ABSTRACT

Vicari, Andrea Sebastiano. Quantitative Methods for Hazard Characterization of Food-Borne Pathogens. (Under the guidance of Drs. Peter Cowen and Lee-Ann Jaykus.)

Over the last decade, heightened awareness about the consequences of foodborne illnesses has fomented the application of quantitative risk assessment to food safety issues. A four-step paradigm – hazard identification, exposure assessment, hazard characterization, and risk characterization – is commonly followed. Hazard characterization is meant to consider the multifaceted interaction between pathogen, host, and food matrix, but it is reduced in practice to a mathematical function linking an exposure dose to an infection/illness probability. Such an approach has obviously a limited capacity to weigh into a risk assessment potential sources of uncertainty (lack of knowledge) and variability (population heterogeneity).

This study develops an analytical framework that makes possible the quantitative consideration of selected uncertainty and variability elements in microbial hazard characterization. Firstly, the bootstrap method is applied to quantify the sampling error associated with fitting dose-response functions to data from volunteer feeding trials with *Campylobacter jejuni* and *Shigella dysenteriae*. The results show that the relevance of theoretical considerations regarding the form of the dose-response function or the resampling scheme depends on the dose range of interest. Further, an epidemiological analysis of FoodNet surveillance data is carried out to estimate the effect of the covariates age and gender on the occurrence of foodborne infection. Specifically, Poisson regression analysis is applied to model *Campylobacter*, *Salmonella*, and *Shigella* rates.

While gender does not cause significant differences, the analysis characterizes the risk associated with specific age groups. Setting young adults as the reference group, the highest relative risks of *Campylobacter* and *Salmonella* infection are in infants (1.87 and 9.15, respectively), while teenagers and the elderly are associated with the lowest relative risks (0.43/0.51 and 0.71/0.70, respectively). Results for *Shigella* are less reliable due to questionable model fit. The final part of the study integrates the previous findings into a probabilistic risk assessment that, with risk management in mind, keeps uncertainty and variability separate. Using two-dimensional Monte Carlo simulation and stratification into homogeneous population subgroups, the risk of foodborne *Campylobacter* infection is calculated for eight different age groups taking into consideration the sampling error attendant to the dose-response function. While age variability turns out to have only a minimal relevance, uncertainty associated with the dose-response function has a major impact on the results.

Overall, this study shows that biological plausibility and epidemiological evidence do not necessarily translate into risk assessment relevance. It is advanced that, in microbial hazard characterization, sources of variability have to be explicitly modeled only to the extent that the magnitude of their effect is large or that they are fundamental to the needs of the risk manager. In contrast, characterization of key sources of uncertainties – in particular of the sampling error associated with the dose-response function – and their consistent propagation throughout a microbial risk assessment appear to be of great importance.

QUANTITATIVE METHODS FOR HAZARD CHARACTERIZATION OF FOOD-BORNE PATHOGENS

by

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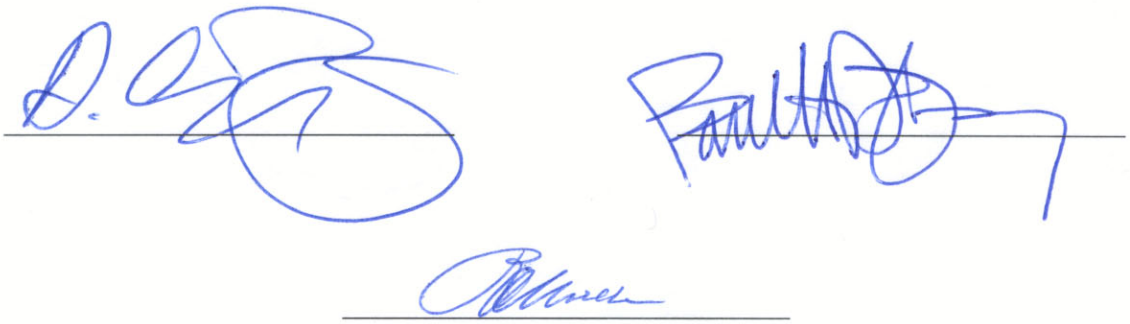

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During my five-year "career" as graduate student (that started at the University of Maryland in College Park, and concludes at NCSU), I was blessed with having not one, but four very special advisors, Drs. Roberta Morales, Peter Cowen, Lee-Ann Jaykus, and Will Hueston. Nobel Laureate Peter Medawar wrote that, "among scientists, many are detectives by temperament and many are explorers; some are artists and other artisans." Instead of molding me into any one of those categories, Roberta, Peter, Lee-Ann, and Will – by the example of their intelligence and open-mindedness – made me understand the benefits of being a bit of everything. Through that, they helped me becoming not only a more skilled epidemiologist, but also a more conscious person. I will always be grateful to Roberta, Peter, Lee-Ann, and Will for that.

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1 HAZARD CHARACTERIZATION IN FOOD SAFETY RISK ASSESSMENT: BACKGROUND, THEORY, AND CURRENT PRACTICE

1.1 Background and Historical Perspective on Food Safety Risk Assessment

Quantitative microbial risk assessment offers a formal approach to evaluate human health risks caused by foodborne pathogenic microorganisms (Jaykus, 1996). Challenged by an apparent emergence of previously unrecognized microbial foodborne disease hazards, regulatory officials in industrialized nations have recognized that risk assessment coupled with risk management can be an effective tool for implementing science-based food safety policies. In the international arena, microbial risk assessment is a central element in the resolution of agricultural and food-related trade disputes.

A widely accepted framework for the conduct of risk assessment relating to foodborne pathogens has been elaborated by the Codex Alimentarius Commission of the U.N. Food and Agriculture Organization (Codex Alimentarius Commission, 1999). The framework includes four very distinct steps, i.e. hazard identification, exposure assessment, hazard characterization, and risk characterization. An essentially similar document had previously been developed in the United States by the National Advisory Committee on Microbiological Criteria for Foods (Buchanan, 1997). A further framework by the Risk Science Institute of the International Life Sciences Institute (ILSI/RSI) provides more guidance on actual methodological considerations (ILSI/RSI, 1996; ILSI/RSI, 2000). Specifically, for hazard characterization, three components are proposed as crucial: host characterization, evaluation of health effects, and quantification of the dose-response relationship. These elements are to be integrated in a host-pathogen profile, which is the outcome of the process. In light of the ILSI/RSI framework, hazard characterization can thus be seen as a more detailed approach to dose-response assessment. More recently, the Codex Alimentarius Commission has initiated an extensive consultation which also aims at establishing precise guidelines on the conduction of hazard characterization for pathogens in food and water (Codex Alimentarius Commission, 2000).

As it will be discussed in subsequent sections, hazard characterization of foodborne pathogens has mainly been limited to a dose-response assessment with dose as the major factor determining the probability of infection or illness. Other components comprising host, agent and environment factors (as implicitly advocated by the ILSI/RSI framework) have not made their way into hazard characterizations to any great extent. Indeed, some authors have viewed this situation as a shortcoming (Jaykus, 1996; Buchanan et al., 2000). Although the need to further develop microbial dose-response models is generally recognized when research resources are allocated in the United States, it is questionable whether there has been a conscientious commitment towards a holistic approach similar to the one proposed by ILSI/RSI. Along other elements, the U.S. National Food Safety Initiative advocated in May 1997 the development and validation of models for dose-response assessment (Anon., 1997). Specifically, this document cited the need to identify which type of models (i.e. threshold or non-threshold) are more appropriate for describing low-dose infectivity rates of infectious and toxico-infectious microorganisms. Strictly speaking, this does not amount to a rebuttal of the prevailing practice. More recently, besides a commitment to develop modeling techniques to assess microbial dose-response relations, the research priorities for the fiscal year 2001 of the Center for Food Safety and Applied Nutrition of the U.S. Food and Drug Administration list the need to establish population trends with respect to behavioral risk factors associated with foodborne illness (FDA/CFSAN, 2000).

In the United States, performance standards are a component of food safety objectives. With regard to the microbial content of food, this essentially translates into establishing tolerance thresholds. In such a strongly prescriptive setting, it is evident that dose will have to remain a major consideration, if not the cardinal one, of any hazard characterization. Under these circumstances, the focus is clearly on predictive models (i.e. models that are as simple as possible) rather than explanatory ones (i.e. models that are as simple as necessary). It should, however, be recognized that, if dose was no longer the principal interest, hazard characterization could take on forms very different from the current one.

1.1.1 Historical Perspective

The current practice in microbial dose-response assessment can be better understood when put into the historical perspective of the evolution of risk analysis. Such an understanding is important because it suggests future avenues for the development of microbial hazard characterization.

Covello and Mumpower (1985) identified a two-fold root of modern risk assessment in probability theory and analytical methods for establishing causal factors of diseases. It is interesting to note that the former still had an important link to medicine. The concept of calculating empirical probabilities was, in fact, introduced by John Gaunt with his "Bills of mortality" published in 1662 (Bernstein, 1998).

Formal risk assessment was initiated in the United States as early as the 1930s with a focus on noxious agents in industry. Risks posed by ionizing radiation were assessed starting in the 1960s, and carcinogens in food in the 1970s. In the mid-1970s, the Environmental Protection Agency and the Occupational Safety and Health Administration were established, and have become major proponents and users of health risk assessments within the U.S. government (U.S. Congress/OTA, 1993; Boroush et al., 1998).

The body of risk assessment experience in the United States was eventually unified in a 1983 document of the National Research Council entitled "Risk Assessment in the Federal Government: Managing the Process" (National Research Council, 1983), which has become known as the "Red Book". Given its genesis, it is not surprising that the focus is on environmental concerns, in particular those posed by carcinogens. A direct inheritance of the industrial setting and consequent engineering leaning is the tendency to view complex situations as modular, mechanistic systems. Other disciplines, in particular toxicology and epidemiology, are seen more as sources of data than of methods.

The legacy of the Red Book is still apparent in several fields, including food safety. Specifically, the four-step approach advocated by the Codex Alimentarius

Commission parallels the Red Book paradigm. However, even more than on specific methodological approaches and methods, the most important influence of the Red Book can perhaps be in conceptual terms. This might explain why, despite the facts that risk analysis has its ancient roots in medicine and the concept of risk is very much present in medicine (especially in epidemiology), the "biomedical dimension" of current microbial risk assessments is often subsidiary to an inherently biomathematical approach. This seems to be particularly evident in hazard characterization.

1.2 Frequency and Burden of Foodborne Illnesses in the United States

Foodborne diseases are commonly regarded as some of the most widespread health problems (Motarjemi & Kaferstein, 1997). It is nonetheless difficult to make an accurate estimation of their incidence because surveillance of foodborne illnesses is complicated by several factors (Mead et al., 1999). Although diarrheal diseases can be severe or even fatal, milder cases usually do not require medical care and thus go underreported. Secondly, the role of foodborne transmission is obscured by the fact that many pathogens transmitted through food are also spread through water or from person to person. Finally, some proportion of foodborne illness is likely to be caused by microorganisms whose role as foodborne pathogens has yet to be recognized. For instance, the roles of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Cyclospora cayetanensis* as causes of foodborne illness were unrecognized just two decades ago.

In the United States, the Centers for Disease Control and Prevention (CDC) located in Atlanta have a leading role in collating data on foodborne illnesses. Those data are generated from monitoring activities at local and national levels. Historically, statistics on foodborne illness have relied on "passive" surveillance, i.e. clinical microbiology laboratories have reported cases of foodborne disease to state health departments which have in turn made communication to CDC. Several systems based on this principle exist at the national level (CDC, 1997; Mead et al., 1999). For instance, the national surveillance of *Salmonella* is conducted through the Public Health Laboratory Information System. Approximately 40,000 culture-confirmed cases are reported each

year through this system to CDC. The Foodborne Disease Outbreak Surveillance System collects data on recognized foodborne illness outbreaks. These data are reviewed in detail in the next section (Section 1.2.1). A feature common to all passive surveillance systems is basically the limited effort by public health officials to guarantee a reliable detection of cases and transmission of information. While such systems are able to monitor trends in the number of illness, it is commonly accepted that their absolute figures result in large underestimation of the actual incidence of foodborne illnesses. In fact, as shown in Figure 1.1, a complex chain of events must occur before a case is counted within the CDC data (CDC, 1997).

To obviate to these shortcomings, an "active" surveillance system – the Foodborne Diseases Active Surveillance Network (FoodNet) – was established in 1996 as a collaborative effort by the CDC, the U.S. Department of Agriculture, the U.S. Food and Drug Administration, and selected state health departments. FoodNet represents an active surveillance system in the sense that public health officials regularly contact microbiology laboratory directors to find new cases of selected diseases thought to be primarily foodborne and report these cases to CDC. It is designed to monitor each of the events that occur along the foodborne diseases pyramid (see "Evaluated by" column of Figure 1.1). FoodNet is discussed further in Section 1.2.2.

1.2.1 Foodborne Disease Outbreak Surveillance System

The Foodborne Disease Outbreak Surveillance System collects data on recognized foodborne illness outbreaks. Its working definition for an outbreak is two or more cases of a similar illness resulting from ingestion of a common food (Bean et al., 1990; Bean et al., 1996; Olsen et al., 2000). A similar system also exists for waterborne disease outbreaks (Barwick et al., 2000). The number of foodborne outbreaks reported in the United States from 1988 to 1997 is shown in Figure 1.2, while the total burden of illness associated with these outbreaks is represented in Figure 1.3.

While the cause of the majority of foodborne outbreaks is unknown, the number of outbreaks with definitive etiology remains relatively constant (Figure 1.2). In contrast,

the temporal trend of the illness number appears to be essentially driven by bacterial outbreaks (Figure 1.3). Figure 1.4 shows further that *Salmonellae* cause the vast majority of illnesses related to bacterial outbreaks. Based on these observations, it can be speculated that the number of illnesses specifically related to foodborne outbreaks is primarily determined by the episodic occurrence of large *Salmonella* outbreaks. Since the data are published in a cumulative form, this hypothesis cannot be further tested. Interesting considerations concerning the setting and vehicle of foodborne outbreaks can also be made (Figure 1.5 and Figure 1.6). While the importance of public food consumption is asserted, the data also indicate the variety of food items that can potentially function as vehicles for the transmission of foodborne pathogens.

Although outbreaks are often the newsworthy effect of foodborne diseases, the total number of cases that they cause is merely a fraction of all foodborne illnesses that occur each year. Hence, "sporadic" cases – that is, cases of foodborne illnesses that are not or cannot be linked to an outbreak – make up the majority of foodborne illnesses. This point is illustrated in the case of *Salmonella* isolates (Figure 1.7). In the period from 1988 to 1997, more than two thirds of the *Salmonella* isolates that were reported to CDC were from sporadic cases (range: 68% in 1996 to 93% in 1988, Figure 1.7). The proportion of sporadic cases for other foodborne pathogens is even higher than the one observed with *Salmonella*. Although the distinction between outbreak and sporadic cases is somewhat artificial given the working definition of outbreak used by CDC (i.e. two or more linked cases), the observation is meaningful in the context of microbial dose-response assessment. In fact, a high proportion of sporadic cases may be suggestive of a small attack-rate and thus of low-dose exposure. This conclusion is consistent with the postulate that the actual dose ingested in sporadic cases of human salmonellosis may frequently be the 1% infective dose (ID_1) (Blaser & Newman, 1982).

1.2.2 Foodborne Diseases Active Surveillance Network (FoodNet)

The Foodborne Diseases Active Surveillance Network (FoodNet) records laboratory confirmed cases associated with seven bacteria (*Campylobacter* spp., *Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Vibrio*

spp., *Yersinia enterocolitica*) and two parasitic protozoa (*Cryptosporidium* spp., *Cyclospora* spp.). In 1999, the system covered regions in seven U.S. States for a population of 25.6 million inhabitants (equivalent to 9.3% of the national population).

In addition to measuring longitudinal incidence estimates of foodborne illness, surveys on the frequency of diarrhea in the general population, the proportion of ill persons seeking care, and the frequency of stool culturing by physicians and laboratories for selected foodborne pathogens are investigated within the framework provided by FoodNet. In the context of food safety initiatives, FoodNet can be used to help evaluate the efficacy of enacted regulatory measures, such as the USDA Food Safety Inspection Service's Pathogen Reduction and Hazard Analysis and Critical Control Points (HACCP) Rule, in decreasing the number of cases of foodborne diseases in the United States. To illustrate this point, the incidence rates of foodborne disease per 100,000 inhabitants estimated through FoodNet are presented in Table 1.1. While *Campylobacter* spp. and *Salmonella* spp. remain the leading causes of foodborne diarrheal illnesses, these figures indicate that the total relevance of all considered diseases and the incidence of *Campylobacter* spp. diseases have diminished between 1996 and 1999. Contemporaneously, a staggered implementation of HACCP in poultry and beef slaughter plants occurred over the same period.

While mortality resulting from acute gastroenteritis is generally considered in the overall foodborne illness statistics, no information about chronic manifestations is considered. Also, measures that would allow one to evaluate the clinical course of diseases (e.g. hospitalization rate) or the disease burden (e.g. disability adjusted life years, DALYs) are not routinely recorded, and they can only be extracted from the general literature. A document redacted by a group of experts in 1995 reviews possible sources of these data for the United States (USDA/ERS, 1995), some of which may find application in microbial hazard characterization.

1.2.3 Burden of Foodborne Illnesses in the United States

Several attempts have been carried out over the years to estimate the total burden caused by foodborne diseases in the United States (see Table 1.2). Although inevitably flawed by a paucity of data and thus requiring a large number of assumptions, such estimates have a critical influence in setting public health priorities. A closer look at the processes used to generate such estimates is thus warranted.

By coupling information on underreporting of salmonellosis with data on other foodborne pathogens, Archer and Kvenberg estimated in 1985 that 24 to 81 million illnesses due to all foodborne agents occurred in the United States each year (Archer & Kvenberg, 1985). Illnesses due to known pathogens were estimated at 8.9 million. In 1987, Bennett et al. computed incidence figures for all known infectious diseases and for different transmission modes. It was concluded that foodborne transmission of known pathogens caused 6.5 million illnesses and up to 9,000 deaths. Todd (1989) employed a combination of methods, including extrapolation from Canadian surveillance data, to derive an estimate of 12.5 million foodborne illnesses and 522 related deaths. A group of experts convened by the Council for Agricultural Science and Technology concluded in 1994 that illness cases likely ranged between 6.5 and 33 million and that deaths might be as high as 9,000 (CAST, 1994). Most recently, Mead et al. (1999) compiled and analyzed information from multiple surveillance systems, including FoodNet. The analysis entailed three basic assumptions that concerned the degree of underreporting, the proportion of foodborne transmission for the individual pathogens, and the frequency of acute gastroenteritis in the general population. It was concluded that foodborne diseases cause approximately 76 million illnesses and 5,200 deaths. Known pathogens were estimated to account for 14 million illnesses, and 1,800 deaths. The same authors recognized two limitations in these estimates. Firstly, separate calculation methods were necessary for estimates specific to bacterial, parasitic, and viral pathogens because of different surveillance information. Secondly, some occasionally infectious pathogens, such as *Plesiomonas*, *Aeromonas*, and *Edwardsiella*, as well as noninfectious agents, such as mushroom or marine biotoxins, metals, and other inorganic toxins, were not

considered because of a lack of surveillance information. Mead et al. (1999) also discussed possible explanations for the discordance among estimates obtained by different authors. Firstly, it is noted that the various figures often refer to a different group of pathogens, i.e. either to known pathogens or to all causes of foodborne illnesses (i.e. known and unknown, infectious and noninfectious). Secondly, the single analyses used data from different sources. Finally, different rates of foodborne transmission were assumed.

An estimation of the global costs caused by seven foodborne pathogens (*Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Toxoplasma gondii*) has been attempted for the United States (Buzby & Roberts, 1997). Based on the assumption that those microorganisms yearly cause an estimated 3.3-12.3 million cases of foodborne illness and up to 3,900 deaths, a total cost of $6.5-$34.9 billion per year was calculated (in 1995 US dollars). This figure does not account for the consequences caused outside the United States, such as the cost in countries importing U.S. food products.

1.3 Select Aspects of the Manifestation and Pathogenesis of Foodborne Illnesses

1.3.1 Clinical Manifestations of Foodborne Infections

Since it encompasses a variety of clinical conditions with diverse etiology, the term foodborne disease is rather generic. Acute gastrointestinal manifestations associated with diarrhea and vomiting are the most readily recognized subset, and are often linked to an infectious etiology. However, it is seldom grasped that clinical manifestations affecting organ systems other than the gastrointestinal tract may accompany acute gastroenteritis or occur independently (Lindsay, 1997). The variety of human responses to foodborne pathogens – from overt illness to chronic carrier status – as well as the variability of such responses among individuals is undoubtedly intriguing.

In acute gastroenteritis, the character of diarrhea varies from watery to bloody depending on microorganism. Acute bacterial gastroenteritis can be classified into toxigenic and invasive (Hamer & Gorbach, 1997). In the toxigenic type, the major

pathogenic effect of the microorganisms is exercised by an enterotoxin, which either injures the mucosal cell or causes the secretion of intracellular fluid. The prototype microorganisms in this group are *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC). Invasive pathogens, such as non-typhoid *Salmonella*, *Shigella*, enteroinvasive *E. coli* (EIEC), *Campylobacter* and *Yersinia*, impact the host by invading the intestinal epithelium. In this case, relatively little is known about the precise mechanisms of fluid production. Whereas toxigenic microorganisms characteristically exert their effect on the small intestine, invasive pathogens usually target the distal ileum and colon. As causal agents of gastroenteritis, four virus families are of particular interest: rotaviruses, enteric adenoviruses, caliciviruses including Norwalk virus, and astroviruses. They attack the upper small intestines, where patchy mucosal lesions are caused (Hamer & Gorbach, 1997).

Bacteremia and septicemia can be a direct, systemic complication of acute infections. Conversely, several long-term complications, which may be unrelated to an acute illness, have been described. These include arthropathies, renal disease, cardiac and neurologic disorders, and nutritional and other malabsorption disorders (Archer & Young, 1988; Bunning et al., 1997; Lindsay, 1997; McDowell & McElvaine, 1997). Table 1.3 lists some complications of foodborne infections and their potential etiological agent. In truth, the evidence for a direct or indirect association of such chronic sequelae to foodborne pathogens or their toxins/antigens ranges from convincing to circumstantial. Such a partial lack of evidence has several explanations. Firstly, the complication often occurs weeks or months after the specific gastrointestinal incident (which may itself have gone unnoticed). A temporal link may thus be missed. Furthermore, the clinical signs caused by a specific pathogen or its product are often uncharacteristic, and overlap with those of other etiologies. Finally, several sequelae have an autoimmune genesis, and often occur after the primary infection has been successfully eliminated.

The ability of a foodborne pathogen to cause clinical manifestations other than acute gastroenteritis is illustrated with the example of nontyphoidal *Salmonella*. Using predominantly outbreak data, several authors have attempted to establish the prognosis

and frequency of such manifestations. Mattila et al. (1998) considered a *S. bovismorbificans* outbreak that occurred from sprouted alfalfa seeds, noting 83% (159) of the respondents reported diarrhea, 57% fever, 54% abdominal pains, 43% fatigue, 35% articular symptoms and 10% with no symptoms. At medical examination, 12% (22) fulfilled the criteria for reactive arthritis (ReA). Joint symptoms were mainly oligoarticular and mild in severity. These lasted less than two months in 11 patients, two to four months in 7, and more than four months in 4. Sixty-one percent of all the respondents required a physician visit due to intestinal or extra-intestinal symptoms, and 11% needed hospitalization. In a similar outbreak caused by *Salmonella enterica* serovar 4,5,12:b:- originating from sprouted mung beans (Mattila et al., 1994), 91% (224) of respondents reported having had enterocolitis, and 7% (16) fulfilled the criteria for ReA. Subjects older than 16 years of age were at a significantly greater risk to develop ReA. The joint symptoms were oligoarticular in 10 patients, monoarticular in 5, and polyarticular in 1. ReA was mild in the majority of the patients. Joint symptoms persisted for less than one month in 6 patients, two to six months in 5, and more than six months in 5. Following a *S. typhimurium* outbreak linked to milk, a 2.3% incidence of ReA was established by physical examination of patients with *Salmonella*-positive stools (Ike et al., 1986). ReA occurred 10-times more frequently than Reiter's syndrome. In a follow-up, 20 patients out of 29 still reported persistent ReA symptoms one year later, and severity had actually worsened in 6 cases. A large outbreak of *S. typhimurium* PT 22 occurred in a group of police officers given a prepackaged box lunch (Inman et al., 1988; Thomson et al., 1995). Out of 340 respondents, 58% of the individuals experienced extra-enteric symptoms including headaches (54% of respondents), joint pain (31%), redness or soreness in the eyes (11%), soreness in the mouth (4%), and skin rash (3%). A positive correlation between duration of gastrointestinal symptoms and duration of joint symptoms was noted. Out of 19 subjects reporting joint pain at medical examination, ReA was diagnosed in 13 patients, and Reiter's syndrome in 6 patients. A follow-up was conducted on 15 patients, and found that arthritis symptoms persisted twelve months after the outbreak in 7 patients. In a *S.* Enteritidis outbreak involving scientists that attended a

medical symposium, 96% (108) of the respondents developed gastroenteritis and 15% developed ReA (Locht et al., 1993). A follow-up after six months involving 13 of the 17 individuals who had developed ReA showed arthritis persisting in 8 patients. In a prospective study of *S.* Enteritidis infection in 9 children, 4 patients required hospitalization, and arthritis developed in all patients within one to three weeks following the diarrheic episode (Kanakoudi-Tsakalidou et al., 1998). This juvenile ReA lasted between one and twelve months. Among the 83 participants who attended a luncheon in which a potato salad contaminated with *S. heidelberg* and *S. hadar* was served, 88% subjects contracted Salmonellosis and 7% developed ReA (Thomson et al., 1992). In the 6 individuals who developed ReA, the symptoms ranged from one to six months in 4 individuals, and more than six months in two. In a review of several published outbreaks totaling 5525 patients with salmonellosis, Maki-Ikola (1992) estimated an incidence of ReA from 1.2% to 7.3% (mean 3.5%). It is commonly considered that 1% to 2% of the subjects infected with any of the enteric pathogens known to trigger reactive arthritis or Reiter's syndrome will develop the disease (Smith et al., 1993; Keat, 1983). However, it has been proposed that, in HLA-B27-positive subjects, as many as 20% of the exposed will develop ReA, Reiter's syndrome or ankylosing spondylitis (Aho et al., 1974; Archer & Young, 1988). In contrast, a lack of correlation between ReA and HLA-B27 has been observed after outbreaks caused by *S. typhimurium*, *S. heidelberg, S. hadar, Salmonella enterica* serovar 4,5,12:b:- (Inman et al., 1988; Thomson et al., 1992; Mattila et al., 1994; Thomson et al., 1995).

Acute gastroenteritis undoubtedly remains a leading cause of morbidity and mortality for children in developing countries. It is also of particular concern for any individual with an underlying immunocompromising condition. Yet, as discussed above, the relevance of chronic manifestations is likely to be underestimated since a link to a prior foodborne infection is often unrecognized. An estimated 2% to 3% of foodborne infections eventually results in chronic sequelae (Archer & Young, 1988). Given their duration and the therapeutic intensity that they require, the long-term burden to human health is likely to be more detrimental than the one caused by acute non-fatal disease. In

a risk assessment of foodborne pathogens, this consideration implies that the endpoints of interest have to be carefully defined during the hazard identification. However, it must be noted that the definition of infection as well as consideration of endpoints other than acute gastroenteritis and death have seldom received detailed consideration.

1.3.2 Interaction between Human Hosts and Foodborne Microorganisms

The human host and its microbial flora constitute a complex ecosystem which is permanently in a delicate balance (Tancrede, 1992). In particular, the gastrointestinal immune system has the ability to mount a defensive response against pathogens while avoiding an inappropriate action against normal bacterial flora (Duncan & Edberg, 1995). The occurrence of gastrointestinal infection can thus be thought of as a disruption in the balance between a microorganism's virulence characteristics and host defense mechanisms. For that to happen, a sufficient quantity of the pathogen must be present in the target section of the gastrointestinal tract, the microorganism must express specific virulence factors, and the gastrointestinal immune defenses must be overcome. Such an occurrence is formally expressed as the ratio of the ingested pathogens and their virulence characteristics to the immune status of the host:

$$\text{Infection} \approx \frac{\text{Number of pathogens} \times \text{Virulence characteristics}}{\text{Immune status of host}}$$

Our understanding of this complex relation as well as our knowledge of the host and pathogen factors that concur in defining it is imperfect. In spite of this uncertainty, the prevailing opinion among microbial risk assessors is that these factors need to be included in dose-response models (Coleman & Marks, 1998b; Buchanan et al., 2000). For instance, the ILSI/RSI framework lists several elements that may be included in the host or pathogen characterization (ILSI/RSI, 1996; ILSI/RSI, 2000). At least in descriptive terms, the components of the host defense system as well as the mechanisms followed by pathogens to avoid them have been previously described (CAST, 1994; Coleman & Marks, 1998). However, referring specifically to risk assessment of foodborne and waterborne pathogens, Duncan and Edberg (1995) pointed out that the

human gastrointestinal tract is rather resilient, and that very profound and specific immunological changes must occur in order to put the host at risk. Hence, they consider the general definition of "immunosuppressed host" or "immunocompromised host" – often used in human health risk assessments – as meaningless unless a specific immune defect related to the gastrointestinal tract is present. It can be concluded that, while there is little doubt that a variety of host and pathogen characteristics concur in defining a dose-response relation, their actual relevance in the practical context of a risk assessment has not been studied nor proved.

Virulence factors of foodborne bacterial pathogens are essentially targeted at accomplishing three main goals: adherence to intestinal wall, production of enterotoxin, and invasion of the enteric epithelium. For each of those tasks, common virulence factors among microorganisms within and across genera have been identified based on the conservation of similar mechanisms and their regulatory elements (Finlay & Falkow, 1989; Finlay & Falkow, 1997). However, complex regulatory circuits that respond to various environmental signals tightly control the timely expression of those virulence factors. Furthermore, maintenance of virulence factors on mobile genetic elements and pathogenicity islands ensure that new strains of pathogens evolve constantly. While comprehension of these common themes in microbial pathogenicity is critical to the understanding and study of bacterial virulence mechanisms, the application of such new knowledge in microbial risk assessment is far from being evident.

With respect to microbial risk assessment, findings of epidemiologic studies probably offer the best opportunity to identify host factors worth considering in the dose-response relation. A review of two dozen analytical studies, i.e. case-control and cohort studies, related to *Salmonella* infections is presented in continuation. On one hand, the review points to a myriad of risk factors (Table 1.4). Based on calculated odds ratios or relative risks, a prioritization of the factors is possible. On the other hand, since studies often differ in the factors considered or the magnitudes of effects vary from one study to another, the resolution of inconsistencies among studies requires a significant amount of

judgment. Methods employed in meta-analysis could help to systematically address this difficulty.

Age. A common observation is that age of patients with *Salmonella* infections is distributed according to a bimodal distribution with peaks in children and the 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*. Additionally, both serovars were more likely to lead to bacteremia in middle and older age groups than in those younger than 5 years of age (Le Bacq et al., 1994), confirming a previous observation made in the United States (Blaser & 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 most common intestinal isolates, while *S. typhi*, *S. typhimurium*, and *S.* Enteritidis were the most common extraintestinal isolates. In a British population-based study, the 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; CDC, 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 titers 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 might be spurious. It is likely that children and the elderly with diarrhea are more frequently cultured than other age groups (Banatvala et al., 1999). Further, age influences the relative exposure to specific serovars. This reason possibly explained an increased risk of infection with resistant *Salmonella* that was observed in infants (Lee et al., 1994). Moreover, age association

may reflect behavioral characteristics. For instance, eating snow, sand, or soil – a behavior more likely in children – was found to be associated with *S. typhimurium* O:4-12 infection (Kapperud et al., 1998).

Gender. In terms of number of isolates, men seem to be generally more affected than are women. A male-to-female ratio of 1.1 has been reported in various occasions (Blaser & 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 studies, 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. A potential role of race and ethnicity has seldom been considered. 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, an 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 populations living in such areas more frequently ingested vehicles harboring *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 diarrheal diseases. In particular, there were nearly twice as many cases of diarrhea 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 Panama, six representative dwelling types were considered as proxies for social and economic influences on the prevalence of specific enteric pathogens among infants with diarrheal disease (Kourany & 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 improved waste water management and drinking water quality consequent to economic development has sensibly diminished the importance of those factors in some countries.

A French study on sporadic *S.* Enteritidis infections in children investigated the influence of diarrhea in another household member in the 3-10 days before a child showed 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

household contact, while children between 1 and 5 years of age are more likely to 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 with both *S.* Enteritidis and serovars other than Enteritidis, although to a greater extent for the latter case. While patients with *S.* Enteritidis were more likely to have traveled within Europe, the majority of non-Enteritidis infections were apparently 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 based on residents of Norway (Kapperud et al., 1998). 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 neighboring countries was the only risk factor considered in the study that remained independently associated with 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* is concerned, no genetic factors related to the host have been reported. As mentioned previously, reports concerning race and ethnicity should be considered in light of eating habits as opposed to genetic risks.

A 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 (Khan, 1996). 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). 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).

Immune status. The host immune status is, as in any other infectious disease, a very important factor 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.

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 & 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. Of these, diabetes was the only condition that was independently associated with infection after exposure to the contaminated meal. Although diabetic cases tended to be more likely to develop symptomatic illness compared to non-diabetics, 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 the only two out of fourteen health conditions that were associated with clinical illness (Kass et al., 1992). Nongastrointestinal medical conditions and, to a larger extent, a recent history of gastrointestinal disorder were associated with sporadic *S. typhimurium* O:4-12 infection in Norway (Kapperud et al., 1998). 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).

Concurrent medications. Although the use of gastric acidity reducers and antimicrobial medication are often considered, the evidence found in the literature concerning their association with human Salmonellosis is conflicting. Some studies find associations with antacid use (Banatvala et al., 1999), others do not (Telzak et al., 1991; Kapperud et al., 1998). 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., 1998; Banatvala et al., 1999). However, having a resistant *Salmonella* infection has been associated with previous antibiotic use (Lee et al., 1994). Additionally, antibiotic use during acute Salmonellosis gastroenteritis can lead to a prolonged clinical course and higher rate of carriage, and is only indicated in the case of possible bacteremia.

Among the 11 different medical therapies considered by a Northern California study on sporadic, clinical Salmonellosis (which also included antacids and antibiotics), only hormonal replacement therapy – principally conjugated estrogen – in older women was associated with 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 factor for *S. typhimurium* O:4-12 infection in Norway (Kapperud et al., 1998). In the same study, use of antacids and antibiotics were not risk factors.

In addition to host and pathogen characteristics, factors related to the meal composition and its ingestion time are possibly involved in influencing the dose-response relation. The *Salmonella* case is again considered for illustration. A survey of major outbreaks (Table 1.5) shows that foodborne Salmonellosis can be related to a variety of food items (D'Aoust, 1997). In spite of the numerous, potential pathogen-commodity combinations, the more recent literature 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 also contribute to their frequent involvement. For instance, live poultry as a 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.

Gastric acidity is an important defense against food-borne pathogens. A variety of pathogen, host, and food factors interact in determining whether a sufficient number of bacteria are able to withstand stomach acidity and 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 the 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 the food, time of the meal, and nature of contamination. The reference to food rather than to food items emphasizes the importance of considering 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 homogenous distribution of the pathogen among the chicken pieces is unlikely (Glynn & 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 infective 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. heidelberg*, 1-6 cells of *S. typhimurium*). Consequently, microorganisms trapped in hydrophobic lipid moieties may be more likely to survive the acidic conditions of the stomach, and the fat content of contaminated foods may thus play a significant role in human salmonellosis. It is, however, unlikely to be a sufficient cause, because experimental evidence in rats shows that *Salmonella* infection is not affected by milk fats (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 & Small, 1998). The same study showed that the pH of the microenvironment occupied by the bacteria on the surface of a food source was critical to their survival.

The effect of substrate was studied in volunteers challenged with *Vibrio cholerae* fed in a buffered medium (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 & 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 consideration 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 occur. In such a case, it can be speculated that the outer layers of bacteria could protect the inner ones, further facilitating at least some pathogen survival during gastric passage.

1.3.3 Hypotheses on Mechanisms of Microbial Infection

The use of dose-response models based on sound biological foundations has been advocated, in particular when there is the need to extrapolate to low doses. This has often been the case in health risk assessment (Gaylor & Razzaghi, 1992). Several microbial dose-response models have been developed from specific hypotheses concerning the mechanisms of infection. Such hypotheses are reviewed in continuation.

A common observation in experimental challenges of a host with bacteria has been that inoculation with a single pathogenic microorganism is unlikely to cause disease or death. In contrast, challenge with many cells often leads to such outcomes (Armitage et al., 1965; Rubin, 1987). As early as 1935, Halvorson advanced two distinct hypotheses to explain such a finding, i.e. the independent-action hypothesis and the cooperative-action hypothesis. The independent-action hypothesis (also called single-organism hypothesis) postulates that individual bacterial cells within an inoculum act independently, i.e. the fate of each cell in the inoculum is unaffected by the other cells. If a likelihood *p* is defined as the mean probability per inoculated bacterium of multiplying to such an extent as to cause disease/death, the hypothesis states that *p* is independent of the dose (the number of microorganisms inoculated). The cooperative-action hypothesis, also known as synergistic-action hypothesis, suggests that a response can only result from the collaboration among inoculated bacteria. In this case, the above-mentioned probability *p* increases as the dose increases (Meynell & Stocker, 1957).

Experimental results have been evaluated with respect to their support for either one of those two hypotheses. Four lines of evidence point to the independent-action hypothesis as the most plausible (Rubin, 1987). The first one rises from the challenge of laboratory animals with equal mixtures of similarly virulent variants of the same bacterial strains. In such circumstances, the single-organism hypothesis predicts that a pure culture (or a predominant culture) of one or the other variant is obtained at the time of a determined response (if the inoculum size is close to the 50% infectious dose, ID_{50}), while a mixture of the variants would be expected following the cooperative-action hypothesis. In experiments with *Salmonella paratyphi* B (Meynell & Stocker, 1957), *S. typhimurium* (Meynell, 1957), *Haemophilus influenzae* type b meningitis (Moxon & Murphy, 1978), *Escherichia coli* K1 (Pluschke et al., 1983), and *Streptococcus agalactiae* (Ferrieri et al., 1980), laboratory rats or mice were inoculated by oral, subcutaneous, intraperitoneal, or intranasal route, and blood cultures were obtained for dead or bacteremic animals. All experiments showed that, when the inoculum size was less than the 2 ID_{50}, blood cultures contained at least a 20-fold excess of one specific

variant. At higher inoculum size, both pure cultures and mixed cultures were obtained. The same observation was made in pharyngeal cultures of rats after intranasal challenge with *H. influenzae* type B meningitis (Rubin & Moxon, 1984). Given their accordance with prediction of the independent-action hypothesis, these results are generally considered to support that hypothesis. Two facts are nonetheless worth mentioning. In the experiment with *S. typhimurium*, although one variant was often predominant, pure cultures were rarely found (Meynell & Stocker, 1957). The authors attributed such an occurrence to a terminal breakdown in host resistance that permitted the multiplication and entry into the blood of other bacteria still surviving in the host. An extension of the independent-action hypothesis as to make allowance for such a resistance breakdown was thus advanced. Although all colonies recovered from the blood of infant rats challenged orally with two variants of *E. coli* K1 were of the same variant, stool as well as mesenteric lymph node cultures yielded both variants (Pluschke et al., 1983). Strictly speaking, only in the case of blood cultures, it can be concluded that the isolated bacteria were descendants of a single cell. The second line of evidence derives from mathematical equations relating the inoculated dose to the probability of host death. Meynell and Stocker (1957) drew log-dose by probit-mortality curves for the above-mentioned parenteral challenges of mice. In the mortality range of 5-95%, i.e. where the curves approximated straight lines, slopes of 1.8 and 0.7 were observed for *S. paratyphi* B and *S. typhimurium*, respectively. An earlier theoretical argument had suggested that, for the independent-action hypothesis to hold true, a slope of less than 2 should be observed at ID_{50} (Peto, 1953). The experimental observation was thus considered to be consistent with such an expectation. Goldberg et al. (1954) developed a model based on the assumption of independent action, and tested its validity by challenging mice with different doses of *Klebsiella pneumoniae*, *Streptococcus zooepidemicus*, *Yersinia pestis*, and a meningopneumonitis virus given by inhalation. For all four pathogens, the log-log mortality was linearly related to the log-dose, which validated the model and thus supported the independent-action hypothesis. In the same experiment, it was also observed that the period of time (1 hour, 6 hours, and 30 days) over which the dose was

administered did not affect the response rate. This result is viewed as the third line of evidence in favor of the independent-action hypothesis. In fact, under the alternate hypothesis of cooperative action, higher mortality rates were to be expected in the shorter inhalation period, since the higher dose would have favored more cooperation among microorganisms. The fourth and last line of evidence relates to the concept of minimal infective (or lethal) dose. While the independent-action claims that any single microorganism in a inoculum has a chance of causing the response, the cooperative-action hypothesis indicates that a response only occurs if the number of inoculated microorganisms is greater than the minimal infective dose (Meynell & Stocker, 1957). In other words, the cooperative-action hypothesis requires that a threshold of more than one microorganism exists. Based on the fact that doses of few tens of microorganisms or lower have been recorded in the above-mentioned experiments, Rubin (1987) concluded that the minimal infective dose may actually be a single organism. Such a conclusion evidently supports the independent-action hypothesis. Evaluation of published data appears complicated by the need to distinguish between administered (or ingested) dose and dose at target organ or tissue. In foodborne *Salmonella* outbreaks, the following minimal infective doses have been reported: <100 cells of *S. eastbourne*, 50 cells of *S. napoli*, 100-500 cells of *S. heidelberg*, 1-6 cells of *S. typhimurium* (D'Aoust, 1997). However, D'Aoust (1985) noted that the fat content of the contaminated foods (i.e. chocolate and cheddar cheese) may have played a significant role in allowing bacteria to survive the acidic conditions of the stomach. This observation would indicate that the reported dose must perhaps be considered upper bounds of a hypothetical minimal infective dose. Nonetheless, experiments in laboratory animals which used a parenteral route of administration have shown that a dose of less than 10 microorganisms is sufficient to cause mortality in some animals (Meynell, 1957; Ferrieri et al., 1980). Relatively high doses have generally been administered in human feeding studies. However, a dose as low as 180 organisms of *Shigella flexneri* 2a was able to cause fever and gastroenteritis in 9 of the 36 challenged human volunteers (DuPont et al., 1972).

Results of cell culture experiments have been interpreted as evidence that one virus particle can establish infection in a single cultured cell (Westwood & Sattar, 1974). When well-dispersed suspensions of viruses are employed, cultured cells greatly outnumber virus infective units. In spite of this unfavorable ratio, plaques develop, and the possibility that more than one virus particle can be involved in the initiation of such infection is considered very unlikely. This conclusion obviously provides direct support to the independent-action hypothesis in the case of viral infections.

As far as the independent-action hypothesis is accepted, each microorganism has thus an inherently fixed, though minute chance to cause a response. However, as the number of inoculated organisms is increased, the likelihood that one of them cause a response becomes progressively more tangible. It must be noted that the relevance of this hypothesis is not limited only to the development of dose-response models, but has also clinical implications (Rubin, 1987). In spite of a recurrent exposure, cases of *Salmonella* and *Campylobacter* enteritis as well as of invasive disease due to *Neisseria meningitidis*, *H influenzae,* and *Streptococcus pneumoniae* are sporadic. Although host defenses are critical in determining whether infection and clinical manifestation will occur, such mechanisms cannot provide a complete explanation. The fact that only a small fraction of exposed individuals become ill indicates the exposure dose is commonly lower than an experimental ID_{50}. It is the small dose in addition to the low probability of any single microorganism to survive the host defenses that explain the infrequent occurrence of clinical manifestation. Given a susceptible population, a clinical case is thus more likely to reflect the chance event of several individuals being exposed to a low pathogen dose rather than of one individual exposed to a large dose. Coincidentally, Blaser and Newman (1982) have postulated that the actual dose ingested in sporadic cases of human salmonellosis may actually be the 1% infective dose (ID_1).

When it comes to describing the theoretical foundations of microbial dose-response models, it appears that researchers have tended to dichotomize between the two hypotheses of independent-action and cooperative-action (Haas, 1983). Although supported by a large, if still inconclusive, body of experimental evidence, such a

tendency may be excessive. For instance, partial synergism has also been advanced. Under this postulate, inoculated microorganisms act independently at any given dose but the probability *p* increases as dose increases. Interestingly, partial synergism and complete independence can only be distinguished if the hosts are of similar resistance (Meynell & Stocker, 1957), but this factor has never been considered experimentally. It must be noted that, in purely theoretical terms, synergism among the microorganisms of an inoculum is as conceivable as antagonism (for instance due to competition for same nutrients). If the universe of possible hypotheses was to be seen as a continuum from complete synergism to complete antagonism, the single-organism hypothesis could then be considered a neutral case between those two extremes.

1.4 Microbial Dose-Response Modeling

In several branches of biology, the effects observed in an individual at a given time after dosage with a drug or exposure to a toxic substance have been defined as either graded or quantal (Hewlett & Plackett, 1979). A response is graded when a quantitative measure, such as body weight or blood pressure, is observed in the single individual. In contrast, a response is quantal when the individual is classified as having responsed or not. Quantal responses have otherwise been called all-or-none or binary responses. While quantal responses are a qualitative phenomenon at the individual level, they can nonetheless be employed to obtain quantitative results at the population level. Within a group, the percentage of individuals responding generally increases with increasing dose or intensifying exposure. Hartung (1987) even goes so far as stating that responses must be classified as quantal when the primary goal of a dose-response curve is to display the incidence of responses. This claim is, however, incorrect when the underlying response at individual level is continuous and can reliably be recorded as such. Analytical techniques for those measurements are actually available, and should be employed because post-experimental categorization of an inherently continuous response leads to loss in information. Data used in dose-response modeling of foodborne pathogens have almost exclusively been obtained from human feeding studies. Given the nature of these data, the current models essentially deal with quantal responses.

Microbial dose-response models appear to have been classified in diverse ways. However, it will be shown that the difference is rather one of nomenclature than of concepts. Armitage et al. (1965) noted that the hypothesis of independent-action is stochastic in nature, while the hypothesis of cooperative-action is deterministic. This remark has led some researchers to distinguish between "stochastic models" and "deterministic models" depending on which hypothesis a model is based upon (Haas, 1983). This denomination may nevertheless induce some confusion because it may be interpreted as describing a feature of the mathematical model formulation. For instance, the exponential model is based on the independent-action hypothesis, and would thus be accounted among the stochastic models. However, no stochastic element is contained in its mathematical formulation.

Models based on the cooperative-action hypothesis have also been defined as "threshold models". Accordingly, models based on the single-hit hypothesis have been called "non-threshold models". Although it makes an explicit reference to the biological hypothesis, the concept of threshold is controversial. Acceptance of the single-hit hypothesis is often understood as a refutation of the existence of a threshold. However, the concept of a threshold need not refer to the individual infectivity probability. A threshold at population level can also be envisaged (Kodell et al., 1999).

An additional classification criterion is linearity of the dose-response curve at low dose in the log-log scale. A popular belief is that non-threshold models are always linear at such conditions, while threshold models never are. As it has been shown in chemical risk assessment, threshold models can be linear, and non-threshold models can be non-linear. It is important to note that the concept of linearity essentially relates to the shape of the curve. A first concern is how and at which dose level graphical linearity should be evaluated. Furthermore, linearity is, in standard statistical terminology, a property of the model parameters. In this sense, all stochastic models are non-linear, while deterministic models are linear or can be made linear with simple transformations.

In a review on dose-response modeling in developmental toxicology, Gaylor (1994) distinguished between empirical models and biologically based models.

Empirical models are data-based, i.e. they simply pursue an adequate mathematical fit of the model to the data. In contrast, biologically based models attempt to translate the underlying biological mechanisms into mathematical terms. The distinction is not clear-cut. It could be argued that empirical models are also based on some biologic consideration since they reflect the nature of the data. Likewise, parameter estimation of the biologically based models is essentially an empirical exercise. Nonetheless, the distinction between empirical and biologically based models is the most truthful. It merely reflects the process followed in the model genesis without making assertions on the characteristics and value of the single models. This classification is followed in this study. For clarity sake, it is pointed out that, within the context of microbial dose-response assessment, "deterministic models" fall into the category of empirical models while "stochastic models" are usually biologically based.

1.4.1 Empirical Dose-Response Models

Empirical models essentially originated from experimental observation (Hewlett & Plackett, 1979). When large groups of randomly selected animals are exposed to different doses of a toxic substance, plotting the response proportion of each group against the exposure dose usually results in asymmetric, cumulative curves. Because the left-hand, lower limb of the curve is shorter than the upper, right-hand one, the point of inflexion of the curve is lower than the 50% response level. When log-dose is used instead, the x-axis becomes increasingly compressed as dose increases. The resulting sigmoidal curve is frequently symmetric, and has its point of inflexion at 50% response.

In the pharmacological field, the concept of individual tolerance offers an elegant interpretation of the obtained curve (Hewlett & Plackett, 1979). The tolerance is that specific dose that is just insufficient to cause a quantal response in the individual. A usual observation has been that the distribution of the individual log-tolerances for a population tends to normality. The cumulative normal distribution of those log-tolerances is then the symmetric sigmoidal curve relating percent response to log-dose. Consequently, variation in the curve position can be interpreted as variation in the mean log-tolerance (and hence in the median effective dose), and variation in steepness implies

variation in the standard deviation of the log-tolerances. An analogy can intuitively be drawn between the tolerance concept and the concept of resistance to microbial infection. Dose-response curves of foodborne pathogens could possibly be interpreted in a way similar to the one presented here.

Responses can be transformed in terms of standard deviations of the cumulative normal curve (Hewlett & Plackett, 1979), and the scale so generated is known as that of normal equivalent deviates or normits. Since measures of response that are all positive have generally been preferred, probits are obtained by adding five to the normits. An important outcome of the transformation is that response probits (as well as normits) are linear in log-dose. This means that, when response probits are plotted against log-dose for empirical data, an approximate linear curve is often obtained. In addition to the normit and probit, other transformations have been advanced. The logistic transformation is perhaps the most popular one. A plot of logit responses against log-dose usually also produces a linear relation. The probit and logit transformations agree closely for responses in the range of 1-99%. In practical terms, this means that statistical analyses based on these two transformations lead to essentially the same conclusions, and a difference can only be detected by experiments using extremely large samples. Nonetheless, the logit transformation is often preferred to the probit because it has a simpler formulation and offers computational advantages.

From the previous discussion, the mathematical formulation of the relative models should be relatively intuitive. In particular, it should be noted how a linear dose-response curve is obtained through a specific transformation of the responses and the use of log-dose. Mathematical modeling is simplified by such linearity in parameters.

The probit model formally expresses the probability of a response P_{res}, such as the probability of infection, as follows (Covello & Merkhofer, 1993):

$$P_{\text{res}} = \Phi[\alpha + \beta \text{ dose}]$$

where Φ represents the cumulative proportion of a normal distribution. There are two parameters α and β, of which β must > 0. In practice, dose is often expressed in a base 10 logarithmic form, and the model is then known as log-probit or log-normal.

The logit or logistic model is formulated as follows (Covello & Merkhofer, 1993):

$$P_{\text{res}} = \frac{1}{1 + e^{-[\alpha + \beta \text{ dose}]}}$$

where β must > 0, and is referred to as the "shape parameter". When dose enters the model in logarithmic form, the log-logistic model results. While the log-normal model is always sub-linear at low dose, the log-logistic model can be supra-linear (β < 1), linear (β = 1), or sub-linear (β > 1) (Edler & Kopp-Schneider, 1998).

Recently, Coleman and Marks (1998) have proposed a further empirical model, the Gompertz model. It is formulated as follows:

$$P_{\text{res}} = 1 - e^{-e^{\alpha + \beta \text{ dose}}}$$

Dose has been expressed in base 10 logarithmic form as well. In the view of its proponents and similarly to the probit and logit models, the Gompertz model offers qualities of ease and speed when performing in-depth data analysis and deriving estimates.

Besides the advantages with respect to mathematical formulation, the linearity between response probits and log-dose offers a useful ground for a visual interpretation of dose-response curves. Since this subject has apparently never been treated in microbial risk assessment, the following discussion is drawn from the toxicological field. It has been mentioned that dose-response curves with a shallow slope indicate a high degree of tolerance variability in a population, while a steep slope implies low variability and therefore a relatively uniform tolerance within a population. A first caveat to such a generalization is that lack of experimental precision widens the confidence limits around the individual points, and thus tends to flatten the dose-response curve. In practice, the shape of a dose-response curve is determined by both population variability and

experimental uncertainty (Hartung, 1987). Furthermore, conclusions on the slope are conditional on the assumption of a linear dose-response curve. Population variability actually causes a departure from linearity in the dose-response curve (Hewlett & Plackett, 1979). This point is best illustrated considering the case of a heterogeneous population for which a variability element is sufficiently characterized to allow recognition of two subpopulations. That is, it is possible to divide the population into a fraction of susceptible individuals (m_s) and a fraction of resistant individuals (m_r). For any given dose, the proportional response in the heterogeneous population (P_h) is the sum of the proportional responses in the two subpopulations (P_s and P_r, respectively) each weighted for the fraction of the relative subpopulations. This can be described as:

$$P_h = m_s P_s + m_r P_r$$

The proportional responses can be used to draw dose-response curves of probit responses to log-dose. Figure 1.8 illustrates such curves for varying susceptibility differences and subpopulation fractions. The first graph (Graph A) considers the case of a heterogeneous population in which one subpopulation is twice as susceptible as the other. Although the two subpopulations differ appreciably in response, the population curve does not greatly deviate from a straight line. In fact, large experiments would be required to demonstrate such a deviation. Under other circumstances, the departure from linearity becomes manifest. When one subpopulation is 20 times as susceptible, a markedly nonlinear curve for the whole population results (Graph B). The lower portion of the curve is dominated by the susceptible subpopulation, while the upper portion depends on the resistant subpopulation. Finally, the influence of the proportion of each subpopulation is considered (Graph C). In this case, while departure from linearity essentially remains unchanged, the curve for the whole population sweeps nearer to the larger subpopulation, and its middle portion is nearly level with the lower level. This discussion shows that variability of susceptibility within a population influences the dose-response relation in a complex yet predictable fashion.

1.4.2 Biologically Based Dose-Response Models

For the sake of constructing biologically based microbial dose-response models, foodborne infection and its consequences have been partitioned into a sequence of events (Haas, 1983; Teunis et al., 1996). Figure 1.9 shows the postulated chain of events leading from a foodborne exposure to diverse endpoints. At a given microbial concentration in the food, the probability of ingesting a number *j* of microorganisms is defined as P_{exp}. These pathogens may cause infection with probability P_{inf}, which in turns may result in illness or death with probability P_{ill} and P_{dth}, respectively.

