is not likely to reduce human salmonellosis acquired from exposure to contaminated chicken until these issues are confronted more systematically.

INTRODUCTION

Many reviews on the topic of minimizing *Salmonella* on raw poultry are not much more than exhortatory lists of best practices in which greater levels of effort and vigilance are urged in the application of remarkably similar recommendations. Such papers have been available for 40 years, with presentations on this topic a regular feature of poultry meetings during that time. Many of these reviews have been useful for thinking about the *Salmonella* problem and possible interventions, but we doubt that we can do a significantly better job of writing such a review than has already been done by many of our colleagues.

The drawback of best practices lists is that they address *Salmonella* contamination in a general way that may be more effective over the long-term, but they do not assure consistent *Salmonella* control for every flock that is produced. In particular, continued *Salmonella* problems are seen in parent and grandparent flocks, which are more valuable and thus receive more conscientious and consistent levels of best-practice care than do flocks of production birds. If five years from now, control efforts in production flocks are at the same level as those devoted to breeder flocks today, there will still be *Salmonella*-positive flocks, even though all of them will have received an improved standard of care. It is also likely that public and regulatory standards will be more demanding, whatever degree of success the worldwide poultry industry may have in reducing *Salmonella* contamination in its products relative to today. The purpose of this review is to examine some of the assumptions behind efforts to reduce *Salmonella* contamination of raw poultry, with the ultimate goal of reducing the risk of human salmonellosis. Reduced incidence of human salmonellosis will be only an accidental product of more demanding standards for *Salmonella* on raw poultry unless those standards are scientifically linked to reduced risk.

ATTRIBUTION OF HUMAN SALMONELLOSIS FROM EXPOSURE TO RAW POULTRY MEAT

> It is widely known that *Salmonella* is associated with poultry products, but we do not know what proportion of human salmonellosis cases are caused by chicken, turkey, eggs, or non-poultry sources, so we do not know how much disease FSIS actions could theoretically prevent. Similarly, we do not know what proportion of cases are associated with undercooking, cross-contamination, or other means of transmission. Without such information, it is difficult to design the most effective strategies to control *Salmonella* contamination in poultry to reduce the pathogen level in the end product or to evaluate the efficacy of FSIS control strategies after they have been implemented.
>
> National Research Council 2009

The possibility of reducing human salmonellosis by targeting raw poultry meat is limited to the amount of illness associated with exposure to *Salmonella* on poultry meat and any related cross-contamination during food preparation. Many studies and expert reports have concluded, however, that only a small amount of data is available for attributing illness to specific foods or for developing effective intervention strategies (Batz et al. 2005, Hald et al. 2004, NRC 2009). Human salmonellosis will not decrease until the risk of acquiring the illness is reduced, and any scientific approach to controlling risk will be constrained by the quality of available information. A science-based food safety policy requires a scientific link between microbiological criteria that are being established and the public health problem that justifies the policy (ICMSF 2006, IOM/NRC 2003, Todd 2004).

Estimates of the percentage of salmonellosis caused by exposure to contaminated food vary from 55% to 96% in different countries (Havelaar et al. 2008, Mead et al. 1999, Sumner et al. 2003).

People in different parts of the world have characteristic ways of producing and preparing foods, so it is unlikely that all parts of the world have similar proportions of foodborne salmonellosis. Moreover, food systems and consumer practices change over time and attribution percentages change and need periodic reexamination (Greig & Ravel 2009).

Methods for attributing illness to specific food sources can be classified into four main approaches: epidemiology, microbiology, risk assessment, and expert elicitation, with all methods having advantages and disadvantages (Batz et al. 2005, EFSA 2008, NRC 2009, Pires et al. 2009, USDA/FSIS 2008). Elicitation of expert opinion tends to produce the highest estimates for human cases of salmonellosis associated with exposure to poultry meat (such as 35% in Hoffman et al. 2007); although, international outbreak data can also yield estimates in the same range (Greig & Ravel 2009). Whether outbreak data can be extrapolated to all cases of salmonellosis is not certain.

Over the past 40 years, rates of human salmonellosis have declined in most developed countries, even as consumption of poultry meat has increased considerably. Current estimates of the percentage of human cases of salmonellosis attributed to exposure to poultry meat generally range from 10% to 22% in developed countries (Dalton et al. 2004, EFSA 2008, Havelaar et al. 2008, Karns et al. 2007, Kirk et al. 2008, Mullner et al. 2009, Pires et al. 2010, Ravel et al. 2009, USDA/FSIS 2008, Van Asselt et al. 2009). According to some authorities, however, the rate of nonfoodborne transmission is underestimated (Barber et al. 2003, Mead et al. 2010).

In the United States, the source of *Salmonella* is identified in only about 0.13% of the estimated 1,400,000 annual human cases of salmonellosis (Barber et al. 2003), leaving considerable uncertainty regarding sources of exposure. If there are more than a million cases annually, then at any moment there are tens of thousands of human carriers (Barber et al. 2003). Several reviews have emphasized the carrier role of humans and the need to isolate poultry from humans (Aho 1992, Barber et al. 2003). Kinde et al. (1996) identified sewage effluent as the likely source of a *Salmonella* infection in a California layer flock, and several surveys have isolated *Salmonella* from human sewage sludge or effluent, including 54 serotypes from 11 sewage treatment plants in the United States (Kinde et al. 1997) and 49 serotypes from eight plants in Sweden (Sahlstrom et al. 2004, 2006).

