Scientific and Technical Factors Affecting the Setting of *Salmonella* Criteria for Raw Poultry: A Global Perspective

Geoffrey Mead[1], Anna Lammerding[2], Nelson Cox[3], Michael P. Doyle[4*], Florence Humbert[5], Alex Kulikovskiy[6], Alex Panin[7], Vladimir Pinheiro do Nascimento[8], Martin Wierup[9] and the *Salmonella* on Raw Poultry Writing Committee[10]

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[*]Corresponding author:
Tel: 770-228-7284
Fax: 770-229-3216
e-mail: mdoyle@uga.edu

[1]Consultant Microbiologist, Somerset, U.K.; [2]Public Health Agency of Canada, Guelph; [3]USDA-ARS, Athens, GA; [4]UGA Center for Food Safety, Griffin, GA; [5]Flowbio-Veto, France; [6]Ministry of Agriculture, Moscow, Russia; [7]VGNKI, Russia; [8]Brazilian Ministry of Education, Brazil; [9]Swedish University of Agricultural Sciences, Uppsala, Sweden

[10]In addition to principal authors listed above, members of the *Salmonella* on Raw Poultry Writing Committee include:
Raphael Andreatti Filho, Sao Paulo State University, Brazil
Roy Biggs, Tegel Foods Ltd, Auckland, New Zealand
Jeff Buhr, USDA-ARS, Athens, GA, USA
Sarah Cahill, Food and Agriculture Organization of the United Nations, Rome, Italy;
John Cason, USDA-ARS, Athens, GA, USA
Thongchai Chalermchaikit, Chulalongkorn Univeristy, Bangkok, Thailand
Hector Hidalgo, University of Chile, Santiago, Chile
Charles Hofacre, Univeristy of Georgia College of Veterinary Medicine, Athens, GA, USA

Henk Hupkes, Meyn Food Processing Technology, Oostzaan, The Netherlands

Mogens Madsen, Incuba Science Park Skejby, Brendstrupgaardsvej, Denmark

Roel Mulder, Spelderhold Poultry, Burg, The Netherlands

Lars Plym Forshell, National Food Administration, Uppsala, Sweden

Martha Pulido Landinez, Universidad National de Colombia, Colombia

Jason Richardson, The Coca-Cola Co., Atlanta, GA, USA

Douglas Smith, North Carolina State University, Raleigh, NC, USA

Yvonne Vizzier Thaxton, Mississippi State University, Mississippi State, MS, USA

Hajime Toyofuku, National Institute of Public Health, Japan

Pirkko Tuominen, Finnish Food Safety Authority, Helsinki, Finland

Mieke Uyttendaele, Ghent University, Gent, Belgium

Sian Ming Shi, Shanghai Jiaotong University, Shanghai, China

Marcel Zwietering, Wageningen University, Wageningen, The Netherlands

SCIENTIFIC AND TECHNICAL FACTORS AFFECTING THE SETTING OF *SALMONELLA* CRITERIA FOR RAW POULTRY: A GLOBAL PERSPECTIVE

Abstract

Concerns about foodborne salmonellosis have led many countries to introduce microbiological criteria for certain food products. If such criteria are not well-grounded in science, they can be an unjustified obstacle to trade. Raw poultry products are an important part of the global food market. Import / export ambiguities, as well as regulatory confusion resulting from different *Salmonella* requirements, were the impetus for convening an international group of scientific experts from 16 countries to discuss the scientific and technical factors that affect the setting of a microbiological criterion for *Salmonella* contamination of raw chicken. A particular concern for the group was the use of criteria implying a 'zero tolerance' for *Salmonella* and suggesting complete absence of the pathogen. The notion can be interpreted differently by various stakeholders and was considered inappropriate because there is neither an effective means of eliminating *Salmonella* from raw poultry nor any practical method for verifying its absence. Therefore, it may be more useful at present to set food-safety metrics that involve reductions in hazard levels. Using terms such as 'zero tolerance' or 'absence of a microbe' in relation to raw poultry should be avoided unless defined and explained by international agreement.

Risk assessment provides a more meaningful approach than a zero-tolerance philosophy and new metrics, such as performance objectives that are linked to human health outcomes, should be utilized throughout the food chain to help in defining risk and identifying ways to reduce adverse effects on public health.

Introduction

The association between poultry and *Salmonella* has a long history. More than 50 years ago, pullorum disease and fowl typhoid were common causes of mortality in chicken and turkey flocks, and development of the industry was delayed until these diseases were brought under control (147). Subsequently, a different problem emerged with the increasing isolation of non-host-specific salmonellae from both poultry products and cases of human salmonellosis. Because of an apparent linkage between the two, fuelled by the intensive nature of poultry production and processing, which was seen to facilitate pathogen transmission, global efforts to control *Salmonella* in the poultry industry have increasingly gathered pace and particularly in the years following the pandemic spread of *Salmonella* Enteritidis in the late 1980s. However, fulfillment of this goal has not been easy. In the production of raw foods, such as chicken meat, there are multiple constraints in attempting to eliminate microbial health hazards, and these are both socio-economic and scientific (i.e., biological, technological and analytical). Food animal production and processing in different parts of the world are faced with similar challenges, such as the frequent presence of potentially pathogenic microorganisms that rarely cause disease in food animals but may do so in humans, along with the very nature of an industry in which environmental contamination with a variety of microorganisms is inevitable. There are inherent limitations in sampling schemes and analytical methods that determine the levels of contamination which can be detected, and different schemes and methods are used by various countries. The global efforts to control *Salmonella* in poultry are further complicated by differences that can be observed in relation to predominant pathogen species /serovar, pathogen prevalence, extent of regulatory control, and the nature, size, and logistic complexity of the industries concerned. Linking the presence and numbers of a particular pathogen in a specific food with the proportion of illnesses caused in a human population constitutes a further challenge, but this information is needed to estimate the magnitude of risk and establish clear

goals for public health protection which can be communicated to industry and the public alike (154). Sound risk management requires allocation of resources that are proportional to the magnitude of the risk and the feasibility and effectiveness of risk-reduction measures.

Raw chicken products are an important part of international food trade. As the world becomes a global market for the exchange of foods, many countries are attempting to prevent foodborne salmonellosis by developing microbiological criteria for control purposes and these may or may not be risk-based. An example is the different criteria (and subsequent actions in the case of non-compliance) that exist for *Salmonella* presence on raw chicken, all of which depend on the stage in the food chain, the sensitivity of the sampling plan and method, and the analytical method used.

International trade agreements have fostered new ways of thinking about food safety (200). Specific requirements for product acceptance criteria may be used as trade barriers. Therefore, such requirements must be scientifically defensible in terms of protecting the health of a nation's consumers, they must be applied equally to domestic as well as imported product, and the prohibitive effect on trade must be kept to a minimum. Global advances towards risk-based management systems and the adoption of an integrated, whole-chain approach have led to new food-safety metrics for the use of industry and regulators in relation to food production and processing. These offer the possibility of linking food-safety control measures to public health outcomes. As the concepts evolve, challenges arise to some of the traditional qualitative approaches to food safety management which focus particularly on end-product control. Risk managers need to consider the relevant scientific evidence, including the quantitative effects of interventions along the food chain and the public health impacts of control measures. Increasingly, risk-based concepts are being adopted for both domestic policy and international trade, despite sometimes being poorly understood and not always applied consistently or with

transparency. Risk-based approaches that utilize the best available scientific information offer objective means to achieve the goals of public health protection, optimal exploitation of resources, continuing access to an adequate food supply, and prevention of unjustifiable trade barriers. A risk-based approach, which may include the use of microbiological criteria, implies that controls and actions are selected and implemented with the understanding and knowledge of the risks to life and health (69).

In November 2008, an international group of experts was convened in Peachtree City, Georgia, USA, to consider the principal scientific and technical factors that affect the setting of a microbiological criterion for *Salmonella* contamination of raw chicken. The group also discussed the impact on this exercise of global variations in commercial production practices, control strategies, and testing regimes. The outcome of the meeting was a scientific report that is presented herein.

Salmonella: public health impact and association with poultry

Salmonella continues to be a leading cause of foodborne enteric disease in many countries and is responsible for significant human suffering, loss of productivity and mortality. Although the disease is under-reported, it has been estimated that 1.4 million people are affected annually in the USA alone, with some 400 fatalities (192). There are also major cost considerations, estimated at 1 – 2.3 billion US dollars per year, including medical expenses, lost productivity, costs to food producers and caterers, as well as investigational costs (18).

The organism occurs commonly in livestock, including poultry, and can also be isolated from wild animals, including rodents, reptiles and birds. When poultry flocks become infected on the farm, *Salmonella* is normally carried asymptomatically in the gastrointestinal tract of a proportion

of the birds and subsequently may be transferred to carcasses during processing via fecal contamination. Further spread may occur during processing due to cross-contamination. In relation to food safety, control measures may be implemented throughout the food chain, and effective control of end-product contamination with *Salmonella* is an important goal for industry, regulators and consumers, to ensure that the product is safe for consumption. However, determining either the true risk to public health from *Salmonella*-contaminated poultry meat or the benefit of reducing contamination is complicated by a number of factors that are discussed in the following.

Although poultry meat is known to be a significant vehicle of foodborne *Salmonella* infections in man, the true proportion of all cases of salmonellosis associated with poultry consumption, or the attributable risk, is difficult to quantify. Currently there is wide variation in the approaches used globally to do so (12, 59, 144). Examples of different approaches include the analysis of outbreak data, case-control studies, microbial sub-typing, source-tracking methods and expert judgment. Considerable effort is now being made to identify the relative contributions to illness from different sources and vehicles of human infection, although a method that is appropriate in one country may be unsuitable in another. The choice will depend on population, infrastructure for surveillance / monitoring, resources available for healthcare, laboratory facilities for sub-typing isolates from animals, human cases and food samples, as well as integration of database systems – if such systems exist. Improved knowledge of the key sources and routes of transmission for pathogens significant in human disease will be vital for evaluating the benefits of expenditures on interventions.

Many countries/regions are moving towards reducing levels of foodborne pathogens using programs based on hazard-control targets, which will be linked to public health objectives. There are challenges to do so from the lack of adequate foodborne illness/source attribution data, and

particularly for *Salmonella*, as it is associated with many different environments, foodstuffs, and transmission routes. The complexity of collecting good attribution data is compounded by variable food handling and cooking practices. Nevertheless, quantitative risk assessments can provide some indication of relative risk by linking the level of a pathogen to some measure of human health impact.

