Microbiological Risk Assessment Series 1

Risk assessments of *Salmonella* in eggs and broiler chickens

Interpretative summary

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World Health Organization
Food and Agriculture Organization of the United Nations

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For further information on the joint FAO/WHO activities on microbiological risk assessment, please contact:

Food Quality and Standards Service
Food and Nutrition Division
Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
I-00100 Rome, Italy

Fax: +39 06 57054593
E.mail: nutrition@fao.org

Web site: http://www.fao.org/es/esn

or

Food Safety Department
World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland

Fax: +41 22 7914807
E.mail: foodsafety@who.int

Web site: http://www.who.int/fsf

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Responsible technical units:

Food Safety Department
World Health Organization

Food Quality and Standards Service
Food and Nutrition Division
Food and Agriculture Organization of the United Nations

RISK ASSESSMENT DRAFTING GROUP

(in alphabetical order)

Wayne ANDERSON
Food Safety Authority of Ireland,
Ireland

Eric EBEL
Office of Public Health and Science, Food Safety and Inspection Service,
United States Department of Agriculture, United States of America

Aamir M. FAZIL
Health Protection Branch, Health Canada,
Canada.

Fumiko KASUGA
National Institute of Infectious Diseases, Department of Biomedical Food Research,
Japan.

Louise KELLY
Department of Risk Research, Veterinary Laboratories Agency,
United Kingdom

Anna LAMMERDING
Microbial Food Safety, Risk Assessment, Health Protection Branch, Health Canada,
Canada

Roberta MORALES
Health and Human Resource Economics, Centre for Economic Research,
Research Triangle Institute, United States of America

Wayne SCHLOSSER
Office of Public Health and Science, Food Safety and Inspection Service,
United States Department of Agriculture, United States of America

Emma SNARY
Department of Risk Research, Veterinary Laboratories Agency,
United Kingdom

Andrea VICARI
FAHRM, College of Veterinary Medicine, North Carolina State University,
United States of America

Shigeki YAMAMOTO
National Institute of Infectious Diseases, Department of Biomedical Food Research,
Japan.

REVIEWERS

The risk assessment was reviewed on several occasions both during and after its elaboration, including through expert consultations and selected peer reviews, and by members of the public in response to a call for public comments.

PARTICIPANTS IN EXPERT CONSULTATIONS

Amma Anandavally, Export Quality Control Laboratory, Cochin, India.

Robert Buchanan, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, United States of America.

Olivier Cerf, Ecole Nationale Vétérinaire d'Alfort (ENVA), France.

Jean-Yves D'Aoust, Health Protection Branch, Health Canada, Canada.

Paw Dalgaard, Department of Seafood Research, Danish Institute for Fisheries Research, Ministry of Food Agriculture and Fisheries, Denmark.

Michael Doyle, Center for Food Safety, University of Georgia, United States of America.

Emilio Esteban, Food Safety Initiative Activity, Centres for Disease Control and Prevention, United States of America.

Lone Gram, Department of Seafood Research, Danish Institute for Fisheries Research, Ministry of Food Agriculture and Fisheries, Denmark.

Steve Hathaway, MAF Regulatory Authority (Meat and Seafood), New Zealand.

Matthias Hartung, National Reference Laboratory on the Epidemiology of Zoonoses, Joint FAO/WHO Collaborating Centre, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany.

Inocencio Higuera Ciapara, Research Centre for Food and Development (CIAD), Mexico.

John Andrew Hudson, The Institute of Environmental Science and Research Ltd, New Zealand.

David Jordan, New South Wales Agriculture, Wollongbar Agricultural Institute, Australia.

Julia A. Kiehlbauch, Microbiology Consultant, United States of America.

Günter Klein, Division of Food Hygiene, Joint FAO/WHO Collaborating Centre, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany.

Susumu Kumagai, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Japan.

Roland Lindqvist, National Food Administration, Sweden.

Xiumei Liu, Department of Microbiology and Natural Toxins, Institute of Nutrition and Food Hygiene, Chinese Academy of Preventative Medicine, Ministry of Health, China.

Carol Maczka, Office of Public Health and Science, Food Safety and Inspection Service, United States Department of Agriculture, United States of America.

Patience Mensah, Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana.

George Nasinyama, Department of Epidemiology and Food Safety, Faculty of Veterinary Medicine, Makerere University, Uganda.

Gregory Paoli, Decisionalysis Risk Consultants Inc., Canada.

Irma N.G. Rivera, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil.

Son Radu, Department of Biotechnology, Faculty of Food Science and Biotechnology, University Putra Malaysia, Malaysia.

Tom Ross, School of Agricultural Science, University of Tasmania, Australia.

Dulce Maria Tocchetto Schuch, Laboratorio Regional de Apoio Animal - Lara / RS, Agriculture Ministry, Brazil.

Eystein Skjerve, Department of Pharmacology, Microbiology and Food Hygiene, The Norwegian School of Veterinary Science, Norway.

Ewen C.D. Todd, The National Food Safety and Toxicology Center, Michigan State University, United States of America.

Robert Bruce Tompkin, ConAgra Inc., United States of America.

Suzanne Van Gerwen, Microbiology and Preservation Unit, Unilever Research, The Netherlands.

Michiel Van Schothorst, Wageningen University, The Netherlands.

Kaye Wachsmuth, Office of Public Health and Science, Food Safety and Inspection Service, United States Department of Agriculture, United States of America.

Helene Wahlström, National Veterinary Institute, Sweden.

Richard C. Whiting, Center for Food Safety and Applied Nutrition, Food and Drug Administration, United States of America.

Charles Yoe, Department of Economics, College of Notre Dame of Maryland, United States of America.

REVIEWERS IN RESPONSE TO CALL FOR PUBLIC COMMENT

The United States Food and Drug Administration.

The Industry Council for Development.

Professor O.O. Komolafe, Department of Microbiology, College of Medicine, Malawi.

PEER REVIEWERS

Fred Angulo, Centers for Disease Control and Protection, United States of America.

Dane Bernard, Keystone Foods, United States of America.

Tine Hald, Danish Veterinary Laboratory, Denmark.

Thomas Humphrey, University of Bristol, United Kingdom.

Hilde Kruse, National Veterinary Institute, Norway.

Geoffrey Mead, Food Hygiene consultant,United Kingdom.

Robert T. Mitchell, Public Health Laboratory Service, United Kingdom.

Maarten Nauta, RIVM, The Netherlands.

Terry A. Roberts, Consultant, Food Hygiene and Safety, United Kingdom.

John Sofos, Colorado State University, United States of America.

Katharina Stark, Swiss Federal Veterinary Office, Switzerland.

Isabel Walls, International Life Sciences Institute, United States of America.

THE JOINT FAO/WHO SECRETARIAT ON RISK ASSESSMENT OF MICROBIOLOGICAL HAZARDS IN FOODS

Lahsen Ababouch, FAO

Peter Karim Ben Embarek, WHO

Sarah Cahill, FAO

Maria de Lourdes Costarrica, FAO

Françoise Fontannaz, WHO

Allan Hogue, WHO

Jean-Louis Jouve, (from June 2001) FAO

Hector Lupin, FAO

Jeronimas Maskeliunas, FAO

Jocelyne Rocourt, WHO

Jørgen Schlundt, WHO

Hajime Toyofuku, WHO

EDITOR

Thorgeir Lawrence, Technical editor, Iceland.

ABBREVIATIONS USED IN THE TEXT

AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of variance
CAC	FAO/WHO Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CDC	United States Center for Disease Control and Prevention
CFIA	Canadian Food Inspection Agency
CFU	Colony-forming unit
EGR	Exponential growth rate
EU	European Union
FDA	Food and Drug Administration [of the United States of America]
FSIS	Food Safety and Inspection Service [USDA]
HIV	Human Immunodeficiency Virus
HLA-B27	Human Leukocyte Antigen B27
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IUNA	Irish Universities Nutrition Alliance
MPN	Most probable number
MRA	Microbiological risk assessment
SE	*Salmonella enterica* serotype Enteritidis (*S.* Enteritidis)
US SE RA	USDA-FSIS *Salmonella* Enteritidis Risk Assessment
USDA	United States Department of Agriculture
YMT	Yolk membrane breakdown time

FOREWORD

The Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food both at the national and the international levels. Increasing foodborne disease incidence over the last decades seems, in many countries, to be related to an increase in disease caused by microorganisms in food. This concern has been voiced in meetings of the Governing Bodies of both Organizations and in the Codex Alimentarius Commission. It is not easy to decide whether the suggested increase is real or an artefact of changes in other areas, such as improved disease surveillance or better detection methods for microorganisms in foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately new tools, which can facilitate actions, seem to be on their way.

Over the past decade Risk Analysis, a process consisting of risk assessment, risk management and risk communication, has emerged as a structured model for improving our food control systems with the objectives of producing safer food, reducing the numbers of foodborne illnesses and facilitating domestic and international trade in food. Furthermore we are moving towards a more holistic approach to food safety where the entire food chain needs to be considered in efforts to produce safer food.

As with any model, tools are needed for the implementation of the risk analysis paradigm. Risk assessment is the science based component of risk analysis. Science today provides us with indepth information on life in the world we live in. It has allowed us to accumulate a wealth of knowledge on microscopic organisms, their growth, survival and death, even their genetic make-up. It has given us an understanding of food production, processing and preservation and the link between the microscopic and the macroscopic world and how we can benefit from as well as suffer from these microorganisms. Risk assessment provides us with a framework for organising all this data and information and to better understand the interaction between microorganisms, foods and human illness. It provides us with the ability to estimate the risk to human health from specific microorganisms in foods and gives us a tool with which we can compare and evaluate different scenarios as well as identify what type of data is necessary for estimating and optimising mitigating interventions.

Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by food-borne pathogens and in the elaboration of standards for food in international trade. However, undertaking a microbiological risk assessment (MRA), particularly quantitative MRA, is recognized as a resource-intensive task requiring a multidisciplinary approach. Yet, food-borne illness is among the most widespread public health problems creating social and economic burdens as well as human suffering, making it a concern that all countries need to address. As risk assessment can also be used to justify the introduction of more stringent standards for imported foods, a knowledge of MRA is important for trade purposes, and there is a need to provide countries with the tools for understanding and, if possible, undertaking MRA. This need, combined with that of the Codex Alimentarius for risk based scientific advice led FAO and WHO to undertake a programme of activities on MRA at the international level.

The Food Quality and Standards Service, FAO and the Food Safety Department, WHO are the lead units responsible for this initiative. The two groups have worked together to develop the area of MRA at the international level for application at both the national and international levels. This work has been greatly facilitated by the contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology to name but a few.

This Microbiological Risk Assessment series provides a range of data and information to those who need to understand or undertake MRA. It comprises risk assessments of particular pathogen-commodity combinations, interpretative summaries of the risk assessments, guidelines for undertaking and using risk assessment and reports addressing other pertinent aspects of MRA.

We hope that this series will provide a greater insight into MRA, how it is undertaken and how it can be used. We strongly believe that this is an area that should be developed in the international sphere, and have already from the present work clear indications that an international approach and early agreement in this area will strengthen the future potential of use of this tool in all parts of the world as well as in international standard setting. We would welcome comments and feedback on any of the documents within this series so that we can endeavour to provide Member States, Codex Alimentarius and other users of this material with the information they need to use risk based tools with the ultimate objective of ensuring that safe food is available for all consumers.

Jean-Louis Jouve
Food Quality and Standards Service
FAO

Jørgen Schlundt
Food Safety Department
WHO

EXECUTIVE SUMMARY OF THE MAIN REPORT

BACKGROUND

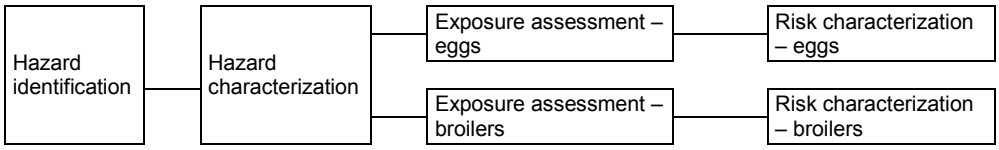
FAO and WHO undertook a risk assessment of *Salmonella* in eggs and broiler chickens in response to requests for expert advice on this issue from their member countries and from the Codex Alimentarius Commission. Guidance on this issue is needed, as salmonellosis is a leading cause of foodborne illness in many countries, with eggs and poultry being important vehicles of transmission.

The risk assessment had several objectives.

1. To develop a resource document of all currently available information relevant to risk assessment of *Salmonella* in eggs and broiler chickens and also to identify the current gaps in the data that need to be filled in order to more completely address this issue.
2. To develop an example risk assessment framework and model for worldwide application.
3. To use this risk assessment work to consider the efficacy of some risk management interventions for addressing the problems associated with *Salmonella* in eggs and broiler chickens.

This document could be used as a resource document that includes currently available information relevant to risk assessment of *Salmonella* in eggs and broiler chickens. Although a cost–benefit analysis of potential mitigations would assist risk managers in determining which mitigations to implement, it was not within the scope of this work and is not considered here.

In order to develop the model, the risk assessment was divided into two risk assessments with a shared hazard identification and hazard characterization. These two risk assessments included the four steps of risk assessment: hazard identification, hazard characterization, exposure assessment, and risk characterization.



One hazard identification and one hazard characterization, including a dose-response model, and two exposure assessment models – one of *Salmonella* in eggs and one of *Salmonella* in broiler chickens – were elaborated. For *Salmonella* in eggs, the risk characterization estimates the probability of human illness due to *Salmonella* following the ingestion of a single food serving of internally contaminated shell eggs, consumed as either whole eggs, egg meals or as ingredients in more complex food (e.g. cake). This work addressed selected aspects of egg production on farms; further processing of eggs into egg products; retail and consumer egg handling; and meal preparation practices. For S*almonella* in broiler chickens, the risk characterization estimates the probability of illness in a year due to the ingestion of *Salmonella* on fresh whole broiler chicken carcasses with the skin intact, and which are cooked in the domestic kitchen for immediate consumption. This work commenced at the conclusion of slaughterhouse processing and considers in-home handling and cooking practices. The effects of pre-slaughter interventions and the slaughter process are not currently included in this model.

The inputs for this risk assessment were obtained from a variety of sources. Information was compiled from published literature, national reports and from unpublished data submitted to FAO/WHO by various interested parties.

The main outputs from the risk assessment are summarized below. It should also be noted that, in the course of the work, efforts were made to identify features that have an impact on the acceptability of findings and the appropriateness of extrapolating findings to scenarios not explicitly investigated in the risk assessments, and these are identified in the risk assessment document.

HAZARD IDENTIFICATION

During the past two decades, *Salmonella* Enteritidis has emerged as a leading cause of human infections in many countries, with hen eggs being a principal source of the pathogen. This has been attributed to this serovar's unusual ability to colonize ovarian tissue of hens and be present within the contents of intact shell eggs. Broiler chicken is the main type of chicken consumed as poultry in many countries. Large percentages are colonized by salmonellae during grow-out and the skin and meat of carcasses are frequently contaminated by the pathogen during slaughter and processing. Considering the major role eggs and poultry have as vehicles of human cases of salmonellosis, an assessment of different factors affecting the prevalence, growth and transmission of *Salmonella* in eggs and on broiler chicken carcasses and the related risk of human illness would be useful to risk managers in identifying the intervention strategies that would have the greatest impact on reducing human infections.

HAZARD CHARACTERIZATION

The hazard characterization provides a description of the public health outcomes, pathogen characteristics, host characteristics, and food-related factors that may affect the survival of *Salmonella* through the stomach. It also presents a review of information on relevant dose-response models describing the mathematical relationship between an ingested dose of *Salmonella* and the probability of human illness. An extensive review of available outbreak data was also conducted. From these data, a new dose-response model was derived using a re-sampling approach, and this was used in both risk characterizations in preference to existing models that are defined within this component of the risk assessment. Finally, an attempt was made to discern whether separate dose-response curves could be justified for different human sub-populations defined on the basis of age and "susceptibility", and whether a dose-response for *S.* Enteritidis was distinguishable from a dose-responses for other *Salmonella.*

Three existing dose-response models for *Salmonella* were identified:

1. Fazil, 1996, using the Beta-Poisson model (Haas, 1983) fitted to the naive human data from *Salmonella* feeding trials (McCullough and Eisele, 1951a, b, c).
2. United States *Salmonella* Enteritidis Risk Assessment (US SE RA) (USDA-FSIS, 1998), based on the use of human feeding trial data for a surrogate pathogen (*Shigella dysenteriae*) with illness as the measured endpoint to describe the dose-response relationship.
3. *Salmonella* Enteritidis Risk Assessment conducted by Health Canada (2000, but unpublished) based on a Weibull-Gamma dose-response relationship. The model uses data from many different pathogen-feeding trials and combines the information with key *Salmonella* outbreak data, using a Bayesian relationship.

These dose-response models for *S.* Enteritidis and *Salmonella* were found to inadequately characterize the dose-response relationship observed in the outbreak data. A new dose-response model was developed in the course of this work. It was derived from outbreak data and was considered to be the most appropriate estimate for the probability of illness upon ingestion of a dose of *Salmonella*. The model was based on observed real world data, and as such was not subject to some of the flaws inherent in using purely experimental data. Nevertheless, the current outbreak data also have uncertainties associated with them and some of the outbreak data points required assumptions to be made. The outbreak data are also from a limited number of developed countries and may not be applicable to other regions.