In the Netherlands, the major risk for Typhimurium infections in children from ages 4 to 12 was playing in a sandbox (Doorduyn et al. 2006). Working in a garden was one of the leading risk factors in Canada for the months of June and July (Ravel et al. 2010). Despite the widespread perception of poultry meat and eggs as the predominant sources of *Salmonella*, recent outbreaks in the United States have been caused by contaminated jalapeno peppers (the largest outbreak in a decade), peanut butter, tomatoes, ground beef, puffed vegetable snacks, and pets such as turtles and African dwarf frogs (CDC 2005, 2008a, 2010). The wide variation in numbers of cases coming from nonfood and nonpoultry sources makes it extremely difficult to attribute a specific amount of human salmonellosis to raw poultry meat, even though we know that poultry meat is a source of *Salmonella*.

RELATIONSHIP BETWEEN *SALMONELLA* ON RAW POULTRY AND ACQUISITION OF HUMAN SALMONELLOSIS

> There exist only limited data that provide evidence for the success (or failure) of national and international control programs in terms of reducing human salmonellosis.
>
> Hald et al. 2004

If the amount of salmonellosis caused by exposure to raw poultry meat cannot be determined with a reasonable degree of certainty, is it possible to estimate how much of a reduction in

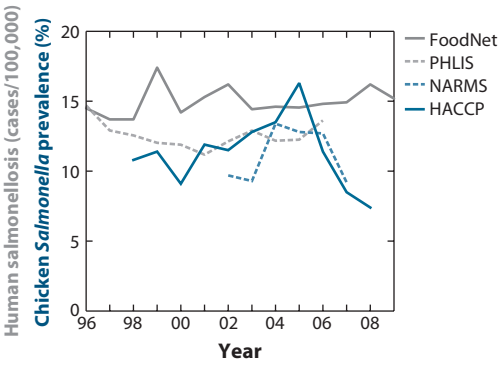

Figure 1

Human salmonellosis data (cases per 100,000) from FoodNet and the Public Health Laboratory Information System (PHLIS), *Salmonella* prevalence data (%) from retail chicken breasts [National Antimicrobial Resistance Monitoring System (NARMS)], and chilled chicken carcasses [Hazard Analysis Critical Control Point (HACCP)] from 1996 to 2009.

Salmonella contamination of raw poultry is needed to observe an associated decrease in the rate of human illness? Can the contribution of nonfood and nonpoultry-related sources be adequately determined to enable an accurate determination of the number of human cases of salmonellosis associated with raw poultry exposure? The ability to correlate reductions in *Salmonella* contamination of raw poultry meat with human salmonellosis is limited by the imprecision of the data collected and the large annual, seasonal, and geographic variation in rates of illness.

Examples of various national and international surveillance systems have been reported by Doyle & Erickson (2006). In the United States, the Public Health Laboratory Information System (PHLIS) and FoodNet are the primary sources of national data regarding the incidence of human salmonellosis. Information about *Salmonella* on raw chicken is available from Hazard Analysis Critical Control Point (HACCP) data collected by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) and the National Antimicrobial Resistance Monitoring System (NARMS) program, which samples retail chicken breasts in areas that provide FoodNet human data. **Figure 1** provides HACCP data for the prevalence of *Salmonella* on chilled chicken carcasses (USDA/FSIS 2010), NARMS data for the prevalence of *Salmonella* on retail chicken breasts (US FDA 2010), and Public Health Laboratory Information System (CDC 2008b) and FoodNet data (CDC 2009) of the incidence of human salmonellosis (cases per 100,000).

Analysis of the data in **Figure 1** reveals why attribution of human illness to a specific food commodity is problematic. There is no significant correlation (J.A. Cason, N.A. Cox, and L.J. Richardson, unpublished data) between the two measures of *Salmonella* prevalence on raw chicken, between the two measures of incidence of human salmonellosis, or between any combination of the chicken and human data. Perhaps there are too many other sources of *Salmonella* contributing to human salmonellosis that mask the relationship between human illness and prevalence on chicken, or prevalence of *Salmonella* on raw chicken is not the best measure of risk of human salmonellosis from chicken, or *Salmonella* prevalence on chicken is not measured in a way that relates to risk of human illness, e.g., many *Salmonella* serotypes from poultry, such as Kentucky strains, rarely cause human illness.

Data from studies using different sampling methods are available from some European countries. Human salmonellosis rates from 2008 (EFSA 2010a) can be compared to processing plant data of *Salmonella* prevalence on chickens that were sampled in the same year (EFSA 2010b). Data are not available for all member countries of the European Union (EU), but illustrative results

Table 1 Prevalence of *Salmonella*-positive chickens at slaughter, human cases of salmonellosis, and imported cases of human salmonellosis in countries of the European Union and the United States, for 2008

Country	Neck skin % *Salmonella*-positive[1]	Human cases per 100,000[2]	Percentage of imported human cases of salmonellosis[2]
Austria	2.4	27.7	19.4
Belgium	16.3	35.9	NR
Bulgaria	22.5	19.8	NR
Czech Republic	5.5	103.1	2.2
Denmark	0	67.0	19.0
Estonia	0	48.2	6.5
Finland	0	59.0	59.0
Germany	15.0	52.2	5.8
Italy	13.5	5.4	0.5
Netherlands	9.5	15.5	10.6
Slovenia	1.7	51.0	NR
Spain	14.9	8.5	0
Sweden	0.2	45.6	82.1
	Whole carcass rinse: % *Salmonella*-positive	Human cases per 100,000	
United States	7.3[3]	16.2[4]	NR

NR = not reported.
[1]EFSA 2010b.
[2]EFSA 2010a.
[3]USDA/FSIS 2009.
[4]CDC 2009.

are shown in **Table 1**, along with the 2008 results from the United States. **Table 1** also includes estimates of the percentage of salmonellosis cases attributed to international travel (not acquired domestically) (EFSA 2010a).