Relevant information can be gained from outbreak surveillance and reporting, although it is acknowledged that this information provides only a partial picture of total disease burden. Outbreak data for the USA between 1990 and 2006 showed that 22.3% of cases of human salmonellosis could be attributed to poultry consumption and about 10% of all investigated cases resulted from consumption of intact chicken (183). On the other hand, expert elicitation estimated that some 22% of US cases could be attributed to intact chicken (108). In the European Union (EU), poultry and pork have been implicated more often in human salmonellosis than beef or lamb (62). Data from England and Wales during the years 1996 and 2000 showed that contaminated chicken was the most important cause of all outbreak-related foodborne illnesses with 398,420 cases and 141 deaths from this particular vehicle, including those due to *Salmonella* (4). From a summary of international data, 4093 reported foodborne outbreaks occurring during 1988 and 2000 showed that 47% of outbreaks were attributable to *Salmonella* and, of these, 34% were associated with chicken meat (78).

Public health and regulatory scientists are attempting to match *Salmonella* serovars from human illness with those obtained from various food sources. However, despite the clear links between food animals and many cases of foodborne salmonellosis, some studies have shown that the predominant serovars detected in poultry are not always those that predominate in human cases (89, 107, 146, 156, 158, 172). In most cases, laboratory procedures seek to recover the broadest possible spectrum of *Salmonella* serovars, but some methods have been developed that focus on

recovering only specific serovars (71, 173, 186). Due to the poor performance of many selective agars in detecting *Salmonella* from non-clinical samples, food microbiology laboratories usually use two or more plating media to reduce the likelihood of false negative results (41). Most laboratories performing *Salmonella* isolation select and identify only a single suspect colony of *Salmonella* per sample and thus risk missing other serovars that also may be present. The *Salmonella* serovars recovered from certain cultivation procedures are highly dependent on the media and methodology procedure employed, introducing a potential confounding variable (29, 82, 120, 164). This bias introduced by culture methods could be potentially influencing results obtained through *Salmonella* surveillance systems and hindering accurate traceback investigations (134). In the analysis of poultry samples, no one method is ever likely to give a true picture of the *Salmonella* serovar distribution. This makes it difficult to use these data for epidemiological and risk assessment purposes (75). Nevertheless, for control purposes, standardized isolation methods in current use are sufficiently sensitive for detecting *Salmonella* in general when present on raw poultry.

A key public health issue is the *Salmonella* contamination level on any positive carcasses at the end of the processing operation. Evidence suggests that, when present, Most Probable Number (MPN) counts are generally low and often no more than 100 cells / carcass (105, 194). Baseline studies carried out in Canada and the USA (33, 178), using the same methods for sampling and testing, revealed that 4 / 2071 carcasses (0.2%) had greater than 12,000 colony-forming-units (CFU) of *Salmonella*. A similar situation was observed for cut portions of chicken sampled at retail in the Netherlands (171). In the latter survey, breast fillets were examined and 8.6% of samples were found to be *Salmonella*-positive, with MPN counts varying from ten to > 1000 per fillet (corresponding to a range of 0.05 – 5.5 CFU / g for a mean fillet weight of 182 g). In total, 0.8% of samples yielded *Salmonella* counts greater than 1000 per fillet. When the data were used in a risk assessment model, it was estimated that more than two-thirds of predicted annual

cases of human salmonellosis from this vehicle were caused by the small proportion of fillets with the highest *Salmonella* contamination. A recent risk assessment on *Salmonella* in Belgian chicken-meat preparations revealed that levels of contamination greater than 1 CFU/ g were most likely to be associated with human salmonellosis (184). At present, enumeration of *Salmonella* is too laborious, costly and time-consuming for routine use, but this situation may change as better methods become available (74).

Salmonella prevalence within broiler flocks varies widely from one country to another. In a recent EU survey, 24 countries were compared using the same sampling protocol and methodology (58). Using a boot swab sampling technique to acquire five pooled fecal samples per flock, national flock prevalence was found to vary from 0 – 65.7% (Table 1). Only in Sweden were no *Salmonella*-positive flocks detected. These results suggest that any common reduction target for prevalence of *Salmonella* in poultry flocks will take longer to achieve in some countries than in others and the costs involved are likely to vary accordingly (185). To address the problem, the EU has made producers more responsible for product safety and is setting specific targets for *Salmonella* reduction in each Member State. For this purpose, legislation has been introduced that makes testing compulsory and specifies deadlines for establishing the required targets in breeders, layers, broilers and turkeys (52, 53). These targets relate to the *Salmonella* serovars that currently predominate in human disease, specifically *S.* Enteritidis, Typhimurium, Hadar, Infantis and Virchow, and may be considered an intermediate step in controlling salmonellae as a whole.

Few studies have been done to evaluate the impact on public health from *Salmonella* control in poultry. However, within the low-prevalence countries, Finland and Sweden, where effective control of *Salmonella* in the industry has been in place for a long time, there is a low prevalence of product contamination, which has considerably reduced consumer exposure to the pathogen in

these countries (62). Examination of the cost-benefit relationship of the Finnish national control program for *Salmonella* in broilers, using a simulation model, led to the conclusion that, in 2000, there was a large saving on public health costs as a result of the program (106). The authors noted that the estimated value of only one death avoided by the control system in place exceeded the cost of the entire program. Cost-benefit studies in Sweden also demonstrated a positive economic and public health effect of reducing *Salmonella* contamination in animal-derived food products (65). Interestingly, in Finland and Sweden, more than 80% of human cases of salmonellosis are attributed to visits abroad, in contrast to countries with a higher prevalence of *Salmonella* in poultry, such as Denmark and The Netherlands, where the majority of cases are associated with domestic food consumption (145). The former situation reflects the efficiency of the overall *Salmonella* control program in those countries and suggests that consumers are mainly infected with *Salmonella* when exposed to food from less well-controlled sources. It is evident that such contaminated food will also include products other than poultry, but the data for salmonellosis acquired domestically indicate clearly the benefit of reducing consumer exposure to *Salmonella* in all food products. However, it is difficult to compare data for foodborne illness, including salmonellosis, for different countries due to differences in surveillance programs.

Control of *Salmonella* spp. in broiler production

In many countries, knowledge of the epidemiology and biology of *Salmonella* has led to the development of mitigation strategies for the organism in commercial poultry-meat production. Thus, it is appropriate to consider the options presently available for this purpose and their application at different stages of the supply chain. Many different interventions have been described and, despite a lack of quantitative data on their ultimate effects, especially as they relate to public health outcomes, it is generally accepted that suitable combinations of measures, implemented throughout the broiler production and processing continuum, can minimize

contamination of the end-product with *Salmonella* (15, 70, 133, 134). Currently, the Codex Committee for Food Hygiene is developing guidelines for controlling *Campylobacter* and *Salmonella* spp. in chicken meat, using a three-tiered approach that describes measures based on: 1) GHP, including biosecurity, cleaning and disinfection; 2) intervention measures that are based on quantitative levels of hazard control, such as a carcass decontamination treatment; and 3) measures based on risk assessment or other information on risk. These guidelines will be applicable from 'farm-to-fork' (27).

For any individual enterprise, the degree of integration is an important factor, because a fully-integrated company should be in the best position to coordinate its control activities and implement traceability. There are many poultry companies around the world that have their own breeding, rearing and hatching facilities, produce their own feed, and slaughter and process their birds for meat. In all cases, however, there is a need to recognize the major epidemiologic risk factors for introducing *Salmonella* into broiler-meat production and allowing the infection to persist: contaminated feed, infected breeder flocks, a lack of effective biosecurity on farms, including inadequate cleaning and disinfection of houses between crops, poor control of hygiene during harvest and transport of broilers, fecal leakage, and cross-contamination of carcasses during slaughter, processing, and further processing of raw product.

Serovar-specific control measures. In some parts of the world, strategies have been adopted to target specific *Salmonella* serovars that are associated with both poultry and human salmonellosis. This has been the case for serovar Enteritidis, which caused a pandemic of human illness from infected layer and broiler flocks, beginning in the 1980s (3). Particular strains of *S.* Enteritidis with an apparent predilection for the reproductive tract of the laying hen were responsible for contamination of egg contents and therefore vertical transmission. Among the measures used successfully to prevent infection of production flocks is stringent biosecurity

for breeding stock and vaccination against the organism in question (3). Testing of breeding flocks and culling of those found to be infected with any serovar of special concern is another means of preventing transmission along the food chain.

The targeting of specific serovars may be justified for those of particular economic or public health significance, especially in relation to production of table eggs, which can be consumed raw or with minimal cooking (76). Also, many of the serovars that predominate in human cases are common in commercial poultry (72. 187). For Enteritidis and Typhimurium, in particular, there is a clear linkage. Conversely, all *Salmonella* serovars are considered to be potentially pathogenic to man, although some of those found in poultry are rarely, if ever, associated with human illness. A classical example is serovar II 1,4,12,[27]:b:[e,n,x], also known as *S.* Sofia, that is often isolated from chicken in Australia but rarely from human cases there (146).

There are, however, potential disadvantages in developing a control strategy for only some serovars at the expense of other food-poisoning salmonellae that also may occur in poultry flocks. Not only do the predominant poultry serovars vary between countries but, even within a single country or region, they can change over time (76). Furthermore, successful control of one serovar may allow another to predominate. For example, epidemiological evidence suggests that Enteritidis may have filled the ecological niche occupied previously by the antigenically-related serovar, Gallinarum, which was eradicated in most of the major poultry-producing countries by the 1970s (148). In Israel, where human salmonellosis has declined since 1995, especially that due to Enteritidis and Typhimurium, a new clone of *S.* Infantis is emerging in human cases that is also found in poultry (8). Although this phenomenon may be due simply to the inherent characteristics of the 'new' strain, it could be the result of intensive efforts to control Enteritidis and Typhimurium in the poultry industry. Clearly, the most rapid means of combating such a problem is to use a serovar-independent approach (76). Most known risk factors for *Salmonella*

infection and available pre-harvest interventions are in this category, and the principal risk factors and interventions are discussed below.

Feed. Together with breeding stock, manufactured feed is a major risk factor for introducing *Salmonella* into the broiler-meat supply chain. The feed can be a latent source of *Salmonella* for food animals, because it is made from a wide range of potentially contaminated ingredients (44, 151). When present in dry feed, *Salmonella* can survive for more than a year and even low numbers may be significant since, for some strains, a level of < 1 cell / g is sufficient to colonize young chicks (157). There are well-documented instances of contaminated feed leading to outbreaks of human salmonellosis from infection of recipient birds, followed by contamination of carcass meat. A classical example was fishmeal imported into the USA, which contained *S.* Agona and led to a rapid increase in human infections with this serovar between 1968 and 1972 (35). It is estimated that *S.* Agona has caused more than one million human cases in the USA alone since it was introduced into the food chain (44). More recently, raw wheat containing *S.* Typhimurium DT1 resulted in poultry-associated human illness in New Zealand (38).

Feed ingredients can be classified according to risk and those presenting the lowest risk should be used wherever possible, especially in feeds for breeding stock. Animal-derived proteins and certain vegetable proteins, such as soybean and sunflower meal, are in the highest-risk category and are heat-treated, whereas rice, for example, is a much lower-risk material and is not heat-treated (61).