From the outbreak data used to examine the dose-response relationship, it could not be concluded that *S.* Enteritidis has a different likelihood from other serovars of producing illness. In addition, comparing the attack rates of *Salmonella* for children less than five years of age, against those for the rest of the population in the outbreak database, did not reveal an overall trend of increased risk for this subpopulation. Although some indication for a difference in attack rates for the two populations had been noted in two of the outbreaks examined, the database of outbreak information might lack the potential to reveal the existence of any true differences. Severity of illness as a function of patient age, *Salmonella* serovar or pathogen dose were not evaluated, although severity could potentially be influenced by these factors and by pathogenicity. However, the current database of information was insufficient to derive a quantitative estimate for these factors.

EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION OF *SALMONELLA* ENTERITIDIS IN EGGS

The exposure assessment section for *S.* Enteritidis in eggs compares and contrasts previously completed models. It describes the general framework of these models, the data used, and the analysis completed for modelling analysis. Generally, these models comprise a production module, a module for the processing and distribution of shell eggs, a module for the processing of egg products, and a module for preparation and consumption. The production module predicts the likelihood of a *S.* Enteritidis-contaminated egg occurring. This depends on the flock prevalence, within-flock prevalence, and the frequency that infected hens lay contaminated eggs. The flock prevalence (i.e. the likelihood of a flock containing one or more infected hens) further depends on factors that serve to introduce *S.* Enteritidis into flocks (e.g. replacement pullets, environmental carryover from previously infected flocks, food contamination, etc.). The shell egg processing and distribution, and preparation and consumption modules predict the likelihood of human exposures to various doses of *S.* Enteritidis from contaminated eggs. The dose consumed in an egg-containing meal depends on the amount of *S.* Enteritidis growth between the time the egg was laid and when it was prepared, as well as how the egg was prepared and cooked. Growth of *S.* Enteritidis in contaminated eggs is a function of storage time and temperature. The output of the exposure assessment, in general, feeds into the hazard characterization to produce the risk characterization output. This output is the probability of human illness per serving of an egg-containing meal.

The exposure assessment included consideration of yolk-contaminated eggs and growth of *S.* Enteritidis in eggs prior to processing for egg products. These issues have not been previously addressed by exposure assessments of *S.* Enteritidis in eggs. Yolk-contaminated eggs might allow more rapid growth of *S.* Enteritidis inside such eggs compared with eggs that are not yolk-contaminated.

This risk characterization of *S.* Enteritidis in eggs was intentionally developed so as not to be representative of any specific country or region. However, some model inputs are based on evidence or assumptions derived from specific national situations. Caution is therefore required when extrapolating from this model to other countries.

Key findings

The risk of human illness from *S.* Enteritidis in eggs varies according to the different input assumptions in the model. The risk of illness per serving increases as flock prevalence increases. However, uncertainty regarding the predicted risk also increases as flock prevalence increases. **Reducing flock prevalence results in a directly proportional reduction in human health risk. For example, reducing flock prevalence from 50% to 25% results in a halving of the mean probability of illness per serving. Reducing prevalence within infected flocks also results in a directly proportional reduction in human health risk. For example, risk of illness per serving generated from eggs produced by a flock with 1% within-flock prevalence is one-tenth that of a flock with 10% within-flock prevalence.**

Adjusting both egg storage time and temperature profiles for eggs from production to consumption was associated with large effects on the predicted risk of human illness. **The risk of human illness per serving appears to be insensitive to the number of *Salmonella***

Enteritidis in contaminated eggs across the range considered at the time of lay. For example, whether it is assumed that all contaminated eggs had an initial number of 10 or 100 *S.* Enteritidis organisms, the predicted risk of illness per serving was similar. This may be because the effect of *S.* Enteritidis growth is greater than the initial contamination level in eggs.

As an example of how the efficacy of interventions aimed at reducing flock prevalence may be assessed the risk assessment examined the effect of a "test and divert" programme. Two protocols were assumed, with either one (at the beginning of egg production) or three (beginning of egg production, four months later & just before flock depopulation) tests administered to the entire population of egg production flocks and their effectiveness was estimated over a four-year period. Testing three times per year for four years reduced the risk of human illness from shell eggs by more than 90% (i.e. >1 log). Testing once a year for four years reduced risk by over 70%.

Other potential interventions evaluated included vaccination and refrigeration. To evaluate the effectiveness of vaccination against *S.* Enteritidis a single test, or two tests four months apart, with 90 faecal samples per test, was considered. The vaccine was assumed to be capable of reducing the frequency of contaminated eggs by approximately 75%. The effects of time and temperature restrictions were evaluated assuming a flock prevalence of 25%. Restricting shelf-life to less than 14 days reduced the predicted risk of illness per serving by a negligible amount (~1%). However, keeping retail storage temperature at no more than 7.7°C reduced risk of illness per serving by about 60%. Were shelf-life to be reduced to 7 days, risk per serving would also be reduced by about 60%.

Limitation

The available data on which this risk assessment was based was limited. For example, evidence regarding enumeration of the organism within eggs was based on only 63 *S.* Enteritidis-contaminated eggs, and in part on estimates of the concentration of the organism in contaminated eggs. It is difficult to represent uncertainty and variability with such limited data. Apparently, there is a lot of uncertainty and it is difficult to quantify. In addition, statistical or model uncertainty was not fully explored.

Much uncertainty attends the effectiveness of various management interventions for controlling *S.* Enteritidis. The magnitudes of uncertainty regarding test sensitivity, effectiveness of cleaning and disinfecting, and vaccination efficacy have not been measured. Some data were available to describe these inputs, but the data may not be relevant to all regions or countries where such interventions might be applied.

Statistical or model uncertainty was not fully explored in this risk characterization. For example, alternative distributions to the lognormal for within-flock prevalence were not considered. In addition, the predictive microbiology used in this model was dependent on very limited data pertaining to *S.* Enteritidis growth inside eggs. Alternative functional specifications for *S.* Enteritidis growth equations were not pursued in this analysis.

EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION OF *SALMONELLA* IN BROILER CHICKENS

The risk assessment model is defined in terms of a number of parameters that describe the processes of broiler chicken carcass distribution and storage, preparation, cooking and

consumption. Some of these parameters can be considered general in that they can be used to describe the situation in many countries. At the same time, some parameters are country specific, such as the prevalence of carcasses contaminated with *Salmonella* at the completion of processing. Predictions of risk for a particular country are best obtained from data relevant to that country.

The exposure assessment of *Salmonella* in broiler chickens mimics the movement of *Salmonella*-contaminated chickens through the food chain, commencing at the point of completion of the slaughter process. For each iteration of the model, a chicken carcass was randomly allocated an infection status and those carcasses identified as contaminated were randomly assigned a number of *Salmonella* organisms. From this point until consumption, changes in the size of the *Salmonella* population on each contaminated chicken were modelled using equations for growth and death. The growth of *Salmonella* was predicted using random inputs for storage time at retail stores, transport time, storage time in homes, and the temperatures the carcass was exposed to during each of these periods. Death of *Salmonella* during cooking was predicted using random inputs describing the probability that a carcass was not adequately cooked, the proportion of *Salmonella* organisms attached to areas of the carcass that were protected from heat, the temperature of exposure of protected bacteria, and the time for which such exposure occurs. The number of *Salmonella* consumed were then derived using a random input defining the weight of chicken meat consumed per serving and the numbers of *Salmonella* cells in meat as defined from the various growth and death processes. Finally, in the risk characterization, the probability of illness was derived by combining the number of organisms ingested (from the exposure assessment) with information on the dose-response relationship (hazard characterization).

Key findings

The *Salmonella* in broiler chickens risk assessment does not consider all parts of the production-to-consumption continuum, and this limits the range of control options that can be assessed. This is primarily due to the lack of representative data to analyse how much change in either the prevalence or level of *Salmonella* in poultry could be attributable to any specific treatment or action. However, the establishment of a baseline model provided a means to compare the effects on risk when prevalence and cell numbers were changed. The model parameters can be modified to evaluate the efficacy of risk mitigation strategies that target those parameters. For example, the parameter describing prevalence of *Salmonella*-contaminated broiler chickens exiting processing can be modified to evaluate the effectiveness of a processing measure such as chlorination of the chilling water to reduce the prevalence of *Salmonella*-contaminated carcasses.

Reduction in the prevalence of *Salmonella*-contaminated chicken was associated with a reduction in the risk of illness. A one-to-one relationship was estimated, with a percentage change in prevalence, assuming everything else remains constant, reducing the expected risk by a similar percentage. **For instance, a 50% reduction in the prevalence of contaminated poultry (20% to 10%) produced a 50% reduction in the expected risk of illness per serving. Similarly, a large reduction in prevalence (20% to 0.05%) would produce a 99.75% reduction in the expected risk of illness.** If management strategies are implemented that affect the level of contamination, i.e. the numbers of *Salmonella* on chickens, the relationship to risk of illness is estimated to be greater than a one-to-one relationship. **A shift in the distribution of *Salmonella* cell numbers on broiler chickens exiting the chill tank at the end of processing, such that the mean number of cells is**

reduced by 40% on the non-log scale, reduces the expected risk of illness per serving by approximately 65%.

A small reduction in the frequency of undercooking and the magnitude of the undercooking event results in a marked reduction of the expected risk of illness per serving. The important caveat here is that altering cooking practices does not address the risk of illness through the cross-contamination pathway. The strategy of changing the consumer's cooking practices needs to be tempered by the fact that cross-contamination may in fact be the predominant source of risk of illness, and the nature of cross-contamination in the home is still a highly uncertain phenomenon.

Limitations and caveats

It was not possible to provide a perfect representation of growth of *Salmonella* in raw poultry and seasonal variations in ambient temperature were not accounted for. The model adopted also assumed that ambient temperature had no impact on the rate of change for storage temperatures used for predicting growth, and this is intuitively inappropriate in some circumstances. Similarly, limitations were present in the way the model predicts the death of *Salmonella* in broiler chicken carcasses during the cooking process.

At several steps, reliance was placed on expert opinion to estimate the value of model inputs. While often easily accessible and sometimes sufficiently accurate, occasionally, expert opinion might reduce transparency and introduce an unacceptable bias that may not be detected by the risk assessors.

Surveillance data from some countries often show a marked seasonality in the number of notifications of human salmonellosis, with peak incidence occurring in the warmer months and the current model cannot account for or explain this important phenomenon.

A lack of detailed understanding of all aspects of cross-contamination in the home hampered the ability of the risk assessment to address this process. While the uncertainty associated with several parameters in the consumption portion of the risk assessment was accounted for, a full analysis of statistical and model uncertainty was not done. Thus, the influence of uncertainty in the cross-contamination pathway was not explored.

CONCLUSIONS

This *Salmonella* risk assessment provides information that should be useful in determining the impact intervention strategies may have on reducing cases of salmonellosis from contaminated eggs and poultry. In the risk assessment of *Salmonella* in broiler chickens, for example, it was determined that there is a relationship between changing the prevalence of *Salmonella* on the broiler chickens and reducing the risk of illness per serving. In the risk assessment of *S.* Enteritidis in eggs, reducing the prevalence of *S.* Enteritidis in poultry flocks was directly proportional to the reduction in risk to human health. The model can also be used to estimate the change in risk of human illness from changing storage times or temperature of eggs. However, comparison of effects of intervention measures, i.e. sensitivity analysis, cannot be done because this risk assessment is not conducted for a specific region or country, or for global settings. Data was collected from different countries for different input parameters. If those data were changed reflecting a specific national situation, the impact of a measure would also be changed. Therefore, caution would be needed in interpreting the results of this risk assessment in Codex activities.

REFERENCES CITED IN THE EXECUTIVE SUMMARY

Fazil, A.M. 1996. A quantitative risk assessment model for salmonella. Drexel University, Philadelphia PA. [Dissertation].

Haas, C.N. 1983. Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *American Journal of Epidemiology*, **118**: 573–582.

Health Canada. [2000]. Risk assessment model for *Salmonella* Enteritidis. Unpublished Health Canada Document.

McCullough, N.B., & Eisele, C.W. 1951a. Experimental human salmonellosis. I. Pathogenicity of strains of *Salmonella* Meleagridis and *Salmonella anatum* obtained from spray-dried whole egg. *Journal of Infectious Diseases*, **88**: 278–289.

-- 1951b. Experimental human salmonellosis. II. Immunity studies following experimental illness with *Salmonella* Meleagridis and *Salmonella anatum*. *Journal of Immunology*, **66**: 595–608.

-- 1951c. Experimental human salmonellosis. III. Pathogenicity of strains of *Salmonella* Newport, *Salmonella derby*, and *Salmonella* Bareilly obtained from spray dried whole egg. *Journal of Infectious Diseases*, **89**: 209–213.

USDA-FSIS. 1998. *Salmonella* Enteritidis Risk Assessment. Shell Eggs and Egg Products. Final Report. Prepared for FSIS by the *Salmonella* Enteritidis Risk Assessment Team. 268 pp. Available on Internet as PDF document at: www.fsis.usda.gov/ophs/risk/contents.htm.

1. RISK ASSESSMENTS OF *SALMONELLA* IN EGGS AND BROILER CHICKENS – INTERPRETATIVE SUMMARY

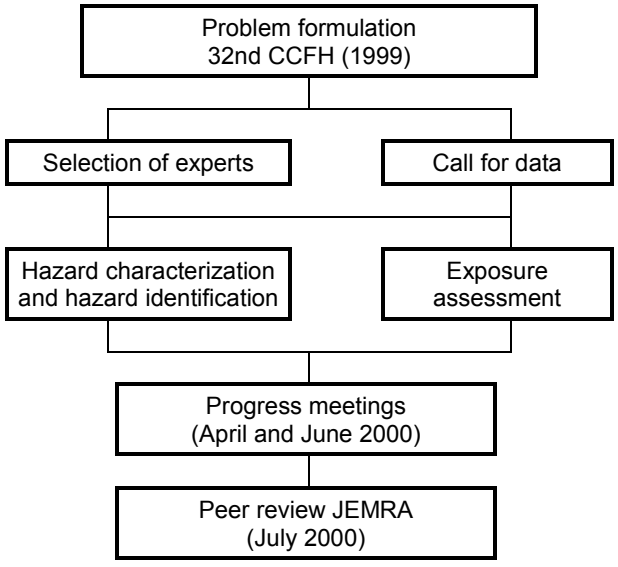
1.1 SCOPE

This chapter summarizes the results of the FAO/WHO Risk Assessment of Salmonella in Eggs and Broiler Chickens, noting the sources used and techniques applied, and drawing out the main conclusions. Following an itemized response to the questions posed by the 33rd CCFH, a number of recommendations are made. Attention is drawn to areas where further research and data collection are required for extending the risk assessment to provide a more comprehensive and reliable tool for risk management.

1.2 HISTORY

FAO and WHO have attempted to meet the expressed needs of their member countries and the Codex Alimentarius Commission (CAC) for guidance in conducting risk assessments of *Salmonella* in eggs and broiler chickens. On the one hand, CAC requested scientific advice as the basis for the development of guidelines and recommendations for the management of risks posed by these microbiological hazards. On the other hand, member countries required adaptable models to use in conducting their own national assessments. A series of topic-specific modules would satisfy a great need, and particularly dose-response modules that could be adapted and used with exposure assessments within national or regional boundaries.

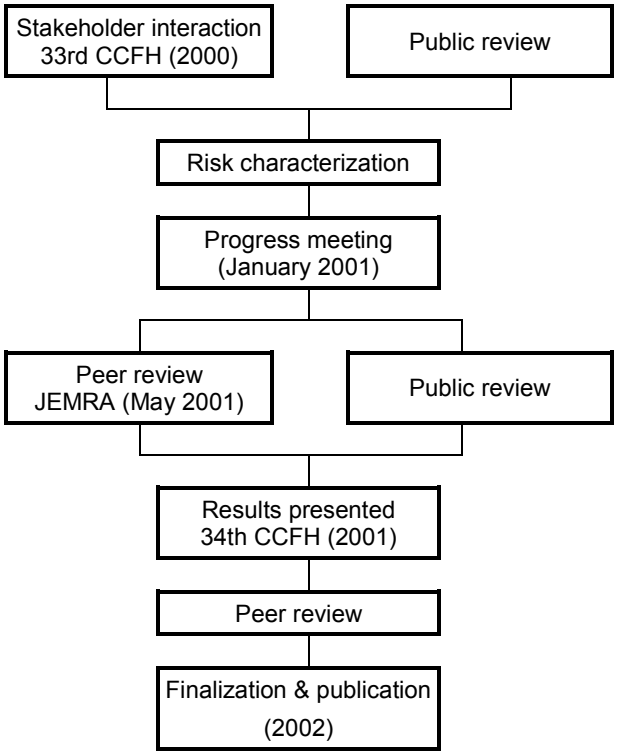
Figure 1. Year 1 of the FAO/WHO process for micro-biological risk assessment of *Salmonella* in eggs and broiler chickens.



FAO and WHO developed a process for conducting micro-biological risk assessments at the international level. The process incorporated essential principles regarding functional separation of risk assessment from risk management; transparency; and freedom from bias (Figures 1 and 2). At its 32nd Session, in December 1999, the Codex Committee on Food Hygiene (CCFH) prioritised pathogen-commodity combinations of concern to public health and international trade in food. CCFH identified *Salmonella* in eggs and in poultry as the top two priorities on their list of 21

pathogen–commodity combinations of concern in food. FAO and WHO were requested to convene ad hoc expert consultations to provide risk assessment advice.

Figure 2. Year 2 of the FAO/WHO process for microbiological risk assessment of *Salmonella* in eggs and broiler chickens.