The correlation between *Salmonella* prevalence on raw chicken and salmonellosis in humans in **Table 1** is not significant, whether calculated for all reported human cases or only for cases attributed to domestic origin, nor are there significant correlations if the three countries with a prevalence of zero *Salmonella* in neck skin samples are left out of the calculations (J.A. Cason, N.A. Cox, and L.J. Richardson, unpublished data). Problems similar to those mentioned in the analysis of the United States data may exist for the European data set as well. The lack of correlation between the prevalence of *Salmonella* in poultry and the incidence of human salmonellosis in both the United States and Europe illustrates the difficulty of attributing human salmonellosis to specific foods, as discussed above.

In addition to the nonfood and nonpoultry sources of human exposure that complicate efforts to define the relationship between *Salmonella* contamination of poultry and associated human illness, there is a strong seasonal component to human salmonellosis. In recent FoodNet data for the United States, the peak-to-trough ratio is more than two, with the peak in cases occurring in the summer months (CDC 2007), a pattern reported by many other countries as well (Ravel et al. 2010). *Salmonella* prevalence data from broiler chicken HACCP samples analyzed by FSIS show a lower peak-to-trough ratio, with peak prevalence occurring between August and November (Lange 2006). *Salmonella* prevalence on broiler chicken farms in the Netherlands also peaks during the months of July to December (van der Fels-Klerx et al. 2008), although seasonal differences for on-farm *Salmonella* were not so apparent in another study (van de Giessen et al. 2006), and

there was no seasonal effect seen in processing plants (van der Fels-Klerx et al. 2008). A study conducted in Canada found little seasonality in *Salmonella* prevalence on retail chicken and no correlation with the seasonal pattern in human illness (Ravel et al. 2010). Less seasonal variation has been reported for the more serious cases of human salmonellosis (Gradel et al. 2007), with further complicating factors such as increased likelihood of antibiotic use in the weeks before human illness (Gradel et al. 2008).

SAMPLING ISSUES

> Much of the research efforts, however, involve only the final portion of the procedure, i.e., the detection or identification of the microorganisms. The upstream portions that deal with sampling and sample preparation are often overlooked.
>
> Brehm-Stecher et al. 2009

After more than a half century of sampling poultry carcasses and environments for *Salmonella*, sampling and experimental design issues still exist. Recent reviews have addressed sample preparation (Brehm-Stetcher et al. 2009), the quality of experimental design and reporting (Sargeant et al. 2009), the quality of statistical analysis (Gardner 2004, Ogliari et al. 2007), and the best practices for investigating and reporting the relationship between food animals and food safety (O'Connor et al. 2010). Many reviews are available for microbiological sampling of poultry (Aho 1992, Capita et al. 2004, Carrique-Mas et al. 2008, Davies 2005, Mead 2007). A recent review of measurement uncertainty in sampling of food for bacteria reports that bias can be introduced by the choice of sampling method, in the way that the original sample is collected, transported, and stored, in subsampling methods, or by the accuracy of all of these procedures (Corry et al. 2007).

An expert consensus on the most appropriate sampling sites and methods for determining the *Salmonella* status of poultry flocks and poultry meat has never been reached, because comparing results between different methods is not a simple matter (Mead et al. 2010). The lack of uniformity in pre- and postharvest sampling methods can lead to misinterpretation of results when comparing *Salmonella* prevalence across different studies. In a recent compilation of data on the prevalence of *Salmonella* on poultry meat, for example, a summary table revealed that reporting countries used either 1, 25, 50, or 60 gram samples of meat (EFSA 2010b). The likelihood of isolating *Salmonella* increases with a larger sample weight and with increasing amounts of skin included in the sample (Jorgenson et al. 2002).

In general, sampling methods were developed based largely on ease of performance and cost, and the relationship of results to human illness was not an important consideration. There are many potential sampling sites and types of sampling methods used in feed mills, hatcheries, farms, processing plants, and distribution channels. Some typical sample types are discussed in Mead et al. 2010 and other previously cited reviews. On farms in Europe, sampling of fecally contaminated litter is done using boot swabs (Davies 2005). Several reports indicate that intestinal tract samples from birds identify more *Salmonella*-positive flocks (Rasschaert et al. 2007, Van Hoorebeke et al. 2009), but it has never been shown whether flocks identified as *Salmonella*-positive by more intensive sampling approaches are more likely to cause human illness.

During processing, several different carcass and meat sampling methods are frequently used. Neck skin sampling and meat samples of 10 or 25 grams are commonly used in Europe. Whole carcass rinsing with 400 or 500 milliliters of rinse liquid per carcass is used in countries such as the United States and Australia with 7.5% or 20% of the rinse liquid cultured for *Salmonella* (USDA/FSIS 1996b, FSANZ 2010). Chilling carcasses in air, as is a common practice in the EU,

or in water, which is commonly done in the United States, may affect the results if drying of the skin enables *Salmonella* cells to better attach to the carcass.

The European Food Safety Authority's Panel on Biohazards has recently suggested that more data are needed from additional studies on the "possible correlation between *Salmonella* targets in broiler flocks and expected contamination levels in the resulting poultry meat," indicating that such a correlation needs further refinement (EFSA 2010b). The panel also stated that it is not possible to establish a quantitative link between the *Salmonella* criteria for poultry meat and any risk or implication for public health.