During the manufacturing process, broiler feed invariably receives heat treatment. The heat-sensitivity of non-sporulating bacteria, including *Salmonella*, is influenced by the temperature and time, as well as the prevailing water activity (a_w) of the feed. The heating regime aims to eliminate *Salmonella* during pelleting, expansion or extrusion processes, and minimize any

adverse effect on the nutritional quality of the feed (42, 50, 104, 119, 127). However, there is a significant risk of recontamination during post-pelleting stages of the milling operation, as well as during storage and transport of feed. Because of this risk, various chemical treatments have been considered e.g. certain short-chain fatty acids, such as formic and propionic acids. These have many of the attributes that are desirable in a feed treatment (92, 113, 151, 188, 195).

Because *Salmonella* tends to occur in very low numbers and is usually distributed unevenly in any contaminated batches of feed material, feasible sampling and testing schemes have a low probability of detecting the organism (126). Instead of depending on extensive product testing, a better alternative is to apply Good Manufacturing Practice (GMP) / Hazard Analysis Critical Control Point (HACCP) principles to the manufacturing process. The superiority of this approach has been demonstrated in Sweden (64, 123), where mills that showed persistent environmental contamination with *Salmonella* were not being identified by end-product testing alone (197). Effective implementation of the HACCP system requires measures to prevent recontamination of the feed following heat treatment. As with raw ingredients, this involves good storage conditions, including rigorous dust control, appropriate control of transport vehicles, regular cleaning and disinfection of the vehicles, and protection of the load up to and including the point of delivery.

Breeding stock. *Salmonella* can spread easily from infected breeding stock to other parts of the production pyramid and it is therefore of utmost importance to ensure that breeding flocks are free from infection. This category of bird includes elite, grandparent and parent flocks, for which, at the top of the breeding pyramid, preventive measures against *Salmonella* infection are the most rigorous. Special precautions have been adopted in different countries for primary breeding and grandparent flocks (46). Firstly, the feed is treated at a higher temperature and for a longer period than usual, and organic acids may be incorporated to combat any residual

Salmonella contamination. The water supply, too, is checked to ensure freedom from *Salmonella,* and there is frequent and comprehensive monitoring of the flock and its environment. Some countries base their production systems on the importation of day-old grandparent chicks and thorough testing of the birds before use was found to be an effective control measure (197). In all cases, biosecurity is normally maintained at a high level. When primary breeder and parent birds come into lay, the eggs are collected regularly and often treated at the earliest opportunity to eliminate any *Salmonella* on the shell surface. While awaiting transport to the hatchery, eggs are held in a pest-proof, temperature-controlled environment.

Hatchery. Good hatchery hygiene is an essential part of a *Salmonella* control program. Even a single infected chick can transmit the organism to many other chicks during the hatching period. The key elements for effective hygiene control are hatchery design, ventilation, isolation, cleaning and disinfection, waste handling, microbiological monitoring, and good communication between management and staff (199). Biosecurity measures should include an integrated pest management system (11, 47, 88, 111, 113), and staff should be properly trained and instructed in hygiene control and Sanitation Standard Operating Procedures (SSOPs). Regular cleaning and disinfection of equipment and implements is needed, but hatcheries themselves are not the easiest of premises to clean, because the setters are in almost constant use and the hatching cabinets are usually empty for only a few hours at a time. Nevertheless, they need to be cleaned and sanitized between hatches, therefore strong disinfectants are required. Even so, it is not unusual to find 'resident' *Salmonella* strains in incubators and chick-tray washing machines (46).

Egg handling practices are highly important. On arrival at the hatchery, the eggs may be treated with a suitable disinfectant. However, labor intensity, fear of affecting hatchability, and cost factors limit the scope for implementation of this control measure. Eggs are sometimes hatched in an atmosphere containing formaldehyde, which is provided by trays of undiluted formalin that

is removed by ventilation before the chicks are collected. However, since formaldehyde is recognized as a carcinogen, the use of alternative fumigants is preferable.

Although every hatchery should aspire to the highest standards of hygiene control, those dealing with eggs from breeder flocks tend to have more rigorous biosecurity requirements, including the need for staff (and any visitors) to shower before entering the building. When a *Salmonella*-positive flock is identified, but the serovar present is not one that requires culling of the flock, the eggs can be handled and incubated separately, and are usually hatched last in the working day.

Special pre-harvest measures. Apart from the above biosecurity requirements, there are various other preventive measures that can be used to reduce the risk of *Salmonella* infection.

Vaccination. One that is widely used in some countries is vaccination that targets the invasive serovars *S.* Enteritidis and *S.* Typhimurium (77, 124), and involves both live, attenuated and inactivated vaccine strains. Such vaccines are not generally used for broilers but, following protection of parent stock with an injectable vaccine, stimulation of maternal immunity may help to protect broiler progeny. Although live vaccines may give superior protection (189, 202), concerns about their safety and persistence (161) have led to the development of more attenuated vaccine strains that could be less effective. A possible advantage in the oral administration of a live vaccine is that *Salmonella* may be excluded initially by competitive inhibition, and a combination of both live and killed preparations may be beneficial (10, 201). Future developments could include specific antibodies, administered via transgenic crop plants incorporated in the feed (14), and dietary immuno-modulators, such as β-glycan, to prime the immature immune system in chicks (121).

Competitive exclusion (CE). CE treatment is another type of preventive measure that may be used to control *Salmonella* in poultry. It is based on the fact that young chicks are particularly susceptible to *Salmonella* colonization, because they lack a fully developed intestinal microflora that would otherwise prevent the pathogen from becoming established (139). Currently-available treatment products contain many of the elements of a normal adult microflora, but their exact composition is usually unknown and therefore they are not accepted for commercial use in all countries. Nevertheless, such products have a long history of safe use in parts of Scandinavia. CE treatment is most effective when used as part of an overall control program that includes comprehensive biosecurity measures and, although *Salmonella* is rarely excluded completely, there is usually a significant reduction in prevalence and in levels of intestinal carriage among positive birds (159). For older birds that may be given a therapeutic dose of antibiotics to clear an existing *Salmonella* infection, CE treatment can be used to regenerate the intestinal microflora and reduce the risk of reinfection (150).

Probiotics and prebiotics. Probiotic preparations containing defined mixtures of treatment organisms have also been developed to control *Salmonella* in poultry. These usually contain one or more strains belonging to a limited range of microbial genera, such as *Lactobacillus* and *Enterococcus* (57). Their purpose is to improve the balance of the intestinal microflora so that conditions become less favorable for the establishment of pathogens. For most defined preparations, however, tests have rarely been carried out under field conditions and, even in experimental studies, the observed reductions in *Salmonella* colonization have been less than those obtained with undefined CE products (128). Prebiotics, too, aim to beneficially influence the balance of the intestinal microflora. These are dietary ingredients that are not digested or degraded by the host and selectively enrich for one or a limited number of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus* spp. (142). Thus, their effects on invading pathogens are likely to be similar to those of probiotics. Most prebiotics are carbohydrates of different

kinds, such as fructo-oligosachrides, which may reduce *Salmonella* colonization of chicks, especially when administered together with a CE preparation (9). However, as with probiotics, few proper field trials have been carried out.

Bacteriophages. The use of lytic bacteriophages to reduce intestinal carriage of *S.* Enteritidis or *S.* Typhimurium in artificially-challenged chicks has been investigated by several research groups (7, 13, 165, 177). The phages were obtained from a variety of sources, including human sewage, and used as 'cocktails'. Although some reduction in *Salmonella* colonization was observed, the effect was generally small and often did not persist. Since phages are relatively robust and can be administered to chickens via feed or drinking water, they are attractive candidates for control purposes, once the necessary treatment conditions have been established; however, there is still a need for better knowledge of phage replication kinetics and the influence of factors such as inoculum size and timing of the treatment (37). In addition, target bacteria such as *Salmonella* develop resistance to phages following repeated exposure. The efficacy of phages in controlling *Salmonella* colonization under field conditions has yet to be fully determined.

Chemical treatments. Chemical treatment of feed at the mill has been described above. Acid treatment of the drinking water given to chicks was found to be an effective method of water sanitization, but had no effect on *Salmonella* carriage in birds that were already infected (5). On the other hand, water acidification used in another study (110) reduced horizontal transmission of *Salmonella* from artificially-challenged chicks to others in the same group that had not been challenged. There was no effect on *Salmonella* prevalence in the inoculated birds.

Antimicrobial treatments. Antimicrobials such as ampicillin, tetracyclines and spectinomycin may be used under veterinary supervision to eliminate *Salmonella* infections that produce no clinical disease in the birds. The treatment is used mainly for breeding flocks infected with *S.*

Enteritidis or *S.* Typhimurium that otherwise would have to be slaughtered. A disadvantage of the treatment is disruption of the intestinal microflora that may result in re-infection from a contaminated environment, and hence the need to administer a CE preparation in order to repair the damage (159). It is also known that *Salmonella* is not necessarily eliminated permanently from treated birds (93). When used at sub-therapeutic levels to improve the growth performance of broilers, antimicrobials can have varying effects on *Salmonella* infection and sometimes, as with avoparcin, increase shedding due to disturbance of the ecological balance in the alimentary tract (125). Also, their use for this purpose may contribute to the growing problem of antimicrobial resistance in human pathogens (122, 174) and is now prohibited or being phased out in many parts of the world.

Broilers. Depending on the prevailing climatic conditions, meat flocks are usually reared on litter in either open, curtain-sided or completely closed, environmentally-controlled houses. For the last-mentioned, biosecurity requirements are given in many national and international codes of practice, standards and guidelines. Although the requirements are less stringent than those described above for primary breeding stock, the measures aim to minimize the risk of *Salmonella* gaining access to the flocks. Other recommendations include an all-in, all-out stocking policy and an adequate time allowance for thorough cleaning and disinfection of each house between flocks. The cleaning and disinfection strategy depends upon the system of managing litter *in situ*. There are different ways of doing this on broiler farms. Either the litter from a previous flock is re-used for successive flocks, with fresh material being added as required, and only replaced periodically, up to once a year, or it is removed after each flock, so that full cleaning and disinfection of the house and its equipment can take place. However, some evidence suggests that *Salmonella* survives less well in built-up litter than in fresh material (66), *Salmonella*-contaminated litter can infect the next batch of chicks to be reared in the house. Also, litter used

for long periods is readily colonized by rodents that may carry *Salmonella* themselves and act as an additional source of transmission to other flocks and the surrounding environment (88).

