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Risk Assessment: *Salmonella* spp. in broilers and eggs

- Preliminary Report-

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Hazard Identification and Hazard Characterization of *Salmonella* in broilers and eggs

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SALMONELLA IN BROILERS AND EGGS HAZARD IDENTIFICATION AND HAZARD CHARACTERIZATION

Final Draft

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EXECUTIVE SUMMARY

Introduction

This document focuses on evaluating the nature of the adverse health effects associated with foodborne non-typhoid and non-paratyphoid *Salmonella* spp. and how to quantitatively assess the relationship between the magnitude of the foodborne exposure and the likelihood of adverse health effects occurring.

Objectives

The objective and scope of the *Salmonella* Hazard Characterization document is to provide:

- A review of the characteristics of the host, organism and food matrix.
- A summary and review of available data and information on adverse health effects.
- A summary and evaluation of existing dose-response models with respect to assumptions, sources of uncertainty, strengths and limitations.
- A description of the use of available outbreak data to evaluate published dose-response models.
- An evaluation of the outbreak data for evidence of a difference between susceptible and normal populations and between *Salmonella* Enteritidis and other strains.

Approach

Information was compiled from published literature and from unpublished data submitted to FAO/WHO by public health agencies and other interested parties. The first section of the document provides a description of the public health outcomes, pathogen characteristics, host characteristics, and food-related factors that may affect the survival of *Salmonella* in the human gastrointestinal tract.

The second section of the hazard characterization document presents a review of the background and rationale for different models that have been reported and used to estimate the dose-response relationship of *Salmonella*. These models mathematically describe the relationship between the numbers of organisms that might be present in a food and consumed (dose), and the human health outcome (response). There are three different models for salmonellosis that have been published or reported: the USDA-FSIS-FDA *Salmonella* Enteritidis model, the Health Canada *Salmonella* Enteritidis model, and a beta-Poisson model fit to human feeding trial data for various *Salmonella* species.

An extensive review of available outbreak data was also conducted, and data appropriate for dose-response estimations were summarized. The dose-response curves reviewed were then compared with the outbreak data to equate the model with observed information. Where

possible, the outbreak data were also used to characterize the differences that may exist between the potential for infection in susceptible and in normal segments of the population. Finally, the outbreak data were used to estimate additional dose-response models.

Overall, the document on "Hazard identification and hazard characterization of *Salmonella* in broilers and eggs" provides a summary of a vast amount of literature available on this subject.

Key findings

In most people, the gastroenteritis lasts 4 - 7 days and patients fully recover without medical treatment. However, some people may develop more severe illness, including potentially fatal infections of the bloodstream or other parts of the body, or long-term syndromes such as reactive arthritis and Reiter's syndrome.

Clinical manifestations of *Salmonella* infections in animals generally differ from the typical gastroenteritis and other sequelae produced in humans, therefore, extrapolations of disease in animals to disease in humans must be done with great caution.

In the case of *Salmonella*, unlike most other bacterial pathogens, there is a reasonable amount of human data. As a result, it was felt that the inclusion of additional information from animal data may contribute to increasing the uncertainty rather than improving the dose-response relationship.

Insight into the potential for some segments of the population to be more susceptible to *Salmonella* infection than others was provided by data extracted from two outbreaks. Assuming children under 5 years of age represented a more susceptible population, it was estimated that at the doses observed in these outbreaks (approximately 2 and 4 log CFU/g), the susceptible population was 1.8 to 2.3 times more likely to get ill.

A review of currently available outbreak data did not produce any evidence to support the hypothesis that *Salmonella* Enteritidis has a higher likelihood of causing illness upon ingestion than a similar dose of another serovar.

The outbreak data indicate that the dose-response relationship (or infectivity/pathogenicity) for all non-typhoid and non-paratyphoid *Salmonella* spp. are similar and could theoretically be characterized using a common model. Specifically, the epidemiological data does not offer any evidence to conclude that different serotypes are more or less pathogenic than others.

Complete outbreak data are sparse and important information for the calculation of dose-response assessments is often missing from outbreak reports. In particular, enumeration of organisms in the implicated food vehicle is frequently not carried out in many outbreak investigations. Valuable data for this report was provided by Japan[*], where since 1997, all large

[*] In accordance with Japanese notification released on March 1997, large scale catering facilities (> 750 meals per day or > 300 dishes of a single menu) have been advised to save food for future examination in the case of illness being associated with the food. Fifty gram aliquots of each raw food material and cooked dish should be saved for a minimum of 2 weeks at temperatures below –20 ºC. Although this notification is not

foodservice establishments have been advised to keep frozen portions of prepared foods for a minimum of 2 weeks for subsequent testing if illness is associated with the food. These data allowed significant insights to be made into the hazard characterization of *Salmonella*.

Five models are summarized below and in Figure 5.1. Three models are published or documented in official reports and two new models were generated from the collected outbreak data. They are:

i. Naïve human feeding trial data beta-Poisson model
The model suffers from the nature of the feeding trial data (i.e. the subjects used were healthy male volunteers) and may not reflect the population at large. The model tends to greatly underestimate the probability of illness as observed in the outbreak data, even if the assumption is made that infection, as measured in the dose-response curve will equate to illness.

ii. USDA-FSIS-FDA *Salmonella* Enteritidis beta-Poisson model
The model uses human feeding trial data for *Shigella dysenteriae* as a surrogate pathogen with illness as the measured endpoint in the data. The appropriateness of using *Shigella* as a surrogate for *Salmonella* is questionable given the nature of the organisms in relation to infectivity and disease. Compared to the outbreak data, and on a purely empirical basis, this curve does tend to capture the upper range of these data.

iii. Health Canada *Salmonella* Enteritidis beta-Poisson model
To date this model has not been fully documented and lacks transparency. The model uses data from many different bacterial pathogen-feeding trials and combines this information with key *Salmonella* outbreak data using Bayesian techniques. Using data from many bacterial feeding trials and the current lack of transparency is a point of caution. Empirically, the curve describes the outbreak data at the low dose well but tends towards the lower range of response at higher doses.

iv. Outbreak data exponential model
The exponential model fit to the outbreak data does not produce a statistically significant fit. The curve does provide an adequate description of the data at the mid- and high-dose ranges, however, it underestimates the low-dose observed data.

v. Outbreak data beta-Poisson model
Similar to the exponential function, the beta-Poisson model when fit to the outbreak data does not produce a statistically significant fit. The curve does produce an adequate characterization of the observed data in the low to mid-dose range. The low-dose range of the dose-response relationship is an especially important area.

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mandatory, it is also applicable to smaller catering facilities with social responsibility such as those in schools, day-care centres, and other child-welfare and social-welfare facilities. Some local governments also have regulations relating to food saving, but the required duration and the temperature of storage vary.

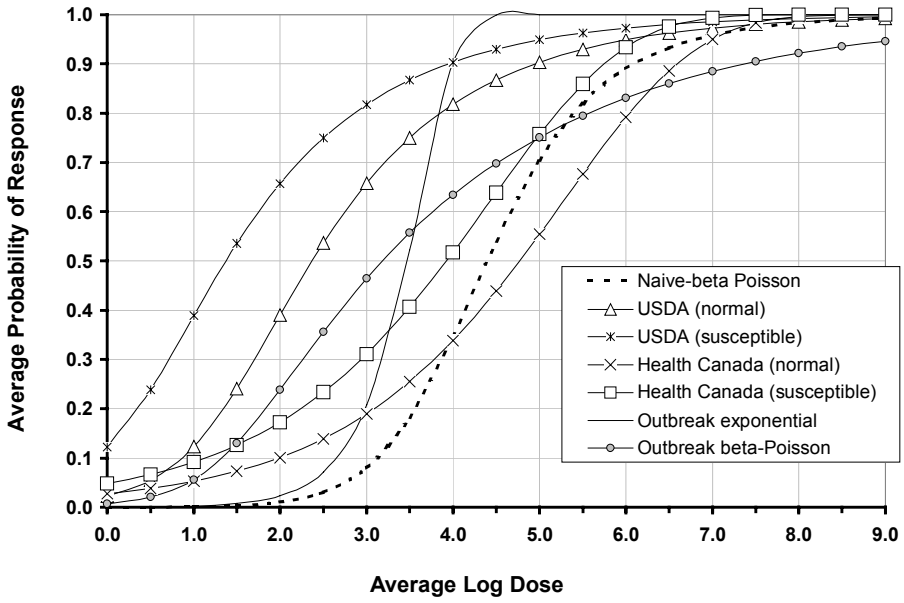


Figure 5.1: Comparison of *Salmonella* dose-response models.

NOTE: The points on the curves do not represent data points and are used only for legend purposes.

Gaps in the data

- Outbreak and epidemiological data, specifically indicating: concentration in the implicated food, amount of food consumed, accurate numbers on ill and exposed populations, accurate characterization of the population including age profiles, medical status, sex and other potential susceptibility factors.
- Quantitative data measuring the impact of the food matrix effects on the probability of infection.
- Quantitative information to facilitate estimating the probability of developing sequelae following illness.
- Characterization and quantification of the relationship between susceptibility factors and increased likelihood of infection.

Conclusions

The derivation of any one of the models is based on many assumptions, such as the use of *S. dysenteriae* or other surrogates for *Salmonella*, combining results of feeding studies for different pathogens, the relevance of infection versus illness as endpoints, and the study design and health status of the test subjects in the human feeding trials. The outbreak data revealed several uncertainties and several assumptions had to be made to derive some of the outbreak estimates subsequently used to fit new dose-response curves.

At present, a single model representation for the relationship between dose and response can not be highlighted as vastly superior to any other model. Compared to the reported outbreak data, the naïve beta-Poisson model is the least desirable since it vastly underestimates the probability of illness and tends towards the lower bound even when the assumption is made that all infections lead to illness. The remaining models were relatively reasonable approximations, with different degrees of under- or over-prediction of illness based on the outbreak data described in this report. The models fit to the outbreak data appear to offer reasonable potential given that they qualitatively, though not statistically significantly, describe observations in a real world environment.

Recommendations

- Consideration should be given to the inclusion of *S. typhi* and *S. paratyphi* in future hazard characterizations. A dose-response relationship for all *Salmonella* spp. could prove to be of great utility, and the added information from *S. typhi* could also serve to expand the current information.
- This document did not consider a quantitative evaluation of secondary transmission (person-to-person) or chronic outcomes. In addition, the impact of the food matrix was not incorporated into the assessment. These may be considerations for future document development.
- Additional data will help to refine the information currently known and ideally support the development of better risk assessments to help make more accurate predictions regarding the safety of foods contaminated with *Salmonella* and other pathogens of public health concern.
- The importance of accurate and complete epidemiological data collection during outbreak investigations should also be communicated and encouraged.

INTERPRETIVE SUMMARY

Introduction

Historically, food safety evaluations have been qualitative rather than quantitative endeavours. Consequently, many decisions about managing food safety have been based on subjective observations and evaluations of available information. Recent initiatives, prompted by scientific bodies and international trade agreements, have led to efforts to quantify the risks associated with foods.

The Codex Alimentarius Commission has developed guidelines and a framework for the conduct of quantitative risk assessment of microbial hazards. Risk assessment is defined as a scientifically based process that includes the following steps: Hazard Identification, Exposure Assessment, Hazard Characterization, and Risk Characterization. These steps describe, respectively: the agents that can cause adverse human health effects and which may be present in a food; an evaluation of the likely intake of such agents in a food when eaten; an assessment of

the nature and magnitude of the adverse effects; and finally an overall estimation of the probability and severity of foodborne disease in a given population because of the food/pathogen combination. An evaluation of the degree of certainty in the information used to arrive at a risk estimate, and how much natural variability occurs in the organism, host, and food should also be considered. In quantitative risk assessments, the information is evaluated in terms of mathematical relationships between the relevant factors. A dose-response assessment is part of Hazard Characterization when sufficient data are available, and is a mathematical interpretation of the relationship between exposure to a pathogen in a food (or a toxin produced by a pathogen), and the likelihood and extent of an effect in the host.

This document provides a brief Hazard Identification and a comprehensive Hazard Characterization for *Salmonella* in broilers and eggs. The Hazard Characterization/Dose-Response step is actually independent of the specific food(s) that is the focus of the risk assessment. Hazard Characterizations for any one pathogen can be constructed and considered as an independent module of the risk assessment process. The combination of information from a Hazard Characterization with the information from an Exposure Assessment on a specific food, likely levels of contamination with the hazard and likely consumption of that food, produce the risk estimates in the Risk Characterization step for the specific hazard/food combination of concern.

This document describes the outcome of exposure to *Salmonella* when ingested, regardless of the food in which it is delivered, and reviews current work that attempts to define a predictable relationship between numbers of organisms ingested and human illness. *Salmonella* can cause more than one type of disease in people, however this document focuses on gastroenteritis (i.e. commonly recognized as a diarrhoeal disease). Chronic disease outcomes beyond gastroenteritis are described, and may warrant further analysis in the future.

Project Objectives and Scope

The expert drafting group for *Salmonella* Hazard Characterization was requested to prepare a brief hazard identification write-up and a hazard characterization in accordance with Codex guidelines that included:

1. A review of basic characteristics of the host, organism and food matrix.
2. A summary and evaluation of available data and information as it applies to estimating the probability of infection, illness, sequelae and secondary transmission, including information on:

    Human clinical feeding studies

    Surrogate pathogens

    Epidemiological data

    Animal studies
3. A summary and evaluation of mathematical microbial dose-response models in conjunction with the *Listeria* Hazard Characterization drafting group.
4. A summary and evaluation of existing models with respect to assumptions, sources of uncertainty and strengths and limitations.

5. The assumption of similar infectivity for *Salmonella* Enteritidis and other strains in the dose-response relationships, absent sufficient evidence based on currently available outbreak investigation data to characterize any differences in the public health outcomes of concern.
6. Use available outbreak data to provide a measure of validation for candidate dose-response models.
7. Incorporate outputs from Exposure Assessment example(s) for *Salmonella* in broilers into hazard characterization. The remit to the drafting group was modified during the course of the work, and thus the outputs from Exposure Assessment and Hazard Characterization were not combined, and are at these time separate documents.

Approach

Information was compiled from published literature, and from unpublished data contributed by countries and/or public health agencies. A description of the public health outcomes, pathogen characteristics, host characteristics and food-related factors that may affect the survival of the organism in the gastrointestinal tract are reviewed in a narrative section. The second section of Hazard Characterization presents the background and rationale for three different models that have been developed in other studies. These models mathematically describe the relationship between the numbers of organisms that might be present in a food and consumed, i.e., the dose, and a human health outcome. These models are graphically compared with data from outbreak investigations in which efforts were made to estimate the number of *Salmonella* in the foodstuff, the numbers of people exposed to the contaminated food, and the numbers of people becoming ill. Finally, the outbreak data were used to generate new dose- response models.

Key findings

1. **Hazard Identification** and the narrative section of **Hazard Characterization** provide a summary of a vast body of literature. *Salmonella* was recognized as a food-borne pathogen before the 1900's, and any one of over 2,000 serovars of *Salmonella* can cause diarrhoeal illness in humans. It is one of the most frequently reported foodborne diseases in developed and developing countries. In most people, the gastroenteritis lasts 4 - 7 days, and patients fully recover without medical treatment. Some people may develop more severe illness, including potentially fatal infections of the bloodstream or other parts of the body, or long-term syndromes such as reactive arthritis or Reiter's syndrome. *Salmonella typhi*, which predominantly causes typhoid fever rather than gastroenteritis, is not included in this assessment.
2. Investigators have produced a large body of research on the genetics of *Salmonella* pathogenesis using inbred mice. However, these studies do not show all aspects of the disease since *Salmonella* produces a typhoid-like disease in the mouse. Less is understood about the factors involved in diarrhoeal illness, and hence mouse data are of limited application. Other animal models in which *Salmonella* has been studied include calves and poultry, which were also deemed to be limited for purposes of extrapolating to humans. We therefore focused on human data where there is also a considerable amount of information. However, only a few studies are complete enough to help characterize the relationship between dose and response. Human data came from controlled human feeding studies, and

reports from food- or water-borne outbreak investigations. The use of surrogate data, i.e. to derive conclusions about *Salmonella* from observations in studies using other pathogens, was not considered directly although such information has been used to derive two of the models reviewed.

3. The number of microorganisms entering the digestive tract per exposure is expressed as a mean number of functional particles of the pathogenic organism, i.e., cells or colony-forming-units (CFU), spores, oocysts, etc. This is the dose, a quantitative measure of the intensity of the exposure. At a certain dose, certain effects in the host occur. The frequency within the exposed population of hosts at which this occurs constitutes the response. The effect may be more or less well defined but generally there will not be a one-to-one relationship between the size of the dose and the specific kind and frequency of the biological effect it produces. Furthermore, pathogenic microorganisms generally produce an array of effects or conditions within an affected host. Thus, instead of a single dose-effect relation there will be a series of dose-response relations that describe the association between the various biological effects and the magnitude of the dose. These effects which can be measured, also referred to as biological end points, include infection, acute illness, degrees of more severe outcomes, or death.

   For purposes of this document, infection is considered to be the result of a process by which a microorganism establishes itself in a host, including transmission, invasion, and multiplication. The evidence of infection is recovery of the organism from the host (usually excretion in faeces) but not necessarily with any other manifestations of disease. Illness is considered to be a symptomatic disease, i.e., deviation from normal health conditions. For enteric pathogens, this generally includes any number of the following symptoms: diarrhoea, abdominal pain, fever, nausea, muscle pain, vomiting, headaches, and/or blood in stools. For the most part, these are the major symptoms constituting a diagnosis of food borne illnesses. However, chronic disease may occur when the organism continues to invade susceptible tissues to cause other adverse outcomes.

   The likelihood that any specific individual will become ill due to an exposure to a foodborne pathogen is dependent on the integration of host, pathogen, and food composition effects. These interactions are often referred to as the "infectious disease triangle" for foodborne disease, which parallels the traditional epidemiological triad of host, agent and environment. Thus, it can be assumed that the relationship between the dose and the response is a function of the organism itself in terms of its virulence properties and its survival characteristics, the food in which it resides, and the susceptibility of the host. A mathematical relationship between the dose and the response should ideally be able to describe the interactions between all these factors. Several investigators have examined dose-response information, and evaluated the usefulness of a range of mathematical models to be able to describe the relationship and predict the outcome for a specific ingested dose for various pathogens. Broadly, two different views have been taken: models that describe a threshold or minimum number of organisms (MID) that have to be ingested before infection or illness occurs; and, models that are non-threshold, in essence stating that even one bacterial cell can cause infection/illness in some proportion of the population. As a result of a meeting of the drafting groups for *Salmonella* and *Listeria monocytogenes* Hazard Characterization, it was generally agreed that the most useful mathematical model(s) are those that do not assume a threshold. However, it was also acknowledged that biological phenomena could impose pressures on

microorganisms that might result in a minimum number of cells that would need to be ingested. This would vary depending on the characteristics of the pathogen, the host and the food.

4. Three different dose-response models were reviewed, two of them with representative relationships for normal and susceptible subpopulations (i.e., probability of illness expected for normal healthy people, and a second derivation to account for immunocompromised populations when exposed to the same dose). The very young, the elderly, pregnant women, and people with medical conditions or treatments are generally considered to be more susceptible to infection or illness from microbial pathogens. These models were derived for risk assessments for *S*. Enteritidis in eggs by the USDA/FDA and by Health Canada. The USDA/FDA model is a beta-Poisson function, derived using the results of feeding studies of *Shigella dysenteriae,* with illness as the biological endpoint. The Health Canada model is based on the Weibull function, derived using data from human feeding studies for several different bacterial pathogens, and data from two *Salmonella* outbreaks. The third model, also a beta-Poisson model, was derived from human feeding study data alone. There have been a total of nine published studies of experimentally induced salmonellosis conducted between 1936 and 1970. Not all these data are suitable for analysis because of limitations in the study designs, which include the use of young healthy subjects, typically male, and only a very few *Salmonella* strains being tested. Nevertheless, these controlled studies do provide some insight into the relationship between a pathogen and the human host.

   Clearly, the derivation of each of the models examined is based on many assumptions, including the use of *S. dysenteriae* or other surrogates for Salmonella, combining results of feeding studies for several different pathogens, modelling infection versus illness as the relevant public health endpoint, and the study design issues in human feeding trials.

5. It has been postulated that 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 virulent than other serovars of *Salmonella*. From the outbreak data used to examine the dose-response relationship, there was no evidence that *S*. Enteritidis had a different likelihood of producing illness than other serovars. In total, 14 sets of data were evaluated for *S*. Enteritidis, against 12 sets of data for other serovars. However, increased severity of illness once infected was not evaluated.

6. Complete outbreak data are sparse. The important information for hazard characterization which is typically not reported includes: the numbers of the pathogen in the food that caused the outbreak (since often the food has been consumed or discarded by the time of the investigation), how much of the food was eaten, how many people actually ate the contaminated food and how many people became ill. Information about the age and health status of the people who ate the food and either became ill or remained healthy, is also frequently lacking. Valuable data for this project were provided by one country that has, since 1997, advised all large foodservice establishments to keep frozen portions of prepared foods for a minimum of 2 weeks for later testing if illness is associated with the food.

   Critical examination for completeness of data provided in each outbreak resulted in excluding some observations from further analysis. In other cases, if it was possible to logically infer something about the incomplete information, assumptions were made. For example, in some cases, the most likely total number of people exposed to the food was estimated, based on the total potentially exposed population. Children under 5 years of age

were regarded as being more susceptible for salmonellosis, and the data were separated accordingly. Single cases were excluded from analysis. The outbreak data were used to fit new dose-response models.

CONCLUSIONS AND FUTURE WORK

At this time, no one model is clearly the best representation of the epidemiological data. All are relatively reasonable approximations, but with differing degrees of under- or over-predicting gastrointestinal illness based on the outbreak data described in this report.

Derivation of current dose-response models is based on many assumptions, such as extrapolating information based on surrogates to the organism of interest or to the human host, combining results of feeding studies for different pathogens, using infection versus illness as endpoints to predict relevant public health outcomes, and accounting for the inherent biases in many human feeding trials. In addition, the outbreak data may also have its own shortcomings. Indeed, several assumptions had to be made to derive some of the outbreak estimates used in developing new dose-response curves. Methodology for sampling and testing the food in the outbreaks was not critically assessed. Reported exposed populations tended to be the maximum number of people that could be exposed. Therefore, the outbreak data represents significant uncertainty.

The drafting group did not consider a quantitative evaluation of secondary transmission (person-to-person) or chronic outcomes in this draft. These may be considerations for future document development.

Several countries responded to the call for data issued by WHO and FAO to support this work. Such collaboration from scientists and public health agencies around the world should continue to be encouraged. More data will help to refine the information currently known and ideally support the development of better risk assessments to help make more accurate predictions regarding the safety of foods contaminated with *Salmonella* and for other pathogens. The importance of collecting accurate and complete epidemiological data during outbreak investigations whenever possible should also be communicated and encouraged.

HAZARD IDENTIFICATION

Salmonellosis is one of the most frequently reported foodborne diseases worldwide. Poultry and poultry products are common food vehicles of the disease. Each year, approximately 40,000 *Salmonella* infections are culture-confirmed, serotyped, and reported to the US Centers for Disease Control and Prevention (CDC), which estimates an annual rate of 1.4 million cases, 16,430 hospitalizations, and 582 deaths in the U.S. alone (Mead *et al*., 1999). Of total cases, 96% are estimated to be caused by foods. International data summarized by Thorns (2000) provides estimated incidences of salmonellosis per 100,000 people for the year 1997: 14 in the USA, 38 in Australia, and 73 cases per 100,000 in Japan. In the Europe Union, the estimates range from 16 cases per 100,000 (The Netherlands) to 120 cases in parts of Germany. The number of foodborne disease cases in Germany between 1993 and 1997 were summarized by Schmidt (1999), 94794 cases of salmonellosis excluding S. typhi and S. paratyphi were reported in 1997 and 140435 cases were reported in 1993. Costs of foodborne salmonellosis have been calculated for the US population, and are estimated as high as $2,329 million annually (in 1998 US dollars) for medical care and lost productivity (Frenzen *et al*., 1999).

Salmonella typically causes gastroenteritis characterized by diarrhoea, fever, abdominal cramps, and dehydration. Individuals suffering from salmonellosis usually recover uneventfully within a week without antibiotic treatment. In some cases, severe diarrhoea requires medical interventions such as intravenous fluid rehydration. Occasionally, the infection may result in bacteremia, meningitis, osteomyelitis, and abscesses. Severe cases may end in death. It is estimated that 93% of patients do not visit a physician and recover fully, 5% visit a physician and recover fully, 1.1 - 1.5% of patients require hospitalization and 0.04 to 0.1% of patients will die (Buzby *et al*., 1996; Mead *et al*., 1999). A small percent of cases are complicated by chronic reactive arthritis.

Over 2,000 *Salmonella* serovars are known to cause illness in humans. It is noted that most, if not all of these, are capable of causing systemic disease (typhoid, or enteric fever) in immunocompromised patients. However, only a few host-adapted serovars typically cause the more severe form of disease in an immunocompetent patient. Of note is *S. typhi*. While foodborne transmission is believed to account for most of the estimated 824 cases of typhoid fever reported in the US, 70% of which have been associated with foreign travel (Mead *et al*., 1999), the principal source of *S. typhi* is human contamination of water or prepared foods. Only one-third of patients suffering from typhoid fever develops diarrhoea. Genetically, *S. typhi* differs from the majority of *Salmonella* serovars which typically cause gastroenteritis, and it has distinctly different virulence attributes (Bäulmer *et al*., 2000). For these reasons, *S. typhi* is not considered in this assessment.

A wide range of foods has been implicated in foodborne illness due to *Salmonella* with poultry as a principal source (Bryan and Doyle, 1995; Humphrey, 2000). Chicken, turkey and eggs were responsible respectively, for 8.6%, 4.7% and 4.3% of 465 foodborne outbreaks caused by bacterial pathogens for which a vehicle was identified, and that were reported to the US CDC during the years 1988-1992 (Bean *et al*., 1997). When a bacterial agent could be identified,

Salmonella caused 12 of 18 outbreaks attributed to chicken, 6 of 12 turkey associated, and 19 of 19 egg-related outbreaks.

The evolution of the *S*. Enteritidis pandemic beginning in the 1980s led to increased foodborne illnesses associated with poultry in many countries and particularly outbreaks and single cases associated with eggs and egg products (Thorns, 2000). During the years 1988-92, *S*. Enteritidis was responsible for the largest number of outbreaks, cases and deaths reported in the US (Bean *et al*., 1997).

A better understanding of the factors associated with contamination of poultry and which influence the outcome of disease will lead to better information for food safety risk managers. There is a need to target effective measures that can be feasibly implemented to reduce, prevent or eliminate human salmonellosis, and lower the estimated costs to society.

HAZARD CHARACTERIZATION

SECTION 1: PUBLIC HEALTH OUTCOMES AND RISK FACTORS

This section presents the scientific literature on the public health outcomes associated with Salmonella, as well as review of the basic characteristics of the organism, risk factors in the human host, and factors related to the food matrix.

PUBLIC HEALTH OUTCOMES

Acute gastrointestinal manifestations

Salmonellosis generally manifests as a self-limiting episode of enterocolitis, whose symptoms resolve in 5 days. Incubation period is generally 8-72 hours; watery diarrhoea and abdominal pain are common symptoms. Susceptibility is highest in infants, elderly people and immunocompromised hosts. Occasionally, systemic infections can occur, particularly with *S. dublin* and *S. cholerasuis* infections which exhibit a predilection toward septicaemia (D'Aoust, 1997). Supportive therapy (fluid and electrolyte replacement) may be required. An intermittent period of faecal shedding may follow the acute illness, lasting from days to years. Buchwald and Blaser (1984) reviewed 32 reports and showed that the median duration of shedding following acute disease was 5 weeks, with less than 1% of patients becoming chronic carriers. Children may shed up to 10^6 to 10^7 salmonellae per gram during convalescence (Cruiskshank and Humphrey, 1987).

Mattila *et al*. (1998) described a 1994 outbreak of *S. bovismorbificans* in southern Finland from sprouted alfalfa seeds. Out of 191 respondents, 117 (61%) of cases required a physician visit due to intestinal or extra-intestinal symptoms. Twenty-one (11%) individuals were hospitalized with a median hospital stay of 9 days. The authors state that most hospitalized patients were over 65 years of age. Ninety-four (49%) subjects received antimicrobials (fluoroquinolones, primarily), with a majority (78/94 cases or 83%) requiring antimicrobial treatment because of diarrhoea, fever, or a *Salmonella*-positive urine sample. Duration of antimicrobial therapy (known for 70 patients) was 2 weeks or more in 44%, 10-12 days in 34% and 1 week or less in 21% of patients.

Kanakoudi-Tsakalidou *et al*. (1998) conducted a prospective study of *S.* Enteritidis infection in 9 children. Diarrhoea lasted for 3-7 days accompanied by fever in all cases. Four patients required hospitalization because of severe dehydration or bloody stools. Arthritis developed in all patients within 1 to 3 weeks following the diarrhoeic episode.