Research has historically focused on the development of models characterizing the probability of infection. Under the proposed conceptual framework, the investigators hypothesize that two steps are basically necessary for infection to occur: 1) one or more pathogenic microorganisms are ingested by a susceptible host, and 2) one or more microorganisms colonize the gut by surviving the host defenses. Assuming that microorganisms are randomly distributed within the food vehicle, and their mean number per exposure is μ, the probability of ingesting *j* microorganisms follows a Poisson distribution:

$$P_{exp}(j) = \frac{\mu^{j}}{j!} e^{-\mu}$$

The hypothesis of independent action postulates that any of the *j* microorganisms has an equal probability *r* of surviving and causing infection. In such case, the probability of infection after exposure to *j* microorganisms is:

$$P_{inf}(j) = \sum_{k=1}^{j} P_{sur}(k \mid j) = \sum_{k=1}^{j} \binom{j}{k} r^{k} (1-r)^{j-k}$$

where $P_{sur}(k \mid j)$ is the probability of *k* microorganisms surviving out of the *j* ingested. The probability of infection after exposure to *at least one* microorganism is consequently:

$$P_{inf}(j \geq 1) = P'_{inf} = \sum_{j=1}^{\infty} P_{exp}(j) \sum_{k=1}^{j} P_{sur}(k \mid j)$$

As a side note, it is interesting that Vose (1998) reached the same conclusion with a possibly more intuitive approach. As each of *j* ingested microorganisms has the equal probability *r* of causing an infection in a consumer, the process is essentially binomial. The probability that a consumer becomes infected is then given by:

$$P'_{\text{inf}} = 1 - (1 - r)^j$$

Starting from the above-mentioned framework, two particular dose-response models – the exponential model and the beta-Poisson model – for calculating the probability of microbial infection P'_{inf} have been derived. Their basic distinction essentially lies in whether $P_{\text{sur}}(k \mid j)$ is assumed constant or is stochastically formulated.

The exponential model assumes a constant probability for any ingested microorganisms to survive and cause infection. Although formally expressed in the seminal work by Haas (1983), a similar equation for calculating the probability of host survival was previously reported in the original description of the single-action hypothesis (Meynell & Stocker, 1957). Its formulation is:

$$P'_{\text{inf}} = 1 - e^{-r\mu}$$

where *r* is the probability for an ingested microorganism to cause infection, and μ is the mean dose (mean number of microorganisms per ingested food portion). In the derivation of this model, it was assumed that: 1) the independent-action hypothesis is true (i.e. a single microorganism is potentially able to cause infection); 2) *r* is small; and 3) μ is large.

Alternatively, *r* has been expressed as a probability distribution with density function $f(r;\theta)$. In this case, the whole universe of pathogen-host interactions is considered (Haas, 1983). This leads to the following formulation of the dose-response model:

$$P'_{\text{inf}} = \int_{r=0}^{1} \left(1 - e^{-r\mu}\right) f(r;\theta)\, \mathrm{d}r$$

The beta distribution has been considered the most plausible specification for $f(r;\theta)$ (Moran, 1954). The solution of the previous integral for the case of a Poisson-distributed dose and a beta-distributed probability of microorganism survival leads to the hypergeometric model:

$$P'_{\text{inf}} = 1 - {}_1F_1(\alpha, \alpha+\beta, -\mu)$$

where ${}_1F_1(\alpha, \alpha+\beta, -\mu)$ is the Kummer confluent hypergeometric function.

By making some binding assumptions concerning the values of α and β, a convenient approximation of the hypergeometric model was derived for a plant virus by Furumoto and Mickey (Furumoto & Mickey, 1967). This approximation was called the beta-Poisson model, and Haas (1983) later applied it to human pathogens. The beta-Poisson model has the following formulation:

$$P'_{\text{inf}} \approx 1 - \left(1 + \frac{\mu}{\beta}\right)^{-\alpha}$$

which holds true only if $\beta >> 1$ and $\beta >> \alpha$. While μ is the mean dose, a simple definition of the two parameters α and β has not been proposed. Both parameters determine the shape and position of the dose-response curve. For instance, a change in β causes the curve to be shifted along the dose axis, without changing the curve shape (Teunis & Havelaar, 2000). Most authors have often referred to α and β simply as "slope" or "fitting" parameters, a circumstance criticized by Vose (1998).

The fitting of the beta-Poisson model to data from human feeding studies has often led to estimates of α and β that are both less than unity (Vose, 1998; Teunis & Havelaar, 2000). Such estimates are not compatible with the assumptions governing the beta-Poisson model as derived by Furumoto and Mickey (Furumoto & Mickey, 1967), and a specific consequence is that the results of uncertainty analysis become biased (Teunis & Havelaar, 2000). In particular when the experimental data contain limited low-dose information, this error leads to relevant overestimation of the risk at low doses,

which is the region of interest in most risk assessments. The hypergeometric dose-response model should be preferred to the beta-Poisson in those cases. Furthermore, Vose (1998) has noted that α and β values between zero and one generate a U-shaped beta distribution for the probability of infection given exposure. This would imply a polarization of the volunteers into those who would almost certainly get infected and those who would not. Given that the volunteers are often regarded as a fairly homogenous group compared to the general population, such an eventuality is unlikely, and casts doubts on the generalization of the model to the population at large.

Although the exponential and the beta-Poisson models have been the most popular for use in microbial dose-response assessment, alternative models have also been proposed. Holcomb et al. (1999) renamed the exponential model as the "single-hit exponential model", and proposed two variants. The first variant is designated the "single exponential model", and it differs from the original in that the $\log_{10}$ of the dose is used instead of the dose (Rose et al., 1991). Although the difference with the original is essentially one of scale, the variant appears to fit some data sets better than others. However, the single-hit exponential and the single exponential models both force x-axis interception at 1 and 0, respectively. Their capacity to fit sets with data points in the low dose region is thus limited. To obviate this problem, the "flexible exponential model" was proposed as an extension of an animal growth model (Bertalanffy, 1957; Richards, 1959). This model is not forced through the x-axis at $\log_{10} = 0$. The formulations of the single exponential model and the flexible exponential are, respectively:

$$P'_{\text{inf}} = 1 - e^{-r \log_{10} \mu}$$

$$P'_{\text{inf}} = \beta\left[1 - e^{-\varepsilon(\log_{10} \mu - \chi)}\right]$$

where r is the probability of any microorganism surviving and causing infection, μ is the mean dose, β is the asymptotic value of r as dose approaches infinity, χ is the predicted dose at a specified value of $1 - P'_{\text{inf}}$, and ε is the curve rate value affecting spread of the curve along the x-axis.

The beta-binomial model was developed from the beta-Poisson model, and has illness as an endpoint (Cassin et al., 1998). It allows for variability in the probability of illness at a given dose in contrast to the beta-Poisson model (which only specifies a mean population risk). The beta-binomial model is formulated as follows:

$$P'_{ill} = 1 - \left[1 - P_{\text{ill}}(1)\right]^{\mu}$$

where $P_{\text{ill}}(1)$ is the probability of illness from ingestion of one microorganism. This probability is assumed to be beta-distributed with parameters α and β.

The Weibull-gamma model was derived from the popular Weibull model (Farber et al., 1996). Similar to the beta-Poisson, it describes the pathogen-host interaction as a distribution, specifically a gamma distribution with parameters α and β. Its approximate formulation is as follows:

$$P'_{\text{inf}} \approx 1 - \left(1 + \frac{\mu^{\chi}}{\beta}\right)^{-\alpha}$$

where α, β, and χ are all parameters affecting the shape of the dose-response curve. The parameter α is related to the probability of infection given exposure to a single organism, while β determines the shape of the individual dose-response curve. Of the presented models, the Weibull-gamma is the only three-parameter model. It is noteworthy how the Weibull-gamma model reduces to a beta-Poisson for $\chi = 1$, and to a log-logistic for $\alpha = 1$ (Farber et al., 1996; Holcomb et al., 1999b).

Following the conceptual framework mentioned in the beginning of this section, the probabilities of developing disease and death after exposure to *at least* one microorganism have been defined as follows (Teunis et al., 1996):

$$P'_{ill} = P'_{\text{inf}} P_{ill}$$

$$P'_{dth} = P'_{\text{inf}} P'_{ill} P_{dth}$$

Dose-response models have often been used to calculate these probabilities. Nonetheless, no reference seems to be available on whether such an approach would correctly conform to the theoretical basis governing the models. Likewise, it is unproven whether the conditional nature of the events is adequately taken into account.

With a more refined approach, the probability of gastroenteritis (i.e. an illness endpoint rather infection) has been calculated by means of a hazard function (Teunis et al., 1999). The approach builds on the notion that, in an infected individual, the probability of becoming ill depends on the duration of the infection episode. This time period essentially mirrors the balance between host defenses and pathogen growth. The latter may be dose-dependent. If the hazard function for illness in an infected individual – the instantaneous chance of becoming ill at any given time t – is defined as $h_i(t)$, then the illness probability given infection at time t can be expressed as:

$$P(\text{ill} \mid \text{inf}; t) = 1 - e^{-\int_{u=0}^{t} h_i(u)du}$$

Through mathematical manipulations and assumptions, this equation can be reduced to:

$$P(\text{ill} \mid \text{inf}) = 1 - (1 + \gamma\lambda)^{-r}$$

where r is the shape of the parameter of the Gamma distribution of the infection duration, and $\gamma\lambda$ may be considered a scale parameter. As long as the illness process depends on dose, the parameter λ is expressed as a function of the ingested dose. Studying the behavior of this parameter (increase, decrease, no change) sums up to testing the dependence form of the P(ill|inf) on the ingested dose.

1.4.3 General Mathematical Formulation of Dose-Response Models

Parameters of microbial dose-response models have usually been estimated by fitting the proposed relation to data from human feeding studies. For such analyses, Kodell et al. (1999) have proposed the following generalization of empirical and

biologically based dose-response models:

$$P(d_i;\theta) = \sum_{j=1}^{\infty} g(d_i, j) \int_{-\infty}^{\infty} p(t, j)\, f(\theta;t)dt$$

where $P(d_i;\theta)$ is essentially the probability of infection in the i^{th} dose group after exposure at a nominal level d_i. The single components of the equation can formally be described as follows:

- $g(d_i, j)$ defines the probability mass function of the dose received by hosts in the i^{th} group;
- $p(t, j)$ is the probability mass/density function that an individual with infectivity parameter t becomes infected after exposure to j microorganisms; and
- $f(\theta;t)$ characterizes the probability density function of t in the population, essentially the host susceptibility variation in the population.

Similarities to the framework presented in the previous section are evident. While the first part of the generalized formulation describes exposure, the integral part establishes the expected proportion of individuals in the population becoming infected after exposure. In fact, the integration over all values of t (i.e. different degrees of susceptibility present in the population) leads to the cumulative function of $p(t, j)$ and $f(\theta;t)$, which essentially represents a population dose-response function $r(\theta, j)$.

Different choices in the specification of the single components – summarized in Table 1.6 – essentially leads to the different dose-response models for the probability of microbial infection. The relevance of the two contrasting hypotheses of independent action and of cooperative action essentially comes into play for the specification of $p(t, j)$. The single-hit hypothesis views the infectivity parameter, t, as the constant probability with which a single microorganism causes infection. In the case of the

cooperative-action hypothesis, t becomes the threshold tolerance that each individual in a population has to the infection. Consequently, $p(t, j)$ is specified either as:

$$p(t, j) = 1 - (1 - t)^j \qquad \text{for the single-hit hypothesis, or as}$$

$$p(t, j) = I_{[t,\infty]}(j) = \begin{cases} 1, & \text{if } t \le j \\ 0, & \text{if } t > j \end{cases} \qquad \text{for the cooperative-action hypothesis}$$

These equations suitably show whether a threshold exists in the dose-response model.

Based on the general formulation and by choosing different distributions of $g(d_i, j)$ and $f(\theta; t)$, Kodell et al. (1999) have proposed further three-parameter dose-response models.

1.4.4 Human Feeding Studies

Data from feeding studies of human volunteers have played a major role in microbial hazard characterization. Consideration of the implied limitations that those data carry in the context of risk assessment is important.

Human feeding studies have been carried out for a variety of foodborne pathogens (Table 1.7). Since most of these studies were originally designed as vaccine trials, the administered dose ranges were generally high enough as to induce immunological protection in a measurable portion of the volunteers without causing illness. Often, a study would gradually have zeroed in on a protective dose, producing a unbalanced experimental design for dose-response modeling. Unfortunately, the doses of interest in microbial risk assessments are generally well below the ones considered by feeding studies. The difference between the doses considered by the human feeding studies and the doses of interest in microbial risk assessment essentially explains the major source of uncertainty in employing those data, i.e. low-dose extrapolation. Further limitations in the experimental design of *Salmonella* feeding have been reviewed by Blaser and Newman (1982). In particular, it is significant that the feeding occurred at a time of high gastric acid levels, that some volunteers had undergone multiple challenges, and that the

lowest dose was administered to only a few volunteers. Pertaining more to microbial risk assessment, risk assessors frequently mention additional considerations, such as the predominant use of healthy and male volunteers. Those limitations not withstanding, data from human feeding studies still provide the most direct means of observing the dose-response relationship of microorganisms in human subjects.

1.4.5 Current Practice of Microbial Hazard Characterization

Hazard characterization in microbial risk assessment has been limited to dose-response assessment. In practice, the proposed models have been fitted to data from human feeding studies of human volunteers. Research has essentially focused on the choice of data subsets appropriate to the situation at hand and on the statistical discrimination among models applied to such data. In part because of the need for low-dose extrapolation, the biologically based exponential and the beta-Poisson models have been the most popular.

While human feeding studies have been carried out for a variety of microorganisms (Table 1.7), foodborne pathogens that are life threatening or that cause disease only in high-risk subpopulations are not amenable to volunteer studies. In particular, human feeding studies for the currently most relevant pathogens, such as *Salmonella* Enteritidis, *Escherichia coli* O157:H7, and *Listeria monocytogenes*, are not available. To obviate this shortcoming, different approaches have been followed.

In the case of *S.* Enteritidis, researchers have considered either combining data from other *Salmonella* human feeding studies or employing data from *Shigella* studies. The use of pooled data from all *Salmonella* feeding studies has been considered inappropriate to characterize illness following a challenge with S. Enteritidis. Firstly, models fitted to such pooled data would underestimate the actual attack rates observed in outbreaks associated with *S.* Enteritidis (USDA/FSIS, 1998). Secondly, although two distinct pathogenicity patterns have been suggested (low pathogenicity for *S. anatum* strain II and *S. meleagridis* strain I; moderate pathogenicity for *S. anatum* I, *S. bareilly*, and *S. newport*), the data from the single *Salmonella* human feedings still indicate the

need of higher doses in order to cause attack rates similar to the ones observed in *S.* Enteritidis outbreaks (Morales et al., 1996; Jaykus et al., 1997). In contrast, high attack rates at low doses were found in human feeding studies with *Shigella dysenteriae* (Levine et al., 1973). This microorganism was thus selected as a surrogate for *S.* Enteritidis in a U.S. risk assessment on shell eggs and egg products, and the relative data were used in a beta-Poisson model (USDA/FSIS, 1998). Buchanan et al. (2000) mention a Canadian risk assessment in which the information from a large *S.* Enteritidis outbreak following ice-cream consumption (Hennessy et al., 1996) was explicitly used in the dose-response model (a reparameterized Weibull model). The use of human feeding studies was thus avoided altogether.

Data from human feeding studies with *Shigella flexneri* (DuPont et al., 1969; DuPont et al., 1972) and *Shigella dysenteriae* (Levine et al., 1973) were used in a Canadian risk assessment on *Escherichia coli* O157:H7 in hamburger. A beta-binomial model was fit to those data (Cassin et al., 1998). The U.S. Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) currently carries out a similar risk assessment. Although it was considered that conclusive evidence on the suitability of *Shigella* dose-response relationships as a surrogate for *E. coli* O157:H7 was lacking, the *Shigella* data was selected. It was, however, warned that the invasive nature of *Shigella* implies a greater pathogenicity than a similar dose of the non-invasive *E. coli* O157:H7. It was thus concluded that the dose-response model based on the *Shigella* surrogate provides an unknown level of conservatism for modeling illness associated with the non-invasive pathogen *E. coli* O157:H7. In order to account for model uncertainty, the beta-Poisson model was contrasted to the Gompertz model (Marks et al., 1998).

The lack of a suitable surrogate microorganism makes the *Listeria monocytogenes* case more complex. In a risk assessment performed in Canada, the Weibull-gamma model was chosen because of its ability to accommodate qualitative information specific to *L. monocytogenes* (Farber et al., 1996). In the United States, no specific dose-response model has been applied. Nonetheless, by coupling data on the annual national incidence to survey data on the frequency and extent of contamination of a ready-to-eat food, a

conservative estimate of the dose-response relations for *L. monocytogenes* in high risk populations was generated (Buchanan et al., 1997).

Once a plausible dataset from human feeding studies has been identified, Holcomb et al. (1999) have proposed that the choice of dose-response model depends on four considerations: goodness-of-fit, parameter parsimony, range reliability (range of conditions over which the model gives good predictions), and flexibility to fit data from various microorganisms. The statistical fit of several dose-response models has been the subject of extensive study (Fazil, 1996; Teunis et al., 1996; Holcomb et al., 1999; Kodell et al., 1999). Models have been fitted to the data by the maximum likelihood method. The adequacy of the single model fit as well as comparison of the fit of different models has then been evaluated by means of the deviance. Fazil (1996) fitted the lognormal, the exponential, and the beta-Poisson models to the data of all *Salmonella* human feeding studies. Considered endpoint was infection. When the data were pooled, the observations relating to three dose groups (i.e. dose $9.3 \cdot 10^5$ of *S. anatum* I, $1.6 \cdot 10^5$ *S. meleagridis* III, and $6.4 \cdot 10^6$ *S. derby*) were defined as outliers. Once these data points had been excluded from the analysis, it was found that both the log-normal and the beta-Poisson models fit the data well. Because of its biological plausibility, the beta-Poisson was eventually proposed as the more adequate *Salmonella* dose-response model. In a more recent, unpublished analysis, the same author analyzed the same data selectively excluding the observations from volunteers that were challenged multiple times. The obtained dose-response curve for the beta-Poisson predicted a marginally greater probability of infection at doses above 10^4. This was interpreted as a sign of greater susceptibility in the previously unexposed volunteers. Teunis et al. (1996) extensively evaluated the fit of the exponential and the beta-Poisson models to the challenge study data from a variety of microorganisms (6 bacterial genera, 3 viral, and 3 parasitic). When data on both infection and illness are available, the infection endpoint was retained. Parameters and confidence intervals for the different pathogens were reported in detail. While the exponential model appeared suitable for the protozoa *Cryptosporidium parvum* and *Giardia lamblia*, the bacterial and viral data were better modeled by the beta-Poisson

model. A more general conclusion was that this model in comparison to the exponential model allows for a flatter dose-response curve and a larger confidence range. Both these circumstances would indicate that the beta-Poisson model is better suited for conservative estimates of the infection risk. Holcomb et al. (1999) compared the fit of six dose-response models (log-normal, log-logistic, simple exponential, flexible exponential, beta-Poisson, Weibull-gamma) to the either infection or illness frequencies originating from human feeding studies with four distinct bacteria (*Shigella flexneri*, *Shigella dysenteriae*, *Campylobacter jejuni*, *Salmonella typhi*). The curves for *C. jejuni* are depicted in Figure 1.10. Although large differences in prediction at low dose were observed for the six models, no model was consistently the most conservative. Moreover, it was found that only the Weibull-gamma model achieved a satisfactory fit for all four data sets. This finding was interpreted as a sign of flexibility of this function in modeling data on foodborne microbial infections. This conclusion was confirmed by Kodell at al. (1999), who fit the Weibull-gamma model as well as other newly developed three-parameter models to the data sets previously considered by Teunis et al. (1996). For the 25 data sets that had enough data points to allow the fitting of a three-parameter model, the Weibull-exponential model and the shifted Weibull model generated a fit comparable to the one of the Weibull-gamma model. Two other three-parameter models – the exponential-exponential and the exponential-gamma – showed no improvement over two-parameter models.

In all models, the ingested dose is considered to follow a Poisson distribution. The assumption justifying this practice rests on a random distribution of the microorganisms in the food vehicle. However, it has been shown that viruses tend to clump (Westwood & Sattar, 1974). In such cases, the distribution of count data is considered to show overdispersion, and a negative binomial distribution has been proposed as a corrective solution (Marks & Coleman, 1998).

It should finally be noted how the current practice has mainly focused on characterizing the probability of intestinal infection. The leaning toward such an endpoint is probably due to the central role that colonization of the gastrointestinal tract

has in the occurrence of further events. However, from a clinical point of view, knowledge on the infection endpoint is marginal to the characterization of the probabilities of illness and death. Microbial risk assessors have often insufficiently recognized the significance of this consideration in the prediction of overall disease risk.

1.4.6 Uncertainty in Microbial Dose-Response Models

Modeling is a powerful tool that can greatly contribute to our understanding of complex situations. However, any model is necessarily a biased representation of reality, and scientific soundness requires transparent consideration of that uncertainty. Their nature can be grasped by considering the elements contributing to the modeling process. Dose-response modeling, as modeling in many other fields, can essentially be viewed as a decomposition and aggregation process (Morgan & Henrion, 1990). A problem is decomposed into its components that can be better understood and for which information is available. The single components need then to be aggregated into an overall prediction. It has to be recognized that the process has in fact two basic elements, i.e. modeling itself and information. Consequently, errors can originate from three distinct sources:

- *modeling errors* are due to inaccuracy in the model itself;
- *parameter errors* are due to scarcity or inaccuracy of the available information;
- *aggregation errors* result from the aggregation process.

While consideration of parameter uncertainty is a common feature of most dose-response models, less attention has been reserved to modeling errors. When different sub-populations have been considered in microbial dose-response assessment, the eventuality of aggregation errors has never been considered.

As a side note, it should be noted that uncertainty should be differentiated from variability (Frey, 1992; Frey & Rhodes, 1999; Morgan & Henrion, 1990). In the specific case of microbial hazard characterization, both elements concur in defining the state of knowledge about the population and pathogen characteristics that determine the dose-response relation. Variability characterizes the degree of knowledge about the

characteristics within a population or across pathogens. In practice, it amounts to the heterogeneity in characteristics that we can understand and quantify. In contrast, uncertainty represents lack of knowledge about those characteristics. This ignorance generates from various sources, such as incomplete understanding and measurement errors, and needs to be formally expressed.

Parameter Uncertainty

It has been mentioned that microbial dose-response models have usually been fitted by the maximum likelihood (ML) method. This procedure is asymptotic, i.e. ML-based test statistics are reliable only if the sample is large. When the sample size is small, as in the analysis of human feeding studies, ML-based test statistics may deviate significantly from those based on other methods, and often are biased. Conditional ML procedures are a possible alternative for dealing with small samples (Kleinbaum et al., 1998). Crump and Howe (1984) have reviewed the methods for calculating statistical confidence limits in low dose extrapolation of toxicological hazards. They specifically pointed out that confidence limit methods based upon ML estimates and likelihood ratios are only asymptotically correct as the sample size approaches infinity. In microbial dose-response modeling, this requirement seems to have been neglected. Although referring more properly to model uncertainty, ML-based test statistics have also routinely been employed to contrast the fit of the different microbial dose-response models (deviance differences as shown in the previous section). The reliability of this evaluation under the usual limited size of human feeding studies has not been characterized either.

As a passing note, it must be noted that ML estimates obtained by different authors using the same data sets and models are often in disagreement. The case of *Campylobacter jejuni* and the beta-Poisson model is considered as an example. While Teunis et al. (1996) established an α of 0.15 and a β of 7.59 (deviance 2.4), Holcomb et al. (Holcomb et al., 1999) found that those parameters were 0.12 and 2.46, respectively (deviance 1.4). Discrepancies in algorithm implementation among software packages may explain such an observation.

Several microbial dose-response models have two or more parameters. Consequently, a confidence interval of the dose-response curve cannot be calculated directly from that of the parameters (Teunis et al., 1996). In developing methods for waterborne pathogens, Regli et al. (1991) calculated ML estimates of one parameter for different values of the other parameter. Confidence intervals were subsequently shown as contour plots of the two parameters. A similar output was obtained by using simple bootstrap estimation procedures (Haas et al., 1993). The experimental data were iteratively sampled to generate a series of hypothetical data sets, and the dose-response model was then fitted to each resampled data set by ML. The obtained parameter pairs were finally used to draw a contour plot. Fazil (1996) contrasted the two approaches in his analysis of the data from *Salmonella* human feeding studies. Based on a visual evaluation, it was judged that both methods produced a similar representation of the parameter uncertainty. Teunis et al. (1996) in their extensive analysis of human feeding studies also used bootstrapping. The same researchers recently reviewed the inefficiency of this approach (Teunis & Havelaar, 2000). Firstly, bootstrapped data can be considerably dichotomized as a consequence of few observations per dose group. Requiring a large number of ML optimizations, the computation can be demanding. Finally, it was recognized that, for small data sets, ML-based methods can produce incorrect confidence intervals. In substitution, a Markov chain Monte Carlo technique was employed to calculate Bayesian posterior distributions for the parameters (USDA/FSIS, 1998; Gilks et al., 1996). Based on this approach, the validity of the beta-Poisson model as an approximation of the exact hypergeometric model was reassessed. While the difference in terms of prediction is limited, the magnitude of the confidence bands varies significantly. It was thus concluded that, in addition to curve fitting consideration, the choice of the model should be evaluated in light of the consequent parameter uncertainty.

An alternative approach has been followed in the risk assessment on *Escherichia coli* O157:H7 in hamburger carried out in Canada (Cassin et al., 1998). In this circumstance, a beta-binomial dose-response model was developed that shows the

estimated uncertainty in the average probability of illness for a given ingested dose. The differences between feeding studies with *Shigella flexneri* and *Shigella dysenteriae* was used as a proxy for pathogen variability.

Model Uncertainty

In contrast to parameter uncertainty, relatively less attention has been reserved in microbial risk assessment to model uncertainty. In particular, a lack of formalism in the way this topic has been treated is apparent.

Kodell et al. (1999) have advanced that the degree of model uncertainty can be quantified by the range of risk estimates resulting from a number of plausible models. These authors also considered important the consideration of threshold models in a formal strategy for characterizing model uncertainty. For instance, a threshold has been incorporated into the originally non-threshold beta-Poisson model (Marks et al., 1998). As mentioned earlier, Teunis and Havelaar (2000) have recently warned about the use of the beta-Poisson model without careful consideration of the assumptions upon which the model was developed.

A topic related to model uncertainty has been the attempt to represent heterogeneity of the dose-response relation within a general population. For instance, the existence of a susceptible subpopulation was modeled in the risk assessment on *Salmonella* Enteritidis in shell eggs and egg products carried out in the U.S. (USDA/FSIS, 1998). In this study, the beta-poisson model fitted to the *Shigella dysenteriae* data was plotted in two forms. The first form was intended to represent the healthy population, and considered an ID_{50} as estimated from the experimental data. Since clinical and laboratory evidence indicated that the susceptible subpopulation is from 10 to 100 times more likely to experience infection, the model form for susceptible people was estimated from an ID_{50} reduced by a factor of 10. To account for uncertainty in this dose-response modeling, the parameter referring to the ID_{50} was further introduced as a probability distribution in the beta-Poisson model rather than in its usual constant form.

Part of this approach, in addition to further innovation, has been carried over to a recent risk assessment on *Salmonella* Enteritidis associated with the consumption of raw shell eggs (Latimer et al., 2001). As in a previous section, data from *Salmonella* and *Shigella* human feeding studies were used as surrogates for *S.* Enteritidis, and were classified into three pathogenicity categories. The three pooled data sets were then used to fit three models, i.e. the exponential model, the beta-Poisson model, and a newly developed "two-subpopulation" exponential model. This last model is essentially the sum of the illness probabilities in a normal subpopulation and a susceptible subpopulation weighted for their proportional representation in the whole population. Maximum likelihood deviance of the single models from the saturated model was calculated for two purposes. Firstly, as usual in microbial dose-response modeling, it was used the check the model's goodness-of-fit to the data. Secondly, it was taken as a measure of model confidence. The exponential and the beta-Poisson model resulted in 50% confidence for the low and moderate pathogenicity categories, while the "two-subpopulation" model and the beta-Poisson model were respectively assigned 9.8% and 90.2% confidence for the high pathogenicity category. To characterize the pathogenicity of different *S.* Enteritidis strains, several dose-response curves were eventually generated as the sum of illness probabilities obtained from varying proportions attributed to three pathogenicity categories and from a different model choice within pathogenicity category. They found that the proportion attributed to each pathogenicity category influenced more the overall dose-response curve than did the model choice within pathogenicity category.

In a U.S. risk assessment on *E. coli* O157:H7 in ground beef (USDA/FSIS, 1998), model uncertainty was depicted graphically using boundary analysis. A distribution of model output was generated via Monte Carlo simulations, within upper and lower bounds which had been established by fixing inputs at the 10^{th} and 90^{th} percentile levels of their uncertainty distributions. Specifically, a general domain of the dose-response curve for *E. coli* O157:H7, called "envelopes", was obtained.

1.5 Concluding Remarks

Over the past decade, microbial risk assessment has increasingly been applied to food safety hazards. As is often the case in any rapidly developing field, the process seems to have been driven by the desire to obtain applicable results. Perhaps justifiably so, scrutiny of the employed methods has taken back seat. While significance for policy-making is undoubtedly the main reason for conducting a risk assessment, it should nonetheless be recognized that the long-term credibility of such an approach heavily relies on the soundness of the employed methods. In hazard characterization of foodborne pathogens, risk assessors are confronted with an allegedly complex interaction of host, microorganism, and food vehicle factors. Knowledge of the single factors as well as the understanding of their interaction is limited. Confronted with this uncertainty, researchers have often opted for pre-made, one-fits-all solutions. Whereas there is no hard evidence indicating that such an approach is unsuitable, no positive proof of its soundness exists either. If not at least for ethical reasons, scrutiny of the current practice in microbial hazard characterization is warranted.

Research has essentially focused on comparing the fit of statistical models to the data from human challenge studies (Haas, 1983; Holcomb et al., 1999; Teunis et al., 1999). By merely evaluating the "internal" validity of a model with regard to the available data, these analyses critically lack in scope when it comes to establishing the soundness of a microbial hazard characterization. To this goal, the "external" validity, such as the plausibility of a low-dose extrapolation and adequate consideration of population heterogeneity, is paramount. Its evaluation will likely require novel approaches.

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Table 1.1. Incidence rates per 100,000 inhabitants of selected pathogens detected by FoodNet, 1996-1999 (CDC, 2000)

Pathogen	1996	1997	1998	1999
Campylobacter	23.5	25.2	21.4	17.3
Salmonella	14.5	13.6	12.3	14.8
Shigella	8.9	7.5	8.5	5.0
Cryptosporidium	n/a	3.0	3.4	2.9
Escherichia coli O157	2.7	2.3	2.8	2.1
Yersinia	1.0	0.9	1.0	0.8
Listeria	0.5	0.5	0.6	0.5
Vibrio	0.2	0.3	0.3	0.2
Cyclospora	n/a	0.3	<0.1	<0.1
Total	51.2	50.3	46.9	40.7

Table 1.2. Estimated number of illnesses and death due to foodborne hazards in the United States

Source	Number of illnesses (in millions)	Number of deaths
Archer & Kvenberg (1985)	24 to 81	--
Bennett et al. (1987)	6.5	8,980
Todd (1989)	12.5	520
CAST (1994)	6.5 to 33	9,000
Mead et al. (1999)	76	5,200

Table 1.3. Select clinical manifestations of foodborne pathogens other than acute gastroenteritis (Archer & Young, 1988; Lindsay, 1997)

Clinical manifestations	Foodborne bacterial etiology (confirmed or speculated)
Bacteremia, septicemia	Nontyphoidal *Salmonella* (*S. dublin* and *S. choleraesuis*), *Campylobacter jejuni*, *Listeria monocytogenes*
Enteric fever	Typhoid and paratyphoid *Salmonella*
Meningitis, encephalitis	Nontyphoidal *Salmonella*, *Listeria monocytogenes*, *Toxoplasma gondii*
Osteomyelitis, arthritis (septic)	Nontyphoidal *Salmonella*
Miscarriage, stillbirth, premature birth, neonatal disease	*Listeria monocytogenes*, *Toxoplasma gondii*
Reactive arthritis, ankylosing spondylitis, Reiter's syndrome	Nontyphoidal *Salmonella*, *Shigella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *E. coli*
Guillain-Barré syndrome (demyelinating polyradiculoneuropathy)	*Campylobacter jejuni*
Hemolytic-uremic syndrome	Verotoxin-producing *E. coli* (*E. coli* O157:H7)
Gastritis	*Helicobacter pylori*
Inflammatory bowel disease (granulomatous colitis or Crohn's disease; ulcerative colitis)	*Mycobacterium paratuberculosis*, *Pseudomonas* spp., *E. coli*
Hepatitis	Hepatitis A virus
Malabsorption	*Enterobacteriaceae*, Rotavirus, *Amoeba* spp., *Cryptosporidium parvum*, *Giardia lamblia*
Erythema nodosum	Nontyphoidal *Salmonella*, *Yersinia enterocolitica*
Graves disease (autoimmune Thyroid disease)	*Yersinia* spp.
Hypothyroidism	*Giardia lamblia*

Table 1.4. Risk factors for foodborne nontyphoidal Salmonellosis reported in case-control and cohort studies

Factor category	*Reported factors*
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

Table 1.5. Major foodborne outbreaks of human Salmonellosis and implicated food items (Adapted from D'Aoust, 1997)

Year	Country	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*

Table 1.6. Components of common microbial dose-response models according to the generalization by Kodell et al. (1999)

	# parameters	Dose $g(d_i, j)$	Pathogen-host interaction [1] $p(t, j)$	Population host susceptibility $f(\theta; t)$
Exponential (Haas, 1983)	1	Poisson distribution with mean d_i	Single-hit (non-threshold)	Point-mass distribution $I_{[t=\theta]}$
Beta-Poisson (Furumoto & Mickey, 1967; Haas, 1983)	2	(See Exponential)	Single-hit (non-threshold)	Beta distribution with parameters α and β
Lognormal	2	Point-mass distribution $I_{[j=d_i]}$	Cooperative (threshold)	Lognormal distribution with mean μ and deviation σ
Log-logistic	2	(See Lognornal)	Cooperative (threshold)	Log-logistic distribution with mean μ and deviation σ
Weibull-gamma (Farber et al., 1996)	3	Poisson distribution with mean d_i^{γ}	Single-hit (non-threshold)	Gamma distribution with parameters α and β

1) See text for functional forms.

Table 1.7. Human feeding studies with foodborne pathogens

Pathogen	References
Campylobacter jejuni	Black et al., 1988
	Mawer, 1988
Cryptosporidium parvum	DuPont et al., 1995
	Chappell et al., 1996
	Okhuysen et al., 1998
	Chappell et al., 1999
Escherichia coli, enteroadherent	Mathewson et al., 1986
Escherichia coli, enterotoxigenic	Evans et al., 1978
Norwalk-like virus	Thornhill et al., 1975
	Gary et al., 1987
	Graham et al., 1994
Rotavirus	Ward et al., 1986
Salmonella anatum	Varela & Olarte, 1942
	McCullough & Eisele, 1951
Salmonella bareilly	McCullough & Eisele, 1951
Salmonella bovismorbificans	Mackenzie & Livingstone, 1968
Salmonella derby	McCullough & Eisele, 1951
Salmonella meleagridis	McCullough & Eisele, 1951
Salmonella newport	McCullough & Eisele, 1951
Salmonella pullorum	McCullough & Eisele, 1951
Salmonella sofia	Mackenzie & Livingstone, 1968
Salmonella typhi	Tigertt, 1959
	Hornick et al., 1966
	Sprinz et al., 1966
	Hornick et al., 1970
	Hornick et al., 1976
	Woodward, 1980
	Glynn et al., 1995
Salmonella typhimurium	Hormaeche et al., 1936
Shigella flexneri	DuPont et al., 1969
	DuPont et al., 1972
Shigella dysenteriae	Levine et al., 1973
Vibrio cholerae	Cash et al., 1974
	Levine et al., 1984
	Levine et al., 1988
Yersinia enterocolitica	Agner et al., 1981
	Ostroff et al., 1994

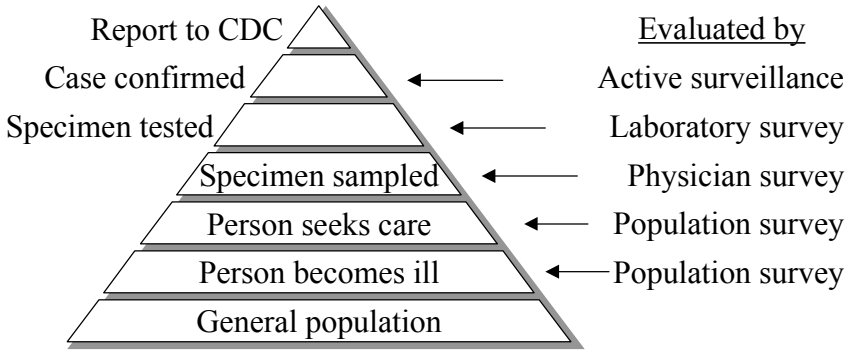


Figure 1.1. "The foodborne diseases pyramid": Organization of the data collection on foodborne diseases in the United States (CDC, 1997)

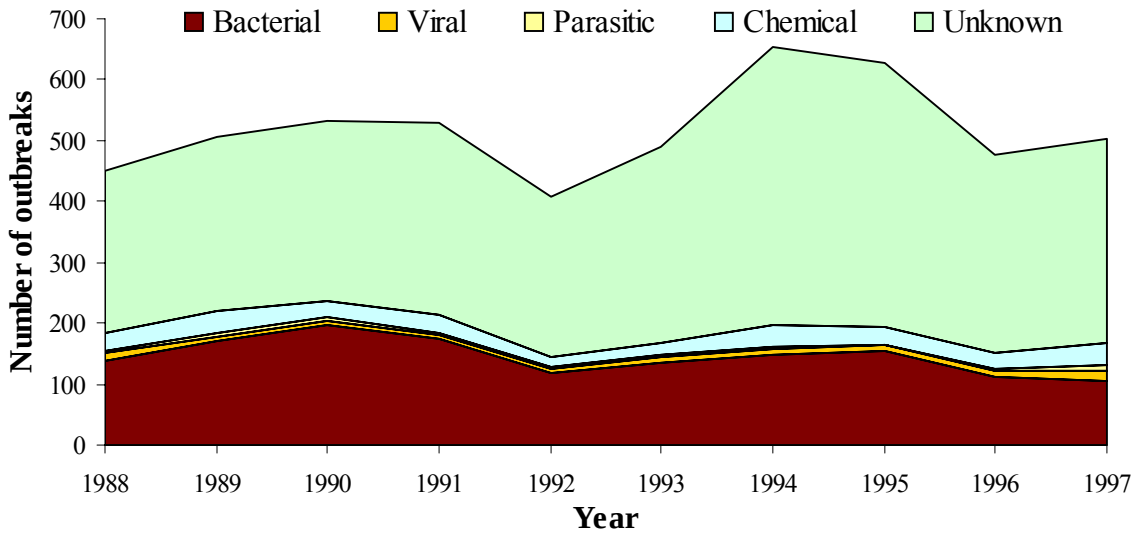


Figure 1.2. Number of foodborne outbreaks, United States 1988-1997 (Bean et al., 1996; Olsen et al., 2000)

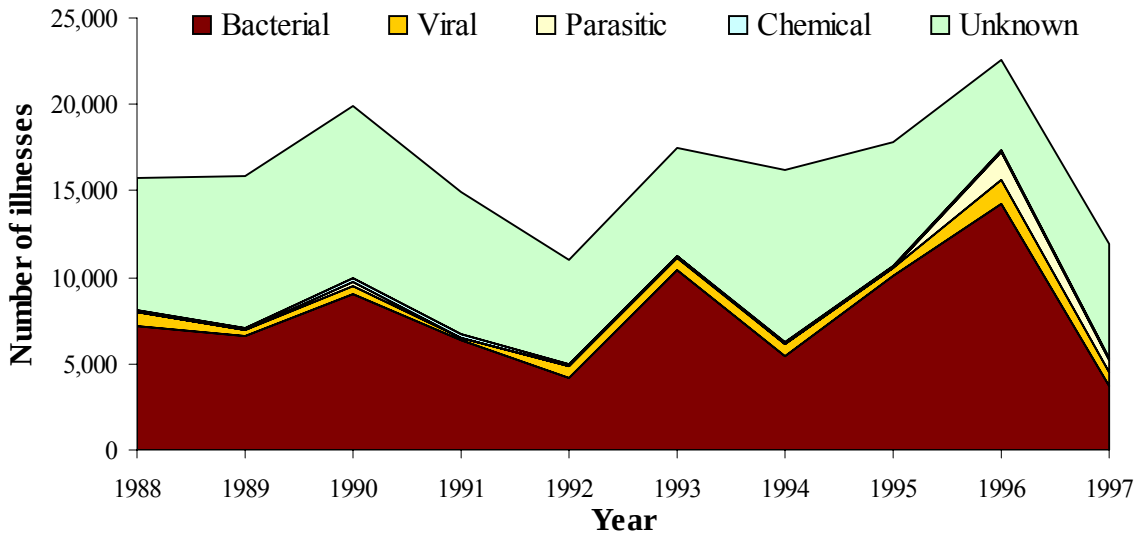


Figure 1.3. Number of illnesses caused by foodborne outbreaks, United States 1988-1997 (Bean et al., 1996; Olsen et al., 2000)

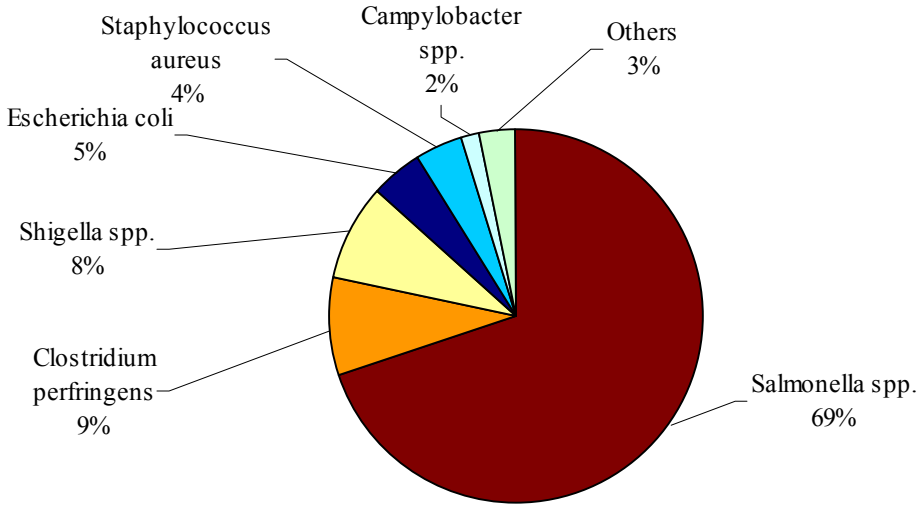
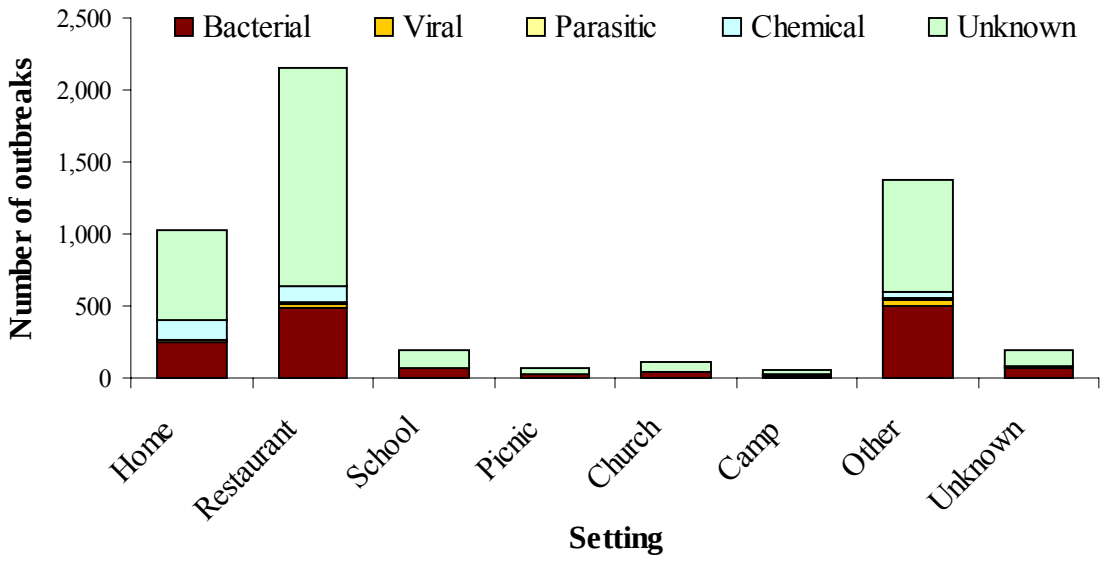


Figure 1.4. Etiology of reported illnesses related to foodborne bacterial outbreaks, United States 1988-1997 (Total isolates = 77,027)



Note: the nature of the category "other" is not specified in the data sources (Bean et al., 1990; Bean et al., 1996; Olsen et al., 2000).

Figure 1.5. Setting of foodborne outbreaks, United States 1988-1997 (Total outbreaks = 5,174)

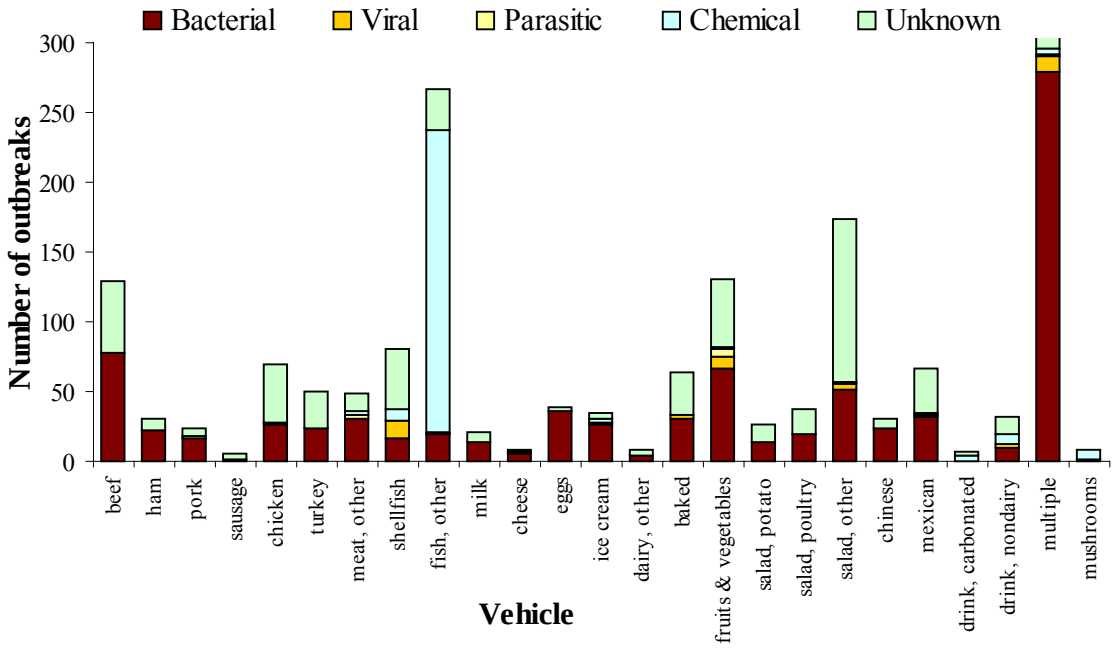


Figure 1.6. Vehicle of foodborne outbreaks, United States 1988-1997 (Total outbreaks = 5,174)

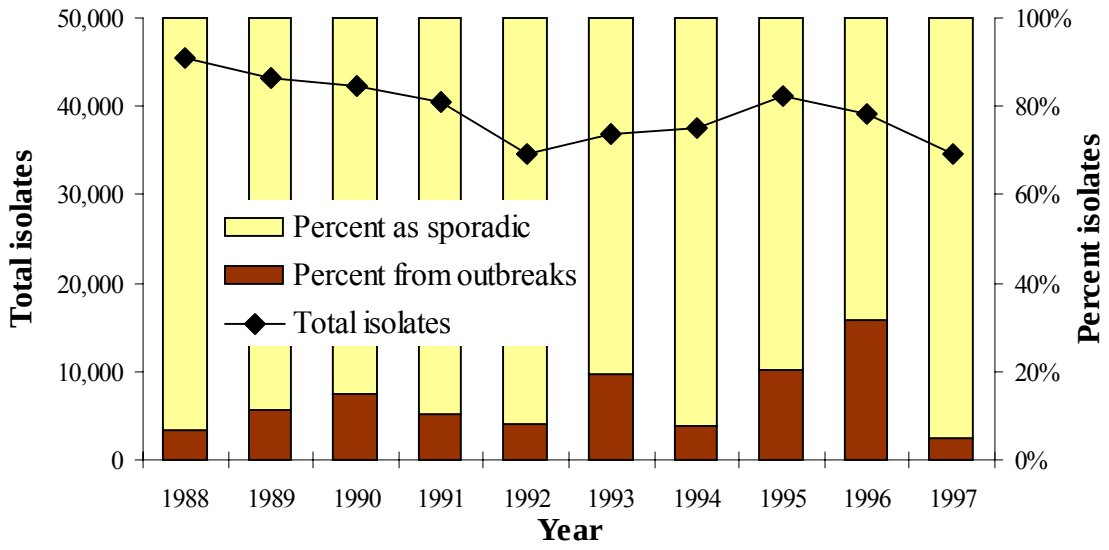


Figure 1.7. Total *Salmonella* isolates and percent distribution between outbreak and sporadic cases, U.S. 1988-1997

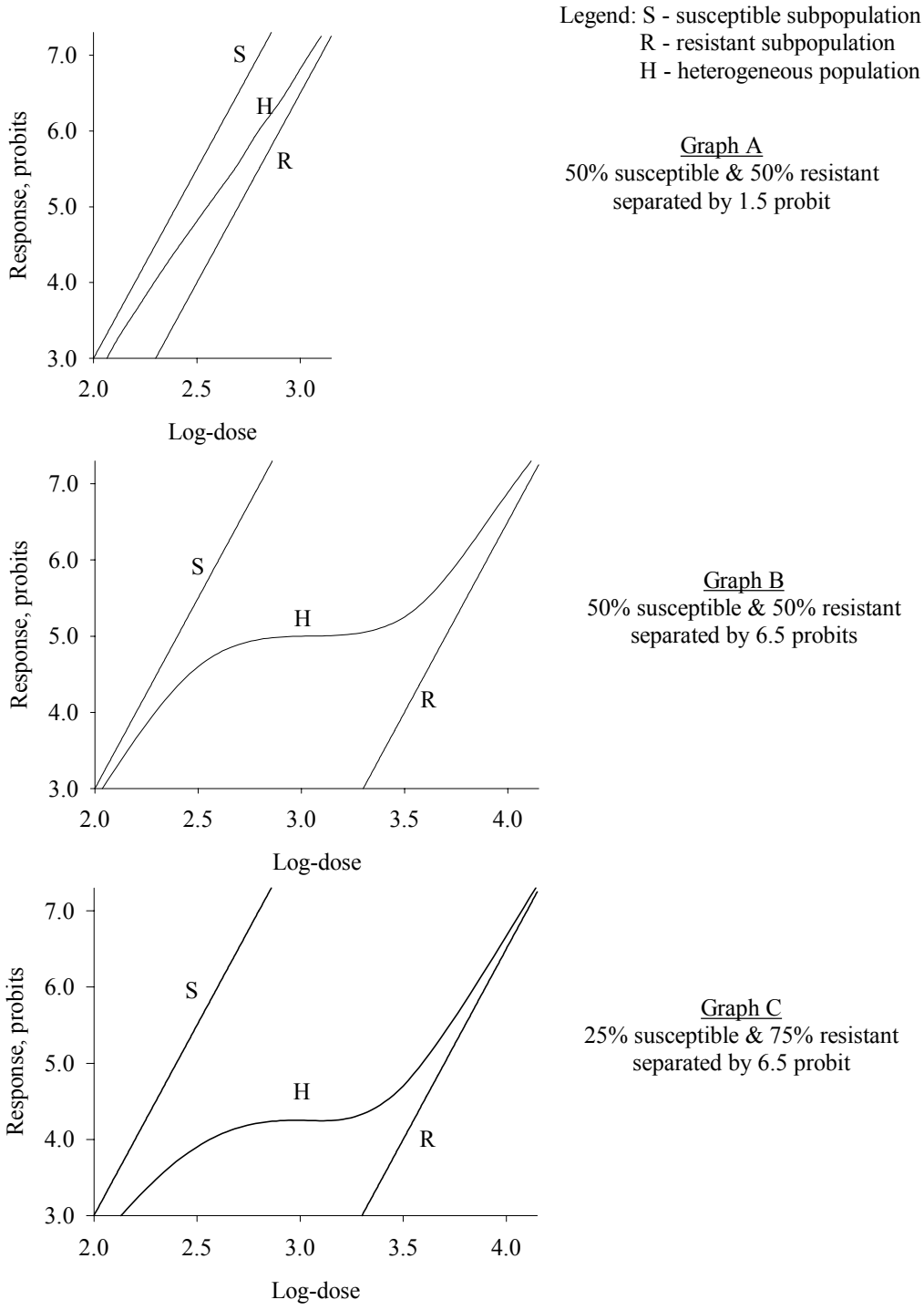
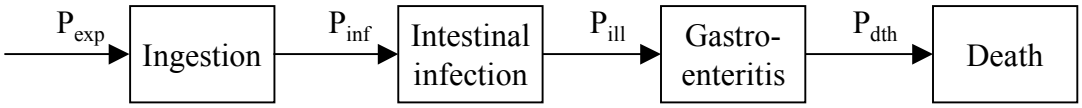


Figure 1.8. Dose-response curves for heterogeneous population



Legend: P_{exp}, P_{inf}, P_{ill}, and P_{dth} as defined in the text.