In January 2000, to conduct these risk assessments, FAO and WHO mobilized an international team of scientists with documented expertise in microbiological risk assessment. The team prepared technical documentation on the hazard identification, exposure assessment and hazard characterization components of the risk assessment. These documents were reviewed and evaluated during a Joint Expert Meeting on Microbiological Risk Assessment (JEMRA) held at FAO, Rome, 17–21 July 2000. The consultation identified two important issues for discussion with the CCFH: the lack of clear risk management questions; and limitations in the usefulness of a global risk estimate. FAO and WHO presented the draft risk assessment and the report of the expert consultation to CCFH at its 33rd Session (Washington DC, 23–28 October 2000) in order to inform risk managers of the progress of the risk assessment and to seek more precise guidance on the needs of the Committee. In response to the request for further elaboration of the risk managers' questions, the CCFH provided a list of risk management questions (Tables 6 and 7).

In the next year, the team of scientists developed the risk characterization element of the assessment. A second expert consultation was held at FAO headquarters (Rome, 30 April – 4 May 2001) to review the work. The report of the Expert Consultation, which included preliminary answers to the questions posed by the CCFH, was presented to the 34th session of CCFH at its meeting in Bangkok (8–13 October 2001). The draft risk assessment was then made available for public comment and also sent for peer review by scientists in a number of countries. The risk assessment was subsequently revised and finalized.

The development of the risk assessments thus took two years, and the peer review required an additional year. Draft documents were reviewed twice by the CCFH and by two joint FAO/WHO expert consultations. In addition, peer review was employed to gather technical comments, and comments were solicited from the general public. This rigorous review process promoted transparency, as well as the involvement of all stakeholders in the process.

1.3 OBJECTIVES

The objectives of the risk assessments of *Salmonella* in eggs and broiler chickens were:

1. To develop a resource document of all currently available information relevant to risk assessment of *Salmonella* in eggs and broiler chickens and also to identify the current gaps in the data that need to be filled in order to more completely address this issue.
2. To develop an example risk assessment framework and model for worldwide application.
3. To use this risk assessment work to consider the efficacy of some risk management interventions for addressing the problems associated with *Salmonella* in eggs and broiler chickens.

Although a cost–benefit analysis of potential mitigations would assist risk managers in determining what measures to implement, it was not within the scope of this work and is not considered here.

1.4 HAZARD IDENTIFICATION

Salmonellosis is one of the most frequently reported foodborne diseases worldwide. International data (where available) indicate an estimated incidence of salmonellosis from 14 to 120 per 100 000 people in 1997 (Table 1). The US Centers for Disease Control and Prevention (CDC) estimate 1.4 million cases, 16 430 hospitalizations, and 582 deaths in the United States of America annually (Mead et al., 1999). Of the total number of cases, 96% are estimated to be caused by foods. Costs of foodborne salmonellosis for the United States of America population are estimated to be as high as US$ 2 329 million annually (in 1998 US dollars) for medical care and lost productivity. Over 2 000 serotypes of *Salmonella* have been identified, the most prevalent of which are *S.* Enteritidis, *S.* Typhimurium and *S.* Heidelberg.

Table 1. Estimated annual incidence of salmonellosis

Country	Cases per 100 000 population
Australia	38
Germany	120
Japan	73
The Netherlands	16
USA	14

SOURCE: Thorns, 2000.

Salmonellosis is characterized by diarrhoea, fever, abdominal pain or cramps, vomiting, headache and nausea. The incubation period ranges from 8 to 72 hours. Symptoms can last up to a week. Salmonella infections vary from mild to severe, and are occasionally fatal. Fatalities are more often seen in susceptible populations, which include infants, the elderly and the immuno-compromised. In the United States of America between 1985 and 1991, there were 54 reported *S.* Enteritidis outbreaks occurring in hospitals or nursing homes, accounting for 90% of all *Salmonella*-associated deaths, but only 12% of all cases. A small proportion of infected individuals may develop Reiter's syndrome, an arthritic disease characterized by symptoms of joint pain, eye irritation and painful urination.

Poultry have a major role as vehicles of transmission in human cases of salmonellosis. An assessment of factors affecting the prevalence and growth of *Salmonella* on broiler chicken carcasses would be useful to risk managers in identifying the intervention strategies that would have the greatest impact on reducing human infections. Broiler chicken is the main type of chicken consumed as poultry in many countries. A large percentage of poultry is colonized by salmonellas during grow-out, and the skin and meat of carcasses are frequently contaminated by the pathogen during slaughter and processing.

Since the late 1970s, *S.* Enteritidis has emerged as the major cause of salmonellosis in North America, Europe and South America. A significant increase in the incidence of *S.* Enteritidis infection has also been reported in Yugoslavia, Finland, Sweden, Norway and the United Kingdom. Hen eggs have become a principal source of the pathogen. The emergence of *S.* Enteritidis as the leading cause of human salmonellosis in many countries is attributed to this serovar's unusual ability to colonize the ovarian tissue of hens and to be present within the contents of intact shell eggs.

Most foodborne *S.* Enteritidis infection is associated with the consumption of raw eggs and foods containing raw eggs, such as homemade egg nog, biscuit batter, homemade ice cream, mayonnaise, Caesar salad dressing and Hollandaise sauce. In fact, 77% to 82% of *S.* Enteritidis outbreaks have been associated with grade A shell eggs, or egg-containing foods Undercooked eggs and products containing undercooked eggs, such as soft custards, French toast, soft-fried and poached eggs, are also significant sources of *S.* Enteritidis. According to a recent USFDA report, between 128 000 and 640 000 *Salmonella* infections are annually associated with the consumption of *S.* Enteritidis-contaminated eggs, and the CDC estimates that 75% of all *Salmonella* outbreaks are due to raw or inadequately cooked Grade A whole shell eggs.

Salmonella is transmitted to eggs by two routes: transovarian (vertical transmission) or trans-shell (horizontal transmission). In vertical transmission, *Salmonella* are introduced from infected ovaries or oviduct tissue to eggs prior to shell formation. Horizontal transmission is usually derived from faecal contamination on the eggshell. It also includes contamination through environmental vectors, such as farmers, pets and rodents. Vertical transmission is considered to be the major route of *Salmonella* contamination and is more difficult to control, while horizontal transmission can be effectively reduced by cleaning and disinfection of the environment.

1.5 HAZARD CHARACTERIZATION

1.5.1 Sources of data

FAO and WHO requested data from member countries through Codex Circular Letters. Data on salmonellosis outbreaks were obtained from a variety of sources, including published literature, national reports and unpublished data. The Ministry of Health and Welfare in Japan provided unpublished data on 16 outbreaks that the agency had investigated since 1997. This information was especially useful because it contained data on the number of organisms present in food implicated in human illness.

1.5.2 Description of the database

Of the 33 outbreak reports that FAO and WHO received, 23 contained sufficient data on the number of people exposed, the number of people that became ill, and the number of organisms in the implicated food to enable calculation of a dose-response relationship. Three of the 23 outbreaks were excluded because the immune status of the persons exposed could not be determined. The remaining 20 outbreaks formed the database used to calculate a dose-response relationship.

Of the 20 outbreaks in the database, 11 occurred in Japan and 9 occurred in the United States of America. The number of people exposed in Japanese outbreaks (≈14 037; 52%) was about the same as that in United States of America outbreaks (≈12 728; 48%). These numbers are approximate because in some cases the number of people exposed had to be estimated from the outbreak report. The overall attack rate in the data was 21.8% (26 765 exposed, 5 636 ill). The attack rate among Japanese outbreaks (27.4%) was higher than that of United States of America outbreaks (15.6%). This was due in part to one large outbreak in the United States of America (8 788 people exposed) with an attack rate of 11.7%, and one large outbreak in Japan (5 102 people exposed) with an attack rate of 26.9%. Several serotypes were associated with the outbreaks, including Enteritidis (12), Typhimurium (3), Heidelberg, Cubana, Infantis, Newport and Oranienburg. Several vehicles were implicated, including food (meat, eggs, dairy products and others), water, and a medical dye capsule (carmine dye).

Reports provided by the Ministry of Health and Welfare of Japan represented a valuable source of information on the real-world dose-response relationship and considerably expanded the database of *Salmonella* pathogenicity. The data in these reports were generated as part of the epidemiological investigations that take place in Japan following an outbreak of foodborne illness. In accordance with a Japanese notification (from March 1997), large-scale cooking facilities that prepare more than 750 meals per day or more than 300 dishes of a single menu at a time are advised to save food for future possible analysis in the event of an outbreak. The notification is also applicable to smaller-scale kitchens with social responsibility, such as those in schools, daycare centres and other child-welfare and social-welfare facilities. Fifty-gram portions of each raw food ingredient and each cooked dish are saved for more than 2 weeks frozen at a temperature lower than -20ºC. Although this notification is not mandatory, the level of compliance is high. Some local governments in Japan also have local regulations that require food saving, but the duration and the storage temperature requirements vary.

1.5.3 Description of the dose-response relationship

The availability of a reasonably large data set representing real-world observations for the probability of illness upon exposure to *Salmonella* (outbreak data) provided a unique opportunity to develop a data-based dose-response relationship. A beta-Poisson model was used as the mathematical form for the relationship, and this was fitted to the outbreak data.

The maximum likelihood technique was used to generate the curve best fitting the data. The fit was optimized using an iterative technique that minimized the deviance statistic, which is based upon a binomial assumption.

The uncertainty in the outbreak data set was incorporated into the fitting routine by reviewing the outbreak information and assigning an uncertainty distribution on observed variables that were potentially uncertain. A detailed summary of the assumptions associated with each outbreak and the estimation for the range of uncertainty for each of the variables were described. A summary of the data set, with uncertainty for the variables, is given in Table 2.

In order to fit the dose-response model to the uncertain outbreak data, the data were re-sampled based on the uncertainty distributions, generating a new data set at each sample. The dose-response model was then fitted to each of the re-sampled data sets. This procedure was repeated approximately 5000 times, generating 5000 dose-response data sets, to which 5000 dose-response curves were fitted. The fitting procedure used places a greater emphasis on fitting the curve through the outbreaks with larger numbers of people exposed compared to the smaller outbreaks. This is primarily a result of the binomial assumption and the greater variance associated with data from a small observation compared with a large one.

Table 2. Uncertainty ranges assigned to the variables in reported outbreak data

Out-break	Serovar	Log Dose (Uncertainty)		Response [Attack Rate] (Uncertainty)	
		Min	Max	Min	Max
1	*S.* Typhimurum	1.57	2.57	11.20%	12.36%
2	*S.* Heidelberg	1.48	2.48	28.29%	36.10%
3	*S.* Cubana	4.18	4.78	60.00%	85.71%
4	*S.* Infantis	6.06	6.66	100.00%	100.00%
5	*S.* Typhimurium	3.05	4.05	52.36%	57.64%
7	*S.* Newport	0.60	1.48	0.54%	2.59%
11	*S.* Enteritidis	4.00	5.00	100.00%	100.00%
12	*S.* Enteritidis	1.00	2.37	6.42%	7.64%
13	*S.* Typhimurum	8.00	8.88	100.00%	100.00%
18	*S.* Enteritidis	5.13	5.57	60.00%	60.00%
19	*S.* Enteritidis	6.03	6.48	87.70%	103.51%
20	*S.* Enteritidis	2.69	3.14	18.61%	36.41%
22	*S.* Enteritidis	6.02	6.47	52.17%	61.32%
23	*S.* Eteritidis	5.53	5.97	84.62%	84.62%
24	*S.* Enteritidis	1.45	1.89	12.19%	23.96%
25	*S.* Enteritidis	3.36	3.80	39.85%	39.85%
30	*S.* Enteritidis	3.53	3.97	60.14%	70.90%
31	*S.* Enteritidis	2.37	2.82	25.62%	30.04%
32	*S.* Enteritidis	1.11	1.57	26.92%	26.92%
33	*S.* Oranienburg	9.63	10.07	100.00%	100.00%

NOTE: "Outbreak" refers to the number of the outbreak as listed in the main report..

It was not possible to get a statistically significant single "best fitting" curve to the expected value of all the outbreak data points. However, the characterization of the observed outbreak data by the fitted dose-response model was better than that of other published dose-response models. It is important to note that the range of possible responses at any one given dose shown in the background of Figure 3 do not represent the statistical confidence bounds of the dose-response fit, but rather the best fit of the beta-Poisson model to different realizations of the observed data, given its uncertainties.

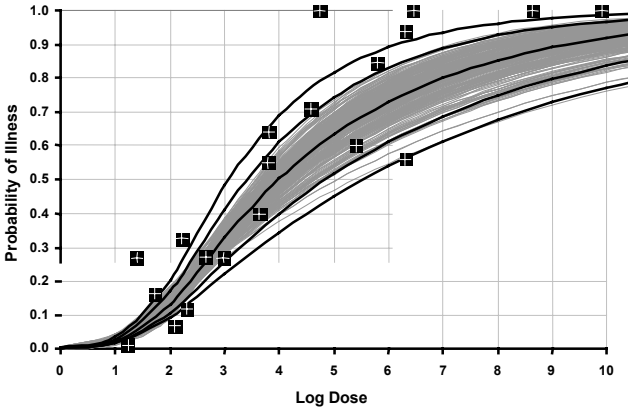


Figure 3. Uncertainty bounds for dose response curves superimposed on the dose-response curves generated by fitting to samples from uncertain outbreak observations

Figure 3 compares the fitted curves and the expected values. The upper bound, lower bound, expected value, 97.5th percentile and 2.5th percentile for the dose-response curves fitted to the 5000 data sets are also shown. The fitted dose-response range captures the observed outbreak data quite well, especially at the lower and mid-dose ranges. The greater range at high dosages is due to the existence of several large-scale outbreaks at the lower- and mid-dose levels through which the curves attempt to pass, while the two high-dose data points are for relatively small-scale outbreaks that allow greater "elasticity" in the fit.

Table 3. Beta-Poisson dose response parameters that generate the approximate bounds shown in Figure 3.

	Alpha	Beta
Expected Value	0.1324	51.45
Lower Bound	0.0763	38.49
2.5th Percentile	0.0940	43.75

Since the fitting procedure generated a dose-response curve for each of the 5000 data sets, there are also 5000 sets of beta-Poisson dose-response parameters (alpha & beta). In order to apply the dose-response relationship in a risk assessment, the ideal approach would be to randomly sample from the set of parameters that are generated, thereby recreating the dose-response curves shown. As an alternative, it is also possible to use the upper, lower, expected value, 2.5th percentile or 97.5th percentile to represent the uncertainty ranges in the dose-response relationship, as opposed to a full characterization resulting from the sampling of the parameter sets. The parameters that generate dose-response curves that approximate the bounds shown in Figure 3 of the dose-response relationship are summarized in Table 3.

In dose-response analysis, the critical region is the lower dose region, as these are the doses that are most likely to exist in the real world. Unfortunately, this is also the region where experimental data is least available. The outbreak data extend to a much lower doses than is common in experimental feeding trials, and consequently may offer a greater degree

of confidence in the lower dose approximations generated by the outbreak dose-response model.

1.5.4 Analysis of the dose-response relationship

Some strains of *S.* Enteritidis, particularly the phage-types isolated from the increased number of egg-related outbreaks seen in recent years, may be more infectious than other serotypes of *Salmonella*. Twelve sets of data were evaluated for *S.* Enteritidis, against 8 sets of data for other serotypes. From the outbreak data used to examine the dose-response relationship, it could not be concluded that *S.* Enteritidis had likelihood of producing illness different from other serotypes. However, increased severity of illness once infected was not evaluated.

An attempt was made to discern whether separate dose-response curves could be justified for different subpopulations, defined on the basis of age and 'susceptibility'. Comparing the attack rates of *Salmonella* for children less than five years of age with those for the rest of the population did not reveal an increased risk for this subpopulation. It is important to note that the database of outbreak information may lack the power to reveal the existence of true differences. Severity could potentially be influenced by patient age or *Salmonella* serotype. However, the current database of information was insufficient to derive a quantitative estimate of these factors.

The dose-response model fitted to the outbreak data offers a reasonable estimate for the probability of illness upon ingestion of a dose of *Salmonella*. The model is based on observed real-world data, and, as such, is not subject to some of the flaws inherent in using purely experimental data. Nevertheless, the current outbreak data also have uncertainties associated with them, and some of the outbreak data points required assumptions to be made. Overall, the dose-response model generated in the current exercise can be used for risk assessment purposes, and generates estimates that are consistent with those that have been observed in outbreaks.

1.6 *SALMONELLA* IN EGGS

For *Salmonella* in eggs, the risk assessment estimates the probability of human illness due to *Salmonella* following the ingestion of a single food serving of internally contaminated shell eggs, either consumed as whole eggs, egg meals, or as ingredients in more complex food (e.g. cake). This work addressed selected aspects of egg production on farms, further processing of eggs into egg products, and retail and consumer egg handling and meal preparation practices.

1.6.1 Exposure assessment

The exposure assessment for *Salmonella* in eggs consists of a production module, a module for the processing and distribution of shell eggs, a module for the processing of egg products, and a module for preparation and consumption. The production module predicts the probability of a *S.* Enteriditis-contaminated egg occurring. The shell egg processing and distribution, and preparation and consumption modules predict the probability of human exposures to various doses of *S.* Enteriditis from contaminated eggs. The model developed combines existing models that have been elaborated at the national level. The output of the exposure assessment, in general, feeds into the hazard characterization to produce the risk

characterization output. This output is the probability of human illness per serving of an egg-containing meal.