Non-gastrointestinal sequelae

Inman *et al*. (1988) reported on a large outbreak in September 1984 of *S. typhimurium* PT 22 in a group of police officers given a prepackaged box lunch. Four hundred seventy three (473) individuals fit the case definition and were mailed a questionnaire inquiring about symptoms

associated with the gastroenteritis with a 72% respondent rate. Out of 340 responders, 196 individuals experienced associated extra-enteric symptoms including headaches (182 or 53.5%), joint pain (106 or 31.2%), redness or soreness in the eyes (37 or 10.9%), soreness in the mouth (15 or 4.4%), and skin rash (10 or 2.9%).

Mattila *et al*. (1998) identified a total of 210 cases with positive stool samples for *S. bovismorbificans* for questionnaire follow-up regarding symptoms. Of the 191 (91%) respondents, 66 (35%) had articular symptoms, 52 (27%) experienced headaches, 8 (4%) had eye symptoms, and 7 (4%) had cutaneous symptoms including one child who experienced erythema nodosum (a dermatological disorder characterized by the formation of tender, red nodules usually located on the front of the legs). Cortazar *et al*. (1985) have likewise noted the association of erythema nodosum with *Salmonella* gastroenteritis.

Maki-Ikola and Granfors (1992) summarized the clinical, epidemiological and laboratory data on *Salmonella*-triggered reactive arthritis. A review of extra-articular manifestations reported in 55 journal publications showed that these included urethritis, conjunctivitis, entesopathy, myalgia, weight loss of over 5 kg., dactylitis, erythema nodosum, oral ulcers, myocarditis, acute anterior uveitis, iritis, cholecystitis, keratitis, pharyngitis, and pneumonia.

Reactive arthritis and Reiter's syndrome. *Salmonella* has been implicated as a triggering organism for reactive arthritis (ReA) and Reiter's syndrome. Reactive arthritis is characterized as the development of synovitis (joint swelling and tenderness) within a few weeks after the occurrence of gastroenteritic symptoms. Reiter's syndrome is defined as the occurrence of arthritis with one or more extra-articular symptoms typical of the disease such as conjunctivitis, iritis, urethritis, and balanitis. The outlook for ReA is usually favourable with symptoms lasting for <1 year in most persons, although 5 to 18% may have symptoms that last more than 1 year and 15 to 48% may experience multiple episodes of arthritis.

Generally, 1 to 2% of a population infected by triggering organisms will develop ReA or Reiter's syndrome (Keat, 1983; Smith *et al*., 1993). Maki-Ikola (1992) reviewed several published outbreaks totalling 5525 patients with salmonellosis and estimated an incidence of reactive arthritis from 1.2% to 7.3% (mean 3.5%). Several researchers (Aho *et al*., 1985; Archer, 1985; Calin, 1988) assert that HLA-B27-positive individuals are at higher risk for developing ReA , Reiter's syndrome and ankylosing spondylitis after an enteric infection with triggering organisms, estimating that approximately 20% of the exposed HLA-B27-positive population develop these chronic sequelae. However, a lack of correlation between ReA and HLA-B27 has been observed after *S. typhimurium* and *S. heidelberg* / *S. hadar* outbreaks in Canada (Inman *et al*., 1988; Thomson *et al*., 1992; Thompson et. al., 1995).

Ike *et al*. (1986) reported an incidence of ReA on physical examination in 2.3% of patients with *Salmonella*-positive stools following the 1985 Chicago milk outbreak of *S. typhimurium* gastroenteritis. Reiter's syndrome occurred approximately 10-fold less often than ReA. In a follow-up study of the Chicago patients, Ike *et al*. (1987) found that 20 of 29 reported persistent symptoms of ReA after one year, and symptoms had actually worsened in six cases.

In September 1984, a Canadian outbreak of *S. typhimurium* PT 22 occurred in 473 out of 1608 police officers given a prepackaged box lunch (Inman *et al*., 1988). A cohort of 137 out of 196 individuals experiencing extra-enteric manifestations agreed to participate in a follow-up. Questionnaires were mailed out to their physicians and were returned for 116 (85%) volunteers further describing the acute phase of the illness. Nineteen were reported by the physician to have

experienced joint pain. Inman *et al*. (1988) noted a positive correlation between duration of gastrointestinal symptoms and duration of joint symptoms. In thirteen patients, symptoms were restricted to ReA, while Reiter's syndrome was present in 6 patients (Inman *et al*., 1988).

An outbreak in Sweden in 1990 involved 113 medical scientists attending a radiology symposium in Sweden who were exposed to food contaminated with *S.* Enteritidis (Locht *et al*., 1993). Ninety-six percent (108) developed symptoms of salmonellosis and 17 (15%) of the 108 developed ReA. Of the individuals developing ReA, 9 (53%) were men and 8 (47%) were women, with a mean age of 48.5 years (range 34-60) (Locht *et al*., 1993; Smith, 1994).

In another Canadian outbreak (Thomson *et al*., 1992), 79 women and 4 men in attendance at a luncheon were exposed to *S. heidelberg* and *S. hadar* from eating contaminated potato salad, and 73 subsequently developed Salmonellosis. *S. heidelberg* and *S. hadar* were isolated from the stool of 21 patients in addition to *S. thompson*. Six of the 73 ill individuals developed ReA (Thomson *et al*., 1992; Smith, 1994). Ages of individuals who developed ReA were not significantly different than those cases that did not develop ReA (Thomson *et al*., 1992).

A 1994 outbreak in Finland from sprouted alfalfa seeds contaminated with *S. bovismorbificans* was recently reported by Mattila *et al*. (1998). Questionnaires were sent to all 210 subjects with positive stool cultures. Median age in the 191 (91%) respondents was 32 years (range 1-90) with 80% being older than 16 years of age; 130 (68%) were female. A total of 66 (35%) subjects reported articular symptoms. Fifty-one cases reporting articular symptoms were examined and 13 were contacted by telephone. A total of 12% (22/191) fulfilled the criteria for ReA, 19 adults and 3 children. The incidence of ReA was not significantly different between children (8%) and adults (12%) (Mattila *et al*., 1998).

Kanakoudi-Tsakalidou *et al*. (1998) followed 9 cases of juvenile ReA prospectively concluding that the disease in children is generally mild, transient and self-limiting. Five out of 9 patients carried the HLA-B27 antigen and experienced a prolonged course for arthritis (mean duration 9.5 months).

The duration of ReA illness was evaluated in several studies:

- Radiology symposium in Sweden (Locht *et al*., 1993): A 6-month follow-up assessment on 13 of the 17 individuals who developed ReA showed 5 patients having complete resolution of symptoms, but arthritis persisting in 8 patients six months after the outbreak.
- Canadian outbreak among policemen (Inman *et al*., 1988): Out of 19 patients experiencing arthritis, a 12-month follow-up assessment was conducted on 15 patients. Symptoms resolved in 8 out of 15 patients within 12 months, while symptoms persisted in 7 patients twelve months after the outbreak.
- Canadian outbreak at a women's luncheon: Duration on illness in the 6 individuals who developed ReA ranged from 4-24 weeks in 4 individuals to greater than 6 months in the other two.
- Sprout outbreak in southern Finland: The median onset of joint symptoms was 8.5 days (range 3-30) after the first symptoms of diarrhoea. Joint symptoms lasted less than 2 months in 11 (50%) subjects, 2-4 months in 7 (32%) and more than 4 months in 4 (18%) individuals.

- Prospective study of 9 children: Juvenile ReA has been reported to have a milder course with duration varying from 1-12 months. In contrast to adult ReA, it seldom recurs or falls to chronicity (Kanakoudi-Tsakalidou *et al*., 1998).

CHARACTERISTICS OF THE ORGANISM

General characteristics of non-typhoid *Salmonella*

In order for infection with a non-typhoidal *Salmonella* to occur, the organism must survive a rather hostile environment. It must adapt to differences in growth conditions between the outside environment and the host and within highly variable microenvironments within the host. The invasive journey towards illness in the host must negotiate distinct temperature differences, osmolarity, oxidation-reduction potentials, environmental iron concentrations, pH and organic and inorganic nutrient environments (Slauch *et al*., 1997). An infective *Salmonella* must then survive peristalsis, the epithelial surface and the host immune response.

Non-typhoid *Salmonellae* possessing certain adaptive characteristics are more likely to produce foodborne disease. First, they must be acid tolerant to survive the pH of the stomach. They must also be able to attach themselves to and invade the intestinal epithelia and Peyer's patches (D'Aoust, 1997). Bacterial virulence factors include those that promote adhesion to host cells in the intestines: specific fimbriae, chromosome-coded bacterial surface adhesins, hemagglutinins and epithelial cell induction of bacterial polypeptides can promote colonization and adhesion.

Resistance of *Salmonellae* to lytic action of complement varies with the length of the O side chains of lipopolysaccharide (LPS) molecules (D'Aoust, 1991). Smooth varieties are more resistant than rough types. O side chains of the LPS also has been shown to affect invasiveness and enterotoxin production (Murray, 1986).

Siderophores, which chelate iron, are necessary for the accumulation of sufficient environmental iron to allow growth of *Salmonellae*. Siderophores include hydroxamate, phenolate, and catechol types. Porins are hydrophobic bacterial cell proteins which enhance the virulence of *Salmonella* by repression of macrophage and polymorphonuclear-dependent phagocytosis. *Salmonella* porins may however have a limited importance in pathogenicity. Chromosomal determinants include specific virulence genes whose potential for action is tightly controlled by regulatory genes. Expression of the genes are determined by the environment and invasion occurs by the two-component regulatory system PhoPQ which enables survival of *Salmonellae* within the hostile environment of phagocytes (Slauch *et al*., 1997).

Virulence plasmids in the range of 50-100 kb have been associated with the ability to spread after colonization, invasion of the intestine, ability to grow in the spleen, and a general suppression of the host immune response (Slauch *et al*., 1997). The presence of virulence plasmids in *Salmonellae* is limited. Chiu *et al* (1999) studied virulence plasmids in 436 clinical human samples in Taiwan: 287 isolates were from faeces, 122 from blood and the remaining were isolated from other sites. Sixty-six percent of the non-faecal isolates compared with 40% of the faecal isolates contained a virulence plasmid. All the isolates (n=50) of the three highly invasive serotypes - S. Enteritidis, *S. dublin* and *S. choleraesuis* contained virulence plasmids. Virulence plasmids have also been confirmed in *S. typhimurium, S. gallinarum-pullorum* and *S. abortusovis*, but are notably absent in *S. typhi*, which is host-adapted and highly infectious.

Other factors that affect the ability of the organism to cause disease include the presence of cytotoxins and diarrhoeagenic enterotoxins. The enterotoxin is released into the lumen of the intestine and results in the loss of intestinal fluids (D'Aoust, 1991).

Antimicrobial resistance of the organism may also affect the severity of the outcome. The effects of underlying illnesses often complicate evaluation of the added clinical impact of resistant *Salmonella*. In a study referring to the U.S. and the years 1989-90, after accounting for prior antimicrobial exposure and underlying illness, patients with resistant *Salmonella* were more likely to be hospitalized (Lee *et al*., 1994). A longer duration of illness and hospitalization was also noted for resistant infections. Although black race and less than one year of age appeared to be host characteristics associated to a resistant infection, differences in the distribution of infecting serovars among ethnic and age groups contributed to the occurrence of such effects. Varying food preferences or methods of food preparation might have been at the basis of different serovar distribution. The same consideration may explain the results of an earlier study which associated infection with *S. heidelberg*, penicillin intake, Hispanic origin, more than 60 years of age, and antacid use to infection with a multi-resistant *Salmonella* (Riley *et al*., 1984). The conclusion of this study, that multi-resistant organisms are more dependent on host characteristics than sensitive organisms to cause disease, should be qualified accordingly.

HOST CHARACTERISTICS

Literature tends to be biased towards reporting statistically significant and positive results. This review can only reflect such a bias, and the focus is evidently on host factors for which a statistically significant association to *Salmonella* gastroenteritis and related complications has been reported. Where clear indication on a non-significant finding is made in the original study, such finding is reported. Also, since not all studies considered the same factors, the significance of a factor in a given study may merely depend on the presence or absence of other ones. For instance, while a Swiss study considered travel abroad an important source for resistant *Salmonellae* (Schmid *et al*., 1996), such an association was not seen in a U.S. study (Lee *et al*., 1994). This apparent inconsistency may have various explanations whose discussion is beyond the scope of this review.

The following risk factors are considered:

➢ Demographic and socioeconomic factors	Age Gender Race & ethnicity Nutritional status Social/economic/environmental factors Travel abroad
➢ Genetic factors	HLA-B27 gene
➢ Health factors	Immune status Previous exposure Concurrent infections Underlying diseases Concurrent medications

Demographic and socioeconomic factors

The following factors are considered in this section: age; gender; race and ethnicity; nutritional status, socioeconomic and environmental factors, travel abroad, and pets and farm animals.

Age. A common observation is that age of patients with *Salmonella* infections is distributed according to a bimodal distribution with peaks in children and elderly. In a Belgian hospital-based study covering isolates for a 20-year period (1973-1992), *S. typhimurium* and *S.* Enteritidis were mainly isolated in children of less than 5 years of age (Le Bacq *et al*., 1994). The age distribution was, however, less accentuated for *S.* Enteritidis than for *S. typhimurium*. Both serovars were more likely to lead to bacteremia in middle and older age groups than in younger than 5 years of age (Le Bacq *et al*., 1994), confirming a previous observation made in the United States (Blaser and Feldman, 1981). Another study reports on *Salmonella* isolates of a Hong Kong hospital for the period 1982-1993 (Wong *et al*., 1994). Among both intestinal and extraintestinal isolates, *S. typhimurium*, *S. derby* and *S. saintpaul* predominated in infants. In patients older than 1 year of age, *S. derby* and *S. typhimurium* remained the commonest intestinal isolates, while *S. typhi*, *S. typhimurium*, and *S.* Enteritidis were the most common extraintestinal isolates. In a British population-based study, highest age-specific isolation rates for *S.* Enteritidis were observed in children aged under 2 years and for *S. typhimurium* in those under 1 year (Banatvala *et al*., 1999).

In children younger than one year of age, the peak incidence is generally observed in the second and third months (Ryder *et al*., 1976; Davis, 1981, 1983). The study from Hong Kong showed, however, a peak at 12 months of age (Wong *et al*., 1994). In a study on Peruvian children, the IgG and IgM titres against *Salmonellae* serogroups AO, BO and DO were higher at 12 months of age than at 2 or 3 months of age, which was interpreted as an indication of acquired immunity (Nguyen *et al*., 1998).

It should be pointed out that association with age may be spurious. Children and the elderly with diarrhoea may be expected to be more frequently cultured than other age groups (Banatvala *et al*., 1999). As mentioned earlier, differences in the distribution of infecting serovars among age groups was considered the reason for an apparent increased risk of resistant *Salmonella* infection in infants (Lee *et al*., 1994). Moreover, age association may reflect behavioural characteristics. For instance, eating snow, sand, or soil - a behaviour more likely in children - was found to be associated to infection with *S. typhimurium* O:4-12 (Kapperud *et al*., 1998a).

Gender. In terms of number of isolates, men seem to be generally more affected than women are. A male-to-female ratio of 1.1 has been reported in various occasions (Blaser and Feldman, 1981; Le Bacq *et al*., 1994; Wong *et al*., 1994). The significance of such a finding does not appear to have been addressed. Several factors, such as proportion of the two genders as well as different age distributions for males and females within a country or hospital catchment area, may play an important role. In the evaluation of single study, it should be pointed out that the occurrence of other factors, e.g. use of antacids or pregnancy, relates to one gender more often or exclusively, and gender may thus have the effect of a confounder.

Race and ethnicity. Potential role of race and ethnicity has seldom been considered. As mentioned above, an association with black race and Hispanic origin was reported for resistant *Salmonella* infections (Lee *et al*., 1994; Riley *et al*., 1984). In the former case, the association

was explained by differences in the distribution of infecting serovars among ethnic groups, which in turn depended on varying food preferences or methods of food preparation.

Nutritional status. An association between altered nutritional status and acute gastroenteritis has been shown in AIDS patients (Tacconelli *et al*., 1998). Apart from this report, no direct reference to the role of nutritional status was found in the literature.

Social/economic/environmental factors. Isolation rates of several *Salmonella* serovars among groups of different socioeconomic extraction have been compared on the basis of the Townsend score, a index for deprivation (Banatvala *et al*., 1999). While isolation rates for *S. typhimurium* were not related to the Townsend score, highest isolation rates of *S.* Enteritidis were observed in more prosperous areas. It was advanced that population living in such areas more frequently ingested vehicles harbouring *S.* Enteritidis.

Sanitation deficiencies have been associated with high rates of enteric disease, but direct reference to the potential role of *Salmonella* spp. is scarce. In the 1950s, lack of sanitation, poor housing, limited water supply, and poor personal hygiene were associated with high *Shigella* rates in Guatemala (Beck *et al*., 1957). A similar observation was made in the United States where, in areas of inadequate sanitary facilities, poor housing, and low income, *Shigella* infections were the major causes of diarrhoeal diseases. In particular, there were nearly twice as many cases of diarrhoea among persons living in dwellings having outhouses than among those whose houses had indoor restrooms (Schliessmann *et al*., 1958). In certain Guatemalan villages, the habits of the people and the density of the population were found to be more important determinants than type of housing (Bruch *et al*., 1963). In a study conducted in Panamá, six representative types of dwelling were considered as an index of social and economic influences on the prevalence of enteric pathogens among infants with diarrhoeal disease (Kourany and Vasquez, 1969). Each dwelling type differed characteristically from one another, but five of the six types were considered substandard and their occupants were of low socioeconomic status. Infection rates for enteropathogenic *Escherichia coli*, *Shigella*, and *Salmonella* among infants from the various groups of substandard dwellings ranged from 6.0 to 10.2%, in contrast to the zero infection rate observed in infants from the better-type housing. It is worth noting that the literature on sanitation and housing was mainly published in the 1950's and 1960's. It is possible that safety improvement in the water supply consequent to economic development has sensibly diminished the importance of those factors in several countries.

A French study on sporadic *S.* Enteritidis infections in children investigated the influence of diarrhoea in another household member in the 10-3 days before a child shows clinical symptoms. The strength of the association with such a factor appeared stronger for cases in infants (1 year of age or less) as compared to cases in children between 1 and 5 years of age (Delarocque-Astagneau *et al*., 1998). On the basis of this observation as well as other results of the study, it was postulated that *S.* Enteritidis infection in children of less than 1 year of age is mainly related to exposure to a contaminated person while children between 1 and 5 years of age contract the infection by consuming raw or undercooked egg products or chicken.

A seasonal pattern in isolations, which generally shows increased rates during hotter months, has been documented. For instance, increased isolation rates for *S.* Enteritidis, *S. typhimurium*, *S. virchow*, and *S. newport* were observed in summer in a British study (Banatvala *et al*., 1999). The French study mentioned in the previous paragraph noted that the association between *S.* Enteritidis infection and prolonged storage of eggs was stronger during the summer period.

Travel abroad. Travel abroad is a risk factor for *Salmonella* gastroenteritis that has been consistently demonstrated in both North America and Europe. For California residents, Kass *et al*. (1992) demonstrated an association between sporadic salmonellosis and travel outside the United States within 3 weeks prior to the onset of illness. Possible variations related to serovar in sporadic Salmonellosis were indicated by a study concerning residents of Switzerland (Schmid *et al*., 1996). Having been abroad within three days prior to clinical onset of the illness was found to be associated to both *S.* Enteritidis and serovars other than Enteritidis, although to a greater extent for the latter case. Little difference was seen between the results of all *S.* Enteritidis phage types and of *S.* Enteritidis PT4. While most patients with *S.* Enteritidis were more likely to have travelled within Europe, the majority of non-Enteritidis infections might have been imported from outside Europe. Individuals of a British region with *Salmonella* infection were more likely to have reported travel abroad in the week before the onset of illness (Banatvala *et al*., 1999). Frequency of overseas travel between patients with *S.* Enteritidis or *S. typhimurium* was not different, but it was among patients with other serovars. Indication of how travel abroad may lead to Salmonellosis can be found in a study referring to residents of Norway (Kapperud *et al*., 1998b). This study suggested that about 90% of the cases from whom a travel history was available had acquired their infection abroad, but failed to show an association to either foreign travel among household members or consumption of poultry. However, consumption of poultry purchased abroad during holiday visits to neighbouring countries was the only risk factor considered by the study that remained independently associated with the disease. Only cases of *S. typhimurium* allowed for a separate analysis, which showed an association with both poultry purchased abroad and foreign travel among household members.

Genetic factors

As far as acute gastroenteritis caused by *Salmonella*, no genetic factors related to the host have been reported. Reports concerning race and ethnicity should be considered in light of eating habits.

The putative association of the gene Human Leukocyte Antigen B27 (HLA-B27) for patients with spondyloarthropathies, in particular reactive arthritis and Reiter's syndrome, has been described above. The HLA-B27 gene has a very high prevalence among the native peoples of the circumpolar arctic and sub-arctic regions of Eurasia and North America, and in some regions of Melanesia. In contrast, it is virtually absent among the genetically unmixed native populations of South America, Australia, and among equatorial and southern African Bantus and Sans (Bushmen) (Khan, 1996). Fifty percent of Haida Indians living on Queen Charlotte Islands of the Canadian province of British Columbia have the HLA-B27 gene, which is the highest prevalence ever observed in a population. The prevalence among Americans of African descent varies between 2 to 3%, while 8% of the Americans of European descent posses the gene (Khan, 1995).

Health factors

Immune status. The host immune status is, as in any other infectious disease, a very important factors in determining both infection and clinical illness. In general terms, its importance does not seem to have been the direct goal of any formal work, and has thus to be indirectly assessed though other factors, e.g. age and HIV infection. (To be added: protection across serovars, influence of previous exposure.)

Concurrent infections. Persons infected with Human Immunodeficiency Virus (HIV) tend to have recurrent enteric bacterial infections. Such infections are often virulent and associated with extraintestinal disease (Smith *et al*., 1988; Angulo and Swerdlow, 1995). The following six risk factors for enteric Salmonellosis have been identified in HIV-infected patients: increasing value on the prognostic scoring system APACHE II (Acute Physiology and Chronic Health Evaluation); altered nutritional status; previous antibiotic therapy; ingestion of undercooked poultry/eggs or contaminated cooked food; previous opportunistic infections; stage C of HIV infection (Tacconelli *et al*., 1998).

Underlying diseases. The significance of Acquired Immunodeficiency Syndrome (AIDS) has been discussed in the previous paragraph. The risk represented by other underlying conditions was evaluated in a large nosocomial foodborne outbreak of *S.* Enteritidis that occurred in 1987 in New York (Telzak *et al*., 1991). Gastrointestinal and cardiovascular diseases, cancer, diabetes mellitus, and alcoholism as well as use of antacids and antibiotics were the factors considered. However, diabetes was the only condition that was independently associated with infection after exposure to the contaminated meal. Although diabetic cases were more likely to develop symptomatic illness compared to nondiabetic, the difference was not statistically significant.. Decreased gastric acidity and autonomic neuropathy of the small bowel (which leads to reduced intestinal motility and prolonged gastrointestinal transit time) are the two biologically plausible mechanisms for the increased risk of *S.* Enteritidis infection among diabetics. Among patients with sporadic Salmonellosis in Northern California, diabetes mellitus and cardiac disease were both associated to clinical illness (Kass *et al*., 1992). This study contemplated 14 health conditions. Nongastrointestinal medical conditions and, to a larger, a recent history of gastrointestinal disorder were associated with sporadic *S. typhimurium* O:4-12 infection in Norway (Kapperud *et al*., 1998a). It was, however, noted that physicians are more likely to require a stool culture for patients with preceding illness. In a British epidemiologic study, cases of *Salmonella* infection were more likely to report a long term illness (including gastroduodenal conditions) than controls (Banatvala *et al*., 1999). All individuals with diabetes mellitus, malignancy, or immunodeficiency were cases.

Concurrent medications. Although the uses of gastric reducing and antimicrobial medication are often considered, the evidence found in the literature concerning their association with human Salmonellosis is contrasting. While some studies have shown an association with antacid use (Banatvala *et al*., 1999), other have fail to do so (Telzak *et al*., 1991; Kapperud *et al*., 1998a). A similar situation is found for the use of antibiotics in the weeks/days preceding the infection or disease onset: some studies have demonstrated an association (Pavia *et al*., 1990; Kass *et al*., 1992; Bellido Blasco *et al*., 1998),but other have not (Telzak *et al*., 1991; Kapperud *et al*., 1998a; Banatvala *et al*., 1999). Having a resistant *Salmonella* infection has been associated to a previous antibiotics use (Lee *et al*., 1994). Antibiotic use during acute Salmonellosis gastroenteritis can lead to a prolonged clinical course and higher rate of carriage, and it has generally been considered only in case of possible bacteremia.

Among the 11 different medical therapies considered by the North California study on sporadic, clinical Salmonellosis (which also included antacids and antibiotics), only hormonal replacement therapy - principally conjugated estrogen - in older women was found to be associated to clinical Salmonellosis (Kass *et al*., 1992). An association between serovars other than *S.* Enteritidis and intake of medications other than antacids was shown in Switzerland (Schmid *et al*., 1996). Regular use of medications was a risk factors for *S. typhimurium* O:4-12 infection in Norway

(Kapperud *et al*., 1998a). (In the same study, use of antacids and antibiotics were not risk factors.)

FACTORS RELATED TO THE MATRIX/CONDITIONS OF INGESTION

Empirical observation, mainly deriving from outbreak investigations, shows that foodborne Salmonellosis can be related to a variety of food items. Table 1 lists major foodborne outbreaks of human Salmonellosis and shows the wide range of foods implicated in these outbreaks (D'Aoust, 1997). In spite of the numerous, potential pathogen-commodity combinations, the more recent literature body decisively points to avian-related products, whether eggs or poultry meat, as the most often implicated food items. In particular, the attention to *S.* Enteritidis and eggs has been striking during the past decade (Rodrigue *et al*., 1990). Besides the intrinsic characteristics of those products, several other factors could actually concur in determining their involvement. For instance, live poultry as prevalent bacterial reservoir, relative frequency of egg and poultry in diet, prevailing preparation method, and publication bias are all elements that should be considered in evaluating the involvement of avian-related food. To date, it appears that little research effort has been conducted to disentangle those factors.

Table 1. Major foodborne outbreaks of human Salmonellosis and implicated food items (Adapted from D'Aoust, 1997)

Year	Country(ies)	Vehicle	Serovar
1973	Canada, United States	Chocolate	*S. eastbourne*
1973	Trinidad	Milk powder	*S. derby*
1974	United States	Potato salad	*S. newport*
1976	Spain	Egg salad	*S. typhimurium*
1976	Australia	Raw milk	*S. typhimurium PT9*
1977	Sweden	Mustard dressing	*S.* Enteritidis *PT4*
1981	The Netherlands	Salad base	*S. indiana*
1981	Scotland	Raw milk	*S. typhimurium PT204*
1984	Canada	Cheddar cheese	*S. typhimurium PT10*
1984	Canada		*S. typhimurium PT22*
1984	France, England	Liver pate	*S. goldcoast*
1985	United States	Pasteurized milk	*S. typhimurium*
1985	Scotland	Turkey	*S. thompson, S. infantis*
1987	Republic of China	Egg drink	*S. typhimurium*
1987	Norway	Chocolate	*S. typhimurium*
1988	Japan	Cuttlefish	*S. champaign*
1988	Japan	Cooked eggs	*Salmonella spp.*
1988	England	Mayonnaise	*S. typhimurium DT49*
1990	Sweden		*S.* Enteritidis
1991	Germany	Fruit soup	*S.* Enteritidis
1993	France	Mayonnaise	*S.* Enteritidis
1993	Germany	Paprika chips	*S. saintpaul, S. javiana, S. rubislaw*
1994	United States	Ice cream	*S.* Enteritidis
1994	Finland, Sweden	Alfalfa sprouts	*S. bovismorbificans*
1998	United States	Breakfast cereal	*S. agona*
1998	England	Chopped liver	*S.* Enteritidis *PT4*
1999	United States	Orange juice	*S. muenchen*

Gastric acidity is recognized as an important defence against food-borne pathogens. A variety of pathogen, host, and food factors interacts in determining whether a sufficient number of bacteria is able to withstand stomach acidity and to go on to colonize the gut. Such an interaction appears extremely dynamic. Although *Salmonellae* prefer to grow in neutral pH environments, they have evolved complex, inducible acid survival strategies that allow them to face the dramatic pH fluctuations encountered in nature and during pathogenesis (Bearson *et al*., 1997). While the

human stomach normally has a pH of 2, several host factors may cause decreased gastric acidity. Examples reported in the previous section are older age, diabetes mellitus, and use of antacid drugs. As for factors specifically related to food, it appears that a systematic treatment of this topic has not yet been carried out. Circumstantial evidence suggests that at least four elements would be of particular relevance: amount of ingested food, nutrient composition of food, time of the meal, and nature of contamination. The reference to food rather than to food item emphasizes the importance to consider the whole meal.