Figure 1.9. Events after foodborne exposure to pathogenic microorganism (Teunis et al., 1996)

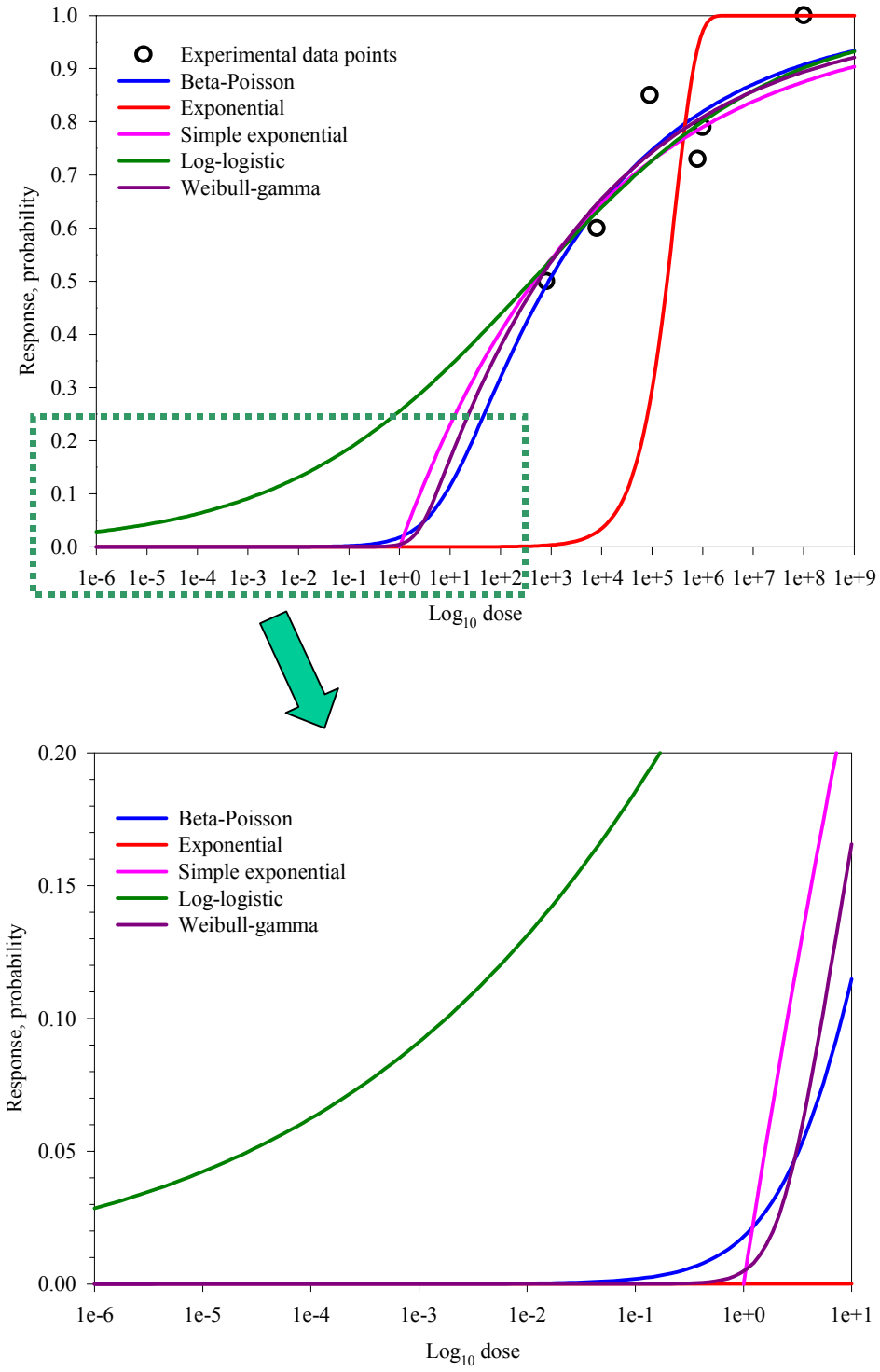


Figure 1.10. Dose-response models for *Campylobacter jejuni* (Holcomb et al., 1999b)

2 UNCERTAINTY OF MICROBIAL DOSE-RESPONSE MODELS, WITH A PARTICULAR REFERENCE TO THE APPLICATION OF BOOTSTRAP SIMULATION TO CHARACTERIZE SAMPLING ERROR

2.1 Problem Definition, Motivation, and Objectives

Over the last decade, quantitative risk assessment has increasingly been applied to microbial food safety issues. With regard to dose-response assessment, mathematical models have been fitted to data from feeding trials of human volunteers (Haas, 1983). As the prevailing practice has made extrapolation at low doses necessary, biologically based models, such as the exponential model and the beta-Poisson model, have been preferred to empirical ones (e.g. log-logistic model). Goodness-of-fit statistics have been the quantitative criterion to discriminate among plausible models (Teunis et al., 1996).

Perhaps owing to a result-oriented focus, microbial risk assessors have often gone to extreme lengths in justifying the choice of a particular dose-response model, and eventually in stating the reliance of their model's prediction. In doing so, consideration of the uncertainty potentially inherent to a specific choice has not been the object of the same scrutiny. The Codex Alimentarius Commission, which is the body setting international standards in food safety, started in 2000 an effort to elaborate guidelines for the conduct of microbial risk assessment (CAC, 2000). As for dose-response modeling, these guidelines essentially suggest pre-made, one-fit-all solutions. Little discussion is reserved to the uncertainty linked to such choices.

This study examines and quantifies select aspects of uncertainty involved in microbial dose-response modeling. Firstly, the concepts of uncertainty and variability are reviewed as they relate to the fields of risk assessment, epidemiology, and statistics. Bootstrap simulations are then carried out to represent those uncertainty elements that are quantifiable. Specifically, two data sets from human feeding trials with *Campylobacter jejuni* and *Shigella dysenteriae*, three dose-response models (exponential, beta-Poisson, and log-logistic models), and four resampling schemes – of which three are first applied

here – are considered, and their combinations are represented. Finally, the relevance of the findings in the context of microbial risk assessment is discussed.

2.2 The Concept of Uncertainty and its Treatment by Bootstrap Simulation

2.2.1 Uncertainty in Risk Assessment, Epidemiology, and Statistics

Risk assessment can be viewed as an exercise of integration. Data and select methods from diverse disciplines are aggregated in order to answer questions that, because of their complexity, could hardly be dealt with by a single discipline. While such an interdisciplinary approach has the potential to create the basis for sound policy-making, the fact that any risk assessment implicitly carries over or, even, compounds the limitations of the original data and methods ought to be acknowledged. Furthermore, the aggregation process, as well as the interpretation of scientific findings, can introduce another layer of limitations. It appears that these possibilities have been recognized to varying degrees in the different application fields of risk assessment. Microbial risk assessment has only started to come to terms with the issue, and it does so essentially on the basis of the prevailing paradigm from the environmental risk assessment arena (Nauta, 2000). This archetype rotates around the concepts of uncertainty and validity.

The Concepts of Uncertainty and Validity

Uncertainty refers to lack of knowledge about specific factors, parameters, or models (Bogen & Spear, 1987). It is a property of the risk assessors, and can be reduced by further or more accurate research (Morgan & Henrion, 1990). In contrast, variability relates to interindividual, temporal, or spatial differences attributable to true heterogeneity (Bogen & Spear, 1987). It is a property of the population under study, and can be treated by stratifying the population of individuals that are heterogeneous with regard to the characteristic of interest into homogeneous subpopulations (Morgan & Henrion, 1990). This has been interpreted to mean that, even in principle, variability is irreducible since it represents an inherent property of the population under study (USEPA, 1997; Frey & Rhodes, 1999; Kelly & Campbell, 2000).

Frameworks for Uncertainty Analysis

Literature on uncertainty analysis is rich in the environmental risk assessment field, and includes several attempts of developing a unified framework. Bogen and Spear (1987) advanced the notion of "uncertain variables" and "empirically specific demographic variables", and provided an analytical framework to analyze them. Among the kinds of quantitative attributes recognizable in policy analysis models, Morgan and Henrion (1990) defined empirical parameters as the measurable properties of the real-world systems being modeled. They proceeded to categorize the sources of uncertainty in such quantities as follows: random error and statistical variation; systematic error and subjective judgement; linguistic imprecision; variability; inherent randomness; disagreement between scientific experts; and reality approximation. In addition, uncertainty about model form was also introduced. Those concepts were further considered in a publication of the National Research Council on the state of environmental risk assessment (NRC, 1994). This report first contrasted the notions of "parameter uncertainty" and "model uncertainty", but also widely treated the concept of variability. Examples of parameter uncertainty are measurement errors, use of generic or surrogate data, response misclassification, random sampling error, and nonrepresentativeness. Components of model uncertainty are errors in model specification, such as omission of relevant variables and inclusion of irrelevant ones. Perhaps owing to the influential nature of the report in which it appeared, the triad of parameter uncertainty, model uncertainty, and variability has become in recent years the prevailing framework of reference in environmental risk assessment and, by reflection, in microbial risk assessment. In Bailar and Bailer's view (Bailar & Bailer, 1999), such conceptualization heavily reflects a toxicologist's perspective. This should be of little surprise considering the context in which it was developed.

Other frameworks have been advanced, and each has specific merits. Some examples are examined. Bogen (1990) listed the generic sources of uncertainty inherent to each step of an environmental risk assessment, thus providing a specific and practical approach. A decomposition of uncertainty into four distinct classes – temporal,

structural, metrical, and translational – was proposed by Rowe (1994). The merit of this classification is that it explicitly recognizes temporal extrapolation as well as the difficulties inherent to risk communication.

A final framework is to partition uncertainty into bias, randomness, and true variability (NRC, 1994). Bias is considered to be almost entirely a product of study design and efficiency, and randomness is a problem of sample size and measurement error. Some research methodologists have apparently preferred this last approach since it might be more productive intellectually. For instance, Frey and Rhodes (1996 & 1998) present a comprehensive quantitative analysis of variability and uncertainty in environmental data and models. They describe uncertainty as either inaccuracy (bias) or imprecision (random error). As long as bias and randomness refer to systematic error and random error respectively, their location within the framework proposed by Morgan and Henrion (1990) is evident. In contrast, many aspects of uncertainty for which quantification is less evident seem to remain untouched. Nevertheless, the specific appeal of this last framework is that the concepts of bias and random error find direct reference in the medical and statistical literature.

Validity and Precision in Epidemiology

As in any other scientific endeavor, obtaining unbiased results is the goal of medical research. Epidemiology is the medical discipline that has the more systematic treatment of the topic, which is likely justified by the necessity to come to terms with the realities of observational studies. In contrast, random error seems to be generally seen by epidemiologists and other scientists alike as a nuisance factor that merely qualifies the strength of the found "true estimate". This is a major difference compared to the mind-set of risk assessors.

The concepts of bias and random error are well established in epidemiology. Bias has been defined as "any systematic error in the design, conduct or analysis of a study that results in a mistaken estimate of an exposure's effect on the risk of disease" (Schlesselman, 1982). Using different terminology, Rothman and Greenland (1998)

distinguish validity (lack of systemic error) and precision (lack of random error). Validity is further divided into two components: validity of the inferences about the population under study (internal validity), and validity of the inferences when the results are extrapolated to the population of interest (external validity or generalizability). Selection bias, confounding, and information bias are the main elements of internal validity. Sample size and study efficiency are the two elements determining precision. In particular, study efficiency refers to modifications in the study design (e.g. planned cross-over) that maximize the information obtained for a given sample size.

In epidemiological terms, human feeding trials with microbial pathogens would be classified as clinical trials. Gordis (1996) summarizes the important issues that must be considered in the design and conduct of such trials. Some of these elements are: selection of subjects (inclusion/exclusion criteria); presence of a control/placebo group; underlying profile of individuals at study entry; allocation of subjects to treatment groups (randomization); masking/blinding; and classification of outcome. Clearly, all these issues relate to validity, but they cannot be fully solved in practice. While compliance with good epidemiological practice can improve the validity of an actual clinical trial, a varying degree of bias needs to be assumed. In contrast, the extent of the random error is essentially linked to the sample size of the study. At the stage of study design, sample size determination is essentially a trade-off between the targeted power and the number of individuals that are available or that can be handled. While it is commonly considered a desirable feature, a large number of subjects in the study can pose practical difficulty which can eventually lead to bias.

Within the typical framework of human feeding studies (i.e. no covariates recorded), bias can only be evaluated qualitatively from the description of the study design and conduct. On the other hand, random error essentially relates to sample size, and its quantitative characterization relies on statistical methods.

Systematic Error and Random Error in Statistics

In statistical terms, systematic error – also referred to as bias or inaccuracy – is the difference between the true value of the quantity of interest and the value to which the mean of the measurements converges as more measurements are taken (Morgan & Henrion, 1990). Systematic error can be the result of biased measurements, non-representative data, or model uncertainty. In contrast, random error – also called statistical variation or imprecision – is the magnitude of random variation from one observation to the next. It arises from the necessity of estimating the true value of an unknown quantity using a limited number of sample data or imprecise measurement techniques. While bias is often difficult to evaluate, precision is routinely quantified by measures of dispersion and represented through confidence intervals.

The standard approach in microbial dose-response modeling is to fit a non-linear model to data from human feeding studies. In this process, the sources of bias are potentially numerous (e.g. unreliable data, inappropriate model, inefficient estimation process), and they can only be assessed in qualitative terms. The same is true for one component of the random error, i.e. measurement error. In contrast, random error due to limited sample size – also called random sampling error – can be assessed quantitatively through a variety of statistical techniques. The bootstrap method is one such approach.

2.2.2 Bootstrap Simulation

Principles of the Bootstrap Method

Data relative to a small sample of individuals are commonly used to make inference for the population from which those individuals originate. Often, the resulting sample statistic is merely an estimate of the population statistic. Analytical solutions have been devised to characterize the relation between sample and population statistics, and constitute the main body of what is frequently perceived as "statistics". Numerical methods – such as the bootstrap – stand as an alternative by remedying shortcomings of the analytical solutions. Specifically, the bootstrap broadens the spectrum of statistical problems that can be handled, makes many assumptions superfluous, and eliminates

routine but tedious theoretical calculations. While it requires intensive computation, this drawback has become less of an obstacle with the advent of powerful and affordable personal computers. The bootstrap was first proposed by Bradley Efron at the end of the '70s (Efron, 1979), and is the subject of a monograph (Efron & Tibshirani, 1993).

With regard to risk communication, an appealing characteristic of the bootstrap method is that its principle can be understood intuitively. Let the observed measurements be *n* data points $x_1, x_2,\ldots, x_n$ contained in data set **x**. When these data are randomly sampled *n* times with replacement, a *bootstrap sample* $\mathbf{x^*} = (x_1^*, x_2^*,\ldots, x_n^*)$ is obtained. The bootstrap sample **x*** is a randomized, or resampled, version of **x**. The first step of the method is to generate a large number *b* of independent bootstrap samples $\mathbf{x^{*1}}$, $\mathbf{x^{*2}}$,…, $\mathbf{x^{*B}}$. The sought statistic *s* is then calculated for each bootstrap sample, and a series of *bootstrap replications* $s(\mathbf{x^{*b}})$ is the result. As the number of bootstrap samples increases, the distribution of the bootstrap replications becomes a reliable estimate of the population statistic. For instance, Efron and Tibshirani (1993) show that 50 to 200 bootstrap samples are generally sufficient to adequately estimate standard errors. More complicated accuracy measures, such as confidence intervals and prediction errors, usually require a few thousand samples.

The crucial step of the bootstrap method is the process by which the observed data set is resampled, i.e. how bootstrap samples are generated. Efron and Tibshirani (1993) discuss the bootstrap algorithm for general data structures, and Figure 2.1 is the schematic diagram of such a situation. In this figure, the crucial step corresponds to the estimation of the entire probability mechanism *P* from the observed data **x**, which is depicted by the bold arrow linking **x** and $\hat{P}$. While this can be surprisingly easy to do, the probability model $P \rightarrow \mathbf{x}$ can often be interpreted in different ways, and, consequently, several implementations may be plausible. As the authors point out (p. 115), the bootstrapping is not a uniquely defined concept.

The bootstrapping of linear regression models (as applied to continuous data, and estimated by least squares) well illustrates this point. A first approach is to bootstrap

observed (X,Y) pairs, while another is based on bootstrapping residuals (Efron & Tibshirani, 1993). Neither approach is inherently better. Bootstrapping residuals assumes that the regression model is correct (Efron & Tibshirani, 1993). In particular, the residuals need to be independent from the explanatory variables and identically distributed, but this condition often fails in practice. In contrast, bootstrapping pairs requires that: the observed (X,Y) pairs are a random sample from a population where X and Y are independent; X values are randomly assigned to sample units and the Y values are an observed treatment response; or the mechanism generating the data makes X and Y values potentially independent. These conditions are hardly compatible with the usual framework to which regression models are applied (Manly, 1997). Also, the number of observed pairs needs to be relatively large (Efron & Tibshirani, 1993).

It is worth noting that, since it is based on resampling observations (i.e. the empirical distribution of the observed data is sampled with replacement), the bootstrapping of pairs is an example of what has been referred to as *resampling* or *nonparametric* bootstrap (Chernick, 1999). By smoothing the empirical distribution of the original data, a *smoothed* bootstrap has also been proposed. In contrast, the *parametric* bootstrap is the case in which a parametric model is used in substitution of the empirical distribution to obtain the bootstrap samples (Efron & Tibshirani, 1993). The bootstrapping of residuals represents an intermediary case, and has thus been called *semiparametric* bootstrap (Davison & Hinkley, 1997).

Bootstrap Method Applied to Microbial Dose-Response Modeling

Estimation of model parameters is the goal of microbial dose-response modeling. The bootstrap method extends the standard approach of obtaining a single, "best" estimate (e.g. maximum likelihood estimate) by generating a distribution of the parameter values. In the case of models with two or more parameters, a joint distribution of the parameter values that maintains the inherent correlation between parameters is generated.

As previously discussed, the main question that needs resolving is the probability model upon which bootstrap samples from data of human feeding trials are generated.

An important consideration is that such data are generally sparse (i.e. few dose-response pairs), dose has to be viewed as a fixed factor, and the response has a domain between 0 and 1. This type of data is not directly treated in the monograph by Efron and Tibshirani (1993), nor is the fitting of nonlinear regression models by maximum likelihood estimation – the standard approach of microbial dose-response modeling.

A bootstrap procedure for quantal dose-response data has been proposed by Crump and Howe (1985). For each dose group, a binomial distribution – whose two parameters are the total number of exposed individuals in the dose group and the experimental infection/illness probability – generates a random value for the number of infected/ill individuals. That is, a bootstrap sample merely differs from the observed data set with regard to the number of infected/ill individuals. After fitting the dose-response model to each bootstrap sample, the resulting series of parameter values (i.e. the bootstrap replications) represents the parameter distribution. Haas et al. (1993) first applied this procedure to microbial dose-response assessment in an example that considers a waterborne exposure to rotavirus. Fazil (1996) employed the same procedure for data sets from diverse *Salmonella* spp. human feeding trials. In a comprehensive work that consider bacterial, viral, and parasitic pathogens, Teunis et al. (1996) also used bootstrapping, but the bootstrap procedure employed is not explained.

Haas et al. (1999) proposed an extension of bootstrapping residuals. This approach substitutes the experimental infection/illness probability in the binomial distribution with a probability that is both predicted by the model and "randomly corrected" with a residual. It is assumed that the residuals are asymptotically normally distributed with mean zero and unit variance. An adjustment to constrain the value of the probability between 0 and 1 is sometimes required.

All mentioned studies do not discuss shortcomings of applying the bootstrap method to dose-response data. In contrast, Teunis and Havelaar (2000) concluded that bootstrap simulation can be inefficient in such cases. Firstly, they noted that bootstrapped data could be considerably dichotomized as a consequence of few observations per dose group. Furthermore, the computation was deemed demanding

since a large number of maximum likelihood optimizations is required. These observations apparently refer to the bootstrap algorithm proposed by Haas (1993).

The bootstrap method proposed by Crump and Howe (1985) originally referred to toxicological dose-response data. A review of more recent literature shows that the main interest in applying the bootstrap method in developmental toxicology lies in interval estimation (Guerra et al., 1997; Aerts & Claeskens, 2001; Huang, 2001). In particular, bootstrap samples are generally generated through the mentioned approach employing the binomial distribution. However, other fields provide useful suggestions. Davidson and Hinkley (1997) consider a randomized block experiment that tests the resistance of sugar cane varieties to a plant disease. To model the number of diseased shoots in a given plot and variety, a binomial model similar to the one applied to dose-response data is proposed. The authors concluded that the binomial model understates the variability of the data, and thus suggest the use of a beta-binomial model. This approach substitutes the fixed probability in the binomial distribution with a beta distribution of the probability.

2.2.3 Parameter Estimation

Given the non-linear character of microbial dose-response models, maximum likelihood (ML) estimation is generally proposed as the method of choice for calculating the values of model parameters.

McCullagh & Nelder (1989, p. 118) proposed the following log likelihood function for binary data:

$$l(\hat{\pi};\mathrm{y}) = \sum_i \{y_i \log \hat{\pi}_i + (m_i - y_i)\log(1-\hat{\pi}_i)\} \qquad \text{(eq. 2.1)}$$

where m_i is the number of trials in group i, y_i is the number of successes, and $\hat{\pi}_i$ is the fitted probability. The ML estimates are those parameter values that maximize this equation. The deviance is defined as twice the difference between the maximum achievable log likelihood (i.e. the one of a saturated model) and that attained under the

fitted model, and can be written as follows:

$$D(\mathrm{y};\hat{\pi}) = 2\sum_i \left\{ y_i \log\left(\frac{y_i}{\hat{\mu}_i}\right) + (m_i - y_i)\log\left(\frac{m_i - y_i}{m_i - \hat{\mu}_i}\right)\right\} \qquad \text{(eq. 2.2)}$$

The deviance is commonly regarded as being asymptotically distributed according to a χ^2 distribution with degrees of freedom equal to the difference between the number of groups *n* and the number of model parameters *p*, and is used as a goodness-of-fit statistic of the fitted model. This is also customary in microbial dose-response modeling (Teunis et al., 1996; Holcomb et al., 1999; Haas et al., 1999). However, McCullagh & Nelder (1989) point out that proofs of the limiting $\chi_{n-p}{}^2$ distribution are based on two assumptions. Firstly, observations are assumed to be independently and binomially distributed. The second assumption is that $m_i\pi_i(1-\pi_i) \to \infty$ and, thus, that m_i tends to infinity. In human feeding studies, m_i (the number of individuals in a dose group) is very limited, and one has to conclude that the second assumption is violated. In such a circumstance, McCullagh & Nelder (1989) consider that a large deviance is not necessarily an indication of a poor fit.

2.3 Data and Methods

Data from human feeding trials with *Campylobacter jejuni* strain A3249 (Black et al., 1988) and *Shigella dysenteriae* 1 strain M 131 (Levine et al., 1973) were considered. These experimental data are reported in Table 2.1 and Table 2.2, respectively. The *Campylobacter jejuni* data are comprised of six dosage groups, while the *Shigella dysenteriae* data have four groups. The beta-Poisson model (Haas, 1983), the exponential model (Haas, 1983), and the log-logistic model (Covello & Merkhofer, 1993) were studied. These models have the following mathematical formulations:

- beta-Poisson model $\quad P_{response} = 1 - \left(1 + \frac{dose}{\beta}\right)^{-\alpha} \qquad \text{(eq. 2.3)}$

- exponential model $P_{\text{response}} = 1 - e^{-r \times dose}$ (eq. 2.4)

- log-logistic model $P_{\text{response}} = \frac{1}{1 + e^{-[\beta_0 + \beta_1 \times \log(\text{dose})]}}$ (eq. 2.5)

While the exponential model only has one parameter, the beta-Poisson model and the log-logistic model both have two parameters. The beta-Poisson model represents a generalization of the exponential model that holds true for α and β greater than 0, and for $\beta >> \alpha$ (Haas, 1983).

Offering both reliance in parameter estimation and flexibility in data management, the software SAS/STAT v8.1 (SAS Institute, Cary, North Carolina) was employed in all computations. In particular, dose-response models were fitted by means of the routine PROC NLIN. To obtain ML estimates, the default least-squares estimation was overridden by specifying the special variables _WEIGHT_ and _LOSS_. The latter in particular used a build-in function for the binomial distribution, and thus virtually coded the deviance function proposed by McCullagh & Nelder (1989, see Section 2.2.3). An example of this SAS/STAT code – as well as the following ones – is reported in the Appendix (see Section 2.7.1).

A first set of analyses was intended to recreate the standard approach in microbial risk assessment and thus to generate a baseline case. ML parameter estimates were calculated for each combination of three dose-response models and the two data sets. Wald's confidence limits as computed by the routine were recorded as well as the deviance value. Comparison of the latter to a critical χ^2 with degrees of freedom equal to the difference between number of dose groups and number of parameters (e.g. beta-Poisson model fitted to *Campylobacter jejuni* data, $\chi^2_{.05,6-2} = 9.49$) was the criteria to establish goodness-of-fit of the models. In addition, grid searches of the parameter estimates led to the construction of joint confidence regions (two-parameter models) and confidence intervals (exponential model).

In a second set of analyses, bootstrapping was carried out for the two-parameter models. (The exponential model was excluded because of the behavior of its deviance function, see Results and Discussion.) This essentially involved the resampling of the experimental data sets according to specific schemes (as described below), and then fitting of the dose-response models to each one of the resampled data sets. The former was achieved through an iterative loop in which random-number routines were nested. In particular, 1,000 resamples of the two experimental data sets were obtained for each of four resampling schemes (i.e. eight distinct files each with 1,000 resampled data sets). To illustrate the sampling algorithms (see also Figure 2.2), the following variables are defined as they refer to the experimental data:

- r_i is the number of infected or ill in the *i* dosage group;
- n_i is the total number of exposed in the same group; and
- p_i is the observed infection/illness probability (i.e. $p_i = r_i / n_i$).

The outcome of all four resampling algorithms was essentially a resampled number of infected (*Campylobacter jejuni* case) or ill (*Shigella dysenteriae* case) for each of *i* dosage groups, and this variable is denominated r_i*. For two resampling schemes, a resampled infection/illness probability – called p_i* – was used in generating r_i*.

The baseline resampling scheme is the one proposed by Crump and Howe (1985), and first applied to microbial dose-response by Haas et al. (1993). The variable r_i* was obtained by sampling a binomial distribution with parameters n_i and p_i, and will be designated "binomial resampling". By using a different second term in the binomial distribution, three new schemes were developed. The predicted value of a dose-response model at dose *i*, i.e. the probability $\hat{p}_i$ as obtained by using the ML estimates from the first set of analyses, replaced p_i in the resampling scheme denominated "binomial resampling with predicted *p*". As in the baseline case, the first term remained constant for all the iterations. A third scheme substituted p_i by sampling p_i* at each iteration from a beta distribution with parameters $\alpha = r_i + 1$ and $\beta = n_i - r_i + 1$. Such a beta distribution essentially reflects the uncertainty of p_i (Vose, 1998). Under this scheme, called "beta-

binomial resampling", the second term varied at each iteration. The fourth and last scheme replaced p_i by sampling p_i* at each iteration from the fitted dose-response models (the first term, n_i, was kept constant at 10). This scheme is termed "parametric resampling" because of the analogy with bootstrap approaches already used in other fields. (Strictly speaking, the other schemes are also "parametric" as they are also based on the parametric binomial and beta distributions.) In summary, the four resampling schemes can be summarized as follows:

- Binomial resampling — r_i* = binomial (n_i , p_i)
- Binomial resampling with predicted p — r_i* = binomial (n_i , $\hat{p}_i$)
- Beta-binomial resampling — r_i* = binomial (n_i , p_i*), where p_i* = beta ($r_i + 1$, $n_i - r_i + 1$)
- Parametric resampling — r_i* = binomial (10 , p_i*), where p_i* = $f_{\text{dose-response}}$ (β_1 , β_2)

For each combination of the two experimental human feeding data sets, two dose-response models (beta-Poisson, log-logistic), and four resampling schemes (i.e. 16 combinations in total), the fitting of the dose-response models to each of 1,000 resampled data sets was done by using the PROC NLIN routine described above. By specifying OUTEST and BY statements, the process was computationally efficient, and produced a file containing 1,000 triplets of two parameter values and the corresponding deviance. Those 16 files constituted the basis for further analyses.

Confidence limits of the parameters were calculated based on two approaches discussed by Efron and Tibshirani (1993). The first method – called "percentile method" – is based on percentiles of the empirical cumulative distribution of the bootstrapped parameters. The second method represents an extension of the percentile method in that it handles both bias as well as transformation (i.e. it is indifferent to the scale used to construct the interval). It is referred to as the "bias-corrected and accelerated method" (BC_a method). Furthermore, the joint distribution of the parameter pairs was compared

graphically to the joint confidence regions obtained earlier with grid searches. Also, the distribution of the parameter pairs obtained following the beta-binomial resampling was graphically contrasted to the distribution from the binomial resampling.

A third set of analyses involved calculating the predicted response for each parameter pair at each 1E+1 dose interval between dose 1E-6 and dose 1E+10. The 2.5^{th} and 97.5^{th} percentiles of these responses at each dose were recorded, and graphically contrasted to the predicted values calculated from the initial ML parameter pair. Empirical cumulative distributions of the responses at select doses (1E-6, 1E-4, 1E-2, 1E+0, 1E+3, 1E+6, and 1E+9) were obtained with the Hazen's plotting position function (Harter, 1984), and plotted for contrasting predictions at either different doses or different model/resampling combinations. Finally, in an attempt to investigate a plausible parametric model, responses at some select doses (1E+0, 1E+3, 1E+6, and 1E+9) were fitted to a beta distribution by means of the routine PROC UNIVARIATE with the HISTOGRAM statement. This approach estimates parameters by ML. Goodness-of-fit was evaluated through three tests offered by the routine, i.e. the Anderson-Darling test, the Cramer-von Mises test, and the Kolmogorov-Smirnov test.

2.4 Results

Parameter and deviance estimates based on ML estimation are reported in Table 2.3. While the parameter values are mainly of interest as reference to the further results, the goodness-of-fit of the different data/model combinations is worth considering. As shown by the significance level of the observed deviances, the beta-Poisson model and the log-logistic model result in a similarly adequate fit for both the *Campylobacter jejuni* and *Shigella dysenteriae* data. In contrast, the fit of the exponential model for either data set is rejected.

Joint confidence regions (two-parameter models) and the confidence interval (exponential model) of the model parameters are the graphical representation of the deviance function (Figure 2.3 to Figure 2.8). While no particular influence of the data set is evident (with the obvious exception that the different data sets lead to different

parameter estimates), a pattern linked to each of the three dose-response models emerges. The log-logistic model generates fairly regular elliptic regions with negative correlation between the two parameters β_0 and β_1 (Figure 2.5 and Figure 2.6). The ML estimates fall at the center of those ellipses. In contrast, the beta-Poisson model produces highly irregular confidence contours (Figure 2.3 and Figure 2.4). The correlation between the parameters α and β is positive and the value range of the parameter β is orders of magnitude broader than the one of parameter α. The location of the ML estimates is shifted with respect to the approximate center of the confidence regions. A log transformation of the axes of Figure 2.3 and Figure 2.4 would make evident that the parameter β is smaller than the parameter α for a sizeable sector of the confidence regions (graphs not shown). While this concerns only the 95% confidence contour in the *Shigella dysenteriae* case, the 50% confidence region also intersects such implausible area for the *Campylobacter jejuni* data.

Figure 2.3 through Figure 2.6 indicate that, over the displayed range of parameter values, the two-parameter models (beta-Poisson, log-logistic) generate a single (global) minimum region in the deviance function (though of very dissimilar shape). In contrast, the deviance function for the exponential model produces several local minima. Specifically, five deviance minima are evident for the *Campylobacter jejuni* data (Figure 2.7), and four for the *Shigella dysenteriae* data (Figure 2.8). In both cases, the minimum that corresponds to the ML estimates is the farthest to the left of the graphs. While the deviance function in the sector of the ML estimates draws a smooth arch, the other minima are spike-like in their lower boundaries. For both data sets, the exponential model's ML parameter estimates do not correspond to a global minimum. Over the considered range of parameter values, the absolute deviance minimum for the *Campylobacter jejuni* data (deviance = 15.95, its significance = .007) corresponds to a parameter value of 4.21E-4, and that for the *Shigella dysenteriae* data (deviance = 10.22, its significance level = .017) to a value of 1.90E-2. As the significance level of these deviances indicates, the fit of the exponential model would still be rejected. The curves based on the parameter values obtained at the different deviance minima are graphed in

Figure 2.9 and Figure 2.10. The curves to the farthest right are the ones corresponding to the ML parameters, and the curves to the farthest left are based on the limiting parameter value of 1. Was it not that only five curves result for the *Campylobacter jejuni* data, a curve would exist for each data point, and it would lay to the left of such a point. In other words, it would seem that each data point "anchors" a curve, or, conversely, curves "snap" from one data point to another.

Figure 2.11 to Figure 2.16 show how the different resampling schemes affect the bootstrapped parameters of the dose-response models for a given data set. (The experimental values are reported in the last column of Table 2.1 and Table 2.2.) In each figure, the distribution of 1,000 resamples at each dose level is contrasted for the binomial resampling scheme and one of the remaining schemes. While the mode is a rough indication of the spread (the scheme with the lower mode has more spread), one should also try to visualize the means of the contrasted distributions. It should then be apparent that the beta-binomial resampling scheme in contrast to the binomial one (Figure 2.11 and Figure 2.12) consistently produces distributions with greater spread. Keeping in mind that dose 800 represents the experimental infectious dose 50 (ID_{50}) in the *Campylobacter jejuni* data and that dose 200 is the ID_{50} for the *Shigella dysenteriae* data, the central location of the distributions from the beta-binomial resampling scheme seems to increasingly move away from the extremes the farther the dose level is from the ID_{50}. In other words, the beta-binomial resampling scheme causes the expected infection/illness probability at each dose level to get closer to 0.5. In contrast, the parametric resampling schemes – whether based on the beta-Poisson model or the log-logistic model (Figure 2.13 to Figure 2.16) – do not produce a clear pattern when compared to the binomial one. Both central tendency and spread of the distributions generally look similar. Differences are larger the more the infection/illness probability predicted by the dose-response model differs from the experimental probability (see *Campylobacter jejuni* data at dose 9E+4 and 8E+5).

The bootstrapped parameter pairs are graphed in Figure 2.17 through Figure 2.20. (A $\log_{10}$ transformation of the parameter values was necessary to improve the graphical

representation in Figure 2.17 and Figure 2.18.) The 95% joint confidence regions presented above (Figure 2.3 to Figure 2.6) are given as reference. Characteristics that are typical of the single resampling scheme are evident. The parametric resampling generates the least dispersion, and the pairs are tightly clustered around the ML pair estimates. The patterns resulting from the binomial resampling and the binomial resampling with predicted *p* are very similar, and largely correspond to the joint confidence regions. The beta-binomial resampling produces the largest spread. When applied to the beta-Poisson model (Figure 2.17 and Figure 2.18), it produces a comet-like tail in the lower, left-hand quadrant. In the log-logistic model (Figure 2.19 and Figure 2.20), the pair cloud lies lower than the joint confidence region. The only effect that seems to arise from the data set (i.e. that is independent from both the dose-response model and the resampling scheme) is visible in the figures related to the *Shigella dysenteriae* data. Scattered pairs lay in the upper, right-hand quadrant following the fitting of the beta-Poisson model (well outside the joint confidence region, Figure 2.18), and in the upper, left-hand quandrant in the case of the log-logistic model.

The binomial and the beta-binomial resampling schemes are contrasted in Figure 2.21 to Figure 2.24. With regard to the beta-Poisson model (Figure 2.21 and Figure 2.22), both schemes generate parameter pairs in which the parameter α is greater than parameter β (i.e. the comet-like tail mentioned above), and are thus outside of the plausible region. The beta-binomial resampling produces such pairs with a higher frequency than the binomial one. In the case of the log-logistic model (Figure 2.23 and Figure 2.24), the fact that the pairs from the beta-binomial resampling tend to lay lower than those from the binomial resampling is again evident. However, the higher magnification brings to light a streaked pattern in the central region of the graph related to the *Campylobacter jejuni* data (Figure 2.23).

Confidence bands for the single parameter distributions calculated through different analytical and numerical methods are reported in Table 2.4 and Table 2.5. Regardless of the pathogen, three main observations can be made. Firstly, the Wald's analytical method produces confidence intervals that are symmetrical around the ML

parameter estimates. In particular, the lower bounds for the beta-Poisson model are negative, and thus violate the assumptions upon which the model has been derived. Secondly, in contrast to the analytical method, the confidence intervals after bootstrapping are asymmetric, and the lower limits for the beta-Poisson model are always equal to or greater than 0. The confidence bands after parametric resampling are the narrowest, while the range from the binomial resampling tends in general to be narrower than that from the beta-binomial resampling. Finally, no clear pattern emerges as to the percentile method versus the bias-corrected and accelerated method (BC_a). However, the latter produces extreme confidence limits in some instances, such as the upper limits of the beta-Poisson model parameters for *Shigella dysenteriae* (Table 2.5).

The confidence bands obtained with the percentile method are graphed in Figure 2.25 to Figure 2.28. For each pathogen, Figure 2.25 and Figure 2.26 contrasts dose-response models for a given resampling scheme. These graphs are best evaluated by distinguishing three areas. At doses below the lowest experimental dose, the log-logistic model consistently generates predicted values and confidence bands that are more conservative (i.e. indicate a higher infection/illness probability) than those obtained with the beta-Poisson model. However, when one looks at the upper limit, the difference is sometimes relative (e.g. upper graph of Figure 2.25). Within the range of the experimental data, the two models produce prediction and confidence bands that are virtually indistinguishable. At doses around or higher than the maximal experimental dose, no clear pattern emerges: the confidence bands of the log-logistic model are either more conservative or embrace the confidence bands of the beta-Poisson model. Figure 2.27 and Figure 2.28 take a different look at essentially the same information: they compare resampling schemes within each dose-response model. The first clear pattern is that the prediction curve (the middle one) is essentially the same for all but the beta-binomial resampling scheme. This tends to generate a steeper prediction curve, i.e. less conservative results at low doses and more conservative results at high doses. As for the confidence bands, it is also evident that, especially outside of the range of experimental data, the beta-binomial resampling produces the widest range. The binomial resampling

and the binomial resampling with predicted *p* essentially give the same bands, and the parametric resampling produces the narrowest bands.

To investigate the behavior of the results between the confidence limits, cross-sections of the middle graphs of Figure 2.27 and Figure 2.28 at select doses are graphed in Figure 2.29 through Figure 2.36. While the infection/illness probability now lies on the abscissa, the ordinate shows the empirical cumulative probability for the given probability. The 2.5^{th} and the 97.5^{th} percentiles in the latter figures correspond to the lower and upper confidence limits, respectively. In order to distinguish among lines at low doses (doses 1E-6, 1E-4, and 1E-2), a $\log_{10}$ transformation of the probability is necessary. However, the distribution at dose 1 (1E+0) as graphed in the lower graph can serve as reference on the shape of the cumulative distributions at low doses without such a transformation (i.e. the distributions at doses lower than 1 would line to the left of such a dose). Figure 2.29 to Figure 2.36 need to be evaluated as to the extent of the symmetry between the tails at low and high infection/illness probability and for the location of the steeper part of the lines. The main observation is that a shape common to all distributions cannot be identified. While the bulk of the response at low doses tends to stack up close to zero, long tails into high infection/illness probability occur. The situation at high doses is mirror-reflected: results tend towards 1, but can reach into the 0.4-0.5 probabilities. Only at dose 1E+3, is a tendency towards symmetric lines consistent. Single effect of dose-response model and resampling scheme are difficult to tease out in these figures. The exception is that, at low doses (upper graphs), the lines are farther apart in the results from the beta-Poisson model (Figure 2.29 to Figure 2.32) as compared to the corresponding ones from the log-logistic (Figure 2.33 to Figure 2.36).

In an attempt to provide a parametric model of the previous empirical distributions, Table 2.6 to Table 2.9 report goodness-of-fit statistics related to a beta distribution. A significance level greater than 0.25 indicates that a reasonable beta model exists. While the fit is questionable between 0.05 and 0.25, it is rejected at significance level lower than 0.05. The results show that the fitting of a beta distribution generally fails. However, regardless of the resampling scheme, a beta model for the responses of

the beta-Poisson model in the case of the *Campylobacter jejuni* data exists at doses 1E+3, 1E+6, and 1E+9.

The last set of figures (Figure 2.37 and Figure 2.38) contrasts the four possible combinations of two dose-response models (beta-Poisson and log-logistic models) and two resampling schemes (binomial and beta-binomial resampling schemes). The fact that the abscissa at dose 1E-2 reflects a $\log_{10}$ transformation of the infection/illness probability should be noted. A first observation is that, with increasing doses, the difference in prediction between the two models decreases (vertical dashed/dotted lines). In the case of the *Campylobacter jejuni* data, the difference gradually vanishes. In contrast, it stabilizes at 0.03 for the *Shigella dysenteriae* data.

Furthermore, an important pattern emerges when one considers the change in the order of the lines from low to high doses. While the dose-response model essentially influences the uncertainty at low doses, the difference at high doses is determined by the resampling scheme. At an intermediate dose (1E+3), the lines are remarkably similar, and no particular influence of dose-response model or resampling scheme can be spotted. Incidentally, this intermediate dose level is the closest to the experimental ID_{50} (800 in the *Campylobacter jejuni* trial, 200 in the *Shigella dysenteriae* trial). While the log-logistic model overall produces more conservative results at dose 1E-2 in the case of the *Campylobacter jejuni* data (Figure 2.37), the predictions of the beta-Poisson model are linked to much larger imprecision (the distributions span over 6 orders of magnitude instead of the 3 of the log-logistic model). In the case of the *Shigella dysenteriae* data (Figure 2.38), the difference essentially relates to the location of the longer tails (at lower cumulative probability for the log-logistic model, at higher cumulative probability for the beta-Poisson model). Considering that, in doses other than 1E-2, the abscissa does not reflect a $\log_{10}$ transformation, the differences at higher doses seems less relevant.

2.5 Discussion and Conclusion

Differentiation between uncertainty and variability not only fulfills a need for scientific credibility, but also has important risk management implications (Morgan &

Henrion, 1990). In particular, identification of key sources of uncertainty provides perspective for the dependability of a risk assessment's results and leads to prioritization of further research, while specification of variability sources allows analyses specific to particular population subgroups. Nonetheless, such a differentiation is not straightforward in both theoretical and practical terms.

A first consideration is that grasping the difference between the concepts of variability and uncertainty is a difficult exercise from a cognitive point of view. The mind-set of a bench scientist is very different from that of a risk assessor. The former is usually called to defend the goodness of the results, while the latter has a duty to represent and discuss the uncertainty of the predictions. It is not surprising then that bench scientists and decision-makers often interpret such an uncertainty as a weakness. But, the contrary is also true. When a risk assessment fails to discuss its limitations and thus purports to be more certain than it truly is, the backlash is usually greater. Obviously, consideration of uncertainty imposes the necessity to do an even better job in terms of risk communication and representation.

As presented in Section 2.2.1, several frameworks for partitioning variability and uncertainty have been proposed in risk assessment. Each has its merits. Furthermore, similar notions are available in almost any scientific discipline, for instance epidemiology and statistics. While core concepts are similar across frameworks and disciplines, it is not immediately evident that talking of uncertainty creates in itself elements of uncertainty. For instance, it has been argued that the distinction between uncertainty and variability is overdrawn. Bailar and Bailer (1999) state that whether variation will be ascribed to either variability or uncertainty depends on the risk assessor's state of knowledge. Indeed, in almost any situation dealing with health hazards, knowledge to adequately characterize the elements causing population heterogeneity is almost always imperfect. One could research such factors, but such an exercise would be onerous and possibly make a risk assessment superfluous. In practice, a risk assessor would revert to the use of surrogate variables – typically demographic factors such as age, gender and race – as well as less parsimonious models. Uncertainty would ultimately result from the

inclusion of those imperfect parameters and relations, and it is difficult to evaluate whether the gain obtained by reducing the variability could offset the loss due to an increase in uncertainty.

Variability is perhaps the concept that is easier to grasp, and is discussed first. It has been defined as the interindividual, spatial, and temporal heterogeneity (Bogen & Spear, 1987). The previous paragraph mentioned that, unless there is a relevant effort at data collection, our ability to characterize and measure the elements governing such heterogeneity is often imperfect in the case of health hazards. Consequently, variability should only be dealt with to the extent that it is necessary and feasible. Whether it is necessary essentially depends on the risk assessment questions. In the case of food safety risk assessment, it is increasingly recognized that there are risk management and communication benefits in calculating risk estimates specific to certain population subgroups (e.g. children, elderly, immunocompromised individuals). The need being established, the knowledge of the elements and mechanisms determining the population heterogeneity decides the feasibility. In the specific case of microbial pathogens, a multitude of factors relating to the pathogen, the host, and the food matrix is generally thought to interact in determining whether an individual will be infected and become ill. Our knowledge of the single factors (let alone their interrelationship) is imperfect. Were one to approach this experimentally, human feeding studies requiring a nearly infinite combination of virulent pathogens, population subgroups, and food media would be required. This is obviously an untenable proposition in both ethical and cost terms.

As for microbial dose-response modeling, one has to assume that the currently available human feeding studies provide the only available quantitative data. Given the uniformity in characteristics of the volunteers in these studies (discussed further below), these data do not allow us to differentiate population heterogeneity. It is thus advanced that, within the framework of food safety risk assessment, consideration of variability inherent to dose effects needs to be deferred from the hazard characterization phase and to, under consideration of the exposure pattern, the risk characterization step. As discussed later, this proposition stands in contrast to a long-standing tradition in

microbial dose-response modeling that aspires to describe the whole host-pathogen interrelationship with a concise and elegant mathematical function.

From a theoretical point of view, there are potentially innumerable sources of uncertainty in microbial dose-response assessment. Sources of biases are discussed first in succession for data, dose-response models, and parameter estimation.

Microbial risk assessors have often pointed out the shortcomings of human feeding trials (Buchanan et al., 2000). At issue is the fact that those studies have mainly employed healthy, young, male volunteers, that only less pathogenic agents have been tested, that only relatively high doses have been used, and that the food matrix has been poorly considered. Strictly speaking, those characteristics do not constitute a source of uncertainty per se. Uncertainty generates when the risk assessor fits a dose-response model to these data, and extrapolates the resulting dose-response curve as to being representative of the whole population, of different pathogens, and of all types of food media. In other words, the shortcomings of human feeding trials as applied to microbial risk assessment are really a function of the prevailing practice of dose-response assessment, i.e. the expectation that this equates to hazard characterization. The real concern with human feeding trials ought to be that the experimental design and conduct is often difficult to evaluate. Since most human feeding studies were originally carried out as vaccine trials (Buchanan et al., 2000), researchers zeroed in on those doses that provide the highest protection. Under these conditions, the implementation of procedures that guarantee adequate randomization of the volunteers – a key requirement to avoid bias in clinical trials – is expected to be challenging. This feature as well as others (e.g. selection/exclusion criteria, blinding) is often poorly described in the references, and it is thus difficult to assess the extent to which data of human feeding studies are biased. The alternatives – animal feeding studies and epidemiological investigations – create their own set of issues (e.g. animal-to-human extrapolation, reliable recording of exposure), and thus do not appear to offer real advantages in terms of bias.

An issue that has often occupied microbial risk assessors is the pooling of human feeding data across experiments with either the same pathogen serotype/strain or different

serotypes/strains of the same pathogen species. Besides creating less sparse data sets (more data points available), pooling tends to generate dose-response curves that are species-specific rather than serotype/strain-specific. It has been performed either based on mere statistical consideration (Fazil, 1996; Teunis et al., 1996) or after a combination of statistical analyses and professional judgment (Jaykus et al., 1997; Latimer et al., 2001). In the feeding studies considered in our work, a further group would have been available for the *Campylobacter jejuni* strain A3249 data, i.e. four individuals that were given an inoculum with sodium bicarbonate instead of the usual of nonfat milk (Black et al., 1988). Analogously, the *Shigella dysenteriae* feeding study also reports two further groups that were challenged with strain A-1 instead of the strain M 131 (Levine et al., 1973). The peculiarity of those groups (i.e. different food media and different pathogen strain, respectively) indicates that these results essentially represent different experimental conditions, and the pooling could inherently compound experimental biases inherent in the single data sets. As this case indicates, generalization (i.e. a dose-response model that is representative of different exposure types) potentially comes at the expense of increasing uncertainty. A risk assessor needs to be aware of this circumstance and to use his/her judgment as to whether the trade-off is worthwhile. The alternative would be to adjust for serotype/strain effects in other phases of the risk assessment. A related question is the use of surrogate data for highly virulent pathogens. For instance, the *Shigella dysenteriae* data have been used as a surrogate for *Salmonella* Enteritidis (USDA/FSIS, 1998a). Coleman and Marks (1998) spells out three qualitative criteria for selecting adequate surrogate data. In particular, a surrogate pathogen is expected to be similar to the agent of interest in terms of taxonomic classification, microbial genetic mechanisms of pathogenicity, and epidemiology of the health effects. The degree of bias that results from using data from a surrogate pathogen is in direct relation to how such criteria can be fulfilled.

Specification of the dose-response model has been the pivotal research interest in microbial risk assessment arena. In a patently deterministic tradition, researchers seem to have been fascinated by the quest of describing the host-pathogen interrelationship with a

concise and elegant formula. A perceived need for low-dose extrapolation has led to a strong preference for models that were developed on the basis of theoretical considerations regarding the infection process (e.g. exponential model, beta-Poisson model) over empirical ones (e.g. log-logistic model). It is our belief that the requirement for low-dose extrapolation is a direct reflection of how paradigms of the toxicological risk assessment have too readily been translated to microbial risk assessment, and that this is fatally flawed. Justification of such a statement goes beyond the purpose of this study but is summarized in Appendix 2.7.2. It suffices to say that, within the range of the fitted data, models commonly viewed as being implausible – as the log-logistic model generally is – produce risk estimates that are very similar to those of biologically based models. Pragmatically, those models would then be acceptable. As it will be shown, they actually offer a number of advantages.

Recently, there have been attempts to quantify model uncertainty. A method that incorporates model uncertainty by weighting averages of estimates from several models has been developed (Kang et al., 2000). Specifically, the Akaike's information criterion (essentially the model's log likelihood adjusted for the number of parameters) is used to calculate model weights. In an analogous approach, Latimer et al. (2001) proposed a weighted composite dose-response model for *Salmonella* species. The weighting factor is in this case the "degree of model confidence" which is calculated as the ratio between the log likelihood of the model being evaluated and the log likelihood of the saturated model. The composite feature of the method is that it models *Salmonella* serotypes of varying pathogenicity. Morgan and Henrion (1990) have pointed out that assigning probabilities (another word for weights, really) to different models is inappropriate. Since any model is inherently false, no model can be more probable than the others. As an alternative, the use of a general metamodel that contains the single models as a special case is suggested. Model uncertainty would then be converted into parameter uncertainty. It is noteworthy that, in microbial dose-response assessment, the Weibull-Gamma model is a generalization of both the log-logistic model and the beta-Poisson, i.e. two models that represent opposite theories of the infection process.

Table 2.3 lists the ML parameter estimates for the two data sets and the three dose-response models considered. Other researchers have reported estimates for those data sets and models, for instance Teunis et al. (1996) and Holcomb et al. (1999). Both references considered the beta-Poisson model, while Teunis et al. also considered the exponential model and Holcomb et al. the log-logistic model (though in a different parameterization). The values for the *Shigella dysenteriae* data set listed in Table 2.3 are equal to those reported in the two references. In contrast, the estimates for the *Campylobacter jejuni* data set are only equal to those of Teunis et al. The description of the data and methods as well as the *Shigella dysenteriae* results indicate that the data, dose-response models, and the applied parameter estimation algorithm are manifestly the same in the three cases. Consequently, an explanation for the discrepancy of the *Campylobacter jejuni* estimates by Holcomb et al. is not immediately evident. Assuming that no human error is involved (e.g. the used data were different to those reported), differences in the implementation of the parameter estimation algorithm by different software could be advanced as an explanation in the case of specific data sets (the dose-response trend in the *Campylobacter jejuni* data is less consistent than that in the *Shigella dysenteriae* data). This emphasizes the need to carefully document the employed software as well as to possibly verify/validate the computations under the specific circumstances.

Similar to what is customary in microbial risk assessment (Teunis et al., 1996; Holcomb et al., 1999; Haas et al., 1999), goodness-of-fit was evaluated by comparing the deviance value to a critical χ^2. Given the limited number of challenged individuals in human feeding studies, such a practice likely violates an important theoretical assumption (see Section 2.2.3). Under these conditions, a large deviance is not necessarily an indication of a poor fit (McCullagh & Nelder, 1989). This consideration leads one to potentially question the rejection of the fit for the exponential model (Table 2.3), and indicates that more reliable methods for establishing goodness-of-fit of microbial dose-response models need to be investigated.

The ML parameter estimates are, by definition, those parameter values for which the log likelihood is maximized. Conversely, the deviance function reaches a minimum. The behavior of the deviance function over varying parameter values – graphically represented by means of joint confidence regions or confidence intervals – offers an invaluable insight into the parameter estimation process of the single microbial dose-response models. The log-logistic model fitted to both the *Campylobacter jejuni* and the *Shigella dysenteriae* data sets (Figure 2.5 and Figure 2.6, respectively) generates ellipsoidal confidence contours, which are indicative of an efficient estimation process (Ratkowsky, 1990). In addition, the negative correlation between the parameters β_0 and β_1 seems to assure that a self-limiting mechanism for their values is built into the model.

Neither feature is apparent for the beta-Poisson model (Figure 2.3 and Figure 2.4), and it thus seems that the estimation process of the beta-Poisson model is less efficient. Strictly speaking, estimation efficiency is merely an indication of the speed with which ML estimates are found. However, the evidence presented here intuitively suggests that the estimation process for the log-logistic model offers better warranties of stability across different data sets and software. In comparison to the beta-Poisson model, the parameter estimates of the log-logistic model would thus incur a lesser chance of being biased. Coleman and Marks (1998) list other advantages of empirical models. Another key consideration with regard to the beta-Poisson model is that relevant portions of the confidence regions intersect an implausible sector (parameter α > parameter β). For both the *Campylobacter jejuni* and the *Shigella dysenteriae* case, therefore, one cannot reject the possibility that the theoretical assumptions governing the derivation of the beta-Poisson model are violated. In such a case, the beta-Poisson model is devoid of its claimed biological fundaments, and reverts to an empirical model very much as is the log-logistic model. Confidence regions for beta-Poisson models fitted to a variety of data sets have been previously presented (Fazil, 1996; Teunis et al., 1996), but – though often applicable – the issue highlighted here was not noted.

In the case of the exponential model, the behavior of the deviance function is decidedly surprising (Figure 2.7 and Figure 2.8). In contrast to the two-parameter models which only have one minimum region, the exponential model generates several minima. In particular, the ML estimates of Table 2.3 are roughly a factor 2 smaller than those relative to the global deviance minimum. It is thus advanced that the parameter estimates commonly reported by microbial risk assessors for exponential dose-response models are greatly underestimated (i.e. biased downward). The impact of such a bias can be appreciated from Figure 2.9 and Figure 2.10, where the parameter bias is translated into overestimating the ID_{50} by approximately a factor 2. Within the context of a risk assessment, the bias depends on the considered exposure dose, but one would tend to underestimate the infection/illness probability and ultimately the risk. From a computational point of view, lack of sensitivity of the iterative process for abrupt, spike-like deviance minima explains why a parameter value linked to the wide and smooth deviance minimum is reported as the final estimate. This occurs even when an initial parameter estimate that is very close to the global deviance minimum is specified. Unfortunately, a mathematical interpretation for the presence of several deviance minima exceeds our capability. In particular, it would be interesting to test theoretical fundaments of the alleged "anchor and snatch" effect.

The results discussed in the two previous paragraphs clearly indicate that, when selecting a dose-response model, microbial risk assessors ought to weigh the underlying biological plausibility of a model against the model behavior. The potential influence on parameter validity is great. Owing to the sparseness of the human feeding data, the fulfillment of the asymptotic approximation (large sample size quality) that governs much of the ML estimation process is doubtful. This fact is of particular concern because the nonlinear nature of dose-response models implies both difficulties in parameter estimation and undesirable statistical properties of the estimators (Ratkowsky, 1990). In this regard, the log-logistic model is the least complicated of the three models considered in this study, while the beta-Poisson seems to be the most complicated. Even if there was a compelling reason for a specific model, the choice of parameterization is often open.

For instance, Ratkowsky (1990) describes three different parameterizations of the exponential model. The one commonly employed in microbial dose-response assessment – and used in this study – is considered to be the least efficient one.

So far, the discussion on uncertainty has dealt with bias. The second aspect of uncertainty –random error or imprecision – is treated in continuation. As already mentioned, the two components of imprecision are measurement error and random sampling error. Measurement error is also a source of bias.