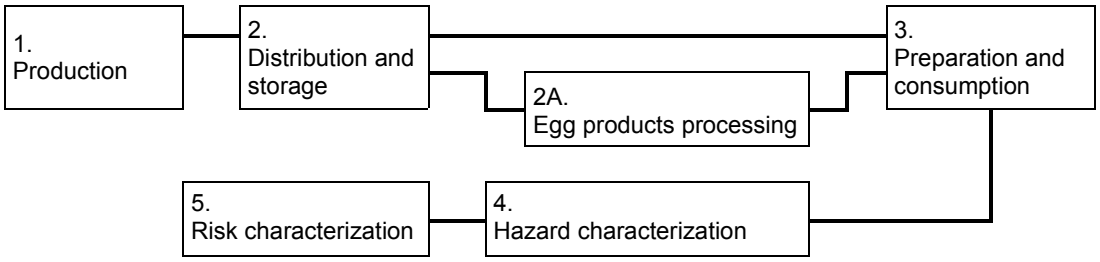


Figure 4. Schematic diagram showing the stages of the risk assessment for *Salmonella* in eggs.

1.6.2 Risk characterization of *Salmonella* in eggs

This risk characterization of *Salmonella* in eggs was intentionally developed so as not to be representative of any specific country or region. However, some model inputs are based on evidence or assumptions derived from specific national situations. Caution is therefore required when extrapolating from this model to other, country-specific situations.

The exposure assessment included consideration of yolk-contaminated eggs and growth of *Salmonella* in eggs prior to processing for egg products. These issues have not been previously addressed by exposure assessments of *Salmonella* in eggs. Yolk-contaminated eggs might allow for more rapid growth of *Salmonella* inside such eggs compared with eggs that are not yolk-contaminated.

The output of the shell egg model is the probability that a serving of an egg dish results in human illness. This probability is determined as the weighted average of all egg servings (contaminated and not contaminated) in a population. Clearly, the risk per serving is variable when we consider individual egg servings (e.g. a serving containing 100 organisms is much more likely to result in illness than a serving containing just a single organism), but the meaningful measure is the population likelihood of illness. This risk per serving can be interpreted as the likelihood of illness given a person consumes a randomly selected serving.

The range in risk of illness predicted by this model extends from at least 0.2 illnesses per million shell egg servings to 4.5 illnesses per million servings. The scenarios considered represent a diversity of situations that approximate some countries or regions in the world. Nevertheless, no specific country is intentionally reflected in this model's inputs or outputs.

Three values for flock prevalence (5%, 25% and 50%) were considered, with three levels of egg storage time and temperature (reduced, baseline and elevated).

The lowest risk of illness is predicted when flock prevalence is 5% and storage times and temperatures are reduced (Table 4). In this scenario, the calculated risk is 2 illnesses in 10 million servings (0.00002%). The highest risk is predicted when flock prevalence is 50% and storage times and temperatures are elevated. In this case, the calculated risk is 4.5 illnesses in each million servings (0.00045%).

Table 4. Predicted probabilities of illness per egg serving, reflecting different flock prevalence settings and egg storage time and temperature scenarios.

Flock prevalence	Time-temperature scenarios		
	Reduced	**Baseline**	**Elevated**
5%	0.00002%	0.00002%	0.00004%
25%	0.00009%	0.00012%	0.00022%
50%	0.00017%	0.00024%	0.00045%

Changes in risk are approximately proportional to changes in the flock prevalence. For example, 5% flock prevalence is one-fifth of 25%. Correspondingly, the risk of illness for scenarios with 5% flock prevalence is one-fifth that of scenarios with 25% flock prevalence. Similarly, doubling flock prevalence from 25% to 50% also approximately doubles the risk of illness, if all other inputs are constant.

Although the degree of change in risk reflects change from baseline conditions, these simulations demonstrate, for example, that changing storage times and temperatures from farm to table implies disproportionately large effects on risk of illness. In addition. the calculated probability of illness per serving can be used to estimate the number of illnesses in a population. For example, a region with 100 egg production flocks of 10 000 hens each could expect about 1300 cases per year.

The output of the egg products model is a distribution of the numbers of *Salmonella* remaining in 4500 litre containers of liquid whole egg following pasteurization. The *Salmonella* considered in this output are only those contributed by internally contaminated eggs. This output serves as a proxy for human health risk until the model is extended to consider distribution, storage, preparation – including additional processing – and consumption of egg products. Figure 5 shows the output for the 25% flock prevalence, baseline scenario. About 97% of the pasteurized lots are estimated to be *S.* Enteritidis-free, and the average level is about 200 *Salmonella* remaining per lot.

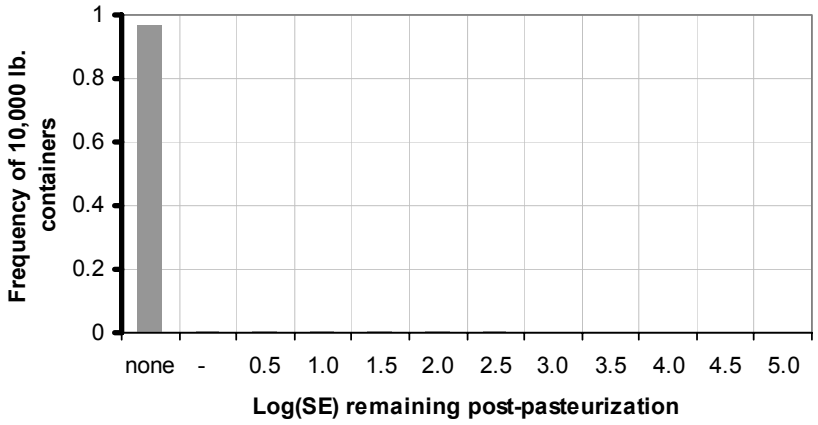


Figure 5. Predicted distribution of *S.* Enteritidis (SE), contributed by internally contaminated eggs, remaining in 4500 litre containers of liquid whole egg after pasteurization. This distribution is predicted based on an assumed 25% flock prevalence and the baseline egg storage times and temperatures used in the model.

The risk of human illness per serving appears to be insensitive to the number of *Salmonella* in contaminated eggs across the range considered at the time of lay. For example, whether it is assumed that all contaminated eggs had an initial number of 10 or 100 *Salmonella* organisms, the predicted risk of illness per serving was similar. This may be because the effect of *Salmonella* growth is greater than the initial contamination level in eggs under the storage conditions considered in this model.

It should be noted that the data available upon which to base this risk assessment was limited. For example, evidence regarding enumeration of the organism within eggs was based on only 63 *Salmonella*-contaminated eggs, and in part on estimates of the concentration of the organism in contaminated eggs. It is difficult to represent uncertainty and variability with such limited data. In addition, neither statistical nor model uncertainty was fully explored.

1.6.3 Discussion

Although this model was deliberately configured and parameterized so that it did not reflect any specific country or region, its results might be indicative of many country situations. A generic risk assessment such as this one provides a starting point for countries that have not developed their own risk assessment. It can serve to identify the data needed to conduct a country-specific risk assessment, as well as to provoke thinking in policy development and analysis.

Control of prevalence, either proportion of flocks infected or proportion of hens that are infected within flocks, has a direct effect in reducing probability of illness per serving. On the whole, egg storage times and temperatures can disproportionately influence the risk of illness per serving. The numbers of organisms initially in eggs at the time of lay seems less important.

Testing flocks, combined with diversion of eggs from positive flocks, is predicted to reduce public health risk substantially. In the scenarios considered, diversion of eggs from test-positive flocks also reduced the apparent risk from egg products. Vaccination might reduce risk of illness by about 75%, but is typically less effective because producers would only vaccinate test-positive flocks.

Biological inputs may be constant between models for different countries or regions, yet little else is likely to be similar. The predictive microbiological inputs, the distribution of within-flock prevalence, and the frequency at which infected hens lay contaminated eggs are examples of biological inputs that might be constant (although not necessarily), and the effect of uncertainty regarding these biological inputs to the model was considered. Nevertheless, there are many aspects of uncertainty not fully considered (e.g. alternative statistical distributions were not evaluated for the predictive microbiology equations or within-flock prevalence distributions). Furthermore, many of the inputs are both highly uncertain and variable within and between countries (e.g. times and temperatures of egg storage may vary considerably). It is difficult for any country to know precisely its distributions for storage times and temperatures.

The model introduces two new concepts not included in previous exposure assessments of *Salmonella* in eggs. First, it considers the possibility of eggs being laid with *Salmonella* already inside the yolk. Such eggs defy previous models' description of the time and temperature dependence of *Salmonella* growth in eggs. Although predicted to be uncommon, yolk-contaminated eggs can support rapid growth of *Salmonella* in much shorter times than eggs contaminated in the albumen.

Second, this model considers the role of *Salmonella* growth in eggs destined for egg products. While most eggs are modelled as being shipped very quickly to egg products plants (i.e. nest run eggs), some eggs can experience moderate or high levels of growth before being broken and pasteurized.

Many of the results generated by this model are contingent on epidemiological assumptions, namely:

- it is assumed that infected hens produce contaminated eggs at a constant frequency that is independent of host, bacterial strain or environmental factors;
- a homogeneous population of layer flocks is assumed (e.g. same size, same basic management and environment). This model also ignores the effect of moulting practices on egg contamination frequency; and
- it is assumed that within-flock prevalence is random and independent of hen age or other host, bacterial strain or environmental factors.

These may be reasonable default assumptions, but more research is needed to determine their appropriateness. Changing these assumptions can generate different results from the model, although the model can be adapted to consider such changes.

1.7 *SALMONELLA* IN BROILER CHICKENS

For *Salmonella* in broiler chickens, the risk characterization estimates the probability of illness in a year due to the ingestion of *Salmonella* originating from fresh whole broiler chicken carcasses with the skin intact and which are cooked in the domestic kitchen for immediate consumption. Due to a lack of suitable data, particularly enumeration data, this work commenced at the conclusion of slaughterhouse processing (i.e. at the end of stage 2 in Figure 6) and considers in-home handling and cooking practices. The effects of pre-slaughter interventions and the slaughter process are not currently included in this model.

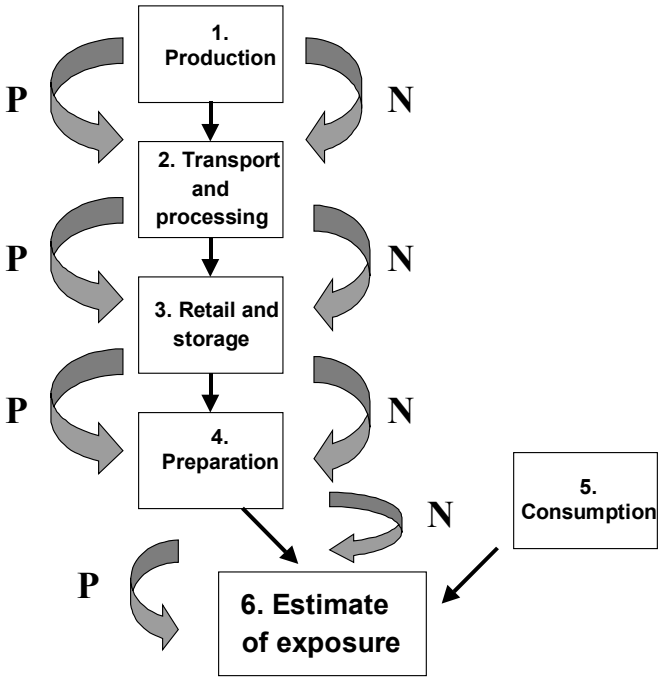


Figure 6. Modular pathway to describe the production-to-consumption pathway for broiler chickens. Each step describes the changes to prevalence (P) and numbers of *Salmonella* (N) that occur within that specific module.

1.7.1 Exposure assessment

The exposure assessment of *Salmonella* in broiler chickens mimics the movement of *Salmonella*-contaminated chickens through the food chain, commencing at the point of completion of the slaughter process. Based on an assumed level of infection, a chicken carcass was allocated an infection status and those carcasses identified as contaminated were assigned a number of *Salmonella* organisms on each iteration of the model, using available data. From this point until consumption, changes in the size of the *Salmonella* population on each contaminated chicken were modelled using equations for growth and death. The growth of *Salmonella* was predicted using inputs for storage time in retail stores, transport time, storage time in homes, and the temperatures the carcass was exposed to during each of these periods. Death of *Salmonella* during cooking was predicted using inputs describing the probability that a carcass was not adequately cooked, the proportion of *Salmonella* organisms attached to areas of the carcass that were protected from heat, the temperature of exposure of protected bacteria and the time for which such exposure occurs. The number of *Salmonella* consumed were then derived using an input defining the weight of chicken meat consumed per serving and the numbers of *Salmonella* cells in meat as defined from the various growth and death processes. Exposure via cross contamination was modelled as well as exposure as a result of consuming undercooked chicken. In particular ingestion of organisms transferred from the raw poultry and onto hands and uncooked food was described using transfer and frequency rates. Outputs from the model relate to exposure via undercooked chicken and exposure via cross-contamination. In both cases, the probability of the event and the numbers of organisms are output.

The exposure assessment is defined in terms of a number of parameters that describe the processes of broiler chicken carcass distribution and storage, preparation, cooking and consumption. Some of these parameters can be considered general in that they can be used to describe the situation in many countries. In contrast, some parameters are country specific, such as the prevalence of carcasses contaminated with *Salmonella* at the completion of processing. Predictions of risk for a particular country are best obtained from data relevant to that country.

It should also be noted that, in the course of the work, efforts were made to identify features that have an impact on the validity of findings and the appropriateness of extrapolating findings to scenarios not explicitly investigated in the risk assessments. These are identified and discussed in the risk assessment document.

1.7.2 Risk characterization of *Salmonella* in broiler chickens

In the risk characterization stage, the outputs from the exposure assessment were combined with the dose-response model to produce two estimates of risk: the risk per serving and the risk via cross-contamination. The risk estimates for probability of illness were first derived using a set prevalence for the presence of *Salmonella* in chilled, raw broiler chickens. At a prevalence of 20% contaminated carcasses (the baseline case), and based on the other model parameters, including the probability that the product would be undercooked, approximately 2% of the broilers prepared for consumption in the home could potentially contain viable cells of *Salmonella*. Figure 6 shows the distribution of average doses (colony-forming units (CFUs)) per serving for contaminated chicken that is subsequently undercooked.

Since the data shown in Figure 7 represent the average dose per serving, the interpretation of values of less than 1 CFU per serving is 1 CFU per multiple servings, e.g., an average dose of 0.01 cells per serving translates to one in 100 servings contains a single cell.

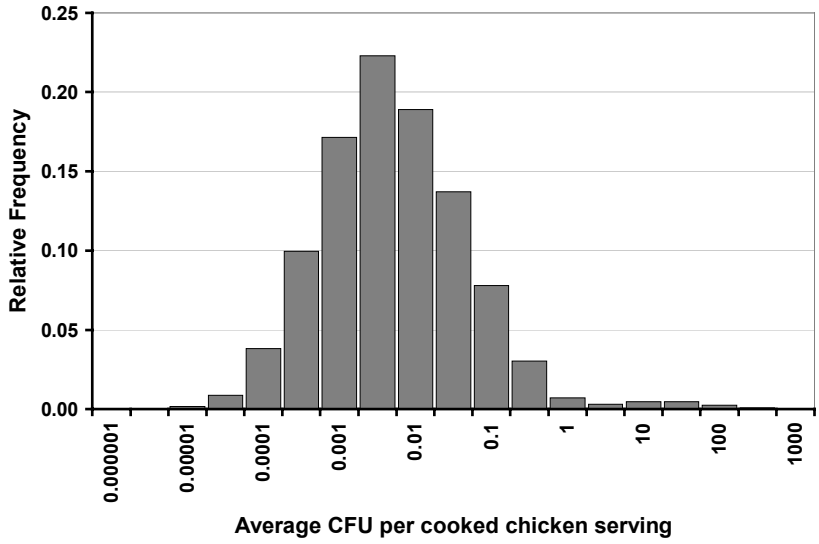


Figure 7. Average dose (colony-forming units (CFUs) of *Salmonella*) per serving in meals prepared from contaminated broiler chickens.

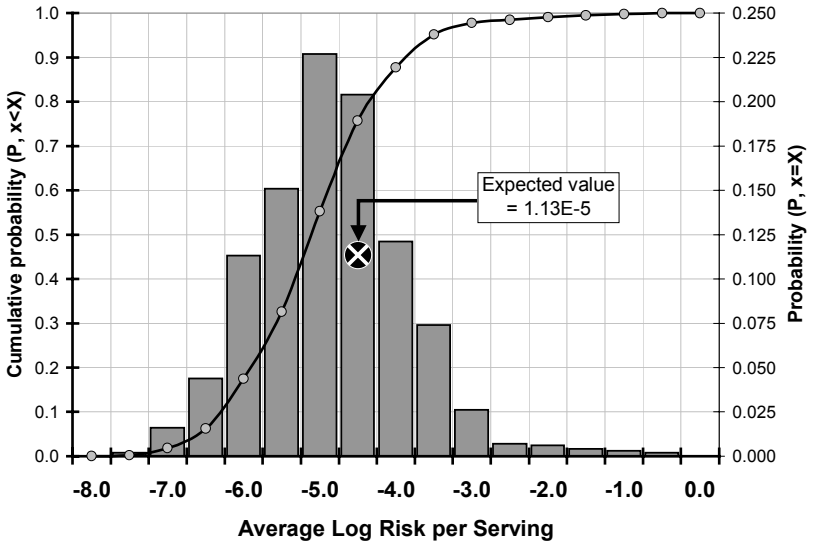


Figure 8. Distribution of average risk per serving.