In a *S. typhimurium* outbreak, it was observed that persons who had eaten two or more pieces of chicken tended to have shorter incubation periods. However, both attack rate and illness severity did not appear to be a function of the amount of chicken consumed. It was concluded that the amount of food consumed provides only a crude estimate of dose because a homogenous distribution of the pathogen among the chicken pieces is unlikely (Glynn and Palmer, 1992). This also means that, since infectivity is not uniformly distributed within a food, a larger meal may increase the chances of ingesting an infected portion. D'Aoust (1985) noted that, in foodborne outbreaks involving fatty vehicles, low infective doses had been reported (chocolate: <100 cells of *S. eastbourne*, 50 cells of *S. napoli*; cheddar cheese: 100-500 cells of *S. heildelberg*, 1-6 cells of *S. typhimurium*). Consequently, microorganisms trapped in hydrophobic lipid moieties may survive the acidic conditions of the stomach, and the fat content of contaminated foods may thus play a significant role in human salmonellosis. In contrast, experimental evidence in rat shows that *Salmonella* infection is not affected by milk fat (Sprong *et al*., 1999). *Salmonellae* were actually protected from acid killing when inoculated onto boiled egg white, a food source high in protein and low in fat (Waterman and Small, 1998). The same study shows that the pH of the microenvironment occupied by the bacteria on the surface of a food source is critical for their survival.

The effect of substrate was studied in volunteers challenged with *Vibrio cholerae* fed in a medium with buffering capacity (Cash *et al*., 1974). The group of subjects that overcame the effect of a bicarbonate vehicle in less than 30 minutes (approximately half of the challenged individuals) experienced a lower attack rate than the group experiencing a prolonged buffering effect. Low oral infecting doses of *Salmonella* were observed in association with the ingestion of the inoculum between meals (Mossel and Oei, 1975). It was postulated that, at such moments, the pyloric barrier would initially fail. The authors also speculated that some food items, such as chocolate and ice cream, are more likely to be ingested between meals, and thus lead to illness even with only a few organisms. A protective effect of alcoholic beverages was observed in a *S.* Enteritidis outbreak (Bellido Blasco *et al*., 1996). Besides the direct effect of ethanol on bacteria, alcohol may stimulate secretion of gastric acid. Last but not least, an important in determining the survival of bacteria in the stomach may be how uniformly a food is contaminated. Although a uniform distribution is usually assumed, the very nature of bacterial growth in colonies would suggest that agglomerations of bacteria within the food actually result. It can be speculated that the outer layers of bacteria would protect the inner ones, allowing some pathogen to survive the gastric passage.

SECTION 2: DOSE-RESPONSE ASSESSMENT

This section presents the quantitative information that is available for *Salmonella* infectivity or illness, from which dose-response relationships can be estimated. An overview of the different types of data and mathematical dose-response models is included in the Appendix.

HUMAN FEEDING TRIALS

There have been a total of nine published studies of experimentally induced salmonellosis conducted between 1936 and 1970 using a variety of serotypes and strains. Serotypes and strains used in these series of feeding trials are listed in Table 2.

Table 2: Summary of human feeding trials that have been performed.

	Serotype	Strains	Reference
1	*S. typhimurium*		(Hormaeche *et al*., 1936)
2	*S. anatum*		(Varela and Olarte, 1942)
3	*S. meleagridis*	I, II & III	(McCullough and Eisele, 1951a)
	S. anatum	I, II & III	(McCullough and Eisele, 1951a)
4	*S. newport*		(McCullough and Eisele, 1951b)
	S. derby		(McCullough and Eisele, 1951b)
	S. bareilly		(McCullough and Eisele, 1951b)
5	*S. pullorum*	I, II, III & IV	(McCullough and Eisele, 1951c)
6	*S. typhi*		(Sprinz *et al*., 1966)
7	*S. sofia & S. bovis-morbificans*		(Mackenzie and Livingstone, 1968)
8	*S. typhi*	Quailes, Zermatt, Ty2V, 0-901	(Hornick *et al*., 1970)
9	*S. typhi*	Quailes	(Woodward, 1980)

Although the list of human feeding trials for *Salmonella* in humans is more extensive than may exist for other bacterial pathogens, some of these studies were deemed to be unsuitable and were not used in further analysis to derive conclusions about the pathogenicity of *Salmonella* in general in humans. The earliest study used 5 subjects who were all fed a dose of approximately 9-logs in water and all exposed individuals were subsequently infected (Hormaeche *et al*., 1936). In a later study, (Varela and Olarte, 1942) apparently only one volunteer was used and became ill after ingesting a dose of 10-logs in water. The study conducted by MacKenzie and Livingstone (Mackenzie and Livingstone, 1968) involved a nasal inoculation of approximately 25 cells in one volunteer who subsequently became ill. These three studies were not informative due to the use of only large doses with 100% attack rates, the testing of only one dose with one subject, or the method of inoculation. Studies conducted using *S. typhi* (Sprinz *et al*., 1966; Hornick *et al*., 1970; Woodward, 1980), were considered to be inappropriate in the current analysis, primarily because of the difference between the illnesses caused by typhoid and non-typhoid salmonellae. *S. typhi* is highly invasive and causes typhoid fever, a systemic bacteremic illness as opposed to

non-typhoid salmonellosis characterized by gastroenteritis and marked by diarrhoea, fever, and abdominal pain with rare systemic invasion.

The most extensive human feeding trials of non-typhoid *Salmonella* were conducted in the late 1940s to early 1950s (McCullough and Eisele, 1951a; McCullough and Eisele, 1951b; McCullough and Eisele, 1951c). A total of six different *Salmonella* serotypes were used with up to 3-4 different strains of some of the serotypes. The subjects used in the feeding trials were healthy males from a penal institution. Feeding trials using *S. pullorum* I, II, III & IV were considered to be inappropriate for deriving estimates about the infectivity of non-typhi *Salmonella* for humans, because, as noted by other researchers (Blaser and Newman, 1982; Coleman and Marks, 1998) this is primarily a fowl-adapted strain. It was noted that a dramatically higher dose was required to produce illness using *S. pullorum* and the clinical picture of illness, when it did occur, was characterized by an explosive onset and fast recovery (McCullough and Eisele, 1951c). At dosages producing illness the organism could only be isolated from the stools for the first day or two and not thereafter. In addition, Fazil (1996) conducted an evaluation of the feeding trial data and found that the dose-response relationship for *S. pullorum* was significantly different from the other strains used in the feeding trials.

In order evaluate the data derived from the human feeding trials the experimental design used by these researchers is briefly described (McCullough and Eisele, 1951a; McCullough and Eisele, 1951b):

Human Volunteers

- The subjects selected for the experimental feeding trials were healthy males from a penal institution
- According to the authors, chronic complainers and those who had frequent gastrointestinal disturbances in the past were eliminated from the trials.
- After an initial selection of volunteers, at least three weekly stool cultures were done.
- Only those individuals with no *Salmonella* or other easily confused organisms in the stools were carried further in the experiment
- An initial serum agglutination test was done against the organism to be administered.
- Subjects that showed a moderate or high agglutination titre against a particular organism were in general not used in the experiments with that species.

Source of *Salmonella* Strains

- Strains of *Salmonella* used in the feeding trials were obtained from market samples of high moisture spray-dried whole egg powder.

Method of feeding

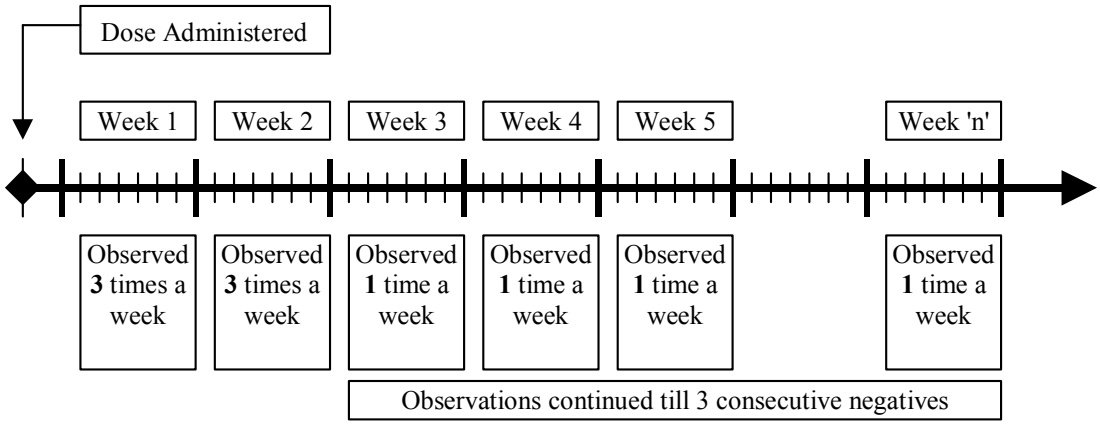
- Cultures for feeding trials were sub-cultured on trypticase soy agar.
- After 24 hours of incubation, the resulting growth was suspended in saline and standardized turbidimetrically.
- The dose was administered in a glass of eggnog shortly following the noon meal.
- A group of men usually consisting of 6, received the same experimental feeding dose.

- Control feedings were provided by eggnog alone or by prior feeding of the test organisms at what the authors observed to be non-infective levels.

Observations after feeding

- Following the feeding, men were interviewed and observed three times a week for a period of two weeks and once a week thereafter
- Additional visits were made when required by the condition of the volunteer
- Men were questioned with regard to symptoms.
- Temperatures were recorded
- Faecal cultures were obtained
- When indicated, blood counts and cultures were also done.
- Blood samples for agglutination were drawn at weekly intervals for 4 weeks longer
- Faecal samples were collected and cultures were done on all men 3 times a week for the first 2 weeks, after that once a week until at least three consecutive negative samples had been obtained.

Figure 1: Schematic for observations during human feeding trial experiment



Infection definition (faecal shedding)

- Infection was defined as the recovery of the administered strain from faecal samples.

Illness definition criteria

- Illness was characterized by the existence of the following two conditions:
  - Documentation of symptoms
  - Recovery of the organism from stool (infection)
  - And one or more of the following:
    - diarrhoea or vomiting,
    - fever

- rise in specific agglutination titre
- or, other unspecified signs.

The feeding trial data (McCullough and Eisele, 1951a; McCullough and Eisele, 1951b) has been reviewed and critiqued by various researchers. Blaser and Newman (1982) reviewed the infective dose of salmonellosis and identified several deficiencies:

1. The feeding of the pathogen to the volunteers was conducted after their noon meal when gastric acid was probably high.
2. It was observed that over half the volunteers who became ill had earlier been fed lower doses of the same serotype. These earlier feedings may have confounded the results by introducing a degree of immunity thus making infection less likely, or, alternatively the earlier feedings may have had a cumulative effect that made infection more likely.
3. A failure to assess the minimal infective dose
4. The use of too few volunteers at low doses.

In the US *Salmonella* Enteritidis risk assessment report (USDA-FSIS, 1998) some additional deficiencies in the feeding trial data were identified:

1. The use of healthy male volunteers could likely underestimate the true pathogenicity to the overall population.
2. The size of the groups used at each of the doses was relatively small, with 18 of the 22 test-doses using less than 6 people.
3. There were no low doses tested, the smallest dose that was tested was greater than 10^4 CFU *Salmonella* bacteria
4. The lowest dose that caused an infection was also the lowest dose tested.

Some additional points related to some of the critiques should also be noted. While it is true that the feeding of the dose after the noon meal when gastric acid was high could potentially reduce the estimated infectivity of the pathogen (Blaser and Newman, 1982), the dose was administered using eggnog, a high fat content medium. The eggnog could have conferred a level of protection against the effects of gastric acid thus potentially negating the acid effects. It seems reasonable however to assume that given the fact that the subjects used in the feeding trials were healthy males, the infectivity estimated for this population will be some factor less than for the general population and more so for the more susceptible members of the general population. Overall, the criticisms of the feeding trial data are for the most part fair in their assessment of the potential biases in the results that may be expected.

The human feeding trial, as described earlier, measured both infection and illness. Most dose-response relationships are developed using infection (faecal shedding) as the dependent variable, primarily out of necessity due to the nature of the data. It should be noted that the use of the infection endpoint in deriving a dose-response relationship could introduce a level of conservatism into the dose-response relationship depending on how the conditional dependence of illness, which is essentially the output of ultimate interest, following infection is treated. In the human feeding trial it was also pointed out that approximately 40% of the volunteers that were shedding were reported to be last positive on or before the second day following administration, apparently clearing the infection two days post administration (Coleman and

Marks, 1998). These authors noted that there is some ambiguity in estimating infection based on faecal shedding for less than two days. The available data measuring illness as the endpoint is sparse, without any response being observed until a dose of approximately 6-logs. It has been noted (Blaser and Newman, 1982) that the strict criteria used by the researchers to define illness may have resulted in volunteers with mild complaints being classified as asymptomatic excretors rather than ill subjects. Although concerns have been raised as to the experimental design of the human feeding trials, it is appropriate to consider it at this juncture as still holding value in providing a basis upon which to at least start exploring the dose-response relationship.

Tables 3a to 3e present the original data from the McCullough and Eisele studies. These data are also summarized in Figure 2.

Table 3a: Feeding trial data for *S. anatum* I, II and III (McCullough and Eisele, 1951a)

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. anatum I	1.20E+04	4.08	2	5	0.40
S. anatum I	2.40E+04	4.38	3	6	0.50
S. anatum I	6.60E+04	4.82	4	6	0.67
S. anatum I	9.30E+04	4.97	1	6	0.17
S. anatum I	1.41E+05	5.15	3	6	0.50
S. anatum I	2.56E+05	5.41	5	6	0.83
S. anatum I	5.87E+05	5.77	4	6	0.67
S. anatum I	8.60E+06	6.93	6	6	1.00
S. anatum II	8.90E+04	4.95	5	6	0.83
S. anatum II	4.48E+05	5.65	4	6	0.67
S. anatum II	1.04E+06	6.02	6	6	1.00
S. anatum II	3.90E+06	6.59	4	6	0.67
S. anatum II	1.00E+07	7.00	6	6	1.00
S. anatum II	2.39E+07	7.38	5	6	0.83
S. anatum II	4.45E+07	7.65	6	6	1.00
S. anatum II	6.73E+07	7.83	8	8	1.00
S. anatum III	1.59E+05	5.20	2	6	0.33
S. anatum III	1.26E+06	6.10	6	6	1.00
S. anatum III	4.68E+06	6.67	6	6	1.00

Table 3b: Feeding trial data for *S. meleagridis* I, II and III (McCullough and Eisele, 1951a)

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. meleagridis I	1.20E+04	4.08	3	6	0.50
S. meleagridis I	2.40E+04	4.38	4	6	0.67
S. meleagridis I	5.20E+04	4.72	3	6	0.50
S. meleagridis I	9.60E+04	4.98	3	6	0.50
S. meleagridis I	1.55E+05	5.19	5	6	0.83
S. meleagridis I	3.00E+05	5.48	6	6	1.00
S. meleagridis I	7.20E+05	5.86	4	5	0.80
S. meleagridis I	1.15E+06	6.06	6	6	1.00
S. meleagridis I	5.50E+06	6.74	5	6	0.83
S. meleagridis I	2.40E+07	7.38	5	5	1.00
S. meleagridis I	5.00E+07	7.70	6	6	1.00
S. meleagridis II	1.00E+06	6.00	6	6	1.00
S. meleagridis II	5.50E+06	6.74	6	6	1.00
S. meleagridis II	1.00E+07	7.00	5	6	0.83
S. meleagridis II	2.00E+07	7.30	6	6	1.00
S. meleagridis II	4.10E+07	7.61	6	6	1.00
S. meleagridis III	1.58E+05	5.20	1	6	0.17
S. meleagridis III	1.50E+06	6.18	5	6	0.83
S. meleagridis III	7.68E+06	6.89	6	6	1.00
S. meleagridis III	1.00E+07	7.00	5	6	0.83

Table 3c: Feeding trial data for *S. newport* (McCullough and Eisele, 1951b)

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. newport	1.52E+05	5.18	3	6	0.50
S. newport	3.85E+05	5.59	6	8	0.75
S. newport	1.35E+06	6.13	6	6	1.00

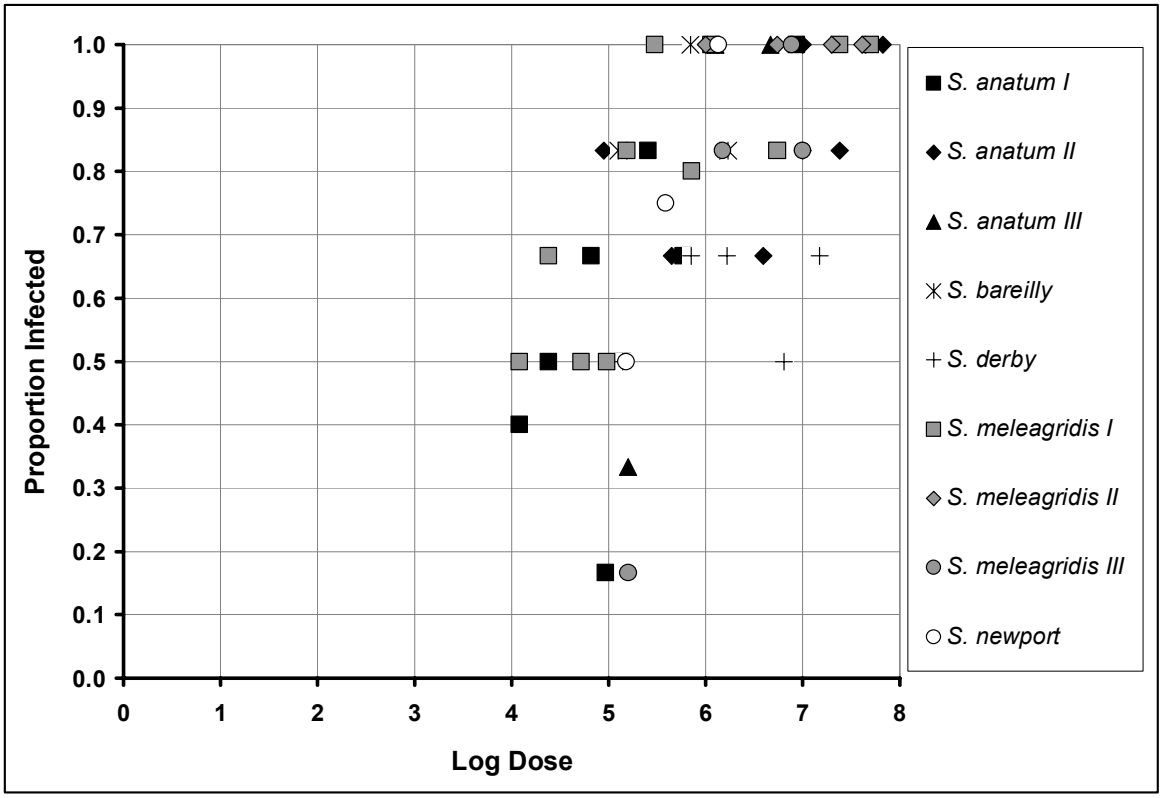
Table 3d: Feeding trial data for S. bareilly (McCullough and Eisele, 1951b)

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. bareilly	1.25E+05	5.10	5	6	0.83
S. bareilly	6.95E+05	5.84	6	6	1.00
S. bareilly	1.70E+06	6.23	5	6	0.83

Table 3e: Feeding trial data for *S. derby* (McCullough and Eisele, 1951b)

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. derby	1.39E+05	5.14	3	6	0.50
S. derby	7.05E+05	5.85	4	6	0.67
S. derby	1.66E+06	6.22	4	6	0.67
S. derby	6.40E+06	6.81	3	6	0.50
S. derby	1.50E+07	7.18	4	6	0.67

Figure 2: Summary of feeding trial data (McCullough and Eisele, 1951a; McCullough and Eisele, 1951b)



It has also been noted that in the feeding trials, some of the volunteers were administered doses more than once. The earlier doses, which were lower and at which no response was observed may have resulted in either a cumulative or immunity effect occurring. In order to attempt to remove this bias, the doses and subjects at which repeat feedings were conducted were edited out and the data re-evaluated. The edited data for naïve subjects only are presented in Tables 4a to 4e, and summarized in Figure 3.

Table 4a: Feeding trial data for *S. anatum* I, II and III for naïve subjects

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. anatum I	1.20E+04	4.08	2	5	0.40
S. anatum I	6.60E+04	4.82	4	6	0.67
S. anatum I	5.87E+05	5.77	4	6	0.67
S. anatum I	8.60E+06	6.93	4	4	1.00
S. anatum II	8.90E+04	4.95	3	4	0.75
S. anatum II	4.48E+05	5.65	4	6	0.67
S. anatum II	2.39E+07	7.38	3	3	1.00
S. anatum II	4.45E+07	7.65	3	3	1.00
S. anatum III	1.59E+05	5.20	2	3	0.67
S. anatum III	1.26E+06	6.10	6	6	1.00
S. anatum III	4.68E+06	6.67	3	3	1.00

Table 4b: Feeding trial data for *S. meleagridis* I, II and III for naïve subjects

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. meleagridis I	1.20E+04	4.08	3	6	0.50
S. meleagridis I	2.40E+04	4.38	4	6	0.67
S. meleagridis I	5.20E+04	4.72	3	6	0.50
S. meleagridis I	1.15E+06	6.06	6	6	1.00
S. meleagridis I	5.50E+06	6.74	5	6	0.83
S. meleagridis I	2.40E+07	7.38	4	4	1.00
S. meleagridis II	1.00E+06	6.00	6	6	1.00
S. meleagridis II	5.50E+06	6.74	6	6	1.00
S. meleagridis II	2.00E+07	7.30	3	3	1.00
S. meleagridis III *	1.58E+05	5.20	1	3	0.33
S. meleagridis III	1.50E+06	6.18	5	6	0.83
S. meleagridis III	7.68E+06	6.89	4	4	1.00

Table 4c: Feeding trial data for *S. newport* for naïve subjects

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. newport	1.52E+05	5.18	3	6	0.50
S. newport	3.85E+05	5.59	4	4	1.00
S. newport	1.35E+06	6.13	3	3	1.00

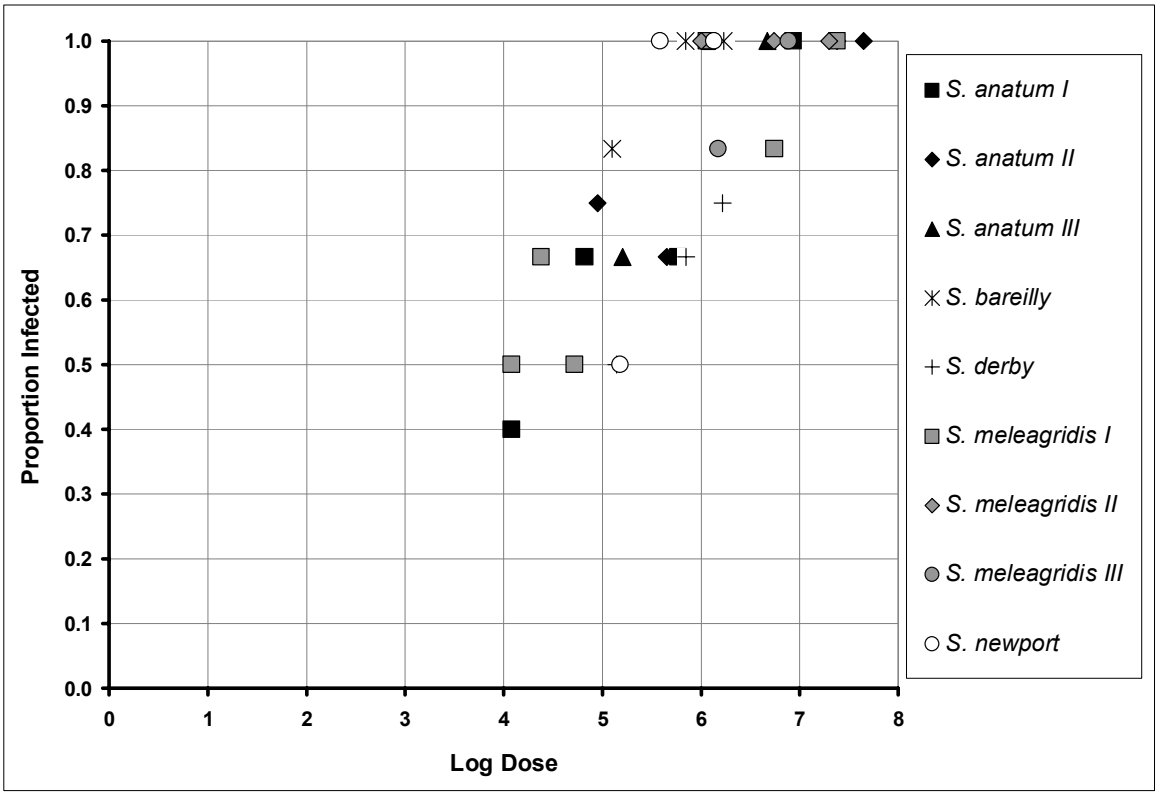
Table 4d: Feeding trial data for *S. bareilly* for naïve subjects

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. bareilly	1.25E+05	5.10	5	6	0.83
S. bareilly	6.95E+05	5.84	3	3	1.00
S. bareilly	1.70E+06	6.23	3	3	1.00

Table 4e: Feeding trial data for *S. derby* for naïve subjects

Serotype	Dose	Log Dose	Positive (Inf)	Total	Proportion
S. derby	1.39E+05	5.14	3	6	0.50
S. derby	7.05E+05	5.85	2	3	0.67
S. derby	1.66E+06	6.22	3	4	0.75
*S. derby**	6.40E+06	6.81	1	3	0.33

Figure 3: Summary of feeding trial data for naïve subjects.



DOSE-RESPONSE MODELS FOR *SALMONELLA*

Several approaches and models to characterize the dose-response relationship for *Salmonella* have been presented in the literature or in official reports and documents. This draft document discusses three different approaches for modelling *Salmonella.* The first model is the beta-Poisson model fit to the human feeding trial data for *Salmonella* (Fazil, 1996). The second model was proposed in the US *Salmonella* Enteritidis risk assessment (USDA-FSIS, 1998) and was based on the use of a surrogate pathogen to describe the dose-response relationship. The third

model, introduced in the Health Canada *Salmonella* Enteritidis risk assessment, used a Weibull dose-response relationship updated to reflect outbreak information using Bayesian techniques. In addition to these models, the current analysis also explores the effect of fitting the beta-Poisson model on the human feeding trial data for naïve subjects only.

Dose-response model fit to non-typhi *Salmonella* human feeding trial data

The human feeding trial data have been analyzed using the beta-Poisson, lognormal (log-probit) and exponential dose-response functional forms (Fazil, 1996). Three doses in the data set were identified as "outliers" (i.e. *S. anatum* I: 9.3E+5; *S. meleagridis* III: 1.58E+5; *S. derby* - 6.4E+6) and were subsequently removed from the analysis. The analysis concluded that both the lognormal and beta-Poisson functional forms fit the majority of the data. However, based upon theoretical considerations (threshold vs. non-threshold) the beta-Poisson model was proposed as the model to describe the dose-response relationship for *Salmonella*. In addition it was reported that all the serotypes could be adequately described using a single beta-Poisson dose-response curve. The parameters of the beta-Poisson dose-response model for non-typhi *Salmonella* in general were reported as alpha = 0.3136, and beta = 3008. The uncertainty in the parameters was estimated using a bootstrap approach, which generated sets of parameters that satisfied the model fitting conditions. The potential for a greater probability of illness for susceptible and normal populations was not addressed in the analysis.

Model Used:	Beta-Poisson
Parameters:	*Alpha = 0.3136* *Beta = 3008*
Comment:	Uncertainty in the parameters estimated using a bootstrap approach which generated a set of alpha and beta parameters that could be randomly sampled in order to incorporate uncertainty.