Dose and outcome are the two measurements performed in human feeding trials. Elements of the innate immune system, such as the gastric acidity, mean that the administered dose is invariably an upwardly biased estimate of the dose reaching the intestinal tract (i.e. the effective dose). This fact is somewhat counterbalanced by the tendency of some pathogens, viruses in particular, to clump together (Teunis & Havelaar, 1999). Inocula are prepared through a dilution process, which certainly introduces imprecision. The dose reported within each dose group really represents an expected value, but it is difficult to advance exactly how variable is the dose administered to the individual subjects. Definition of a health outcome, such as establishing whether infection/illness has occurred following foodborne exposure to a pathogen, is an inherently biased process. Health and disease are extremes of a continuum. When defining binary outcomes, a viable trade-off between sensitivity (the ability to detect true positives) and specificity (the ability to avoid false positives) is struck. More recently, microbial risk assessors have attempted to obviate such a shortcoming by using continuous measurements, such as stool volume or antigen levels in feces (USDA/FSIS, 1998b; Teunis et al., 2002). In addition to introducing imprecision, the approach does not really address the issue of bias because the considered variables only partially mirror the outcome of interest. Although a definitive statement on its extent is impossible, measurement error needs to be assumed in microbial dose-response assessment, and has a potential influence on both bias and imprecision.

Random sampling error arises from the necessity of making inferences regarding a population from a sample of limited size. In this context, the bootstrap method is a

statistical approach that describes the sampling distribution of an uncertain parameter (Efron & Tibshirani, 1993). Specifically, the technique is applied in this study to characterize the sampling error of the parameters of microbial dose-response models, and, by reflection, of the dose-response curves. Instead of zeroing in on a "correct" approach (as other microbial risk assessors have so far tended to), four different resampling schemes are contrasted. Efron and Tibshirani (1993) have pointed out that, in many situations, bootstrapping is not a uniquely defined concept. This should not be taken to mean that the technique is somehow subject to the analyst's choices. Rather, the notion reflects the fact that the probability model governing the data (i.e. the $P \rightarrow \mathbf{x}$ of Figure 2.1) can often be characterized in different ways. At least three of the four resampling schemes employed in this study can be viewed as representing different components of the sampling error involved in microbial dose-response assessment. The results from the resampling scheme termed "parametric resampling" show the sampling error that is inherent in estimating a continuous dose-response curve based on few data points (four for the *Shigella dysenteriae* data, six for the *Campylobacter jejuni* data). It could be argued that, while the parametric resampling looks at the sampling error linked to interpolation, the primary focus of the "binomial resampling" and the "beta-binomial resampling" is extrapolation. The "within-trial" sampling error that arises from the limited number of individuals in the human feeding studies (between 4 and 10 volunteers in the dose groups for the *Shigella dysenteriae* data, six for the *Campylobacter jejuni* data) is depicted through the "binomial resampling". By considering the infection/illness probability stochastically, the "beta-binomial resampling" characterizes a "between-trials" sampling error as well as the within-trial sampling error. Especially within the context of a risk assessment, the purpose of a microbial dose-response model is future prediction of an infection/illness probability. That is, the interest is not only in translating the information of a single experiment to a large population, but also in evaluating the variation that would result from repeating the experiment. While the binomial resampling only considers the first aspect, the beta-binomial resampling treats both elements. Together they concur in partitioning a conceptual domain from no uncertainty

(a stepwise vertical line from 0 to 1) to an uninformative, spurious relation (ideally, a horizontal line with slope 0), in which any dose-response curve is constrained. One is hard pressed to conclude that the beta-binomial resampling is the "correct" method to bootstrap a dose-response model. As the results show, the relevance of theoretical considerations depends in practice on the dose range of interest. As long as one is true to the inherently pragmatic nature of risk assessment, the binomial resampling may remain an equally acceptable approach because its results differ little from those of the beta-binomial resampling within the range of the observed data.

The "binomial resampling with predicted *p*" has some resemblance to the "bootstrapping of residuals" proposed by Haas et al. (1999). In particular, the residual specific to the dose group rather than a fraction (less than 0.15 for the *Campylobacter jejuni* data, less than 0.25 for the *Shigella dysenteriae* data) of a residual randomly sampled from all available ones is chosen to adjust the infection/illness probability. When the slight differences between the binomial resampling and the binomial resampling with predicted *p* are also considered, one can expect that the results of the residual bootstrapping would be very close to the ones of the binomial resampling. This fact alone questions whether the complexity of the residual bootstrapping (which makes programming errors more likely) is worth pursuing. More importantly, the assumption that the variance asymptotically tends to unit is undemonstrated within the nonlinear framework, and the need for an adjustment to limit the probability between 0 and 1 makes the approach odd. While it is not possible to state that any of resampling schemes previously discussed is inherently the best, it has to be concluded that the residual bootstrapping has too many shortcomings for the limited benefits that it seems to offer.

The results of the bootstrap simulation tell a consistent story. Whether one looks at the resampled data sets (Figure 2.11 through Figure 2.16), compares resulting parameter pairs (Figure 2.17 through Figure 2.20), or considers confidence bands of the dose-response curves (Figure 2.25 through Figure 2.28), it is evident that the parametric, binomial, and beta-binomial resampling schemes describe a continuum of sampling error. The binomial resampling with the predicted *p* produces results that are virtually

indistinguishable from the binomial resampling. The least sampling error is present in the parametric resampling, while the beta-binomial reflects the most. The binomial scheme, which has to be viewed as the "gold standard" scheme because of its previous application in the microbial risk assessment field, takes an intermediary position. This generalization is coherent with the theoretical expectation discussed above.

Three specific observations are also worth mentioning. Whether in concomitance with the binomial or the beta-binomial resampling schemes, parameter pairs in the implausible region are evident for the beta-Poisson model (Figure 2.17, Figure 2.18, Figure 2.21, Figure 2.22). As they reflect a specific realization of the bootstrap process, they are perfectly legitimate. This result reaffirms the previous concern on whether the application of the beta-Poisson model to the *Campylobacter jejuni* and the *Shigella dysenteriae* data actually fulfills the model assumptions. The reason why the combination with the beta-binomial resampling increases the frequency of implausible pairs is not immediately evident. This resampling scheme tends to even out differences in the number of infected/ill individuals among dose groups, and the resulting flatter dose-response curves need to be described by lower parameter values (as noted earlier, α and β are positively correlated). In contrast to analytical solutions, the bootstrap method produces parameter confidence limits that are both plausible and realistic (i.e. greater than zero and asymmetric, Table 2.4 and Table 2.5). Within the bootstrapping framework, several techniques for simulating those limits are available, and sometimes lead to dramatic differences in the upper confidence limit. While the outputs of the bias-corrected and accelerated (BC_a) method are superior, it is stressed that the gain in terms of bias and precision comes at the expense of much increased computational complexity. In situations in which establishing accurate confidence limits is critical, a risk assessor may want to either increase the number of simulations in relation to the percentile method or use the BC_a method. Finally, the relevance of two specific patterns – the shifting of the pair cloud resulting from the combination of log-logistic model and beta-binomial resampling applied to the *Campylobacter jejuni* data (Figure 2.19), and the streaked

pattern of Figure 2.23 – is an open question. These instances may require further elucidation.

Figure 2.25 through Figure 2.28 show that sampling error greatly increases outside the range of experimental data. This observation suggests that a simple way to limit the inclusion of sampling error into a risk assessment would be to avoid extrapolation by means of the dose-response model. While the log-logistic model consistently produces more conservative predictions than the beta-Poisson model, the difference between the models becomes relative when the confidence limits of such predictions are considered. The common practice among microbial risk assessors to contrast dose-response models purely based on their predictions is highly questionable. However informative confidence bands of the dose-response curves may be, they give no insight into the behavior of the uncertainty between the extremes. To this purpose, empirical distributions of the response were developed (Figure 2.29 through Figure 2.36). These graphs make evident that the distributional shape of the response uncertainty varies depending on the considered doses. Unfortunately, a generally applicable parametric model, which could be used to simulate response uncertainty in a risk assessment, cannot be advanced. In particular, the beta model – a parametric model that would be both plausible and flexible – fails to fit the responses in most of the cases (Table 2.6 to Table 2.9). Other parametric models, such as the uniform model, the triangular model or a model unbounded at either 0 or 1 (e.g. some form of the exponential model), do not appear to fit the data better or more consistently.

Figure 2.37 and Figure 2.38 sum up the key quantitative findings of this study. Mere consideration of one model's prediction can be misleading when dose-response models are contrasted. In the example of the *Campylobacter jejuni* data (Figure 2.37, upper left-hand graph), the prediction of the beta-Poisson model at dose 10^{-2} is almost a factor of three smaller than that of the log-logistic model. However, the sampling error magnitude linked to the estimate of the beta-Poisson model is twice than that of the log-logistic model. Furthermore, the implications of the choice of a given dose-response model or bootstrap resampling scheme heavily depend on the considered dose. While the

uncertainty due to model choice dominates at doses smaller than 10, the resampling scheme becomes somewhat more critical at the highest doses ($>10^6$). Within the range of the experimental data, graphical results are virtually equivalent regardless of the dose-response model and/or the resampling scheme. This means that the relevance of the choices made by a risk assessor essentially depends upon the considered dose. In practice, the aspect that most critically determines the dose range in a risk assessment is how the different assessment steps are assembled (i.e. how exposure assessment feeds into dose-response assessment, see Appendix 2.7.2).

The quantitative part of this study is not immune to limitations. A major criticism could be that, for the most part, conclusions are drawn based on graphical illustrations rather than statistical hypothesis testing. In particular, goodness-of-fit methods, such as the chi-squared test, the Kolmogorov-Smirnov test, and the Anderson-Darling test, could have been employed to test the differences between empirical distributions. Since exploration of the underlying uncertainty processes involved in microbial dose-response modeling was the purpose of the simulation, those tests would not have provided the insight that the graphical representation do, and they are thus to be seen as a complement rather than an alternative to the analyses carried out here. A somewhat related limitation is that the relevance of the findings within the context of a complete microbial risk assessment remains to be proven. Given the pivotal role of dose-response models, the expectation is that they will matter. However, sensitivity analysis pertaining to the whole risk assessment can formally test such relevance. A third limitation is that the bootstrap method can produce resampled data sets for which ML estimation fails to converge. While a few iterations out of 1000 showed this behavior for the log-logistic model, a dozen of iterations failed to converge in the case of the beta-Poisson model. The relative parameter pairs are inherently biased. Nonetheless, one cannot simply exclude them because each resampled data set is perfectly legitimate. There is no clear solution to this quandary. It was decided to leave in those few anomalous parameter pairs among our results based on the belief that the relatively large number of iterations provides guarantees of robustness. Comparison of the results with and without those parameter

pairs could formally test this belief. This third limitation also suggests that the application of the bootstrap method to further data sets needs to be verified. The dose-response trend contained in the *Shigella dysenteriae* data is as good as a data set from a human feeding trial gets. While the trend for infection in the *Campylobacter jejuni* data is inferior, it is still better than in many other human feeding studies. It is possible that, when there is either an inconsequential dose-response trend or numerous dose groups with zero response, the rate of computational problems would dramatically increase, and thus put into question the reliability of the approach.

The lack of a pre-existing, similar research body preempts us from putting the results into the perspective of other researchers' conclusions. Indeed, the bootstrap method has previously been applied to microbial dose-response modeling (Fazil, 1996; Teunis et al., 1996; Medema et al., 1996). The purpose of such applications has been descriptive, i.e. to show that the spread of bootstrapped parameter pairs corresponded to the joint confidence regions. Limitations of the bootstrap method itself or the relevance of the represented uncertainty are not discussed in those studies. Teunis and Havelaar (2000) have suggested that bootstrapping applied to microbial dose-response assessment is computationally demanding and that bootstrapped data can be considerably dichotomized. They concluded that Markov Chain Monte Carlo (MCMC) simulation is a better alternative to bootstrapping (Teunis & Havelaar, 2000). In our experience, while the bootstrap method required some up-front programming, computation was astonishingly quick. The results relative to the *Shigella dysenteriae* data, which only have four data points, seem to refute the conclusion regarding a potential dichotomization of the bootstrapped data. Both methods eventually result in a series of parameter pairs that preserves the inherent correlation of the parameters, and can thus be equally useful to propagate dose-response uncertainty into a whole risk assessment. In our limited exposure to the MCMC method (results not shown), we have experienced that the coherent specification of prior distributions for the parameters as well as reliable convergence diagnostics requires an in-depth understanding of the Bayesian framework. Since most risk assessors still are frequentists, that means learning and accepting a new

paradigm of statistical data analysis. It is concluded that, while each one imposes its peculiar challenges to the risk assessor, both the bootstrapping and MCMC methods should lead to comparable results.

In conclusion, this study reviewed the theoretical background regarding the concept of uncertainty and variability in risk assessment, specifically dose-response assessment. This background was used as a guiding framework to evaluating the sources of uncertainty potentially involved in microbial dose-response assessment. In particular, the bootstrap method – partially using novel resampling schemes – was applied to characterize sampling error. While microbial risk assessors have by and large been obsessed with the specification of the dose-response model, the findings of this study show that uncertainty related to parameter estimation and sampling error plays, at the very least, an equally important role. Other non-quantifiable sources of uncertainty, such as biases in human feeding studies, likely exist.

The variety of uncertainty sources that potentially affect microbial dose-response modeling is nearly overwhelming. At first sight, one has to wonder whether the exercise stands any chance of being scientifically sound. The resolution of such a dilemma is, however, a professional judgment that a risk manager, not the risk assessor, has to make. The risk assessor has to resist her/his innate tendency – a clear vestige of the scientific background – of believing that uncertainty will somehow undermine her/his work. As uncertainty is an intrinsic component of risk, it is only by thinking of and dealing with uncertainty that a risk assessor guarantees her/his share of scientific soundness. Sometimes, this may mean finding a way around uncertainty. After assessing the dose-response of *Campylobacter jejuni*, Medema et al. (1996) reached the general conclusion that, "to reduce the uncertainty in the dose-response estimate, especially at low dose, additional dose-response data at low doses are needed." This statement sums up the prevailing wisdom on dose-response modeling among microbial risk assessors. The notion that more and better data from human feeding studies will reduce uncertainty may well be true. However, it is a fact that such data will not be available anytime soon (if ever). Furthermore, other sources of uncertainty would subsist. Microbial risk assessors

ought to move beyond the current paradigm of low dose extrapolation, and look for novel ways to exploit dose-response models at those doses where the least uncertainty is apparent. Hopefully, this study will have shown the way.

2.6 References

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2.7 Appendices

2.7.1 SAS/STAT Code

Relevant excerpts of the STAT/SAS code are illustrated in the example of the *Shigella dysenteriae* data, the beta-Poisson model, and beta-binomial resampling.

The first step of the analysis contemplated the maximum likelihood estimation of model parameters. The corresponding code was (r = number of ill, n = number of exposed, a = parameter α, b = parameter β):

```
proc nlin data=obsdata;
     parms a=.3 b=20;
     p=1-(1+dose/b)**-a;
     model r=n*p;
     _weight_ = 1/(n*p*(1-p));
     _loss_   = deviance('bino',r,n*p,n) / _weight_;
quit;
```

The special variables _WEIGHT_ and _LOSS_ code the deviance function (i.e. the parameter fitting algorithm). Fitting of the other models involved modifying the *p* statement and providing adequate initial parameters estimates (*parms* statement).

The beta-binomial resampling of the experimental data sets was carried out as follows (the variable p_0 corresponds to the beta-binomial resampling probability p_i* illustrated in Figure 2.2):

```
data resample;
     set obsdata;
     do i=1 to 1000;
          call ranuni(892475,u);
          p0=betainv(u,r+1,n-r+1);
          call ranbin(653826,n,p0,y);
          output;
     end;
run;
```

The DO loop iteratively generates 1,000 data sets each identified by the *i* variable and contained in a single file (called RESAMPLE). Other resampling schemes were obtained by modifying the random-number functions and CALL routines contained between DO and OUTPUT statements.

The fitting of the dose-response models to each one of the 1,000 data sets used the PROC NLIN routine described above:

```
proc nlin data=resample noprint
     outest=bootparms(where=(_TYPE_="FINAL" or
       (_TYPE_="ITER" and _STATUS_="3 Error")));
     by i;
     parms a .3 b 20;
     p=1-(1+dose/b)**-a;
     model y=n*p;
     _weight_ = 1/(n*p*(1-p));
     _loss_   = deviance('bino',y,n*p,n) / _weight_;
quit;
```

By specifying OUTEST and BY statements, the process is computationally efficient, and produces a data file (BOOTPARMS) containing 1,000 triplets of two parameter values and the corresponding deviance.

2.7.2 Fallacy of Low-Dose Extrapolation in Microbial Risk Assessment

In microbial risk assessment, a perceived need for low-dose extrapolation has justified the predilection for biologically based dose-response models, such as the exponential model and the beta-Poisson model. However, the requirement for low-dose extrapolation is essentially a direct reflection of how paradigms of toxicological risk assessment have uncritically been translated to the microbial risk assessment context.

Assessment of the chronic effects of carcinogens is the archetype of human health risk assessment. Within this context, the exposure assessment determines a Lifetime Average Daily Dose (LADD, also called Chronic Daily Dose). The LADD is essentially the daily concentration (unit: mg * day * kg body weight) of a carcinogen that is obtained by averaging the total lifetime exposure. The assumption is that exposure is both relatively continuous and constant. Experiments in which animals are exposed to high doses of the carcinogen for an extended period of time generate the data for estimating a dose-response relation, from which a Cancer Potency Factor (PF, unit: 1 / mg * day * kg body weight) is calculated. An Excess Lifetime Cancer Risk, the final outcome of the risk assessment, is calculated by multiplying the LADD and the PF.

Microbial risk assessment is carried out in much the same way. The main difference is that the timeframe of interest, which is often merely implied, commonly

corresponds to one year. The exposure assessment estimates an average dose per exposure (unit: number of pathogen / exposure). Data from trials in which human volunteers were fed once with a specific dose of the pathogen of interest are used to estimate a dose-response function. The final risk of contracting a foodborne infection/illness is finally calculated by inserting the exposure dose into the dose-response model. This process is illustrated with an example reported by Haas et al. (1999, p. 322). In a given drinking water scenario, the exposure to rotavirus is 10^{-3} viruses per exposure. Parameter estimates for a beta-Poisson dose-response model are estimated from experimental data of a human feeding trial with rotavirus. When the exposure dose is plugged into the dose-response model, the risk from a single exposure results in 6×10^{-4}. If 10,000 people were exposed, one would conclude that $6 \times 10^{-4} *$ 10,000 = 6 infections resulted.

The conclusion of this example may surprise some. One has to wonder how it is possible that exposure to a thousandth of a virus – a biologically implausible entity – can eventually result in 6 infections. The fact is that the exposure dose actually represents an averaged dose over all times a person drank water (i.e. exposure to the medium rather than the agent). That is, assuming that a person drank water 3,000 times in a year, the water may have been sterile 2,999 times, and contaminated with three viruses once. This is a very different exposure scenario than the one relative to carcinogen risk assessment. While a relatively constant exposure is assumed in carcinogen risk assessment, only the intermittent presence of a pathogen in a medium can explain a dose that is lower than unity. The exposure to microbial pathogens is acute rather than chronic.

Where the translation of the carcinogen risk assessment into the microbial risk assessment breaks down is in the use of the dose-response function. In both cases, the average exposure dose is used to let the dose-response model predict a response. But, while the dose-response model in the carcinogen risk assessment is based on the chronic exposure of animals, the microbial dose-response model arises from one time exposure of human volunteers. In other words, the abscissa of the microbial dose-response represents

one exposure's dose not an averaged dose over several exposure. This fact has so far been ignored in microbial risk assessment.

Ultimately, this highlights the fallacy of the current approach in microbial risk assessment. The solution is nevertheless simple, and merely requires a rethinking of the outputs of the single risk assessment steps. Exposure assessment should establish consumption pattern (number of contacts with the medium), the contamination pattern (number of exposures to the pathogen given contact), and the degree of exposure (dose given exposure). This last factor would be fed into the microbial dose-response model, so that an estimate of probability of infection/illness only when exposure actually occurs would be calculated (response). Finding an averaged risk estimate for the timeframe of interest (response x number of exposures / number of medium contacts) should eventually be the goal of risk characterization.

Table 2.1. Data from *Campylobacter jejuni* human feeding trial (Black et al., 1988)

Dose (cfu)	Total number of exposed subjects	Number of subjects infected
800	10	5
8,000	10	6
90,000	13	11
800,000	11	8
1,000,000	19	15
100,000,000	5	5

Legend: cfu, colony-forming unit.

Table 2.2. Data from *Shigella dysenteriae* human feeding trial (Levine et al., 1973)

Dose (no. cells)	Total number of exposed subjects	Number of subjects ill
10	10	1
200	4	2
2,000	10	7
10,000	6	5

Table 2.3. Parameter estimates and goodness-of-fit statistic based on maximum likeloohod estimation for Campylobacter jejuni and Shigella dysenteriae data

Model	Parameter/Statistic	Estimates	
		Campylobacter jejuni	*Shigella dysenteriae*
Beta-Poisson	Alpha	0.145	0.277
	Beta	7.590	21.159
	DF	4	2
	Deviance	2.42	0.03
	Pr(deviance)	0.66	0.98
Exponential	Rho	3.52E-06	4.52E-04
	DF	5	3
	Deviance	107.96	13.21
	Pr(deviance)	<.001	0.004
Log-logistic	Beta0	-1.385	-3.248
	Beta1	0.484	1.249
	DF	4	2
	Deviance	2.37	0.19
	Pr(deviance)	0.67	0.91

Legend: DF, degree of freedom; Pr(deviance), significance level of observed deviance.

Table 2.4. Lower and upper confidence limits of the parameter estimates for the *Campylobacter jejuni* data according to different analytical and numerical methods (Legend: BCa, bias-corrected accelerated)

Model	Beta-Poisson		Exponential	Log-logistic	
Parameter	Alpha	Beta	Rho	Beta0	Beta1
Maximum likelihood estimate	0.145	7.590	3.52E-06	-1.385	0.484
- Lower confidence limit (2.5th percentile)					
Wald's analytical solution	-0.030	-69.440	1.12E-06	-3.502	0.058
Percentile method after binomial resampling	0.036	0.000	3.78E-03	-3.673	0.098
BCa method after binomial resampling	0.036	0.000	1.70E-06	-3.785	0.086
Percentile method after beta-binomial resampling	0.006	0.000	1.04E-03	-4.011	-0.194
BCa method after beta-binomial resampling	0.042	0.000	8.85E-08	-5.111	0.035
Percentile method after parametric resampling	0.114	1.169	n/a	-1.843	0.414
BCa method after parametric resampling	0.050	0.002	n/a	-1.738	0.404
- Upper confidence limit (97.5th percentile)					
Wald's analytical solution	0.320	84.619	5.93E-06	0.732	0.911
Percentile method after binomial resampling	0.301	484.465	9.75E-02	0.674	0.992
BCa method after binomial resampling	0.302	461.538	4.79E-05	0.589	0.961
Percentile method after beta-binomial resampling	0.295	612.229	9.75E-02	2.153	0.988
BCa method after beta-binomial resampling	0.444	1901.692	4.25E-04	0.925	1.281
Percentile method after parametric resampling	0.193	25.645	n/a	-1.143	0.625
BCa method after parametric resampling	0.182	20.252	n/a	-1.100	0.579

Table 2.5. Lower and upper confidence limits of the parameter estimates for the *Shigella dysenteriae* data according to different analytical and numerical methods (Legend: BCa, bias-corrected accelerated)

Model	Beta-Poisson		Exponential	Log-logistic	
Parameter	Alpha	Beta	Rho	Beta0	Beta1
Maximum likelihood estimate	0.277	21.159	4.52E-04	-3.248	1.249
- Lower confidence limit (2.5th percentile)					
Wald's analytical solution	-0.330	-121.967	-8.04E-05	-8.716	-0.634
Percentile method after binomial resampling	0.102	0.680	2.59E-02	-8.635	0.561
BCa method after binomial resampling	0.029	0.238	1.87E-04	-9.327	0.561
Percentile method after beta-binomial resampling	0.021	0.000	2.06E-02	-9.060	0.117
BCa method after beta-binomial resampling	0.053	0.075	1.26E-04	-121.131	0.504
Percentile method after parametric resampling	0.176	5.465	n/a	-7.547	0.910
BCa method after parametric resampling	0.045	2.689	n/a	-5.945	0.887
- Upper confidence limit (97.5th percentile)					
Wald's analytical solution	0.884	164.285	9.85E-04	2.221	3.133
Percentile method after binomial resampling	10.651	15162.423	1.79E-01	-1.380	2.952
BCa method after binomial resampling	0.988	6044.222	1.62E-03	-1.538	2.966
Percentile method after beta-binomial resampling	13.707	11227.630	1.98E-01	-0.417	2.950
BCa method after beta-binomial resampling	8.72E+5	5.80E+11	2.41E-02	-1.228	36.818
Percentile method after parametric resampling	2.944	901.966	n/a	-2.321	2.903
BCa method after parametric resampling	0.574	136.100	n/a	-2.183	2.209

Table 2.6. Beta-Poisson model and *Campylobacter jejuni* data: Goodness-of-fit statistics for the beta distribution fitted to the bootstrap predictions at four given doses

Goodness-of-fit test		Kolmogorov-Smirnov	Cramer-von Mises	Anderson-Darling
- Binomial resampling				
Dose 1E+0	Statistics	0.15	7.06	36.90
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.02	0.08	0.57
	P-value	>0.25	>0.25	>0.25
Dose 1E+6	Statistics	0.03	0.22	1.26
	P-value	0.19	0.24	0.25
Dose 1E+9	Statistics	0.02	0.05	0.30
	P-value	>0.25	>0.25	>0.25
- Beta-binomial resampling				
Dose 1E+0	Statistics	0.12	4.09	24.86
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.04	0.20	1.72
	P-value	0.08	>0.25	0.13
Dose 1E+6	Statistics	0.02	0.16	1.23
	P-value	>0.25	>0.25	>0.25
Dose 1E+9	Statistics	0.02	0.06	0.62
	P-value	>0.25	>0.25	>0.25
- Parametric resampling				
Dose 1E+0	Statistics	0.28	25.28	134.95
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.08	2.20	14.97
	P-value	<0.001	<0.001	<0.001
Dose 1E+6	Statistics	0.14	6.85	42.98
	P-value	<0.001	<0.001	<0.001
Dose 1E+9	Statistics	0.17	9.90	59.61
	P-value	<0.001	<0.001	<0.001

Table 2.7. Log-logistic model and *Campylobacter jejuni* data: Goodness-of-fit statistics for the beta distribution fitted to the bootstrap predictions at four given doses

Goodness-of-fit test		Kolmogorov-Smirnov	Cramer-von Mises	Anderson-Darling
- Binomial resampling				
Dose 1E+0	Statistics	0.04	0.44	2.75
	P-value	0.06	0.06	0.04
Dose 1E+3	Statistics	0.98	331.93	9699.81
	P-value	<0.001	<0.001	<0.001
Dose 1E+6	Statistics	0.04	0.32	1.87
	P-value	0.12	0.12	0.11
Dose 1E+9	Statistics	0.97	331.80	n/a
	P-value	<0.001	<0.001	n/a
- Beta-binomial resampling				
Dose 1E+0	Statistics	0.47	90.36	1141.69
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.45	82.05	375.63
	P-value	<0.001	<0.001	<0.001
Dose 1E+6	Statistics	0.48	103.88	492.35
	P-value	<0.001	<0.001	<0.001
Dose 1E+9	Statistics	0.51	111.20	1499.09
	P-value	<0.001	<0.001	<0.001
- Parametric resampling				
Dose 1E+0	Statistics	1.00	333.33	190292.69
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.05	0.90	6.13
	P-value	0.02	<0.01	<0.001
Dose 1E+6	Statistics	1.00	333.33	n/a
	P-value	<0.001	<0.001	n/a
Dose 1E+9	Statistics	0.05	0.62	4.23
	P-value	0.03	0.02	0.01

Table 2.8. Beta-Poisson model and *Shigella dysenteriae* data: Goodness-of-fit statistics for the beta distribution fitted to the bootstrap predictions at four given doses

Goodness-of-fit test		Kolmogorov-Smirnov	Cramer-von Mises	Anderson-Darling
- Binomial resampling				
Dose 1E+0	Statistics	0.10	2.73	15.86
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.05	0.32	2.26
	P-value	0.01	0.12	0.07
Dose 1E+6	Statistics	0.14	6.65	37.95
	P-value	<0.001	<0.001	<0.001
Dose 1E+9	Statistics	0.08	1.18	7.46
	P-value	<0.001	<0.001	<0.001
- Beta-binomial resampling				
Dose 1E+0	Statistics	0.13	5.69	29.01
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.05	0.78	4.91
	P-value	0.01	0.01	<0.01
Dose 1E+6	Statistics	0.11	3.75	23.48
	P-value	<0.001	<0.001	<0.001
Dose 1E+9	Statistics	0.06	0.54	4.37
	P-value	<0.01	0.03	0.01
- Parametric resampling				
Dose 1E+0	Statistics	0.14	7.23	n/a
	P-value	<0.001	<0.001	n/a
Dose 1E+3	Statistics	0.21	16.16	87.10
	P-value	<0.001	<0.001	<0.001
Dose 1E+6	Statistics	0.22	16.81	86.73
	P-value	<0.001	<0.001	<0.001
Dose 1E+9	Statistics	0.13	6.22	35.08
	P-value	<0.001	<0.001	<0.001

Table 2.9. Log-logistic model and *Shigella dysenteriae* data: Goodness-of-fit statistics for the beta distribution fitted to the bootstrap predictions at four given doses

Goodness-of-fit test		Kolmogorov-Smirnov	Cramer-von Mises	Anderson-Darling
- Binomial resampling				
Dose 1E+0	Statistics	0.04	0.39	2.38
	P-value	0.04	0.08	0.06
Dose 1E+3	Statistics	0.11	4.09	n/a
	P-value	<0.001	<0.001	n/a
Dose 1E+6	Statistics	0.05	0.50	3.24
	P-value	0.02	0.04	0.02
Dose 1E+9	Statistics	0.15	5.67	30.33
	P-value	<0.001	<0.001	<0.001
- Beta-binomial resampling				
Dose 1E+0	Statistics	0.06	0.60	4.94
	P-value	<0.01	0.02	<0.01
Dose 1E+3	Statistics	0.12	5.90	n/a
	P-value	<0.001	<0.001	n/a
Dose 1E+6	Statistics	0.06	1.02	6.51
	P-value	<0.01	<0.01	<0.001
Dose 1E+9	Statistics	0.14	6.12	31.18
	P-value	<0.001	<0.001	<0.001
- Parametric resampling				
Dose 1E+0	Statistics	0.14	6.40	36.73
	P-value	<0.001	<0.001	<0.001
Dose 1E+3	Statistics	0.27	26.67	n/a
	P-value	<0.001	<0.001	n/a
Dose 1E+6	Statistics	0.11	4.37	26.86
	P-value	<0.001	<0.001	<0.001
Dose 1E+9	Statistics	0.24	18.03	96.14
	P-value	<0.001	<0.001	<0.001

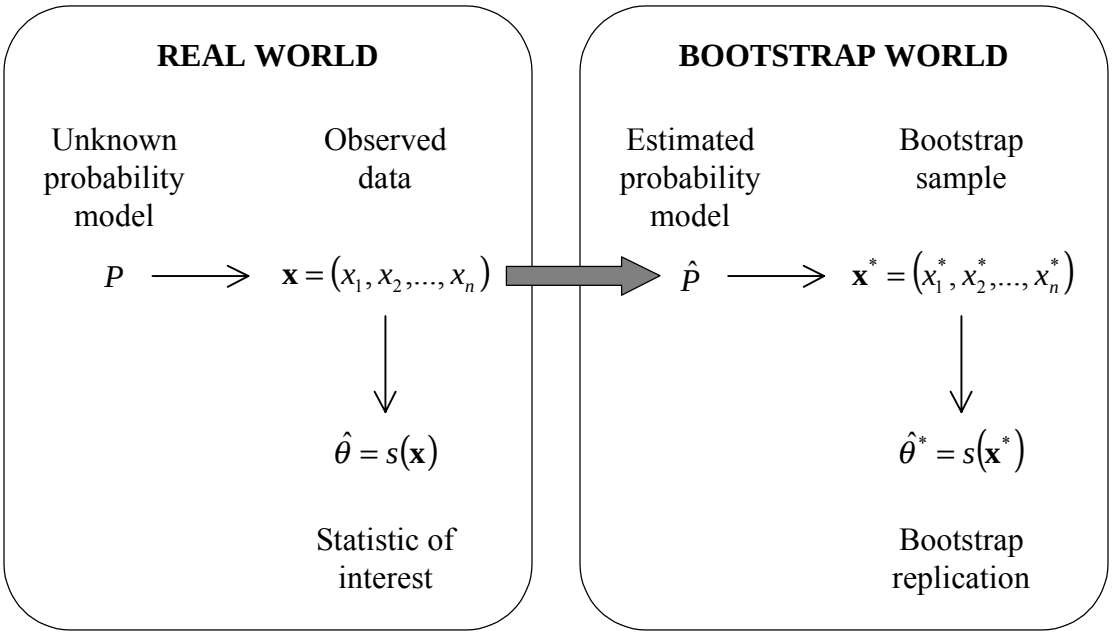


Figure 2.1. Schematic representation of the bootstrap principle (Efron and Tibshirani, 1993). The crucial step $\Rightarrow$ produces an estimate $\hat{P}$ of the entire probability mechanism P from the observed data $\mathbf{x}$.

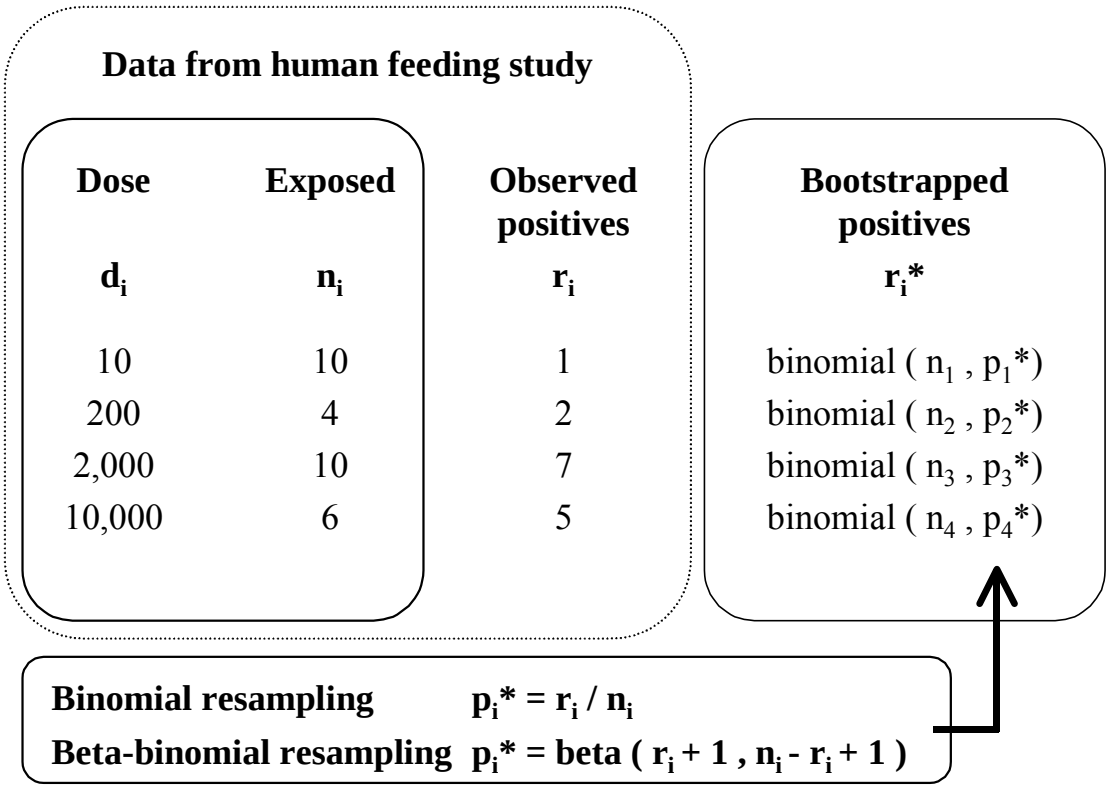
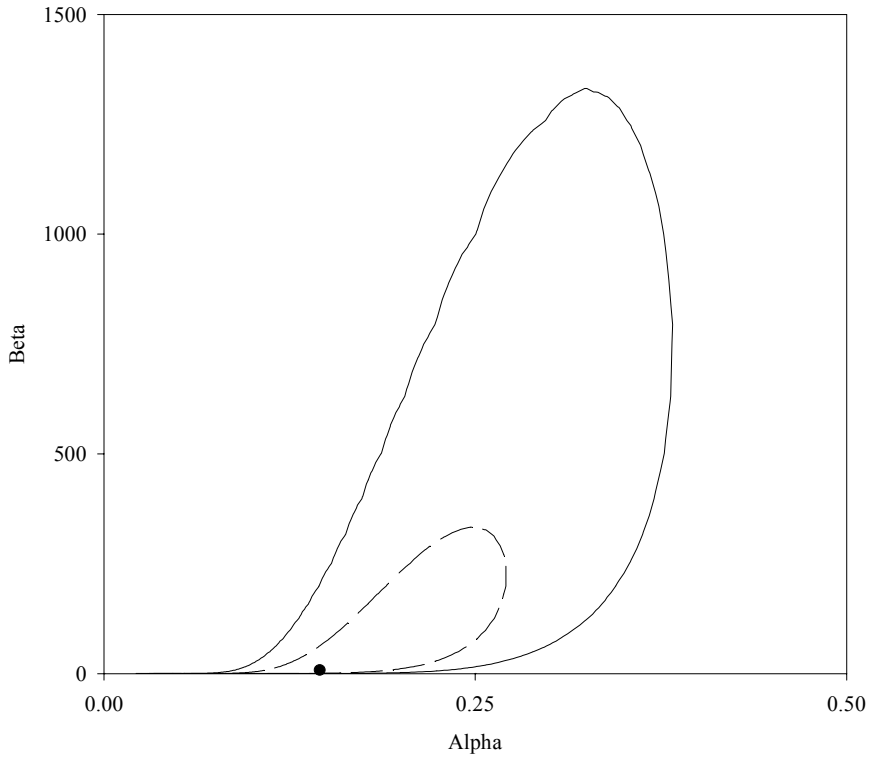
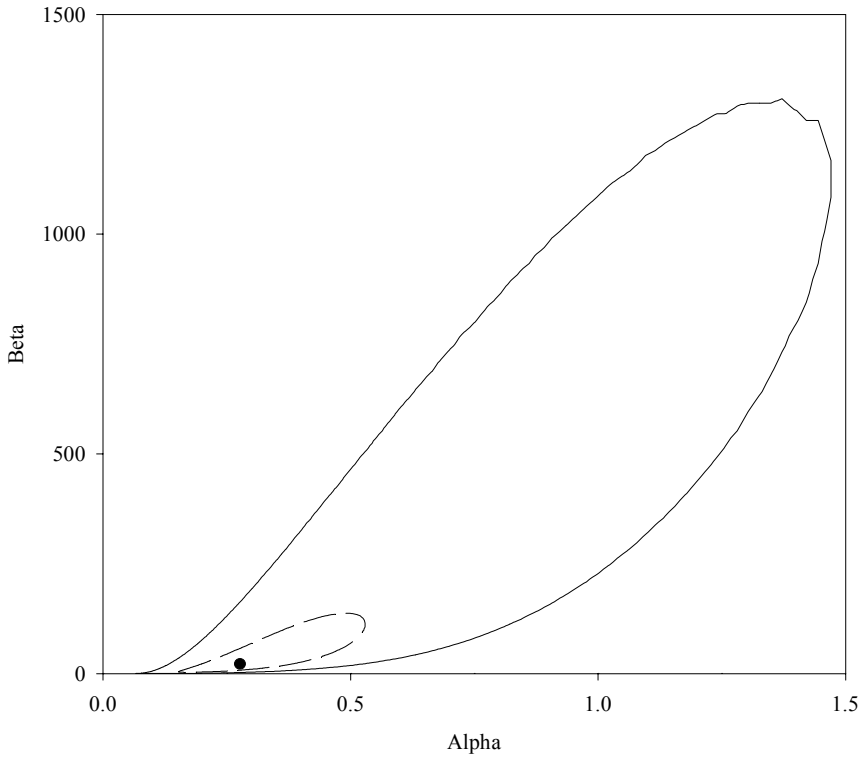


Figure 2.2. Binomial and beta-binomial resampling schemes



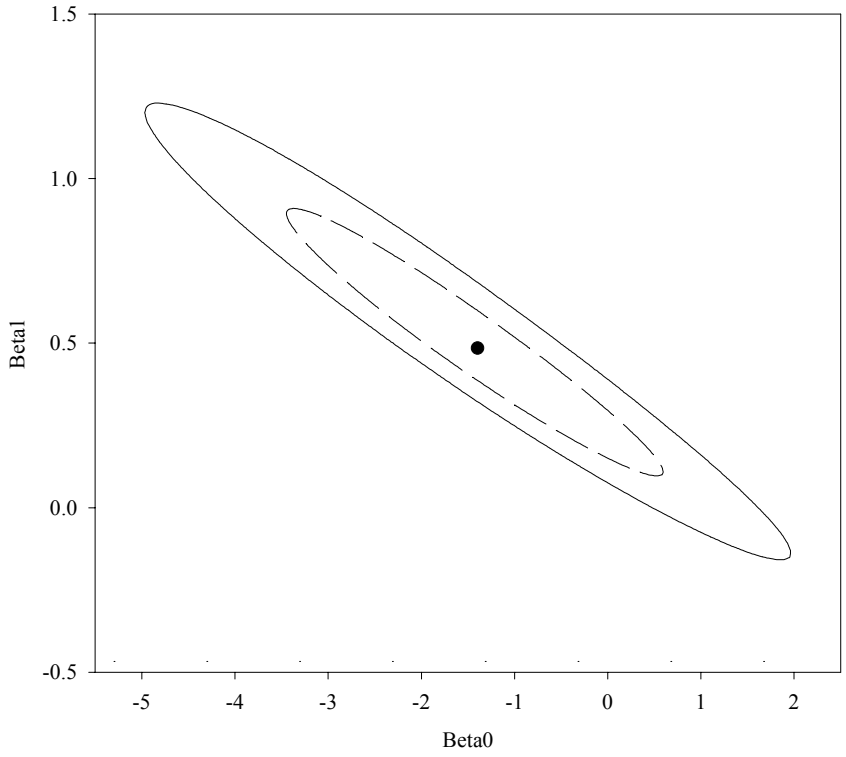
Legend: dot, maximum likelihood estimates; solid line, 95% confidence region; dashed line, 50% confidence line.

Figure 2.3. Joint confidence region of the parameters for beta-Poisson model fitted to *Campylobacter jejuni* data



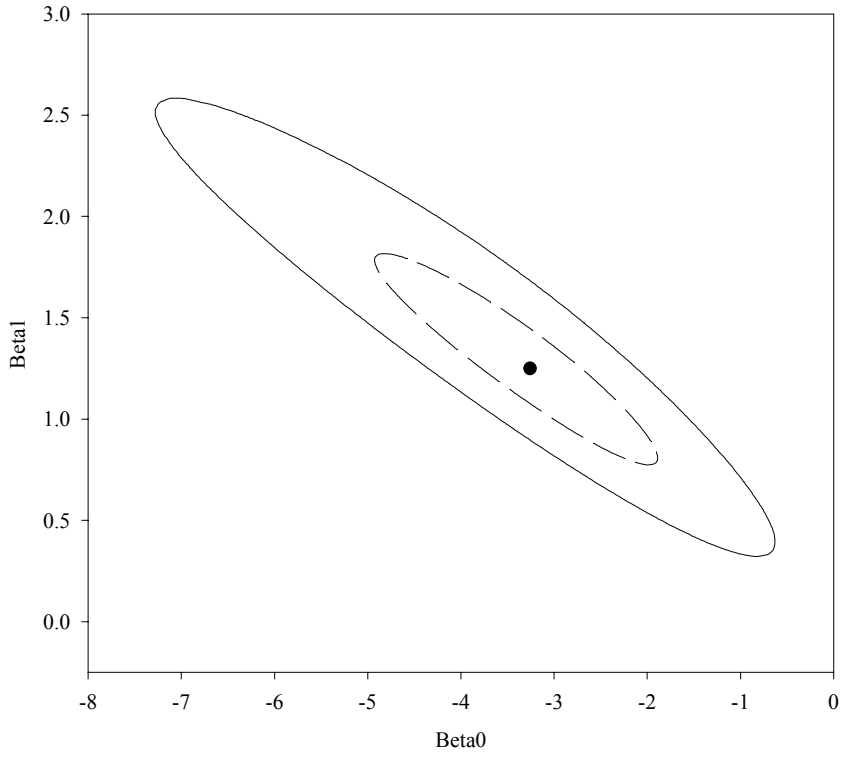
Legend: dot, maximum likelihood estimates; solid line, 95% confidence region; dashed line, 50% confidence line.

Figure 2.4. Joint confidence region of the parameters for the beta-Poisson model fitted to *Shigella dysenteriae* data



Legend: dot, maximum likelihood estimates; solid line, 95% confidence region; dashed line, 50% confidence line.

Figure 2.5. Joint confidence region of the parameters for the log-logistic model fitted to *Campylobacter jejuni* data



Legend: dot, maximum likelihood estimates; solid line, 95% confidence region; dashed line, 50% confidence line.

Figure 2.6. Joint confidence region of the parameters for the log-logistic model fitted to *Shigella dysenteriae* data

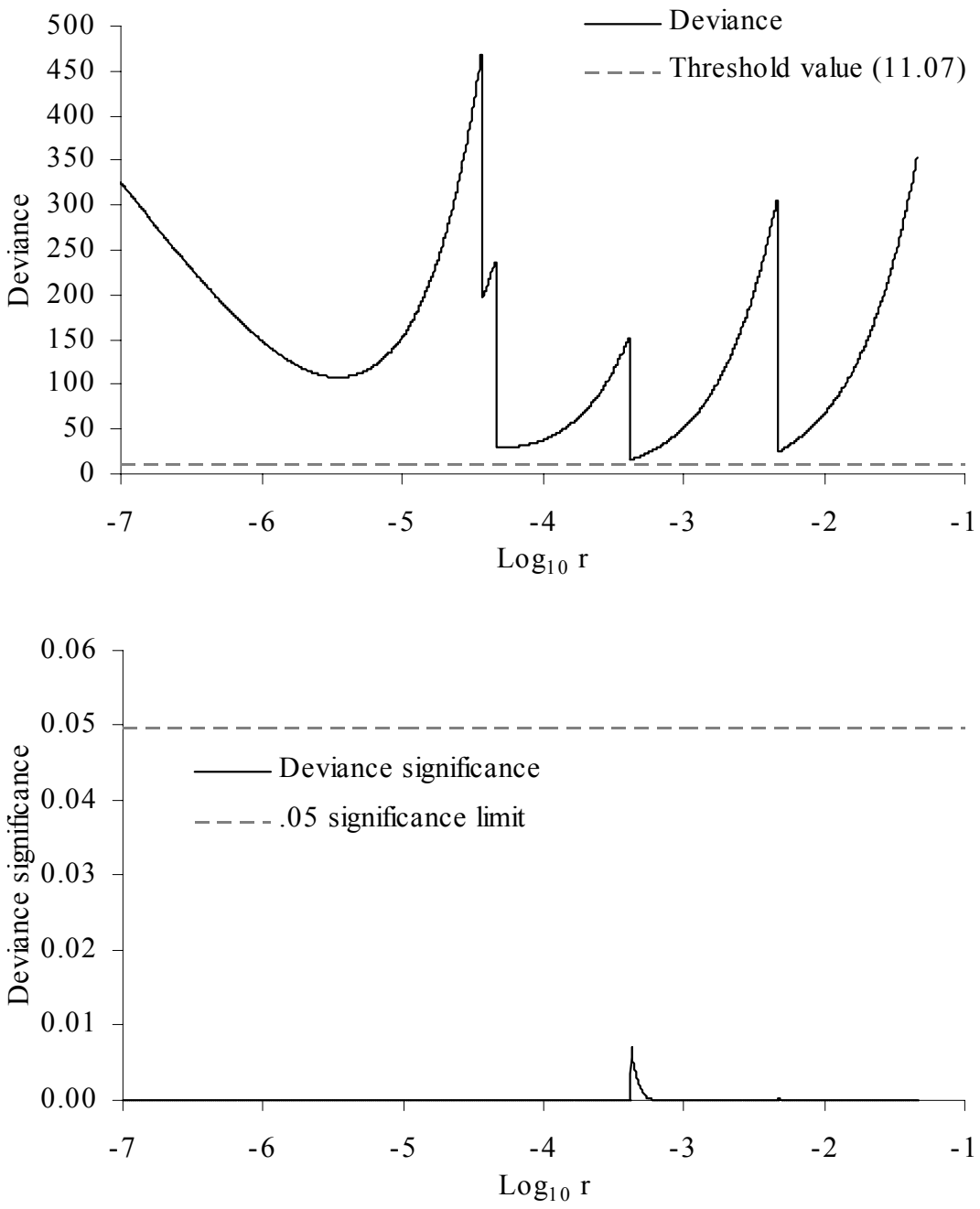


Figure 2.7. Deviance (and its significance) in function of parameter *r* value of the exponential model fitted to *Campylobacter jejuni* data

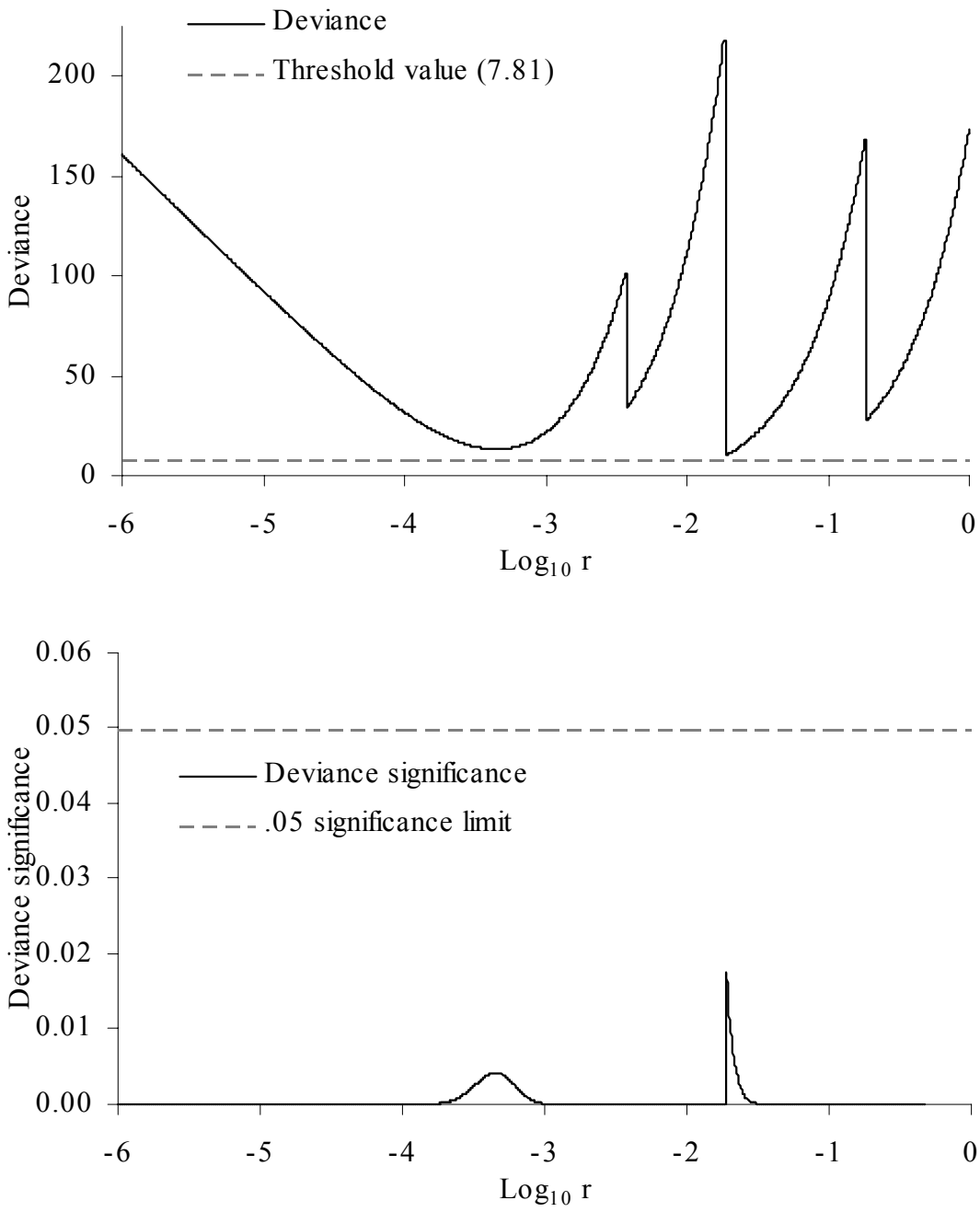


Figure 2.8. Deviance (and its significance) in function of parameter *r* value of the exponential model fitted to *Shigella dysenteriae* data

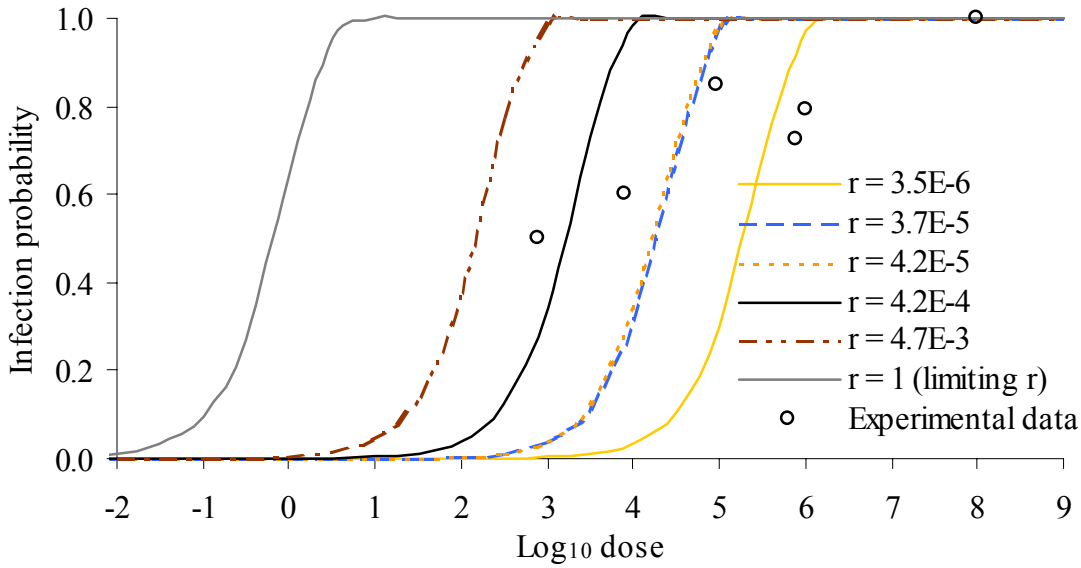


Figure 2.9. Dose-response curves based on the local deviance minima of the exponential model fitted to *Campylobacter jejuni* data