Assuming a 20% prevalence of contaminated broilers, the estimated frequency and cumulative distribution of average risk per serving are shown in Figure 8. The expected risk per serving is 1.13E-5, or 1.13 illnesses per 100 000 servings. This value represents the average risk for all individuals in the population that consume servings of chicken that are stored, transported and prepared in the manner described in the model, and also accounts for the probabilities that the serving was from a chicken contaminated with *Salmonella*, and that the meal was undercooked. It should be recognized that some individuals consuming a serving on certain occasions would experience a much higher risk than others who might be consuming servings with no salmonellosis risk at all, since the serving would be free of the pathogen.

The expected risk per serving can be extended to the expected risk over multiple servings, such as meals consumed in a year. If it is assumed that the risk posed by one exposure (serving) is statistically independent of any other exposure (serving), then the overall risk of infection following a series of exposures (annual risk) can be estimated from risk of infection per exposure (daily or serving risk). In order to estimate the annual risk of infection, two items of information are required: the risk of infection per serving, and the number of servings consumed in a year. The calculation of annual risk based on the estimated average per serving risk and the assumptions for this baseline scenario are shown in Table 5.

Table 5. Calculation of expected annual risk.

Prevalence of contaminated carcasses	20%
Expected risk per serving	1.13E-05 (1.13 illnesses per 100 000 servings)
Number of servings in year	26
Annual expected risk	2.94E-04 (2.94 illnesses per 10 000 servings)
Rate of illness per 100 000	29.38

Illustrative calculation for annual expected number of illnesses for a country or region with this annual expected risk:

Population	20 000 000
Proportion of population that eats chicken	0.75
Potentially exposed population	15 000 000
Expected number of cases in the year	4406

The assumptions inherent in the calculation in Table 5 are that each of the servings consumed during the year have the same expected risk per serving, and that the risk from each exposure is independent of every other exposure. The annual risk was estimated using the assumption that 26 chicken meals were consumed in a year, i.e. chicken was consumed once every 2 weeks. For illustration, a population risk for 20 million individuals was considered, with 75% of that population eating chicken. In this case, the total expected number of salmonellosis cases arising from the model assumptions is estimated to be 4400, equivalent to a rate of 29 cases per 100 000 population. Obviously these statistics need to be tailored for a specific country or region.

In addition to estimating the risk per serving based on consumption of undercooked poultry, the assessment also modelled the risk from cross-contamination. The sequence and nature of events that need to occur in order for the bacteria on raw chicken to be disseminated and ingested via other pathways is complex and difficult to model completely. There is a lack of information to adequately describe cross-contamination, but it is acknowledged that this is an important route for foodborne illness. The following estimates offer an approximation of the magnitude of the problem, although not all potential pathways that could result in exposure and illness were modelled.

In the baseline scenario, the expected risk from cross-contamination (transfer from raw chicken to hands to non-cooked foods, or from raw chicken to cutting board to non-cooked foods) was estimated to be 6.8E-4, or 6.8 illnesses per 10 000 exposures to contaminated material. This is more than an order of magnitude higher than the expected risk from a serving. This estimate is a function of two factors (conditional probabilities) in the current model: first, the expected risk when the event occurs, and, second, the expected probability that the event occurs.

The expected probability that the event occurs is driven by the prevalence of contamination plus the probability of undercooking in the case of consumption, versus the prevalence of contamination plus the probability of not washing hands or not washing cutting boards in the case of cross-contamination. Given the assumptions made in the model, the expected risk from this cross-contamination pathway is equivalent to approximately 60 chicken consumption exposures. Although the frequency with which people do or do not wash their hands can be debated, the ultimate risk from cross-contamination could in fact be even higher than that estimated here, since there are multiple cross-contamination opportunities in the home preparation environment.

The risk assessment of *Salmonella* in broiler chickens does not consider all parts of the production-to-consumption continuum and this limits the range of control options that can be assessed. This is primarily due to the lack of representative data to analyse how much change in either the prevalence or level of *Salmonella*, or both, in poultry could be attributable to any specific treatment or action. However, the establishment of a baseline model provided a means to compare the effects on risk when prevalence and cell numbers were changed. The model parameters can be modified to evaluate the efficacy of risk mitigation strategies that target those parameters. For example, the parameter describing prevalence of *Salmonella*-contaminated broiler chickens exiting processing can be modified to evaluate the effectiveness of a processing measure such as chlorination of the chilling water to reduce the prevalence of *Salmonella*-contaminated carcasses.

Reduction in the prevalence of *Salmonella*-contaminated chicken was associated with a reduction in the risk of illness. A one-to-one relationship was estimated, with a percentage change in prevalence – assuming everything else remains constant – reducing the expected risk by a similar percentage. For instance, a 50% reduction in the prevalence of contaminated poultry (20% to 10%) produced a 50% reduction in the expected risk of illness per serving. Similarly, a large reduction in prevalence (20% to 0.05%) would produce a 99.75% reduction in the expected risk of illness, a risk reduction that perhaps might be obtained by pre-slaughter risk management actions.

If management strategies are implemented that affect the level of contamination, i.e. the numbers of *Salmonella* on chickens, the relationship to risk of illness is estimated to be greater than a one-to-one relationship. A shift of the distribution of *Salmonella* cell numbers on broiler chickens exiting the chill tank at the end of processing, such that the mean number of cells is reduced by 40% on the non-log scale, reduces the expected risk of illness per serving by approximately 65%.

A small reduction in the frequency of undercooking and the magnitude of the undercooking event results in a marked reduction of the expected risk of illness per serving. The important caveat here is that altering cooking practices does not address the risk of illness through the cross-contamination pathway. Any strategy to change consumer cooking practices needs to be tempered by the fact that cross-contamination may in fact be the predominant source of risk of illness, and it must be remembered that the nature of cross-contamination in the home is still a highly uncertain phenomenon.

1.7.3 Summary and recommendations

To date, no full exposure assessments have been undertaken of *Salmonella* in broiler chickens. This report has therefore considered:

- What is required for undertaking such assessments.
- What information is available.
- How the available information meets the requirements.
- Development of a general model using the available data that meets the specified requirements.

The following recommendations are made for directing future work:

(i) Reporting of prevalence at different steps of the full exposure pathway should be encouraged in all regions of the world.

(ii) Reported data should give full details of study methodology, including sampling site, sampling time, how the sample relates to the overall population, and microbiological methods.

(iii) Determination of quantitative data should be encouraged and, if it becomes available, then full exposure assessments could be developed to investigate mitigation strategies (e.g. use of chlorine in chill water) or to compare alternative practices (e.g. air chilling versus immersion chilling).

(iv) Cross-contamination during processing and handling operations should be studied quantitatively, and methodologies for modelling this process developed. Cross-contamination during these stages is a critical factor, which is often associated with outbreaks.

(v) At the national level, the collection of consumption data should be promoted. The design of such studies should accommodate the data requirements for exposure assessments. These requirements include population variability, portion size, and frequency of consumption.

(vi) In predictive microbiology, the area of survival has been less well studied than growth or death. There are few predictive models that describe survival at chill and frozen temperatures, and so further development of such models is essential.

1.8 OVERALL KEY FINDINGS

One of the important outcomes of the risk assessment work was the compilation and collation of a wealth of information on *Salmonella* and broiler chickens, and *Salmonella* associated with eggs. The organization of these data in the structured risk assessment format has led to the identification of significant gaps in the existing data. This provides a guide for future research work to help ensure that such research targets the generation and collection of the most useful and relevant data.

In general the following conclusions could be drawn from the hazard characterization work.

- Existing dose-response models for *Salmonella* were found to characterize inadequately the dose-response relationship observed in the outbreak data.
- The new dose-response model derived from outbreak data is considered to be the best available estimate for the probability of illness upon ingestion of a dose of *Salmonella*. The outbreak data is an extremely useful body of real-life data. Nevertheless, the outbreak data has uncertainties. In particular, the number of people exposed and the exposure dose were not always known with complete certainty. In addition, the outbreak data came from only two countries – Japan and the United States of America.

- The outbreak data did not provide evidence to conclude that the dose of *Salmonella* causing illness is different from the dose of other *Salmonella* serotypes.
- The outbreak database did not reveal an increased risk of illness in children under five years of age compared with the rest of the population exposed to *Salmonella*. The database may lack sufficient power to reveal the existence of true differences that might exist.

2. RESPONSES TO THE QUESTIONS ASKED BY THE CODEX **COMMITTEE ON FOOD HYGIENE**

The Codex Committee on Food Hygiene (CCFH) requested that the expert group provide answers to the questions shown below, in Tables 6 and 7.

Table 6. Risk management questions for *Salmonella* in eggs

1.1 Estimate the risk from *Salmonella* in eggs in the general population and in the various susceptible populations (e.g. elderly, children, immunocompromised patients) at various prevalence and concentration levels of *Salmonella* in contaminated eggs.
1.2 Estimate the change in risk likely to occur from each of the interventions below, including their efficacy.
1.2.1 Reduce the prevalence of positive flocks (Destroy positive breeding or laying flocks, or both; Vaccinate egg laying flocks for *Salmonella*; Competitive exclusion).
1.2.2 Reduce the prevalence of *Salmonella*-positive eggs (Test and divert eggs from positive flocks to pasteurization).
1.2.3 Reduce the number of *Salmonella* organisms in eggs (Heat treatment of egg products; Refrigeration of eggs after lay and during distribution; Requirement for a specific shelf life for eggs stored at ambient temperatures).

Table 7. Risk management questions for *Salmonella* in broiler chickens

2.1 Estimate the risk from pathogenic *Salmonella* in broiler chickens for the general population and for various susceptible population groups (elderly, children and immunocompromised patients) consequent to a range of levels in raw poultry.
2.2 Estimate the change in risk likely to occur for each of the interventions under consideration (see below), including their efficacy.
2.2.1 Reduction in the prevalence of positive flocks (Destruction of positive breeder and chicken (broiler) flocks; Vaccination of breeding flocks; Competitive exclusion (e.g. with *S.* Sofia)).
2.2.2 Reduction in the prevalence of *Salmonella*-positive birds at the end of slaughter and processing (Use of chlorine in water chilling of chicken (broilers); Water chilling versus air chilling for chicken (broilers)).
2.2.3 Evaluation of the importance of various routes by which pathogenic *Salmonella* are introduced into flocks, including through feed, replacement birds, vectors and hygiene.
2.2.4 The impact on risk of change in consumer behaviour (not part of the questions asked by CCCFH but addressed by the Risk Assessment).

Question 1.1 – Estimate the risk from *Salmonella* in eggs in the general population and in the various susceptible populations (e.g. elderly, children or immuno-compromised patients) at various prevalence and concentration levels of *Salmonella* in contaminated eggs

The model was used to estimate the relative effects of different prevalence and concentration levels of *Salmonella* in contaminated eggs. Prevalence can either be the proportion of flocks containing one or more infected hens (i.e. flock prevalence) or the proportion of infected hens within infected flocks (i.e. within-flock prevalence). The risk associated with different flock prevalence levels is illustrated in Table 4. One can also examine the risk of illness per serving for different within-flock prevalence levels, as well as for different starting concentrations of *Salmonella* per egg.

To model the effect of within-flock prevalence on risk, the 1st, 50th, and 99th percentiles of the within-flock prevalence distribution (0.1%, 0.5%, and 22.3%, respectively) were simulated (Figure 9). Flock prevalence was 25% for these simulations. In the baseline time–temperature scenario, risk of illness per serving was 6×10^{-8} (6 per 100 million) 3×10^{-7} (3 per 10 million) and 1×10^{-5} (1 per 100 000) for within-flock prevalence of 0.1%, 0.5% and 22.3%, respectively. The results show that a change in within-flock prevalence will lead to a directly proportional change in the risk of illness per serving. Consequently, risk per serving from a flock whose within-flock prevalence is 10% (i.e. 10 in every 100 hens is infected) poses 100 times the risk to humans relative to a flock whose within-flock prevalence is 0.1% (i.e. one in every 1000 hens is infected).

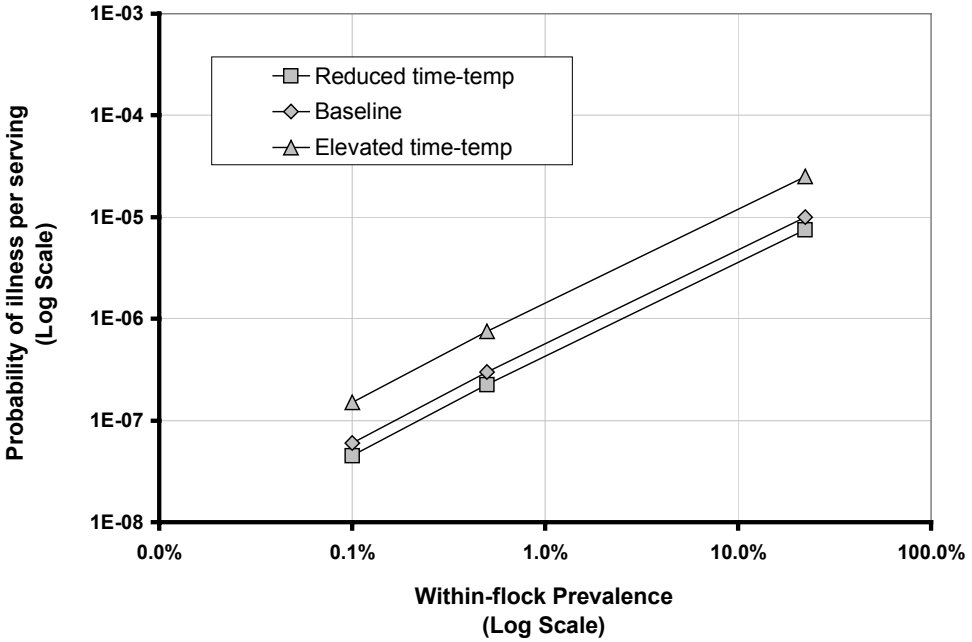


Figure 9. Predicted probability of illness, assuming that within-flock prevalence is either 0.1%, 0.5% or 22.3% (1st, 50th or 99th percentiles of the lognormal distribution used in the model, respectively). Three egg storage time and temperature scenarios are considered. Flock prevalence is assumed to be 25%.

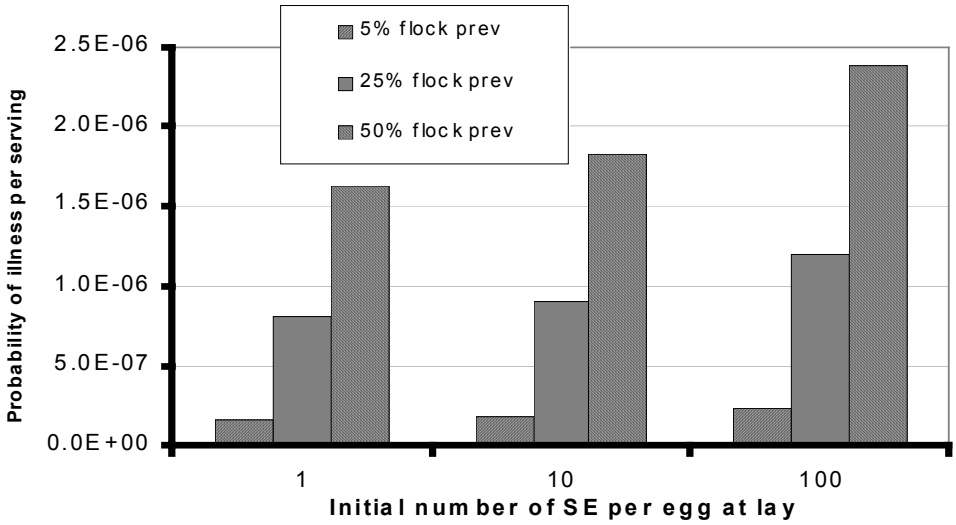


Figure 10. Predicted probability of illness per serving assuming that the number of *Salmonella* per contaminated egg at lay is 1, 10 or 100. Three flock prevalence levels are considered. Egg storage times and temperatures are assumed to be the baseline settings.

The impact of different initial levels of *Salmonella* in eggs at the time of lay have on the probability of illness per serving, assuming that all contaminated eggs started with 1, 10 or 100 organisms are showed on Figure 10. The baseline egg storage time and temperature scenario was assumed, but flock prevalence was varied. For a flock prevalence of 5%, risk per serving was about 2 per 10 million, regardless of whether the initial number of *Salmonella* per egg was 1, 10 or 100. For flock prevalence levels of 25% and 50%, a more detectable change in risk per serving occurs between eggs initially contaminated with 1, 10 or 100 *Salmonella*. For example, at 25% flock prevalence, the risk per serving increases from 8 per 10 million to 10 per 10 million as the number of *Salmonella* in eggs at lay increases from 1 to 100. Nevertheless, for one-log changes in the initial numbers of *Salmonella*, the resulting change in probability of illness is much less than one log.

The dose-response function used in this risk characterization predicts that the probability of illness given an average dose of 1, 10 or 100 organisms is 0.2%, 2.2% or 13%, respectively. If all contaminated eggs were consumed raw immediately after lay, one would expect these probabilities to be appropriate to predict illnesses. The production module predicts that contaminated eggs are produced at a frequency of about 5×10^{-5} (~1 in 20 000) when flock prevalence is 25%. If all contaminated eggs contained just one organism and there was no growth or decline before consumption, the predicted risk per serving should be 1 in 10 million Similarly, the risk per serving if all eggs were contaminated with 10 and 100 organisms is 10^{-6} (1 in 1 million) and $\sim 7 \times 10^{-6}$ (7 in 1 million), respectively.