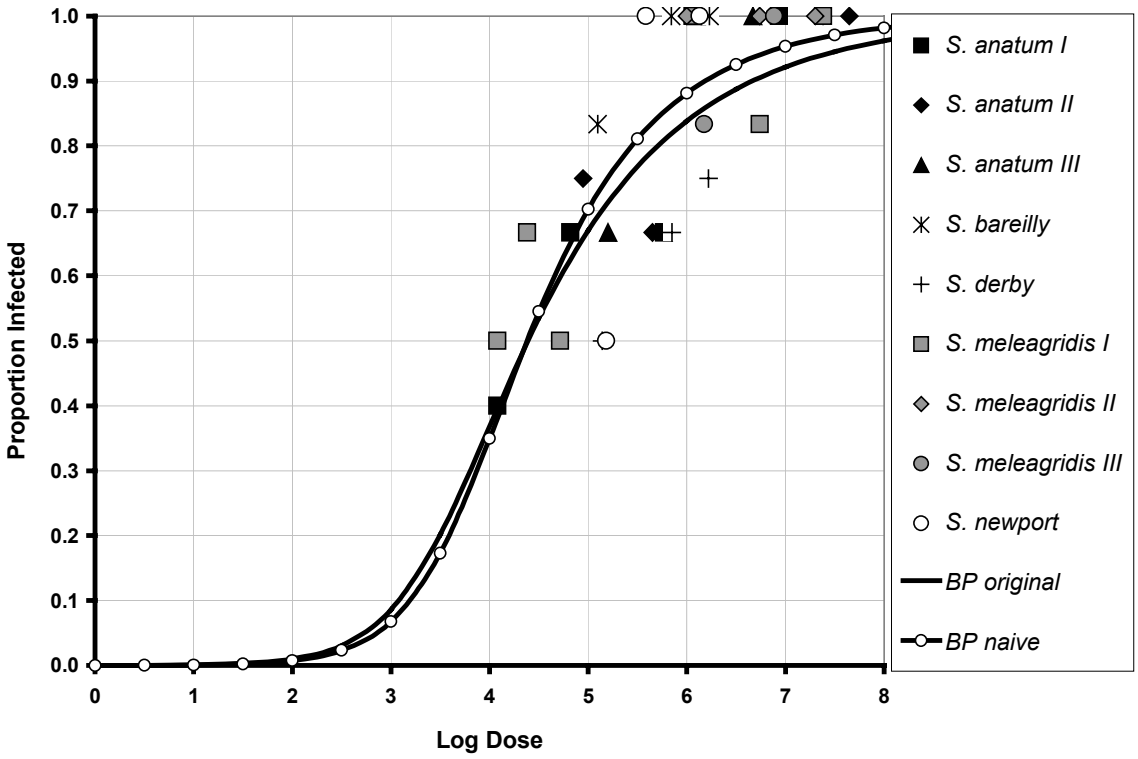
Dose-response model fit to non-typhi *Salmonella* naïve human feeding trial data

The model parameters reported by Fazil (1996) did not consider the effect multiple feedings may have on the dose-response relationship. As a result, for this present review, the data using only naïve subjects (Tables 4a to 4e and Figure 3) were re-fit to the beta-Poisson model and the parameters for this model were estimated. The data was fit using maximum likelihood techniques, as described by various authors (Haas, 1983; Haas *et al*., 1993; Regli *et al*., 1991; Teunis *et al*., 1996). The parameters of the beta-Poisson dose response model fit to the data for naïve subjects was estimated to be alpha = 0.4059, and beta = 5308. The uncertainty in the parameters was estimated using the bootstrap approach.

Model Used:	Beta-Poisson
Parameters:	*Alpha = 0.4059* *Beta = 5308*
Comment:	Uncertainty in the parameters estimated using a bootstrap approach which generated a set of alpha and beta parameters that could be randomly sampled in order to incorporate uncertainty.

The beta-Poisson dose-response curves generated using the original dose-response data and the data edited to reflect only naïve subjects are shown in Figure 4. Also shown in the figure is the feeding trial data to illustrate the fit to the data.

Figure 4: Comparison between dose-response model fit to original feeding trial data and feeding trial data for naïve subjects.



As shown in Figure 4, both models fit the feeding trial data well and the difference between the curves using the original data and the data that reflects only naïve subjects is small. Interestingly, the curve fit to the naive data tends to estimate a greater probability of infection at doses above approximately 10^4, than the curve fit to the original data, perhaps reflecting a tendency in the data for a slightly greater susceptibility for naive subjects. Within the lower dose regions, the two curves are very similar, and the dose translating to a probability of infection for

50% of the population is virtually identical for the two curves (2.44e4 vs. 2.40e4 for the original and naïve models). The low dose extrapolation for the two dose-response curves was also very similar. As a result of the similarities between the models and the concerns that have been raised about potential immunity or cumulative effects, the beta-Poisson model fit to the data of naïve subjects is used in the remainder of this analysis, as the representation of the human feeding trial data fit to the beta-Poisson model.

USDA/FDA *Salmonella* Enteritidis Risk Assessment

The hazard characterization in the U.S. *Salmonella* Enteritidis Risk Assessment (USDA-FSIS, 1998) evaluates the public health impacts of exposure to SE through shell eggs and egg products in terms of numbers of illnesses and specific public health outcomes on an annual basis. Considerations in quantifying the dose-response relationship included the selection of an appropriate functional form, extrapolation of fitted curves to low-dose ranges, and the use of surrogate organisms in the absence of feeding trial data specific for SE.

In the initial quantification of a dose-response relationship for *Salmonella* Enteritidis, a beta-Poisson dose-response curve was initially fitted to the pooled data from all *Salmonella* feeding trials (McCullough and Eisele, 1951a; McCullough and Eisele, 1951a) and the fitted model compared to epidemiological information from available *Salmonella* Enteritidis outbreak data. The model validation on the epidemiological data showed that outbreaks associated with *Salmonella* Enteritidis exhibited a higher attack rate than would be estimated using the pooled human feeding trial data for *Salmonella*. Furthermore, an analysis of variance (ANOVA) on the *Salmonella* human feeding trial data for dose and serotype effects revealed two distinct, statistically significant dose-response patterns (representative of doses $>10^3$ organisms) among the *Salmonella* serotypes in the human feeding studies data (Morales *et al*., 1996; Jaykus *et al*., 1997).

The inability of several dose-response models, fitted to the *Salmonella* data, to predict the high attack rates associated with low doses such as the 1994 *S.* Enteritidis outbreak from ice cream (Hennessy *et al*., 1996) was likewise previously noted by Morales *et al*. (1996). In order to capture the region of concern (i.e., the low-dose range with corresponding high attack rates evident in the outbreak investigation data), human feeding study data utilizing a low-dose organism was selected for subsequent dose-response modelling as a surrogate for SE. The absence of human feeding study data for SE prompted the selection of *Shigella dysenteriae* (Levine and DuPont, 1973) as a proxy for modelling "low-dose" *Salmonella* serotypes (attack rates >0 with doses $\leq 10^3$ organisms).

Epidemiological evidence from outbreak investigations was once again used to conduct a model validation check on the two dose-response models generated (beta-Poisson curves fit to human feeding trial data for pooled *Salmonella* species and to the low-dose proxy *Shigella dysenteriae*). A review of the epidemiological outbreak investigations showed that many of the reported doses resulting in illnesses were several orders of magnitude lower than the doses reported in the *Salmonella* feeding trials. Further, the doses which caused outbreaks were likewise several orders of magnitude lower than the doses which were predicted by the dose-response models constructed from the *Salmonella* feeding trial data. Model validation to the available outbreak investigation data subsequently served as the basis for selection of a dose-response relationship (see Figure 5 below). The outbreak investigation data used for dose-response model validation are detailed in Table 5.

Figure 5: Comparison of available *Salmonella* outbreak investigation data and beta-Poisson dose-response curves for *Shigella dysenteriae* estimated for normal and susceptible subpopulations.

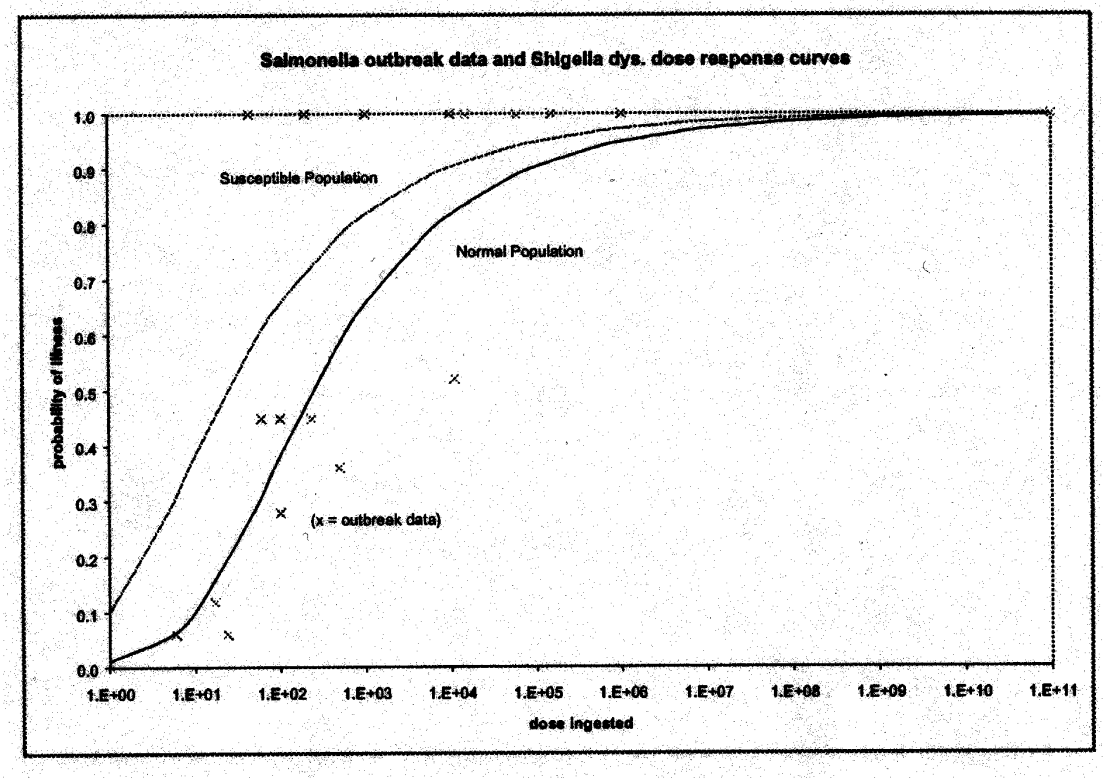


Table 5: Comparison of available *Salmonella* outbreak investigation data and beta-Poisson dose-response curves for *Shigella dysenteriae* estimated for normal and susceptible subpopulations (USDA-FSIS, 1998).

Reference	Serovar	Dose	Log Dose	Number Ill	Attack Rate
Boring *et al*., 1971	Typhimurium	1.7E+01	1.23	16,000	12%
Lipson, 1976	Schwarzengrund	4.4E+01	1.64	1	100%
Fontaine *et al*., 1978	Newport	6.0E+01	1.78	48	45%
D'Aoust *et al*., 1975	Eastbourne	1.0E+02	2.00	95	45%
Fontaine *et al*., 1980	Heidelberg	1.0E+02	2.00	339	28%
George, 1976	Heidelberg	2.0E+02	2.30	1	100%
Fontaine *et al*., 1978	Newport	2.34E+02	2.36	46	45%
Fontaine *et al*., 1980	Heidelberg	5.0E+02	2.70	339	36%
Armstrong *et al*., 1970	Typhimurium	1.1E+04	4.04	1,790	52%
Lang *et al*.. 1967	Cubana	1.5E+04	4.18	28	100%
Lang *et al*., 1967	Cubana	6.0E+04	4.78	28	100%
Reitler *et al*., 1960	Zanzibar	1.5E+05	5.18	6	100%
Angelotti *et al*., 1961	Infantis	1.0E+06	6.00	5	100%
Reitler *et al*., 1960	Zanzibar	1.0E+11	11.00	8	100%
Hennessy *et al*., 1996	Enteritidis	6.0E+00	0.77	>1,000	6%
Vought and Tatini, 1998	Enteritidis	2.4E+01	1.38	>1,000	6%
Levy *et al*., 1996	Enteritidis	1.0E+03	3.00	39	100%
Levy *et al*., 1996	Enteritidis	1.0E+04	4.00	39	100%

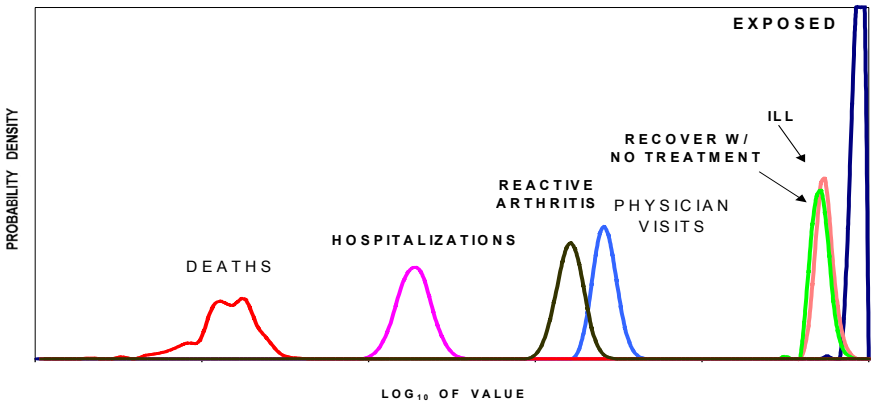
The dose-response relationship subsequently used was a beta-Poisson model fitted to the human feeding trial data for *Shigella dysenteriae* M131 with parameters alpha = 0.2767, and beta = 21.159 (http://www.fsis.usda.gov/OPHS/risk/semodel.htm, July 2000). Uncertainty was introduced into the beta parameter by characterizing it as a normal distribution truncated at zero, with a maximum of 60 and a mean and standard deviation of 21.159 and 20 respectively for the proportion of the population assumed to be in good health (normal subpopulation). In addition, the beta parameter of the *S. dysenteriae* beta-Poisson model was reduced by a factor of 10, thus shifting the curve to the left to estimate a higher probability of illness for susceptible individuals (susceptible subpopulation). Uncertainty in the beta parameter for the susceptible subpopulation was therefore introduced using a normal distribution with a mean and variance of 2.116 and 2.0 respectively and a minimum of 0 and maximum of 6.

The hazard characterization estimated that a given number of people are each exposed to a specific dose of SE bacteria, but assumed that not all individuals exposed to the dose of SE become ill. For each subpopulation (normal and susceptible), the probability of an individual person within a subpopulation becoming ill from exposure to a specified dose of SE was calculated using a stochastic dose-response function that incorporates the uncertainty in parameters of the dose-response function as described earlier. From the number of persons exposed and the computed probability of becoming ill from exposure to a specified dose of SE, the distribution of the number of persons becoming ill in each subpopulation was calculated. The ill persons in each subpopulation were then partitioned into four mutually exclusive groups based on clinical outcomes after exposure to SE and the development of illness. A fifth outcome, long-term or chronic sequelae such as reactive arthritis, was also estimated. The public health outcomes simulated in the hazard characterization, based on the population who become ill given exposure to SE, were:

1. Recovery from illness without medical treatment
2. Treatment by a physician with subsequent recovery from illness
3. Hospitalization with subsequent recovery from illness
4. Death
5. Development of long-term sequelae

These public health outcomes were expressed as probability distributions rather than constants, and thus incorporate the uncertainty contained in the dose-response parameters as well as all other related variables (an example is presented in Figure 6). The number of persons in each of the outcomes of interest was modelled as a normal approximation to the binomial distribution with mean = np and standard deviation = $[np(1-p)]^{½}$. Estimates of the public health outcomes from the simulation were presented in summary statistic form (mean, minimum, maximum, and 90% confidence limits) as well as in graphical form. Sensitivity analysis was also conducted (USDA-FSIS, 1998).

Figure 6: Distribution for exposures and public health outcomes for normal population (total population of 100,000 exposed to 10^3 organisms)



Model Used:	Beta-Poisson	
Parameters:	Normal	*Alpha = 0.2767* *Beta = Normal (μ:21.159, σ:20, min:0, max:60)*
	Susceptible	*Alpha = 0.2767* *Beta = Normal (μ:2.116, σ:2, min:0, max:6)*
Comment:	Human feeding trial data for *Shigella dysenteriae* used as a surrogate. Susceptible population characterized by reducing beta parameter by a factor of 10. Simulation of public health outcomes for normal and susceptible subpopulations incorporates the uncertainty represented in the beta parameters.	

Health Canada *Salmonella* Enteritidis dose-response relationship

The Health Canada *Salmonella* Enteritidis risk assessment used a re-parameterized Weibull dose response model. Bayesian methods were employed as a means to provide a consistent framework for combining information from various sources including feeding and epidemiological studies (Paoli, 2000; Ross, 2000). The Canadian *Salmonella* Enteritidis risk assessment has not currently been published, however a brief description of the procedure used is provided, and the model generated is compared to the other alternatives.

The Canadian model begins with the standard Weibull dose-response model.

$$P = 1 - \exp\left(-\theta \times d^{b}\right)$$

Where "d" is the dose.

The model was re-parameterized as summarized below (Paoli, 2000; Ross, 2000), and this is the equation that is referred to in the remainder of this section.

$$P = 1 - \exp\left(-\exp\left\{b\left[\ln(d) - \kappa\right]\right\}\right)$$

$$\beta = \ln(b)$$

$$\kappa = \frac{-\ln(\theta)}{b}$$

The parameter "b" in the model was characterized by performing a meta-analysis of all the bacterial feeding trial data. This analysis determined that the log transformed value of "b", termed β (β = ln[b]) could be well described using a normal distribution with mean of –1.22 and a standard deviation of 0.025. This characterization of β, for all bacterial pathogens represents between-study variability, which is used as a reference prior (Paoli, 2000; Ross, 2000). Epidemiological data, specifically information generated from the Schwanns ice-cream outbreak (Hennessy *et al*., 1996; Vought and Tatini, 1998), was incorporated into the model by adjusting the parameter "θ".

In order to adjust the parameter "θ", the following equation in terms of epidemiological information was used (Paoli, 2000; Ross, 2000).

$$\theta = \frac{-\ln(1-P)}{X^b}$$

Where "P" represents the attack rate reported in an epidemiological outbreak and "X" represents the dose estimated to have caused the outbreak.

Within the model, the dose ingested was defined stochastically so as to reflect the uncertainty associated with the data. A single value for the attack rate "P" was used, and this was estimated to be 6% (Hennessy *et al*., 1996). The dose was estimated based on the concentration reported and the amount of ice cream consumed. The concentration (CFU/g) was characterized using a lognormal distribution with a mean of 0.15 and a standard deviation of 0.1. The amount of ice cream consumed was estimated using a pert distribution with a minimum of 60, a mode of 130, and a maximum of 260.

A separate dose-response relationship was generated for the susceptible population, which was based on epidemiological information. Specifically, information from a waterborne outbreak of *S. typhimurium* in Riverside, California (Boring III *et al*., 1971), which reported on age specific attack rates, was used to shift the value of "θ"according to the following equation (Paoli, 2000; Ross, 2000; Baumler *et al*., 2000).

$$\theta_{susceptible} = \theta_{normal}\left(\frac{\ln[1-beta_{sus}\{a_s,b_s\}]}{\ln[1-beta_{norm}\{a_n,b_n\}]}\right)$$

Where the parameters ("a" and "b") for the beta distributions are estimated from the reported epidemiological data on the total number of individuals exposed and the number that became ill. The sub-scripts "s" and "n" refer to the data for susceptible and normal populations respectively.

Model Used:	**Re-parameterized weibull**
Parameters:	*Beta = Normal (μ:-1.22, σ:0.025)* *Concentration = Lognormal (μ:0.15, σ:0.1)* *Amount consumed = Pert (min:60, mode:130, max:260)* *Attack Rate = 6.6%* a_s = *231* b_s = *987* a_n = *749* b_n = 5966

Several parameters in the dose-response models described, incorporated uncertainty into their characterization. In order to display the dose-response curves in the following sections, the uncertainty in those parameters has been simulated and the specified moments displayed.

The following abbreviations are introduced and will be used when referring to the dose-response curves: Canadian normal population dose-response ("Can-norm"), Canadian susceptible population dose-response ("Can-susc"), US normal population dose-response ("US-norm"), US susceptible population dose-response ("US-susc"), and beta-poisson dose-response curve fit to naïve subject human feeding trial data ("Naïve-BP").

Figure 7a & b: Dose-response curves for normal (Can-norm) and susceptible (Can-susc) populations as estimated in Canadian *Salmonella* Enteritidis risk assessment

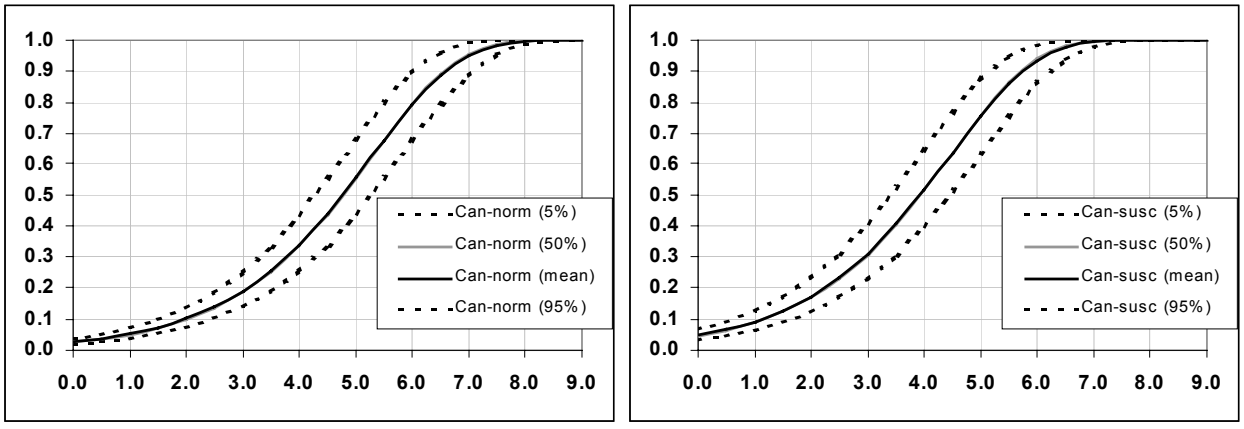


Figure 7c & d: Dose-response curves for normal (US-norm) and susceptible (US-susc) populations as estimated in U.S. *Salmonella* Enteritidis risk assessment

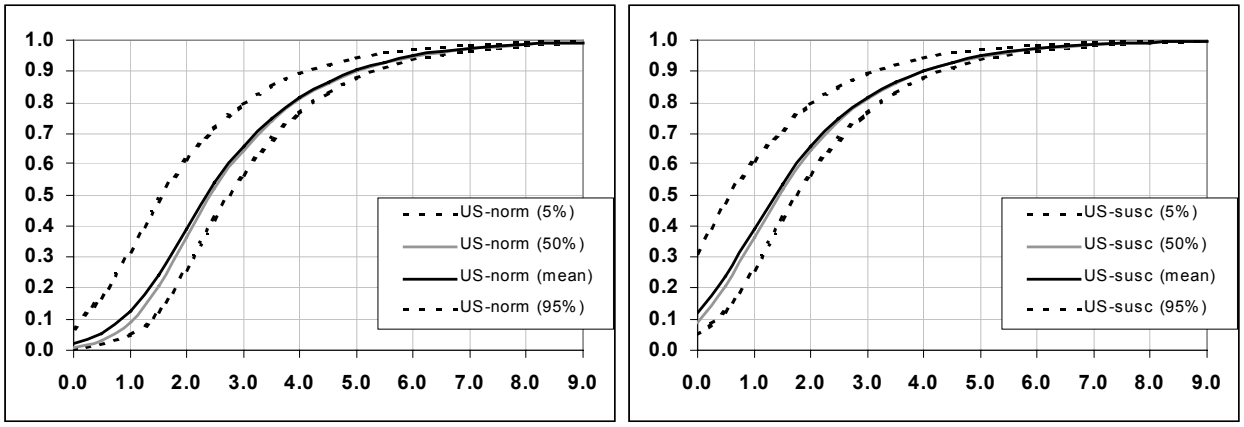
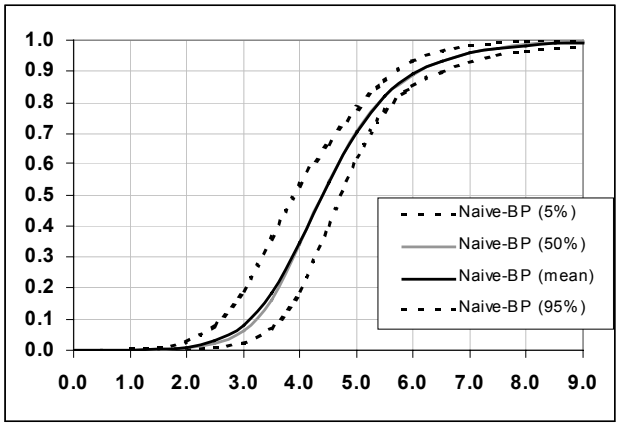
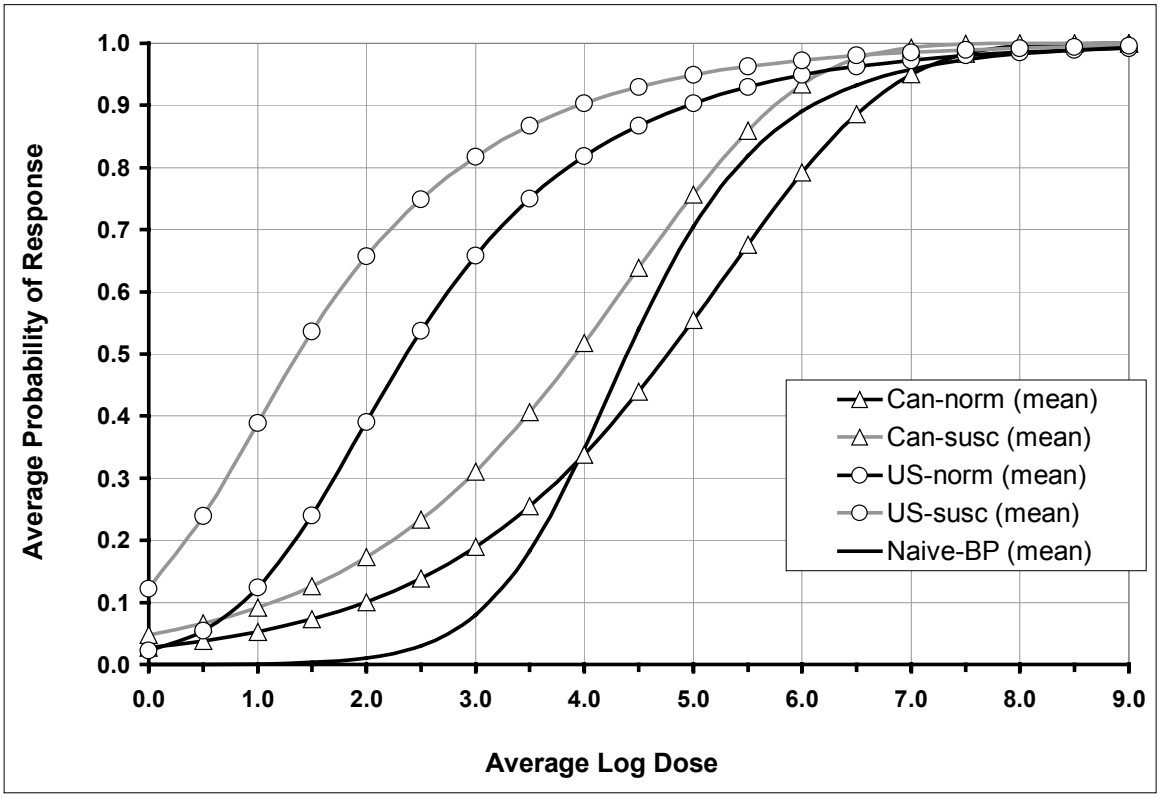


Figure 7e: Beta-poisson dose-response curve fit to naïve subject non-typhi *Salmonella* human feeding trial data (Naïve-BP).



The 5 dose-response curves are plotted together in Figure 8 to assist in the comparison of the curves. Since the 50th percentile and the mean are very similar in all 5 dose-response curves (Figure 7a-e), only the mean value for the curves is plotted. The 95th percentile and 5th percentile boundaries for the curves are omitted from this figure for visual reasons.

Figure 8: Comparison between 5 dose-response curves (Canadian normal population model, Canadian susceptible population model, US normal population model, US susceptible population model, and beta-Poisson model fit to naïve subject data set)



There is some overlap between the "Can-norm" dose-response curve and the "Naïve-BP" dose-response curves. However, the "Naïve-BP" curve estimates a higher probability of response than the "Can-norm" for individuals exposed to a dose greater than approximately 10^4 cells. At an average dose of less than approximately 10^4 cells the "Can-norm" dose response curve estimates a greater probability of response than the "Naïve-BP". In fact, at an average dose of 2 log (100 cells) the "Can-norm" dose-response curve estimates a probability of response of approximately 10% compared to approximately 1% for the "Naïve-BP" dose-response curve. The adjustment of the Canadian dose-response curve to reflect epidemiological information, specifically the 6% response rate at a dose of approximately 1 log (Hennessy *et al*., 1996; Vought and Tatini, 1998) is evident in the behaviour of the curve at that lower dose region.

The "US-norm" and "US-susc" dose-response curves, which are based on using *Shigella* as a surrogate pathogen, estimate a higher probability of illness at a given dose than the other dose-response curves across almost the entire dose range except the lowest (10 or less organisms). At the 2 log (100 cells) average dose level, the normal population using the US dose response curve would be estimated to have approximately a 40% average probability of response and the susceptible population would be estimated to have approximately a 65% probability of response. This can be compared to 10% and 18% for normal and susceptible populations using the Canadian dose-response curves.