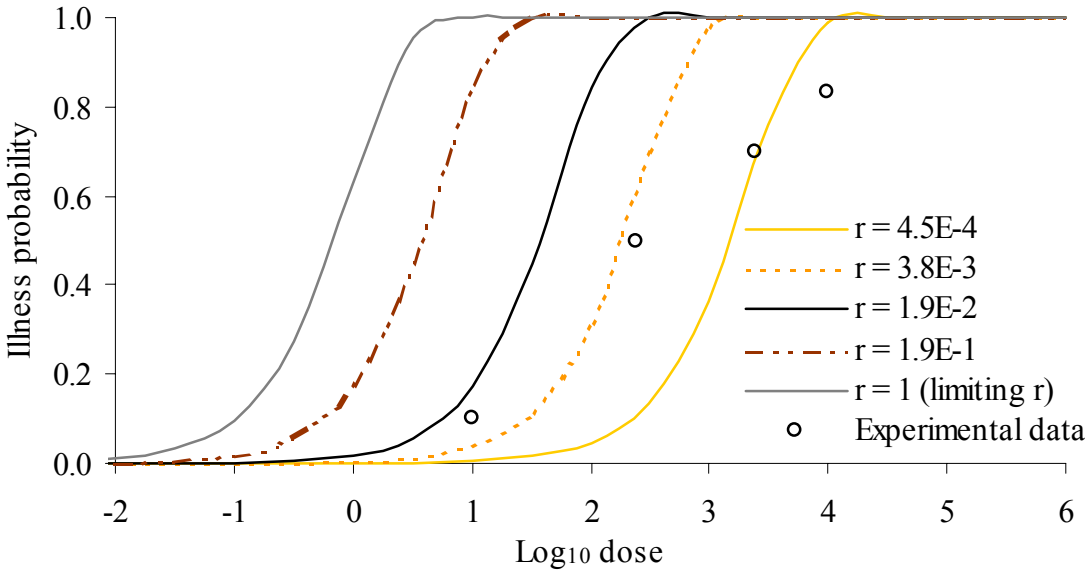


Figure 2.10. Dose-response curves based on the local deviance minima of the exponential model fitted to *Shigella dysenteriae* data

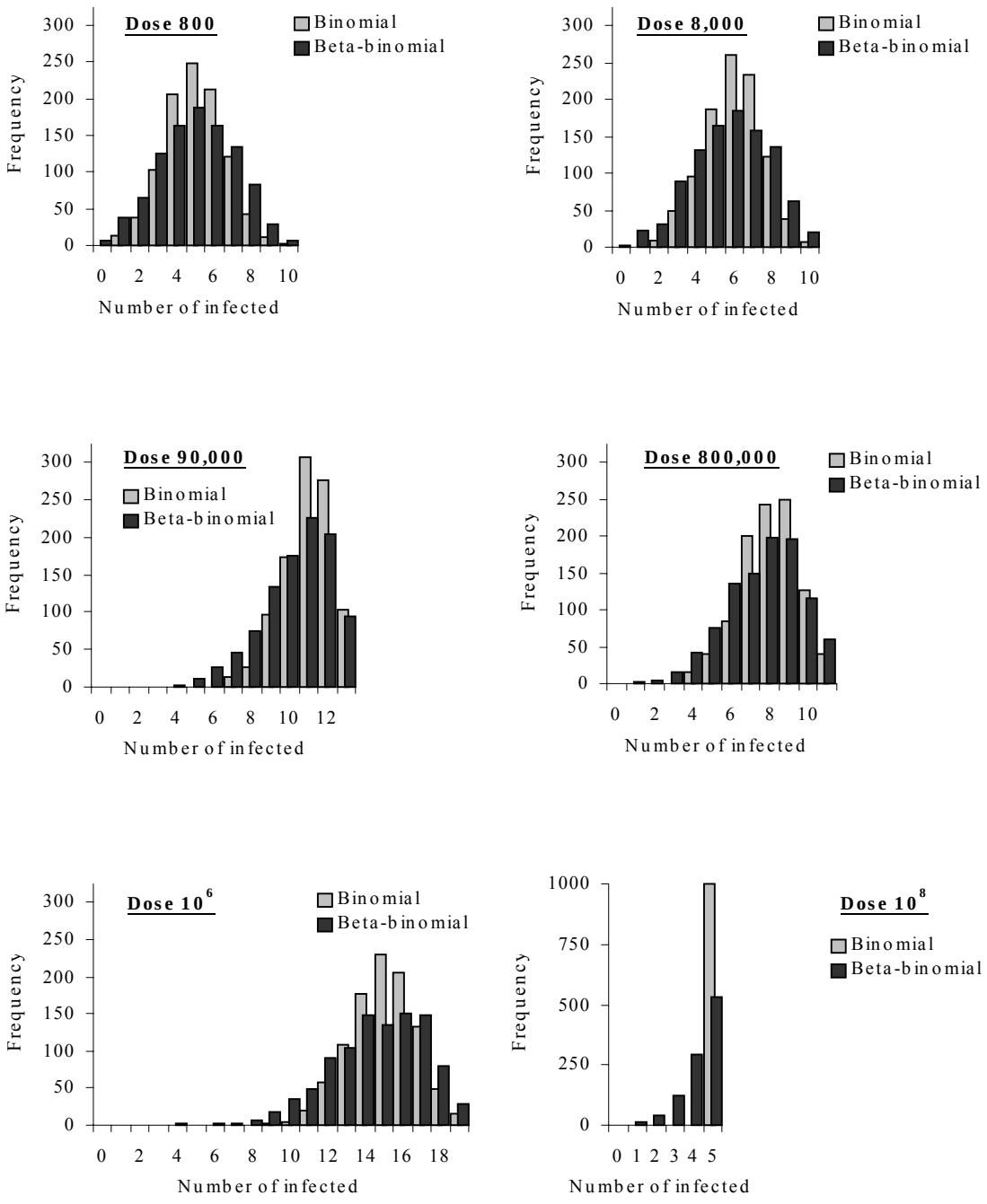


Figure 2.11. Contrast of the binomial resampling and beta-binomial resampling as applied to *Campylobacter jejuni* data (1000 resamples)

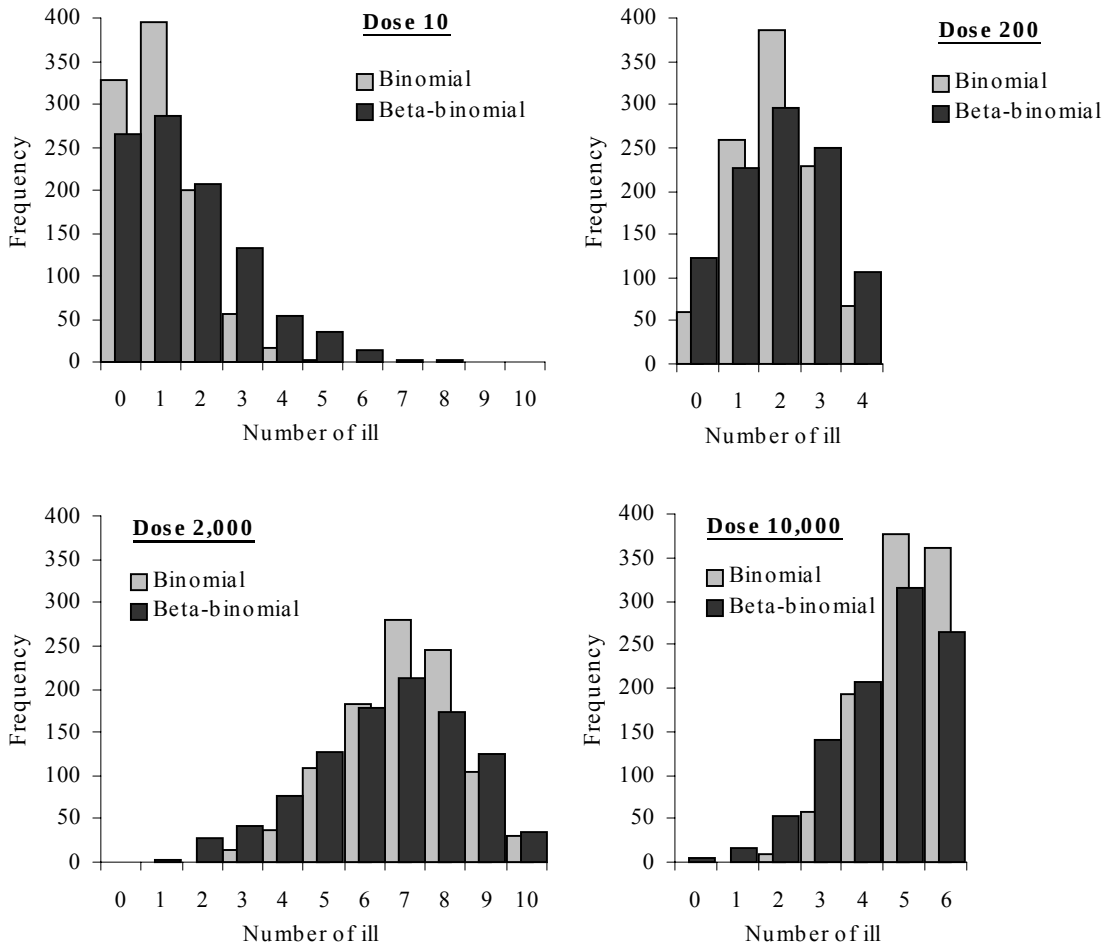


Figure 2.12. Contrast of the binomial resampling and beta-binomial resampling as applied to *Shigella dysenteriae* data (1000 resamples)

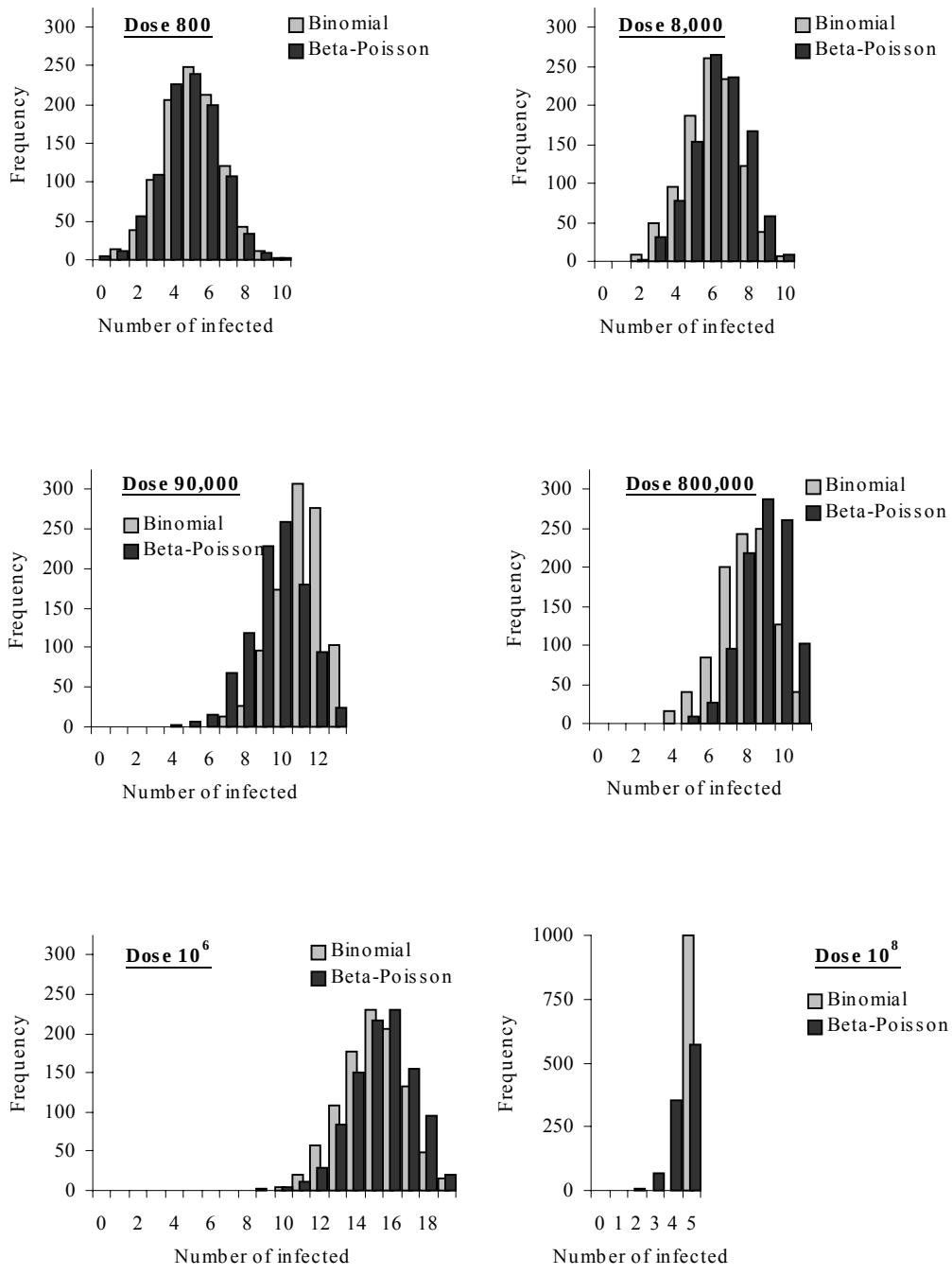


Figure 2.13. Contrast of the binomial resampling and parametric resampling based on beta-Poisson model as applied to *Campylobacter jejuni* data (1000 resamples)

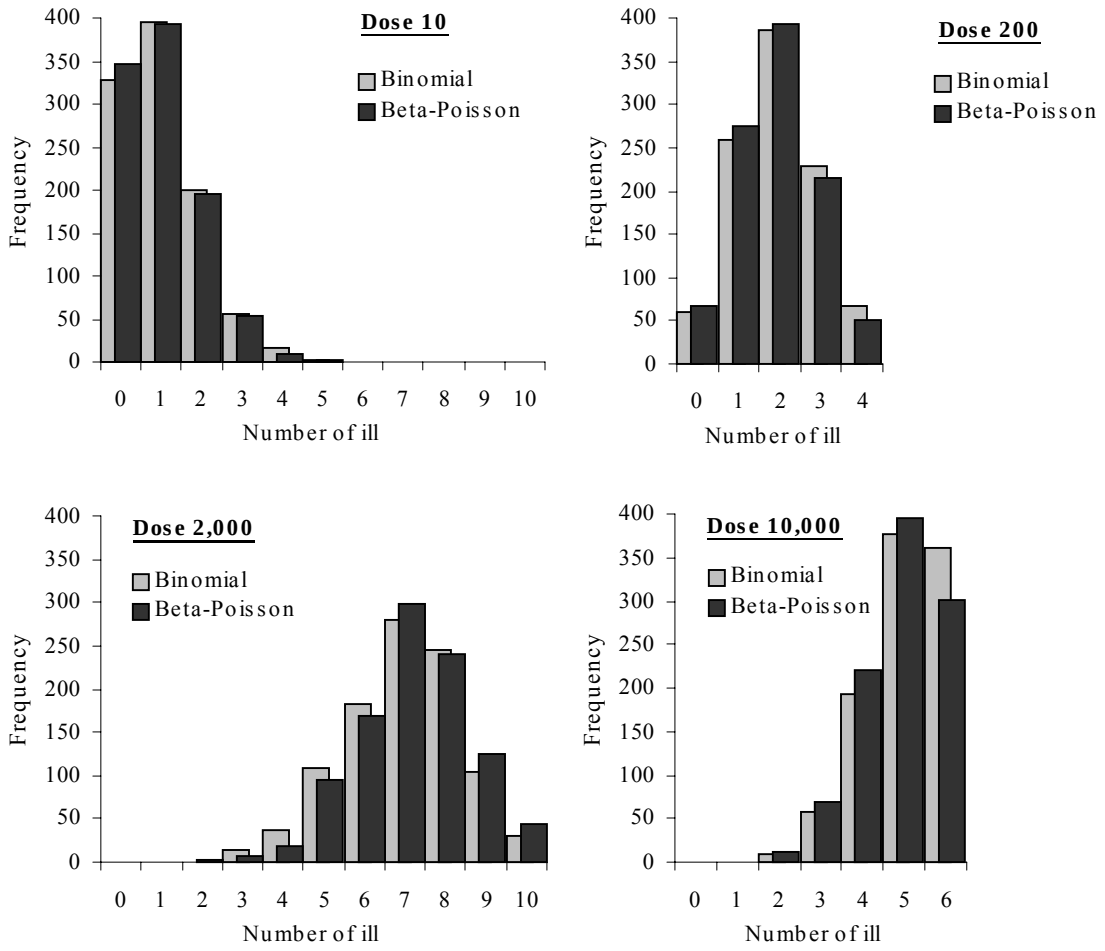


Figure 2.14. Contrast of the binomial resampling and parametric resampling based on beta-Poisson model as applied to *Shigella dysenteriae* data (1000 resamples)

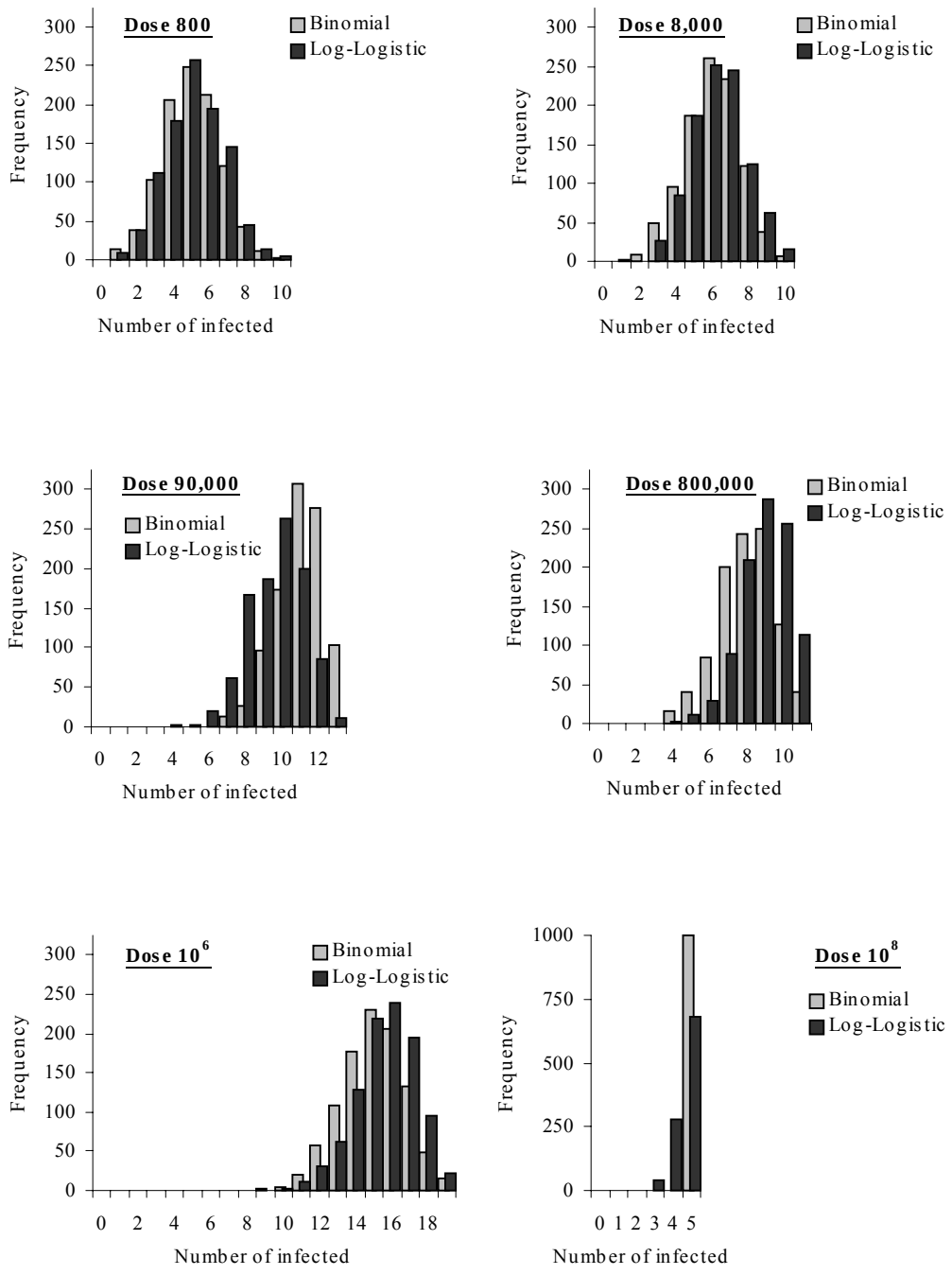


Figure 2.15. Contrast of the binomial resampling and parametric resampling based on log-logistic model as applied to *Campylobacter jejuni* data (1000 resamples)

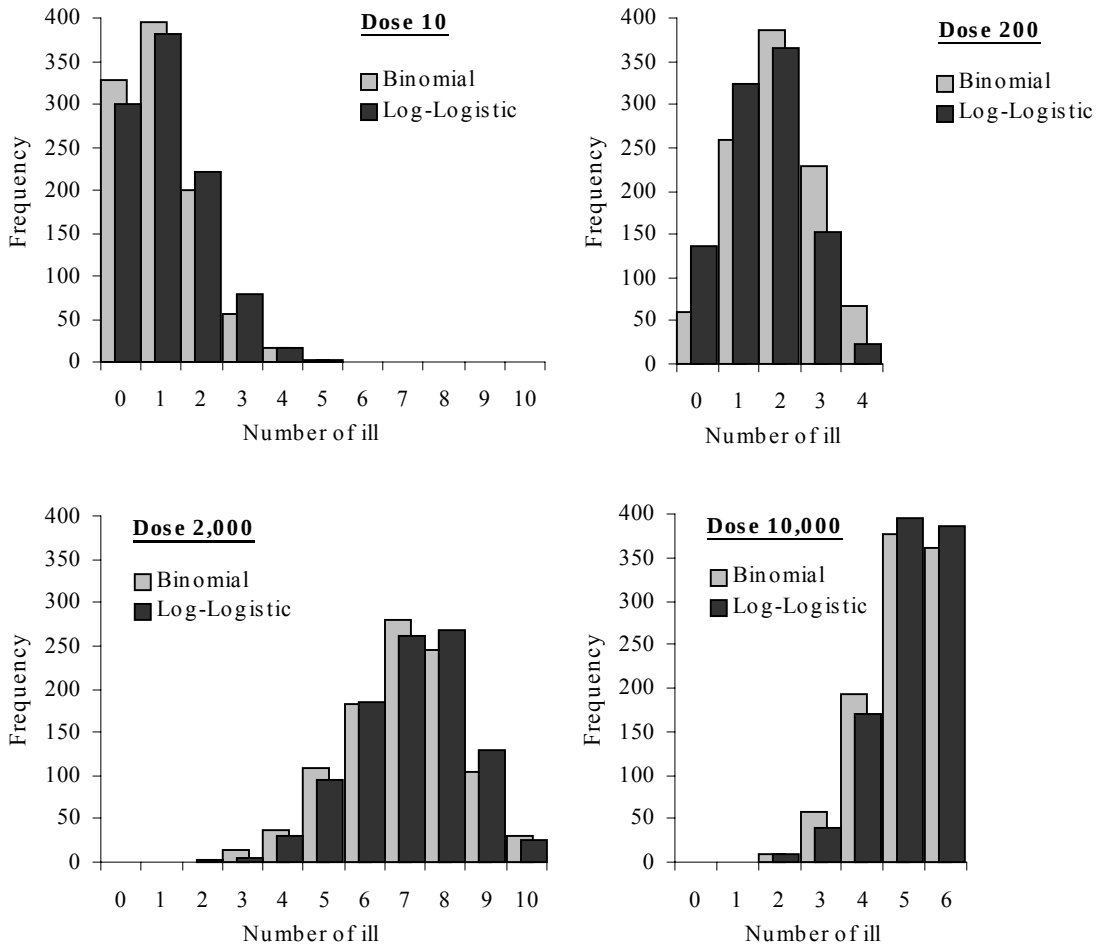


Figure 2.16. Contrast of the binomial resampling and parametric resampling based on log-logistic model as applied to *Shigella dysenteriae* data (1000 resamples)

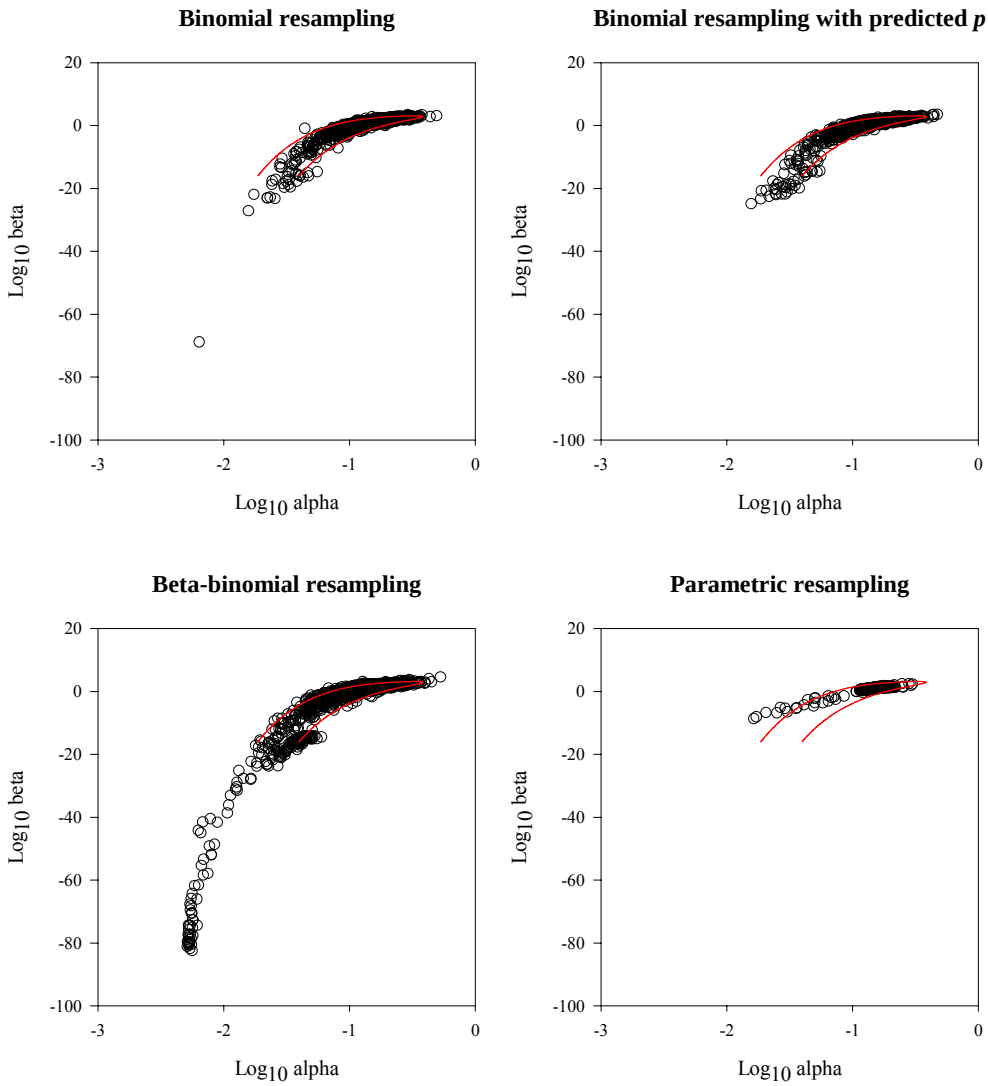


Figure 2.17. Beta-Poisson model and *Campylobacter jejuni* data: Parameter pairs obtained by means of different resampling methods, contrasted to joint confidence region (red line).

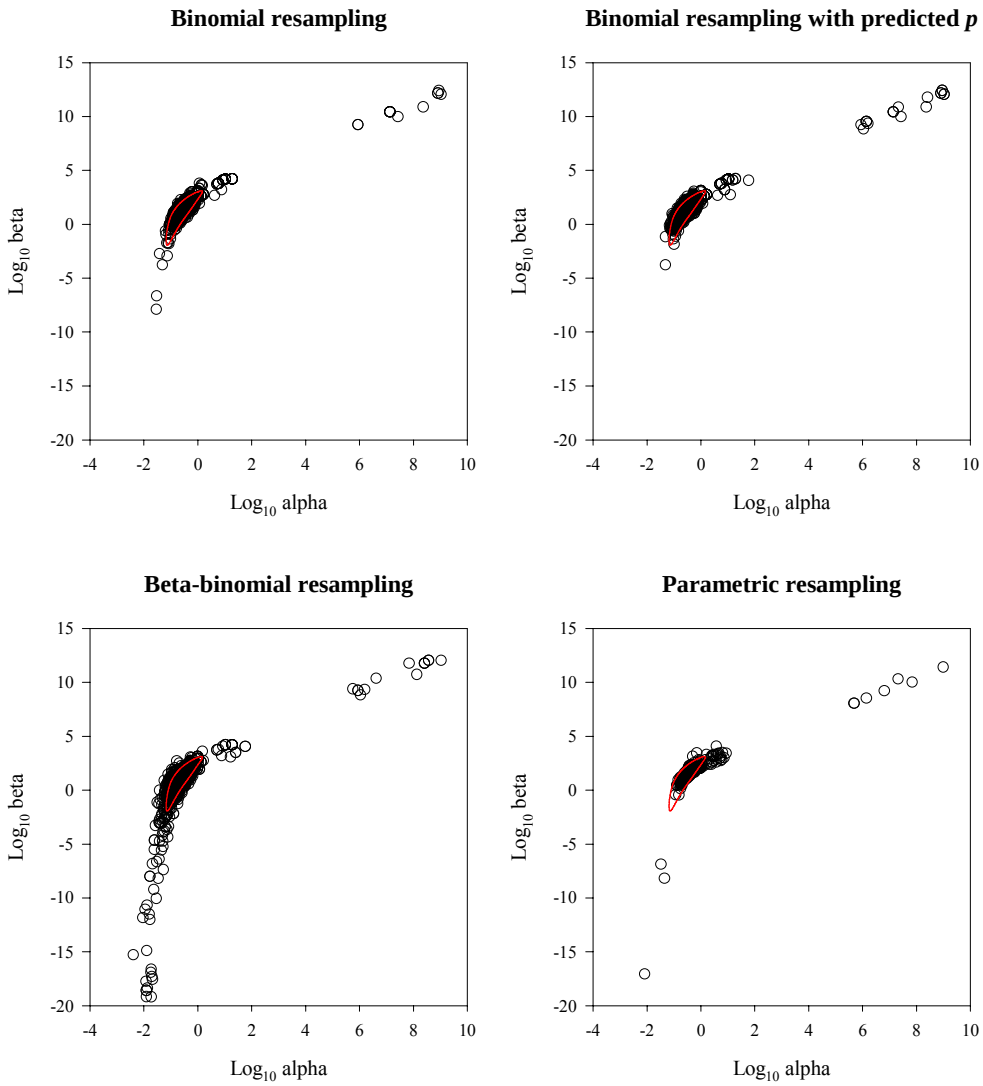


Figure 2.18. Beta-Poisson model and *Shigella dysenteriae* data: Parameter pairs obtained by means of different resampling methods, contrasted to joint confidence region (red line).

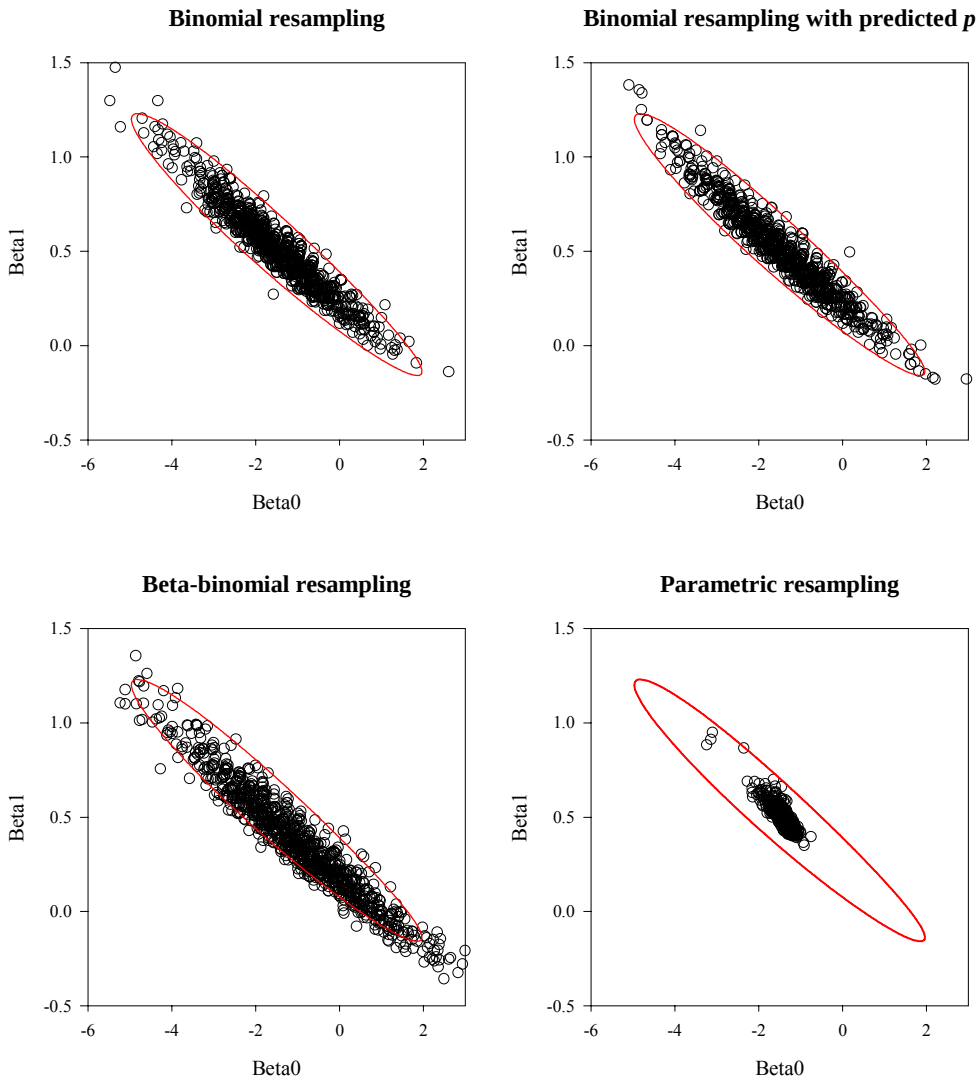


Figure 2.19. Log-logistic model and *Campylobacter jejuni* data: Parameter pairs obtained by means of different resampling methods, contrasted to joint confidence region (red line).

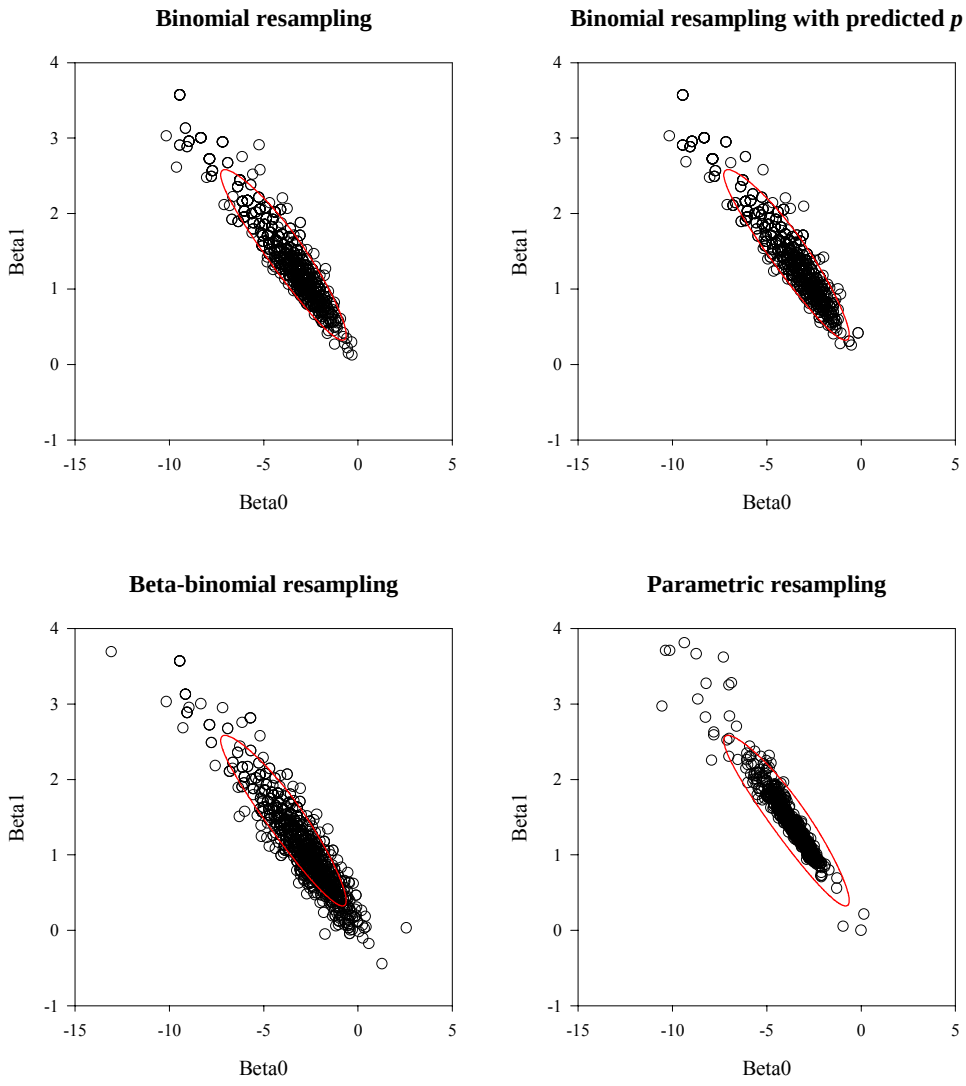


Figure 2.20. Log-logistic model and *Shigella dysenteriae* data: Parameter pairs obtained by means of different resampling methods, contrasted to joint confidence region (red line).

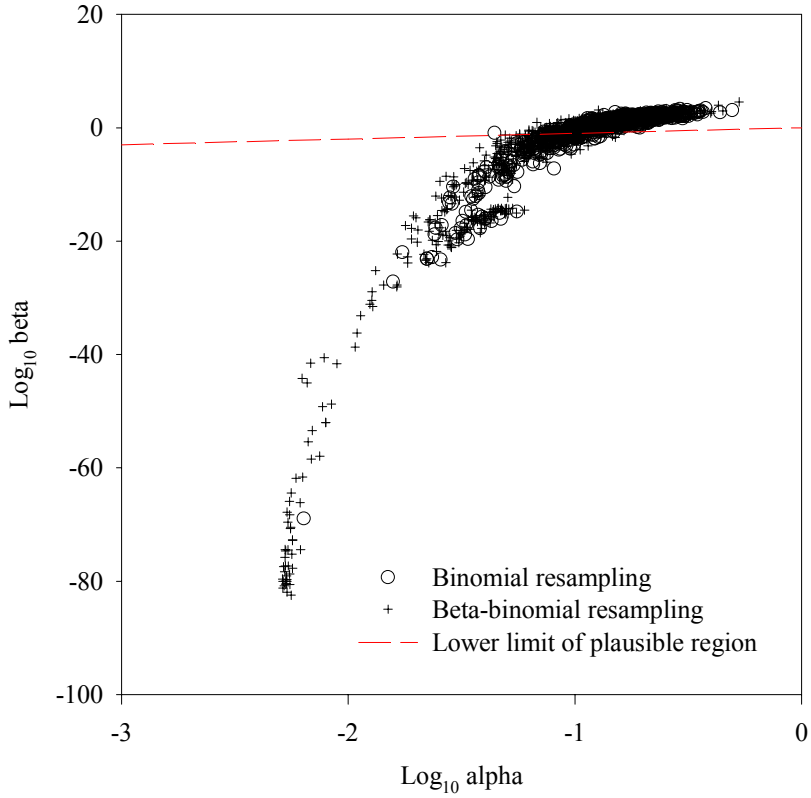


Figure 2.21. Parameter pairs of the beta-Poisson model fitted to *Campylobacter jejuni* data obtained by means of binomial and beta-binomial resampling, respectively

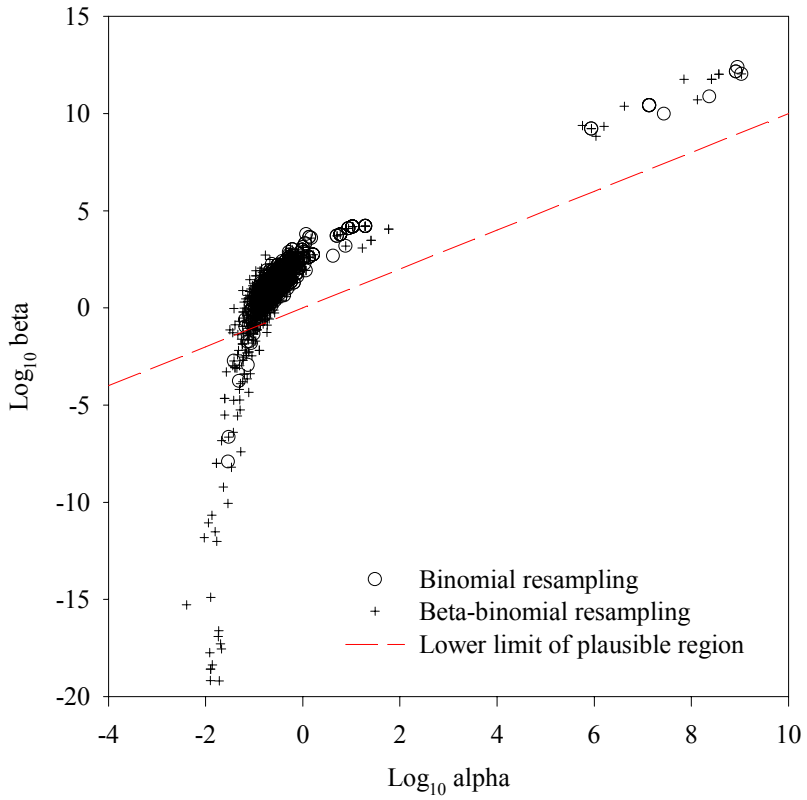


Figure 2.22. Parameter pairs of the beta-Poisson model fitted to *Shigella dysenteriae* data obtained by means of binomial and beta-binomial resampling, respectively

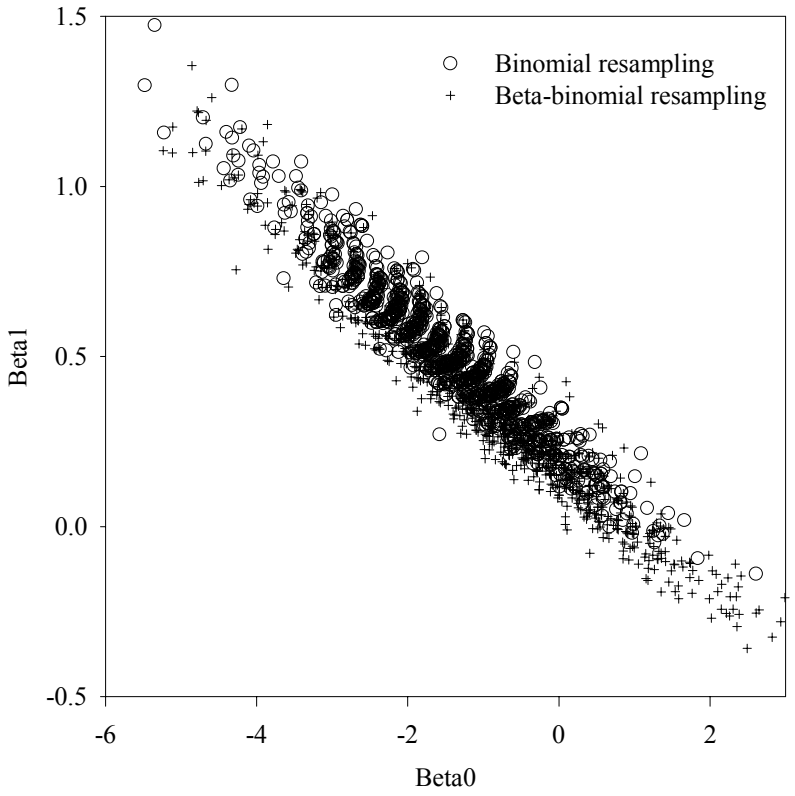


Figure 2.23. Parameter pairs of the log-logistic model fitted to *Campylobacter jejuni* data obtained by means of binomial and beta-binomial resampling, respectively

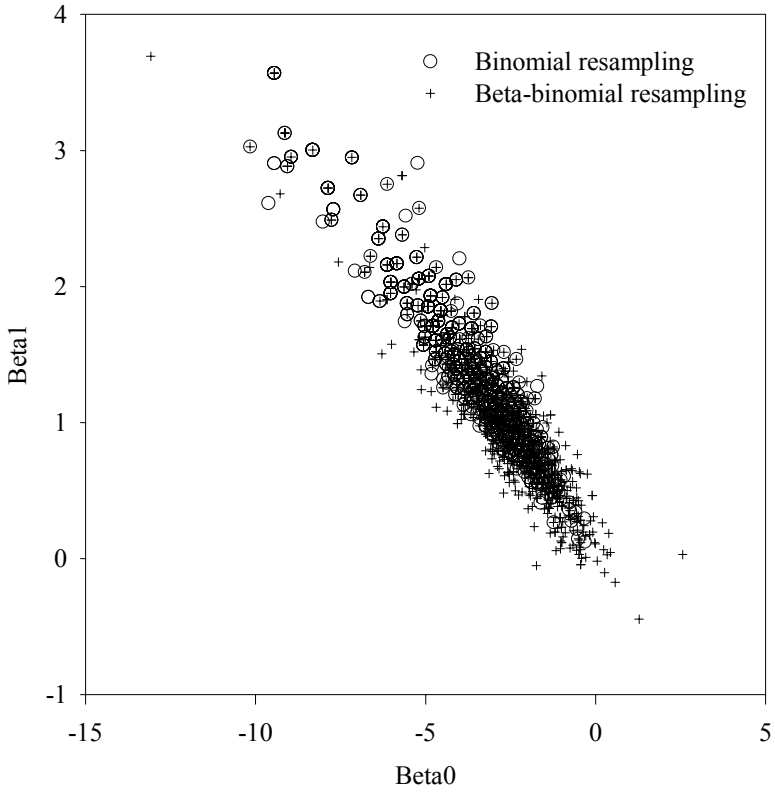


Figure 2.24. Parameter pairs of the log-logistic model fitted to *Shigella dysenteriae* data obtained by means of binomial and beta-binomial resampling, respectively

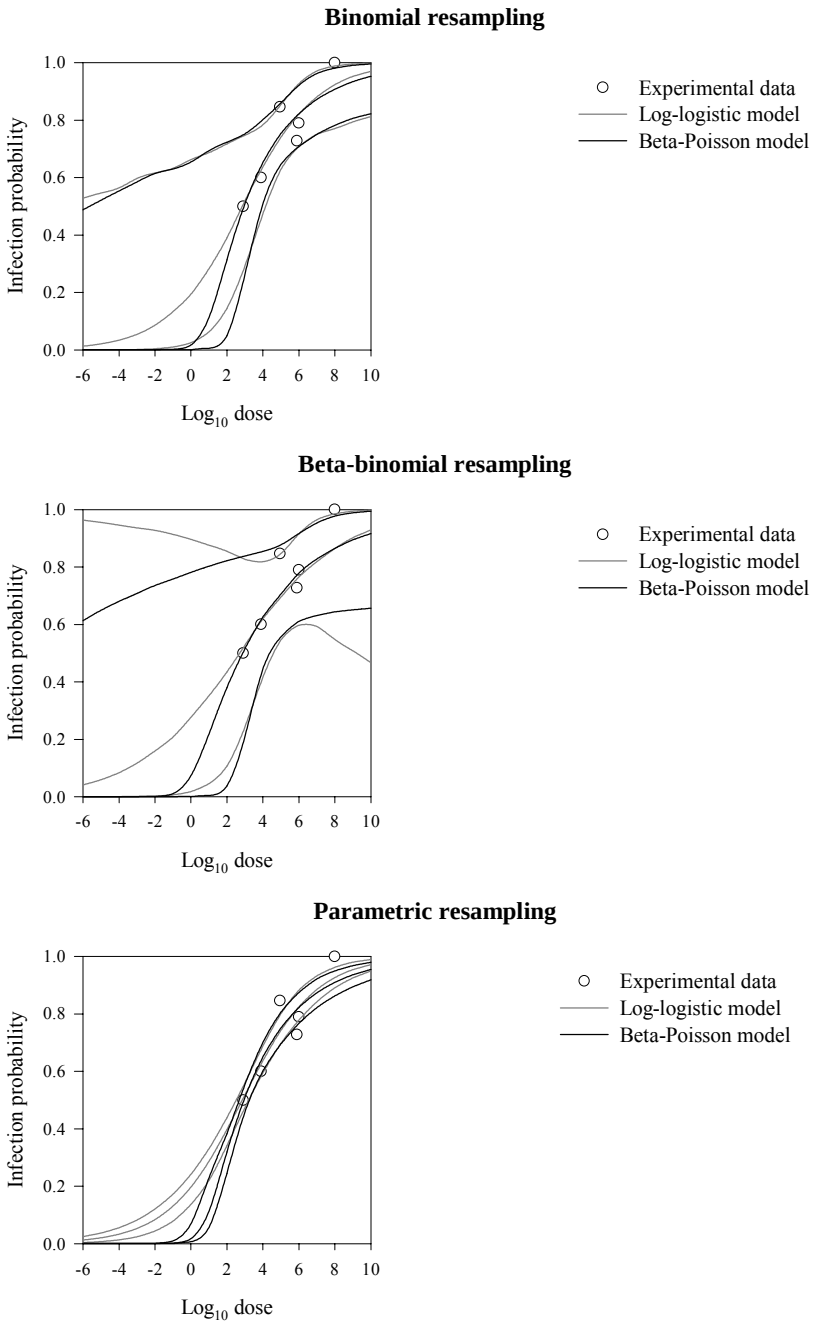


Figure 2.25. Comparison of beta-Poisson and log-logistic models (predicted values and 95% confidence bands) by resampling method for *Campylobacter jejuni* data

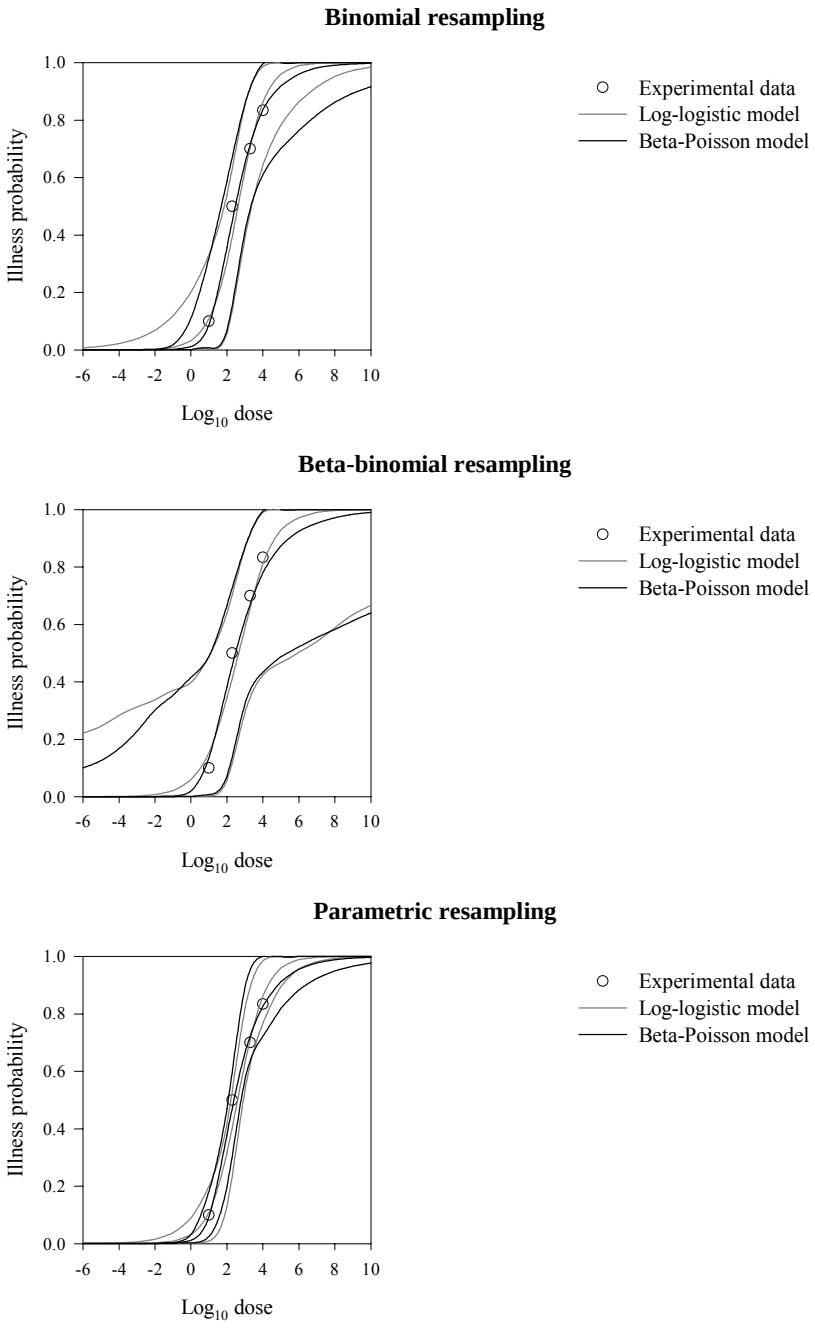


Figure 2.26. Comparison of beta-Poisson and log-logistic models (predicted values and 95% confidence bands) by resampling method for *Shigella dysenteriae* data

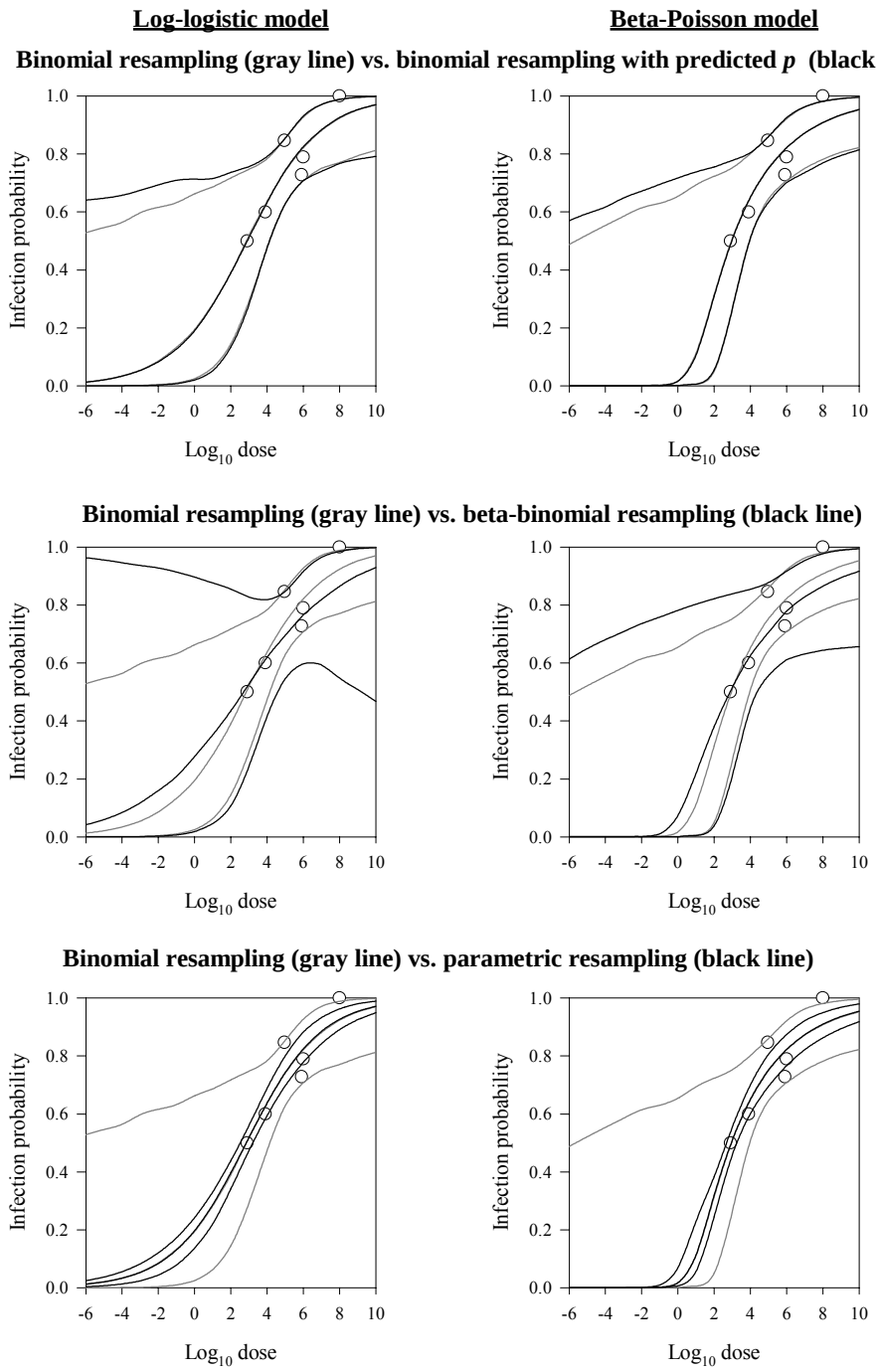


Figure 2.27. Comparison of resampling methods (predicted values and 95% confidence bands) by dose-response model for *Campylobacter jejuni* data