The measures described above, such as flock vaccination, use of a CE preparation or acid treatment of feed, are rarely used for broilers, because of cost, but may be employed on farms where the litter is removed after each flock and persistent problems from *Salmonella* remain despite cleaning and disinfection of the premises. Since the risk of exposure to *Salmonella* cannot be eliminated, broiler flocks can be tested at about two weeks before slaughter as a pre-harvest control measure (197). This allows any *Salmonella*-positive flocks to be processed separately, last in the day or excluded from slaughter. Also, carcass meat from positive flocks may be subjected to a cooking process, if required.

Collecting and transporting birds to the processing plant. In some countries, broiler flocks are partly depopulated (thinned) before reaching their final slaughter age. This is done to provide retailers with carcasses or parts of a specific weight- range and it also allows the house to be stocked more fully when the chicks are placed. A disadvantage is that biosecurity may be breached during the period that the house is opened and a proportion of the flock removed, but older birds are less susceptible than chicks to *Salmonella* infection and may resist colonization by the organism. Nevertheless, special care is needed during thinning to avoid infection of the remaining birds from contaminated catching equipment, transport crates and modules that are brought on to the site for the thinning operation.

Before any birds are sent to a processing plant, feed is withdrawn for 8 – 12 h to reduce the presence of ingesta in the alimentary tract. This facilitates carcass evisceration and reduces the microbial load brought into the processing plant. It also reduces soiling of the containers in

which the birds are transported. Fasting birds longer than 12 h is not recommended due to thinning of the gut wall, while the contents tend to become wetter and spread more easily (196).

The containers used to transport birds from the farm to the processing plant may be contaminated with *Salmonella* on arrival at the farm, in spite of having been cleaned previously (40), and *Salmonella* has been isolated from the feathers of previously negative birds, following transportation (166). The residual fecal material present in transport containers has been identified as a key factor in carcass contamination (90) and necessary improvements in the cleaning process have been described (175).

Control of Salmonella spp. in primary carcass processing.

There is increasing emphasis on the importance of hygiene management in the processing plant and, as described above, data from Finland and Sweden have demonstrated that the benefits of on-farm interventions aimed at reducing *Salmonella* prevalence in poultry flocks can be maintained throughout subsequent transportation, slaughter and processing (62). However, following the introduction of a pre-harvest control program for *Salmonella*, a significant period may be required before such an effect can be seen. From the hygiene viewpoint, processors should seek to minimize both levels of carcass contamination with any pathogens, such as *Salmonella*, and the prevalence of positive carcasses. Both are important in reducing the risk of salmonellosis in consumers (67, 171). The main stages in processing at which contamination problems arise are scalding, plucking (picking), eviscerating and chilling of carcasses. The ways in which hygiene control can be optimized in each case will be considered below.

The purpose of carcass scalding is to loosen the feathers and facilitate subsequent defeathering. Not only do carcasses carry large numbers of microbes on skin and feathers, as they enter the scalding process, but there will be some degree of involuntary defecation that greatly increases the load of fecal organisms in the scald water. Survival of these organisms in the water is influenced by temperature, but even the high temperatures used in 'hard' scalding, usually 58 – 63^{o}C, have little effect on those organisms that are attached to, or entrapped in, the skin. Slavik et al. (167) reported no significant reduction in *Salmonella* contamination of carcasses during hard scalding.

Because carcasses entering the scalder sometimes carry large amounts of adherent organic material, some US companies use a pre-scald brushing and washing process, with super-chlorinated water containing a chlorine residual above that usually permitted in drinking water. The process is said to produce up to a 90% reduction in the amount of extraneous material that can be transferred to the scald water (153). Also, conditions can be improved in a conventional scald tank by using a counterflow system in which a barrier is installed between the lines of carcasses moving in opposite directions, thus preventing microbes that are washed off the carcasses initially from contaminating those leaving the system (153). It is recommended that the flow-rate of water through the tank is kept as high as possible and an acidic disinfectant is added, especially when carcasses are scalded at 50 – 53^{o} C. Under the best operating conditions, scalding can be expected to reduce the initial *Salmonella* prevalence. When scalding was changed to the counterflow configuration in a single tank and a post-scald, hot-water rinse was added, James et al. (103) noted an improvement in the microbiological condition of carcasses. In addition, heating the water to at least 75^{o}C during break periods and then cooling it to the operating temperature avoids the risk of transferring any residual *Salmonella* to a new batch of carcasses (15). Some modern processing plants have multi-stage, counterflow scalding systems that progressively dilute the microbial load in the water, as carcasses pass through the series of

tanks. In a study of a commercial three-tank process, *Salmonella* was isolated with diminishing frequency from the first tank to the last, and the heaviest microbial load was observed in the first unit (31). It was concluded that multi-stage scalding reduces the opportunity for cross-contamination with *Salmonella*, when compared with a single-tank system.

The defeathering process causes some extrusion of residual fecal material from the carcasses, considerable scattering of microbes in the vicinity of the machines, and contamination of the rubber 'fingers' themselves, so that cross-contamination is inevitable. Transmission of *Salmonella* has been studied (36, 102, 116) and a three-fold increase in *Salmonella* prevalence reported (36). The machines are particularly difficult to clean and disinfect properly and, when Campbell et al. (28) examined various items of equipment before the start of processing, *Salmonella* was isolated most often from the pickers. Modern machines provide better access to the banks of fingers, so that cleaning can be done more rapidly and effectively. However, it is also necessary to replace worn fingers regularly, because cracking of the rubber during use allows ingress of microbes, which are then protected from the effects of chemical sanitizers. The use of super-chlorinated water in the defeathering process is likely to have less of an effect on carcass contamination than other sanitizing agents that are less prone to inactivation by organic matter (153).

Carcass evisceration is carried out either manually or with the use of a series of automated machines, each dedicated to a particular function. Careful setting of the machines is necessary to avoid excessive breakage of the intestines and one type of machine immediately transfers the exposed viscera to a separate, parallel line for subsequent inspection. This prevents carcasses from becoming soiled by ruptured or leaking viscera. However, rupture of the crop and spillage of crop contents can occur at a later stage and crops were considered 86 times more likely to rupture than ceca during processing (20, 81). The evisceration stages can contribute

significantly to an increase in *Salmonella* prevalence (155), and control of carcass contamination with ingesta or fecal material is paramount. In the USA, most carcasses with visible contamination can be reprocessed and may be treated with antimicrobial compounds (182).

During the evisceration process, carcasses are frequently subjected to water sprays that remove organic debris and reduce microbial contamination by about one $\log_{10}$ unit. Some of the organisms may be removed in the final inside-outside spray-washer. This is primarily a carcass-cleaning process and, where water-immersion chilling is being used, it reduces the organic loading of the chill water (168), which has a favorable impact on any added chlorine. However, the removal of bacteria from carcasses in the spray-washing process is not enhanced by using chlorine and / or hot water (136), probably because organisms that become firmly attached to the tissues are protected from the effects of these agents and are not easily removed (118, 137).

Chilling poultry carcasses to about 4^{o}C or below ensures that any *Salmonella* present will be unable to multiply. The methods commonly used for this purpose involve immersion in cold water, with or without the addition of ice, or exposure to cold air, either by passing carcasses through an air-blast system or holding them in a chill room. Air chilling may also include the use of water sprays to provide evaporative cooling. For high-rate production, many processors use a system in which carcasses are moved mechanically through one or more tanks where the water is agitated to assist cooling. The continuous immersion system has a washing effect which reduces microbial contamination by up to one $\log_{10}$ unit (132). The accumulation of bacteria in the chill water is partly controlled by the water throughput. However, since large numbers of carcasses share a common waterbath, there are ample opportunities for cross-contamination to occur. Water chilling is considered a major site for flock-to-flock transmission of *Salmonella* (102, 117, 155) and when *Salmonella*-positive flocks are processed, the prevalence of contaminated carcasses increases unless the water is super-chlorinated (32, 102, 103, 116, 135).

Nevertheless, even when added at a level of 50 mg / l, chlorine has little direct effect on carcass contamination, because of the rapid rate of inactivation, and its effect on *Salmonella* prevalence appears to be mainly due to disinfection of the chill water (116). To keep the water free from viable vegetative bacteria, it has been found necessary to maintain a total chlorine residual of 45 – 50 mg / l (131). By contrast, air chilling involves much less contact between carcasses, but there is no washing effect in the chilling process to reduce carcass contamination and a low scalding temperature is required to safeguard the ultimate appearance of the skin. Cross-contamination between carcasses may be less of a hazard, but is still possible *via* air currents and water droplets, if carcasses are sprayed during chilling (130).

Although chemical treatment of carcasses or process water is not currently permitted in the EU, several such treatments are available and are used regularly in the USA and other countries. Chlorine dioxide is seven times more effective than chlorine (115) and therefore may be used at relatively low concentrations in immersion chillers (3 – 5 mg / l). The compound has lower reactivity than chlorine with organic matter and can be effective in reducing *Salmonella* contamination of carcasses (191). Other chemical treatments include those approved by the US Department of Agriculture (USDA) for use in automated re-processing of fecally-contaminated carcasses. An example is acidified sodium chlorite, which also can be added to chill water and is capable of reducing *Salmonella* contamination (109). Trisodium phosphate has been used for the same purpose, but the high pH of the water then has an adverse effect on the efficacy of chlorine, and disposal of large amounts of phosphate may raise environmental issues (19). Another treatment option is lactic acid, which has a delayed bacteriostatic effect during the storage of treated meat (169). In the USA, a sequence of interventions involving washing of carcasses before and after chilling and various chemical treatments reduced the prevalence of *Salmonella* by up to 91% (170).

Food safety management systems

Application of the HACCP system to primary processing of poultry has been widely advocated (2, 95, 133) and is mandatory in both the USA and the EU. In the former, the processing operation is the main focus for legislative control of *Salmonella* in the industry whereas, in the latter, processing is only one stage in the supply chain at which control measures are required by law. Both approaches include a criterion for *Salmonella* and require regular testing of post-chill carcasses as part of the verification of the food-safety management system. The US system (179) also includes a 'zero-tolerance' policy for visible fecal contamination on carcasses entering the chilling process (180) and the need for a HACCP plan to ensure that avoidance of fecal contamination is a CCP (181). Otherwise, the determination of CCPs is a matter for the individual company, their number and location being likely to vary from one establishment to another (179).

Among the prerequisites for an effective HACCP program are GMP, appropriate training of staff and SSOPs that cover plant cleaning and disinfection. Rasschaert et al. (149) sampled processing equipment at three Belgian slaughterhouses after cleaning and disinfection, but before the start of processing. In two cases, *Salmonella* contamination was detected and carcasses in the first *Salmonella*-free flock to be processed acquired the same strains. Furthermore, another study showed that most of the *Salmonella* strains isolated from processed carcasses were different from those that predominated in the pre-harvest environment (91), thus highlighting the importance of hygiene control during processing. Strains acquired during processing, however, would be additional to any that were present pre-slaughter.