The dose-response curves thus have a significant degree of deviation from each other. Selecting a dose-response curve from this information would have to be based on numerous considerations which include: the level of conservatism that one wishes to employ; the theoretical acceptability of using a surrogate pathogen; the biological plausibility of various functional forms for modelling dose-response relationships; the biological endpoint or public health outcome of interest; or the acceptability of the human feeding trial data in capturing the overall response for a population.

In order to gain additional insight into the pathogenesis of *Salmonella*, the available data from epidemiological information was explored.

EPIDEMIOLOGICAL INFORMATION

Epidemiological data can provide valuable insight into the pathogenicity of microorganisms as it applies to the general population. In a sense, outbreaks represent realistic feeding trials with the exposed population often representing a broad segment of society, the doses are essentially real-world levels and the medium carrying the pathogen represents a range of characteristics (protective, fatty, long residence time, etc). Ideally, an epidemiological investigation should attempt to collect as much quantitative information as possible in order to lend itself to better characterizing the dose-response relationship for microbial pathogens. In order to refine the dose-response relationship so that it has greater applicability to the general population, two pieces of information are required in an epidemiological investigation: the dose and the attack rate.

The dose that is suspected to have caused illness in a specific outbreak is often the most difficult measure in an investigation. The lack of dose estimates can be attributed to either the inability to obtain samples of contaminated food or the lack of emphasis being placed on the value of such information. Oftentimes, contaminated food is tested and only the presence or absence of the suspected pathogen is reported. This information is often viewed as sufficient to incriminate the food, however it does little in furthering our knowledge of the dose-response relationship.

The attack rate represents the response in a dose-response relationship. In order to estimate the attack rate, an accurate estimate of the population that was exposed to the contaminated food as well as the number of individuals that got sick is required. In addition, it is valuable to know the characteristics of the exposed and affected population, in order to account for potential susceptibility issues.

Summary of epidemiological and outbreak information

The following sections present and summarize outbreaks found in the literature that included quantitative information from which the dose and attack rate could be estimated. It is important to note that although these outbreaks include quantitative data, some assumptions had to be made depending on the nature of the information. In the interest of transparency, the following sections present the information from the original epidemiological reports in as much detail as possible and where appropriate, the assumptions that are used are clearly indicated. The information used for further analysis from each of the reports is highlighted in the tables.

In addition, reports that are currently unpublished were received from Ministry of Health and Welfare, Japan. Although these reports have not been published, and the details of the methods

used in the investigations have not been stated (other than through personal communication), they represent a valuable source of information on the real world dose-response relationship and expand our database of *Salmonella* pathogenicity considerably. The data in these reports are generated as part of the epidemiological investigations that take place in Japan following an outbreak of foodborne illness. In accordance with a Japanese notification released on March 1997, large scale cooking facilities which 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. Fifty gram portions of each raw food material and each cooked dish are saved for more than 2 weeks at temperature lower than –20ºC. Although this notification is not mandatory, it 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. (Some of the local governments in Japan also have local regulations that require food saving, but the duration and the storage temperature requirements can vary among them).

In the evaluation of the outbreak data, whenever sufficient information was available, susceptible and normal populations were separated out of the database to aid in further analysis. Children ages 5 or younger were considered to be susceptible populations. The criteria or assumptions used to identify potentially susceptible populations are noted in the individual outbreak summaries.

To provide context and more information for the reader, several assumptions may be presented for any one outbreak, with the rationale. However, only the data shown in highlighted rows, bolded comments in the footnotes, were used in further analyses.

Number: 1

Reference: (Boring III *et al*., 1971)

Serovar: *S. typhimurium*

Setting: Citywide municipal water

Medium: Water

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.70E+01	#/Litre	0.75	Litre	1.28E+01	1.11	8788	1035	11.78%
2	1.00E+03	#/Litre	0.75	Litre	7.50E+02	2.88	8788	1035	11.78%
3	1.00E+02	#/Litre	0.75	Litre	7.50E+01	1.88	8788	1035	11.78%
4	1.00E+02	#/Litre	0.75	Litre	7.50E+01	1.88	295	87	29.49%
5	1.00E+02	#/Litre	0.75	Litre	7.50E+01	1.88	389	23	5.91%
6	1.00E+02	#/Litre	0.75	Litre	7.50E+01	1.88	7572	805	10.63%
7	1.00E+02	#/Litre	0.75	Litre	7.50E+01	1.88	1216	230	18.91%

Footnotes

1. Concentration found in tap water using composite sample with reported average attack rate
2. Order of magnitude for concentration found in tap water based on single sample collected independently with reported average attack rate
3. Assumed concentration based on two reported concentrations with reported average attack rate
4. Assumed concentration with highest reported attack rate
5. Assumed concentration with lowest reported attack rate
6. **Assumed concentration with attack rate reported for individuals > 5 years old (assumed "normal" population).**
7. **Assumed concentration with attack rate reported for individuals < 5 years old (assumed "susceptible" population).**

Comments

Composite water samples were collected late in the epidemic (nine days after initial case) and water in the composite sample had been stored for one to four days at room temperature prior to culturing. Varying amounts of water from a few ml to as much as 500ml were pooled from several sample bottles, it is thus possible that numbers in some samples were greatly diluted by negative samples. The pooled sample consisted of water from 74 different samples, and only 5 of the 74 samples were actually positive.

The concentration of 1000/L was estimated from a single isolation made independently from a 1ml sample (suggesting an order of magnitude of 1000 organisms/litre).

The pooled or composite sample indicates an order of magnitude of about 10/Liter, whereas the other individual isolation indicates approximately and order of magnitude of 1000/Liter. A mid-point was selected with an order of magnitude of 100/Liter.

A house to house survey was conducted that comprised 8788 people, with 1035 reporting gastro-enteric illness. The highest attack rate according to census tract was reported in the University of California, 29% (87/295), while the lowest attack rate was approximately 6%, (23/389). It should be noted that the attack rates listed in this table assume exposure to the pathogen only once during the outbreak. The report also identified attack rates according to age, which was used in the current analysis as an estimate of the attack rate for susceptible and normal populations. Children under 5 years old (assumed susceptible) were reported to have an 18.9% attack rate, compared to approximately 11% for the rest of the population.

Number: 2

Reference: (Fontaine *et al*., 1980)

Serovar: *S. heidelberg*

Setting: Restaurant

Medium: Cheddar Cheese

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	3.60E-01	#/100g	28	g	1.01E-01	-1.00	140	50	35.71%
2	1.80E+00	#/100g	28	g	5.04E-01	-0.30	140	50	35.71%
3	1.08E+00	#/100g	28	g	3.02E-01	-0.52	140	50	35.71%
4	1.08E+01	#/100g	28	g	3.02E+00	0.48	140	50	35.71%
5	1.08E+02	#/100g	28	g	3.02E+01	1.48	140	50	35.71%
6	1.08E+03	#/100g	28	g	3.02E+02	2.48	140	50	35.71%

Footnote

1. Concentration reported by Food Research Institute, Wisconsin, US and attack rate for patrons and employees.
2. Concentration reported by CDC in Atlanta, US and attack rate for patrons and employees.
3. Average of two reported concentrations
4. Average concentration adjusted for a 90% die-off prior to culturing
5. **Average concentration adjusted for a 99% die-off prior to culturing**
6. **Average concentration adjusted for a 99.9% die-off prior to culturing**

Comments

Samples analyzed by the CDC in Atlanta were reported to have an MPN of 1.8 organisms/100 grams while the Food Research Institute in Wisconsin reported an MPN of 0.36 organisms /100

grams. According to the restaurant, the serving size was approximately 28 grams of cheese per meal. The potentially very low infectious dose for this outbreak was noted by the researchers, and even if a 99.9% die-off occurred prior to culturing, the dose would have still have been approximately 100 CFU per 28gram serving (360/100 x 28) to 500 CFU per 28grams (1800/100 x 28).

In the current analysis the attack rate was estimated by combining the attack rates reported for employees and patrons of the restaurant. Overall, 46 employees and 94 patrons were reported to have been exposed and 50 individuals becoming ill. It was further assumed that there was a 99% to 99.9% die-off prior to culturing.

Number: 3

Reference: (Lang *et al*., 1967)

Serovar: *S. cubana*

Setting: Hospital

Medium: Carmine dye capsules

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	3.00E+04	#/capsule	0.5	capsule	1.50E+04	4.18	14	12	85.71%
2	3.00E+04	#/capsule	1	capsule	3.00E+04	4.48	14	12	85.71%
3	3.00E+04	#/capsule	2	capsule	6.00E+04	4.78	14	12	85.71%

Footnote

1. Low dose estimate given that some individuals received 1/2 a capsule.
2. **Mid dose estimate based on 1 capsule.**
3. High dose estimate given that some patients received 2 capsules.

Comments

This outbreak involved a susceptible population that consisted of debilitated and aged people, infants and persons with altered GI function. Carmine dye capsules are used as a faecal dye marker for such things as the collection of timed stool specimens, GI transit time and the demonstration of GI fistulas. The number of capsules given to patients ranged from 1/2 to 2. There were a total of 21 recognized cases during this outbreak, however 4 were reported to have been infected prior to admission and 5 cases were suspected to have been secondary transmission. Therefore, there were 12 confirmed cases directly as a result of carmine dye capsule ingestion. Unfortunately, for attack rate estimation the total number of exposed individuals was not determined, however the authors of the report note that there were some people who received carmine but were not infected. It was thus inferred that the attack rate was less than 100%, and as an upper bound it was assumed that 2 additional individuals received dye capsules and did not get infected.

Number: 4

Reference: (Angelotti *et al*., 1961)

Serovar: *S. infantis*

Setting: Home

Medium: Ham

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	2.30E+04	#/g	50	g	1.15E+06	6.06	8	8	100.00%
2	2.30E+04	#/g	100	g	2.30E+06	6.36	8	8	100.00%
3	2.30E+04	#/g	200	g	4.60E+06	6.66	8	8	100.00%

Footnotes

1. Overall attack rate and dose given smallest consumption amount (1 slice of 50 grams)
2. **Overall attack rate and dose given most likely consumption amount (1 large slice of 100 grams or 2 small slices of 50 grams)**
3. Overall attack rate and dose given higher consumption amount (2 large slices of 100 grams)

Comments

This outbreak occurred in a family consisting of adults and at least two grade school age kids. Smoked ham purchased from a supermarket was taken home and refrigerated for approximately 5 hours. 8 people in the family ate either raw or fried slices of ham, and all 8 experienced acute diarrhoea with gastroenteritis symptoms within 8 to 24 hours. An uneaten portion of ham was obtained and examined in laboratory 2 days after the outbreak occurred. Various bacteria were isolated from the raw ham: Total aerobic plate count (268,000,000/gram), Coliform bacteria (15,000/gram), Streptococcus faecalis (31,000,000/gram), Staphylococci (200,000,000/gram) and *S. infantis* (23,000/gram). Staphylococci were negative for coagulase production, and negative for enterotoxin production. Stools from 4 of the 8 persons affected were examined 10 days after the outbreak: mother, father and two grade school age sons. *S. faecaalis* isolated from both parents and one son, *S. faecaalis var. liquefacies* was isolated from other son and *S. infantis* was isolated from both parents but not the sons. The researchers noted that *S. infantis* in the ham, stools and the long incubation period implies infection of *Salmonella* aetiology. However a mixed infection is a possibility.

The weight of a slice of ham was estimated to range from 50 to 100 grams, with 1 to 2 slices consumed. The average consumption was assumed to be 100 grams in this analysis. Furthermore, the exposed population was assumed to be made up of normal individuals. Even if a child younger than 5 years was involved, since the attack rate was 100% for the whole exposed population these assumption does not have a big impact on differentiating between the susceptible and normal population at this dose.

Number: 5

Reference: (Armstrong *et al*., 1970)

Serovar: *S. typhimurium*

Setting: Various parties and banquets

Medium: Imitation ice-cream

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.13E+04	#/75gram	75	grams	1.13E+04	4.05	3450	1790	51.88%
2	1.13E+02	#/75gram	75	grams	1.13E+02	2.05	1400	770	55.00%
3	1.13E+03	#/75gram	75	grams	1.13E+03	3.05	1400	770	55.00%
4	1.13E+04	#/75gram	75	grams	1.13E+04	4.05	1400	770	55.00%

Footnote

1. Reported concentration adjusted for authors' experimental evidence of up to 2-log reduction prior to testing the food, and overall attack rate for all the outbreaks.
2. Reported concentration and specific attack rate for limited menu outbreak
3. **Reported concentration with 1-log adjustment and attack rate for limited menu outbreak**
4. **Reported concentration with 2-log adjustment and attack rate for limited menu outbreak**

Comments

This episode involved 14 outbreaks with a total of 3450 people attending the various events at which imitation ice-cream (chiffonade) was identified as the vehicle of infection. The authors estimated a 52% attack rate based on a survey of persons attending seven of the events. The menus at the various events were relatively extensive, however one of the outbreaks involved a large affair with a limited menu in which the authors cite that nearly all the people had eaten all of the foods offered. Using this outbreak the attack rate was estimated to be 55% (1400 people attending and 770 people sick).

The chiffonades were stored at -20C for 1 month before quantitative cultures were done, and the MPN was reported to be 113 or fewer salmonellae per 75 gram serving. The reduction in numbers that could be expected due to freezing was experimentally determined by artificial inoculation of *S. typhimurium* into chiffonade and storing these samples at -20C. Artificial inoculation experiments indicated that no more than a 2-log reduction occurred during the one-month storage. As a result, the concentration was estimated to be approx. 11300 per serving.

Number: 6

Reference: (Fontaine *et al*., 1978)

Serovar: *S. newport*

Setting: Interstate: (Maryland: households, Colorado: households, Florida: naval base)

Medium: Hamburger

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	6.00E+00	#/100gram	100	gram	6.00E+00	0.78	?	48	?
2	2.30E+01	#/100gram	100	gram	2.30E+01	1.36	?	48	?

Footnote

1. Lowest concentration reported, undetermined attack rate
2. Highest concentration reported, undetermined attack rate

Comments

The concentration of *S. newport* in ground beef was determined from MPN to be between 6 to 23 per 100 grams. Accounting for freezing, the authors cite that experimental evidence would indicate a 1 to 2 log reduction due to freezing which would place the concentration at 60-2300 per 100 gram. However, cooking, even undercooking is likely to produce a reduction prior to consumption. If the effects of cooking are conservatively assumed to be 1-2 log, then the concentration prior to consumption is again estimated to be 6 to 23 per 100grams. Assuming consumption of 100 grams the dose that was capable of causing an infection in some people can be estimated to be approximately 6 to 23 organisms. Unfortunately, this outbreak was geographically widespread and the authors did not report the total number of individuals that were exposed. The attack rate is therefore undetermined in this outbreak.

Number: 7

Reference: (Fazil, 1996)

Serovar: *S. newport*

Setting: Naval Base

Medium: Hamburger

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	6.00E+00	#/100gram	100	gram	6.00E+00	0.78	7254	21	0.29%
2	2.30E+01	#/100gram	100	gram	2.30E+01	1.36	7254	21	0.29%
3	1.50E+01	#/100gram	100	gram	1.50E+01	1.18	7254	21	0.29%
4	1.50E+01	#/100gram	100	gram	1.50E+01	1.18	3627	21	0.58%
5	1.50E+01	#/100gram	100	gram	1.50E+01	1.18	725	21	2.90%
6	1.50E+01	#/100gram	100	gram	1.50E+01	1.18	73	21	28.77%

Footnote

1. Low concentration reported with attack rate based on entire population of recruits being exposed
2. High concentration reported with attack rate based on entire population of recruits being exposed
3. Average concentration with attack rate based on entire population of recruits being exposed
4. Average concentration with attack rate based on 50% population of recruits being exposed
5. **Average concentration, attack rate based on 10% population of recruits being exposed**
6. Average concentration with attack rate based on 1% population of recruits being exposed

Comment

The data in this outbreak is derived from the prior episode described (Fontaine *et al*., 1978). However, Fazil (1996) examined the naval outbreak in greater detail through a series of personal communications with the US Navy to attempt to determine an attack rate. A total of 21 cases occurred at the naval training centre, 2 were asymptomatic food handlers and 19 were trainees. The entire complex had a population of 12483 with the military population listed as 9904 (full time military personnel and trainees). Meals were served at several locations, and included: the galley, the staff galley and the exchange cafeteria. The outbreak was reported to have occurred at the "Training Station", which is a separate area within the centre where training is conducted. There were 7254 recruits who were fed at the galley, servicing the trainees. Therefore, depending on the assumed number of people that ate a contaminated hamburger, an attack rate can be estimated/approximated. Assuming 7254 individuals exposed (all present which is unlikely), the attack rate is estimated to be 0.3% (21/7254). Perhaps a more reasonable estimate is to assume 10% of the individuals were exposed, i.e., attack rate of 2.9% (21/725); if only 1% of the population was exposed then the attack rate is estimated to be 28.8% (21/73).

Number: 8

Reference: (Narain and Lofgren, 1989)

Serovar: *S. newport*

Setting: Restaurant

Medium: Pork and Ham sandwiches

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	4.40E+07	#/gram	75	gram	3.30E+09	9.52	200	105	52.50%
2	4.40E+07	#/gram	75	gram	3.30E+09	9.52	120	105	87.50%
3	4.40E+07	#/gram	75	gram	3.30E+09	9.52	105	105	100.00%

Footnote

1. Reported concentration with attack rate based on restaurant managers recollection of the total number of people served at the restaurant
2. Reported concentration with attack rate based on the assumption that 60% of the people served were actually exposed
3. Reported concentration with attack rate based on worst case scenario of all exposed individuals becoming sick (approximately 53% of those reported to have eaten at the restaurant were exposed).

Comments

A total of 105 people were reported to have become ill during this episode that was attributed to ham and pork sandwiches. The sandwiches were suspected to have been contaminated at the restaurant and a refrigerated portion of a pork sandwich from a patient yielded $44x10^6$ *S. newport* per gram.

The attack rate that might be inferred from information provided in this report would be highly uncertain. The restaurant reported serving approximately 200 people during the period of which 105 got ill. However the information required is an estimate of the number of people that actually ate ham and pork sandwiches and were exposed to contaminated food. It can be assumed that not everyone ate the ham and pork sandwiches, if it is assumed that 60% of the people visiting the restaurant ate the contaminated food, then 120 people may have been exposed. At the other extreme it could also be assumed that only 105 people were actually exposed and 105 got sick, this would place the attack rate at 100%.

Number: 9

Reference: (Craven *et al*., 1975)

Serovar: *S. eastbourne*

Setting: Interstate-Homes

Medium: Chocolate balls

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	2.50E+00	#/gram	450	gram	1.13E+03	3.05	?	80	?
2	2.50E+00	#/gram	225	gram	5.63E+02	2.75	?	80	?
3	2.50E+00	#/gram	45	gram	1.13E+02	2.05	?	80	?

Footnote

1. Reported concentration with dose estimated based on the consumption of an entire bag of chocolates (approximately 50 chocolate balls), indeterminate attack rate
2. Reported concentration with dose estimated based on the consumption of half a bag of chocolates (approximately 25 chocolate balls), indeterminate attack rate
3. Reported concentration with dose estimated based on the consumption of approximately 5 chocolate balls, indeterminate attack rate

Comments

This outbreak involved a potentially susceptible population and involved chocolate balls. The median age of the cases in this outbreak was 3 years old. The attack rate cannot be determined in this case because no information was provided in the report and the geographically widespread nature of the outbreak makes inferences difficult. The outbreak occurred simultaneously in the US and in Canada. The description of the Canadian portion of the outbreak is described in the next section (D'Aoust *et al*., 1975).

The New Jersey health department reported a mean concentration of 2.5 salmonellae per gram of chocolate from samples obtained from homes where cases occurred. A bag of the chocolate was reported to be 1 lb or approximately 450 grams, therefore the maximum dose causing infection in some people was estimated to be no more than approximately 1000 cells (2.5/gram x 450grams). Alternatively, the dose could be as low as 100 cells if only 40 grams was consumed (2.5 x 40).

Number: 10

Reference: (D'Aoust *et al*., 1975)

Serovar: *S. eastbourne*

Setting: National-Homes

Medium: Chocolate balls

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	2.00E+00	#/ball	50	balls	1.00E+02	2.00	?	95	?
2	9.00E+00	#/ball	50	balls	4.50E+02	2.65	?	95	?
3	5.50E+00	#/ball	50	balls	2.75E+02	2.44	?	95	?
4	5.50E+00	#/ball	25	balls	1.38E+02	2.14	?	95	?
5	5.50E+00	#/ball	5	balls	2.75E+01	1.44	?	95	?

Footnote

1. Lower concentration reported and dose estimated from consumption of entire bag of chocolates (50 balls), indeterminate attack rate
2. Higher concentration reported and dose estimated from consumption of entire bag of chocolates (50 balls), indeterminate attack rate
3. Average concentration and dose estimated from consumption of entire bag of chocolates (50 balls).
4. Average concentration and dose estimated from consumption of half a bag of chocolates (25 balls).
5. Average concentration and dose estimated from consumption of 5 chocolate balls.

Comments

This outbreak again involved a potentially a susceptible population, 46% of the cases were in children aged 1-4 years old. There were a total of 95 reported cases. The outbreak was attributed to chocolate balls, each ball was reported to weigh approximately 10 grams, with a bag of chocolate containing approximately 50 balls. The contamination of the chocolate balls was estimated to be 2 to 9 salmonellae per chocolate ball. This outbreak was the Canadian part of the outbreak that also occurred simultaneously in the US and described previously (Craven *et al*., 1975).

The dose causing illness in some of the exposed population was estimated by the authors based on the consumption of a bag of chocolate. This estimate, which may be high considering the consumption of 50 chocolate balls, would place the dose at approximately 100 to 450 cells. Depending on the assumption of the amount of chocolate that was consumed the dose causing illness could be as low as 2 cells if only one ball was consumed at the lowest concentration. However, it is difficult to determine with the given information exactly how much chocolate sick

individuals consumed and what the concentration on those chocolate balls was. The overall attack rate for this outbreak is also difficult to estimate, similar to Craven *et al*. (1975), due to the geographically widespread nature of the outbreak.

Number: 11

Reference: (Levy *et al*., 1996); (USDA-FSIS, 1998)

Serovar: *Salmonella* Enteritidis

Setting: Hotel

Medium: Raw shell eggs (hollandaise sauce)

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.00E+03	#/gram	10	gram	1.00E+04	4.00	39	39	100.00%
2	1.00E+04	#/gram	10	gram	1.00E+05	5.00	39	39	100.00%

Footnote

1. **Concentration reported from informal quantification, dose estimated from consumption of 2 tablespoons of sauce and attack rate estimated from all individuals consuming hollandaise sauce becoming ill**
2. **1-log higher concentration from informal quantification results, dose estimated from consumption of 2 tablespoons of sauce and attack rate estimated from all individuals consuming hollandaise sauce becoming ill**

Comment

In this outbreak a total of 56 persons who ate at a Washington, D.C. hotel had onset of diarrhoea. The D.C. public health department conducted an investigation on the outbreak involving 41 ill patrons and 23 well patrons. The investigation incriminated hollandaise sauce as the likely vehicle after it was found that all 39 persons who had eaten hollandaise sauce became ill. Furthermore, according to the USDA (USDA-FSIS, 1998) only 39 persons ate the hollandaise sauce and all 39 became ill, which would imply a 100% attack rate. It is uncertain if these 39 people were from the 41 ill patrons used in the investigation or of the total 56 people who got sick. If the 39 people were from the investigation of 41 people it is possible that there were individuals in the 15 who were not investigated that ate hollandaise sauce and did not get ill, placing the attack rate at some value below 100%. In the current analysis however, it is assumed that the attack rate was indeed 100%.

The actual concentration of *Salmonella* Enteritidis causing illness in this outbreak was not reported in the publication describing the outbreak (Levy *et al*., 1996), however the USDA-FSIS (1998) reported the results of some testing. This informal quantification, which was not performed to extinction, tested a sample of sauce recovered from a patron who had taken it home in a "doggie bag" and refrigerated it for 72 hours. The concentration in this sample was reported to be 10^3 per gram. It was assumed that 2 tablespoons (approximately 10 grams) were consumed

by the patrons of the restaurant, placing the dose at approximately 10^4 (USDA-FSIS, 1998). The actual concentration, it should be noted, could be higher given the nature of the analysis, or, the attack rate as noted previously could potentially have been lower than 100%.

Number: 12

Reference: (Vought and Tatini, 1998); (Hennessy *et al*., 1996); (USDA-FSIS, 1998)

Serovar: *Salmonella* Enteritidis

Setting: Interstate

Medium: Ice-cream

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	9.30E-02	#/gram	73	g	6.79E+00	0.83		>1000	6.60%
2	4.62E-01	#/gram	73	g	3.37E+01	1.53		>1000	6.60%
3	1.52E-01	#/gram	73	g	1.11E+01	1.05		>1000	6.60%
4	8.94E-01	#/gram	73	g	6.53E+01	1.81		>1000	6.60%
5	1.55E-01	#/gram	73	g	1.13E+01	1.05		>1000	6.60%
6	2.76E-01	#/gram	73	g	2.01E+01	1.30		>1000	6.60%

Footnote

1. Mode of concentration reported from 6 consumer samples using traditional MPN analysis, and reported attack rate.
2. Maximum concentration reported using traditional MPN analysis.
3. Mode of concentration reported using Bayesian analysis of MPN data.
4. Maximum concentration reported using Bayesian analysis of MPN data.
5. Average concentration from traditional MPN analysis results.
6. **Average concentration from Bayesian analysis of MPN data.**

Comment

This outbreak involved an interstate outbreak attributed to ice-cream. The report provides details on the epidemiological characteristics of the outbreak and reports on the concentration found in samples of ice-cream using traditional MPN techniques (Hennessy *et al*., 1996) A re-analysis of the quantitative MPN results was performed using alternative statistical tools to better estimate the concentration in the ice cream(Vought and Tatini, 1998). The attack rate in this outbreak was reported to be 6.6% based on a cross-sectional study of consumers (Hennessy *et al*., 1996).

The concentration in various samples of ice cream was reported by Vought and Tatini (1998), and the effect of storage at -20C on the concentration was also experimentally investigated. The authors found no evidence of a decrease in numbers during storage at -20C for 16 weeks, unlike the work of Armstrong *et al*. (1970), described previously in this document. Concentrations

found in various consumer samples were reported as follows using different analysis methods (Traditional MPN and Bayesian analysis of MPN results):

- Traditional MPN: mode (6 samples) = 0.093 MPN/g (0.03 - 0.38); max = 0.462 MPN/g (0.10 - 2.4)
- Bayesian analysis: mode (6 samples) = 0.152 MPN/g (0.018 - 0.335); max = 0.894 MPN/g (0.087 - 2.06)

Number: 13

Reference: (Taylor *et al*., 1984)

Serovar: *S. typhimurium*

Setting: Home

Medium: Ice-cream

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.00E+06	#/ml	1000	ml	1.00E+09	9.00	1	1	100.00%
2	1.00E+06	#/ml	750	ml	7.50E+08	8.88	2	2	100.00%
3	1.00E+06	#/ml	500	ml	5.00E+08	8.70	3	3	100.00%
4	1.00E+06	#/ml	250	ml	2.50E+08	8.40	1	1	100.00%
5	1.00E+06	#/ml	100	ml	1.00E+08	8.00	1	1	100.00%
6	1.00E+06	#/ml	520	ml	5.20E+08	8.72	8	8	100.00%
7	1.00E+06	#/ml	337.5	ml	3.38E+08	8.53	1	1	100.00%
8	1.00E+06	#/ml	666.7	ml	6.67E+08	8.82	6	6	100.00%

Footnote

1. Reported concentration and dose estimated for 13-year-old boy who consumed 1000ml.
2. Dose estimated for 35 and 22 year old males who consumed 750ml.
3. Dose estimated for 30 year old female and 9 and 8 year old boys who consumed 500ml
4. Dose estimated for 6 year old girl who consumed 250ml
5. **Dose estimated for 2 year old girl who consumed 100ml**
6. Dose estimated assuming an overall average consumption of 520ml
7. Dose estimated for assumed susceptible child
8. **Dose estimated for assumed 6 normal individuals (excluding the 13-year-old boy who received a fatal dose).**

Comments

This outbreak involved a family and one neighbour and was attributed to home made ice cream. The ages of the exposed population were as follows: Father 35, mother 30, sons 13, 9 and 8, daughters 6 and 2, and male neighbour 22 years old. Ice cream was obtained from the freezer at the farm and found to have 10^6 salmonellae/ml. One of the sons, aged 13 years, who ate the most ice cream died from his illness. Various amounts of ice cream ranging from 100ml to 1000ml were reported to have been consumed by the family members. The specific consumption amounts were reported as follows (age/sex): 2F=100ml, 6F=250ml, 30F=500ml, 9M=500ml, 8M=500ml, 35M=750ml, 22M=750ml, 13M=1000ml

In the current analysis the four children, without the 13 year old boy that died, were assumed to represent a susceptible population while the 3 adults were assumed to represent a normal population. Using this assumption the average amount of ice cream consumed by the children was estimated to be approximately 340 ml, while the average amount consumed by the adults was estimated to be approximately 670 ml. It should be noted that the concentration in the ice cream was so high that the difference between these consumption amounts on the dose is minimal.