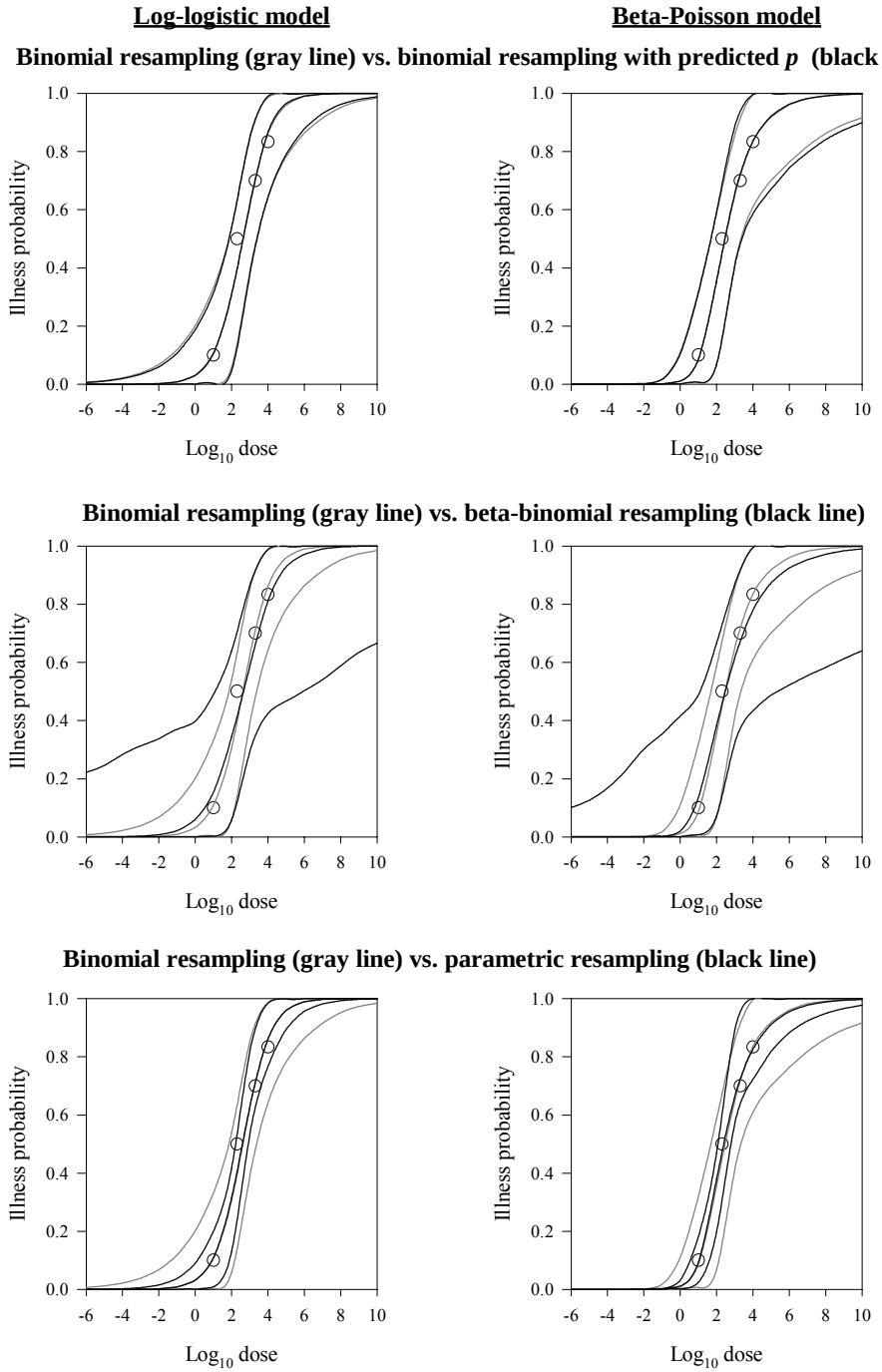
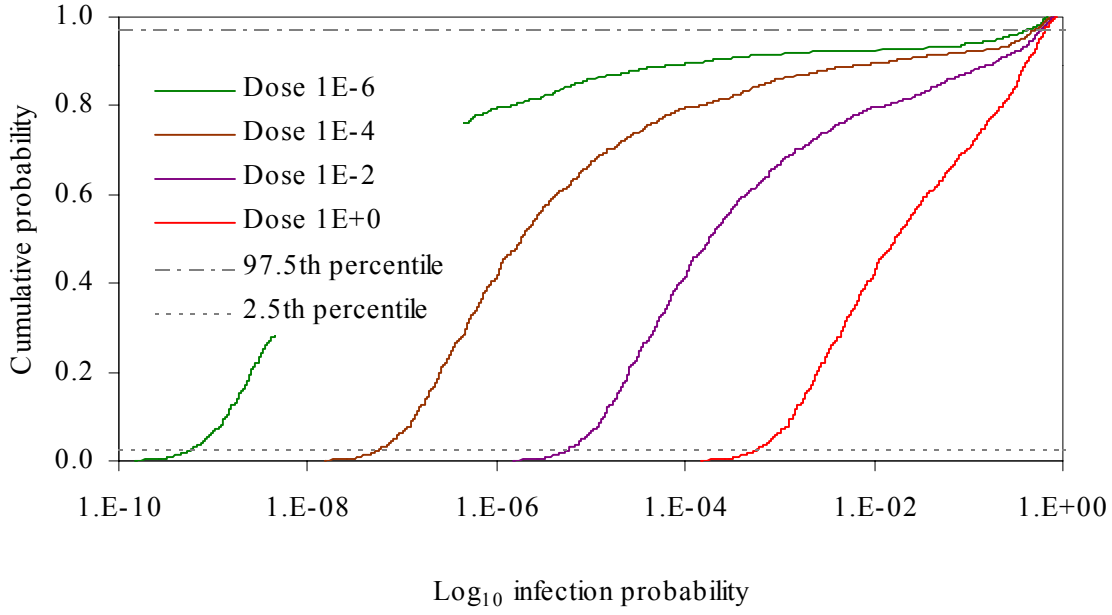


Figure 2.28. Comparison of resampling methods (predicted values and 95% confidence bands) by dose-response model for *Shigella dysenteriae* data



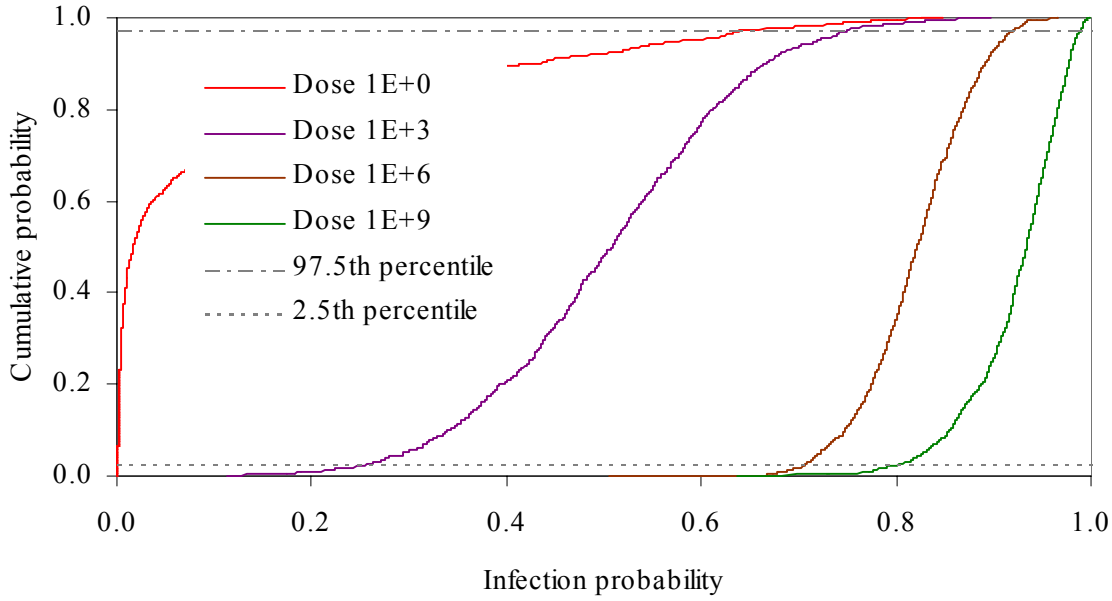


Figure 2.29. Beta-Poisson model, binomial resampling, and *Campylobacter jejuni* data: Uncertainty in response at select doses

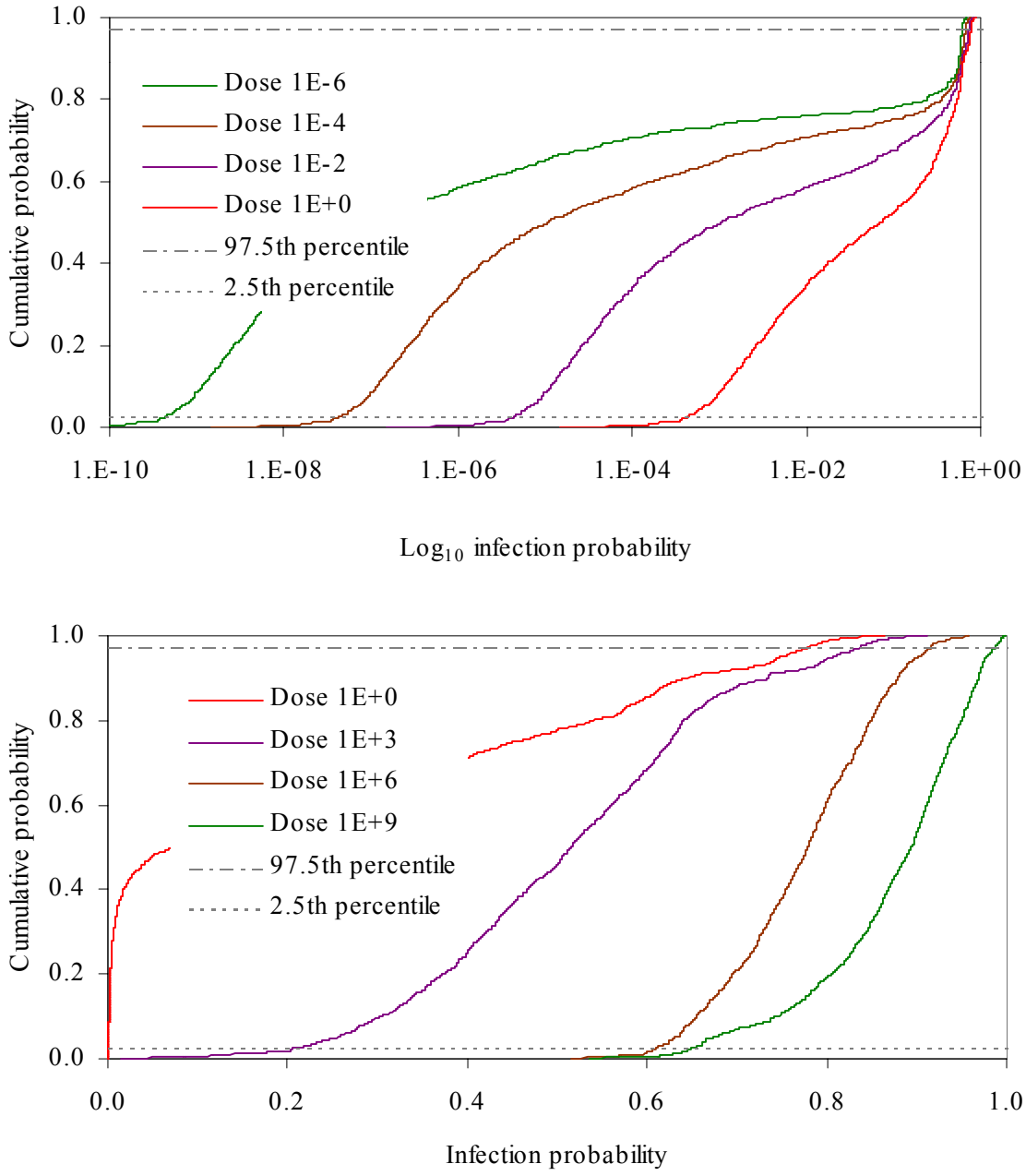


Figure 2.30. Beta-Poisson model, beta-binomial resampling, and *Campylobacter jejuni* data: Uncertainty in response at select doses

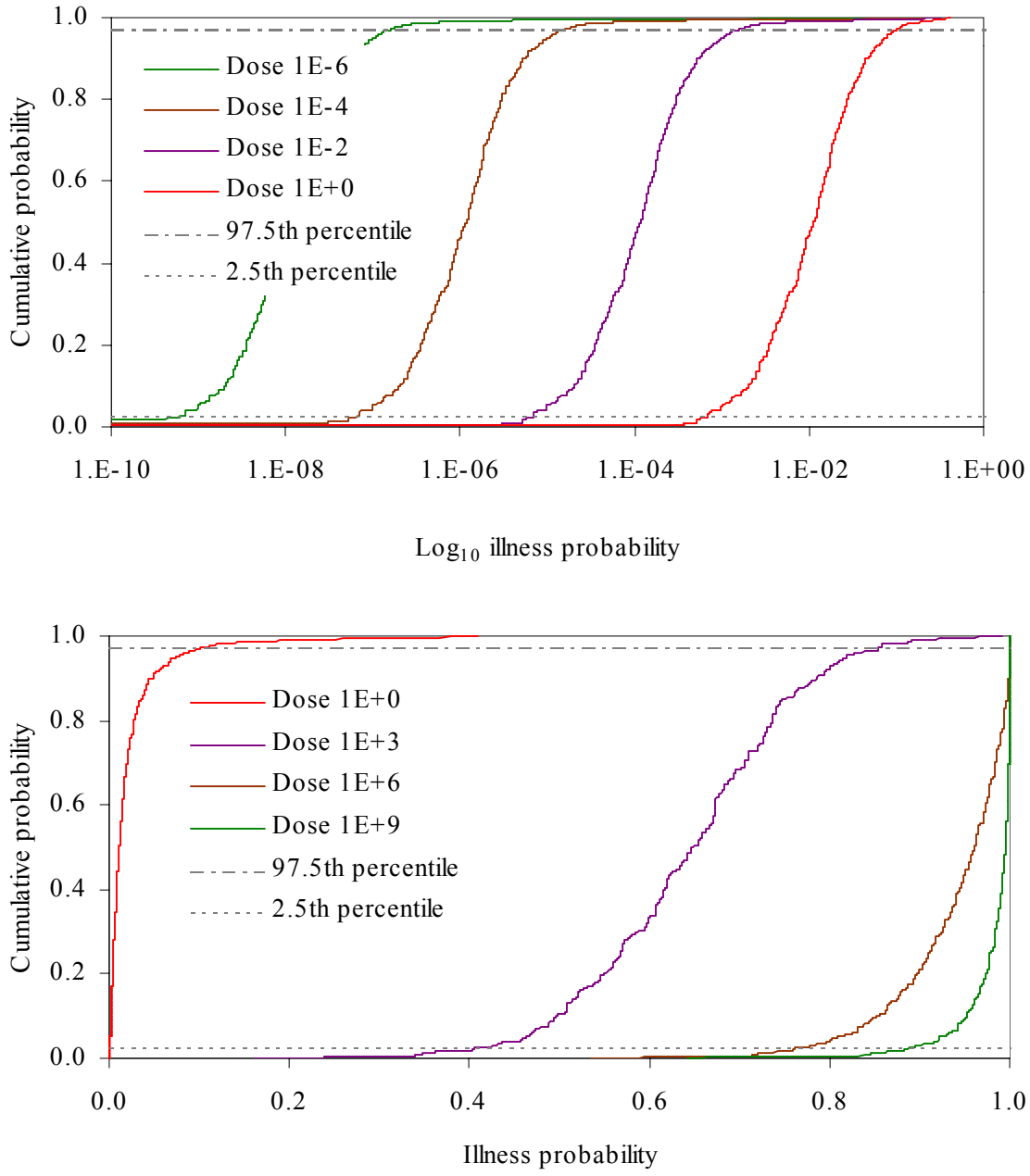


Figure 2.31. Beta-Poisson model, binomial resampling, and *Shigella dysenteriae* data: Uncertainty in response at select doses

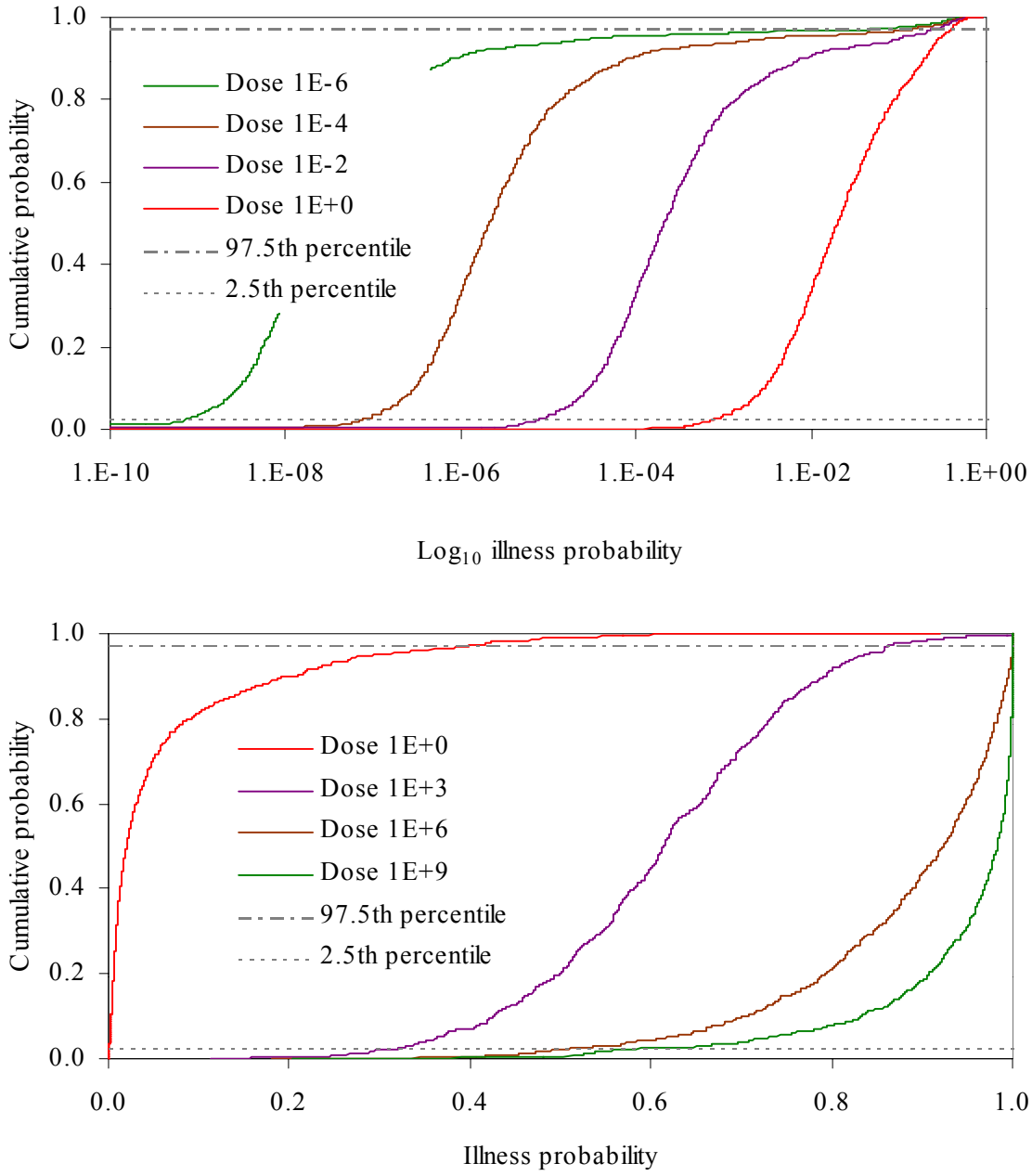


Figure 2.32. Beta-Poisson model, beta-binomial resampling, and *Shigella dysenteriae* data: Uncertainty in response at select doses

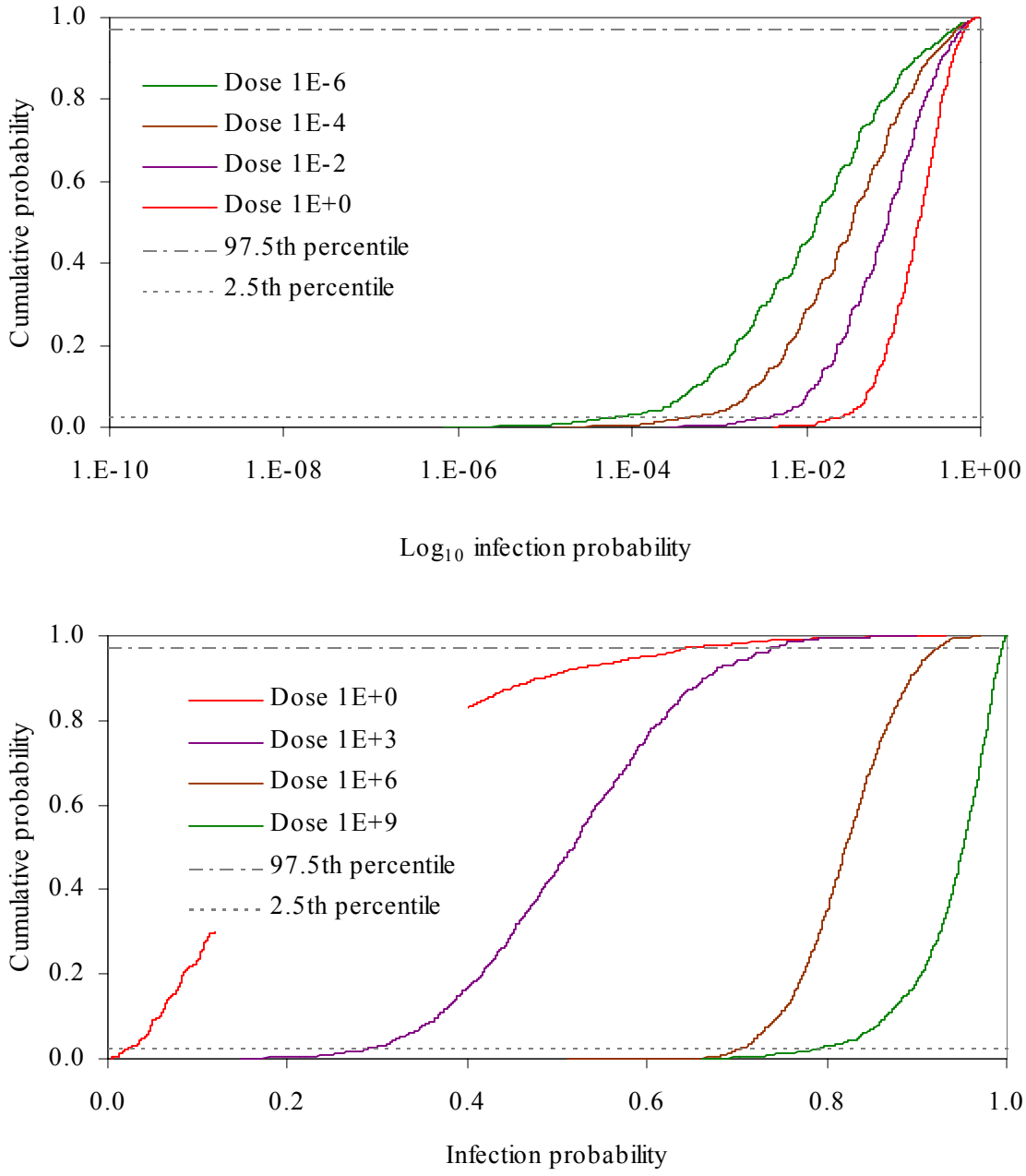


Figure 2.33. Log-logistic model, binomial resampling, and *Campylobacter jejuni* data: Uncertainty in response at select doses

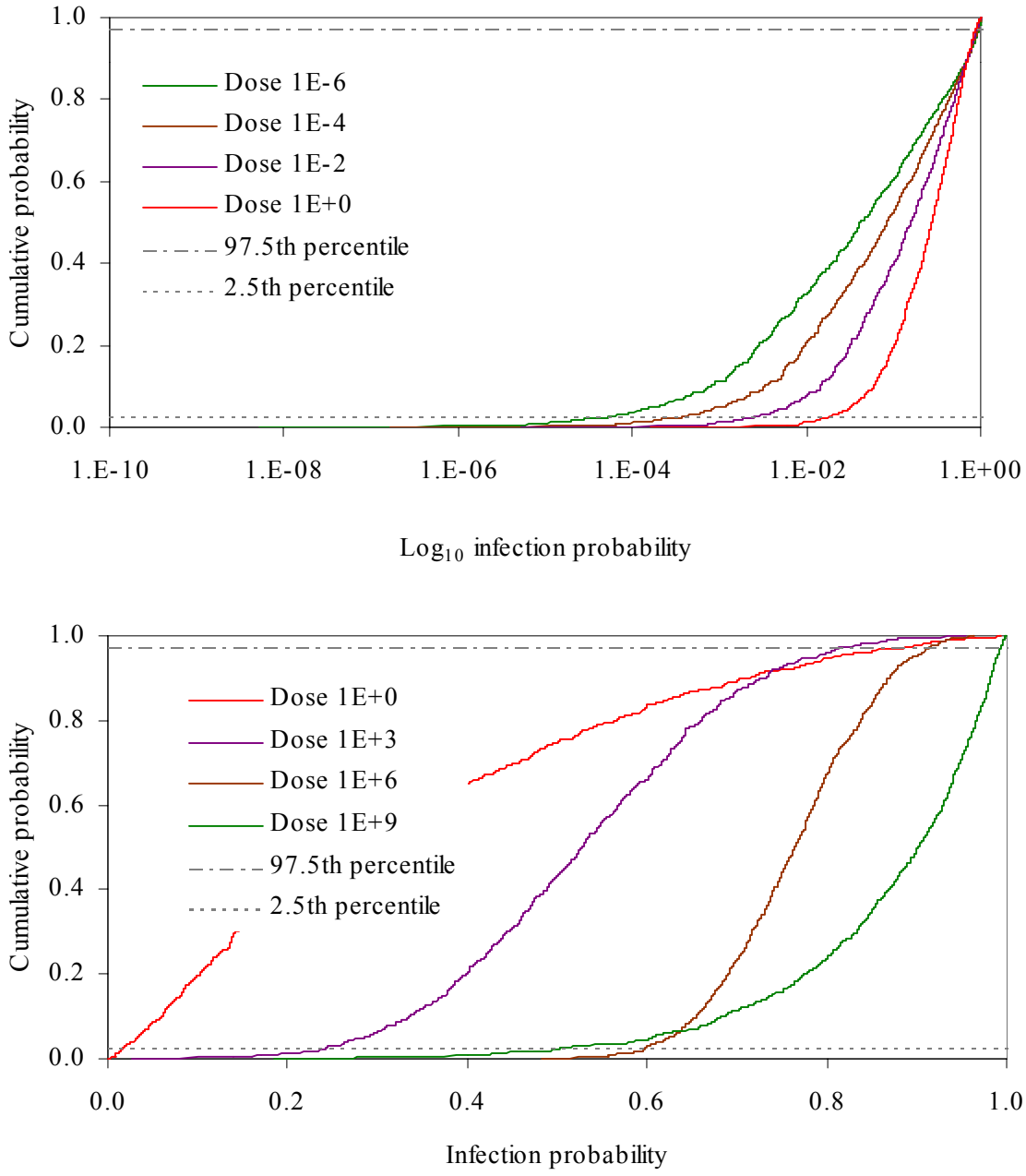


Figure 2.34. Log-logistic model, beta-binomial resampling, and *Campylobacter jejuni* data: Uncertainty in response at select doses

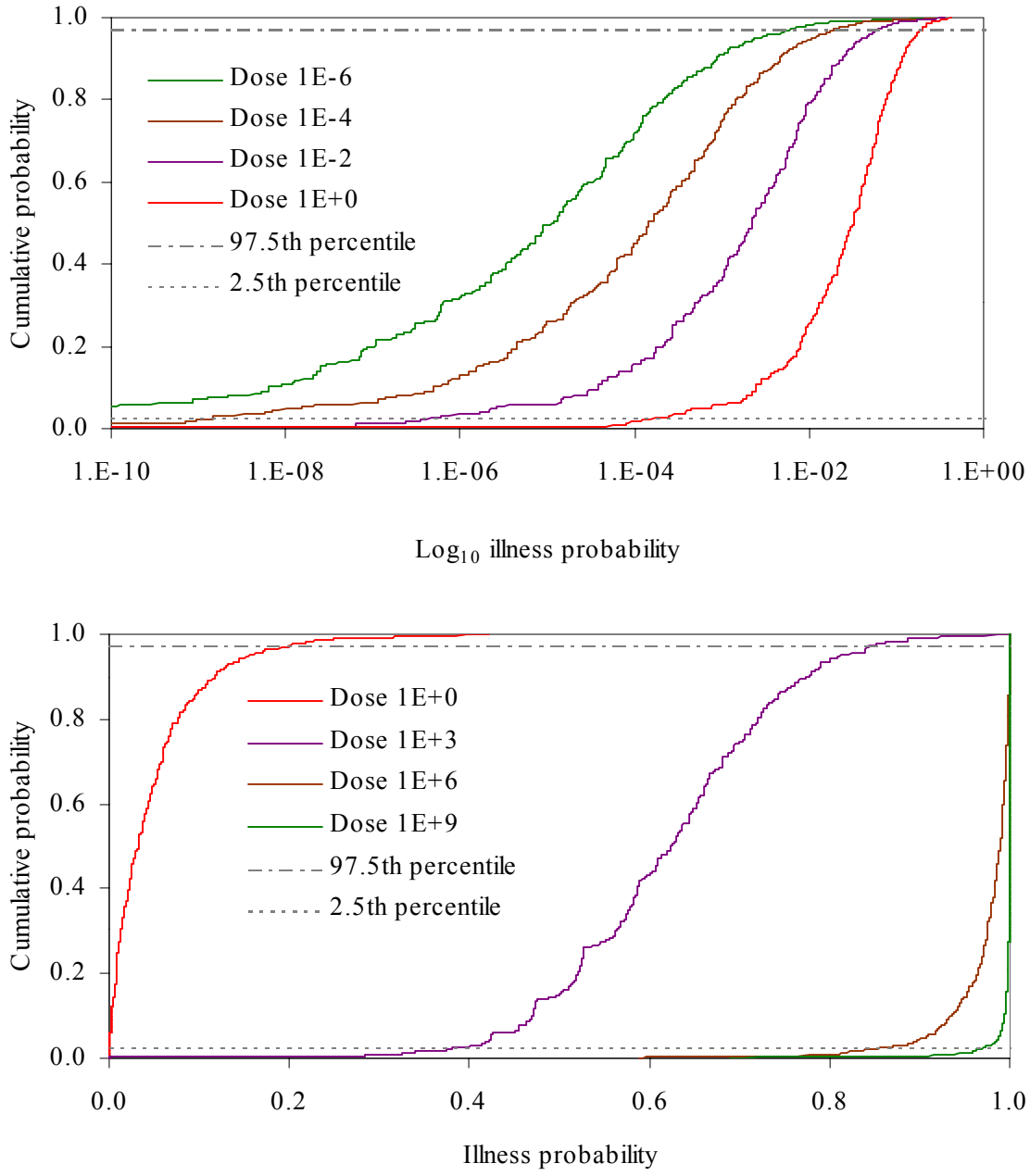


Figure 2.35. Log-logistic model, binomial resampling, and *Shigella dysenteriae* data: Uncertainty in response at select doses

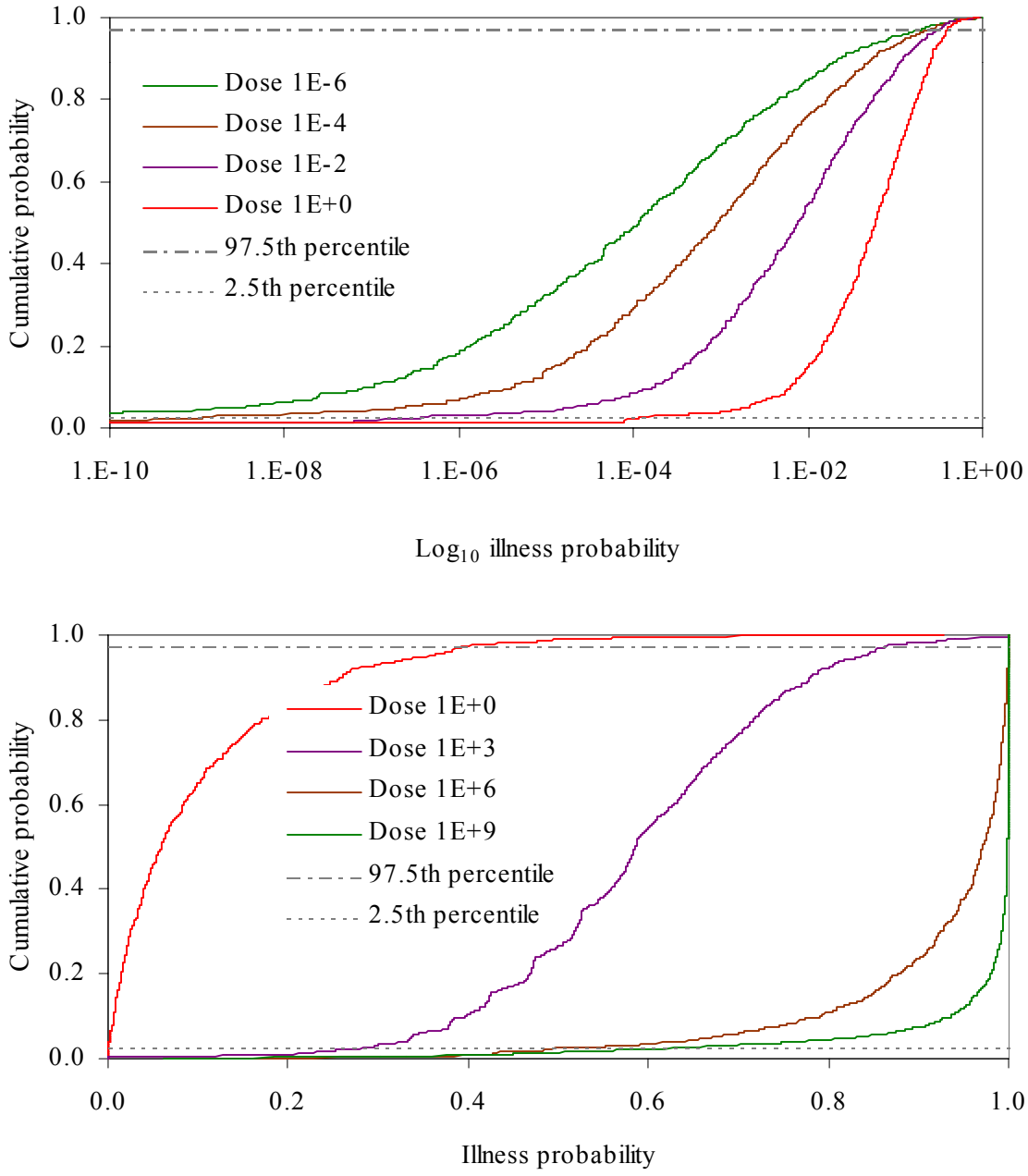


Figure 2.36. Log-logistic model, beta-binomial resampling, and *Shigella dysenteriae* data: Uncertainty in response at select doses

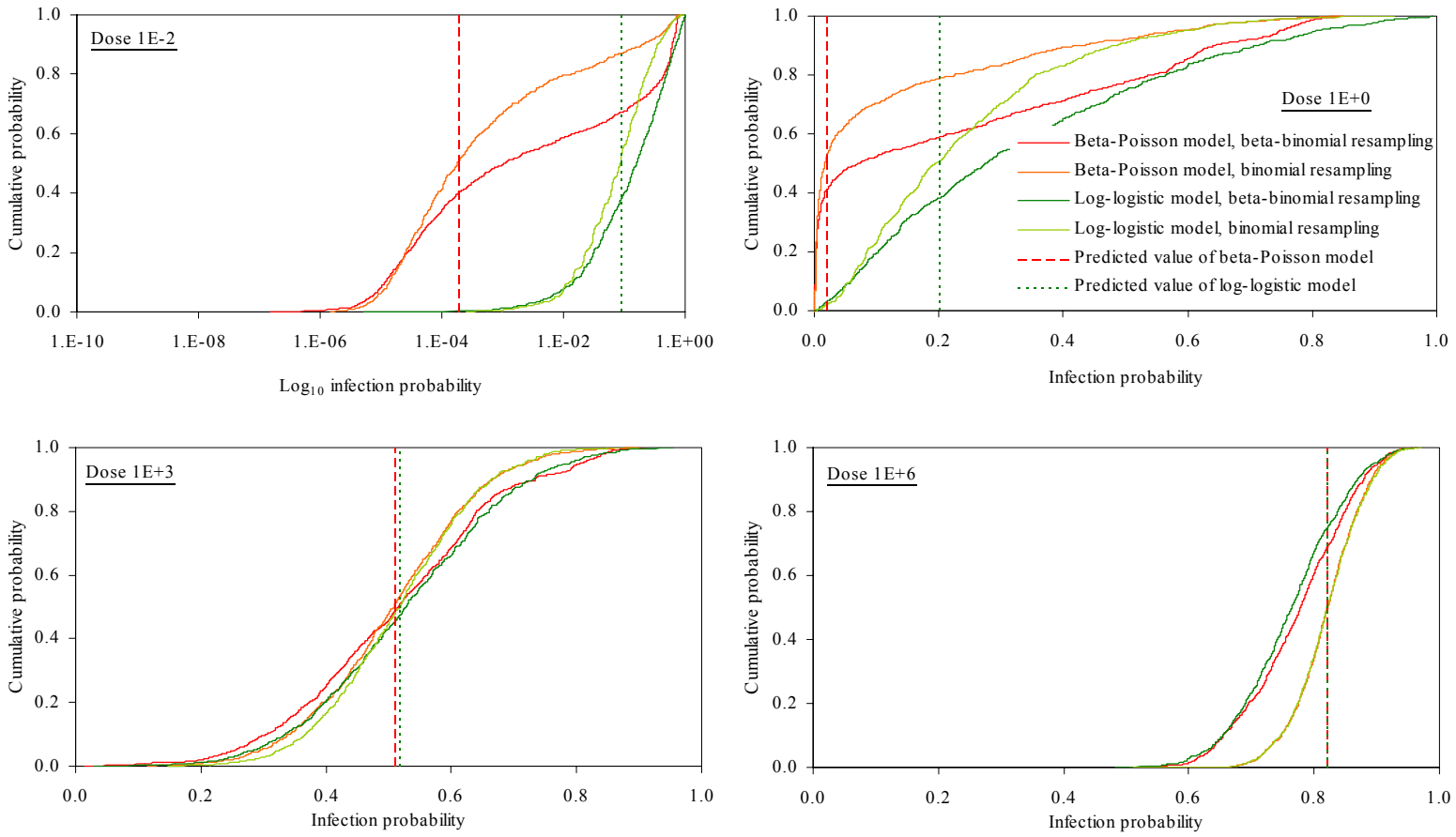


Figure 2.37. Uncertainty in response at select doses for different combinations of dose-response model (beta-Poisson and log-logistic) and resampling method (binomial and beta-binomial), *Campylobacter jejuni* data

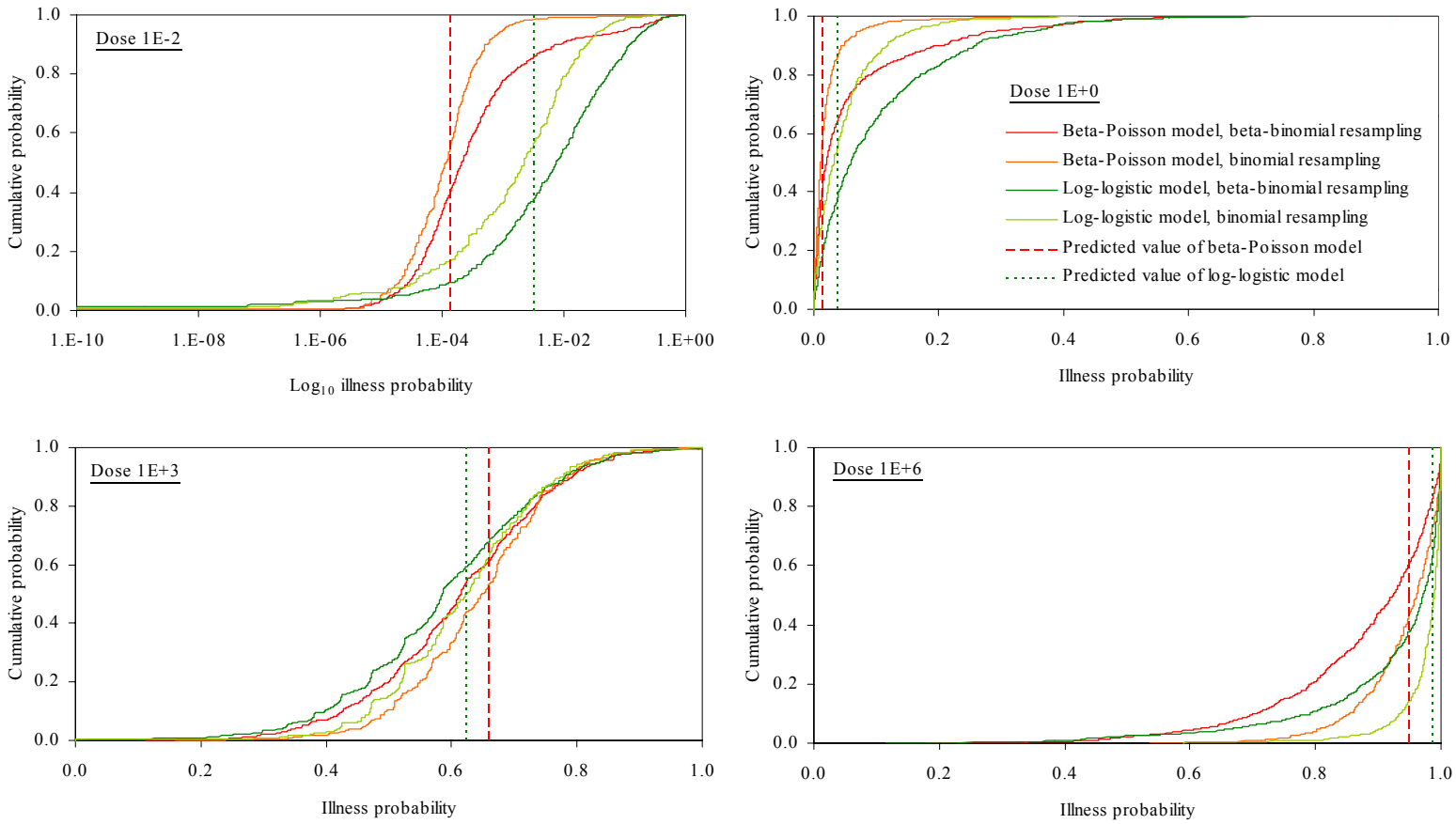


Figure 2.38. Uncertainty in response at select doses for different combinations of dose-response model (beta-Poisson and log-logistic) and resampling method (binomial and beta-binomial), *Shigella dysenteriae* data

3 AGE AND GENDER EFFECTS ON FOODBORNE INFECTION RATES: ANALYSIS OF FOODNET SURVEILLANCE DATA FOR 1996-1999

3.1 Problem Definition, Motivation, and Objectives

Within the framework of the U.S. National Food Safety Initiative, it has been recognized that the availability of reliable epidemiologic data on foodborne disease is an important prerequisite to assessing food hazards and evaluating the cost-effectiveness of prevention programs (Binder et al., 1998). As such, surveillance data ultimately provide the basis for purposeful changes to food safety regulations. In an effort to improve upon existing routine passive surveillance, the Foodborne Diseases Active Surveillance Network (FoodNet) was thus initiated in 1996 as a collaborative effort among several federal agencies and state health departments.

So far, FoodNet surveillance data has essentially been the object of descriptive analyses. For instance, the annual reports describe temporal trends by contrasting yearly rates and interpreting the potential effect of demographic covariates through frequency tables (CDC, 2000a; CDC, 2000b; CDC, 2000c; CDC, 2000d). The availability of an analytical approach that was able to better harness the multivariate and longitudinal nature of the FoodNet data would provide a more formal and powerful insight. Specific challenges are likely to emerge, though. Firstly, surveillance data are less specific or precise than are those from research studies (Buehler, 1998), and may not be amenable to the assumptions constraining statistical analyses. Such a quantitative approach would also have to respect two specific constraints. The first is the discrete count characteristic of the dependent variable (i.e. number of cases of foodborne infections). Secondly, the likely correlation among repeated measurements needs to be handled. Further, exposure may not be well characterized by the available explanatory variables (place, time, covariate), and the interrelationship among these effects may be complex.

This study looks at the application of Poisson regression analysis as an analytical tool for FoodNet surveillance data. Specifically, a Poisson loglinear model is used to model rates of foodborne illnesses as a function of age, gender, site, and year.

Parameters are estimated through the Generalized Estimating Equations (GEE) method. Specific outcomes are incidence rate ratios for the different levels of two covariates (age and gender, if main effect significant) in the case of reported infections of *Campylobacter*, *Salmonella*, and *Shigella*. Such measures of relative risk are intended for use in a subsequent chapter of this dissertation, in which they will be employed to characterize interindividual variability in susceptibility within the framework of microbial risk assessment.

3.2 Characteristics and Analysis of Surveillance Data

Surveillance has been defined as an ongoing and systematic collection, analysis, interpretation, and dissemination of descriptive information on health events (Declich & Carter, 1994). Essentially, surveillance systems focus on describing when and where health problems are occurring and who is affected, i.e. the quintessential epidemiologic information of time, place, and person (Buehler, 1998). In this process, a key constraint is the balance between information needs and feasibility of data collection. As a continuous process, the long-term sustainability of a surveillance system relies on the participation of the health professionals upon whom only a proportionate burden can be put. Surveillance data are thus generally less specific or precise than those from research studies, and their analysis and interpretation imposes caution.

3.2.1 Foodborne Diseases Active Surveillance Network

Although foodborne illnesses are commonly regarded as one of the most widespread health problems (Motarjemi & Kaferstein, 1997), several factors complicate an accurate estimation of their incidence (Mead et al., 1999). While diarrheal diseases can be severe or even fatal, milder cases usually do not require medical care and thus go unreported. As many pathogens transmitted through food are also spread through water or by person-to-person contact, the relative role of foodborne transmission is often unclear. Finally, the infectious etiology of foodborne illness often remains undefined. For instance, the role of *Campylobacter jejuni* was unrecognized just two decades ago.

Statistics on foodborne illnesses have historically relied on "passive" surveillance, and several systems based on this principle exist in the United States (CDC, 1997; Mead et al., 1999). For example, within the framework of the Public Health Laboratory Information System, clinical microbiology laboratories have reported findings on *Salmonella* isolates to state health departments. The Centers of Disease Control and Prevention (CDC) have eventually collated the data at the national level. As this example shows, the hallmark of a passive surveillance system is that there is limited involvement of public health officials in guaranteeing consistent detection of cases and transmission of information. As for foodborne illnesses, while passive surveillance can monitor trends, its absolute figures largely underestimate the actual incidence. In fact, a complex chain of events – called the "prevalence of illness pyramid" – must occur before a case is eventually counted (CDC, 1997).

FoodNet comprises a series of activities, the purpose of which is to collect information along each step of that pyramid. Its four main objectives are to measure the frequency and severity of foodborne diseases, to establish the relative importance of specific food items, to describe the epidemiology of new and emerging foodborne pathogens, and to monitor temporal trends (CDC, 2000d). The core component of FoodNet is a population-based active surveillance at over 300 clinical microbiology laboratories distributed in several states. The word "active" essentially means that FoodNet investigators contact, either weekly or monthly, these laboratories to collect information on all laboratory-confirmed cases of diarrheal infections. Specifically, information is sought on cases associated with seven bacteria (*Campylobacter* spp., Shiga toxin-producing *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Yersinia enterocolitica*) and two parasitic protozoa (*Cryptosporidium* spp., *Cyclospora cayetanensis*). Each case is meant to represent the first isolation of the given pathogen from a specific person by the clinical laboratory (CDC, 2001a). As the type of reporting implies, most specimens are obtained for diagnostic purpose from ill persons. Specific information such as possible vehicle and demographic characteristics are also collected for each case. In 2001, a total population

of 33.1 million persons (about 12% of the national population) in areas of nine U.S. states was under surveillance (CDC, 2001a).

The results of the FoodNet active surveillance are viewed as a comprehensive and timely database of foodborne illness in a well-defined population (CDC, 1997). They are presented in yearly reports (CDC, 2000a; CDC, 2000b; CDC, 2000c; CDC, 2000d; CDC, 2001a), which provided the core of the data analyzed later in this study. Table 3.1 presents the infection rates per 100,000 at the five original FoodNet sites (i.e. California, Connecticut, Georgia, Minnesota, and Oregon) for the period from 1996 to 2000 (CDC, 2001a). These data show that the relative importance of the single pathogens has remained constant over the five-year period. In particular, *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. have been – in decreasing order – the three most frequently reported agents. Important year-to-year and regional fluctuations complicate the identification of secular incidence trends (CDC, 2001a). For instance, an enlargement of the area under surveillance can explain annual variations for some sites. Further, the occurrence of large outbreaks also has an important impact. For instance, the spike in Shigellosis cases registered in 2000 is attributed to outbreaks in California and Minnesota. When the 1996 and 2000 data are compared, the incidence of both *Campylobacter* and *Salmonella* infections declined. While *Salmonella* rates were fewer in all five original sites, *Campylobacter* declined in only four sites. Nevertheless, fluctuations in the years between 1996 and 2000 could also suggest that the decline is merely part of a random process. Especially in the case of *Shigella* infections, the incidence varies substantially from year to year and from site to site. Overall, these few points clearly show that, based solely on a descriptive analysis, the identification of temporal trends is challenging and cannot be established with reasonable certainty.

Besides contrasting the results of different surveillance years, the annual FoodNet reports descriptively discuss the influence that select demographic characteristics have on the number of reported cases. The reports for the years 1997, 1998, and 1999 discuss age and gender effects, and conclude that, especially for *Campylobacter* and *Salmonella* infections, the annual incidence of foodborne illness varies by age and gender. In

particular, the rates in children less than 1 year of age are substantially higher than those of the other age groups. Gender also appears to have an influence, in that males are more likely than females to acquire campylobacteriosis and salmonellosis. The difference is especially marked for the former disease. While detailed data are not reported, the interaction of age and gender is briefly discussed. For *Campylobacter* cases, the incidence rates for males tend to be higher than those for females. In 1997, the rates in infants (children of less than 1 year of age) were similar in both males and females. In contrast, rates of *Salmonella* infection are generally higher for males in the early age groups (infants, children of 1-10 years of age, and possibly young adults up to 19 years of age), while females tend to have higher rates in the remaining age groups. In 1999, *Salmonella* rates were also higher for males of 40-49 years of age. From the text of the FoodNet reports, it is not clear whether the highlighted differences are the results of a statistical analysis. The reports also present data on ethnicity and race, i.e. count of cases and percentage distribution for each pathogen/site. Unfortunately, absence of information on these traits' distribution in the surveyed population makes an analysis impossible.

Main limitations of the FoodNet surveillance have been discussed (CDC, 2001a). Currently the system encompasses approximately one tenth of the U.S. population, and the data may not be representative of the national situation. Secondly, FoodNet does not completely upturn underreporting. Since most foodborne illnesses will not require in-depth medical investigation, data still merely reflect the fraction of cases that are laboratory-confirmed. Even if an illness case becomes the object of a laboratory work-up, procedural and performance differences among laboratories may influence the isolation outcome. For instance, while stool specimens are routinely tested for *Salmonella* and *Shigella* and often for *Campylobacter*, testing for *E. coli* O157 is only carried out half of the time, and other pathogens even less frequently. Further, some reported cases may be the result of an exposure not linked to food, such as drinking water or person-to-person contact. Finally, cases are recorded by site of occurrence, but exposure may well have occurred at another location. In particular, a FoodNet case-

control study completed in 1999 showed that foreign travel was a risk factor for *Campylobacter* infection (CDC, 2000d).

FoodNet is an integral component of former President Clinton's Food Safety Initiative (Binder et al., 1998). In particular, the monitoring of foodborne illness over time – such as provided by the surveillance component of FoodNet – is viewed as an important element in evaluating the effectiveness of new food safety initiatives, such as the USDA Food and Safety Inspection Service's Pathogen Reduction and Hazard Analysis and Critical Control Points (HACCP) Rule. Among its key findings, the 1999 FoodNet report mentions that *Campylobacter* incidence decreased by 18% between 1998 and 1999, and by 26% between 1996 and 1999 (CDC, 2000d). As poultry is the most common source of *Campylobacter* infections, the decline is linked to changes in poultry processing plants required by the implementation of the HACCP Rule. This example suggests that inferences drawn from FoodNet surveillance data have implications that reach into the core of the food safety policy-making process. Understanding the limitations of the surveillance tool, and, therefore, achieving a truthful interpretation of the data has to be viewed as an important element in safeguarding the scientific soundness of that process.