If rearing and processing modifications aimed at reducing *Salmonella* are not tested using appropriate sampling methods, apparent reductions may be misleading and may not reduce human salmonellosis. If conditions experienced by bacteria in the sample during transport to the laboratory are not equivalent to those conditions experienced by the chicken meat destined for the consumer, results from the sample might not be relevant to public health. Currently, there is no validated method for detecting *Salmonella*-contaminated carcasses or flocks that are the greatest risk to human health because risk assessment has not reached that level of sophistication.

CULTURE METHODOLOGY ISSUES

> This survey found major differences in isolation methods. In fact, no two [of 74] laboratories isolated *Salmonella* the same way.
>
> Waltman & Mallinson 1995

No studies have been reported that correlate laboratory culture methods for detecting *Salmonella* in foods with the risk of acquiring human salmonellosis. Current methods were developed to obtain the greatest number of *Salmonella*-positive samples with an acceptable cost and ease of performance, and no consideration was given to determining the relationship of results to the risk of acquiring human salmonellosis. Different types of samples can contain different microbial competitors, nutrients, and antimicrobial inhibitors, and there is a different response regarding *Salmonella* growth, depending on the degree of stress or sublethal injury that the bacteria may have experienced; hence, sample characteristics and history must be considered when selecting a cultural methodology for *Salmonella* detection or isolation. Even for a relatively well-understood sample type such as a chilled poultry carcass, there is no internationally recognized standard methodology for *Salmonella* detection, either for sample size or microbiological methods. Use of different cultural media is not simply a matter of laboratory choice, with some methods delineated by regional, national, or international organizations.

Classical cultural techniques for *Salmonella* detection generally follow a standard sequence, including a nonselective preenrichment (which may be used depending on whether cells are stressed or are present in low numbers), a selective enrichment, isolation on selective agar media, biochemical screening with triple sugar and lysine iron agars, and serological confirmation with poly-O and poly-H antisera. Many different media formulations have been developed for the preenrichment, enrichment, and selective isolation steps in the detection sequence. A survey of diagnostic veterinary laboratories in the United States revealed that 17 different selective enrichment media were being used (Waltman & Mallinson 1995). There were also differences in incubation temperatures, whether samples were incubated for 24 h or 48 h or both, or whether samples were subjected to delayed secondary enrichment conditions (e.g., samples held at room temperature for up to five additional days). Selective enrichment cultures were inoculated onto 14 different plating media, with most labs using two or more different types of media to increase the likelihood of recovering

Salmonella. Finally, the survey revealed considerable variation in numbers of colonies selected for further testing by the different laboratories, with many selecting and identifying only one colony.

Several collaborative studies have been done in 16 national reference laboratories in Europe comparing the efficacy of procedures for *Salmonella* isolation and identification from feces (Voogt et al. 2002a,b). When standard samples were provided to all participating labs, the results revealed significant differences among the labs and within different subsections of the study in the ability to detect *Salmonella* and identify the serotypes that were isolated. Several modified methods were used as the studies progressed, and new methods were developed in the absence of an official reference method for isolating *Salmonella* from fecal samples. Discussions of *Salmonella* detection in control or regulatory samples often assume accurate and reproducible results, but the reality of results of laboratory assays is that considerable variability can occur (Waltman & Mallinson 1995; Voogt et al. 2002a,b).

The commonly used liquid and solid media utilized for isolation of *Salmonella* in food laboratories have changed over time. Lactose broth, Gram negative broth, Rappaport (Vassiliadis) broth, selenite cystine broth, and tetrathionate broth were used years ago. All of these broths are still used to some degree, but the last three have been significantly modified to increase their effectiveness. Plating media used over the years for *Salmonella* isolation included brilliant green, bismuth sulfite, Hektoen enteric, *Salmonella-Shigella*, and XLD agars. Some of these are rarely used today and others have been modified to increase isolation efficiency from different types of samples. Brilliant green with sulfapyridine added is now called BGS. XLD has had many modifications, including addition of sodium thiosulfate and Tergitol 4 to make XLT4 agar. These additions have improved overall performance of these media. Food microbiology laboratories usually use two or more plating media to reduce the occurrence of false-negative results (Cox & Berrang 2000). Rappaport's enrichment medium was modified to become the widely used Rappaport-Vassiliadis (RV) broth (Vassiliadis 1983) and was then further modified with the development of modified semisolid RV (MSRV) for selection of motile *Salmonella* (De Smedt et al. 1986).

The main reason for development and adoption of different media has always been isolation of the maximum number of colonies of *Salmonella* serotypes other than Typhi. The widespread use of MSRV in Europe during the past 20 years, as opposed to less use in the United States and some other parts of world, may have influenced the reported differences in isolation rates and prevalence of different serotypes in various parts of the world.

Polymerase chain reaction (PCR)-based, immunological, and other nontraditional methods skip some of the steps in classical microbiology and are used in many laboratories, but presumptive positives still must be confirmed by traditional cultural methods. In addition to confirmation, this provides an isolate that can be used for serotyping. Even with the development of more rapid methods, such assays usually require the presence of 10^3 or 10^4 cells for detection; hence, enrichment is still required for most food samples, which generally have less than 10^3 salmonellae per 25 g. PCR was determined to be better than traditional culture methods for detecting *Salmonella* in neck skin samples, but both methods together detected more positives than either method individually (Whyte et al. 2002), also a common observation for any two conventional cultural methods. Additional sampling and methodology issues are discussed in a recent publication by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF 2010).