The dose of Salmonella ingested and the attack rates for children under five years of age were compared with the rest of the population exposed in order to compare susceptible and normal populations. The database did not reveal an increased risk of illness in children under five year of age compared with the rest of the population exposed to salmonella. The database may lack sufficient power to reveal true differences that might exist.

Questions 1.2 – Estimate the change in risk likely to occur from each of the interventions, including their efficacy (1.2.1 reduce the prevalence of positive flocks; 1.2.2 reduce the prevalence of *Salmonella*-positive eggs; 1.2.3 reduce the number of *Salmonella* organisms in eggs).

As shown earlier, risk of illness per serving decreases as the percentage of infected flocks (i.e. flock prevalence) decreases. Table 8 illustrates the influence of flock prevalence on risk of illness per serving. Because the model includes uncertain inputs, risk per serving is also uncertain, and this table summarizes uncertainty as the mean, 5th and 95th percentile values (rounded to the nearest significant digit) of the predicted distribution.

Table 8. Predicted uncertainty in risk of illness per egg serving for different flock prevalence levels

Flock prevalence	Mean	5th	95th
0.01%	0.00000005%	0.00000002%	0.00000009%
0.10%	0.0000005%	0.0000002%	0.0000009%
5.00%	0.00002%	0.00001%	0.00004%
25.00%	0.0001%	0.0001%	0.0002%
50.00%	0.0002%	0.0001%	0.0005%

The results in Table 8 can be used to predict the reduction in risk for a country or region that decides to control infected flocks. For example, consider a country with 5% of its flocks containing one or more infected hens. If such a country were to institute a programme (for example destruction of positive flocks) with 98% effectiveness in reducing flock prevalence, then successful implementation of the programme would result in a flock prevalence of about 0.1%. The model predicts, in this case, that the mean risk of illness per egg serving would decrease from 2 per 10 million to 5 per 1000 million. Pre-harvest interventions such as those used in Sweden (destruction of positive flocks) and other countries might result in flock prevalence levels of 0.1% or lower.

Although the model predicts that probability of illness per serving is proportional to flock prevalence, the question remains: how can one reduce prevalence of infected flocks. To accomplish this, one seemingly needs to either prevent uninfected flocks from becoming infected, or to treat infected flocks to render them uninfected.

Treatment of breeding flocks to render them uninfected has been used in The Netherlands (Edel, 1994). Antibiotic treatment of the flock followed by administration of a competitive exclusion culture might succeed in eliminating the organism from infected hens, but environmental reservoirs might still exist to re-infect hens once the effects of the antibiotic have worn off. Furthermore, application of this method to commercial flocks may be neither feasible nor economic.

Preventing uninfected flocks from becoming infected is where most attention is focused in control programmes. Uninfected flocks can become infected via vertical transmission (i.e. eggs infected before hatch result in exposure of a cohort via horizontal transmission following hatching), via feed contamination, or via environmental sources (i.e. carry-over infection from previously infected flocks). Control programmes may attempt to eliminate these avenues of exposure by:

(i) Testing breeding flocks to detect *Salmonella* infection, followed by destruction of the flock, if infected, to prevent it from infecting commercial flocks through its future offspring.
(ii) Requiring heat treatment of feed before sale (thereby eliminating *Salmonella* and other pathogens from chicken feed).
(iii) Intense cleaning and disinfecting of known-contaminated poultry environments after removing an infected flock. Such an approach must also eliminate potential reservoirs (e.g. rodents).

Most control programmes use all three interventions to prevent *Salmonella* infection of flocks. The control programme in Sweden consists of such an approach (Engvall and Anderson, 1999). The Pennsylvania Egg Quality Assurance Program in the United States of America also used such an approach (Schlosser et al., 1999). However, discerning the efficacy of each intervention is difficult. Ideally, one would like to know what percentage of newly infected flocks result from vertical transmission, or feed contamination, or from previously contaminated environments.

Giessen et al. (1994) presented a model for determining the relative contribution of risk of infection from vertical, feed-borne (or other outside environmental sources), and carry-over environmental contamination. Comparing the model to data collected in The Netherlands, it appears that carry-over infection was the dominant contributor to risk of infection. The conclusion was based on the shape of a cumulative frequency curve for flock infection, which suggests that most flocks are infected soon after placement in commercial facilities. There is also evidence that the prevalence of infected breeder flocks is very low in The Netherlands.

Data from the United States of America *Salmonella* Pilot Project (Schlosser et al., 1999) suggest a fairly constant prevalence by age, and that infection did not necessarily increase over time. Nevertheless, these data do not describe the age when infection was introduced. Roughly 60% of the poultry flocks tested in this project were *S.* Enteritidis-positive. Additional evidence presented shows that 6 of 79 pullet flocks (8%) tested were *S.* Enteritidis-positive. These data suggest that the risk of infection from vertical transmission might be about 8%. Furthermore, there is little suspicion that feed contamination is an important source of *Salmonella* for United States of America poultry flocks.

The data from The Netherlands and the United States of America suggest that the carry-over route may account for >80% of the risk of flock infection in countries where *Salmonella* is endemic. If true, then complete control of breeder flocks might only be expected to achieve ≥20% reduction in the prevalence of *Salmonella* infected flocks in such countries.

Results of an aggressive monitoring programme of breeder flocks in The Netherlands between 1989 and 1992 have been reported (Edel, 1994). For egg-sector breeding flocks, there is some suggestion that prevalence of infected flocks was reduced by ~50% per year. Effectiveness was less dramatic for meat-sector breeding flocks. This programme involved regular faecal testing of all breeder flocks, as well as regular testing of hatchery samples from day-old chicks. Positive flocks were depopulated until mid-1992, when treatment with enrofloxacin and a competitive exclusion culture was allowed as an alternative to the expense of prematurely depopulating a breeding flock. If a programme with 50% effectiveness in reducing prevalence of infected flocks each year were implemented for 3 years, one might

predict that prevalence would be about 12% (0.5^3) of the prevalence at the start of the programme.

To reduce the risk of carry-over infection for commercial flocks, it is thought that aggressive cleaning and disinfection must be completed after an infected flock is removed and before another flock is placed to begin a new production cycle. Cleaning and disinfection must also include an effective long-term rodent control programme. Analysis of efforts in Pennsylvania to reduce the prevalence of infected commercial flocks suggest a decline from 38% to 13% during three years of programme operation (White et al., 1997). This programme routinely screened flocks for evidence of *Salmonella* and required thorough cleaning, disinfection and rodent control once positive flocks were removed. Another study in Pennsylvania (Schlosser et al., 1999) found 16 of 34 (47%) poultry environments that were initially *S.* Enteritidis-positive were negative for the pathogen following cleaning and disinfection of the environment.

The effectiveness of "test and divert" programmes depends on the specific testing used in commercial flocks. For example, Sweden collected three pooled samples, each consisting of 30 faecal droppings, during two or more examinations of egg production flocks during each production cycle (Engvall and Anderson, 1999). In their breeder-flock monitoring programme, The Netherlands' testing protocol collects two pools of 50 caecal droppings each, every 4 to 9 weeks of production (Edel, 1994). The *Salmonella* Pilot Project's protocol in the United States of America required collection of swabs from each manure bank and egg belt in a hen house on three occasions during each production cycle (Schlosser et al., 1999).

Regardless of the size or type of sample collected, it would seem that a testing protocol that examines commercial flocks frequently and diverts eggs soon after detection should result in a meaningful reduction in the number of contaminated shell eggs marketed each year.

To examine the effect of a "test and divert" programme using the present model, two protocols were assumed, with either one or three tests administered to the entire population of egg production flocks. The single test is administered at the beginning of egg production. Under the three-test regime, testing at the beginning of egg production is followed by a second test four months later, and the third administered just before the flock is depopulated. Each single test consists of 90 faecal samples randomly collected from each flock. A flock is considered positive if one or more samples contained *S.* Enteritidis.

For the within-flock prevalence distribution used in this model, a single test of 90 faecal samples was likely to detect 44% of infected flocks. This was calculated using an equation which assumed that an infected hen shed sufficient *S.* Enteritidis in her faeces to be detected using standard laboratory methods.

If a flock tested positive (i.e. one or more samples were positive for *S.* Enteritidis), its entire egg production was diverted to pasteurization. It was assumed that the egg products industry normally uses 30% of all egg production (consistent with the United States of America industry). Therefore, eggs going to breaker plants from flocks other than those mandatorily diverted were adjusted to maintain an overall frequency of 30% (i.e. the percentage of eggs sent to breaker plants from test-negative infected flocks and from non-infected flocks was reduced proportionally).

The premises of flocks found test-positive were assumed to be cleaned and disinfected following flock removal. The effectiveness of cleaning and disinfection in preventing re-infection of the subsequent flock was assumed to be 50%. Furthermore, it was assumed that carry-over infection was responsible for flocks becoming infected. Consequently, houses that were not effectively cleaned and disinfected resulted in infected flocks when they were repopulated.

Assuming a starting prevalence of 25% and the baseline egg storage time and temperature scenario, the effectiveness of the two testing protocols was estimated over a four-year period. The probability of illness per shell egg serving for each year was calculated for each protocol (Figure 11). Testing three times per year for four years reduced the risk of human illness from shell eggs by more than 90%. Testing once a year for four years reduced risk by over 70%. At the end of the fourth year, the flock prevalence for the one-test and three-test protocols had fallen to 7% and 2%, respectively. Therefore, assuming the cost of testing three times per year is three times that of testing once a year (ignoring producer costs or market effects from diversion of eggs), then the change in flock prevalence suggests a roughly proportional difference (e.g., $7\% \div 2\% \approx 3$) in the protocols. Nevertheless, the reduction in risk per serving of the one-test protocol is greater than one-third of the three-test protocol. In other words, the one-test protocol achieves a 70% reduction in risk of human illness, while a testing protocol that is three times more costly achieves a 90% reduction. Such a result is not surprising when we consider that a single test at the beginning of the production year most substantially affects risk, as flocks detected on the first test have their eggs diverted for the entire year, while flocks detected on a second test have their eggs diverted for just over half the year. Furthermore, flocks detected on the third test are tested so late in production that diversion of their eggs does not influence the population risk at all.

While egg diversion from positive flocks reduces the public health risk from shell eggs, it might be expected that there is some increased risk from egg products. Mandatory diversion causes more contaminated eggs to be sent to pasteurization. Nevertheless, the average quality of contaminated eggs is improved by diversion in this model.

It was assumed in the model that all diverted eggs were nest run (i.e. stored usually less than 2 days). Without mandatory diversion, 97% of lots were *S.* Enteritidis-free post-pasteurization and the average number of surviving *Salmonella* in a 4500 litre bulk tank was 200 (assuming 25% flock prevalence and the baseline egg storage for time and temperature scenario in the model). If a single test is used to determine which flocks are diverted, there are still 97% of vats that are *S.* Enteritidis-free and they average 140 *Salmonella* per lot. The decrease in the average number of *Salmonella* per lot is due to the increased proportion of nest run eggs that are diverted. Nest run eggs are stored for a shorter period of time and, consequently, contribute fewer organisms. If two tests are used, then there are 97% of vats that are *S.* Enteritidis-free and the average is 130 per lot. If three tests are used, there is no additional effect on egg products beyond the second test because the third test occurs just as the flock is going out of production.

Although not a direct measure of public health risk, these egg products results suggest that the risk from egg products decreases as flocks are detected and diverted. However, this effect is conditional on nest run eggs being substantially less contaminated than restricted or graded eggs. Alternative scenarios to the one considered here may result in some increase in risk from diversion.

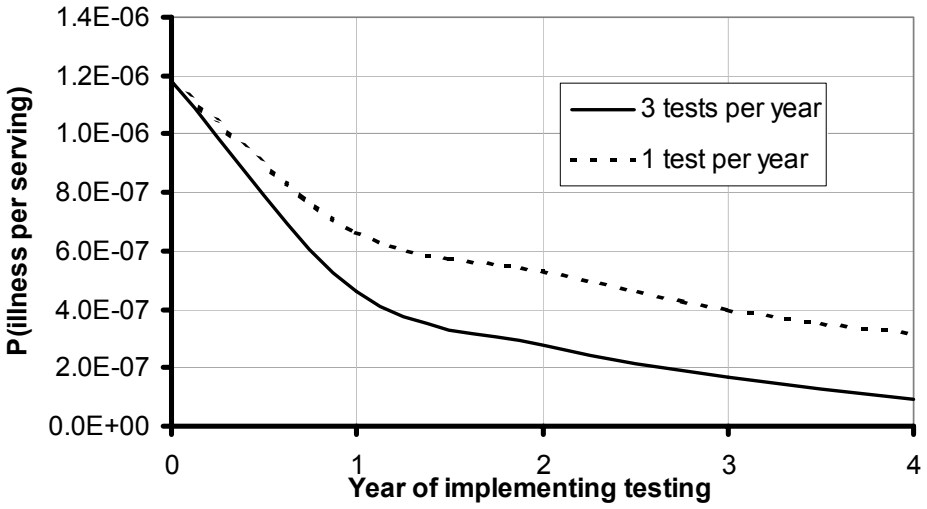


Figure 11. Predicted probability of illness per serving from shell eggs per year following implementation of two testing protocols. It is assumed that all flocks in the region are tested each time. Beginning flock prevalence is assumed 25%. The baseline egg storage time and temperature scenario is used for the four years.

Vaccination for *Salmonella* has been examined extensively in experimental settings, but less so in field trials. Experimentally, several types of vaccines have been evaluated: killed bacterins of various strains, live bacterins of attenuated strains, and surface antigen extracts of various strains. Injected killed bacterins are thought to have limited efficacy in preventing intestinal colonization of hens with *S.* Enteritidis, although such bacterins may reduce internal organ (including ovary) infection via stimulation of humoral antibody. Live bacterins – or surface antigen vaccines – may be more effective at modulating intestinal colonization by *Salmonella* because these products may elicit the cell-mediated immune response needed to resist colonization. Nevertheless, most commercially available vaccines are currently of the killed variety.

Evidence used for this model concerning the effectiveness of *Salmonella* bacterins in controlling infection was from a report for some flocks in Pennsylvania in the United Satates of America (Schlosser et al., 1999). A group of 19 flocks from two farms used a bacterin to control their *Salmonella* infection, and sampling results were compared with 51 flocks that did not use a bacterin. Only a slight difference was noted in environmentally positive samples collected in vaccinated (12%) and unvaccinated (16%) flocks. However, the overall prevalence of *S.* Enteritidis-positive eggs was 0.37 per 10 000 in vaccinated flocks against 1.5 per 10 000 in unvaccinated flocks. These results support the hypothesis that bacterins may not influence risk of colonization, but may reduce systemic invasion of *S.* Enteritidis, and reduce resultant egg contamination. This analysis did not control for confounding factors (e.g. rodent control, adequacy of cleaning and disinfection) that may have influenced the differences between vaccinated and unvaccinated flocks.

To evaluate the effect of vaccination against *Salmonella* using the present model, it was assumed that flocks would need to be tested to determine their status prior to use of a vaccine. A single test, or two tests four months apart, with 90 faecal samples per test, was assumed. The vaccine was assumed to be capable of reducing the frequency of contaminated eggs by

approximately 75% (e.g. 0.37 per 10 000 for vaccinated flocks ÷ 1.5 per 10 000 for non-vaccinated flocks).

Assuming 25% flock prevalence and the baseline egg storage time and temperature scenario, the probability of illness per serving for a single test and vaccination protocol is about 70% of a non-vaccination protocol (Figure 12). Risk is reduced to 60% of the non-vaccination protocol if two tests are applied.

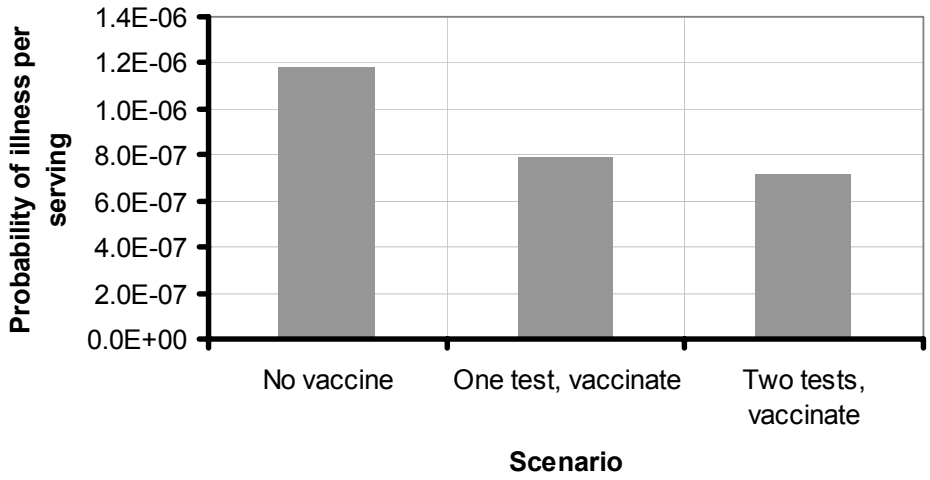


Figure 12. Comparison of predicted probability of illness per serving between scenarios when no vaccination is used; when one test is applied at the beginning of production and positive flocks are all vaccinated; and when a second test is applied four months after the first test and additional test-positive flocks are vaccinated. Flock prevalence is assumed to be 25%, and the baseline egg storage time and temperature scenario is used.