3.2.2 Analytical Framework for Surveillance Data

As shown by the FoodNet data, surveillance data often come in the form of discrete counts of events. When frequencies are contained, an adequate assumption is that the counts follow a Poisson distribution (Stokes et al., 2000). A common analytical goal of surveillance data is to estimate incidences (rates of illness/infection per population at risk) and to establish the potential relation with a set of available explanatory variables (e.g. site, age). The Poisson regression is an analytical method that can achieve that goal while preserving and exploiting as much as possible the discreteness of the count variable. Such an approach has already been applied in epidemiology. For instance, Shahpar and Guoha (1999) performed an age-period-cohort analysis to characterize temporal trends and birth cohort patterns of death rates from homicide in the United States. Other recent examples are analyses of mortality trends for

multiple sclerosis in Italy (Tassinari et al., 2001), of age-incidence relationships in cervical cancer in Sweden (Hemminki al., 2001), and of age, sex, geographical and socio-economic effects in hospital admissions for anaphylaxis in the United Kingdom (Sheikh & Alves, 2001).

Computational implementation of the Poisson regression is carried out within the framework of Generalized Linear Models (GLM) (Stokes et al., 2000). One assumes that the dependent variable Y is Poisson-distributed with mean and variance μ. If only a single explanatory model is considered, the base GLM regression model for μ is written as

$$g(\mu) = \alpha + x\beta$$

where *g* is the link function. The Poisson regression model applies the log function as *g*, and results in the loglinear model

$$\log(\mu) = \alpha + x\beta$$

When the interest lies in modeling rates, one needs to define an exposure variable *N* (e.g. population at risk, time at risk). The rate is then *Y* / *N*. The expected value becomes μ/N, which is modeled as

$$\log\left(\frac{\mu}{N}\right) = \alpha + x\beta$$

This model can be rearranged as follows

$$\log(\mu) = \alpha + x\beta + \log(N)$$

where the term log(*N*) is called an *offset* and is considered in the estimation process. For multiple explanatory variables, the model is written as

$$\log(\mu_i) = \log(N_i) + \mathrm{x}_i'\beta$$

A useful characteristic of the Poisson loglinear model is that, similar to logistic regression, the exponentiation of parameter coefficients leads to measures of relative risk, i.e. the incidence rate ratio (IRR).

With real-life data, the observed variance of counts usually exceeds the nominal variance (i.e. the mean) of a Poisson distribution (McCullagh & Nelder, 1989). This situation is called overdispersion. When a Poisson regression is estimated by maximum likelihood (MLE), overdispersion has an important impact on hypothesis testing: while the parameter estimates are still consistent (provided that the conditional mean is correctly specified), their standard errors will be underestimated (Cameron & Trivedi, 1998). This eventually leads to an overly optimistic conclusion on the statistical significance of the considered parameter. As long as outliers and a misspecified regression model can be excluded, overdispersion in MLE can be accommodated by adjusting the variance of the Poisson distribution with a scaling factor or by applying a more flexible model, such as the negative binomial model (Stokes et al., 2000). Alternatively, other estimation approaches could be applied.

In order to capture the temporal trend of health events, most surveillance systems collect data over consecutive time periods. In the case of FoodNet, the population at each site is observed year after year, and repeated measurements on the number of foodborne infections are recorded. Similar to time-series data, observations within a specific site (one cluster) are likely autocorrelated. In order to draw valid statistical inferences, the longitudinal structure of the data needs to be respected in the analysis (Diggle et al., 1994). If ignored, inefficient estimates of the regression coefficients (i.e. imprecise estimates) and incorrect inferences about those coefficients would result.

The Generalized Estimating Equations (GEE) method is an extension of GLM that provides a semi-parametric approach to longitudinal analysis (Liang & Zeger, 1986). In contrast to GLM, the GEE approach accounts for the structure of the response covariances through its specification in the estimating process. By defining a common link and variance function, the analyst describes the random component of the model for each marginal response. The method then manages the covariance structure as a nuisance

parameter, and models the function of the marginal expectation of the response variable as a linear function of explanatory variables. Although the specification of a working correlation matrix is required, the approach is robust to misspecification of this matrix. Even when the assumed correlation structure is incorrect, the GEE method relies on independence across clusters to consistently estimate the parameter variances.

GEE are ideal for repeated, discrete response data such as binary outcomes and Poisson count (Stokes et al., 2000). In addition to modeling the correlation structure, they are inherently resilient to overdispersion. Further, the GEE method is flexible as it can handle continuous explanatory variables, a moderate number of explanatory categorical variables, time-dependent explanatory variables, and randomly missing data. Likewise to MLE, GEE rely on asymptotic theory, and a sufficiently large number of clusters is needed to produce consistent estimates. With a limited number of explanatory variables, 25 clusters may be enough. With 5-12 explanatory variables, more than 100 clusters (possibly 200) are preferred. This issue also has implications when it comes to assessing the significance of an explanatory variable through Type3 contrasts (i.e. partial analyses). The Z statistic and the Wald statistic require about 200 clusters to be reliable at the 0.05 confidence level. The score statistic is often more conservative in the presence of small numbers of clusters. Finally, unlike MLE, there are no readily specified procedures to assess goodness-of-fit within the GEE framework.

3.3 Data and Methods

Counts of *Campylobacter*, *Salmonella*, and *Shigella* infections corresponding to the years 1996, 1997, 1998, and 1999 were extracted from annual FoodNet reports (CDC, 2000a; CDC, 2000b; CDC, 2000c; CDC, 2000d). Specifically, the frequency tables concerning the two factors age and gender stratified by site were consulted.

Data for the whole four-year period are available for five sites, i.e. California, Connecticut, Georgia, Minnesota, and Oregon. Data for Maryland and New York only exist for the years 1998 and 1999. FoodNet surveillance does not necessarily cover the whole area of a state. The level of data aggregation was not only different for the seven

sites, but it also changed over the four-year period. The 1996 data cover the entire states of Minnesota and Oregon, and selected counties in California, Connecticut, and Georgia (CDC, 2001a). Twelve Georgia counties and one county in Connecticut were added in 1997. In 1998, the surveillance became statewide for Connecticut and it began in selected counties in Maryland and New York. Finally, the remaining counties in Georgia and eight counties in New York were added in 1998. From 1996 to 1999, the total population in catchment areas of the bacterial surveillance went from 14.3 to 25.9 million. State- and year-specific censuses stratified by age and gender were obtained from the U.S. Census Bureau (U.S. Census Bureau, 2002). As counties under surveillance are not specified in the FoodNet reports, state censuses were reduced proportionally to the site-specific populations listed in those reports. It is thus assumed that the age and gender distribution in each site is equal to the distribution at state level. Coincidentally, the sums of the adjusted figures only differed from the one presented in the FoodNet reports by few units (1996 -1, 1997 -4, 1998 +4, 1999 no difference). Eventually, by combining infection counts and census figures, two data sets – one with counts stratified by 8 age categories (data set AGE), the other with counts stratified by gender (data set GENDER) – were obtained. The variables contained in the data set are described in the Appendix.

After calculation of the annual incidence rates, data were explored by means of graphics in which two of the three explanatory variables had been contrasted. Poisson regression was implemented with the PROC GENMOD of the software SAS/STAT version 8.01 (SAS Institute, Cary, NC). Specification of the REPEATED statement in which the variable identifying clusters was the crossing of age group and site resulted in the implementation of the GEE method (independent covariance structure). Univariate analyses were used to screen explanatory variables (state, year, and either age or gender) to be included in the multivariate models. Specifically, those variables with a level of significance smaller than 0.25 were further considered. This arbitrary threshold was chosen in accordance with standard epidemiological practices (Hosmer & Lemeshow, 1989). Multivariate analyses started with the model specifying the retained main effects

and their first-order interactions. Through backward selection, the interaction or main effect term that was the least significant (i.e. highest P-value in Type3 contrasts, yet still at a >0.05 level) was subsequently eliminated. The procedure stopped when no term or effect in the model exceeded the 0.05 significance level. IRR were calculated through exponentiation of the parameter estimates.

Goodness-of-fit for the final model was investigated through residual analysis. The transformation of the dependent variable that is closest to normality and is standardized to mean 0 and variance 1 defines the Anscombe residual (Cameron & Trivedi, 1998). For a Poisson-distributed variable, the transformation $y^{2/3}$ is closest to normality, and the Anscombe residual is calculated as:

$$a_i = \frac{1.5 * \left(y_i^{2/3} - \hat{y}_i^{2/3} \right)}{\hat{y}_i^{1/6}}$$

where y_i is the observed value for a specific combination of levels of the explanatory variables, and $\hat{y}_i$ is the relative predicted value. Plots of the Anscombe residuals against the observed number of cases and levels of the explanatory variables were used to assess the goodness-of-fit of the final models. The normality of the residual was checked through the Kolmogorov-Smirnov test and normal probability plots.

3.4 Results

With regard to the Poisson distribution, the counts of reported foodborne infections show a large degree of overdispersion (Table 3.2). Depending on pathogen and whether data are collated by age or gender, the observed variance is 34 to 84 times larger than the mean. Table 3.3 shows that there are 56 clusters in the data set AGE (age-state strata) and only 14 in the data set GENDER (gender-state strata). Missing observations are present since data for Maryland and New York are unavailable for the year 1996 and 1997. Cases of foodborne infections for a given covariate-state stratum are correlated from one year to another. In the data set AGE, Spearman rank correlations for age-state observations range between 0.70 and 0.91 (Figure 3.1 to Figure 3.3). In

contrast, within the data set GENDER and for the *Salmonella* and *Shigella* data, the level of correlation for gender-state observations appears to regularly diminish with increasing time between measurements of a given cluster (Table 3.4). This pattern is not evident for *Campylobacter* rates, which have a correlation of 0.79-0.92.

3.4.1 Interpretation of Graphics

Graphical representation of the rates offers insight into potential interactions among covariate (age/gender), place (state), and time (year). Figure 3.4 through Figure 3.18 systematically contrast two variables by stratifying for the third one. In each figure, the upper and lower series of graphs essentially show the same information, where the levels of the x-axis variable in the upper series become the lines in the lower set of graphs, and vice versa. Horizontal lines imply that the x-axis variable has no influence on the infection rates; vertical distance among the lines reflect the effect of the other variable. Lines that are parallel between two subsequent levels of the x-axis variable suggest lack of interaction between the two variables. Such parallelism should be evident in both series of graphs. If a similar pattern of lines emerges within each series of graphs, one would infer that infection rates are not affected by different levels of the stratifying variable. The effect of age (data set AGE) is presented first in order for *Campylobacter*, *Salmonella*, and *Shigella* rates.

Rates of *Campylobacter* infection are higher in infants (children less than one year of age) and in adults of 20-29 years of age, and appear fairly similar for the other age groups (Figure 3.4, upper series of graphs). While this pattern is particularly marked for California, the increased risk for infants at that site seems to have diminished from 1996 to 1998. The lower series of graphs shows that, although most evident for infants, California tends to have the highest infection rates across all age groups, and Georgia and Maryland the lowest. Figure 2.21 reaffirms the higher rates for infants and young adults, and suggests that the surveillance year did not affect such a pattern. The exception is California, where the spread among rates of the age groups has gradually diminished between 1996 and 1998 (lower set of graphs). Figure 3.6 shows once again a higher risk for California, but the difference among States is smaller for age groups other than

infants. Overall, Figure 3.4 to Figure 3.6 reveal that *Campylobacter* infection rates are increased for infants and young adults. While the frequencies depend on site, a decline in infection rates over the years is only apparent for California. From these figures, first-order and second-order interactions cannot be excluded.

Rates of *Salmonella* infection are the highest for children less than 1 year of age (Figure 3.7). Frequencies for the age groups 1-9 and 20-29 could possibly be higher than those of the remaining age categories. The lower series of graphs shows that infection rates vary among States only for infants. Unaffected by the surveillance year, Georgia has the highest rates among infants, followed by Maryland and California. For the other age groups, the infection rates are fairly constant across sites. Figure 3.8 confirms the highest risk for infants, but also suggests that such risk can vary across surveillance years in a unpredictable manner (lower set of graphs, e.g. constant decline for California, decline and surge for Georgia, increase for New York). Dependence of *Salmonella* infection rates in infants from the variable state and – to a lesser extent – year is again evident in Figure 3.9. However, this figure clearly shows that, for all other age groups, the frequencies are largely unaffected by those two variables. Overall, Figure 3.7 to Figure 3.9 show that *Salmonella* infection rates are higher in infants than in other age groups. However, location (state) and time (year of surveillance) influence the specific risk in infants, which would suggest interaction of the considered variables.

The dependence of *Shigella* infection rates on age, state, and year is more complex to characterize than that of the two previous pathogens. Generally speaking, Figure 3.10 suggests that rates are increased for age between 1 and 9 years and – for some States – between 20 and 39 years. Georgia and California appear to pose higher infection risk. However, these trends vary depending on the surveillance year. For instance, a peak in the age group 1-9 is absent in two States, where infants were actually at increased risk. For 1999, Minnesota displays a greater risk than that of Georgia in two age groups. The lower set of graphs of Figure 3.11 shows that infection rates for children less than 10 years of age plummeted in Georgia between 1998 and 1999. The risk for adults of the age groups 30-39 has decreased over the surveillance years in California,

while the risk in infants momentarily dropped in 1998. Keeping in mind that only data for 1998 and 1999 are available for Maryland and New York, infection rates at these two sites seem to be relatively unaffected by the variables age and state (the rates in New York infants dropped from 1998 to 1999, thought). Figure 3.12 shows once more that, for given States and years, the rates of *Shigella* infection can be increased. Infection frequency is particularly elevated in Georgia in children of less than 10 years of age. In contrast, California seems to have a moderately increased risk that is constant through the years and age categories. The lower series of Figure 3.12 makes evident that variation from state and year becomes far less important for an age greater than 10 years. Overall, Figure 3.10 to Figure 3.12 show that, while an age between 1 and 9 years generally represents an increased risk for *Shigella* infection, both state and year can play a role as well. This circumstance suggests first- and second-order interactions.

Figure 3.13 to Figure 3.18 investigate the influence of gender on *Campylobacter*, *Salmonella*, and *Shigella* infection rates. The frequency of *Campylobacter* infection tends to be lower in females than males (Figure 3.13, upper series of graphs). However, with perhaps the exception of California, the difference is minimal (if at all present). This effect of gender essentially remained unaltered over the four years of the surveillance (Figure 3.14). The influence of gender on *Salmonella* rates does not follow a clear pattern (Figure 3.15). Depending on the State, the frequencies for females can either be higher, equal or lower. The same consideration is true when the combined effects of age and surveillance year are combined (Figure 3.16). Rates of *Shigella* infection for females and males appear to be equal across most of the States (Figure 3.17). However, similar to *Campylobacter*, females in California seem to be consistently at a lower risk than males. This remark is true for the four surveillance years (Figure 3.18). Overall, differences in rates of *Campylobacter*, *Salmonella*, and *Shigella* infection between the two genders are small. Nevertheless, females in California display a lower risk than males for both *Campylobacter* and *Shigella*. Of the four variables considered in this study (age, gender, state, year), the influence of gender appears to be the smallest.

3.4.2 Analytical Results

The first analytical results are incidence rates for each surveillance year estimated from the data set AGE (Table 3.5). The second column of Table 3.5 presents "observed crude rates", i.e. rates that were calculated based on the number of reported cases with known age and the population in catchment areas as reported in each FoodNet Report for the years 1996 to 1999. (These figures differ from those reported in Table 3.1, where rates for only the five original sites are reported and cases with unknown age are also considered.) "Estimated crude rates" were estimated with a model specifying only year as explanatory variable, and can be directly compared to the observed ones. While, as one would expect, the point estimates are equal, the analytical approach delivers confidence limits (columns 4 and 5 of Table 3.5), and allows one to formally contrast the single estimates. With exception for the contrast of the years 1996 versus 1997 ($p=0.319$), all *Campylobacter* crude rates are statistically different (1996 vs. 1998, $p=0.011$; all other contrasts, $p<0.001$). This means that the decreases in annual *Campylobacter* rates from 1997 to 1998 and from 1998 to 1999 are statistically significant. All possible contrasts of the annual *Salmonella* rates are not significant ($p\geq0.119$), i.e. the annual *Salmonella* rates are similar in statistical terms. Only the *Shigella* rates for year 1999 differ statistically from those of the previous years ($p=0.010$-0.035). This indicates that *Shigella* rates in 1999 are significantly lower than in the previous three years.

By fitting a model with all three main effects (i.e. age, state, year), "age-state adjusted rates" were obtained (columns 6-8 of Table 3.5). While they can no longer be compared to the observed crude rates, these adjusted rates reflect the specific impact of time since age and state effects are controlled for. The adjustment leads to minor modification of the previous conclusions. Based on formal contrasts, the 1996 adjusted *Campylobacter* rates are statistically equal to those of 1997 and 1998 ($p=0.072$ and $p=0.147$, respectively). All other *Campylobacter* rates remain significantly different ($p<0.001$), specifically the 1999 rates are lower than those of the previous years. The *Salmonella* adjusted rates for 1998 become lower than those of 1996 and 1999 ($p=0.012$

and p=0.019, respectively), but the other contrasts are statistically indistinguishable ($p \geq 0.064$). For the *Shigella* adjusted rates, the only difference is that the 1999 rate is no longer statistically lower than that of 1998 (p=0.081). Noteworthy is also the fact that, at least for *Campylobacter* and *Shigella* rates, the estimates are more efficient (i.e. smaller standard errors as evidenced by narrower confidence intervals). This implies that addition of the variables age and state permit a more precise quantification of the time effect.

The IRR for the univariate and multivariate analyses of the data set AGE are reported in Table 3.6 through Table 3.9. In these tables, the arbitrary reference levels of each variable are the age category between 20 and 29 years, site Oregon, and surveillance year 1999 (Oregon in 1999 for Table 3.7). Where the confidence interval does not cover the value 1.00, there is a conservative indication (i.e. not based on specific contrasts as in the previous paragraph) that the given level differs statistically from the reference level. In the univariate analysis (columns 2-4 of Table 3.6), an age of less than 1 year represents an increased risk of *Campylobacter* infection, while the age groups 10-19 and ≥60 are at a lower risk. California is linked to a greater risk, but Georgia and Maryland show a reduced risk. Finally, year 1999 is associated with a lower risk of *Campylobacter* infection than the previous three years. (The IRR for the year effect from the univariate analyses reported in Table 3.6, Table 3.8, and Table 3.9 are equivalent to the ratio of the rates listed in Table 3.5). As the age, state, and year effects are all statistically significant at a level <0.25 in the univariate analysis (p=0.073, p=0.005, and p<0.001, respectively), the three main effects as well as the three first-order interactions were the basis for the backward selection in the multivariate analysis. Eventually, the three main effects and the first-order interaction between state and year were statistically significant at a level <0.05 (main effects p<0.001; state*year p=0.013). As evident from the results of the variable age, the more complex model has little effect on the point estimates of the parameters. However, there is a gain in estimator efficiency as indicated by the narrower confidence intervals. While the age group <1 remains at an increased risk of *Campylobacter* infection, the age group 20-29 is associated in the multivariate analysis

with a significantly greater risk than the remaining six age categories. Although a detailed discrimination would preferably require specific contrasts between specific age groups, the age group 10-19 has the lowest risk and people older than 60 years the second lowest risk. Based on the confidence intervals, these results are statistically significant. Age groups 1-9 and 30-39 have likely similar risk, so do people between 40 and 59 years of age. Owing to the significance of the interaction between state and year, these two variables need to be cross-tabulated (Table 3.7). These results are in close conformity to what one would expect based on the results of the univariate analysis. In comparison to the *Campylobacter* infection risk experienced in Oregon during 1999, the four estimates for California reflect a greater risk. However, California's risk has almost halved over the 4-year period. The rates for Georgia and Maryland in 1999 were lower. Finally, risks experienced in Oregon in 1996, 1997, and 1998 were higher than in 1999. In other words, the multivariate analysis gives a more detailed insight into the role of the variables state and year than that offered by the univariate analysis. The goodness-of-fit of the final multivariate model for the *Campylobacter* data is satisfactory. No particular pattern is evident from the graphical representations of the Anscombe residuals (Figure 3.19). Also, these residuals appear normally distributed with mean 0.044 and standard deviation 1.265 (Figure 3.20, Kolmogorov-Smirnov statistics $p>0.15$).

The relative risks of *Salmonella* infection are presented in Table 3.8. In the univariate analysis, an age of 9 years or younger is linked to a statistically greater infection risk than the reference age group 20-29. With the exception of the age group 30-39, the remaining age groups are at a lower risk. In contrast, all levels of the variables state and year appear to represent an indistinguishable risk of *Salmonella* infection. Overall, the age and year effects are statistically significant at a level <0.25 in the univariate analysis ($p=0.038$, $p=0.202$, respectively). The state effect largely exceeds that threshold ($p=0.567$), and had to be excluded from the multivariate analysis. Completion of the backward selection procedure led to a final model that only contains age as an explanatory variable. This is equivalent to a univariate analysis with that variable. The goodness-of-fit of such a model is passable. The graphical representations of the

Anscombe residuals show that, although the fit is not particularly good for the variables excluded from the model (state and year), a pattern is absent for age, i.e. the main variable of interest (Figure 3.21). Specifically, the residuals seem to be normally distributed with mean -0.047 and standard deviation 2.367 (Figure 3.22, Kolmogorov-Smirnov statistics $p>0.15$). The left-hand, upper graph of Figure 3.21 indicates that there are at least two outliers. Refitting the model upon deletion of all observations that generate Anscombe residuals greater than 2.6 does not sensibly change the values of the parameters (results not shown). It is thus concluded that outliers did not have particular leverage on parameter estimates.

Table 3.9 reports the IRR resulting from the analysis of the *Shigella* data. In the univariate analysis, the category between 1 and 9 years of age shows a significantly increased infection risk when compared to the reference age group 20-29. In contrast, an age between 10 and 19 years and greater than 50 years is associated with a lower risk. California is the only site that appears to have a statistically different risk of *Shigella* infection, specifically an increased one. Further, risks for the years 1996, 1997, and 1998 were significantly increased with respect to year 1999. As the age, state, and year effects are all statistically significant at a level <0.25 in the univariate analysis ($p=0.062$, $p=0.183$, and $p=0.024$, respectively), the backward selection in the multivariate analysis started from the model with the three first-order interactions. The procedure resulted in a model with the two main effects for age and state ($p=0.027$ and $p=0.025$, respectively). While the point estimates for the levels of the age variable are fairly similar for the univariate and multivariate analyses, the estimates for the variable state are perceptibly lower for the levels California and Georgia. The estimators are more efficient for both the variable age and the variable state. While an age between 1 and 9 years remains associated with an increased risk of *Shigella* infection, infants (less than 1 year of age) are also at increased risk with respect to the reference group (age 20-29) according to the multivariate analysis. However, infants' risk is half that of the 1-9 age group. Age between 10 and 19 years and greater than 40 years is associated with a lower risk. The risk for persons older than 60 years seems to be significantly smaller than that of at least

6 out of the 7 other age groups (the exception is age group 50-59). Increased efficiency of the estimators leads us to reassess the influence of the variable state. With respect to Oregon, California and Georgia display an increased risk of *Shigella* infection, while Connecticut, Maryland, and New York are associated with a lower risk. The goodness-of-fit of the final multivariate model for the *Shigella* data is questionable. Although the variable age and state are included, residuals for observations relating to the age group 1-9 and the site Georgia show a much greater dispersion than those of the other levels (Figure 3.23). A decreasing pattern is evident for increasing surveillance year. Furthermore, it cannot be concluded that the residuals are normally distributed (Figure 3.24, Kolmogorov-Smirnov statistics $p<0.01$). While the exclusion of observations with an Anscombe residual >2.6 sensibly affect the values of the parameter estimates, the general inferences on the influence of age and state does not change. In order to obtain a satisfactory goodness-of-fit, a model with all three first-order interactions would be necessary.

In the univariate analysis of the data set GENDER, the gender effect resulted in significance levels that were above the 0.25 significance threshold for further consideration in a multivariate analysis (*Campylobacter*, p=0.373; *Salmonella*, p=0.868; *Shigella*, p=0.783). Nonetheless, females would seem to have been, over the period 1996-1999, at a lower risk of contracting a foodborne infection than were males. The relative IRR were: *Campylobacter* 0.80 (95% confidence interval: 0.49-1.28); *Salmonella* 0.98 (0.81-1.20); *Shigella* 0.91 (0.48-1.73).

3.5 Discussion and Conclusion

Children, elderly, pregnant women, and immunocompromised persons are commonly perceived to be at the greatest risk of serious illness and mortality from food- and waterborne enteric microorganisms (Gerba et al., 1996; Smith, 1998; Smith, 1999). Analogously, a main interest of this study was to investigate the potential influence of the covariates age and gender on the incidence rates of *Campylobacter*, *Salmonella*, and *Shigella* infection recorded through the FoodNet surveillance system. Generally speaking, indications obtained from the graphical representation of the rates (Figure 3.4

through Figure 3.18) find confirmation in the statistical analysis (Table 3.6 through Table 3.9). Results are discussed first for the effect of age in order for *Campylobacter*, *Salmonella*, and *Shigella*.

In our results, infants (children of less than 1 year of age) show *Campylobacter* incidence rates that are 2-3 times greater than those of older people (Table 3.6). The second highest risk is found to be associated with an age between 20 and 29 years. A higher incidence among infants and young adults has been documented earlier. Referring to passive surveillance data collected in the United States between 1982 and 1986, Tauxe et al. (1988) described a bimodal distribution for *Campylobacter* isolates with the highest rates in infancy and a second increase in the early adulthood. Skirrow (1987) proposed that *Campylobacter* generates a similar pattern (bimodal distribution with peaks in children younger than 4 years of age and young adults) in developed countries. Different reasons are commonly advanced to explain the higher *Campylobacter* infection rates in those two age groups. The elevated risk in children has been attributed to susceptibility on first exposure, a higher degree of case ascertainment, and/or a more frequent exposure to pets (Altekruse et al., 1999; Salfield & Pugh, 1987). In contrast, increased infection rates in young adults has been explained with more frequent travel abroad (Kapperud & Aasen, 1992) and recreational activity that would also include aquatic sports (Skirrow, 1987). Further, it is thought that young adults are more frequently exposed to high-risk food items, especially due to their poor food handling practices (Tauxe, 1992; Altekruse et al., 1996). Men are more likely to report such mishandling (Altekruse et al., 1999).

Our results point to a second consideration that has not been documented earlier. Specifically, people that are older than 60 years or in the 10-19 age group show significantly lower rates of *Campylobacter* infection. Teenagers actually have the lowest incidence of all age groups. A closer look at the results of the multivariate analysis (Table 3.6) actually discloses that these two age groups seem to be the endpoints of declines in *Campylobacter* incidence that originated in early infancy and adulthood, respectively. It has been noted that, in spite of allegedly frequent reinfections, adults are often asymptomatic in developing countries (Newell & Nachamkin, 1992). Under these

conditions, an increase in antibody levels directed against *Campylobacter jejuni* has been observed as people aged (Blaser et al., 1986). It has thus been proposed that the development of a protective immune response in developing countries is the result of a persistent exposure to multiple strains (Newell & Nachamkin, 1992). Similarly, one could advance that the two-phase declines in *Campylobacter* infection rates observed in our multivariate analysis reflect exposure to two different populations of the pathogen – one present since very early on and another arising during adolescence. Study of the specific nature of *Campylobacter* isolates could test this hypothesis.

Analysis of *Salmonella* rates show that infants have a 9-fold greater risk of infection than young adults (Table 3.8). Rates for the age group 1 to 9 are twice those of the reference age groups. In contrast, individuals between 10 and 19 years of age and older than 40 years display comparable, lower rates. These results are in accordance with available literature. For instance, 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). 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 in a British population-based study (Banatvala et al., 1999). Similar to *Campylobacter*, the association of age with increased *Salmonella* rates might have several explanations. It has been proposed that children and the elderly with diarrhea are more frequently cultured than other age groups (Banatvala et al., 1999). Further, age influences the relative exposure to specific serovars. This reason possibly explains an increased risk of infection with resistant *Salmonella* that was observed in infants (Lee et al., 1994). Moreover, age association may reflect behavioral characteristics. For instance, eating snow, sand, or soil – a behavior more likely in children – was found to be associated with *S. typhimurium* O:4-12 infection (Kapperud et al., 1998).

Children between 1 and 9 years of age show the highest incidence rates for *Shigella* infection (Table 3.9). In comparison to young adults (20-29 years of age), they are about three times more likely to experience an infection. Infants also appear at

increased risk. People older than 60 years of age display the lowest risk, while age groups 10-19 and 40-49 also have lower infection rates. The increased incidence in children and young adults (20 to 39 years of age) is commonly acknowledged. The vast majority of shigellosis cases in the United States are linked to *Shigella sonnei* outbreaks in child care centers and other settings where maintenance of good hygienic conditions requires special care (CDC, 2001b). Accordingly, CDC views toddlers aged 2 to 4 and their families as the most commonly affected population segment (CDC, 2000e). Higher shigellosis rates has been reported in Iowa for females that are 20-29 years old (Moyer, 2001). The primary role of young women as childcare providers was advanced to explain such an observation. As inferred from passive surveillance data, the greatest increase in isolation rates for *Shigella* during the period 1966-1988 in the United States was in persons that were older than 20 years of age (Lee et al., 1991). Such observation was interpreted to indicate a shift toward infection at older ages. While the graphical representation of the *Shigella* infection rates does not indicate such a trend (Figure 3.11), the limited timeframe under consideration (4-year period from 1996 to 1999) is unlikely sufficient to make such an inference.

Our analysis points to a potentially lower infection risk for females than for males: IRR range from 0.98 for *Salmonella* to 0.80 for *Campylobacter*. However, the differences were not statistically significant. When numbers of *Salmonella* isolates are compared, a male-to-female ratio of 1:1 has been reported on various occasions (Blaser & Feldman, 1981; Le Bacq et al., 1994; Wong et al., 1994). Seemingly, the significance of such a finding has not 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 case of *Campylobacter*, FoodNet reports point out that *Campylobacter* incidence rates for males tend to be higher than those for females in all age groups (CDC, 2000c; CDC, 2000d). This consideration is consistent with an earlier conclusion based on data for the period 1982-1986 (Tauxe et al., 1988). In countries other than the United States, a greater risk of campylobacteriosis has been reported for young boys than for young girls (Skirrow, 1987; Kapperud et al.,

1992). However, this difference appears to vary among countries (CAC, 2001). As discussed below, a limitation of our results is the inability to test for the compounded effect of gender and age. The significance of the observation that, in California, the *Campylobacter* and *Shigella* rates were consistently lower for females than males (Figure 3.14 and Figure 3.18) remains unclear.

The justification for carrying out an analysis that also considers the effects of location and time (variables denominated state and year) is that the obtained estimates are covariate-specific. By controlling for state and surveillance year, one more efficiently assesses the underlying effect of age or gender. (According to the classification by Diggle et al. (1994), the GEE method is actually an example of so-called marginal models, i.e. models whose regression parameters reflect population-averaged effects.) While not the primary interest of this study, some considerations concerning the effects of site and year of surveillance are noteworthy. As for the variable state, the increased rates experienced in California for all three pathogens under consideration are intriguing. For both *Campylobacter* and *Shigella* (the two pathogens for which the variable state appears to have a statistically relevant influence), the infection rates in California are 3 to 6 times greater than those recorded for other sites. (As noted in the previous paragraph, the difference may be greater for males than for females. Overall, Georgia shows a similar risk of *Shigella* infection for the years 1996, 1997, and 1998.) This increased risk may be real rather than the consequence of heightened surveillance intensity. Firstly, California rates for other pathogens are in line with those of other sites. The same can be said for the number of outbreaks with 10 or more ill persons reported for California in 1998 and 1999 (CDC, 2000c; CDC, 2000d). As the median number of ill persons in the two 1999 California outbreaks is comparable to that observed at other sites, one can exclude that large outbreaks caused the elevated rates. Finally, although there is a downward trend, rates were elevated in all four years under consideration. It thus appears that the increased rates observed for California reflect an underlying, widespread risk of *Campylobacter* and *Shigella* infection. The meaning of this piece of evidence is unclear. One could advance that a greater environmental threat, such as waterborne transmission,

exists in California. Wildlife – particularly wild birds – is involved in the ecology of *Campylobacter jejuni*, and the microorganism is found in natural water sources throughout the year (Altekruse et al., 1999). Seven out of 57 *Campylobacter* outbreaks reported in the United States during the period 1978-1986 were actually linked to a common exposure through the community water supply (Tauxe, 1992). One such outbreak affected an estimated 3,000 people (Vogt et al., 1982). Nonetheless, *Campylobacter* cases are far more likely to be sporadic. As much as 85% of the cases are thought to be the outcome of a foodborne exposure (Mead et al., 1999), especially from the consumption of poultry meat (Tauxe, 1992). Perhaps, poultry meat marketed in California is more likely contaminated or is contaminated with more virulent *Campylobacter* strains than that of other sites. Finally, one has to be aware that the variable state does not only represent geographical location. It also reflects demographic factors and behavioral traits that are specific for a given site and that are not specifically controlled for in the analysis. It is perhaps not coincidental that *Shigella* rates are also elevated for California. Humans are the natural reservoir for this pathogen species, and shigellosis cases are closely associated with specific behavioral traits (CDC, 2000e). Once more, this final hypothesis stresses the importance of a multivariate analysis not only to properly characterize the factors influencing the observed infection rates, but also to ultimately point to possible mitigation strategies (e.g. educational campaigns).

As for the effect of surveillance year, the results of Table 3.5 suggest that the *Salmonella* rates remained unchanged over the period from 1996 to 1999. In contrast, the *Campylobacter* rates statistically decreased in 1999 from the previous years, and *Shigella* rates for 1999 were significantly lower than those for 1996 and 1997. However, the relevance of this statistical finding ought to be reconsidered in light of the fact that rates for both *Campylobacter* and *Shigella* increased considerably from 1999 to 2000 (Table 3.1). As data for year 2000 were not yet available for our analysis, the statistical meaning of this increase could not be established. Also, it should be recognized that national figures essentially result from the aggregation of site-specific data. For example, the 1999 FoodNet report asserts that there was a decline in *Campylobacter* infection rates

from 1996 to 1999 (CDC, 2000d). As shown in Table 3.1, such a diminution is essentially linked to a decrease that only occurred in California. A decrease is actually absent for the four other original sites. Unless a trend is consistent across sites, conclusions about national trends do not seem to be warranted. Generally speaking, it would seem that detection of temporal trends has been the main expectation of the FoodNet surveillance data. For instance, the majority of the key findings listed in the 1999 FoodNet report highlight decreases in the incidence of several foodborne pathogens (CDC, 2000d). Over the last decade, there has been an increasing awareness about the public health role of foodborne illness of which President Clinton' Food Safety Initiative is perhaps the most prominent manifestation. From a regulatory perspective, this has translated into regulations, such as through the HACCP Rule, that target the food industry. Understandably, federal agencies are eager to prove the efficacy of the enacted measures. It is a distinct duty of public health professionals to step back and question the significance of short-term improvements. From a public health perspective, drawing premature conclusions that ultimately would turn out to be spurious can only provide a false sense of security. Worse, it can prevent the complementation of current measures with targeted efforts that could better address the multifaceted farm-to-fork continuum.

The results of this study are potentially affected by limitations that concern both the data and methodology used. In the food safety arena, a commonly held perception is that, among others, children and the elderly are more susceptible to foodborne pathogens (Gerba et al., 1996; Smith, 1998). It is not always made clear whether that statement implies susceptibility to illness, infection, or both. Within the context of FoodNet surveillance, a similar ambiguity exists. A case is defined as the first isolation of an enteric pathogen from a given person (CDC, 2001a). While this definition would only reflect infection, it is also thought that, since data are collected through clinical laboratories, most cases also represent a clinical illness. The ambiguity is the extent to which calculated rates represent risk of illness in addition to risk of infection. While our results (and, in general, the literature) point to a higher risk in children, no specific increased risk for the elderly is evident from the FoodNet data. Mins et al. (1995) point

out that the evidence for a general reduction in resistance to infectious disease in elderly people is weak. It may be that the nature of the risk is different in children and the elderly. Children could be more susceptible to both infection and illness (due to a naïve immunity), while the elderly could merely be more likely to incur in a serious illness (due to physical and physiological reasons). As the considered data do not differentiate among degrees of illness severity, our analysis could not have identified the kind of risk typical for the elderly. It has been advanced that case ascertainment in children and, possibly, the elderly is relatively more efficient than that of other age groups (Tauxe, 1992; Banatvala et al., 1999). This eventually would lead to overestimate infection rates for children and elderly.

A further limitation arises from the age classification used in the FoodNet reports. In particular, the age groups comprising children of 1 to 9 years of age and adults older than 60 years may reflect a greater heterogeneity in susceptibility to enteric infection/illness than the remaining age groups. Under these conditions, grouping into a unique age category essentially implies an averaging of the risk over the respective age span. For example, in the case of *Shigella* data, the age group comprising the 1 to 9-year-olds shows the highest rates. As the majority of *Shigella* cases are linked to child care centers (CDC, 2001b), the age classification used in FoodNet potentially causes an underestimation of the risk for children between 1 and 5 years of age. In contrast, the risk for 6 to 9-year-old children would be overestimated. Within the age group ≥60, this issue is potentially greater because increasing age is associated with a diminishing statistical weight of the relative age stratum. That is, even if older people (e.g. older than 80 years of age) had a much increased risk, their numerical proportion would be too limited for the effect to become apparent. This phenomenon alone could explain why, contrary to expectations, no increased rates for the elderly were found in our analysis.

A potential limitation is that, since cross-tabulated data for age and gender are unavailable, the cumulated effect of age and gender cannot be tested. FoodNet reports mention that the incidence rates of *Campylobacter* were higher for males than for females in all age groups (CDC, 2000d). In contrast, *Salmonella* rates were higher for either

males or females depending on age group. As mentioned above, an increased incidence of *Shigella* in young women has been reported in Iowa (Moyer, 2001). As the influence of the age effect seems to be relatively large, this shortcoming could possibly affect only the interpretation of the gender effect. Our results suggest that males are overall at a higher, but not statistically significant risk than females. However, it cannot be excluded that a decreased risk for males in specific age categories is balanced by an increased risk in other age groups.

Undoubtedly, the GEE method has theoretical appeal when it comes to the analysis of surveillance data. Nonetheless, some practical aspects of its implementation are less established than under the MLE framework. Our analysis required making choices regarding the inference and goodness-of-fit tests to be employed. Specifically, the score statistic was used to evaluate the significance of explanatory variables (i.e. Type3 contrasts). This statistic tends to be more conservative than the Wald statistic (Stokes et al., 2000). Had the latter statistics been chosen, more complex models, i.e. models that would contain more interaction terms, would have resulted (results not shown). The graphical representation of the infection rates seems to indicate that such interactions are present. Rather than predictive, the intent of our study was explanatory, i.e. the emphasis was on revealing underlying influences on the modeled variables. This justifies having opted for the less sensitive, yet more specific test.

The model building strategy used in this study did not always lead to adequately fitting models. While goodness-of-fit is acceptable for the *Campylobacter* and *Salmonella* models, the selected model for the *Shigella* data shows a poor fit. The accuracy of the *Shigella* risk estimates is thus less acceptable. Interestingly, for *Shigella* data, only a model that specifies all three first-order interactions would lead to a passable fit.

A varying dependence on outbreak cases distinguishes the quantitative occurrence of the three pathogens under study. On the one extreme, there is *Campylobacter* for which the vast majority of cases are sporadic (Tauxe, 1992). To the other extreme, outbreaks contribute significantly to the overall shigellosis incidence. For example,

outbreaks that occurred in Minnesota and California were the main cause of the substantial increase in shigellosis observed from 1999 to 2000 (CDC, 2001a). The framework of the Poisson distribution is one of rare and random events. Although the difference between sporadic and outbreak cases is admittedly arbitrary, it would seem that cases linked to outbreaks are no longer compatible with a Poisson process. For pathogens like *Shigella*, for which outbreaks drive the number of reported cases, the implementation of more complex models should probably be evaluated. Conversely, one could argue that sporadic and outbreak cases reflect different mechanisms of occurrence, respectively a low-dose, widespread exposure versus a high-dose, punctual exposure. The two mechanisms would likely have different implications in terms of food safety measures. A discrimination of sporadic and outbreak cases in the FoodNet data would set the stage to investigate this hypothesis.

In conclusion, the FoodNet surveillance system is expected to provide epidemiological information important to the food safety policy-making process (Binder et al., 1998). While FoodNet undoubtedly represents a qualitative improvement upon previous passive surveillance systems, it would seem that too much emphasis has so far been put on the data themselves. The issue of how such information ought to be analyzed and interpreted remains largely uncharted. For instance, the 1999 FoodNet report concluded that a decline in *Campylobacter* infection rates observed for the period from 1996 to 1999 was likely related to changes in poultry processing plants due to the HACCP rule (CDC, 2000d). In 2000, *Campylobacter* rates surged from the low of 1999 (CDC, 2001a). The extent to which core facts – whether temporal trends or host characteristics – are teased out from a noisy, multifaceted background largely depends on the availability of an adequate analytical framework.

This study proposed and investigated the application of the Poisson regression model estimated by means of GEE. From a theoretical perspective, such a statistical approach permits a multivariate analysis while respecting the intrinsic characteristics of the FoodNet data. In practical terms, however, its implementation, such as establishing a model's goodness-of-fit, is not yet fully established. The question is not so much whether

the identified, underlying findings are in accordance with the expectations; our results are generally consistent with available literature. The main issue is rather whether the quantitative estimates are sufficiently reliable. Indeed, this is the main justification for a statistical analysis. While the application to the *Campylobacter* and *Salmonella* data is considered adequate, that to the *Shigella* data is less so. What sets *Shigella* apart from the two other pathogens is that outbreaks due to non-foodborne transmission predominantly shape its epidemiological characteristics. The potential inability to accommodate spikes in cases typical of outbreaks has to be viewed as the primary limiting factor for the general applicability of the proposed method to other enteric pathogens.

3.6 References

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3.7 Appendix: Description of Data Sets

Data set	Variables	Attributes
Both data sets	Pathogen	Nominal (3 values): Campylobacter, Salmonella, Shigella
	Year	Discrete (4 values): 1996, 1997, 1998, 1999
	State	Nominal (7 values): California, Connecticut, Georgia, Maryland, Minnesota, New York, Oregon
	Case	Positive discrete value
	Population at risk	Positive discrete value
Data set AGE only	Age category	Nominal (8 values): less than 1 year of age, 1 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, greater than 60
Data set GENDER only	Sex	Nominal (2 values): female, male

Table 3.1. Infection rates per 100,000 at the five original FoodNet sites, 1996-2000 (CDC, 2001a)

Pathogen	1996	1997	1998	1999	2000
Campylobacter	23.5	25.2	21.4	17.5	20.1
Cryptosporidium	--	3.7	2.9	1.8	2.4
Cyclospora	--	0.4	0.1	0.1	0.1
Escherichia coli O157	2.7	2.3	2.8	2.1	2.9
Listeria	0.5	0.5	0.6	0.5	0.4
Salmonella	14.5	13.6	12.3	13.6	12.0
Shigella	8.9	7.5	8.5	5.0	11.6
Vibrio	0.2	0.3	0.3	0.2	0.3
Yersinia	1.0	0.9	1.0	0.8	0.5

Table 3.2. Mean and variance of reported cases

Dataset - Pathogen	Mean	Variance	Variance/Mean
Data set AGE			
- *Campylobacter*	79	2687	34
- *Salmonella*	58	2019	35
- *Shigella*	26	2002	77
Data set GENDER			
- *Campylobacter*	316	21993	70
- *Salmonella*	237	20016	84
- *Shigella*	105	6734	64

Table 3.3. Number of clusters and observations in data sets

Characteristics	Data set AGE	Data set GENDER
Number of clusters	56	14
- with 4 observations	40	10
- with 2 observations	16	4
Number of observations	224	56
- not missing	192	48
- missing	32	8

Notes: cluster, the crossing of a given covariate level (age category, gender) and state; observation, the measurement in a given year relating to a cluster; incomplete cluster / missing observations because data on Maryland and New York only available for 1998 and 1999.

Table 3.4. Spearman correlation matrix of reported rates lagged within each gender-state stratum

(Data set GENDER)	Reference year	Lag 1	Lag 2	Lag 3
Campylobacter				
- Reference year	1.00			
- Lag 1	0.85	1.00		
- Lag 2	0.79	0.82	1.00	
- Lag 3	0.87	0.92	0.90	1.00
Salmonella				
- Reference year	1.00			
- Lag 1	0.63	1.00		
- Lag 2	0.56	0.91	1.00	
- Lag 3	0.41	0.85	0.88	1.00
Shigella				
- Reference year	1.00			
- Lag 1	0.75	1.00		
- Lag 2	0.51	0.76	1.00	
- Lag 3	0.20	0.55	0.81	1.00

Table 3.5. Annual incidence rates (per 100,000) for *Campylobacter*, *Salmonella*, and *Shigella* reported cases

Pathogen - Year	Crude rates	Crude rates (model with year effect)			Age-state adjusted rates (model with all main effects)		
	Observed	Estimated	LCL	UCL	Estimated	LCL	UCL
Campylobacter							
- 1996	23.5	23.5	19.2	28.8	30.7	27.3	34.6
- 1997	24.7	24.7	21.0	28.9	33.4	30.4	36.7
- 1998	19.4	19.4	17.0	22.1	28.4	26.2	30.8
- 1999	14.5	14.5	12.3	17.1	22.8	20.5	25.3
Salmonella							
- 1996	14.5	14.5	12.1	17.3	11.3	8.4	15.2
- 1997	13.7	13.7	11.4	16.5	10.6	7.8	14.3
- 1998	13.7	13.7	11.4	16.4	10.2	7.6	13.6
- 1999	15.2	15.2	11.9	19.4	11.3	8.4	15.1
Shigella							
- 1996	8.9	8.9	5.4	14.6	5.5	4.6	6.7
- 1997	7.9	7.9	4.6	13.5	4.9	4.1	5.9
- 1998	7.1	7.1	4.6	11.1	5.0	4.2	6.0
- 1999	3.8	3.8	2.7	5.4	2.4	1.3	4.3

Legend: Observed, direct calculation based on number of cases with known age listed in FoodNet Reports 1996 to 1999; Estimated, predicted through Poisson regression with mentioned model; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit.

Table 3.6. Incidence rate ratio for *Campylobacter* (Poisson model with generalized estimating equations, independent correlation structure)

Parameter	Univariate analysis			Multivariate analysis		
- Level	IRR	LCL	UCL	IRR	LCL	UCL
Age						
- <1	1.88	1.05	3.34	1.87	1.72	2.04
- 1-9	0.86	0.49	1.51	0.85	0.77	0.94
- 10-19	0.42	0.25	0.73	0.43	0.39	0.46
- 20-29 *	1.00	--	--	1.00	--	--
- 30-39	0.86	0.52	1.43	0.86	0.79	0.94
- 40-49	0.71	0.43	1.16	0.72	0.66	0.78
- 50-59	0.64	0.38	1.08	0.65	0.58	0.73
- >=60	0.51	0.31	0.86	0.51	0.47	0.56
State				See next Table.		
- CA	2.10	1.58	2.79			
- CT	0.89	0.68	1.16			
- GA	0.51	0.37	0.71			
- MD	0.39	0.30	0.50			
- MN	0.98	0.74	1.30			
- NY	0.87	0.68	1.12			
- OR *	1.00	--	--			
Year				See next Table.		
- 1996	1.62	1.41	1.86			
- 1997	1.70	1.54	1.87			
- 1998	1.34	1.23	1.45			
- 1999 *	1.00	--	--			

Legend: IRR, incidence rate ratio; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit; *, reference level.

Table 3.7. Incidence rate ratio (95% confidence interval) by state and year for *Campylobacter* reported cases

State	Year			
	1996	1997	1998	1999
California	3.09	2.67	2.00	1.76
	(1.95-4.92)	(1.66-4.32)	(1.22-3.29)	(1.49-2.07)
Connecticut	0.90	1.20	1.03	0.96
	(0.52-1.59)	(0.73-1.96)	(0.57-1.85)	(0.81-1.15)
Georgia	0.65	0.75	0.68	0.43
	(0.41-1.03)	(0.45-1.26)	(0.38-1.21)	(0.37-0.51)
Maryland	N/A	N/A	0.55	0.34
			(0.30-1.03)	(0.26-0.44)
Minnesota	1.06	1.40	1.19	0.92
	(0.68-1.65)	(0.88-2.21)	(0.72-1.96)	(0.79-1.08)
New York	N/A	N/A	1.11	0.95
			(0.62-1.98)	(0.77-1.18)
Oregon	1.21	1.27	1.19	1.00
	(1.06-1.39)	(1.11-1.46)	(1.01-1.39)	(Reference)

Table 3.8. Incidence rate ratio for *Salmonella* (Poisson model with generalized estimating equations, independent correlation structure)

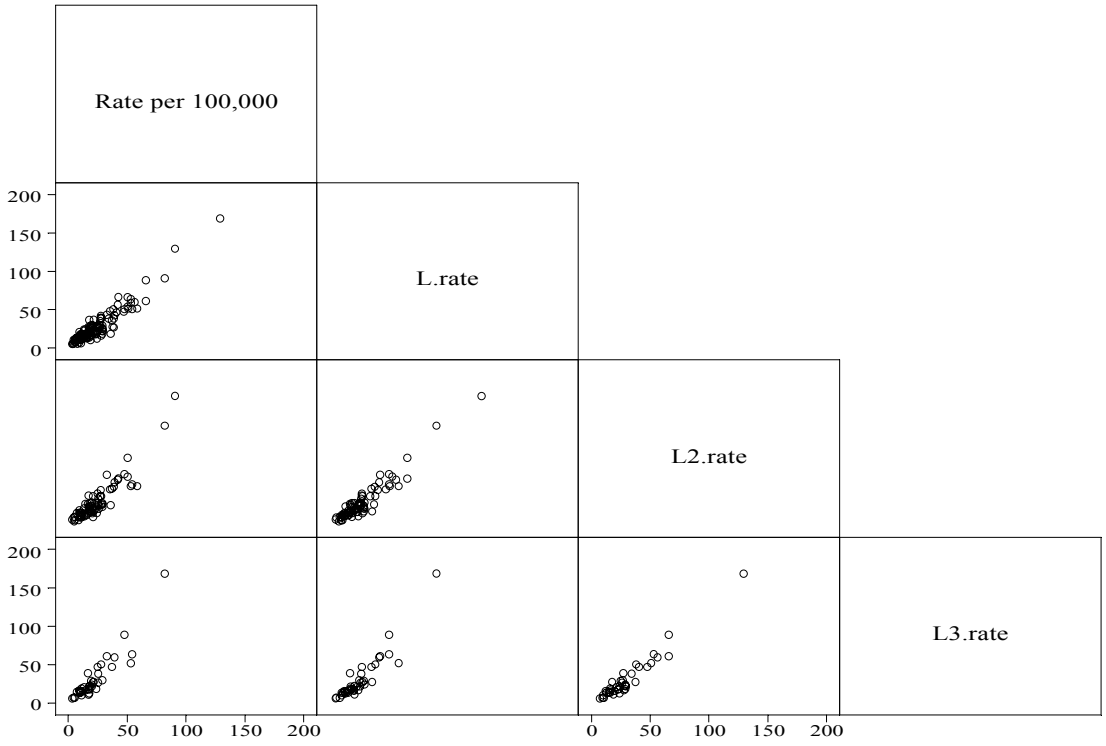
Parameter	Univariate analysis			Multivariate analysis		
- Level	IRR	LCL	UCL	IRR	LCL	UCL
Age						
- <1	9.16	5.71	14.70	9.15	5.83	14.34
- 1-9	1.98	1.40	2.80	1.97	1.43	2.72
- 10-19	0.71	0.53	0.94	0.71	0.53	0.96
- 20-29 *	1.00	--	--	1.00	--	--
- 30-39	0.78	0.59	1.04	0.78	0.58	1.06
- 40-49	0.66	0.50	0.89	0.67	0.50	0.90
- 50-59	0.63	0.47	0.84	0.63	0.47	0.86
- >=60	0.70	0.54	0.91	0.70	0.54	0.92
State						
- CA	1.50	0.99	2.29	1.42	1.17	1.73
- CT	1.48	0.97	2.27	1.49	1.23	1.79
- GA	1.38	0.68	2.80	1.32	0.97	1.80
- MD	1.62	1.00	2.65	1.62	1.37	1.93
- MN	1.20	0.82	1.75	1.19	0.98	1.45
- NY	1.34	0.88	2.03	1.32	1.10	1.57
- OR *	1.00	--	--	1.00	--	--
Year						
- 1996	0.95	0.82	1.10	1.00	0.91	1.09
- 1997	0.90	0.79	1.02	0.94	0.86	1.02
- 1998	0.90	0.79	1.02	0.90	0.84	0.97
- 1999 *	1.00	--	--	1.00	--	--

Legend: IRR, incidence rate ratio; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit; *, reference level.

Table 3.9. Incidence rate ratio for Shigella (Poisson model with generalized estimating equations, independent correlation structure)

Parameter	Univariate analysis			Multivariate analysis		
- Level	IRR	LCL	UCL	IRR	LCL	UCL
Age						
- <1	1.63	0.84	3.16	1.62	1.20	2.19
- 1-9	3.35	1.68	6.68	3.38	2.52	4.54
- 10-19	0.48	0.28	0.82	0.49	0.42	0.57
- 20-29 *	1.00	--	--	1.00	--	--
- 30-39	0.99	0.53	1.85	1.01	0.75	1.36
- 40-49	0.57	0.29	1.11	0.59	0.37	0.96
- 50-59	0.47	0.28	0.79	0.50	0.30	0.83
- >=60	0.22	0.14	0.35	0.24	0.17	0.34
State						
- CA	3.03	1.34	6.88	2.74	1.91	3.94
- CT	0.72	0.29	1.79	0.71	0.61	0.83
- GA	2.46	0.77	7.81	2.29	1.69	3.12
- MD	0.53	0.25	1.15	0.51	0.32	0.82
- MN	1.08	0.45	2.60	1.05	0.87	1.26
- NY	0.61	0.24	1.56	0.60	0.47	0.77
- OR *	1.00	--	--	1.00	--	--
Year						
- 1996	2.33	1.61	3.38			
- 1997	2.07	1.35	3.17			
- 1998	1.87	1.31	2.67			
- 1999 *	1.00	--	--			

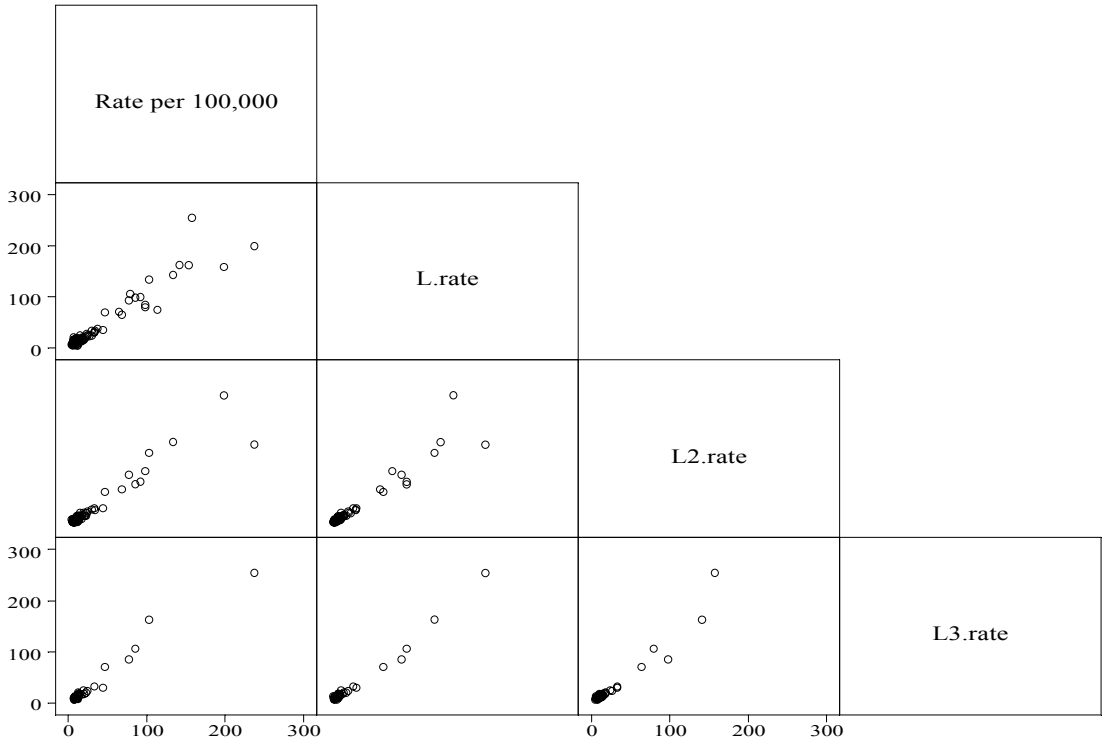
Legend: IRR, incidence rate ratio; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit; *, reference level.