SALMONELLA PREVALENCE ISSUES

> As generally used, enrichment culture effectively erases valuable information about initial microbial numbers within a sample, downgrading a potentially quantitative test into a qualitative one.
>
> Brehm-Stecher et al. 2009

What is the true prevalence of *Salmonella* on poultry? *Salmonella* prevalence rates on chicken carcasses can be increased or decreased depending on the sampling methods used, such as changing the portion cultured after whole carcass rinsing or changing the sample weight in neck skin or meat sampling (Cox & Blankenship 1975; King et al. 2008; Simmons et al. 2003a,b; Surkiewicz et al. 1969). Culturing a smaller sample size can reduce the prevalence of *Salmonella* on poultry, whereas larger-than-typical size samples can increase the prevalence. Sampling methods for determining *Salmonella* prevalence are often based on convenience or previous experience, and prevalence data are often presented and compared with insufficient information regarding testing methods.

Studies at the retail level have revealed that there has been less change in prevalence of *Salmonella* on chicken meat than anticipated (Fletcher 2006). Remarkably, data reported in 81 publications between 1961 and 2004 revealed average *Salmonella* prevalence on chicken meat was similar in different parts of the world, in different decades, and as determined by several different methods. Despite many programs to control *Salmonella* in chickens, not much changed in broad averages of data from supermarkets and shops at the point nearest the consumer, with a mean retail prevalence of about 31%. Fletcher concluded that regulatory pressure to reduce *Salmonella* in processing plant samples would not improve public health unless *Salmonella* prevalence was also reduced at the point of distribution and sale. The location at which prevalence samples are obtained may also be an important factor in determining the relationship between prevalence of *Salmonella* on raw poultry and the risk of acquisition of human salmonellosis.

Compared to other sampling methods, neck skin samples have less variation in indicator bacteria that are present in relatively high numbers (Hutchison et al. 2006). *Salmonella* prevalence results from neck skin samples are sometimes similar to results from whole carcass rinse sampling (Cox et al. 2010c, Sarlin et al. 1998); although, higher prevalence in neck skin samples has been reported in cutting plants (Burfoot et al. 2009) and with retail chicken (Jorgenson et al. 2002). A study in which only one to four grams of neck skin were assayed compared to the 25 grams usually tested today revealed that whole carcass rinses had a significantly higher *Salmonella* prevalence (Cox et al. 1978). The neck skin–whole carcass rinse comparison may be affected by the number of *Salmonella* cells that are present on the carcasses and the extent to which they are attached to the carcass, so that different flocks and different processing methods may affect the results. Three of the cited studies were done with immersion-chilled carcasses (Cox et al. 1978, 2010c; Sarlin et al. 1998), one with air-chilled carcasses (Burfoot et al. 2009), and the chilling method was not apparent in the other paper (Jorgenson et al. 2002).

In whole carcass rinse sampling, the rinse liquid comes in contact with the entire surface of the carcass if the rinse procedure is properly conducted. A neck skin sample of approximately 25 grams is a relatively small percentage of the total skin or surface area of the whole carcass, so the approximate equivalence observed in several studies may imply that there are more than a few *Salmonella* cells that are attached or associated with the skin and carcass and are about as likely to be isolated from macerating the neck skin as from a rinse of the entire carcass. Finding a higher *Salmonella* prevalence in neck skin than in whole carcass rinse samples may indicate that many of the cells are attached or associated to some degree with the skin and are not being recovered by rinsing the carcass. In whole carcass rinse sampling, the first *Salmonella*-positive rinse is found sometimes only after multiple negative rinses of the same carcass (Izat et al. 1991, Lillard 1989).

As indicated by Brehm-Stecher and colleagues (2009), prevalence reports the proportion of samples that are positive without reference to the number and strains of bacteria that are present. This review points out many examples in which prevalence information alone is inadequate for a human health risk assessment.

SALMONELLA ENUMERATION ISSUES

> Most studies that have investigated foodborne pathogen contamination on meat have focused on the prevalence of samples that are positive for the organism. These prevalence measures are largely irrelevant for predicting risks that depend on the quantity of pathogens ingested, as most foodborne illnesses are expected to come from the right tail (i.e., exceptionally high region) of the frequency distribution of microbial loads on meats.
>
> Singer et al. 2007

Enumeration of *Salmonella* is difficult and expensive compared to estimating counts of many common bacteria. As a result of the cost of most-probable-number (MPN) procedures that are largely used to estimate numbers of *Salmonella* in samples, *Salmonella* cell numbers are rarely reported in the scientific literature compared with the number of papers that report *Salmonella* prevalence. A three-tube MPN inoculated at three dilutions will be about nine times more expensive than a prevalence test and will have confidence intervals that are considerably larger than those from counting bacteria on plates.

Enumeration of salmonellae on carcasses or parts of carcasses is done by removing bacteria from the sample and suspending them in liquid. The resulting estimates have an uncertain relationship with the number of salmonellae that remain on the carcass (as in whole carcass rinsing for which the carcass is not incubated) or were not included in the partial sample (as in neck skin sampling for which salmonellae on the remainder of the carcass are not included in the sample). Salmonellae that are not included in the suspension tested in the laboratory may remain on the carcass and reach the consumer. For aerobic bacteria and *Enterobacteriaceae* that are present on poultry carcasses in large numbers, a single whole carcass rinse removes only about 10% of the total bacteria that can be removed with repeated, multiple rinses (Lillard 1988, 1989). The numerical relationship between bacterial cells removed and cells remaining on the carcass is much less certain for bacteria that are present in relatively low numbers. For broiler chickens, the mean MPN value for *Salmonella*-positive whole carcass rinses is often cited as approximately 30, a much lower and more variable number than that of aerobic bacteria or *Enterobacteriaceae* in chicken carcass rinses.