In applying GMP and HACCP principles to the control of *Salmonella* in poultry processing, there are two necessary objectives: firstly, to limit transmission of the organism from contaminated to previously uncontaminated carcasses; and, secondly, to reduce the level of carcass contamination when *Salmonella* is present. Given the nature of the process as a whole, cross-contamination of carcasses is not preventable, but may be diminished by some of the measures described above, such as use of multi-stage, counterflow immersion-scalding (31, 153). The control parameters at this and most other stages of the process are relatively easy to monitor (e.g. temperature, water usage) and any corrections to the operating conditions can be made rapidly, in accordance with HACCP requirements.

Sampling and testing for *Salmonella*

Food processing companies commonly use microbiological testing of finished products to determine conformity with food-safety criteria (e.g., 96, 114). These criteria are set by different stakeholders, including regulatory bodies, but are also used to guide the manufacturing process and to define and verify preventive actions. Although sampling on its own is of limited value, it is indispensable when applied regularly at different stages of the poultry supply chain as an integral part of an implemented control program. No feasible sampling plan can guarantee the absence of *Salmonella*, but sampling on a regular basis will reveal changes in infection or contamination so that corrective action can be taken, as required. The sampling strategy should be defined according to the public health risk involved, the anticipated prevalence of the target organism, the desired level of confidence in the results obtained, and the general principles of statistical control, which will indicate the degree of confidence offered by negative results. Other factors to consider are the stage in the food chain at which samples should be taken, the type of sample in each case, how many samples to take at any one time and how often, and what quantity of the material to collect. Standardized methods of analysis should always be used;

methods advocated for international adoption are provided by organizations such as the International Organization for Standardization (ISO) and the World Organization for Animal Health (OIE). There is also the question of who should carry out the sampling, although regulations may specify that this must be done, at least in part, by the competent authority (68). An effective control strategy requires detailed consideration of the nature of the food chain and the points at which sampling will provide the most meaningful information. No single sampling site is ever sufficient to achieve this. Testing for *Salmonella* at any stage should always have a clear objective that is related to control of the organism, allowing proportionate action to be taken on the basis of the results obtained. Other factors include the likelihood of infection or contamination at a particular stage and whether there are practices or interventions that might minimize the risk. This allows resources to be allocated appropriately and cost-effectively in relation to the risk involved. Even so, feasible levels of sampling are not usually sufficient to determine fully the effectiveness of a specific control measure.

The locations in the broiler production and processing continuum at which samples for *Salmonella* testing may be obtained are shown in Table 2. The table gives examples of the types of sample that may be taken and indicates not only when sampling is most appropriate, but those situations in which more intensive sampling is needed.

Sampling in feed mills. When *Salmonella* is present in manufactured feed, the organism is likely to be low in number and unevenly distributed, so that large amounts of feed need to be analysed to assess the contamination rate with any accuracy. For individual feed ingredients, contamination rates vary from one ingredient to another and between batches, and ingredients used in only small quantities are prone to being overlooked in any testing regime (151). In monitoring the mill environment, Jones and Richardson (104) noted that dust was consistently contaminated with *Salmonella* throughout the mill, especially near pellet coolers, which draw in

large amounts of air. Thus sampling of dust and the mill environment is much more effective than monitoring the end product, with the sampling being done as part of a HACCP program (60, 197).

Sampling on the farm. Strategies used for flock sampling depend on the type of flock, purpose of the exercise, and likely variation in within-flock prevalence of *Salmonella* infection (6). Suitable protocols that are applied to different types of flock in the breeding pyramid and in broilers have been described (197). It is also necessary to take account of changes in colonization behavior as the flock ages. The objective for both grandparent and parent flocks is to prevent and / or control vertical transmission of salmonellae, especially that due to *S.* Enteritidis and *S.* Typhimurium.

The relative merits of different sampling methods are addressed by Davies (46). Sampling of feces or tissues taken from birds post mortem is sometimes appropriate, but this approach is generally considered too costly and laborious for determining the *Salmonella* status of the flock as a whole (49), and it is relatively insensitive. A superior alternative is environmental monitoring, carried out at key contamination points (112), and involving samples of dust that accumulate readily on extractor fans and various ledges within the house. *Salmonella* persists well in such material, while competitors tend to die out (46).

Sampling of litter and feces in the house is carried out in various ways, using swabs made of gauze or cellulose sponge, drag swabs or boot swabs (16). Grab-samples of the litter itself may also be taken. Samples must be collected in a representative manner and a single swab or grab-sample is insufficient. It has been suggested (46) that a combination of boot swabs and dust samples is the most practical and sensitive method for sampling non-caged flocks, with sampling geared to likely peaks of flock infection. When analyzing dust and other materials, the larger the

amount taken, the more sensitive the test is likely to be (163). After clearance of the flock, the spent litter is often removed and the house, as well as its equipment and any external concrete apron, are cleaned and disinfected before the next flock is introduced. Disinfection of the premises is critical if the previous flock was carrying *Salmonella*, and its effectiveness needs to be monitored thoroughly by swab-sampling of surfaces (48).

Sampling in the hatchery. The key samples that are relevant to the hatchery are listed in Table 2. If sampling is restricted to dead-in-shell and culled chicks, *Salmonella* infection in hatchlings is likely to be significantly underestimated (79). Many companies also take samples of hatcher fluff, but box liners and macerated waste are better sources of *Salmonella* contamination (46). For traceback studies, the emphasis may be on eggs from specific flocks and on the individual hatcher cabinet, which is considered to be the main location at which chicks acquire infection. As at other stages of the broiler production and processing continuum, effective monitoring of cleaning and disinfection is essential for preventing cross-infection, despite the limited time available to do so between different batches of hatching eggs.

Sampling of poultry meat. Many different methods have been developed for sampling carcasses (129) and, for use in the processing plant, such methods should be sensitive and easy to apply without causing unacceptable damage to the product. Any method used must take into account that salmonellae and other microbial contaminants are found mainly on carcass surfaces, whether on skin, cut muscle or inside the abdominal cavity. The organisms are often distributed unevenly over the carcass and may be attached to, or entrapped in, the skin and muscle, so that they are difficult to remove without macerating the tissue to release them.

Among the techniques available are those involving surface swabbing, whole-carcass rinsing, tissue excision and maceration, repeated dipping of carcasses in diluent, collection of drip

(weepage), high-pressure spraying or scraping of a defined area of skin, spraying the abdominal cavity and lifting of skin contaminants with an agar contact plate or nitrocellulose membrane. Another technique is to remove an area of skin and sample the underlying muscle. This method may recover some salmonellae that are capable of causing systemic infection in the bird, e.g. *S.* Gallinarum, *S.* Pullorum, but does not capture those on the carcass surface, thereby introducing a bias. Sampling of cut portions includes items both with and without skin and usually involves tissue maceration. Where possible, inclusion of skin generally increases the likelihood of recovering *Salmonella* (105).

Sampling in the processing plant. The techniques most commonly used for sampling carcasses in the plant are those involving surface swabbing (especially for larger carcasses), whole-carcass rinsing, and maceration of skin samples, as described in ISO (100). Rinse sampling is used in the USA within the Food Safety Inspection Service (FSIS) Pathogen Reduction Program (179), whereas sampling of neck skin is preferred in the EU. The latter method represents only a small proportion of the skin as a whole, but has the advantage that carcasses can be sampled without their removal from the processing line. Thus, sample collection is faster and less laborious than it is in the case of rinse sampling. Although carcass swabbing tends to give a lower recovery of *Salmonella*, rinse sampling and neck-skin maceration give comparable results (155). Work by Cox and colleagues confirm these findings (43). However, carcasses obtained either pre-chill or post-chill had significantly greater ($P<0.05$) *E. coli* counts when using the rinse method compared to neck-skin sampling ($\log_{10}$ 2.9 vs 2.4 CFU / ml and $\log_{10}$ 0.65 vs 0.14 CFU / ml, respectively). Although there was no significant difference in *Salmonella* prevalence between the two sampling procedures, both methods resulted in false negatives, which indicate their potential lack of sensitivity. Pre-chill, 37% (66/180), 28% (50/180), and 51% (91/180) of carcasses were *Salmonella* positive by whole-carcass rinse, neck-skin maceration, and both procedures

combined, respectively. Post-chill, the respective figures were 3% (5/177), 7% (12/177), and 10% (17/177) of carcasses positive (43).

The finding that methodological variations affect sensitivity in relation to *Salmonella* detection has been shown in numerous studies (162). Rinse protocols also vary; there are differences in the composition and amount of rinse fluid, the time period of rinsing, and the volume of rinsate used for analysis in relation to the total volume applied. There is also likely to be variation from one operative to another in the degree of vigor with which carcasses are shaken manually to obtain the sample.

Inevitably, the choice of sampling method is a compromise between practicality and sensitivity. In a recent modification (162), the test carcasses were incubated in the rinse fluid for 24 h before aliquots of the fluid were analyzed for *Salmonella*. The results indicated that the method improved recovery and therefore was more sensitive than the traditional whole-carcass rinse when only low numbers of *Salmonella* were present. Most laboratories, however, would lack the large amount of incubator space needed to accommodate whole carcasses. Steps are currently being taken to establish standardized methods of carcass sampling (101) that would avoid much of the uncertainty, be of value for international trade in poultry meat, and facilitate comparison of data from different processing plants. However, the methodology procedure also has to be standardized or validated along with the carcass sampling procedure.

Sampling at retail. Testing products at this stage, rather than during processing, is more relevant to the exposure of consumers to *Salmonella* via raw poultry meat. The results obtained, therefore, can be of greater value in assessing the human health risk, which is required in risk assessments, and in verifying the effectiveness of *Salmonella* control measures for different categories of product. This, in turn, will help provide the scientific basis for any new criteria that

are deemed necessary. The sampling strategy should be statistically-based and related to the sources of *Salmonella* exposure for the majority of the population; hence it should be largely focused on retail products that are on display in major towns and cities, and the principal retail outlets from which most poultry meat is sold. All the main forms in which poultry products are marketed should be sampled, e.g. whole carcasses, portions, meat preparations, fresh and frozen products, and it will be important to distinguish between domestic and imported products.