Number: 14

Reference: (D'Aoust *et al*., 1985), (D'Aoust, 1985)

Serovar: *S. typhimurium*

Setting: Nationwide

Medium: Cheddar Cheese

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	3.60E-01	#/100g	100	g	3.60E-01	-0.44	?	1500	?
2	9.30E+00	#/100g	100	g	9.30E+00	0.97	?	1500	?
3	3.50E+00	#/100g	100	g	3.50E+00	0.54	?	1500	?
4	1.50E+00	#/100g	100	g	1.50E+00	0.18	?	1500	?
5	9.10E+00	#/100g	100	g	9.10E+00	0.96	?	1500	?
6	4.20E+00	#/100g	100	g	4.20E+00	0.62	?	1500	?

Footnote

1. Minimum concentration reported in lots from plant, consumption of 100g approximated from range of consumption reported for 6 cases, indeterminate attack rate
2. Maximum concentration reported in lots from plant.
3. Average concentration for lots from plant.
4. Minimum concentration reported in samples from patients
5. Maximum concentration reported in samples from patients.

6. Average concentration for samples from patient cases

Comments

This outbreak involved more than 1500 people with cheddar cheese implicated as the vehicle of infection. Cheese samples were obtained from the plant as well as from homes of some of the individuals that were ill. The level of contamination on the cheese from the plant was found to be between 0.36 to 9.3 salmonellae per 100 grams (D'Aoust *et al*., 1985), while the level of contamination on cheese from individual homes was found to be between 1.5 to 9.1 salmonellae per 100grams (D'Aoust, 1985). The average concentration from cheese plant samples was estimated to be 3.5 per 100 grams while those from homes was estimated to be 4.2 per 100 grams. The authors noted that the number of salmonellae probably did not change substantially during storage and the levels estimated reflect the levels at the time of consumption. It was estimated that approximately 100 grams of cheese was consumed, based on the level of consumption reported for six individuals that ranged from 20 grams to 170 grams.

The attack rate in this case is again difficult to estimate due to a lack of information on the exposed population and the inability to make reasonable assumptions given the information and the widespread distribution of the outbreak.

Number: 15

Reference: (George, 1976)

Serovar: *S. schwarzengrund*

Setting: Hospital

Medium: Pancreatin

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.00E+03	#/g	0.2	g	2.00E+02	2.30	1	1	100.00%

Footnote

1. Reported concentration and dose estimated from consumption of 200mg by single susceptible individual.

Comments

This case involved a susceptible individual (1-year-old child) who developed diarrhoea when treated with pancreatic extract (pancreatin is an extract from the pancreas of mammals used to assist in the digestion of food) that was contaminated with *S. schwarzengrund*. The pancreatic extract was found to be contaminated with 1000 salmonellae per gram, and the child got sick following ingestion of 200 mg. It should be noted that this case involves only one individual and the 100% attack rate quoted for this dose could skew the true attack rate which could be less for a group of individuals receiving this dose. For example it could be possible that this one individual might be the only one that got sick if 20 similarly susceptible individuals were given the same dose. In that hypothetical situation the attack rate would be estimated to be only 5%.

Number: 16

Reference: (Lipson, 1976)

Serovar: *S. schwarzengrund*

Setting: Hospital

Medium: Pancreatin

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	8.00E+00	#/g	5.6	g	4.48E+01	1.65	1	1	100.00%

Footnote

1. Dose estimated based on last 24 hours of feedings consisting of four 1.4 gram amounts.

Comments

This case involved a susceptible individual (9-month-old child with cystic fibrosis) who was fed pancreatin contaminated with *S. schwarzengrund*. The pancreatin was found to be contaminated at a level of 8 salmonellae per gram. The child was given approximately 700mg with each 6-hourly feed for the first 10 days, increasing to approximately 1.4 g in the 36 hours before the symptoms began. The authors note that he had therefore ingested less than 22 organisms per day initially and less than 44 organisms per day in the last 36 hours. If the dose is not cumulative over 24 hours then the infective dose would be approximately 44 organisms (24 hours, fed every 6 hours which translates to 4 feedings and each feeding is 1.4 grams which translates to 5.6 grams. 5.6 grams x 8 per gram = approx. 44 cells). The points raised about one individual exposed and the attack rate estimates in the previous case (George, 1976) also apply in this case.

Number: 17

Reference: (Greenwood and Hooper, 1983)

Serovar: *S. napoli*

Setting: Nationwide

Medium: Chocolate bars

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.60E+00	#/g	64	g	1.02E+02	2.01	1	1	100.00%
2	2.40E+01	#/g	64	g	1.54E+03	3.19	1	1	100.00%
3	5.85E+00	#/g	64	g	3.74E+02	2.57	1	1	100.00%

Footnote

1. Average concentration reported, consumption of 4 bars and attack rate for that one individual
2. Maximum concentration found in an individual bar.
3. Highest average concentration in packet consisting of 6 bars.

Comments

This was a nationwide outbreak attributed to chocolate bars (16 grams each) contaminated with *S. napoli*. Although the overall attack rate in the population exposed cannot be determined, details were given on three individuals: A mother and two sons. All three ate two bars on the first day, and one son ate two more bars on the second day. The son that ate chocolate bars on two days, and thus received a larger dose, got ill. We can only state that the attack rate for the one child that ate 4 bars was 100%, and this particular case was assumed to represent a potentially susceptible individual.

A box of chocolates, which consisted of 8 packets with 6 bars in each packet, was obtained from a retailer from whom two patients had purchased chocolate. This box of chocolates was analyzed and 42 of the 48 bars examined were positive with the average concentration on the positive bars reported to be 16 organisms per 10 grams. The highest concentration on a bar was 240 organisms per 10 grams and the lowest was 3 organisms per 10 grams. It was also observed that the level of contamination per packet was not consistent. Packets consisting of 6 bars that were all positive also tended to have a higher contamination level. Of the 8 packets examined, the packet with the highest average concentration was 58.5 per 10 grams.

Since information is only known about one case, these data were not considered for further analysis.

Number: 18

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *S.* Enteritidis (PT4)

Setting: Restaurant

Medium: Roasted Beef

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	2.00E+03	#/gram	120	g	2.40E+05	5.38	5	3	60.00%

Footnote

1. Reported concentration, consumption and attack rate

Number: 19

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis

Setting: Caterer

Medium: Grated yam diluted with soup

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	3.20E+04	#/gram	60	g	1.92E+06	6.28	123	113	91.87%

Footnote

1. Reported concentration, consumption and attack rate

Number: 20

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis (PT22)

Setting: School lunch

Medium: Beef and bean sprouts

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	4.00E+01	#/gram	22	g	8.80E+02	2.94	10552	967	9.16%
2	4.00E+01	#/gram	22	g	8.80E+02	2.94	7914	967	12.22%
3	4.00E+01	#/gram	22	g	8.80E+02	2.94	5276	967	18.33%
4	4.00E+01	#/gram	22	g	8.80E+02	2.94	3517	967	27.50%

Footnote

1. Reported concentration, consumption and attack rate
2. Attack rate adjusted, assuming 75% of the reportedly exposed were actually exposed
3. Attack rate adjusted, assuming 50% of the reportedly exposed were actually exposed
4. **Attack rate adjusted, assuming 33% of the reportedly exposed were actually exposed**

Comments

The number of potentially exposed elementary school students (6 –12 years old) was very large since a central cooking facility serves 15 schools. Patients were found from almost all the schools, however, the patient number was especially high in 5 schools. This might suggest the

heterogeneous distribution of the pathogen in causative food. The attack rate used in calculations was based on the assumption that one-third of the total student population was actually exposed.

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Number: 21

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis

Setting: Home

Medium: Egg

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	<3.00E-02	#/gram	60	g	<1.80E+00	<0.26	5	3	[60.00%]

Footnote

1. Concentration reported as <0.03, limit of detection, concentration uncertain data not used

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Number: 22

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis

Setting: Hotel

Medium: Scallop roasted with egg yolk (product 1), Shrimp roll in bread (product 2), Hamburg steak (product 3)

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	4.70E+04	#/gram	40	g	1.88E+06	6.27	115	63	54.78%
2	1.50E+05	#/gram	40	g	6.00E+06	6.78	115	63	54.78%
3	3.00E+04	#/gram	40	g	1.20E+06	6.08	115	63	54.78%
4	7.57E+04	#/gram	40	g	3.03E+06	6.48	115	63	54.78%

Footnote

1. Concentration reported in product 1 and reported amount consumed and attack rate
2. Concentration reported in product 2 and reported amount consumed and attack rate
3. Concentration reported in product 3 and reported amount consumed and attack rate
4. **Average concentration reported in three products, reported amount consumed and attack rate**

Number: 23

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis

Setting: Confectionery

Medium: Cake

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	6.00E+03	#/gram	100	g	6.00E+05	5.78	13	11	84.62%

Footnote

1. Reported concentration, amount consumed and attack rate

Number: 24

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis (PT1)

Setting: School lunch

Medium: Peanut sauce

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.40E+00	#/gram	35	g	4.90E+01	1.69	5320	644	12.11%
3	1.40E+00	#/gram	35	g	4.90E+01	1.69	3990	644	16.14%
4	1.40E+00	#/gram	35	g	4.90E+01	1.69	2660	644	24.21%
5	1.40E+00	#/gram	35	g	4.90E+01	1.69	1330	644	48.42%

Footnote

1. Reported concentration, consumption amount and attack rate
2. Reported concentration and consumption amount. Attack rate adjusted, assuming 75% exposure.
3. **Reported concentration and consumption amount. Attack rate adjusted, assuming 50% exposure.**
4. Reported concentration and consumption amount. Attack rate adjusted, assuming 25% exposure

Comments

The attack rate that was reported for this outbreak was based on the entire school population that receives lunch from the central kitchen being exposed. With a large exposed population like this one, which can be highly uncertain, the estimated attack rate can vary widely. It is highly unlikely that the entire reportedly exposed population was actually exposed to the contaminated food. It was therefore assumed in this analysis that 50% of the individuals were actually exposed.

Number: 25

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis

Setting: Daycare

Medium: Cooked chicken and egg

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	2.70E+01	#/gram	150	g	4.05E+03	3.61	16	3	18.75%
2	2.70E+01	#/gram	150	g	4.05E+03	3.61	117	50	42.74%

Footnote

1. **Average of two reported concentrations (4.00E+00 and 5.00E+01), reported consumption amount and attack rate for adults**
2. **Average dose, and attack rate for children.**

Comments

The food was a rice dish covered with cooked chicken and eggs. Of 133 exposed people, 16 were adults (3 became ill) and 117 were children (50 became ill). Daycare-aged children were assumed to be of increased susceptibility to foodborne pathogens.

Number: 26

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis (PT1)

Setting: School lunch

Medium: Peanut sauce

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	<1.00E+02	#/gram	80	G	8.00E+03	3.90	2267	418	18.44%

Footnote

1. Reported concentration, consumption amount and attack rate

Comments

The attack rate that was reported for this outbreak was based on the entire school population that receives lunch from the central kitchen being exposed. With a large exposed population like this one, which can be highly uncertain, the estimated attack rate can vary widely. It is highly unlikely that the entire reportedly exposed population was actually exposed to the contaminated food. In addition, the reported concentration per gram of food was less than 100 CFUs, which introduces a second significant uncertain parameter.

Number: 27

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis

Setting: Hospital

Medium: Raw egg in natto

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.20E+06	#/gram	50	g	6.00E+07	7.78	191?	45	23.56%
2	1.20E+06	#/gram	50	g	6.00E+07	7.78	47	45	95.74%
3	1.20E+01	#/gram	50	g	6.00E+02	2.78	191	45	23.56%

Footnote

1. Reported concentration, amount consumed and attack rate
2. Attack rate that would be expected at this dose (1/4 of the reported exposed population)
3. Dose that would be expected at this attack rate (5 log lower)

Comments

Eggs were pooled in the preparation of this food. The number of exposed was the number of people who were served with this dish. 128 people from 191 served answered the food-intake examination. (Some of the hospital patients could not talk.) Among 128 responses, 36 did not actually consume this dish. Among the 45 cases, 2 were TB patients and apparently had taken antibiotics. The number of TB patients in the actual exposed population is unknown. This outbreak is highly unusual, the dose is very high while the attack rate is very low. In addition, the outbreak is reported to have occurred in a hospital, an environment in which we would

expect the exposed population to be more susceptible than the overall population. The attack rate at this dose would be expected to be greater than 90%,. which would be calculated only 1/4 of the reportedly exposed population was actually exposed to the contaminated food. Alternatively, the dose that would be expected to produce an attack rate similar to that reported would be almost 5 order of magnitude lower than the dose reported. Because of the uncertainties in these data, this outbreak was not included for further analysis.

Number: 28

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis (PT4)

Setting: Hospital

Medium: Grated yam diluted with soup

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	2.40E+03	#/gram	60	g	1.44E+05	5.16	343?	75	21.87%
2	2.40E+03	#/gram	60	g	1.44E+05	5.16	86	75	87.21%
3	2.40E+00	#/gram	60	g	1.44E+02	2.16	343	75	21.87%

Footnote

1. Reported concentration, amount consumed and attack rate
2. Attack rate that would be expected at this dose (1/4 of the reported exposed population)
3. Dose that would be expected at this attack rate (3 log lower)

Comments

This outbreak is unusual, like the previous hospital-associated outbreak. Eggs were pooled and mixed well in preparing this dish. The actual number of individuals exposed is suspected to be lower than originally reported. The reported attack rate is lower than would be expected at this high dose level. In addition, the exposed population were hospital patients, whom are generally considered to be more susceptible to foodborne disease. The attack rate at this dose would be expected to be greater than approximately 85%, which would occur if only 1/4 of the reportedly exposed population was actually exposed to the contaminated food. Alternatively, the dose that would be expected to produce an attack rate similar to that reported would be almost 3 orders of magnitude lower than the dose reported. Some of the patients had antibiotic treatment, which may be a confounding factor in interpretation of these data.

Number: 29

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis (PT1)

Setting: Hospital

Medium: Tartar sauce

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.00E+02	#/gram	36	g	3.60E+03	3.56	126	36	28.57%
2	1.00E+02	#/gram	36	g	3.60E+03	3.56	63	36	57.14%
3	1.00E+01	#/gram	36	g	3.60E+02	2.56	126	36	28.57%

Footnote

1. Reported concentration, amount consumed and attack rate
2. Attack rate that would be expected at this dose (1/2 of the reported exposed population)
3. Dose that would be expected at this attack rate (1 log lower)

Comment

This outbreak is also unusual, like the previous three hospital outbreaks, although in this case the dose is not as high. Nonetheless the attack rate is lower than would be expected for a population that might be considered susceptible. It is possible that the hospitalized individuals were not more susceptible than the normal population, and in that case the observed attack rate is only slightly lower than what has been observed for similar doses in outbreaks involving a normal population. Calculations referenced in Footnotes 2 and 3 indicate estimates for the expected attack rate at the given dose and expected dose at the given attack rate with respect to the other observed data.

Number: 30

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis (PT1)

Setting: Restaurant

Medium: Cooked egg

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	2.00E+02	#/gram	10	g	2.00E+03	3.30	885	558	63.05%
2	2.00E+02	#/gram	50	g	1.00E+04	4.00	885	558	63.05%
3	2.00E+02	#/gram	30	g	6.00E+03	3.78	885	558	63.05%

Footnote

1. Reported concentration and attack rate and lower reported amount consumed
2. Reported concentration and attack rate and higher reported amount consumed

3. Reported concentration and attack rate and average amount consumed

---

Number: 31

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis (PT4)

Setting: Confectionery

Medium: Cake

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	1.40E+01	#/gram	30	g	4.20E+02	2.62	5103	1371	26.87%

Footnote

1. Reported concentration, amount consumed and attack rate

Number: 32

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *Salmonella* Enteritidis

Setting: Daycare

Medium: Egg salad

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	7.80E-01	#/gram	30	g	2.34E+01	1.37	156	42	26.92%
2	7.80E-01	#/gram	50	g	3.90E+01	1.59	156	42	26.92%
3	7.80E-01	#/gram	40	g	3.12E+01	1.49	156	42	26.92%

Footnote

1. Reported concentration and attack rate and lower reported amount consumed
2. Reported concentration and attack rate and higher reported amount consumed
3. **Reported concentration and attack rate and average amount consumed**

Comments

This outbreak was assumed to represent a susceptible population since the outbreak occurred in a daycare facility.

Number: 33

Reference: Ministry of Health and Welfare, Japan, 1999

Serovar: *S. oranienburg*

Setting: Hotel

Medium: Grated yam diluted with soup

Foot note	Concentration		Amount Ingested		Dose	Log Dose	Exposed	Response	Attack Rate
	Value	Units	Value	Units					
1	5.00E+07	#/gram	150	g	7.50E+09	9.88	11	11	100.00%

Footnote

1. Reported concentration, amount consumed and attack rate

EPIDEMIOLOGICAL DATA SUMMARY AND ANALYSIS

A summary of the doses, attack rates, serovars and characteristics of the exposed population derived from the outbreak reports summarized in the preceding section is given in Table 6 and Figure 9. Data were used from 19 outbreaks of the 33 reports. Six outbreaks yielded 2 data points, representing identified susceptible and normal population information, or when uncertainty in the data required warranted two estimations. The analysis of the epidemiological data was intended to serve three purposes:

1. To determine if there is any epidemiological evidence for greater attack rates in susceptible vs. normal populations.
2. To determine if there is any epidemiological evidence for different attack rates for *Salmonella* Enteritidis vs. other *Salmonella* serotypes.
3. To compare the epidemiological data for dose and attack rate with the estimates generated by the dose-response models.

The data in Table 6 and Figure 9 are coded according to the outbreak number assigned in this document. If additional information related to a specific data point is required, for example the assignment of two data points, the details of the outbreak can be referred to in the previous section.

The related assumptions for inclusion, exclusion, or multiple data points are certainly issues for discussion and debate, and therefore included in the summary of reported outbreaks. For those individuals who were part of the working group meeting held in June, 2000 in Bilthoven and reviewed the first draft of this document, some of the data sets have been modified or deleted, based on questions of inconsistencies that led to obtaining further information about the initial data sets. This includes the exclusion of single case reports, or outbreaks for which little was actually known about one or more of the critical criteria. Estimations of total exposed populations proved to be most perplexing, especially in a potentially large number of people being exposed, but with mitigating circumstances that would negate the actual consumption of a specific food. For example this might occur in a hospital, where all individuals being served a complete meal may not actually consume all dishes served in the meal.

Table 6: Summary of outbreak data[1]

Outbreak #	Serovar	Norm(N)/Susc(S)[2]	Log Dose	Attack Rate
1	*S. typhimurium*	N	1.88	10.63%
1	*S. typhimurium*	S	1.88	18.91%
2	*S. heidelberg*	N	1.48	35.71%
2	*S. heidelberg*	N	2.48	35.71%
3	*S. cubana*	S	4.48	85.71%
4	*S. infantis*	N	6.36	100.00%
5	*S. typhimurium*	N	3.05	55.00%
5	*S. typhimurium*	N	4.05	55.00%
7	*S. newport*	N	1.18	2.90%
11	S. Enteritidis	N	4.00	100.00%
11	S. Enteritidis	N	5.00	100.00%
12	S. Enteritidis	N	1.30	6.60%
13	*S. typhimurium*	S	8.00	100.00%
13	*S. typhimurium*	N	8.82	100.00%
18	S. Enteritidis	N	5.38	60.00%
19	S. Enteritidis	N	6.28	91.87%
20	S. Enteritidis	N	2.94	27.50%
22	S. Enteritidis	N	6.48	54.78%
23	S. Enteritidis	N	5.78	84.62%
24	S. Enteritidis	N	1.69	24.21%
25	S. Enteritidis	N	3.61	18.75%
25	S. Enteritidis	S	3.61	42.74%
30	S. Enteritidis	N	3.78	63.05%
31	S. Enteritidis	N	2.62	26.87%
32	S. Enteritidis	S	1.49	26.92%
33	*S. oranienburg*	N	9.88	100.00%

[1] For details about each outbreak, source and assumptions for data, see text.

[2] Data segregated for Normal (N) populations, considered to be healthy with no underlying medical conditions vs. individuals considered to be of increased susceptibility (S) because of conditions believed to compromise the immune system

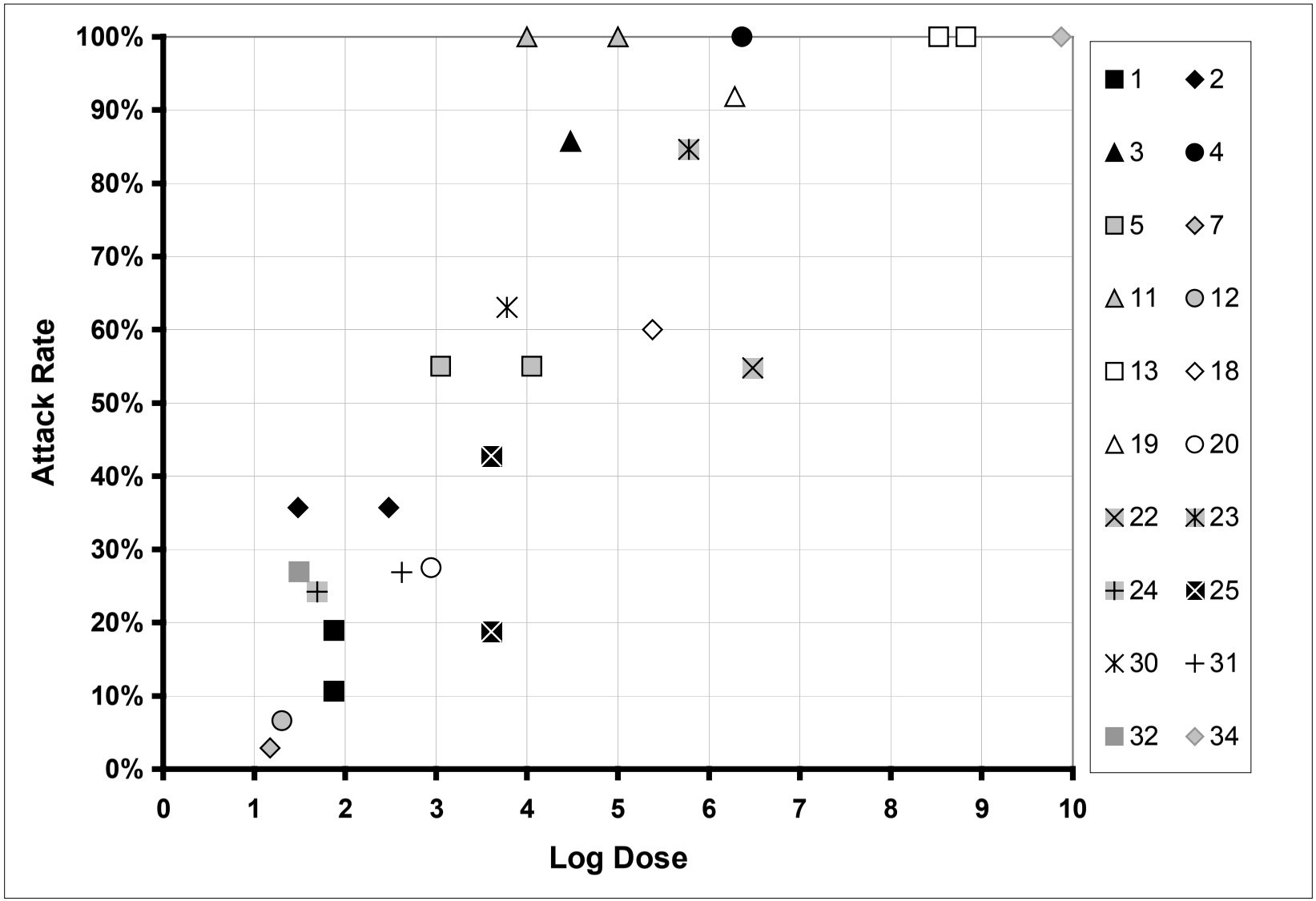


Figure 9: Summary of epidemiological data. Legend numbers indicate outbreak number given in text.

The data shown in Figure 9 appear to reflect our theoretical assumptions regarding the increasing trend in attack rates as dose increases. In addition, although there is a degree of clustering in some of the data points, a dose-response relationship is visually evident.

As noted earlier, some data were excluded from this summary and further analysis. For example, outbreaks # 27, 28 and 29 were attributed to *Salmonella* Enteritidis in a hospital setting, where the exposed population would be expected to be more susceptible. The characteristics of the individuals that were exposed to the food is highly uncertain, so it may in fact be the case that the condition for which they were hospitalized is such that their immunity was not compromised. However even if they are assumed to have normal susceptibility, these outbreaks were still distinctly different from outbreaks with a similar dose level, if the reported exposure were accurate. Alternative explanations for these data sets are that the individuals served the meal did not actually consume it; or that antibiotic therapy prevented the ingested *Salmonella* from colonization and illness production.

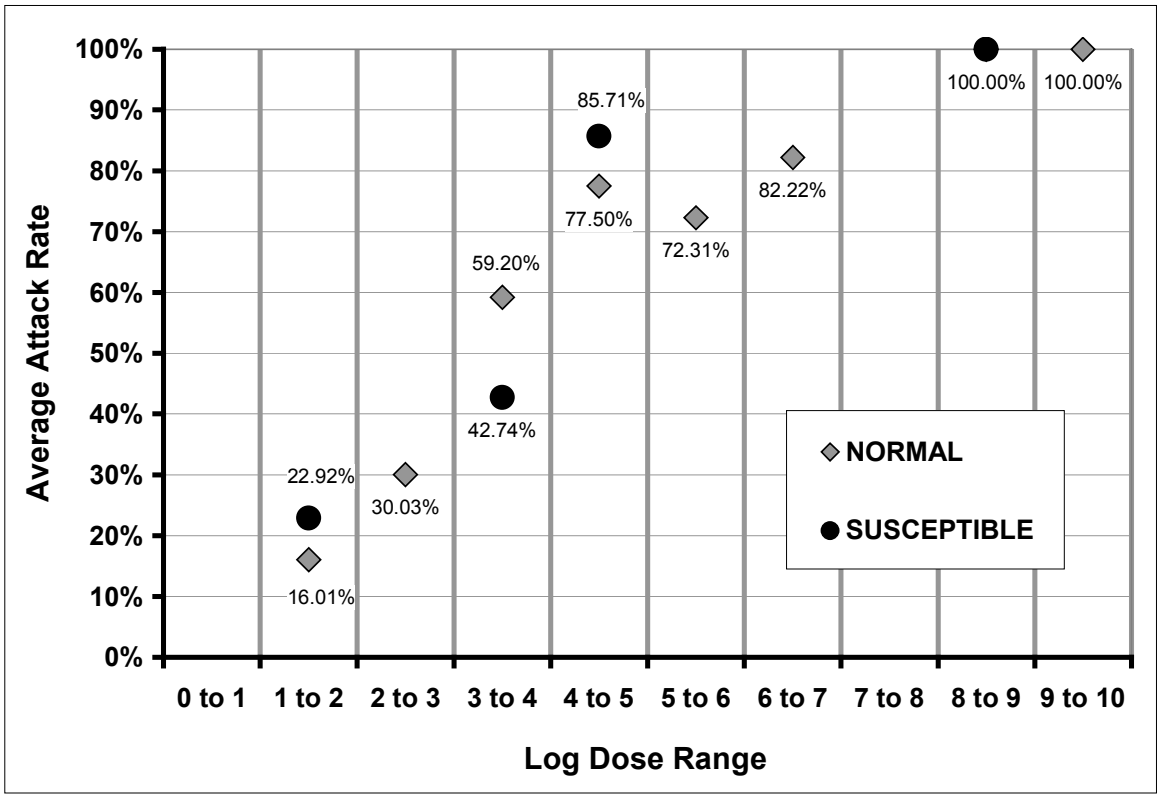
Susceptible vs. Normal Populations

The outbreak data can be used to offer some insight into the potential differences that may exist between susceptible and non-susceptible populations. Several outbreaks were determined to be associated with susceptible and non-susceptible populations. In addition, some outbreak reports described the characteristics of the exposed population allowing inference of the proportion that was susceptible and non-susceptible. This information was included in the summary of the outbreaks (Figure 9) and in the summary shown in Table 6. The susceptible and non-susceptible outbreak information was compared by grouping the outbreaks according to dose and calculating the average attack rate for the outbreaks that fell within the dose range. This information is summarized in Tables 7 and 8, and in Figure 10.

Table 7: Comparison between normal population attack rate and susceptible population attack rate (NA, Not applicable, no data available).