Some information is available, however, concerning the relatively few chicken carcasses or chicken parts carrying high numbers of *Salmonella*. MPN values of 2,071 immersion-chilled chicken carcasses were reported using the same methods (USDA/FSIS 1996a, CFIA 2000). Of the 2,071 carcasses sampled in the two baselines, there were four carcasses that had MPN values greater than 12,000 per carcass (MPN per milliliter multiplied by the number of milliliters of rinse), with the most contaminated sample containing 112,000. In the most recent baseline study of 3,275 carcasses sampled at the rehang point between the kill and evisceration sections of processing plants, the most contaminated carcass had a total *Salmonella* MPN of 1,100 per ml or 440,000 for the entire carcass (USDA/FSIS 2009). A study in England in which 241 whole chickens at retail were sampled revealed two carcasses with approximately 10^4 salmonellae each, as determined by direct plating (Jorgenson et al. 2002). Results of other studies have revealed large numbers in a few samples when *Salmonella* cells on chicken carcasses, chicken breasts, or meat were enumerated (Cason et al. 2007, DuFrenne et al. 2001, Straver et al. 2007, Uyttendaele et al. 2009). If the single-rinse to total-carcass bacteria ratios reported for aerobic bacteria counts and *Enterobacteriaceae* cell numbers hold true for carcasses contaminated with large numbers of *Salmonella*, there are likely many more *Salmonella* cells present on those carcasses that are not recovered in any type of nondestructive sampling.

The importance of enumerating *Salmonella* has been emphasized in an expert report by the American Society for Microbiology (ASM 2006) and the National Advisory Committee on

Microbiological Criteria for Foods (NACMCF 2004). The ASM report indicated that enumeration of *Salmonella* would be "a major scientific step forward." Both organizations reported that the efficacy of pathogen reduction cannot be determined without enumeration, because control efforts could reduce pathogen cell numbers and risk to human health even if prevalence is not changing. Cell numbers are also important for conducting risk assessments, which have shown that risk can be reduced with no change in prevalence (Lammerding 2006, WHO 2002). Risk assessments have also revealed that most of the risk of acquiring human salmonellosis is from exposure to chicken carcasses and parts that are contaminated with large numbers of salmonellae (Straver et al. 2007, Uyttendaele et al. 2009, WHO 2002).

Many experts believe that most poultry-related cases of salmonellosis are caused by cross-contamination in the kitchen from raw poultry meat to other foods or back to cooked poultry, with cross-contamination studies revealing movement of bacteria from chicken skin to other surfaces with relative ease (Chen et al. 2001, Luber 2009, Zhao et al. 1998). Luber (2009) noted that the World Health Organization risk model for chicken meat (WHO 2002) does not include the risk of cross-contamination of *Salmonella* from meat to other surfaces. It is likely that a highly contaminated carcass or portion of meat has a greater probability of transferring *Salmonella* cells to other surfaces than a carcass contaminated with only a few cells of *Salmonella*.

Calculations can be done to relate *Salmonella* cell numbers present in a sample to the likelihood of a *Salmonella*-positive prevalence test result. For whole carcass rinse samples, for example, a portion of the total rinse is cultured for the presence of *Salmonella*, with aliquot volume varying in different testing plans (King et al. 2008, USDA/FSIS 1996a). Assuming that all cells recover and reproduce during enrichment, the probability of a *Salmonella*-positive test can be calculated as $P(+) = 1–(1–30/400)^n$ where n equals the number of suspended cells in 400 milliliters of rinse and a 30-ml aliquot is tested for the presence of *Salmonella*. **Figure 2** shows an approximate histogram of the relationship between cell numbers of *Salmonella* in a whole carcass rinse versus *Salmonella* prevalence based on an aliquot from that rinse, with the x-axis (total cell numbers recovered in a carcass rinse) on a log scale and with observed frequency on the y-axis. With nine cells suspended in the rinse, there is an approximately 50% chance of a *Salmonella*-positive test result, so at the low end of the x-axis there are many false-negative results even though the rinses contain cells of *Salmonella*. However, we believe that these *Salmonella*-negative results have little significance for human illness because risk models indicate that most human salmonellosis is caused by exposure

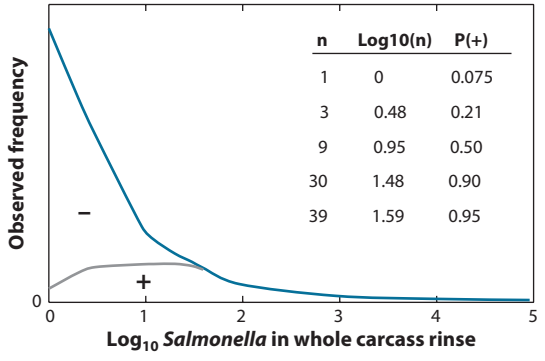

Figure 2

Histogram of log10 *Salmonella* cells in whole carcass rinses (*blue line*) showing the relationship between numbers of cells in 400 ml of rinse and the probability of a positive test when an aliquot of 30 ml is cultured, with the gray line separating test positive and test negative results.

to chicken carcasses and parts contaminated with more than 10^4 cells per carcass (Straver et al. 2007, WHO 2002). Hence, the most contaminated chicken meat products carry a higher risk of causing salmonellosis (Uyttendaele et al. 2009). Heavily contaminated carcasses should also be much more likely to cross-contaminate other foods.