Given the efficacy of bacterin use based on the field evidence, one could assume that universal vaccination might reduce the baseline risk to 25% of the risk resulting from a non-vaccinated population. However, the cost of vaccinating the entire population of laying hens could be high. The scenarios considered here assume that some testing is done first to determine if a flock is infected before that flock is vaccinated. Nevertheless, the cost of testing all flocks must be weighed against the cost of vaccination. Also, more field research concerning the true efficacy of vaccination should be conducted before the cost of vaccination is borne by more than a few producers (i.e. if costs are to be paid by the public or shared across the entire industry).

The effects of competitive exclusion (CE) treatment are difficult to quantify from field evidence. Sweden and The Netherlands for example include the use of CE in their *Salmonella* control programmes. Nevertheless, such treatment is only one component of these programmes and its effect is not clearly separable from other components. Competitive exclusion has been studied in experimental settings for newly hatched chicks. The intent of CE inoculation in chicks is to quickly establish an indigenous intestinal flora that resists *Salmonella* colonization. Efficacy of preventing infection appears to depend on the CE culture used, timing of exposure, dose of exposure, and possibly the addition of lactose (Corrier and Nisbet, 1999). Field evidence of CE efficacy in mature hens comes from the United Kingdom and The Netherlands. In both countries, antibiotic treatment was applied to

flocks known to be infected and the hens were subsequently inoculated with CE cultures. The intent of CE inoculation for hens was to quickly restore intestinal flora – destroyed by the antibiotic treatment – to assist the hens in resisting future *Salmonella* exposures. In the United Kingdom, 20 of 22 trials that combined antibiotic and CE treatments succeeded in preventing re-infection of flocks for a 3-month study period (Corrier and Nisbet, 1999). Infection status was determined from cloacal swab samples in treated flocks. In The Netherlands, combining antibiotic and CE treatments resulted in preventing 72% (n=32) of flocks becoming re-infected. Two such combined treatments prevented re-infection in 93% of flocks.

Interventions intended to minimize the dose of *Salmonella* in contaminated eggs focus on preventing any growth of the pathogen after the egg is laid. Most evidence suggests that naturally-contaminated eggs contain very few *Salmonella* organisms at lay. If eggs are consumed soon after lay, or eggs are kept refrigerated during storage, then the number of *Salmonella* is relatively unchanged prior to preparation of egg-containing meals.

Available predictive microbiology models suggest that eggs stored at 10°C will not grow *Salmonella* for 46 days on average. If most eggs are stored at <10°C and are consumed within 25 days, then interventions intended to improve egg handling will only be influential on the fraction of eggs that are time–temperature abused.

The effect of mandatory retail storage times and temperatures using slightly different baseline assumptions was evaluated. These hypothetical settings used might be typical in a country that does not have egg refrigeration requirements. The effects of time and temperature restrictions were evaluated assuming a flock prevalence of 25%.

Truncating retail storage time to a maximum of either 14 days or 7 days simulated a shelf-life restriction scenario. Truncating the retail storage temperature to less than 7.7°C simulated a refrigeration requirement. The results are summarized in Figure 13.

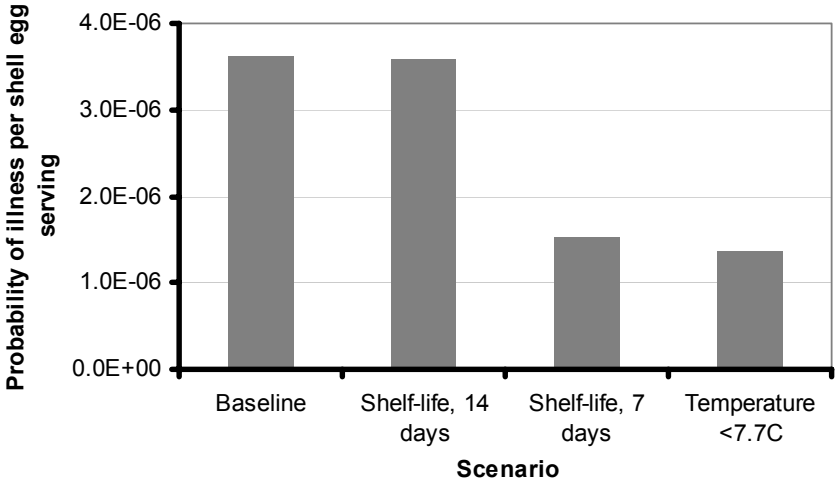


Figure 13. Probability of illness per serving of shell eggs given a mandatory shelf-life of <14 or <7 days at retail, or mandatory retail storage temperature <7.7°C. Egg storage times and temperatures are modelled as the in baseline scenario, except for changes introduced to represent a country or region that does not routinely refrigerate eggs. Flock prevalence was assumed to be 25%.

Restricting shelf-life to less than 14 days reduced the predicted risk of illness per serving by a negligible amount (~1%). However, keeping retail storage temperature at no more than 7.7°C reduced risk of illness per serving by about 60%. Were shelf-life to be reduced to 7 days, risk per serving would also be reduced by about 60%.

Figure 14 compares these predicted risks – when no growth or cooking is assumed – with the predictions shown in Figure 10 for 25% flock prevalence. When just a single *Salmonella* organism is in contaminated eggs, Figure 14 implies that allowing growth inside eggs elevates the risk. Yet, when contaminated eggs contain 10 or 100 organisms, Figure 14 implies that cooking of egg meals substantially reduces the risk. The explanation for these findings is that regardless of the initial contamination, the combined effect of growth and cooking is to stabilize the risk per serving to nearly one per million. It can be concluded from Figures 10 and 14 that the model's output is relatively less sensitive to initial numbers of *Salmonella* than other inputs that influence growth and cooking.

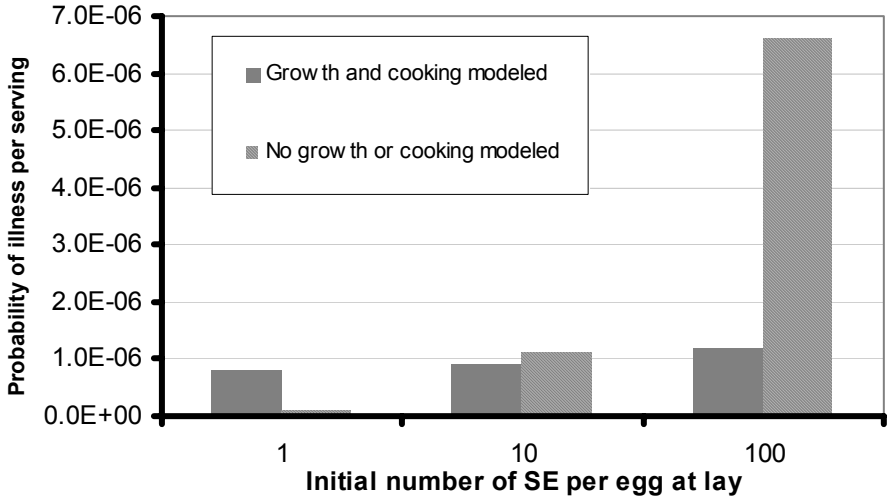


Figure 14. Comparison of predicted risk of illness when exposure assessment model includes effects of growth and cooking, versus when no growth or cooking is modelled, for cases where the initial number of *Salmonella* (SE) in contaminated eggs at lay is 1, 10 or 100. Flock prevalence is assumed to be 25%, and baseline egg storage times and temperatures are assumed when growth and cooking are modelled.

Question 2.1 – Estimate the risk from *Salmonella* in broiler chickens for the general population and for various susceptible populations (e.g. elderly, children or immuno-compromised patients) consequent to a range of levels in raw poultry and question 2.2.1 - reduce the prevalence of positive flocks.

The questions concerning on-farm interventions could not be evaluated due to lack of representative data. It was nevertheless estimated that a reduction in the concentration of

infected birds leaving processing would reduce the risk of illness per serving at least proportionally. The expert group found the available data on the importance of various routes for introduction of *Salmonella* into flocks, including feed, replacement birds, vectors and hygiene. It was not possible, therefore, to evaluate the importance of on-farm routes of introduction of *Salmonella*. Also, the need was identified for better understanding of cross-contamination processes in all the steps in the production chain.

A change in the prevalence of contaminated raw product affects the risk to the consumer by altering the frequency of exposure to risk events, i.e. exposure to the pathogen. The change in risk as a result of a change in the prevalence of *Salmonella*-contaminated broilers was estimated by simulating the model using a range of initial prevalence levels. Seven different prevalence levels were investigated: 0.05%, 1%, 5%, 10%, 20%, 50% and 90%. If the prevalence of contaminated chickens leaving processing is altered, through some management practice either at the farm level or at the processing level, the expected risk per serving is altered. The magnitude of the changes in risk per serving and risk per cross-contamination event as a result of changes in prevalence are summarized in Table 9.

Table 9. Impact on risk. Following change in prevalence

	Prevalence						
	0.05%	**1.0%**	**5.0%**	**10.0%**	**20.0%**	**50.0%**	**90.0%**
Consumption							
Expected risk per serving *	2.81E-08	5.63E-07	2.81E-06	5.63E-06	1.13E-05	2.81E-05	5.07E-05
Number of servings	26	26	26	26	26	26	26
Annual expected risk	7.32E-07	1.46E-05	7.32E-05	1.46E-04	2.93E-04	7.31E-04	1.32E-03
Rate of illness per 100 000	0.07	1.46	7.32	14.63	29.26	73.14	131.61
Calculation of expected number of cases in the year based on assumed population size and exposed population							
Population			20 000 000				
Proportion of population that eats chicken			0.75				
Potentially exposed population			15 000 000				
Expected number of cases in the year	11	219	1 097	2 195	4 389	10 970	19 741
Cross-contamination							
Expected risk per event	1.70E-06	3.41E-05	1.70E-04	3.41E-04	6.81E-04	1.70E-03	3.07E-03

* 2.81E-08 can also be expressed as 2.81 cases per 100 million servings. Similarly for the other risks expressed. E-07 is …per 10 million; E-06 is …per million; E-05 is …per 100 000; etc…

A reduction of 50% in the number of cases of salmonellosis was estimated if a 20% contamination rate at the retail level was reduced to 10% contamination. The relationship between a percentage change in prevalence and expected risk is largely a linear one. Assuming everything else remains constant, it can be expected to reduce the expected risk by the same percentage.

Questions 2.2 - Estimate the change in risk likely to occur from each of the interventions, including their efficacy (2.2.2 Reduction in the prevalence of *Salmonella*-positive birds at the end of slaughter and processing ; and 2.2.3 evaluation of the importance of various route by which pathogenic *Salmonella* are introduced into flocks.

The effectiveness of specific mitigation interventions, either on-farm or as treatments during processing, were not evaluated in the present risk model because lack of representative data precluded analysis of changes in either or both prevalence and level of contamination that might be attributable to a specific intervention. However, the influence of reducing prevalence can be interpreted, although with a high degree of uncertainty given the current state of knowledge, in the context of chlorine addition to the chill tanks during processing. There is little evidence that the addition of chlorine at levels of 50 ppm or less actually decreases the numbers of the pathogen attached to the skin of poultry carcasses. However, available data suggest that chlorine prevents an increase in the prevalence of contaminated carcasses, i.e. a reduction in cross-contamination (Table 10), while one study observed a substantial reduction in prevalence. In Table 10, the factor in the last column is a ratio of the prevalence after chilling to the prevalence before chilling. A ratio greater than 1 indicates an increase in prevalence of contaminated carcasses.

Table 10. Experimental data for effects of chlorine on *Salmonella* prevalence after immersion chill tank.

Source	Amount	Prevalence before chilling			Prevalence after chilling			Ratio[(1)]
		Total	Positive	Prevalence	Total	Positive	Prevalence	
				With Chlorine				
[1]	20–50 ppm (tank)	48	48	100%	103	60	58%	0.58
[2]	4–9 ppm (overflow)	50	21	42%	50	23	46%	1.10
[3]	1–5 ppm (overflow)?	90	18	20%	90	17	19%	0.94
[4]	15–50 ppm (tank)	48	4	8%	96	7	7%	0.88
								0.87
				Without Chlorine				
[5]	–	160	77	48%	158	114	72%	1.50
[6]	–	99	28	28%	49	24	49%	1.73
[7]	–	40	5	13%	40	11	28%	2.20
[7]	–	40	4	10%	40	15	38%	3.75
[7]	–	84	12	14%	84	31	37%	2.58
[8]	–	60	2	3%	120	18	15%	4.50
								2.71

NOTES: (1) Ratio of prevalence after chilling to prevalence before chilling. A ratio >1 indicates an increase in prevalence of contaminated carcasses.

DATA SOURCES: [1] Izat et al., 1989. [2] James et al., 1992a. [3] Cason et al., 1997. [4] Campbell 1983. [5] James et al., 1992a. [6] James et al., 1992a. [7] Lillard, 1980. [8] Campbell, 1983.

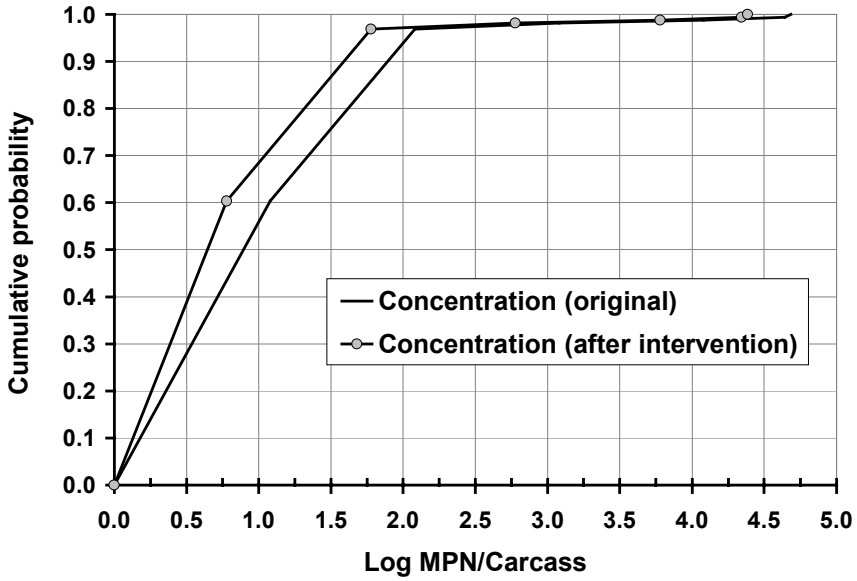


Figure 15. Original and post-intervention concentration distributions.

The effect of reducing the numbers of *Salmonella* on poultry carcasses without changing the prevalence of contaminated carcasses was also assessed, although not specifically noted in the CCFH list of questions. The values of the post-intervention concentration distributions compared to the baseline scenario were reduced by 50% (approximately 0.3 logMPN per carcass; Figure 15). The model was run using the reduced level of contamination while maintaining the prevalence at 20% and with no changes in any of the other parameters. Figure 16 compares the per-serving risk estimates for the modified simulation representing an intervention with the original data representing the baseline situation.

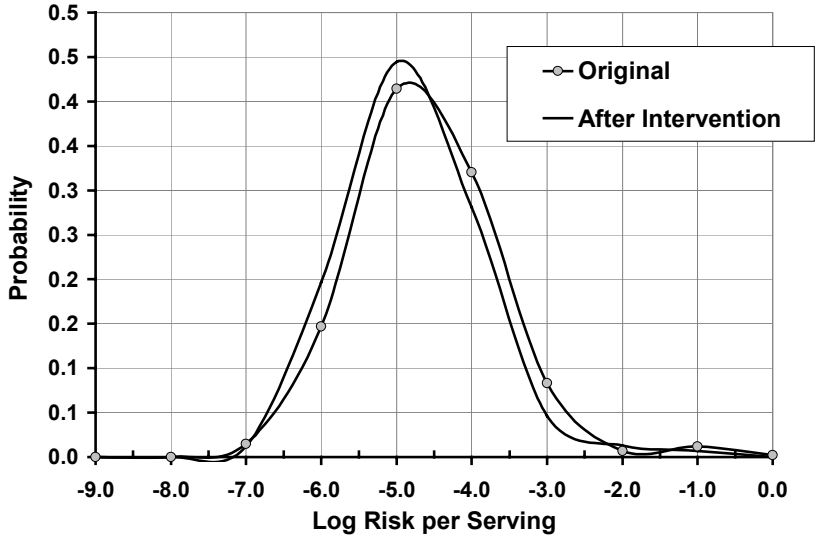


Figure 16. Risk per serving distribution before and after intervention to change concentration.

Unlike a change in prevalence, a change in concentration of the pathogen does not necessarily have a linear relationship with the risk outcome. The distribution of risk shown in Figure 16, is the risk per serving when contaminated. The servings were estimated to be contaminated and potentially undercooked approximately 2% of the time. That statistic remains unchanged even if the level of contamination is reduced.

The expected risk per serving, which incorporates the prevalence of contaminated servings and the probability of undercooking, was estimated to be 11.3 illnesses per million servings in the original case, and 4.28 per million servings in the situation when the level of contamination is reduced. The expected risk per serving is therefore reduced by approximately 62%. A summary of the results is shown in Table 11.

The risk from cross-contamination events is also affected when the level of contamination is reduced.

Table 11. Risk summary before and after intervention to change concentration.