Limitations of international equivalence in product sampling and testing protocols

In the context of global agreements for trade amongst countries, Hathaway (85) noted that: 'The individual sanitary measures that comprise food inspection and certification systems often vary from country to country, and determination of their equivalence is arguably the most important contemporary food-safety issue in international food trade. Development of a framework for judgment of equivalence requires a comprehensive understanding of risk analysis and food safety objectives'. Establishing the legitimacy of diverse approaches to this goal has led to the principle of equivalence being a central tenet of the World Trade Organization (WTO) Agreement on Sanitary and Phytosanitary Measures (200). Under the Codex Alimentarius Commission (CAC), 'equivalence' means that different sanitary measures achieve the appropriate level of sanitary protection in all cases; in other words, the capability of different inspection and verification systems to meet the same objectives (21). 'Inspection' is the examination of food or systems for control of food, raw materials, processing and distribution, including in-process and finished-product testing, in order to verify that they conform to requirements. Harmonization of different approaches must include consideration of methods used for sampling and analysis. Different sampling and testing strategies and methods can achieve similar levels of protection, but this needs scientific validation. Clear guidance on demonstrating equivalence for different microbiological methods is lacking, often because of inherent variation in the performance of

such methods (39). For example, the 95% confidence limit about the mean for a direct plate count is in the order of ± 0.3 $\log_{10,}$ which makes for difficulty in attempting to validate alternative methods. For demonstrating the presence of *Salmonella* by a particular method, it is important to know the smallest number of viable cells that can be detected in a sample with a given probability. For rapid detection methods, however, the international validation scheme described in ISO (100) is the basis for independent validation in relation to the reference method for *Salmonella* (99).

The concept of equivalence allows some flexibility to accommodate control procedures that yield comparable results but would be more suited to the exporting country's conditions. The benefits of applying the principles of equivalence include minimizing costs, maximizing public health outcomes for a given resource input, facilitating trade and decreasing reliance on costly commodity testing (140). For international trade, and in order to avoid unfair trade restrictions, importing countries must not demand a standard of product that is not readily achievable domestically. Microbiological testing and the setting of microbiological criteria are not recommended for raw poultry end-product acceptance testing (97), because of the known limitations of testing, such as choice of a suitable sampling plan and the selectivity and specificity of the isolation method used. However, with the understanding of operational performance characteristics of a sampling plan, and methodology sensitivity and specificity, testing can be valuable in trend analysis to identify significant deviations in a process and/or in a product. The establishment of microbiological criteria should be based on a sound scientific rationale to avoid imposing unwarranted burdens on food-producing industries in all countries.

Establishing equivalence standards for *Salmonella* testing is difficult and requires that 'good science' be recognized in these activities, because successful *Salmonella* isolation is a complex, multifactorial procedure. Microbiological examination of foods involves many processes, and

pathogen prevalence, along with testing-data bias, can be affected by differences in sample type and duration of transportation, as well as storage time and method of analysis (39). Some of the bias can be controlled by microbiologists, but other elements may be out of their hands, due to the lack of scientific information. The 'gold standard' for *Salmonella* detection in food follows a standard protocol of nonselective pre-enrichment, selective enrichment, isolation on selective agar media and biochemical and serological confirmation. Choice of enrichment broth, incubation temperature, inoculum ratio and plating media affect the *Salmonella* detection limit (17, 34, 45, 51, 83, 84, 141, 143, 160, 193). Numerous cultivation methods that are commonly used in *Salmonella* surveillance and outbreak investigations vary significantly in sensitivity and specificity (30, 45, 63, 120, 164, 193). Technological advances are providing more rapid and automated methods that may be appropriate for screening samples, however they must be validated for the specific sample types for which they are used, to an international standard such as ISO and/or Association of Official Analytical Chemists (AOAC).

Global variations in broiler production and processing, and implications for *Salmonella* control

The systems used for large-scale production and processing of poultry are similar throughout the world and much of the specialized equipment used by the industry in each country is supplied by the same manufacturers. Also, a small number of companies provide most of the world's poultry breeding stock. There are, however, differences between countries that are determined by factors such as climate, national or regional legislation, availability of low-cost labor and market demand for particular products. Such differences may have an influence on the strategies used to mitigate contamination of the end-product with foodborne human pathogens, but are not well documented for comparative purposes. The information described in this report was provided mainly by the workshop participants and examples of different production and processing

practices for a selection of countries is given in Table 3. The countries in question cover different climatic conditions and include the world's largest producer of chicken meat, the USA, and Sweden, one of the smallest producers among the developed countries, but one with particularly stringent requirements for *Salmonella* control. Among them, these countries produce a range of fresh and frozen raw products and, in the case of Mexico, a significant proportion of unprocessed carcasses that are sold at local markets. Although Table 3 covers only basic features of the industry in each country, providing additional detail would be difficult because of differences among individual companies within countries.

Climatic conditions are an important factor in poultry production, because they may influence the type of housing used for the birds, whether open-sided (with or without curtains) or houses that are closed and provide a temperature-controlled environment. A closed environment facilitates the application of biosecurity measures and effective between-crop cleaning and disinfection of the premises, although the costs of construction and maintaining the required temperature are greater in this case. Also, the type of climate determines whether *Salmonella* is capable of multiplying significantly in the environment. The availability of low-cost labor may be a key factor in deciding the location of the industry within a country and can provide the workforce for slaughter and processing operations, thus reducing the need for a high degree of process mechanization and automation.

National and regional legislation have a role in establishing the basic elements of the *Salmonella* control strategy. In the EU a 'top-down' pre-harvest approach is taken, involving control of breeding stock, hatcheries and broilers (52, 55). In addition, the strategy in the Scandinavian countries is that all broiler flocks are tested for *Salmonella* before slaughter (pre-harvest control), and if any are *Salmonella*-positive, they are not allowed to enter the slaughterhouse and are destroyed. Sweden is one of the countries listed in Table 3 where all grandparent stock is

imported. To ensure freedom from *Salmonella,* the birds are kept in quarantine and tested intensively before being released for production purposes. For these and other flocks, strict control of feed manufacture, including heat treatment to eliminate *Salmonella,* is among the mandatory requirements in Sweden that are considered key factors in minimizing *Salmonella* contamination of poultry and reduce the need for extensive testing of feedstuffs. Outside the EU, mandatory control of feed production appears to be less common. In many cases, broiler flocks are tested prior to slaughter and some countries favor the rescheduling of positive flocks so that these are processed separately or last in the day. The absence of mandatory control for poultry flocks in some countries reflects the lack of a regulatory process that covers the supply chain as a whole to provide an integrated control system, such as that promulgated in the EU.

Sampling and testing regimes in the different countries reflect the scope and stringency of each mandatory control program. Where the required measures are confined to the processing plant, as in the USA, the only 'official' samples are post-chill carcasses. In contrast, the farm-to-fork approach taken in the EU requires samples of various kinds to be taken throughout the supply chain, although only specific *Salmonella* serovars are targeted in breeding and broiler flocks (55, 56). The control strategy in Sweden and other Scandinavian countries covers all serovars, and measures to prevent *Salmonella*-contaminated broiler flocks from entering the food chain involve a comprehensive sampling program. The intensity of sampling is increased in some circumstances, e.g., when a positive flock is identified and the house is cleaned and disinfected following clearance (198). Other measures taken include those aimed at restricting the spread of *Salmonella* and determining the source of the infection.

Further factors having an influence on *Salmonella* control are mainly operational. The degree of integration between different sectors of a poultry enterprise is widely seen as an advantage in controlling infectious agents, and the industry in most of the major poultry-producing countries is

highly integrated, although broilers are often reared by contract growers. In Russia, the concept of integration is different, and the larger companies usually confine their activities to a single, extensive site. This makes it easier to transfer broilers from the farm to the processing plant, but requires strict biosecurity to prevent transmission of any pathogens from one part of the operation to another. The re-use of litter for successive bird-flocks is a common feature of broiler production in some countries and has implications for *Salmonella* control, because it does not allow thorough cleaning and disinfection of the poultry house once the birds have been sent for slaughter. This feature, combined with the use of open or curtain-sided houses for broilers, appears to indicate that the highest standards of biosecurity are presently impossible. Especially when the houses are opened up in hot weather and wild birds, rodents, insects and other vermin can gain access. Even with closed houses, however, biosecurity may be hampered by the absence of an ante-room in which to place a hygiene barrier, or the presence of an earthen floor, which is more difficult to disinfect after the birds have been sent for slaughter and the house cleared.

Further inter-country differences can be seen in the intervention measures applied to breeding stock (Table 3). In Sweden, CE treatment is never used for breeder flocks because it may mask any low levels of *Salmonella* infection in the birds. By contrast, vaccination, CE treatment, probiotics or dietary organic acids are used in combination with biosecurity measures for example, in Brazil, while vaccination is common in Russia and The Netherlands. Treatment of flocks with undefined CE preparations is not permitted in the USA. Mandatory control of breeding stock in other countries includes regular monitoring for *Salmonella* and culling of infected flocks according to the serovars present.

Some key differences in processing practices among countries are also included in Table 3. It is common to use a fully automatic evisceration system, the exception being Mexico where

evisceration is either less automated or entirely manual. Eviscerating carcasses manually necessarily involves more handling and may increase cross-contamination. Sweden and The Netherlands are typical of most EU Member States in using air chilling, with no processing aids or chemical decontamination treatments, because their use is not currently permitted in the EU. Air chilling usually involves a relatively low scalding temperature to safeguard the ultimate appearance of the chilled carcass and this has little effect on survival of *Salmonella* in the scald water, which then favors cross-contamination. Other countries utilize water immersion chilling and often allow super-chlorination of process water and application of other chemicals. An exception in this respect is Brazil, which exports chicken to the EU and uses water chilling with only low-level chlorination. Of the seven countries listed in Table 3, only Japan and Russia have no requirement for implementation of the HACCP system in all processing plants, but, in the latter, any *Salmonella*-positive flocks are processed last in the day. In the USA, HACCP is the basis for controlling *Salmonella* contamination in the plant, with a 'zero-tolerance' policy for visible fecal contamination and official action taken to ensure that HACCP principles are properly applied (179). The US approach favors the use of chemical decontamination treatments to facilitate meeting a performance standard for *Salmonella*. Whatever control measures are applied, sampling of processed carcasses and testing for *Salmonella* appears to be the normal practice in all the major poultry-producing countries. In no country, however, is it possible to hold chilled carcasses while awaiting results of the tests. In Sweden, such tests are used only as a check on plant hygiene and the efficacy of on-farm control measures, because all flocks will have tested negative before slaughter (Table 3). The consequences of failing to comply with an end-product criterion differ among countries. At one extreme, US processing plants may be closed if there is consistent non-compliance, whereas the EU response to 'unsatisfactory results' is a requirement to improve slaughter hygiene and to review process controls and on-farm biosecurity (54).