SALMONELLA SEROTYPING ISSUES

> . . .The probability of detecting a specific *Salmonella* strain had little to do with its starting concentration in the sample. The bias introduced by culture could be dramatically biasing *Salmonella* surveillance systems. . .
>
> Singer et al. 2009

Although the scientifically conservative position is to regard all *Salmonella* serotypes as human pathogens (all are considered pathogenic at some dose), there are known differences between serotypes in ability to survive stress, colonize animals, invade tissues, and cause disease with varying outcomes. Among the common *Salmonella* serotypes causing human illness, the percentage of cases of salmonellosis that are hospitalized varies from 14% to 67%, with the range of case fatality rates varying by more than 100-fold (Jones et al. 2008). Septecemia caused by different serotypes is strongly influenced by increasing age of the patient, but there are major serotype differences, with Virchow being more invasive in young children and Enteritidis in persons more than 60 years of age (Weinberger et al. 2004). *Salmonella* of different serotypes also differ in ability to survive food processing conditions, with Enteritidis surviving better than Typhimurium and Infantis, and much better than Dublin (Hald et al. 2004).

In a model that incorporated seven major *Salmonella* serotypes (not including Enteritidis) with all serotypes assumed to be equally capable of causing disease, in United States data, there was a mismatch between observed illnesses and expected outcomes (Sarwari et al. 2001). More illnesses than expected were caused by Typhimurium and Newport, with many fewer cases than expected caused by Kentucky and Derby. When a tenfold higher risk was assumed for each major food animal (cattle, swine, and poultry), there was little effect on the results, with each serotype's ability to cause disease having more impact on human illness than the type of serotypes in each animal. In Danish surveillance data, serotypes Newport, Virchow, and Thompson were more virulent than Enteritidis (Pires & Hald 2010).

In general, *Salmonella* methodology has been selected based on maximum recovery of positive colonies and not on finding specific *Salmonella* serotypes within a food sample. Many studies have demonstrated, however, that cultural techniques can influence the serotypes that are recovered from samples (Carrique-Mas & Davies 2008, Harvey & Price 1967, Kinde et al. 2004, Love & Rostagno 2008, Rostagno et al. 2005, Singer et al. 2009). Serotype bias has been documented since the 1950s, but the public health significance is still little known and poorly understood. Enteritidis, for instance, is a major serotype in human illness, but when mixed in equal proportions and incubated overnight, Enteritidis is outgrown by Newport (Singer et al. 2009) and by Heidelberg and Senftenberg (Kinde et al. 2004). Growth of Enteritidis in the presence of other serotypes in feces or hatchery fluff is also reduced by the stress of drying (Cox et al. 2010d). The unequal competition between serotypes may have implications for pooling of samples in monitoring of *Salmonella* in poultry. Some samples are routinely pooled in neck skin and farm sampling in Europe, possibly distorting the picture of what serotypes are present in multi-serotype samples.

Multiple serotypes have been isolated from the same samples in some reports. In a study that sampled retail chicken wings and turkey necks by two culture methods, multiple serotypes were

recovered from a high proportion of *Salmonella*-positive samples (Temelli et al. 2010). In a recent study in our laboratory, 49 of 52 broiler chicken carcasses rinsed immediately after defeathering were *Salmonella*-positive, with one serotype isolated from seven carcasses, two from 19 carcasses, three from 18 carcasses, and four from five carcasses (Cox et al. 2010a). Other studies have revealed isolation of multiple *Salmonella* serotypes from individual samples (FSANZ 2010, Jorgenson et al. 2002). Using multiple isolation media and picking three to five suspect colonies from each plate can result in the isolation of multiple *Salmonella* serotypes from a large percentage of individual samples. Picking only one colony per plate may underestimate the number of *Salmonella* serotypes that are present in a sample.

Two recent research projects in our laboratory revealed different effects of plating media on serotype isolation from hatchery fluff and carcass rinses (Cox et al. 2010a,e). From the fluff samples, 455 presumptive *Salmonella* isolates were subtyped with antisera. Serogroup C1 (later identified as Lille) was isolated 34 times from BGS or Hektoen Enteric plates, but only once from XLT4 plates. In the broiler carcass rinse study, on the other hand, serotype Kiambu was detected 15 times on XLT4 and never on BGS. When the isolates were later restreaked, the Lille was determined to be a weak H_2S producer that did not appear as a typical *Salmonella* on XLT4, and the Kiambu was a strong H_2S producer that formed smaller-than-average colonies on BGS. All of the work was done by technicians well-experienced in isolating *Salmonella*, demonstrating that nontypical *Salmonella* colonies can be overlooked on plates that have many suspect colonies, even when multiple colonies are being selected.

Serotype differences may explain some of the difficulty in attributing human salmonellosis to different foods. Many studies have revealed a poor match between human and animal *Salmonella* serotypes (Heithoff et al. 2008, Jones et al. 2008, Kariuki et al. 2002, Pointon et al. 2008, Ravel et al. 2010, Sarwari et al. 2001, Schlosser et al. 2000, Stevens et al. 2009, Sumner et al. 2004, Todd 2004). One explanation may be that changes in serotype patterns have occurred between the hatchery and farm (Bailey et al. 2002), between the farm and processing plant (McCrea et al. 2006, van der Fels-Klerx et al. 2008, Rasschaert et al. 2008, Volkova et al. 2009), and between the processing plant and retail (Mellor et al. 2010, Van Asselt et al. 2009, Van de Giessen et al. 2006). New serotypes can be introduced into a flock because of contaminated transport cages and because of cross-contamination of serotypes between different flocks via processing equipment (Corry et al. 2002, Rasschaert et al. 2008). Differences in *Salmonella* isolation methods may be part of the reason for serotype changes from hatchery to farm to processing plant. Composition of samples is different (environment and fecal, carcass rinses or neck skin) and stresses experienced by the salmonellae can be different (dry versus wet, temperature changes, chemical stress in some countries). The differential response of *Salmonella* of various serotypes to different laboratory culture methods may also be a factor in the changing serotype patterns reported during rearing and processing of poultry.