	Original	**After Intervention**
Prevalence	20%	20%
Expected risk per serving	1.13 per 100 000	4.28 per million
Number of servings in year	26	26
Annual expected risk	2.94 per 10 000	1.11per 10 000
Rate of illness per 100 000	29	11
Illustrative calculation for annual expected number of illnesses for a country/region with this annual expected risk		
Population	20 000 000	20 000 000
Proportion of population that eats chicken	0.75	0.75
Potentially exposed population	15 000 000	15 000 000
Expected number of cases in the year	4406	1670

The data available were inconclusive concerning the importance of the various routes by which pathogenic *Salmonella* are introduced into flocks – including through feed, replacement birds, vectors and poor hygiene. Interpretations of existing studies and results are confounded because of the number of different sampling protocols, specimen types and laboratory methods, as well as the nature of poultry-rearing operations (e.g. very large versus very small premises; types of waterers or feeders). For these reasons, it was not possible to evaluate the importance of on-farm routes of introduction of *Salmonella*, and this stage was not incorporated into the risk assessment.

Question 2.2.4 – Change in consumer behaviour and its effect on risk (not asked by CCFH)

The consumer represents the final intervention in mitigating risk. However, the effectiveness of strategies aimed at changing consumer behaviour is difficult to anticipate, and difficult to measure. However, for the purposes of this assessment, the potential impact on risk resulting from modifying food preparation practices was investigated by running the simulation assuming the implementation of a strategy that changes consumer behaviour. The assumed changes were:

– probability that product is not adequately cooked:

(OLD):	Min = 5%,	Most likely = 10%,	Max = 15%
(NEW):	Min = 0%,	Most likely = 5%,	Max = 10%

– exposure time (minutes):

(OLD):	Min = 0.5,	Most likely = 1.0,	Max = 1.5
(NEW):	Min = 1.0,	Most likely = 1.5,	Max = 2.0

The changes are thus assumed to reduce the probability of the consumer not adequately cooking the food, and, for those that do tend to undercook, the degree to which they undercook is less.

If the simulation model is re-run with these assumptions, the expected risk is reduced from 11.3 per million, to 2.2 per million. As a result, the changes in consumer practices reduce the expected risk per serving by almost 80%. The changes in consumer practices have an impact on the frequency with which a potentially contaminated product remains contaminated prior to consumption (probability of undercooking) and also reduces the risk when the potentially contaminated product reaches the consumer (longer cooking time). The distribution of risk per serving before and after the intervention is shown in Figure 17.

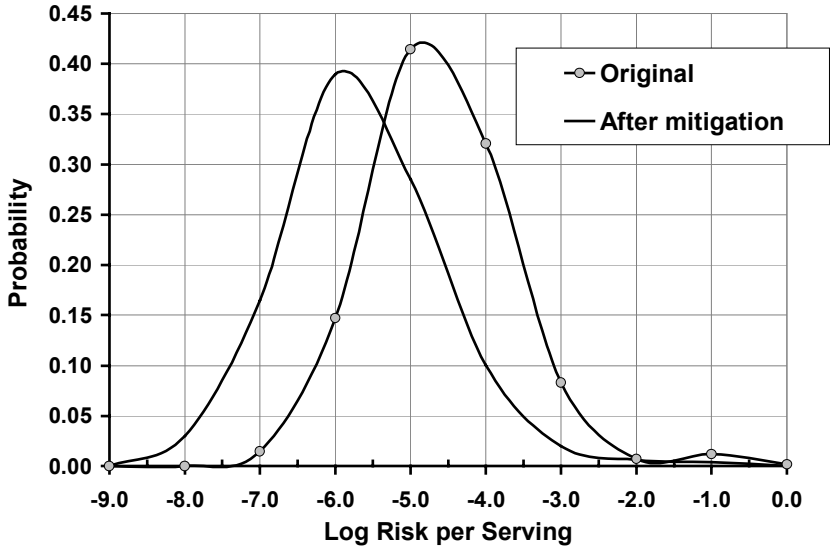


Figure 17. Risk distribution per serving before and after intervention to alter consumer behaviour.

It is important to note that the mitigation strategy to alter cooking practices does not address the risk associated with cross-contamination. In the baseline scenario, the expected risk per cross-contamination event was shown to be much larger than the risk from consumption of under-cooked chicken. As a result, the strategy to change the consumers cooking practices needs to be tempered by the fact that cross-contamination may in fact be the predominant source of risk, and that the nature of cross-contamination in the home is still a highly uncertain phenomenon.

3. DATA GAPS AND FUTURE RESEARCH NEEDS

One of the important outcomes of the risk assessment work was the compilation and collation of a wealth of information on *Salmonella* in eggs and broiler chickens. The organization of these data in the structured risk assessment format allowed the identification of significant gaps that exist in the data. This can guide future research work and help ensure that it focuses on generating and collecting the most useful and relevant data. These data and research needs are outlined below.

3.1 Hazard characterization

In order to improve hazard characterization, additional outbreak and epidemiological data are needed. More specifically these data should indicate cell number in the implicated food, amount of food consumed, accurate estimates of the size of ill and exposed populations, and accurate characterization of the population, including age profiles, medical status, sex and other potential susceptibility factors.

The impact of the food matrix was not incorporated into the hazard characterization due to the limitations of available data. Hence, characterization and quantification are needed of the impact of food matrix effects and also host-pathogen interactions and virulence factors, and their effect on the probability of infection, with or without illness, so that these issues can be more completely addressed in future work. Quantitative information to facilitate estimating the probability of developing sequelae following illness is also required.

As this is a developing science, the optimal models have not yet been developed. Therefore, new dose-response models that improve our ability to estimate the probability of illness would be useful.

3.2 Data for exposure assessment in general

Quantitative modelling of the individual exposure steps requires quantitative information. Data can be collected from a number of sources including, but not limited to,

- national surveillance data,
- epidemiological surveys,
- industrial surveys,
- research publications,
- unpublished research work, and
- government reports.

Often these data are publicly available, appearing, for example, in the published literature. However, other data, such as those collected through industry surveys, are often confidential and thus access becomes difficult. It is vital that confidence be built up between risk managers, assessors and those who can provide valuable data for risk assessment. Confidence building requires discussions and meetings (interactive risk communication) to discuss the type of data needed and for what the data are being used (the risk management

activity). In addition, discussions provide insight into the data and how it has been generated, e.g. sampling strategy, testing methods, etc. Such insight can be important to ensure correct modelling and thus final results. Overall, good communications among all parties is essential.

In certain cases, adequate data may not be available. One way of dealing with this is to use expert opinion. Use of expert opinion introduces several considerations, such as how to choose experts, how to avoid biased judgement, how to elicit information and how to combine information from different experts. For further information of this area of study, see Kahneman, Slovic and Tversky (1982) and Vose (2000).

In risk assessment, and particularly in the development of generic models (i.e. for application in decision-making in general commodity production, processing, distribution and consumption management), data often come from many different sources. Two issues arise from this: first, what data to include within the model, and, second, how to combine such information. Determining what data to include involves considering applicability (e.g. Are the data relevant for a particular country? Are the data representative of the existing situation? Were scientifically and statistically sound sampling and testing methods used in the collection of the data?) Furthermore, regardless of the data selection criteria, the rationale and process for selection must be transparent. The importance of transparency is also emphasized for combining data. Various methodologies, including weighting of information, can be used, but the assessor must clearly set out the methodology to ensure clarity and reproducibility.

Overall, data collection is probably the most resource-intensive part of the modelling of exposure and involves many issues that influence the quality of the risk assessment outcome.

3.3 Exposure assessment of *S.* Enteriditis in eggs

Data relating to the biology of *Salmonella* in eggs is needed. This need is seemingly universal in its application to previous and future exposure assessments.

Additional studies on the numbers, and the factors that influence the survival and growth, of *Salmonella* in naturally (yolk-) contaminated intact shell eggs are needed, as information is currently available for only 63 intact shell eggs. Enumeration data of *Salmonella* in raw liquid egg are also required. Additional data concerning the numbers of *Salmonella* in raw liquid egg before pasteurization would assist in reliably predicting the effectiveness of any regulatory standard concerning egg products.

More data on the prevalence of *Salmonella* in breeder and pullet flocks and the environment, as well as in feedstuffs, is needed to adequately assess the benefit of pre-harvest interventions. In particular, associations between the occurrence of *Salmonella* in these pre-harvest steps and its occurrence in commercial layers should be quantified.

Better data on time and temperature, specifically in relation to egg storage and then preparation and cooking, would serve to build confidence in the modelling results. The importance of time and temperature distributions in predicting growth of *Salmonella* in eggs, combined with the lack of reliable data to describe these distributions, highlights the need for such data. Furthermore, new studies are needed on the relationship between cooking time, cooking method and cooking temperature and the death of *S.* Enteritidis.

More studies are needed on the survival and growth of *Salmonella* in eggs, particularly as a function of egg composition and the attributes of infecting strains (e.g. heat sensitivity).

3.4 Exposure assessment of *Salmonella* in broiler chickens

The lack of good quality data, prior to the end of processing in particular, limited the scope of this exposure assessment. In relation to primary production, the information available was mainly prevalence data, but for some regions of the world, including Africa, Asia and South America, even that was limited. In addition, information was lacking on study design, specificity or sensitivity of the analytical methodologies used. Very few quantitative data were available. A similar situation was observed for the processing stage. In addition, data tended to be old, and knowledge of processing practices was not readily available. In order to address these deficiencies, the areas where data collection and research efforts need to focus are identified below.

- Prevalence data of *Salmonella* in broilers during production and at slaughter, and on carcasses post processing, together with information on study design, are needed for many regions of the world.
- Microbial ecology studies are needed to determine sources and numbers of the pathogen.
- Studies are needed on the correlation between within-flock prevalence levels and the number of *Salmonella* cells either shed in faeces or found on birds.
- Precise estimates are needed of the numbers of organisms per bird for all stages of the exposure pathway, coupled with improvements in sensitivity, and availability of cost-effective methods to enumerate small populations of *Salmonella.*
- Between-bird (bird-to-bird) cross-contamination data are needed in a form suitable for modelling this phenomenon at the pre-harvest, transport and processing stages.
- Data are required on the survival of *Salmonella* under chilling and freezing conditions. These data should improve the predictive microbiology component of exposure assessments relevant to international trade in poultry products.
- Specific consumption data and information about food preparation practices are needed for most geographical locations, preferably presented in terms of portion size and frequency of consumption rather than average consumption per day.
- Information on the distribution of time and temperature for storage and cooking in domestic kitchens in a variety of national environments.
- Data on the magnitude of cross-contamination in the domestic kitchen, and the pathways for such cross-contamination.

If an attempt were made to extend the risk assessment to more fully assess pre-slaughter interventions, then more data would also be required on the prevalence of *Salmonella* in feed and replacement stock, and fasting prior to slaughter. Data on the effect of scalding, de-feathering, evisceration, washing and chilling processes, as well as other decontamination treatments, are needed to effectively model the benefits of control interventions at the processing level.

4. REFERENCES CITED

Campbell, D.F., Johnston, R.W., Campbell, G.S., McClain, D., & Macaluso, J.F. 1983. The microbiology of raw, eviscerated chickens: a ten-year comparison. *Poultry Science*, **62**: 437-444.

Cason, J.A., Bailey, J.S., Stern, N.J., Whittemore, A.D., & Cox, N.A. 1997. Relationship between aerobic bacteria, salmonellae and Campylobacter on broiler carcasses. *Poultry Science*, **76**(7): 1037-1041.

Corrier, D.E., & Nisbet, D.J. 1999. Competitive exclusion in the control of *Salmonella* enterica serovar Enteritidis infection in laying poultry. *In:* A.M. Saeed, R.K. Gast and M.E. Potter (eds). Salmonella enterica *serovar enteritidis in humans and animals: Epidemiology, pathogenesis, and control*. Ames, Iowa IA: Iowa State University Press.

Edel, W. 1994. *Salmonella* Enteritidis eradication programme in poultry breeder flocks in The Netherlands. *International Journal of Food Microbiology*, **21**: 171–178.

Engvall, A., & Anderson, Y. 1999. Control of *Salmonella enterica* serovar Enteritidis in Sweden. *In:* A.M. Saeed, R.K. Gast and M.E. Potter (eds). Salmonella enterica *serovar enteritidis in humans and animals: Epidemiology, pathogenesis, and control*. Ames, Iowa IA: Iowa State University Press.

Giessen, A.W. van de, Ament, A.J.H.A., & Notermans, S.H.W. 1994. Intervention strategies for *Salmonella* Enteritidis in poultry flocks: a basic approach. *International Journal of Food Microbiology*, **21**(1-2): 145–154.

Izat, A., Druggers, C., Colberg, M., Reiber, M., & Adams, M. 1989. Comparison of the DNA probe to culture methods for the detection of *Salmonella* on poultry carcasses and processing waters. *Journal of Food Protection*, **52**: 564–570.

James, W.O., Brewer, R.L., Prucha, J.C., Williams, W.O., & Parham, D.R. 1992a. Effects of chlorination of chill water on the bacteriologic profile of raw chicken carcasses and giblets. *Journal of the American Veterinary Medical Association*, **200**: 60–63.

Kahneman, D., Slovic, P., Tversky, A. 1982. *Judgement under uncertainty: Heuristics and biases.* New York, NY: Cambridge University Press.

Lillard, H.S. 1980. Effect on broiler carcasses and water of treating chilled water with chlorine and chlorine dioxide. *Poultry Science*, **59**: 1761–1766.

Mead, P.S., Slutsker, L., Dietz, V., McCraig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., & Tauxe, R.V. 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases*, **5**: 607–625.

Schlosser, W.D., Henzler, D.J., Mason, J., Kradel, D., Shipman, L., Trock, S., Hurd, S.H., Hogue, A.T., Sischo, W., & Ebel, E.D. 1999. The *Salmonella* enterica serovar Enteritidis Pilot Project. p.353-365, *In:* A.M. Saeed, R.K. Gast and M.E. Potter (eds). Salmonella enterica *serovar enteritidis in humans and animals: Epidemiology, pathogenesis, and control*. Ames, Iowa IA: Iowa State University Press.

Thorns, C.J. 2000. Bacterial food-borne zoonoses. *Revenue scientifique et technique Office international des epizooties*, **19**(1): 226–239.

Vose, D. 2000. *Risk analysis: a quantitative guide*. Chichester, UK: John Wiley & Sons.

White, P.L., Schlosser, W., Benson, C.E., Madox, C., & Hogue, A. 1997. Environmental survey by manure drag sampling for *Salmonella* Enteritidis in chicken layer houses. *Journal of Food Protection*, **60**: 1189–1193.

5. THE APPLICATION OF MICROBIOLOGICAL RISK ASSESSMENT

Quantitative microbiological risk assessment is intended to answer specific questions of importance to public health. For microbiological risk assessment to deliver benefits it needs to be purposefully incorporated into the decision making process. This implies a change in the way nations approach food safety and public health decisions. The novelty of microbiological risk assessment is that it quantifies the hazard throughout the food production chain and directly links this to the probability of food-borne disease. The risk assessments of *Salmonella* in eggs and broiler chickens present an example of the potential of this approach.

The increased use of microbiological risk assessment will result in new capacity building needs. The exercise of producing this risk assessments has been a learning experience and since it is comprehensive, it can also provide a basis for future training efforts and applied research. These risk assessments are a resource that can be used by many parties including the Codex Alimentarius and national authorities. Ensuring their applicability and utility to all regions and countries is a priority for future work in FAO and WHO.

An important prerequisite for microbiological risk assessment is the need for an interdisciplinary approach. There is a dual need to develop the capacity for microbiological risk assessment skills and expertise within all the relevant disciplines (microbiology, modelling, epidemiology, etc.) and to ensure that these disciplines become effectively integrated into the risk assessment process. Transparency must be maintained throughout the risk assessment process from the initial stages of building the risk assessment team, to data collection and analysis.

This exercise in conducting risk assessment at the international level has underlined the need for data to be acquired from all regions and for the development of countries' capacities to conduct risk assessments. The development of these capacities requires an infrastructure for the surveillance of food-borne disease and the monitoring of microbial hazards in foods throughout the food-chain and the effect of processing and other factors on the micro-organism. It also requires human resources with the technical skills needed to conduct microbiological risk assessment.

There is a considerable amount of useful information made available through these risk assessments for both risk assessors and risk managers. The concepts presented are generic, and may be directly adaptable or considered as stand-alone modules. For those planning to undertake a quantitative microbiological risk assessment the models developed can be used as a template for undertaking risk assessment for these pathogen-commodity combinations at regional or national levels. The data used in the models, however, must reflect the food item, raw material, manufacture, retail conditions, and consumption habits as well as the characteristics of the population within the region under consideration.

These *Salmonella* risk assessments provide information that may be useful in determining the impact that intervention strategies have on reducing cases of salmonellosis from contaminated eggs and poultry. This information is of particular interest to the Codex Alimentarius in their work on the elaboration of standards, guidelines and related texts.

Furthermore, in undertaking this work a number of lessons were learned with regard to making optimal use of risk assessment as a decision support tool. In order to meet the needs of risk managers, the risk assessment must be clearly focused. This can be achieved by adequate planning, good communication and a strong interface between the risk assessors and the risk managers. To ensure that risk assessment contributes to management decisions that can be successfully implemented, there needs to be communication from the outset with other relevant stakeholders such as the food industry and consumers.

In conclusion, the risk assessments provide an example of a format for organising the available information in a readable way, and connecting pathogen contamination problems in food with human health outcomes. They provide scientific advice and analysis that may be useful for establishing regulatory policies for control of foodborne disease in different countries. In addition, the risk assessment process has identified important data gaps, and includes recommendations for future research, which can be used to allocate resources to priority areas.

These are the first microbiological risk assessments to be undertaken at the international level. During the course of the work it was recognized that MRA is still a developing science, yet, every effort has been made to provide a valuable and unique resource for those undertaking risk assessments and addressing the problems associated with *Salmonella* in eggs and broiler chickens.