Zero tolerance and related concepts

The adoption of quantitative risk assessment practices in microbiological food safety underscores the reality that 'zero risk' is unattainable for all raw foods, a reality in everyday events and everyday life. The choice of 'zero tolerance', implying the complete absence of a hazard, may be regarded as the expression of a regulatory preference for the precautionary principle, and has little to do with food safety and human health (80, 176). A US committee formed under the National Research Council reported on a 'Review of the Use of Scientific Criteria and Performance Standards for Safe Foods' and noted that the term 'zero tolerance' is commonly used, but generally is poorly, defined or understood (138). Use of this language in expressing objectives is troublesome, in that the terminology has different meanings to different audiences, as underlined by the definition the aforementioned committee offered for its own purposes: "Lay audience perception of the absence of a hazard that cannot be scientifically assured, but is operationally defined as the absence of a hazard in a specified amount of food as determined by a specific method". To some people, 'zero tolerance' implies a notional concept of 'zero risk' associated with the food, or 'zero prevalence' of a pathogen in the food commodity. Such a misunderstanding could easily arise from the pending EU requirement for the absence of *Salmonella* in 25 g of fresh (raw) poultry meat (52), since no details are given on how this requirement would be interpreted. In the absence of any means of eliminating the pathogen from a raw food product, the 'zero' concept is misleading to those consumers who may interpret such regulations as implying 'no-risk', and hence have unrealistic expectations of the effectiveness of regulatory action. If a hazard exists, there is some probability it will cause an adverse effect, no matter how small (85). 'Zero tolerance' may also imply that both minor and major deviations from a policy will be treated with the same severity. This is obviously not a sensible approach to identifying and resolving problems. Internationally, there is no consistency in interpreting the concept, and what action should result from any deviations. The purpose of a so-called 'zero

tolerance' policy should be to provide an alert, leading to a review of control policies and procedures, while permitting distribution of the final product to the marketplace in situations where withdrawal would not give a risk reduction proportional to cost and other practical considerations. Little is to be gained when dealing with food-safety management practices based on microbiological criteria for end-product testing alone (accept/reject) as, even when a process is completely under control, some, albeit small, probability exists for exceeding the established parameters (190, 203). Without knowledge of the degree of variability in a process/product, and knowing where the uncertainties of a food process lie, the likelihood of exceeding the limits is unknown.

Several further challenges exist to applying a 'zero tolerance' policy for *Salmonella* in poultry meat. These include defining the accuracy, sampling intensity, sampling material, and method sensitivity. At which point is the assessment to be made, pre-harvest or post-harvest, who bears the repercussions for enforcement, who has and what is the enforcement capacity? Ultimately, regulatory choices in establishing control policies need to be verified through scientific evidence for their effectiveness in reducing risk so that social costs can be made transparent (80).

International trade, risk analysis and food safety metrics

International trade in foods has led to agreements on global compliance with requirements and internationally harmonized approaches to prevent unwarranted trade barriers. The World Trade Agreement, and specifically the Sanitary and Phytosanitary (SPS) provisions, are significant in driving the development of new approaches and requirements for international trade (200). Sanitary measures "…include all relevant laws, decrees, regulations, requirements and procedures, including…end product criteria; processes and production methods; testing, inspection, certification and approval procedures; quarantine tests…provisions on relevant

statistical methods, sampling procedures and methods of risk assessment; and packaging and labeling requirements directly related to food safety" (200). The SPS rules can be briefly summarized as follows (86):

- SPS measures must be based on scientific evidence, or, where appropriate, scientific risk assessment.
- Application of SPS measures must be non-discriminatory, i.e. consistency is required in risk management decisions.
- Transparency must be maintained with respect to rule-making.
- SPS measures must be used that create the least distortion to trade, i.e., there must be consideration of alternative measures that achieve the same health objective.
- The concept of regionalization must be applied.
- The equivalence of an SPS measure that must be accepted in an exporting country can objectively demonstrate that its controls provide the importing country's desired level of protection.

The WTO's Technical Barriers to Trade (TBT) Agreement also requires that a country must not ask for a higher degree of safety for imported goods than it does for goods produced in its own country (200).

A key element of the WTO/SPS agreements is the principle of equivalence of diverse approaches to assure food safety. Hence, harmonization of sanitary measures is required by basing them on international standards, guidelines or recommendations, where they exist, scientific evidence and risk assessment, and the articulation of an 'appropriate level of protection' (ALOP) to justify the sanitary measures or equivalence required by an importing country to protect its population. The CAC is the recognized intergovernmental body for establishing international food safety

standards, guidelines, codes of practice, and protocols for the conduct of risk assessments. The OIE is the CAC counterpart for animal health issues, including codes of practice for poultry production.

The CAC has established a risk analysis framework and guidelines that provide a structured, systematic process for prioritizing and supporting food-safety risk management activities (23). Risk analysis is defined as a process comprising risk assessment, risk management and risk communication. Risk assessment consists of science-based data analysis and inference to characterize the hazard/food, likely exposure, and the likelihood and nature of adverse human health impacts associated with the product of concern (23, 98). Risk management comprises consideration of the scientific evidence and other relevant factors in selecting appropriate controls, and risk communication is regarded as an interactive exchange of information among all stakeholders, including consumers. An important distinction in the risk analysis approach is the understanding of risk, i.e., the likelihood and magnitude of a public health impact, as a result of a hazard in a food, versus simply the presence of the hazard (85). Risk assessment provides a much more meaningful way of evaluating hazards and interventions than a simple 'zero tolerance'. As working principles, each of the three components of risk analysis should be developed and applied consistently, and should be open, transparent and well documented (25, 26, 68, 69).

From within this context of risk analysis, new operational concepts have emerged. These include the definition of public health goals (e.g., an ALOP), the expression of food safety objectives (FSOs), performance objectives (POs) and performance criteria (PCs), linked together with traditional parameters, including product and process criteria and microbiological criteria (MCs). The move towards a risk-based management approach is a major step in advancing a science-based food-safety system by clearly linking food-safety requirements and criteria to the public health problems they are designed to address.

Countries have traditionally attempted to improve food safety by setting microbiological criteria for raw or finished processed products. However, the frequency and extent of sampling used in traditional food testing programs may not provide a high degree of consumer protection (96). In most cases, a microbiological criterion has been set without estimating its quantitative effect in reducing the risk of foodborne disease. Sometimes microbiological criteria established by national governments for different foods have been viewed by other countries as barriers to international trade. In accordance with the SPS agreement, if a country sets an MC that is imposed upon trading partners for a specific health hazard in a particular food product, they must be able to explain, based on scientific data, consideration of risk and societal implications, the rationale and justification for the criterion.

Appropriate level of protection

The WTO/SPS agreement defines the ALOP as "the level of protection deemed appropriate by the member (country) establishing a sanitary or phytosanitary measure to protect human, animal or plant life within its territory" (200). It should be noted that the term "ALOP' carries legal weight in the international trade arena, under the auspices of the WTO, which specifies that this is a national standard being achieved domestically and is defensible in trade disputes (69). Alternative terminology should be used in referring to public health targets, such as 'acceptable' or 'tolerable' risk, when considered for future national goals, and need not be as stringently validated as would be necessary in international trade disputes.

Although an ALOP or other public health target can be implicit (e.g. reasonable certainty of no harm), effective implementation often requires a more explicit articulation of public health expectations (e.g. number of cases per 100,000 population per year associated with a specific

hazard in a particular food commodity). Other factors may be involved in determining the ALOP, such as nutritional benefits of a product, competing risks that may arise from interventions, feasibility, costs, public preferences and distribution of risks and benefits. An alternative approach for setting an ALOP, which is based on the performance of currently available risk-management options is ALARA (as-low-as-reasonably-achievable; 68), however, some associated level of public health protection must, and will be, articulated through the use of risk assessment to link levels of hazard control that are desired or achieved to an expected public health outcome.

Food safety risk management metrics

The targets for pubic health goals in reducing foodborne illness, typically set by governments, need to be translated into parameters that can be used by industry and assessed by government agencies in assuring food safety. The concepts of FSO and PO are introduced to serve this purpose (23). An FSO is "The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)". The FSO sets a target for the food industry to reach, but does not specify how the target is to be achieved. Hence, the FSO gives flexibility to use different operations and processing techniques, as is feasible and appropriate for individual industries, as long as the maximum hazard level specified at the point of consumption is not exceeded (98).

It is debatable whether an FSO at point of consumption is a useful guideline for the production of raw end-products, and it may not be relevant in producer or regulatory risk management (87). Therefore, a PO may be set, or a series of POs, each being a level defined in terms of prevalence and/or concentration that must be met at earlier steps in the food chain (69, 87). This may include the establishment of a PO for prevalence at the farm level, or for raw materials entering a

processing facility. Similar to an FSO, this target also must provide, or contribute to, the desired ALOP, taking into account the steps between the point where the PO is defined and, ultimately, the point of consumption, and any changes that occur in pathogen concentration or prevalence as a result of subsequent stages. A further metric is a PC, which indicates the effect (e.g. a specified logarithmic reduction or a reduction in prevalence) on a hazard in a food that must be achieved by the application of one or more control measures to provide, or contribute to, the PO or FSO. Traditional metrics are process criteria, product criteria, and MCs, but these too should be linked to the overall achievement of a PO, PC, and/or FSO, and, ultimately, a public health target. The definition of an FSO should integrate three dimensions: the prevalence of contamination, the average concentration and the range of variability of concentration (152). The same logic will apply to establishing targets earlier in the food chain, such as POs, and allowing credible limits to be established and verified by processors to indicate control. Although FSOs and POs are expressed in quantitative terms, they are not MCs, and often are not directly measureable (190). MCs may be established to verify that the FSO/PO is being met at a specific point of the process; however, the limitations of methods of sampling and testing must be considered in determining the usefulness of MCs. Other measurable criteria and validated processes, Good Agricultural Practice (GAP), Good Hygienic Practice (GHP), and HACCP also contribute to ensuring that the targets will be achieved.

The establishment of an MC for a particular food product is a complex issue requiring interaction between scientists, risk managers / control authorities and other stakeholders, especially consumers and food business operators. The parameters of the exercise are well known and include the following: (1) identification of the microorganism of concern; (2) selection of the analytical method for detection / enumeration; (3) development of a sampling plan, including the number of samples to be taken (n), the microbiological limit values (m, a limit that may be permitted, and M, a limit that must never be exceeded) and the number of units (c) that are

allowed to exceed m but not M; (4) a statement of the food to which the criterion applies; (5) determination of the point in the food chain at which the product will be tested; (6) agreement on any action to be taken when the criterion is not met ((22, 94).

Without an intervention, at any point in the production-processing continuum, capable of completely eliminating a pathogen from raw foods intended to be cooked, absolute safety cannot be realized for a raw product. Sampling plans and MCs do not guarantee safety, although absence in defined sample sizes (or within defined critical limits) is a means to identify highly contaminated lots that indicate a deviation. However, routine testing is not recommended for *Salmonella* on raw products and statements of "shall be absent" with no numerical tolerance being expressed should not be used, since this concept is not compatible with the use of FSOs and POs (96). No feasible sampling plan can ensure complete absence of a pathogen and the detection limit of the analytical method needs to be taken into account. However, what can be stated is the probability of acceptance of a lot, also known as the Acceptable Quality Level (AQL), depending on the known defective rate (24).