Mismatches are observed frequently in serotypes of *Salmonella* isolates obtained from food animals and from infected humans. Isolations of Paratyphi B var. L(+) tartrate+ (formerly known as Java) increased explosively in poultry in recent years in the Netherlands and northern Europe (Van Pelt et al. 2003, Van de Giessen et al. 2006, Van Asselt et al. 2009), with few human illnesses caused by this serotype being reported. More isolations of this serotype occur in retail samples than in processing plant samples (Van de Giessen et al. 2006). Kentucky has been isolated from more than 50% of chicken HACCP samples and from NARMS retail chicken breast monitoring samples, but Kentucky typically causes only 0.3% of human cases in the United States (CDC 2008b). Serotype Sofia (now known by its antigenic formula II 1,4,12,27:b:[e,n,x]) has been isolated from more than 90% of poultry isolates during some years in parts of Australia, but has been isolated from few human cases there (Harrington et al. 1991, Mellor et al. 2010, Pointon et al.

2008, Sumner et al. 2004). Sofia attaches to processing plant equipment surfaces more efficiently than other *Salmonella* serotypes (Chia et al. 2009), so it may have properties that make it more likely to be isolated from poultry samples.

SALMONELLA SUBTYPING ISSUES

> At present there is an urgent need for *Salmonella* fingerprinting to determine the true extent of genetic diversity among isolates of the same serotype, whether a limited number of clones are associated with human disease and the molecular basis for virulence of these strains.
>
> Manfreda & De Cesare 2005

Despite the importance of serotyping as a useful tool for understanding the contamination of raw poultry by *Salmonella*, serotype information alone is not sufficient for ecological and epidemiologic investigations to understand the movement of *Salmonella* through the different stages of poultry production or the relationship of those strains to human salmonellosis. Hald et al. (2004) reported that serotype distributions are not enough information and that "discriminatory epidemiological typing methods" are needed for intensive monitoring of animal and human serotypes. Strain differences in virulence in mice of animal and human Typhimurium isolates were the main reason why there was a weak correlation between these strains in animal and human isolations (Heithoff et al. 2008). Antibiotic resistance patterns in isolates from imported meat and returning international travelers were helpful in identifying specific sources of some strains of *Salmonella* (Hald et al. 2007).

Different *Salmonella* isolates of the same serotype found in environmental samples and in a chicken flock are not always the same subtype, with preharvest strains usually not predominant in neck skin samples from processed carcasses (Heyndrickx et al. 2007). There are also important differences in strains of *Salmonella* from different parts of the world, with relatively avirulent strains of Sofia in Australia compared with virulent strains of the same serotype causing substantial human illness in Israel (Harrington et al. 1991).

Enteritidis is a serotype that is responsible for a major percentage of human salmonellosis worldwide, but it is a somewhat unusual *Salmonella* in that it has relatively low genetic variability compared to other serotypes. Liebana et al. (2001) concluded that better methods such as phage typing or multiple molecular methods are needed to differentiate different strains of Enteritidis isolated from poultry. As with many subtyping methods, there is no definitive international agreement on the best molecular methods for subtyping *Salmonella* isolates.

Pulsed field gel electrophoresis (PFGE) and antibiogram patterns can also be used to evaluate the diversity within individual *Salmonella* serotypes. In another study in our laboratories, the PFGE and antimicrobial resistance patterns of *Salmonella* isolates were compared to 4,620 XbaI patterns originating from testing 17,597 isolates in USDA-VetNet (Cox et al. 2010b). The isolates for this study originated from 52 whole carcass rinses that were tested using culture method combinations that are not commonly used by regulatory agencies. Compared to patterns within the USDA-VetNet database, a total of 10 new *Salmonella* PFGE XbaI patterns were identified from only 49 positive samples. The identification of new patterns suggests that further work needs to be conducted on cultural influences that select certain *Salmonella* serotypes and subtypes.

Taking into account the distinctions between *Salmonella* serotypes and strains that have been recognized in recent years, many older publications may need to be reevaluated. Many assumptions concerning generic *Salmonella* at different stages of rearing and processing were likely confounded with different serotypes and strains involved. Serotypes of *Salmonella* isolates obtained from the

farm and during processing may not have been the same strain. Sampling and cultural methods may have skewed the results that were obtained.

SUMMARY POINTS

1. There is a poor correlation between *Salmonella* prevalence in poultry meat and human salmonellosis, indicating that prevalence as measured is not closely related to risk.
2. In several parts of the world, as much as half of *Salmonella*-positive samples from poultry are serotypes that are rarely associated with human disease.
3. Programs to reduce *Salmonella* contamination in poultry meat are not likely to greatly affect the risk of acquiring human salmonellosis unless those serotypes that cause a large proportion of human illness are targeted and reduced.
4. Monitoring programs for *Salmonella* in poultry may not be adequate to detect reductions in the risk of acquiring human salmonellosis.
5. Methods for isolating *Salmonella* from poultry and other foods are not equally effective in obtaining all serotypes, thereby influencing the accurate identification of the *Salmonella* serotypes that are present.
6. Improved *Salmonella* enumeration methods are needed to develop more useful risk assessments.
7. A better understanding of the ecology of *Salmonella* in poultry and humans is needed to develop more strategies to reduce the risk of human salmonellosis.
8. Internationally agreed upon sampling and laboratory culture methods for *Salmonella* in poultry from the farm to retail are needed.

DISCLOSURE STATEMENT

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Errata

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