Log Dose	Normal Population Attack Rate				Susceptible Population Attack Rate			
	Data Pts.	Low	Average	High	Data Pts.	Low	Average	High
0 to 1	*0*	NA	NA	NA	*0*	NA	NA	NA
1 to 2	*5*	2.90%	16.01%	35.71%	*2*	18.91%	22.92	26.92
2 to 3	*3*	26.87%	30.03%	35.71%	*0*	NA	NA	NA
3 to 4	*4*	18.75%	59.20%	100%	*1*	42.74%	42.74%	42.74%
4 to 5	*2*	55.00%	77.50%	100%	*1*	85.71%	42.74%	42.74%
5 to 6	*2*	60.00%	72.31%	84.62%	*0*	NA	NA	NA
6 to 7	*3*	54.78%	82.22%	100%	*0*	NA	NA	NA
7 to 8	*0*	NA	NA	NA	*0*	NA	NA	NA
8 to 9	*1*	100%	100%	100%	*1*	100%	100%	100%
9 to 10	*1*	100%	100%	100%	*0*	NA	NA	NA

Figure 10: Comparison between normal population and susceptible population attack rate



The epidemiological evidence evaluated does not appear to support the hypothesis of a higher attack rate for the susceptible population versus the normal population. However, the outbreak data did not always include age distributions or information about potentially susceptible individuals in the groups that were affected. As noted, a number of hospital situations are not included in these evaluations because of large uncertainties in the exact numbers of people who actually consumed the food that was prepared and served in a meal. It might also be that antibiotic therapy in the hospital setting confounds the interpretation of the data. A final comment is that the data available do not reflect the severity of illness, for example, whether or not the disease contributed to premature death.

Table 8: Difference between observed normal and susceptible population attack rates.

Log Dose	Normal Average	Susceptible Average	Delta (Susc.) - (Norm.)	Ratio (Susc.) : (Norm.)
0 to 1	**NA**	**NA**	**NA**	**NA**
1 to 2	0.16	0.23	0.07	1.43
2 to 3	0.30	NA	NA	NA
3 to 4	0.59	0.43	-0.16	0.72
4 to 5	0.78	0.86	0.08	1.11
5 to 6	0.72	NA	NA	NA
6 to 7	0.82	NA	NA	NA
7 to 8	NA	NA	NA	NA
8 to 9	1.00	1.00	0.00	1.00
9 to 10	1.00	NA	NA	NA

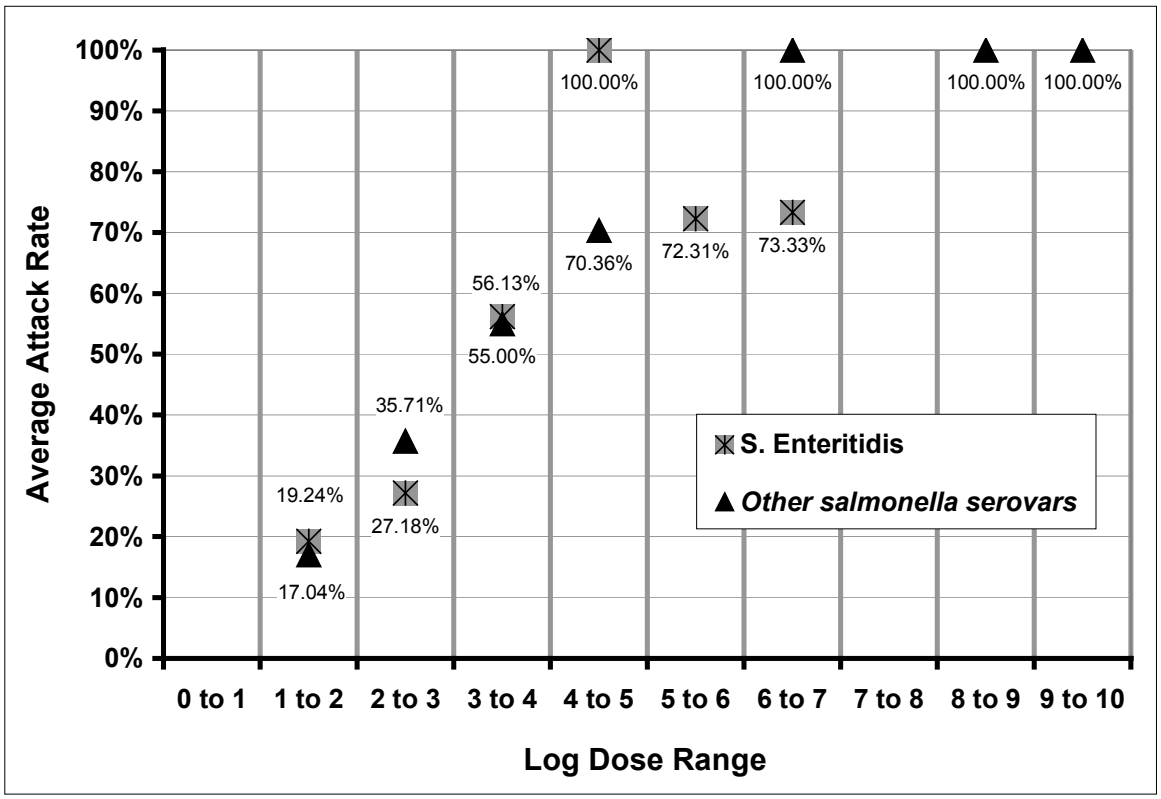
Salmonella Enteritidis vs. other *Salmonella* serovars

In a similar manner as the comparison between susceptible and non-susceptible populations, the difference between the observed attack rate for *Salmonella* Enteritidis and other *Salmonella* species can be compared. The information is summarized in Table 9 and Figure 11.

Table 9: Comparison between *S.* Enteritidis and other *Salmonella* serovar attack rates.

Log Dose	Other *Salmonella* serovars Attack Rate				*S. enteritidis* Attack Rate			
	Data Pts.	Low	Average	High	Data Pts.	Low	Average	High
0 to 1	*0*	NA	NA	NA	*0*	NA	NA	NA
1 to 2	*4*	2.90%	17.04%	35.71%	*3*	6.60%	19.24%	26.92%
2 to 3	*1*	35.71%	35.71%	35.71%	*2*	26.87%	27.18%	27.50%
3 to 4	*1*	55.00%	55.00%	55.00%	*4*	18.75%	56.13%	100%
4 to 5	*2*	55.00%	70.36%	85.71%	*1*	100%	100%	100%
5 to 6	*0*	NA	NA	NA	*2*	60.00%	72.31%	84.62%
6 to 7	*1*	100%	100%	100%	*2*	54.78%	73.33%	91.87%
7 to 8	*0*	NA	NA	NA	*0*	NA	NA	NA
8 to 9	*2*	100%	100%	100%	*0*	NA	NA	NA
9 to 10	*1*	100%	100%	100%	*0*	NA	NA	NA

Figure 11: Comparison between *S.* Enteritidis and other *Salmonella* serovar attack rates



The epidemiological data do not show any discernible trend for a higher attack rate by *Salmonella* Enteritidis compared with other *Salmonella* serovars, or vice-versa. It is possible that the effect of differing attack rates on the susceptible and normal populations may be confounding the comparison between *Salmonella* Enteritidis and the other *Salmonella* serovars. However, the small number of data points currently available does not allow further analysis that might characterize these effects. Overall, there does not appear to be any epidemiological evidence to support the hypothesis that *Salmonella* Enteritidis is more pathogenic than the other *Salmonella* serovars known to cause human illness. Experimental data in animal models suggest that enhanced virulence may be associated with specific strains rather than serotype or phage type (Poppe, 2000).

Epidemiological comparison with dose -response models

The proposed dose response models were compared to the outbreak data complied, and these comparisons are shown in Figures 12 – 15. Based on the data presented, and the functional form and parameters of the dose-response models, there is clearly no one representation that describes all the available data sets. A limitation is in the accuracy of the outbreak data, i.e. sampling methods, testing methods, and reporting. It is also worth considering the original derivations of the proposed dose-response models.

The US model used *Shigella dysenteriae* human feeding trial results for derivation of the Beta Poisson (BP) parameters (US-norm), and adjusted by a factor of 10 for susceptible populations (US-susc). Both curves bound most of the data points in the upper range of probability of illness. This approach was selected because it was deemed a better representation of the available epidemiological data (Table 5) which included several outbreaks with high attack rates including a few which were attributed to low doses of *Salmonella* Enteritidis. The selection of *Shigella* as a surrogate for *Salmonella* was based on the organism's characteristic for producing illness at low doses.

The Canadian re-parameterized Weibull model was constructed based on parameters derived from all available bacterial feeding studies, in addition to *Salmonella* outbreak data for normal (Can-norm) and susceptible populations (Can-susc). These are less conservative than the US models; in fact, the Can-susc is less conservative than the US-norm, except at low doses, less than 1 log. The Can-norm appears to bound most of the data points in the lower range of probability of illness, and is clearly not conservative.

The Naïve BP is based on human feeding trial data for non-typhi *Salmonella*, excluding data from subjects that had been administered repeat doses. This model is more conservative than the Can-norm at doses above 4 logs, and less conservative at doses below. It must be kept in mind that this model is based on studies with healthy adult males; however, the response measured is infection, not illness.

Given the epidemiological data, justification could be made for selection of any one of the five models. Theoretical considerations for the functional form of the dose-response models and derivation of their parameters warrants further discussion.

As a result of the large database of epidemiological data, which represents observed real world data that relates attack rates to dose, and the inability of the existing dose-response models to adequately describe the dose-response relationship, dose-response models were fit to this data. The exponential and beta-poisson models were used as a result of recommendations (MRA006) to use models with linear low dose behaviour. The maximum likelihood technique was used to fit the models to the data. In order to fit the models, the epidemiological data was characterized giving equal weight to large and small outbreaks. This step was taken because the maximum likelihood fitting technique gives greater weight to a data set with a large exposed population. This is appropriate when dealing with experimental data, where there is greater confidence in experiments conducted on a large sample. However, in the case of epidemiological data, an outbreak with a large exposed population does not imply greater confidence in that data set. In fact, as far as attack rate statistics go, it could be argued that the smaller size outbreaks have more accurate data on the number of people that were exposed and the number of people who got sick. The epidemiological outbreak data for normal and susceptible individuals were used directly to derive the parameters for new beta-Poisson and exponential models. The results are shown in Figures 16 to 18. The goodness-of-fit tests for these curves were not statistically significant, however given the nature of real world data, and in the absence of a detailed selection of potential outlier data points, this is not an unexpected result.

Figure12: Outbreak data compared to USDA susceptible dose-response model (US-susc) and USDA normal dose-response model (US-norm).

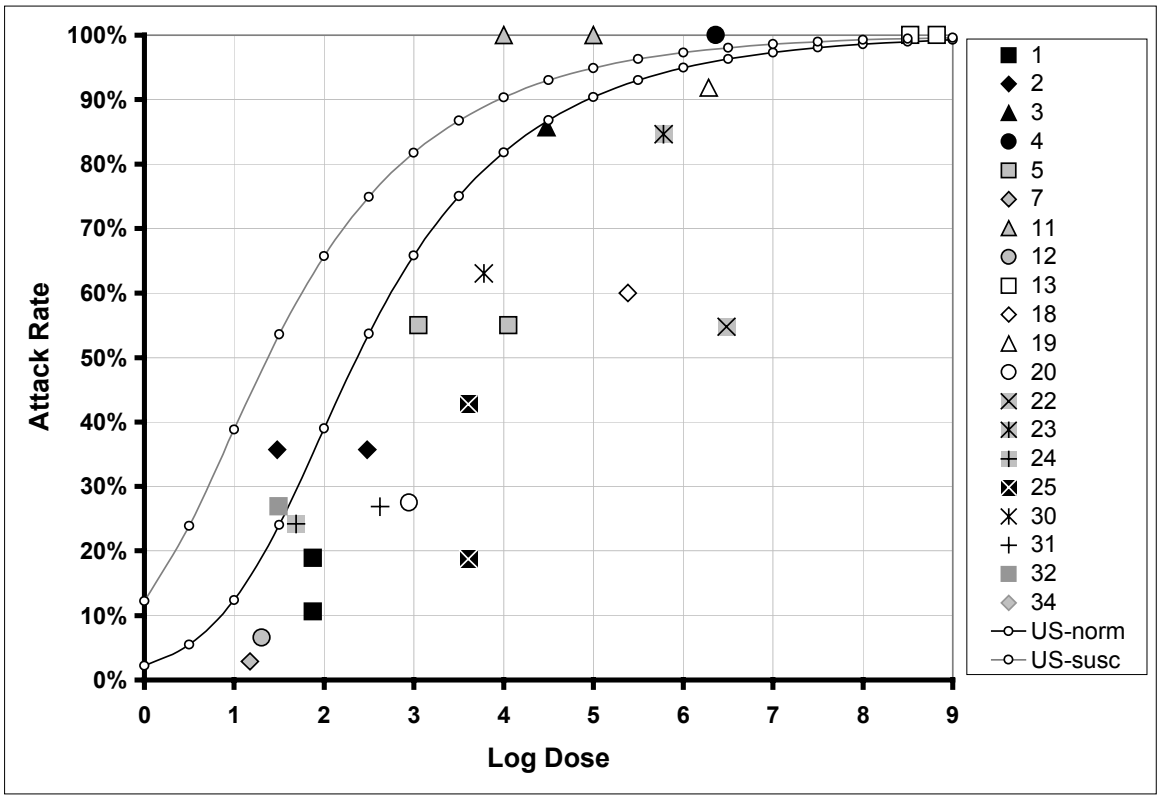


Figure13: Outbreak data compared to Canadian susceptible dose-response model (Can-susc) and Canadian normal dose-response model (Can-norm)

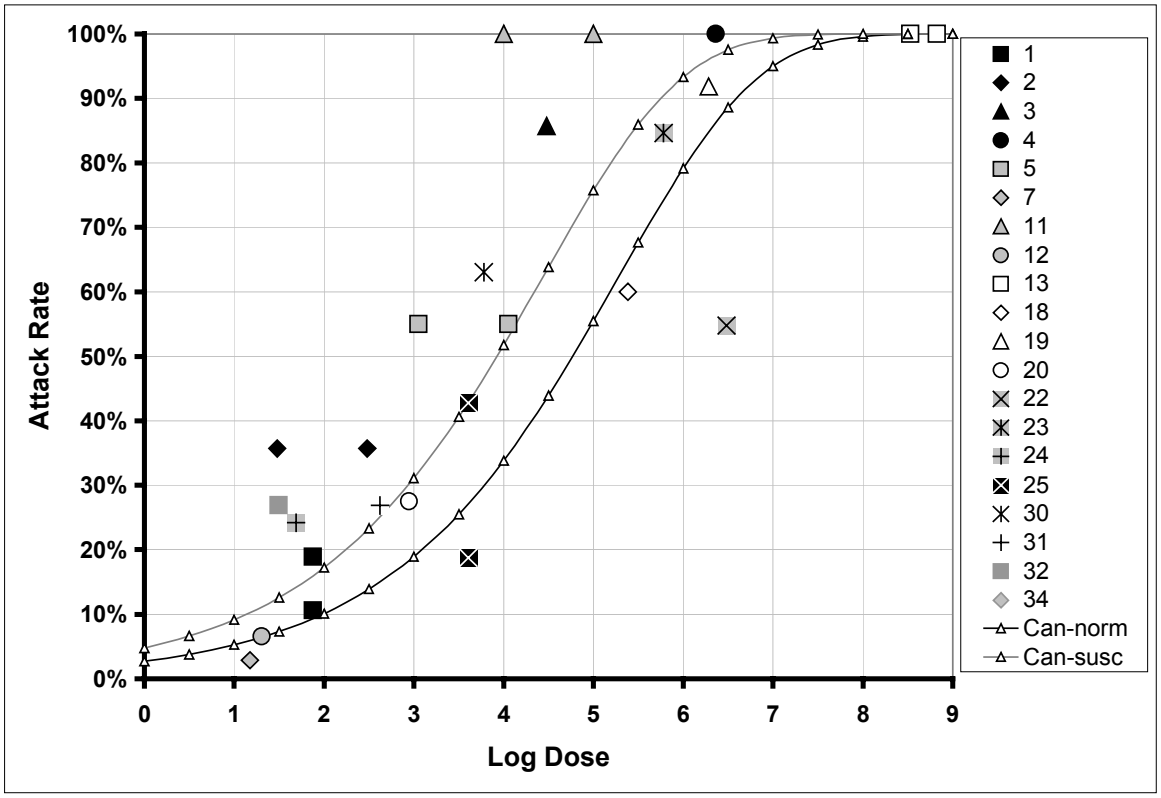


Figure14: Outbreak data compared to dose-response model fit to feeding trial data (Naïve-BP)

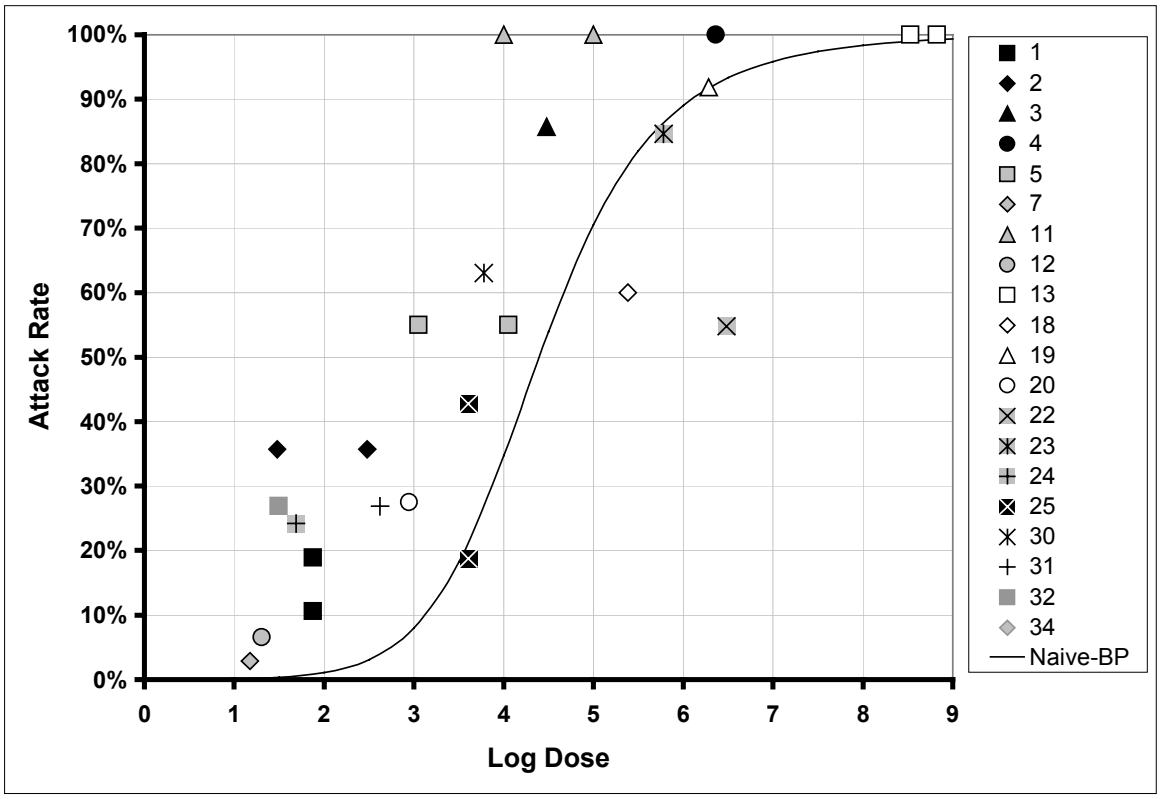


Figure 15: Outbreak data compared to 5 published dose-response curves.

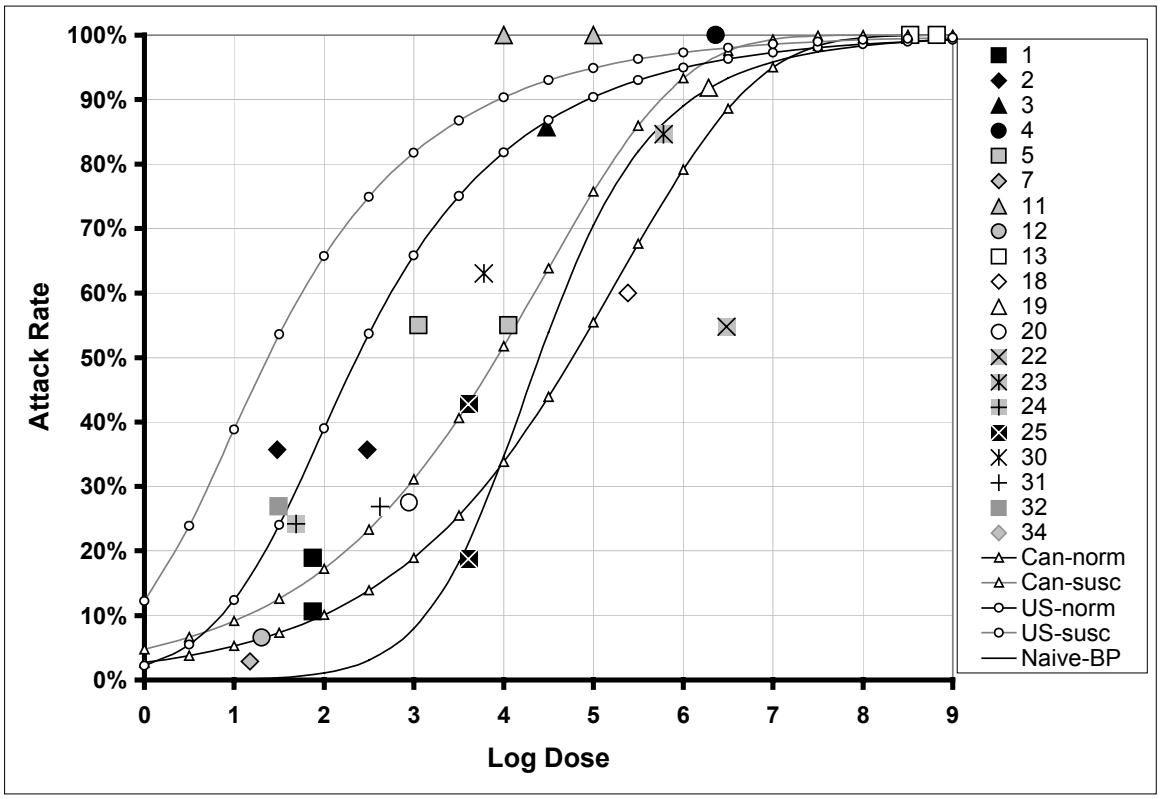


Figure16: Outbreak data compared to beta-Poisson dose-response model fit to "normal population" outbreak data. [Alpha = 0.1672, Beta = 24.437].

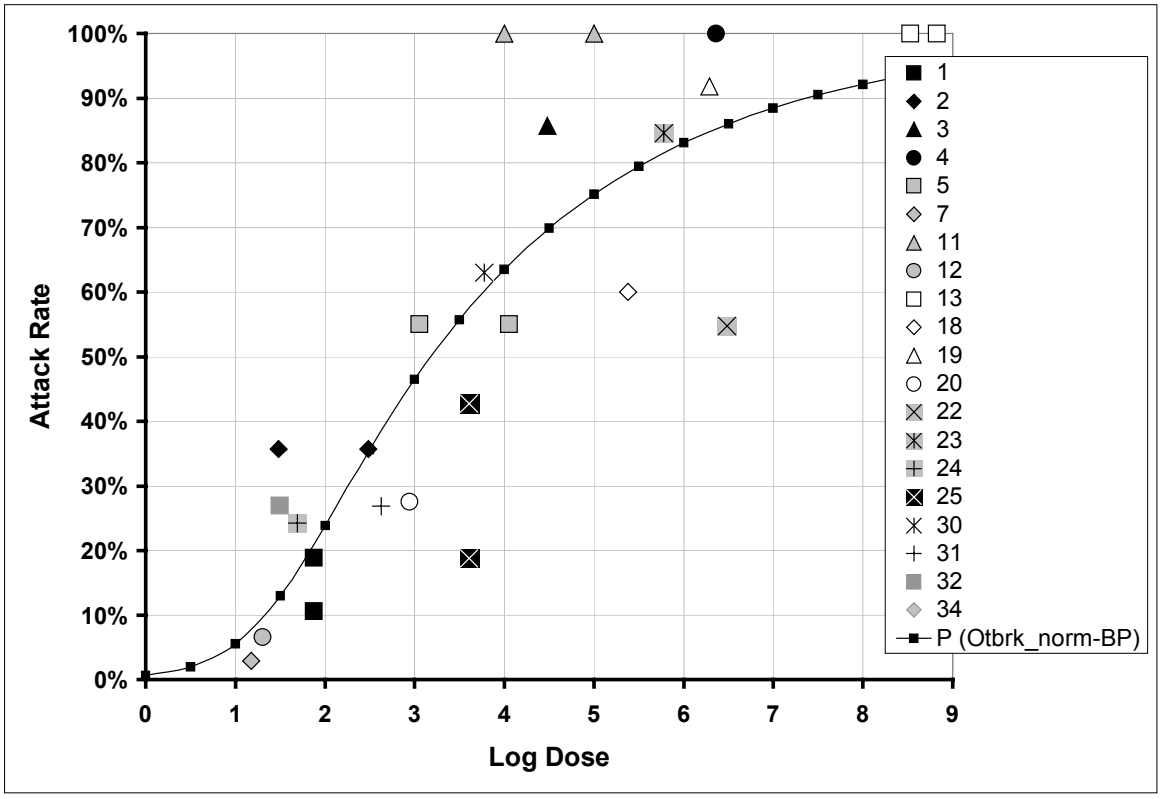


Figure17: Outbreak data compared to beta-Poisson dose-response model fit to "susceptible population" outbreak data. [Alpha = 0.195, Beta = 20.396].