It should be recognized that some level of residual risk, even if quite low, or considered negligible, will remain associated with raw foods despite stringent efforts, short of total elimination by processes such as irradiation. Risk managers should consider additional means of mitigating risk, such as informing consumers how to avoid or manage the risk themselves.

The establishment of appropriate risk-based food safety metrics is not without challenges at this time. The development of robust risk assessments requires sound quantitative data on pathogen prevalence and exposures, and reliable dose-response relationships from, for example, epidemiological studies. Quantifying the impact of interventions along the food chain, in terms of pathogen reduction and subsequently human-health risk reduction, is currently constrained by

a lack of robust, statistically-sound quantitative data on the absolute effectiveness of any one individual intervention in reducing or eliminating a pathogen. The relative impact of one intervention versus that of another is also dependent on true prevalence/incidence in a population, and knowledge of the specificity and sensitivity of sampling and testing methodologies. And finally, the measure of risk reduction in a population requires data for attribution of human illness to specific sources.

There still remain questions that need to be addressed on the issue of defining an ALOP. For example, adverse health effects are not limited to acute gastroenteritis, and can be significantly more severe (e.g., mortality, renal failure, other chronic illnesses) and these need to be taken into account. Further, there are considerations of application to the entire population, or only certain susceptible subgroups. Should 'tolerable risk' be 'divided' among different products where the pathogen may be found and how is this to be done? Other questions are: how to quantify cross-contamination of other foods; and how to account for other transmission routes, such as person-to-person (87)?

The American Academy of Microbiology discusses the concept of 'acceptable risk':
"Acceptable risk can be defined as the level of risk that is protective of public health for a population considering cost, feasibility, and other considerations. Acceptable risk figures may be used to derive water quality standards or other goals. Ideally, these standards should be protective of health goals, understandable, tolerated by the public, scientifically defensible, implementable, and roughly equivalent to the other risks faced by members of the community. In addition, treatment and analytical technologies must exist to make achieving the goal feasible. Although an acceptable risk level can be difficult to identify, it is often necessary so that a management goal can be defined" (1).

Key considerations captured in the foregoing include 'scientifically defensible', and 'roughly equivalent to the other risks faced by members of the community'. These are issues that must be carefully considered when allocating resources for control purposes. Furthermore, in relation to poultry, the evidence suggests that currently, there are no treatments that can be applied in all countries to ensure elimination of the organism from raw poultry products. Instead, efforts should be focused on reducing *Salmonella* contamination at all points of the food chain.

Conclusions and recommendations

It is well documented that *Salmonella* in raw poultry products is an important global cause of human salmonellosis. Increasingly however, it is evident that there are various obstacles to determining the extent to which *Salmonella*-infected / contaminated, chicken is responsible for foodborne human illness, whether directly or indirectly via cross-contamination of other foods. Such information is needed to understand better the true risk to public health and the effectiveness of any interventions. The approaches used for food-source attribution are currently under scrutiny. In the absence of any reliable means of measuring the public-health impact of interventions used in the poultry industry and elsewhere, it is recognized that reductions in hazard levels can be used as a surrogate measure of control effectiveness, where appropriate data are available, but, ultimately, regulatory efficacy should be assessed on the basis of risk outcomes.

Much is known about the epidemiology of *Salmonella* infection in poultry flocks and the measures necessary to minimize transmission of the organism along the food chain. Stringent control of feed and primary breeding stock, linked to intensive surveillance of both the birds and their environment, is essential. Otherwise, *Salmonella* may be transmitted from one generation to another, finally being amplified in broilers. These measures should be implemented consistently

and also include thorough cleaning and disinfection of premises and equipment between flocks, to minimize possible sources of infection for the next flock.

Efforts should be made to reduce the risk of introducing *Salmonella* into feed mills via contaminated feed ingredients and to poultry through use of contaminated feed. HACCP-based controls should be applied both in crushing plants and feed mills, with special emphasis on environmental sampling.

Salmonella contamination is usually expressed in terms of prevalence, but evidence from microbiological risk assessment shows that levels of contamination are also important in relation to public health, and efforts at any stage of production or processing to reduce the numbers of *Salmonella* on the end-product will help to reduce risk. With the development of better means of enumerating *Salmonella* and method(s) that are internationally acceptable, this aspect should receive greater attention in the future, enabling more heavily contaminated items to be identified and suitable interventions developed.

In the case of a country, such as Sweden, with a stringent and successful control program for poultry, the present degree of *Salmonella* control for raw poultry took several decades to achieve and incurred considerable expenditure, which nevertheless was cost-effective. Comparable measures are not likely to be economically or technically feasible for direct application in all countries, but there are lessons to be learned from the Swedish experience, especially about the need for effective control of breeding stock, feed, and application of biosecurity.

Salmonella infection of broiler flocks and therefore contamination of processed carcasses varies widely among different countries and largely reflects the stringency of the measures being taken

to control *Salmonella* in the poultry production-processing continuum. Conditions in production and processing also vary, as do the strategies and methods used for sampling and testing for *Salmonella.* There is a need to standardize these methods, especially those used for end-product testing, in order to ensure consumer safety and facilitate international trade.

Given the nature of the industry, controls that are currently applied will not guarantee the absence of *Salmonella* from raw poultry. At present, there are different approaches among countries in the emphasis placed on pre-harvest and post-harvest control, respectively. However, the most effective strategy for *Salmonella* control would be one that covers all stages of the food chain. Therefore, this strategy should be followed wherever possible.

In order to establish international standards and achieve global compliance, countries and industries within countries must be willing to work towards the adoption of internationally harmonized approaches for data collection and analysis. Baseline studies, as carried out in the EU, are fundamental undertakings prior to setting criteria or targets in order to provide a measure of the magnitude of hazard, exposure and human health risk, against which to consider optimal (cost-effective) points for intervention and then to scientifically evaluate the actual outcomes/impacts following implementation. Performance of methodologies used should be benchmarked in order to determine whether reported statistics represent accurately the status of an industry or country and, when possible, if any estimated change in reported data is due to an intervention, or is merely an artifact of methodological changes in sampling, laboratory testing procedures, or reporting systems.

The term ‘zero tolerance’ for specific pathogens such as *Salmonella* in food products is interpreted differently by both scientists and other stakeholders in different countries and therefore has been confusing, misleading, and misapplied. All countries signing the international

WTO agreements are entitled to establish sovereign levels of protection. However, with regard to sanitary measures that include MCs, the most appropriate and legally defensible approach is to define such criteria by limits of detection according to the analytical method imposed, together with confidence limits of sampling and testing. Using terms such as 'zero tolerance' or 'absence of a microbe' in relation to raw poultry should be avoided, unless defined and explained by international agreement. New metrics, such as POs that are linked to human health outcomes based on risk assessment, should be utilized throughout the food chain and will define the resultant public health risk.

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Prevalence of *Salmonella*-positive broiler flocks in the EU,

Table 1 2005 – 2006 (EFSA, 2007)

Member state	No. of flocks sampled[1]	Positive (%)
Austria	365	7.7
Belgium	373	15.3
Cyprus	248	10.9
Czech Republic	334	22.5
Denmark	295	3.1
Estonia	131	2.2
Finland	360	0.3
France	381	8.9
Germany	377	17.2
Greece	245	27.3
Hungary	359	65.7
Ireland	351	27.9
Italy	313	30.4
Latvia	121	9.1
Lithuania	156	5.1
Poland	357	57.7
Portugal	367	42.8
Slovakia	230	8.3
Slovenia	326	3.1
Spain	388	42.3
Sweden	291	0.0
The Netherlands	362	10.2
United Kingdom	382	10.7

[1] The number of samples taken was statistically-based. Samples were pooled fecal samples, using boot swabs, five per flock tested.

Table 2 Sampling for *Salmonella* at different stages of the supply chain

Stage in supply chain	What to sample?	When?
Feed manufacture	Bulk ingredients Mill environment and equipment Finished feed	Prior to use
Grandparent / parent flocks	Litter Dead birds Dust Feces	More intensive for grandparent stock. Sample before and just after moving to production house
	Surfaces and equipment	After cleaning and disinfection
Hatchery	Internal surface of hatching cabinet Chick box liners Eggshells Meconium Dead-in-shell chicks Culled chicks	After hatching
	Surfaces and equipment	After cleaning and disinfection
Broiler flocks	Litter Dust Feces	Prior to slaughter
	Surfaces and equipment	After cleaning and disinfection
Slaughter and processing	Neck skin or Carcass rinse	After carcass chilling
	Plant environment and equipment	After cleaning and disinfection
Portioning and deboning	Meat surface / skin	As required
	Plant environment and equipment	After cleaning and disinfection
Wholesale (fresh and frozen)	Meat surface / skin	As required
Retail	Meat surface / skin	As required

Examples of inter-country differences in typical production and processing of broilers in relation to *Salmonella* control

Table 3

	Brazil	Japan	Mexico	Netherlands	Russia	Sweden	USA
Annual broiler production [a]	5.28 billion	725million	1.5 billion	406.4 million	1.15 billion	75 million	9.02 billion
Annual poultrymeat production (tonnes) [a]	8.67 million	1.36 million	2.5 million	609,600	1.72 million	97,000	16 million
Vertical integration	yes	mostly	partly	yes	yes [e]	yes	yes
Importation of grandparent stock	no	majority	majority	some	yes	yes	no
Interventions for breeding stock (mandatory or voluntary)	vaccination and others	yes, unspecified [c]	testing only	vaccination and others	vaccination and others	testing only	yes, unspecified [c]
Mandatory *Salmonella* testing of breeding stock	yes	no	no	yes	yes	yes	no
Type of broiler house.	curtain-sided / closed	open-sided	open-sided	closed	closed	closed	curtain-sided / closed
Broilers: all in, all out	yes	yes	yes	no [d]	yes	no [d]	yes
Re-use of litter for broilers [b]	yes	yes	no	no	no	no	yes
Mandatory control of feed	no	no	no	yes	yes	yes [f]	no
Rescheduling of *Salmonella*-positive flocks	yes	no	no	yes	yes	no [g]	no
Slaughter-process automation	yes	yes	partly	yes	mostly	yes	yes
HACCP implementation in processing plants	yes	some	mostly	yes	some	yes	yes
Air or water chilling	mostly water	water	water	air	mostly water	air	mostly water
Use of chemical processing aids	no	yes	yes	no	yes	no	yes

Salmonella testing of carcasses in-plant	yes	no	yes	yes	yes	yes [h]	yes

For each country, inclusion of a superscript provides clarification or is used to denote an exception to typical practice.

[a] FAO data for 2007.

[b] The litter may be re-used for successive flocks, with or without treatment to control disease agents.
[c] No national policy.
[d] Due to partial depopulation of flocks prior to normal slaughter age.
[e] Within a single site.
[f] This involves an obligatory heat treatment.
[g] All *Salmonella*-positive flocks are destroyed.
[h] Carried out as a check on farm controls and plant hygiene.