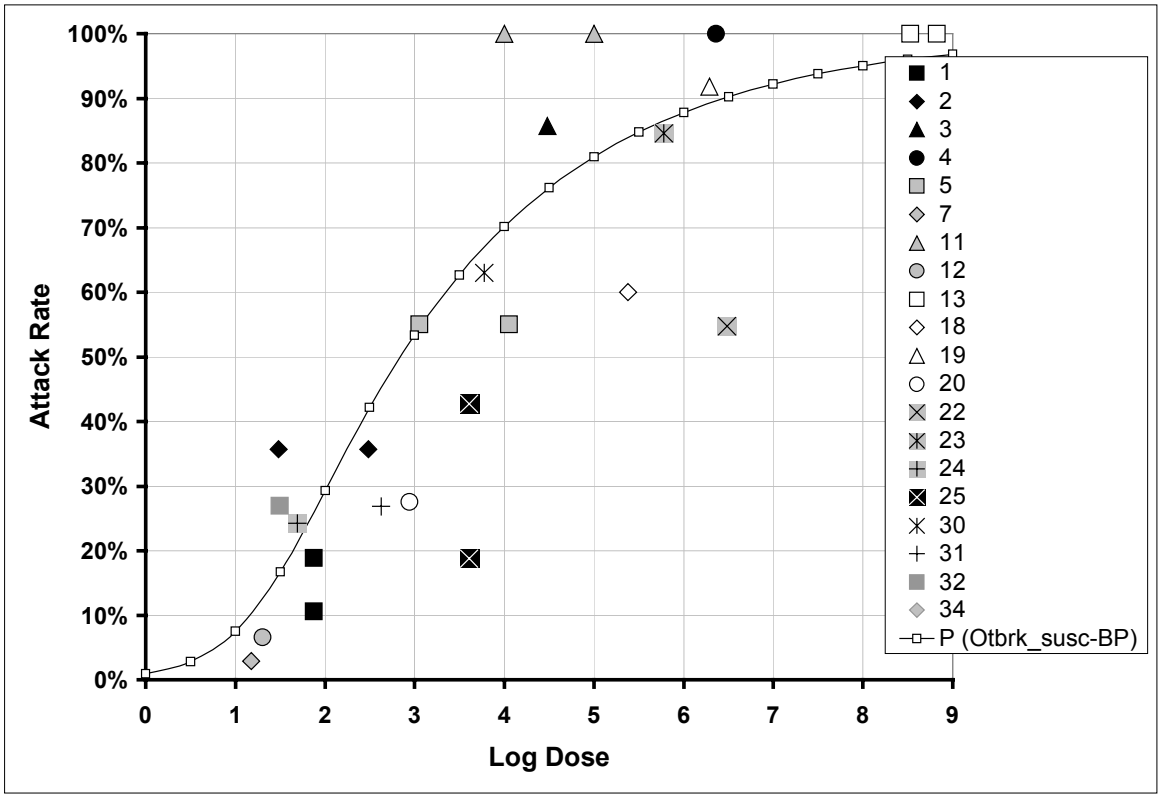


Figure18: Outbreak data compared to exponential dose-response model fit to "normal population" outbreak data. [Exponential parameter "r" = 2.33e-4]

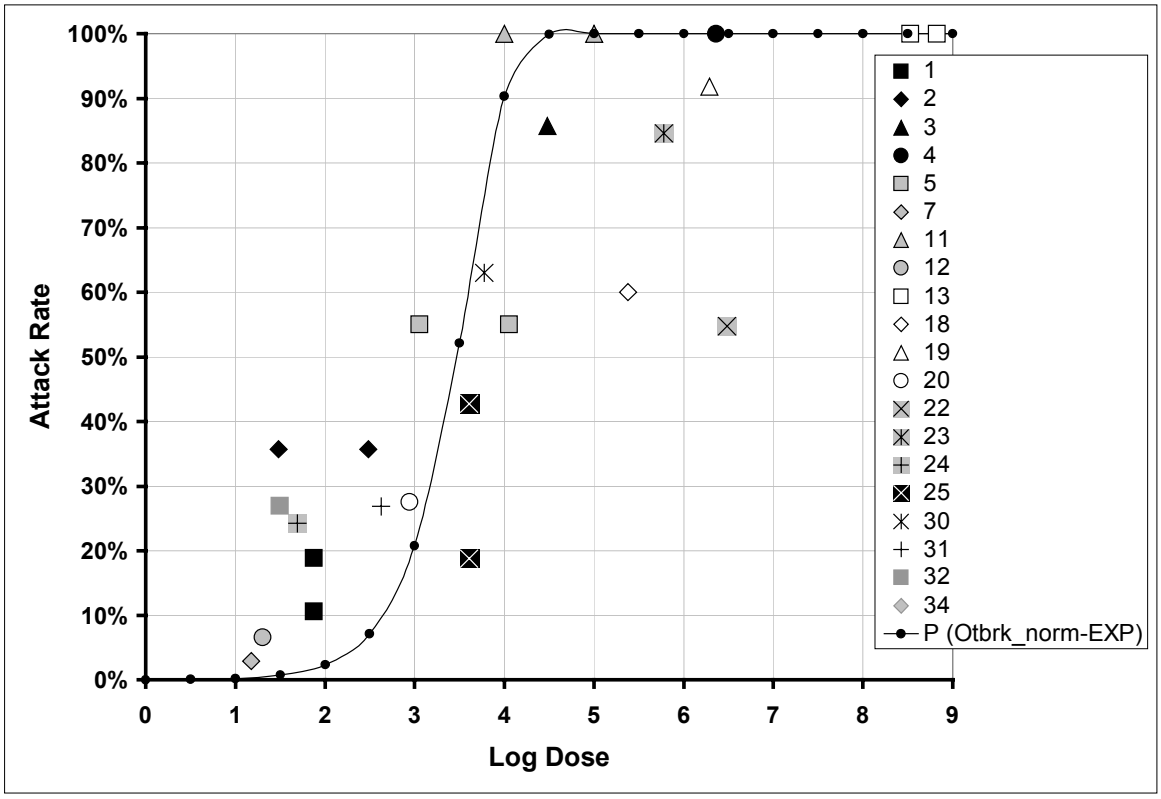


Figure19: Outbreak data compared to exponential dose-response model fit to "susceptible population" outbreak data. [Exponential parameter "r" = 1.28e-4]

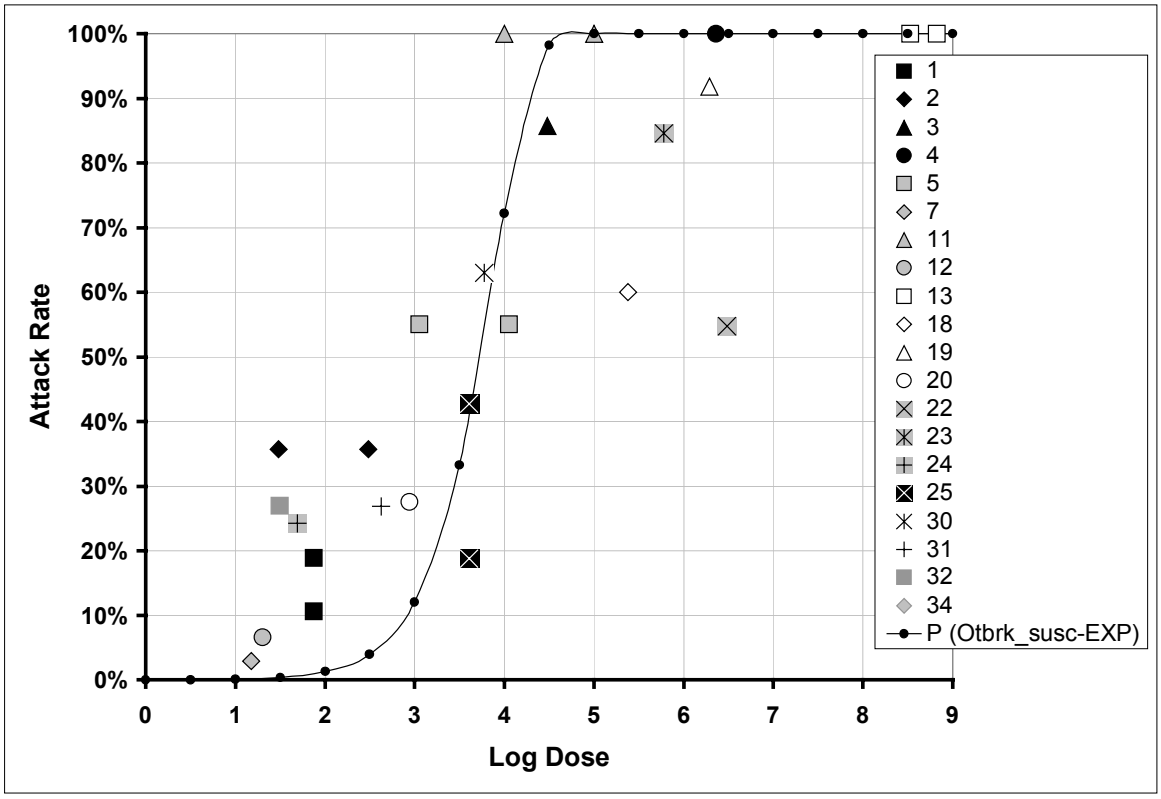
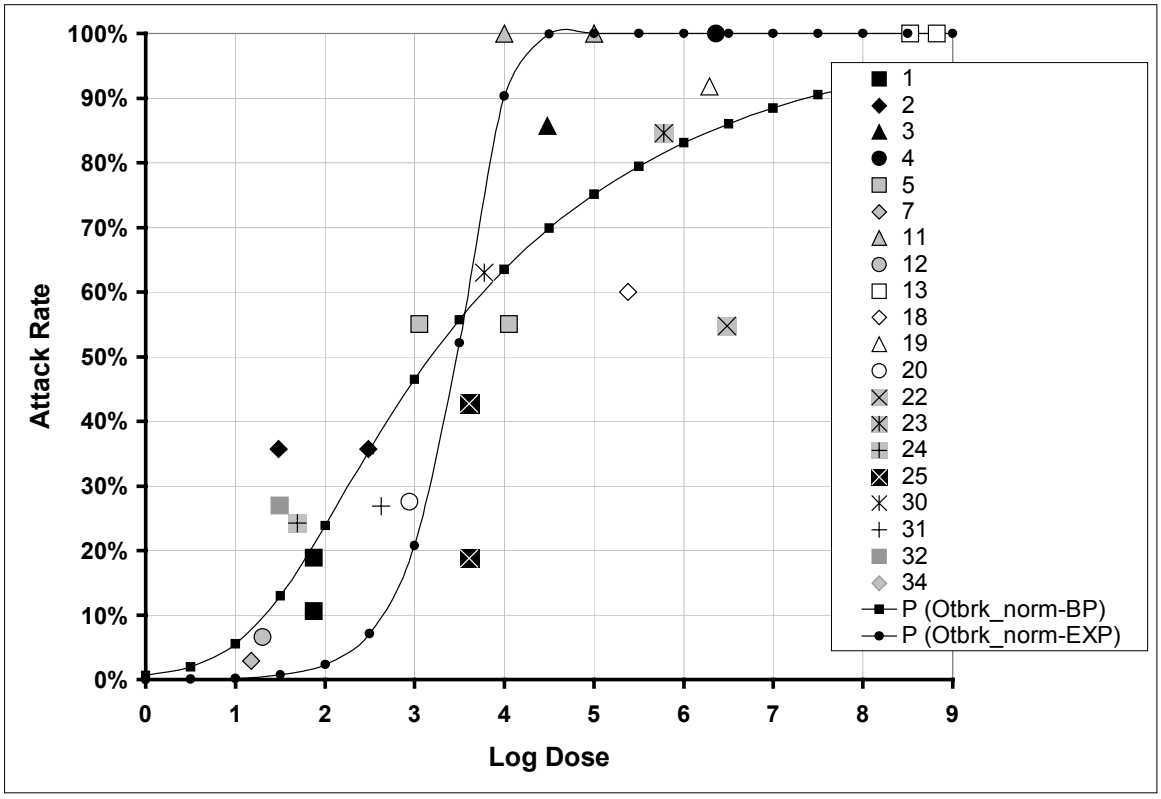


Figure 20: Comparison of outbreak data with two normal population dose-response curves fit (Figures 16 and 18) to outbreak data.



CONCLUSIONS

The derivation of current dose-response models should take into consideration the suitability of assumptions such as extrapolating information based on organism or animal surrogates to the pathogen of interest or to the human host; combining results of feeding studies for different pathogens; using infection versus illness as endpoints to predict relevant public health outcomes; or, accounting for biases which may be inherent in many human feeding trials. In addition, it is important to recognize that outbreak data may also have its own shortcomings. Indeed, several assumptions had to be made to derive the some of the outbreak estimates used in developing new dose-response curves. Methodology for sampling and testing the food in the outbreaks was not critically assessed. Reported exposed populations tended to be the maximum number of people that could be exposed. Therefore, the outbreak data represents significant uncertainty.

At present, a single model representation for the relationship between dose and response can not be highlighted as vastly superior to any other model. Compared to the reported outbreak data, the naïve beta-Poisson model is the least desirable since it vastly underestimates the probability of illness and tends towards the lower bound even when the assumption is made that all infections lead to illness. The remaining models were relatively reasonable approximations, with different degrees of under- or over-prediction of illness based on the outbreak data described in this report. The models fit to the outbreak data appear to offer reasonable potential given that they qualitatively, though not statistically significantly, describe observations in a real world environment.

FUTURE WORK

- Consideration should be given to the inclusion of *S. typhi* and *S. paratyphi* in future hazard characterizations. A dose-response relationship for all *Salmonella* spp. could prove to be of great utility, and the added information from *S. typhi* could also serve to expand the current information.
- This document did not consider a quantitative evaluation of secondary transmission (person-to-person) or chronic outcomes. In addition, the impact of the food matrix was not incorporated into the assessment. These may be considerations for future document development.
- Additional data will help to refine the information currently known and ideally support the development of better risk assessments to help make more accurate predictions regarding the safety of foods contaminated with *Salmonella* and other pathogens of public health concern.
- The importance of accurate and complete epidemiological data collection during outbreak investigations should also be communicated and encouraged.

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APPENDIX: APPROACHES TO MODELING DOSE RESPONSE RELATIONS (FROM LINDQVIST & BUCHANAN)

This Appendix is part of the document "FAO/WHO Risk Assessment of Listeriosis Associated with Ready-to-Eat Foods: Hazard Identification and Hazard Characterisation" authored by Rolland Lindqvist and Robert Buchanan.

General approaches and limitations to Modeling dose response relations for foodborne pathogens

The number of microorganisms entering the digestive tract per exposure is expressed as a mean number of functional particles of the pathogenic organism, CFU, spores, oocysts etc (Teunis *et al*., 1996; Vose, 1998). This is the dose, a quantitative measure of the intensity of the exposure. At a certain dose, certain effects in the host occur. The frequency within the exposed population of hosts at which this occurs constitute the response. The effect may be more or less well defined but generally there will not be a one-to-one relationship between the size of the dose and the specific kind and frequency of the biological effect it produces. Furthermore, pathogenic microorganisms generally produce an array of effects or conditions within an affected host. Thus, instead of a single dose-effect relation there will be a series of dose-response relations that describe the relationship between the various biological effects and the magnitude of the dose (Teunis *et al*., 1996). The effects, which are also referred to as biological end points, that have been considered include infection – for *L. monocytogenes* often measured by counting the number of bacteria in the spleen or the liver of the animal model – and various levels of morbidity, or death (Vose, 1998).

The response of a human population to an exposure to a foodborne pathogen is highly variable. This reflects the fact that the frequency and extent of disease is dependent on a variety of factors such as the virulence characteristics of the pathogen, the numbers of pathogenic cells ingested, the general health and immune status of the hosts, and attributes of the food that alter microbial or host status. The likelihood that any specific individual will become ill due to an exposure to a foodborne pathogen is dependent on the integration of host, pathogen, and food matrix effects. These interactions are often referred to as the infectious disease triangle. Thus, it can be assumed with a high degree of certainty that the relationship between the dose and the response is a function of the *L. monocytogenes* strain in terms of its virulence properties and its survival characteristics, the food in which it resides, and the susceptibility of the host. A mathematical relationship between the dose and the response should ideally be able to describe the interactions between all these factors.

Sources of data: general considerations

An appreciation of the factors described above is critical to a scientifically rigorous consideration of dose-response relations. Equally as important is an appreciation of the uncertainty associated with the different sources of dose-response data.

The primary source of microbiological dose-response data has been human volunteer feeding studies. Such trials provide the most direct measure of human response to pathogens and have been the data of choice for quantitative microbial risk assessments. However, these data do have limitations that must be considered when these dose-response relations are going to be used to

estimate the susceptibility of the entire population. Volunteers for these studies have been almost exclusively limited to healthy adult males. Information on the susceptibility of higher risk subpopulations or potential gender effects is generally not available. Of necessity, volunteer studies have almost always been limited to foodborne diseases that are not considered life-threatening for the test subjects. Thus, volunteer feeding studies are unlikely to be conducted for diseases that are either life threatening (e.g. enterohaemorrhagic *E. coli*) or affect almost exclusively high risk subpopulations (e.g., *L. monocytogenes*). Volunteer studies have often been conducted in conjunction with vaccine trials, which tend to focus on higher dose levels. Typically, there are relatively few test subjects per dose, and because of the small size of the test population, dose levels are used that produce relatively high rates of infection or morbidity. It is usually not possible to evaluate doses that are directly pertinent to the pathogen levels most often associated with human exposures via food. Thus, most dose-response determinations rely on extrapolations of the dose-response relations based on high doses, which can lead to a high degree of uncertainty at the low dose levels.

Since concerns about *L. monocytogenes* are primarily focused on specific more highly susceptible subpopulations, an alternative to human feeding studies is required. The primary alternative has been the use of animal models. The successful use of animal models is dependent on a number of factors, not the least of which is the need for a "conversion factor" that allows the quantitative relations observed in the animal to be correlated with human response to the pathogen. Success is highly dependent on the selection of an appropriate animal model. This can be a significant challenge with many foodborne pathogens. It assumes that the pathogen causes disease by the same mechanism of pathogenicity in both man and animal, that the animal's physiological and immune responses are similar to that of humans, and that quantitative relationships between infectivity, morbidity and mortality are similar for the two species. Further, animal feeding studies have many of the same difficulties as human volunteer studies. For example, most studies are conducted using only healthy animals that are similar in age and weight. In fact, most laboratory animals are so highly inbred that genetic diversity among the animals is negligible. This reduces the variability associated with the testing but brings into question the data's applicability to the general population. While the disease characteristics of *L. monocytogenes* have been examined in a wide range of animals, the primary animal model for dose-response studies with this microorganism has been the mouse, with death being the primary biological end point measured. Care must be taken in reviewing these studies since the dose-response relations vary substantially depending on both route of entry and the variety of mouse employed as the surrogate for humans.

Potentially, epidemiological investigations could be a source for human dose-response information, particularly for outbreaks involving ready-to-eat foods that do support the growth of the pathogenic bacterium. However, to be useful for risk assessments, the investigations would have to be expanded well beyond their current scopes. In addition to detailed information about who became ill, the investigations would also have to acquire information about variety of other factors such as who consumed the food and did not become ill, the amounts of food consumed by both groups, and the frequency and extent of contamination. Regretfully, few epidemiological investigations have been conducted in a manner that provided such data.

As an alternative approach to using epidemiological data to develop dose-response relations for pathogens that are not appropriate for human volunteer feeding studies, Buchanan *et al*. (1997a) proposed that data on the annual national incidence for a disease could be coupled with food

survey data on the frequency and extent of contamination of a ready-to-eat food to produce a conservative estimate of the microorganism's dose-response relations. Assuming that all cases of listeriosis were due to a single food, this approach was used to generate a conservative estimate of the dose-response relations for *L. monocytogenes* in high risk populations.

Mathematical Models

The relation between ingestion of a certain amount of pathogenic micro-organisms of relevance to foodborne illness, N, and the possible outcomes have been quantitatively described in a number of ways (Table 8).

Table 8. Summary of some dose-response models used for foodborne pathogens. The table is adapted and modified from Holcomb *et al*., 1999

Model name	Function Probability=	Where	Reference
Log-normal	$\phi[b_0 + b_1 * \log10(N)]$	ϕ = cumulative normal distribution function b_0 = intercept b_1 = log10(dose) slope parameter	Dupont *et al*., 1972
Log-logistic	$\beta/1+[(1-p)/p] * e^{-\varepsilon\{\log10(N) - \chi\}}$	β = Asymptotic value of probability of infection as dose approaches ∞. β=1 in Holcomb *et al*. (1999). χ = Predicted dose at specified value of p where p = Pr^* ε = Curve rate value affecting spread of curve along dose axis	Levine *et al*., 1973
Simple exponential	$1- e^{- r*\log10(N)}$	r = Reflects host microorganism interaction probability	Rose *et al*., 1991[1]
Flexible exponential	$\beta * [1-p*e^{-\varepsilon\{\log10(N) - \chi\}}]$	β = Asymptotic value of probability of infection as dose approaches β=1 in Holcomb *et al*. (1999). χ = Predicted dose at specified value of p where p = 1 - Pr^* ε = Curve rate value affecting spread of curve along dose axis	Levine *et al*., 1973
Beta-Poisson	$1 - (1 + N/ \beta)^{-\alpha}$	α, β = Parameters affecting the shape of curve[2]	Haas 1983
Beta-Binomial	$1-(1-P_I(1))^N$	$P_I(1)$ = probability of illness from exposure to one organisms. $P_I(1)$ assumed to be Beta(α, β) distributed	Cassin *et al*. 1998
Weibull-Gamma	$1 – [1 + (N)^b/\beta]^{-\alpha}$	α, β, b = parameters affecting shape of curve	Todd and Harwig 1996
Gompertz	$1 – \exp[-\exp(a + bf(x))]$	a = model (intercept) parameter, b = model (slope) parameter, f(N) = function of dose	Coleman and Marks 1998

N = Ingested dose of microorganisms

P^* = Probability of infection

1 Rose *et al*., (1991) used the form $1- e^{- r *dose}$

2 See Vose (1998) for a discussion on the interpretation of α, β

Models may be classified/distinguished in different ways, and depending on the assumptions and parameter values chosen some models may be special cases of other models (Haas 1983, Holcomb *et al*. 1999). One important distinction is between models describing infection as a deterministic and a stochastic process, respectively (Haas 1983). The deterministic view assumes that each organism have an inherent minimal infective dose, i.e. there is a threshold value, below which no response (depending on the endpoint) is seen. The value of the minimal dose in the population may be assumed to follow different distributions. The stochastic view on the other hand holds that the actions of individual pathogenic organisms are independent and that only a single micro-organism need survive until it can infect and provoke a response in the individual, i.e. a single hit, non-threshold model (Haas 1983).

Models can also be differentiated on the basis of whether they are mechanistic or empirical. Buchanan *et al*. (2000) suggested that most dose-response models used currently are empirical, and are limited because they attempt to extrapolate beyond the limits for which there is effective data. Potentially, mechanistic models would be more flexible since they focus on specific physiological or chemical attributes; however, there have been few attempts to develop such models. Buchanan *et al*. (2000) encouraged the development of mechanistic dose-response models, and outlined a simple three-compartment dose-response model for foodborne salmonellosis. No mechanistic dose-response models are currently available for *L. monocytogenes*.

Deterministic - threshold models

These models assume a minimum, threshold dose before the response occurs. In a given population the variation in the minimal dose can be described by a distribution. For instance, the Log-normal model assumes that the minimal dose is normally distributed (Haas 1983), whereas the Log-logistic model assumes that it follows a logistic distribution (Holcomb *et al*., 1999). Both these distributions are symmetrical about the mean, but the Log-logistic distribution allows for more variance away from the mean.

Marks *et al*. (1998) compared a Beta-Poisson model with a Beta-Poisson model that employed a threshold value of 3 bacteria in a risk assessment for *E. coli* O157 in hamburgers. The introduction of a threshold means that the location of the dose response curve is shifted along the x-axis by the threshold amount, and the differences between these models were significant only in the low dose range. The resulting estimates of risk were a 100 to a 1000-fold larger using the non-threshold model depending on the cooking temperature. The authors concluded that the two-parameter Beta-Poisson model appeared insufficient for describing the complexity of dose-response interactions and that it is inadequate as a default model for microbial risk assessment, especially in cooked foods (Marks *et al*. 1998). They also concluded that the consideration of threshold models as alternative dose-response models is of great importance and that additional research is needed in this area.

Stochastic - single hit models

Other researchers have favoured the use of single hit models, which in many instances have described data quite well (Haas 1983, Teunis *et al*. 1996). For instance, dose-response data for protozoan parasites can be well described by the exponential model (Teunis 1997), and bacterial dose-response is generally well described by Beta-Poisson models (Teunis *et al*. 1997, Teunis *et al*. 1999), or by the Weibull-Gamma model (Holcomb *et al*. 1999). The same model may not be

equally effective for all biological end points caused by the pathogen. For example, the FDA *L. monocytogenes* risk assessment reported that the exponential model did not fit mouse infection data (i.e., isolation of *L. monocytogenes* from the spleen and liver), but was among the best models for describing the relationship between dose and the frequency of death (FDA, 2000).

Exponential model:

In the derivation of this model it is assumed that all of the ingested organisms have the same probability, r, of being individually capable of causing an infection to a specific consumer. Also, the distribution of organisms is assumed to follow a Poisson process, with a mean number of organisms N per portion (Haas 1983, Vose 1998).

$$P = 1- \exp^{-r*N}$$

In a few cases, notably for the pathogenic protozoa *Cryptosporidium parvum* and *Giardia lamblia*, this model provides an acceptable fit, but the slope of the model is generally steeper than what is observed from data (Teunis *et al*. 1996). Holcomb *et al*. (1999) modified the form of the exponential model and termed it the simple exponential by using the log10 of the dose instead of the dose directly (Table 8). The reason was because the simple exponential model fitted more of the investigated data sets. Both these forms means that the fitted curve is forced to intercept the x-axis at 1 (or 0, log10(1)=0), which limits their ability to fit data where much greater doses results in 0 percent infection. To circumvent this problem, Holcomb *et al*. (1999) used a flexible exponential model (Table 8), which, while similar to the simple exponential model, does not have this limitation.

Beta-Poisson model:

In this model heterogeneity in the organism/host interaction is introduced and r, the probability of an organism to initiate infection given a successful introduction in the host, is assumed to follow a Beta-distribution. Haas (1983) suggested that this variation reflect the variation in virulence of the individual pathogens, or in the sensitivity of the host, or both. In contrast, Vose´s (1998) interpretation was that the beta-distribution characterised by its α and β values describes the expected probability of each of the consumed microorganisms to cause infection averaged over all volunteers.

In the derivation of this model a complex function results. However, under the assumption that β is much larger than both α and 1 the following approximation can be used:

$$P = 1- [1 + N/\beta)]^{-\alpha}$$

However, in some cases the use of this model to fit dose-response data has not fulfilled this condition (Teunis *et al*. 1996). These authors proposed to use the approximated function in all cases anyway since the influence of using the correct approach was rather insignificant. Vose (1998) criticised the use of α and β just as fitting parameters without any consideration of their interpretation in the beta distribution. For instance, values between 0 and 1 of these parameters

mean that the distribution of probability of infection will peak at both zero and 1. This could be interpreted as a polarisation among volunteers into susceptible and non-susceptible populations. Teunis *et al*. (1996) concluded that the Beta-Poisson model appear to fit most available dose-response data well and has the desired property of being conservative when extrapolated to low doses.

Beta-Binomial model:

Cassin *et al*. (1998) developed a Beta-Binomial dose-response model to assess the risk of *E. coli* O157:H7 in hamburgers. The model reflected the same assumptions used in the original Beta-Poisson model. However, the Beta-binomial model yields variability for probability of illness from a particular dose in contrast to the original model, which only specifies a mean population risk.

$$P = 1- (1-P_I(1))^N$$

$P_I(1)$ is the probability of illness from ingestion of one microorganism, and this probability was assumed to be Beta-distributed with parameters α and β. By fitting the model to data from human feeding studies with *Shigella* it was possible to generate a dose-response curve showing the estimated uncertainty in the average probability of illness verses the ingested dose. The variability between feeding studies was used as a proxy for the uncertainty in the parameters α and β.

Weibull-Gamma model:

This model was chosen by Farber *et al*. (1996) due to its flexibility, i.e. it is possible to accommodate available qualitative dose-response information for *L. monocytogenes*, and to be adaptable for both healthy and high risk groups. The starting point for the derivation is the Weibull model:

$$P = 1 - e^{-a*N\text{^}b}$$

N is the dose ingested and *a* and *b* are parameters. The parameter *a* is related to the probability of illness given exposure to a single organism, whereas *b* determines the shape of the individual dose-response curve. In this model host/pathogen heterogeneity is considered by assuming that *a* follows a Gamma distribution characterised by the parameters α and β. The resulting equation, the Weibull-Gamma model becomes:

$$P = 1 - [1 + (N^b)/ \beta]^{-\alpha}$$

Depending on the parameter values the Weibull-Gamma model can be reduced to both the Beta-Poisson and the Log-logistic models (Farber *et al*., 1996, Holcomb b*et al*. 1999).

Gompertz model:

Recognizing that a number of empirical models adequately may fit observed data Coleman and Marks (1998) in addition to logistic and Beta-Poisson models used a Gompertz model to datasets from human feeding studies.

$$P = 1 - \exp[-\exp(a + bf(N))]$$

Where *a* is a model (intercept) parameter, *b* is a model (slope) parameter, and f(N) is a function of dose.

Choice of dose-response model

The issue what functional form truly describes the reality, i.e. the interactions between the pathogen, the food vehicle and the host remains an open question that needs additional research. For instance, an equally good fit to *Shigella* dose-response data was provided by a Gompertz function as the Beta-Poisson model, but outside the data range the predictions differed greatly (Marks *et al*. 1998). The choice of dose-response model may depend on its applicability, e.g. how well does it fit the available data, the simplicity in model formulation including parsimony in the number of parameters without sacrificing adequacy, and the range of conditions over which the model gives good predictions (Holcomb *et al*. 1999). Holcomb *et al*. (1999) emphasised the flexibility of the dose-response model to fit data from different organisms allowing direct comparisons of infectious doses for use in risk assessment. In their comparison of how well the models in Table 8, fitted different experimental data they reported up to a nine order of magnitude difference in the predicted dose affecting one percent of the population. This illustrates the difficulty of extrapolating from high to low doses. They also concluded that the three-parameter Weibull-Gamma model was the only model capable of describing all data sets.

Although results of dose-response experiments fit a single hit model, i.e. the Beta-Poisson model well, some serious shortcomings have been noted (Teunis 1997, Teunis *et al*., 1999). The models ignore the incubation period and there is no opportunity for generalisation with regard to microorganism, host and vector. Further, the probability of illness changes with dose in a manner different from that of the probability of infection. For instance, a decrease in the probability of illness was noted with a higher dose of *Campylobacter jejuni* (Black *et al*., 1988, Teunis *et al*, 1997). Teunis *et al*. (1999) developed a hazard function for the probability of illness, given successful infection, occurring in the time between onset and clearing of the infection. The duration of the infection period was supposed to follow a Gamma distribution, and the scale parameter, λ, representing the time scale for the primary events (Poisson processes) responsible for clearing the infection, was the authors primary choice for dose dependence. Three possible scenarios were modelled; increasing illness hazard with dose, decreasing illness hazard with dose, and a dose independent illness hazard. Examples of each of these possible scenarios were illustrated with volunteer data from the literature. The different alternatives were suggested to reflect the balance of the interactions between the pathogen and the host (Teunis *et al*. 1999).

In their risk assessment of *L. monocytogenes*, FDA (2000) assumed that there is no *a priori* means of determining which is the "correct" model to fit to a data set. Accordingly, they

employed an alternate approach of fitting several of the dose-response models described above to dose-response data. An integrated dose-response relationship was then derived by combining the individual dose-response curves after weighting to take into account how well each model fit the data. The differences in response values at any single dose predicted by the individual models were used as a means of estimating the uncertainty related to model selection.