Spearman correlation matrix

(Graph label)	Reference year (Rate per 100,000)	Lag 1 (L.rate)	Lag 2 (L2.rate)	Lag 3 (L3.rate)
Reference year	1.00			
Lag 1	0.87	1.00		
Lag 2	0.86	0.87	1.00	
Lag 3	0.89	0.90	0.90	1.00

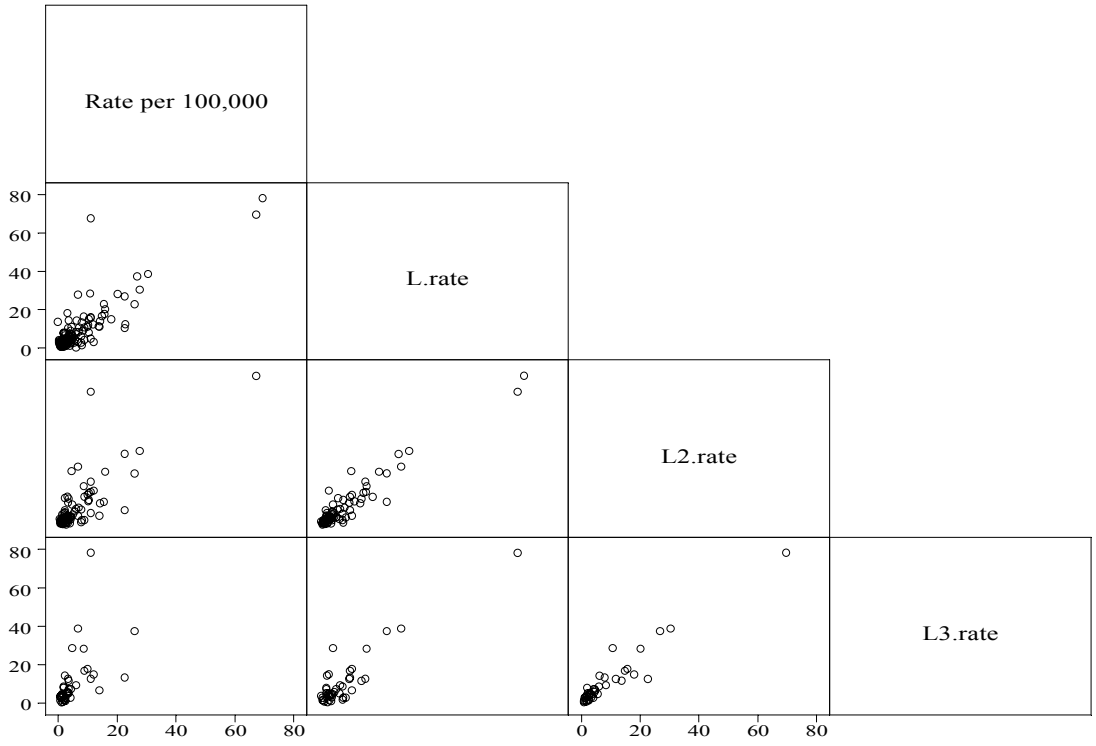
Figure 3.1. Correlogram and correlation matrix of *Campylobacter* reporting rates lagged within each age-state stratum



Spearman correlation matrix

(Graph label)	Reference year (Rate per 100,000)	Lag 1 (L.rate)	Lag 2 (L2.rate)	Lag 3 (L3.rate)
Reference year	1.00			
Lag 1	0.82	1.00		
Lag 2	0.84	0.91	1.00	
Lag 3	0.89	0.86	0.90	1.00

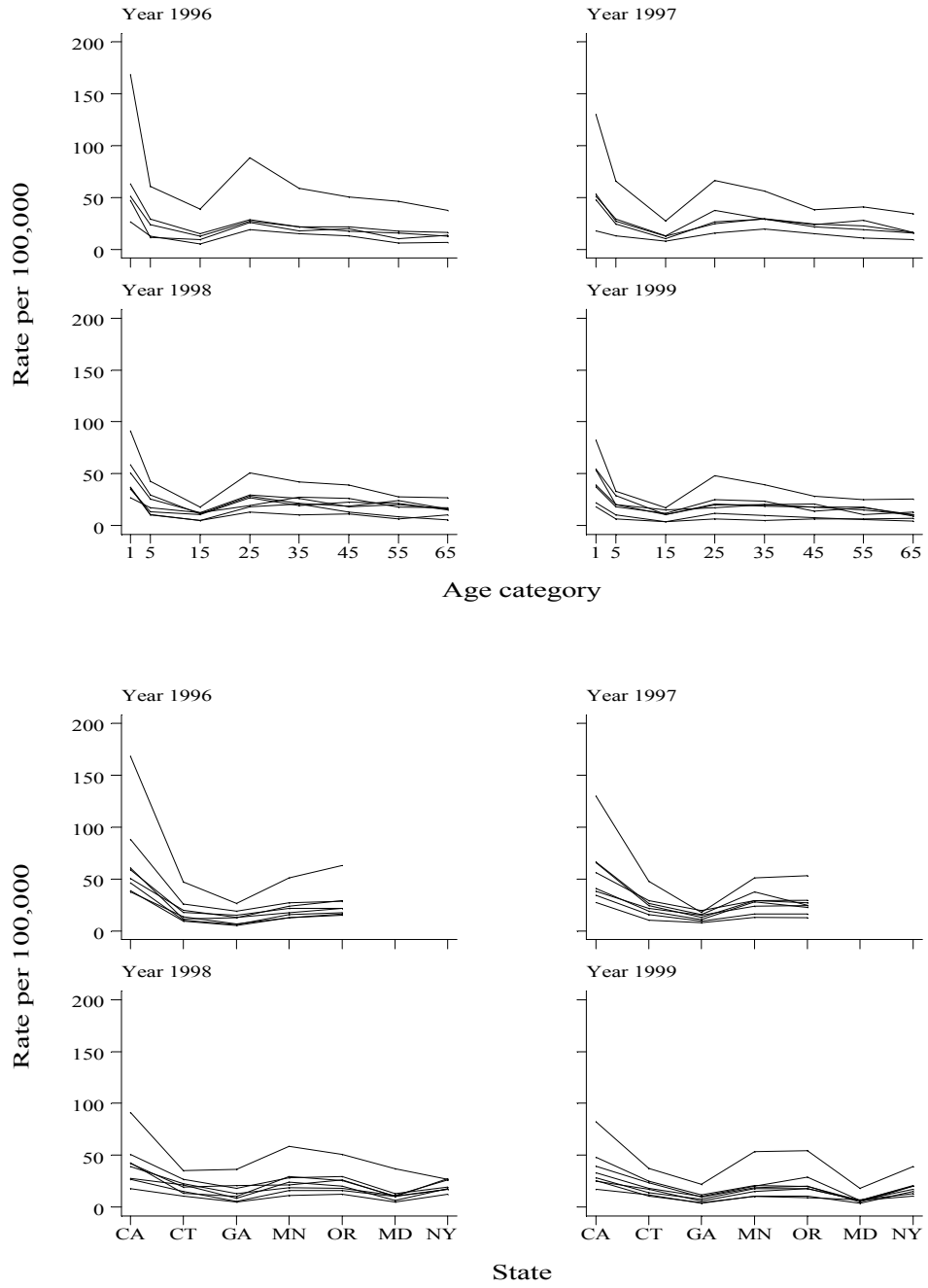
Figure 3.2. Correlogram and correlation matrix of *Salmonella* reporting rates lagged within each age-state stratum



Spearman correlation matrix

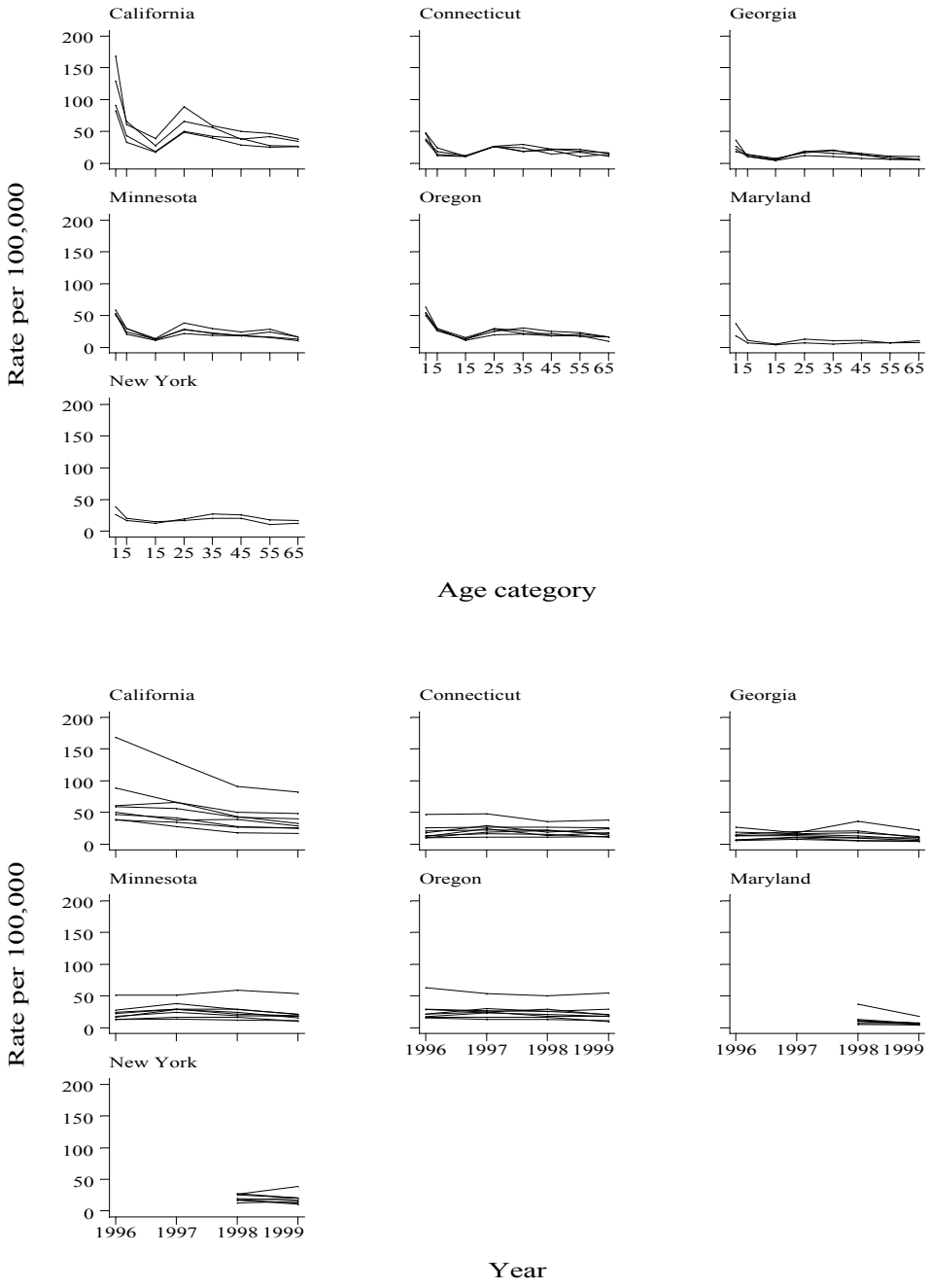
(Graph label)	Reference year (Rate per 100,000)	Lag 1 (L.rate)	Lag 2 (L2.rate)	Lag 3 (L3.rate)
Reference year	1.00			
Lag 1	0.71	1.00		
Lag 2	0.70	0.82	1.00	
Lag 3	0.74	0.62	0.91	1.00

Figure 3.3. Correlogram and correlation matrix of *Shigella* reporting rates lagged within each age-state stratum



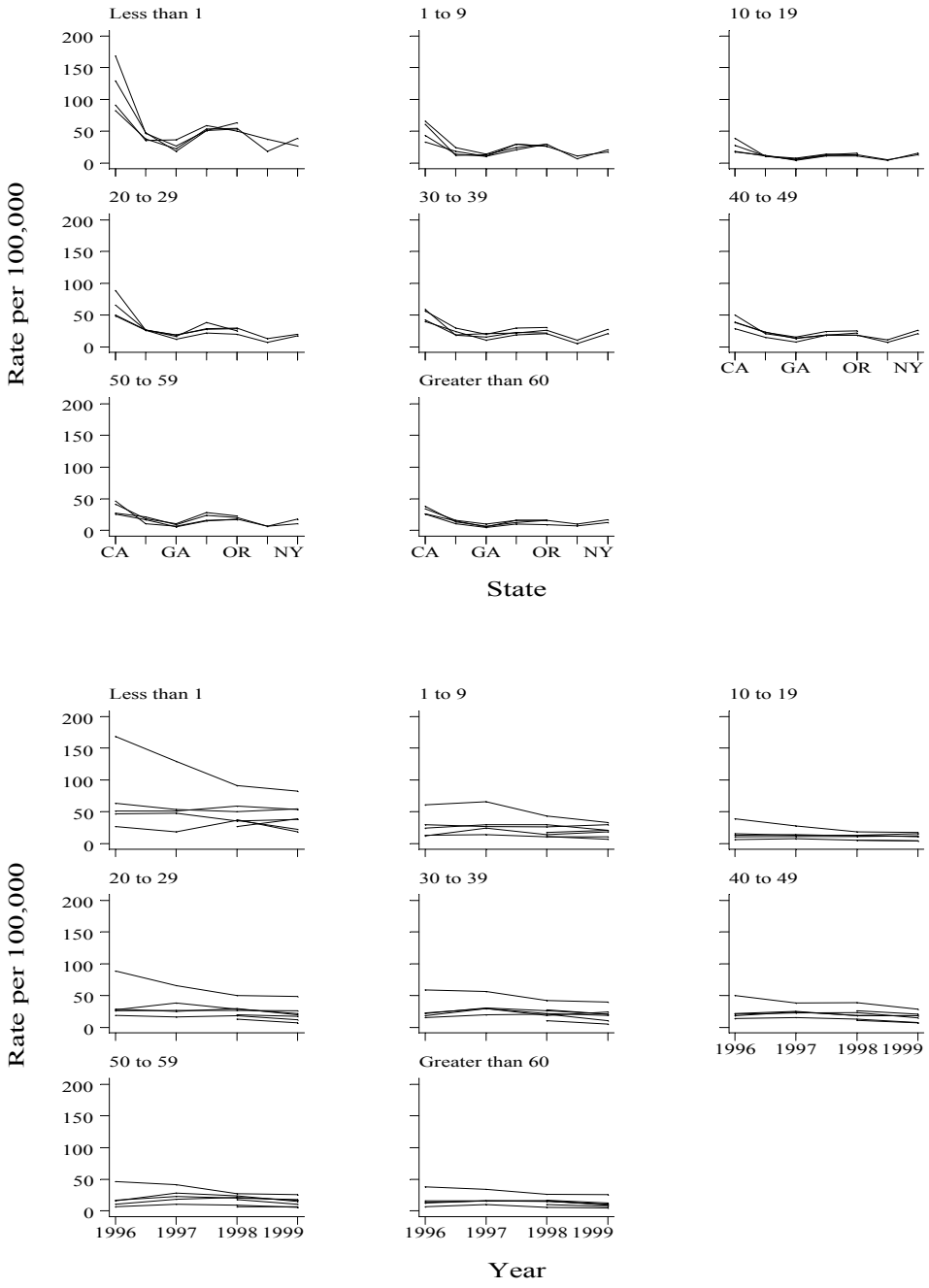
Note: each line connects the rates of a specific state (upper set of graphs) or age category (lower set of graphs).

Figure 3.4. Age and state effects on *Campylobacter* rates



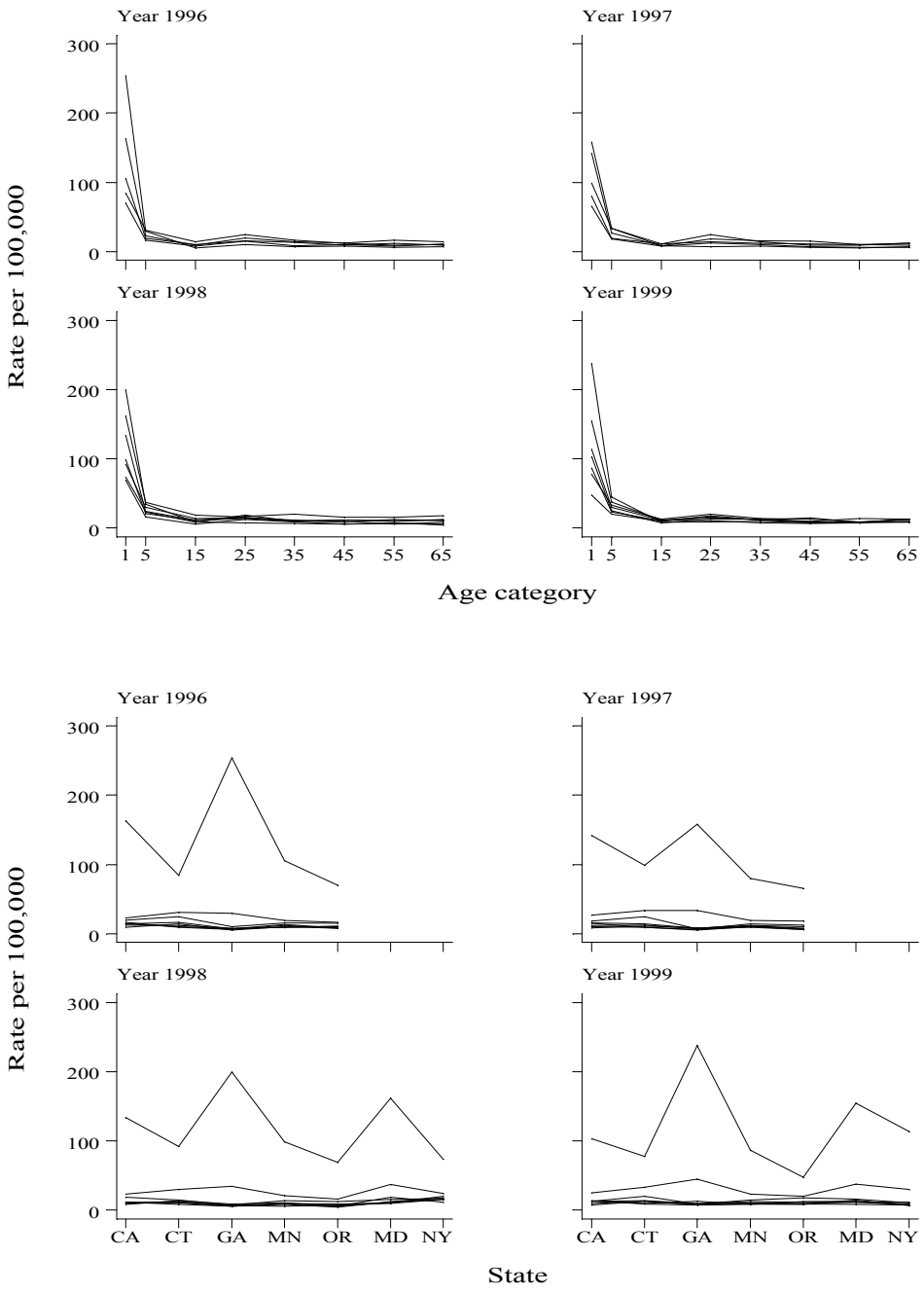
Note: each line connects the rates of a specific year (upper set of graphs) or age category (lower set of graphs).

Figure 3.5. Age and year effects on *Campylobacter* rates



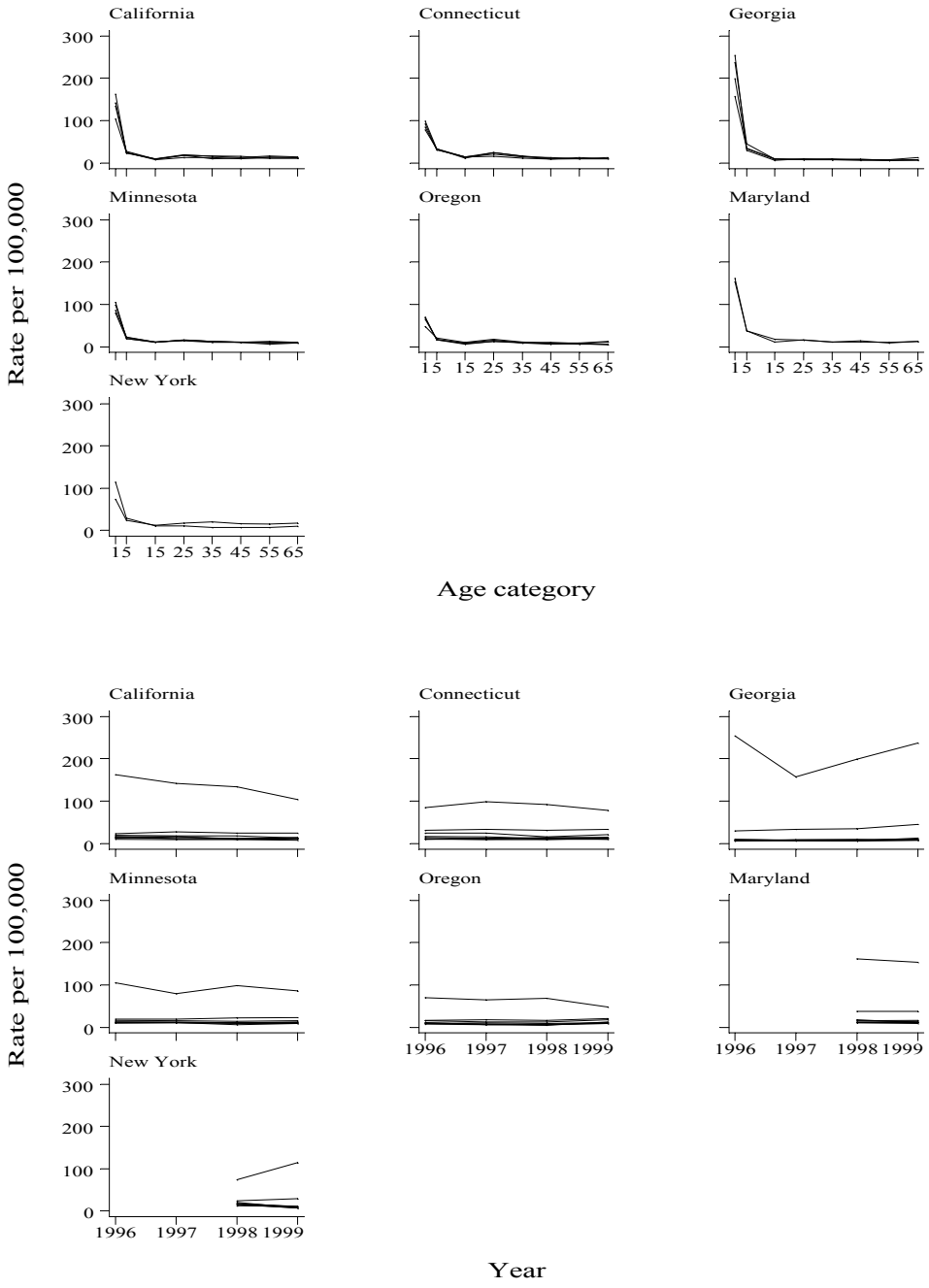
Note: each line connects the rates of a specific year (upper set of graphs) or state (lower set of graphs).

Figure 3.6. State and year effects on *Campylobacter* rates



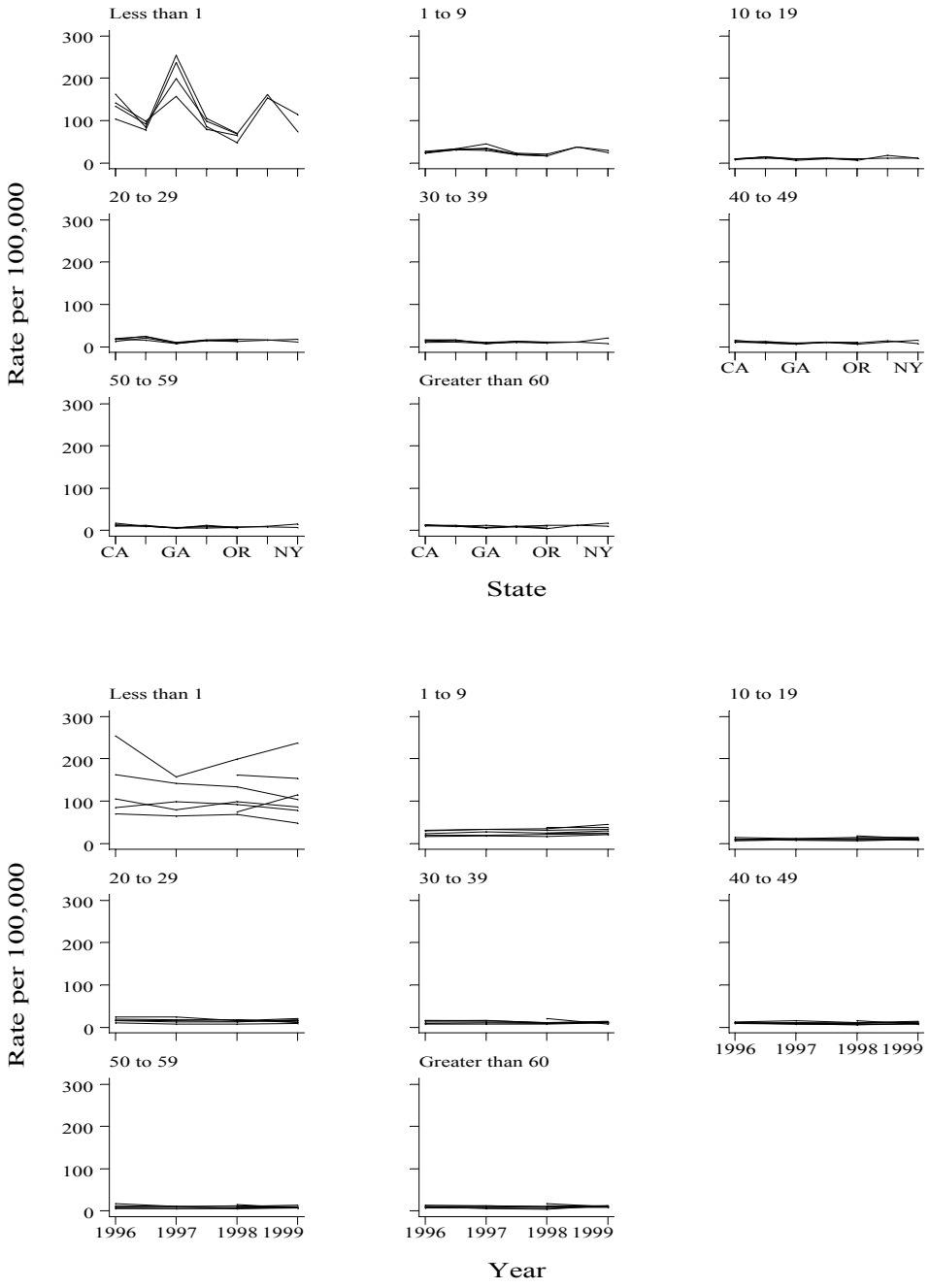
Note: each line connects the rates of a specific state (upper set of graphs) or age category (lower set of graphs).

Figure 3.7. Age and state effects on *Salmonella* rates



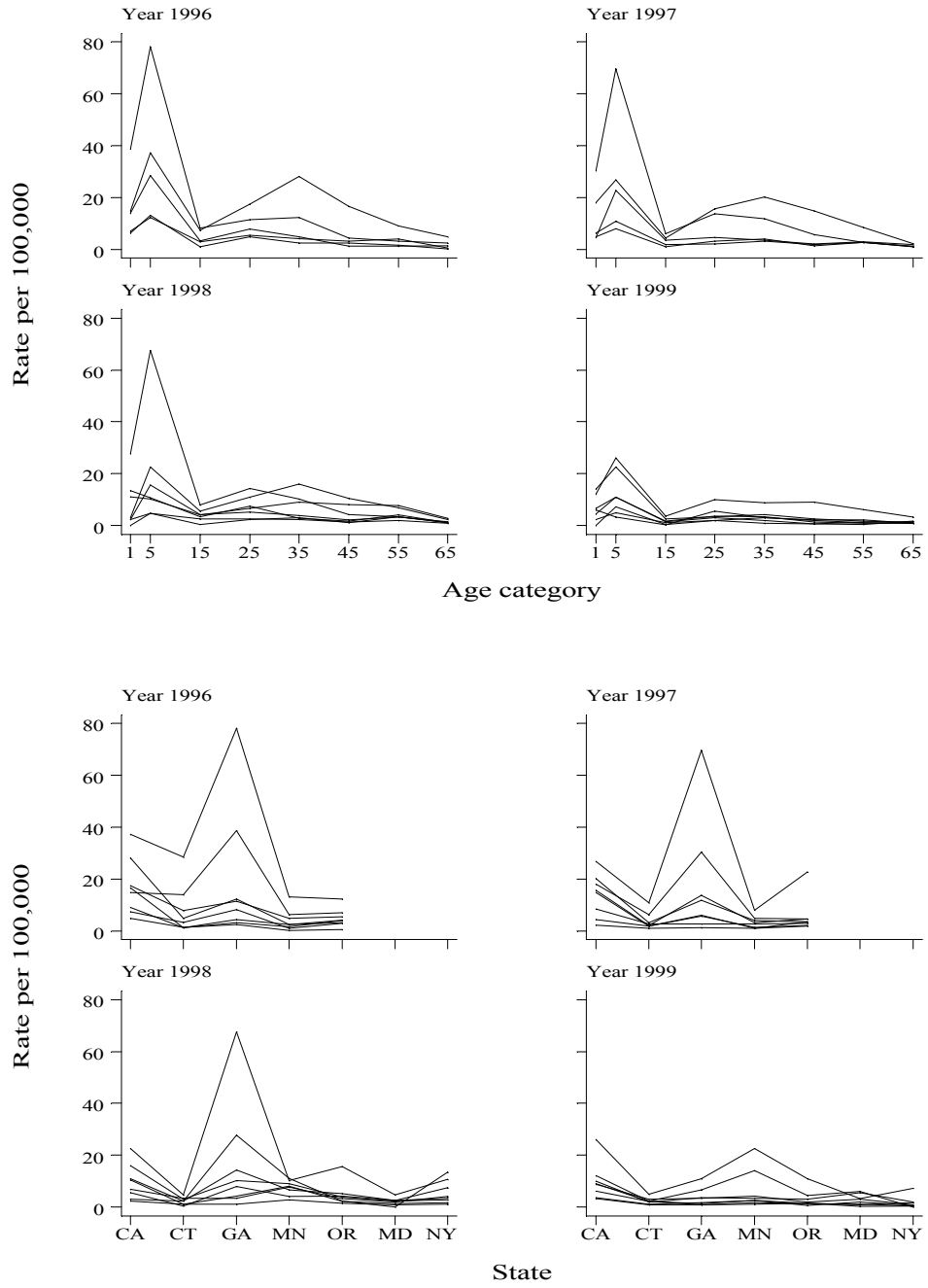
Note: each line connects the rates of a specific year (upper set of graphs) or age category (lower set of graphs).

Figure 3.8. Age and year effects on *Salmonella* rates



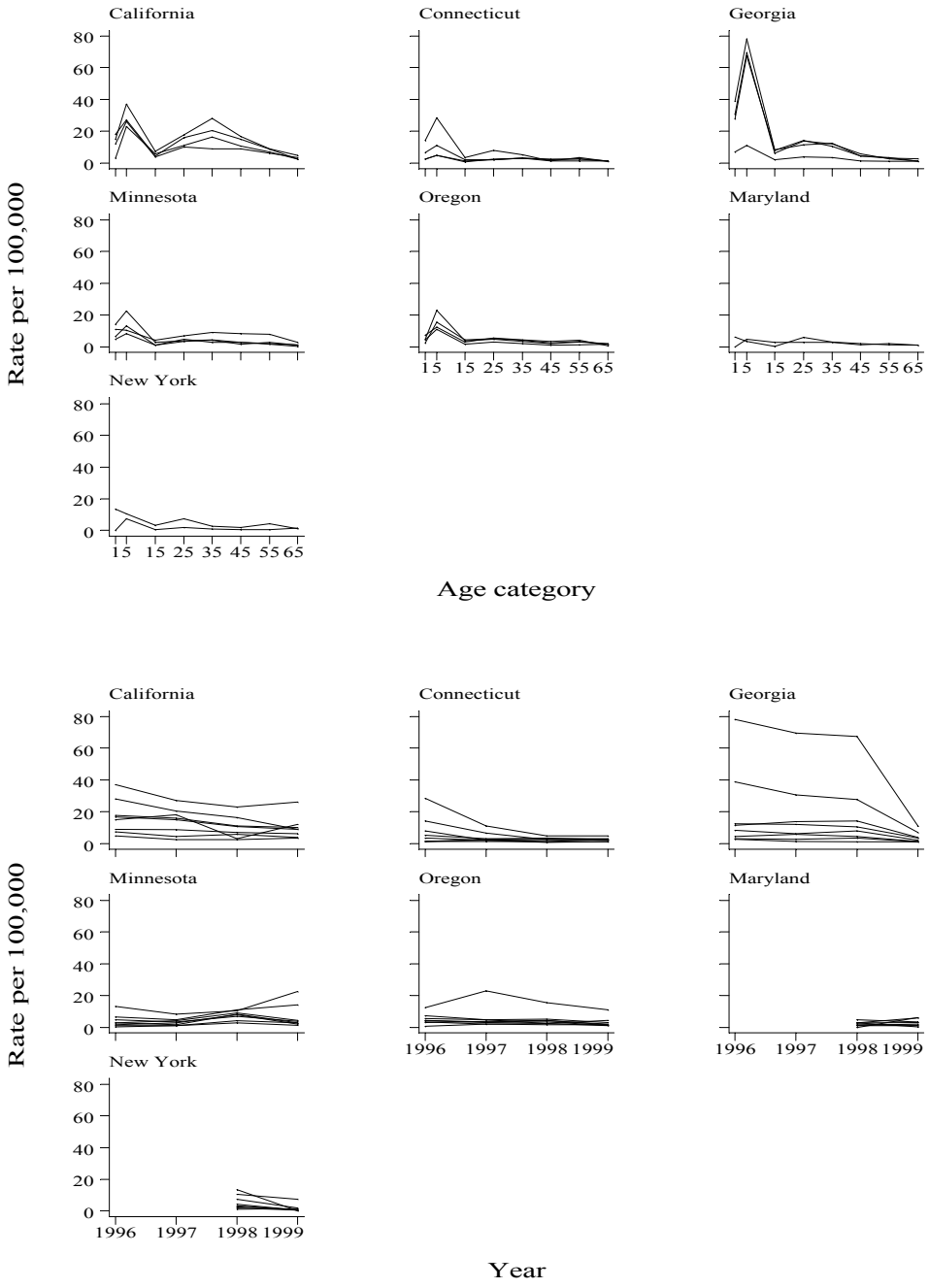
Note: each line connects the rates of a specific year (upper set of graphs) or state (lower set of graphs).

Figure 3.9. State and year effects on *Salmonella* rates



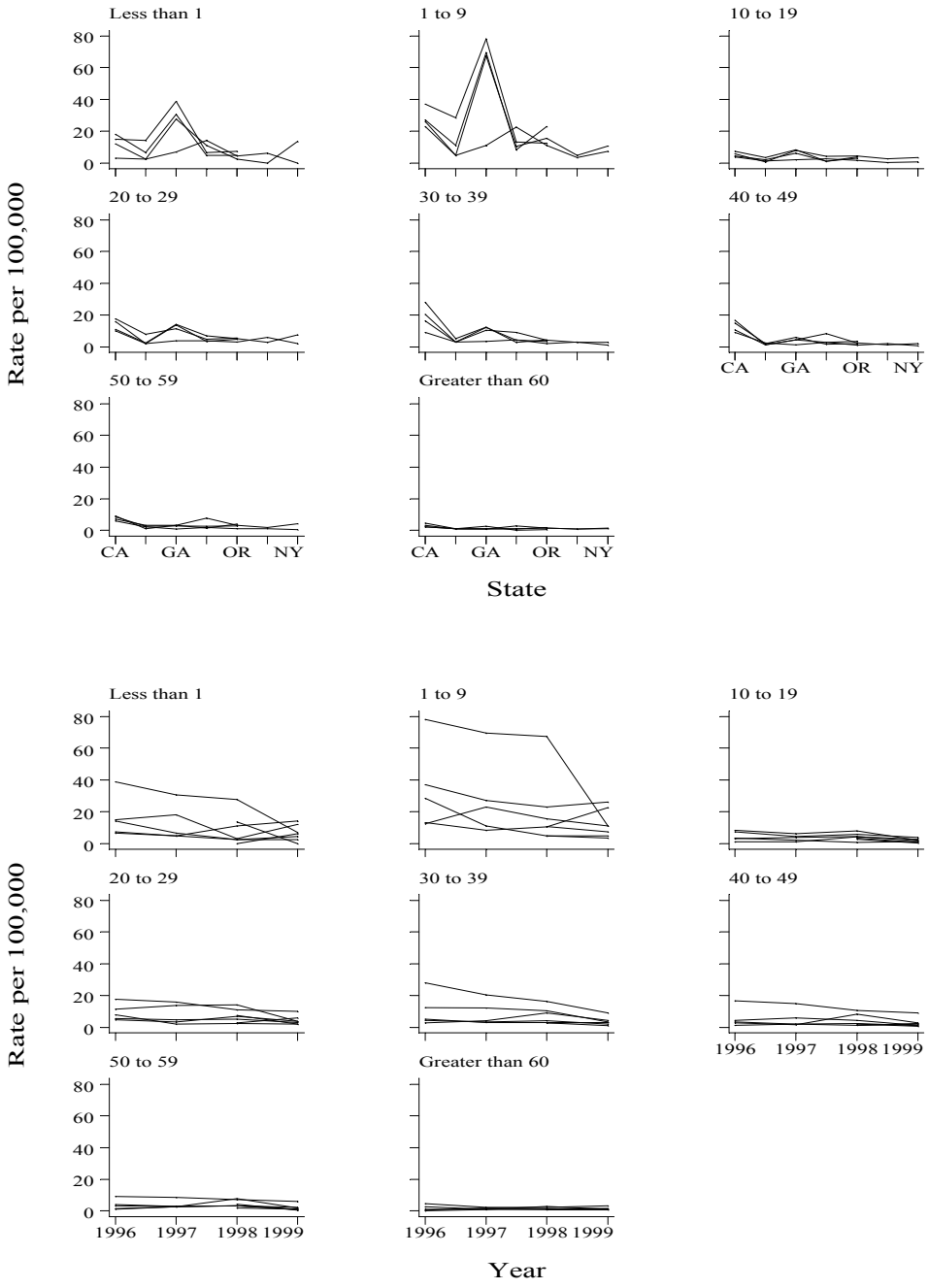
Note: each line connects the rates of a specific state (upper set of graphs) or age category (lower set of graphs).

Figure 3.10. Age and state effects on *Shigella* rates



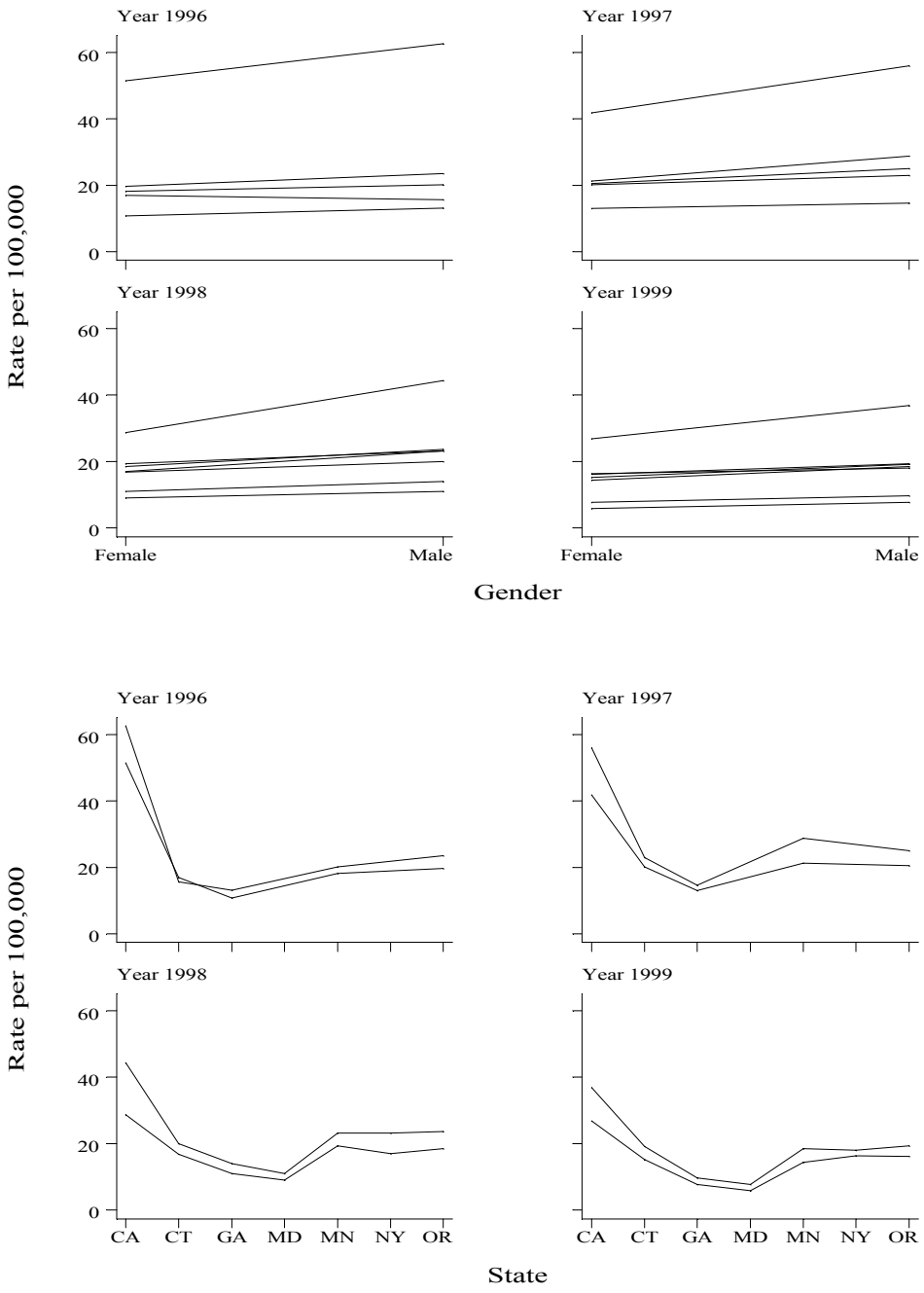
Note: each line connects the rates of a specific year (upper set of graphs) or age category (lower set of graphs).

Figure 3.11. Age and year effects on *Shigella* rates



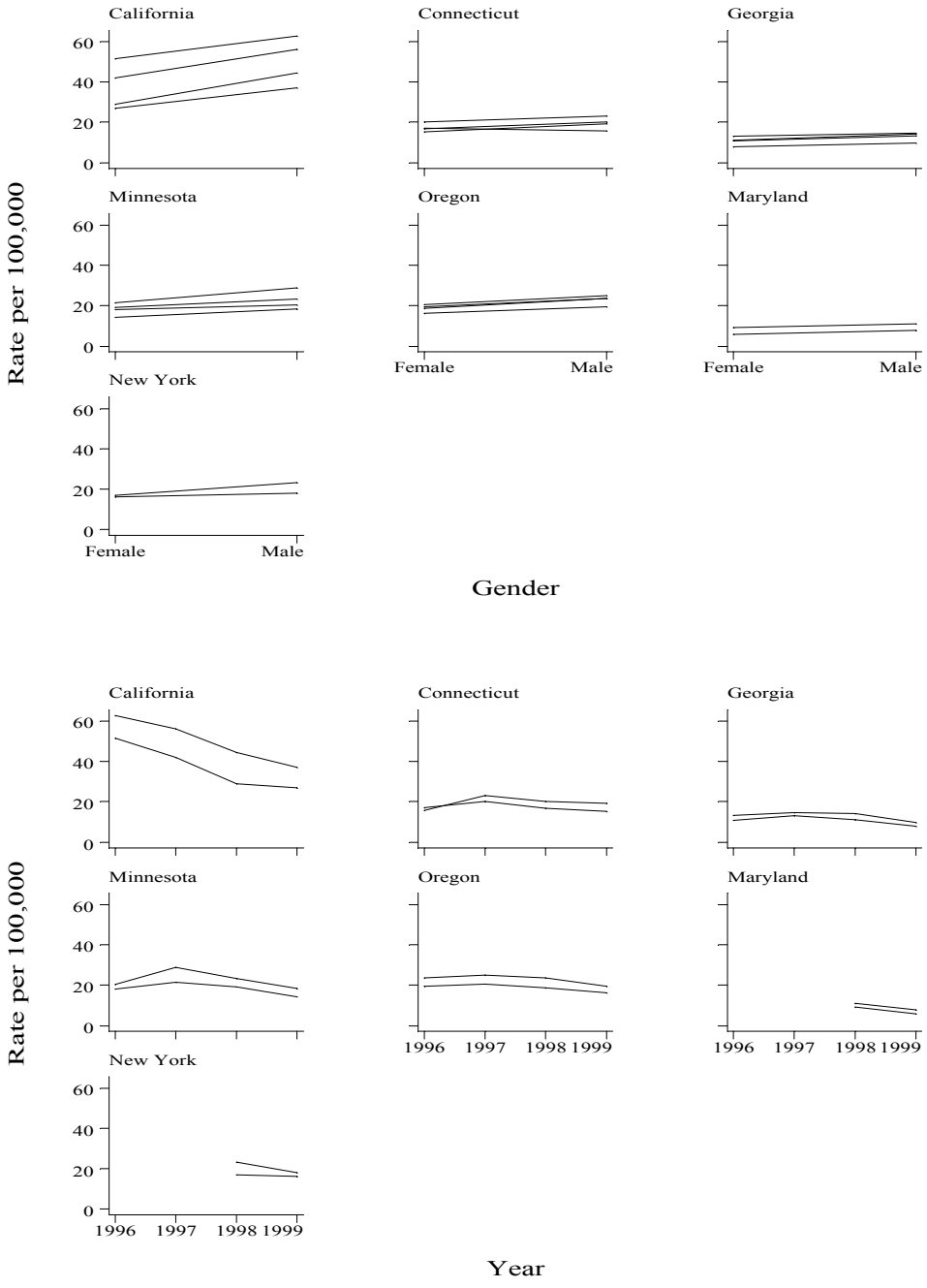
Note: each line connects the rates of a specific year (upper set of graphs) or state (lower set of graphs).

Figure 3.12. State and year effects on *Shigella* rates



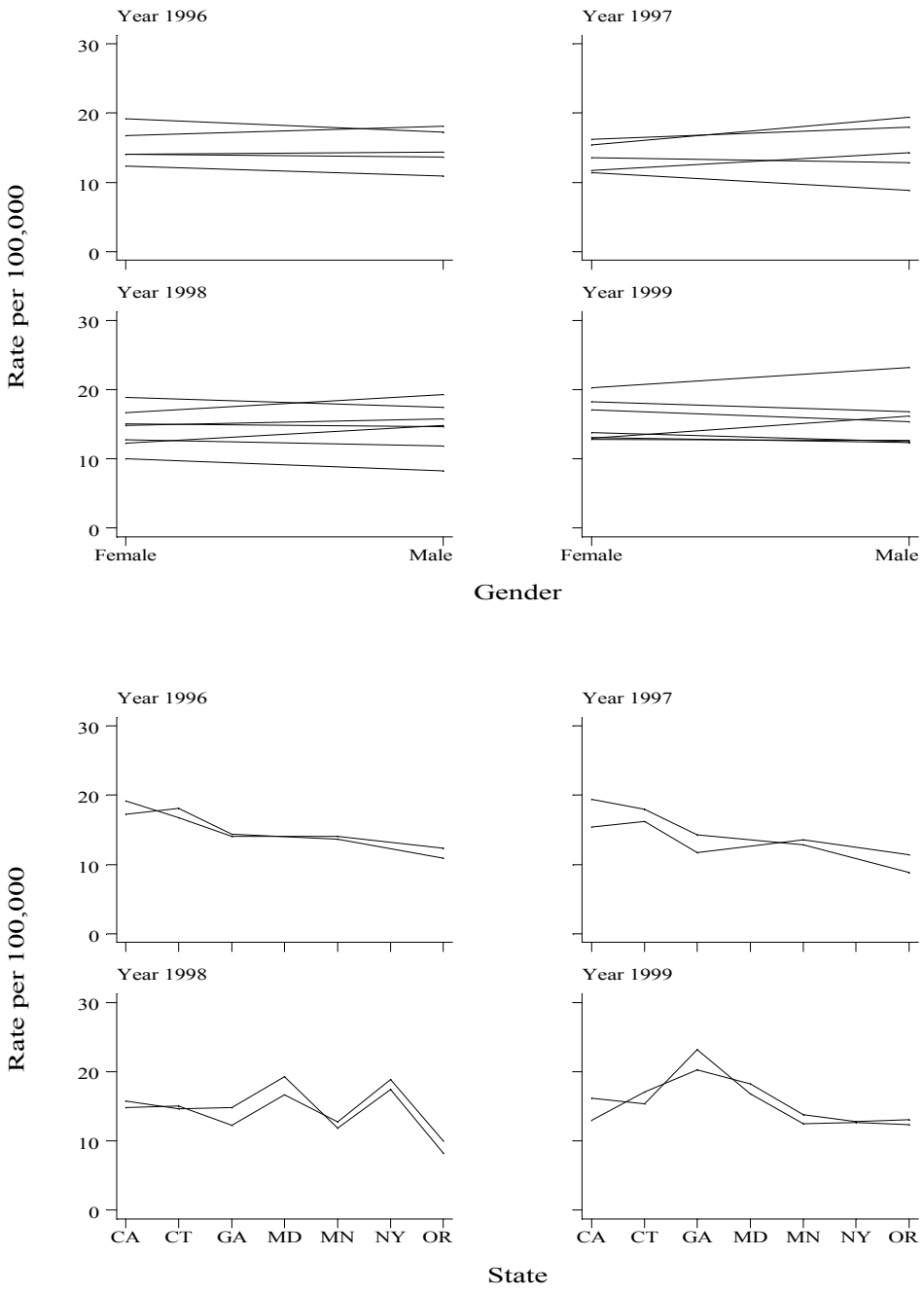
Note: each line connects the rates of a specific state (upper set of graphs) or gender (lower set of graphs).

Figure 3.13. Gender and state effects on *Campylobacter* rates



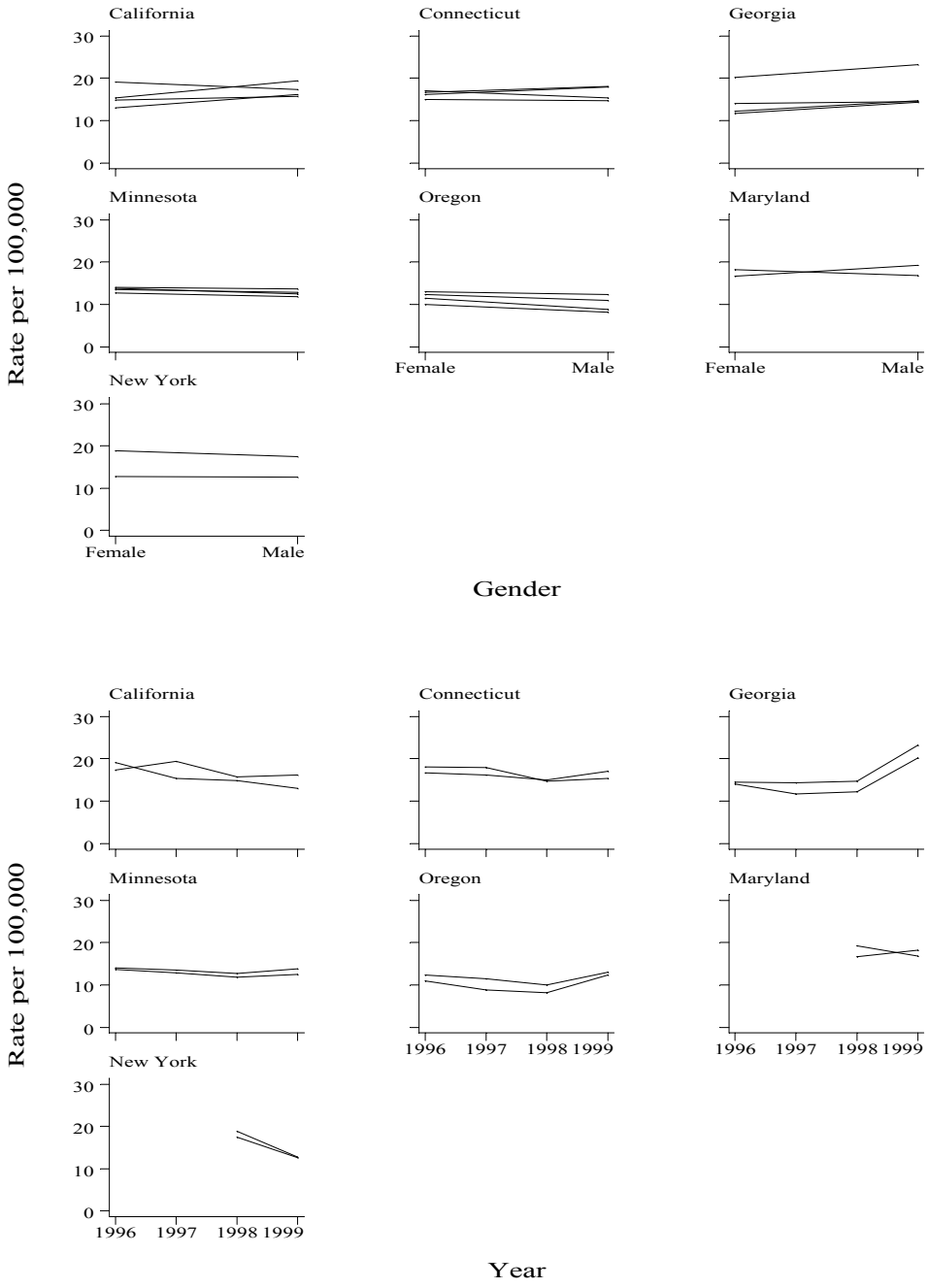
Note: each line connects the rates of a year (upper set of graphs) or gender (lower set of graphs).

Figure 3.14. Gender and year effects on *Campylobacter* rates



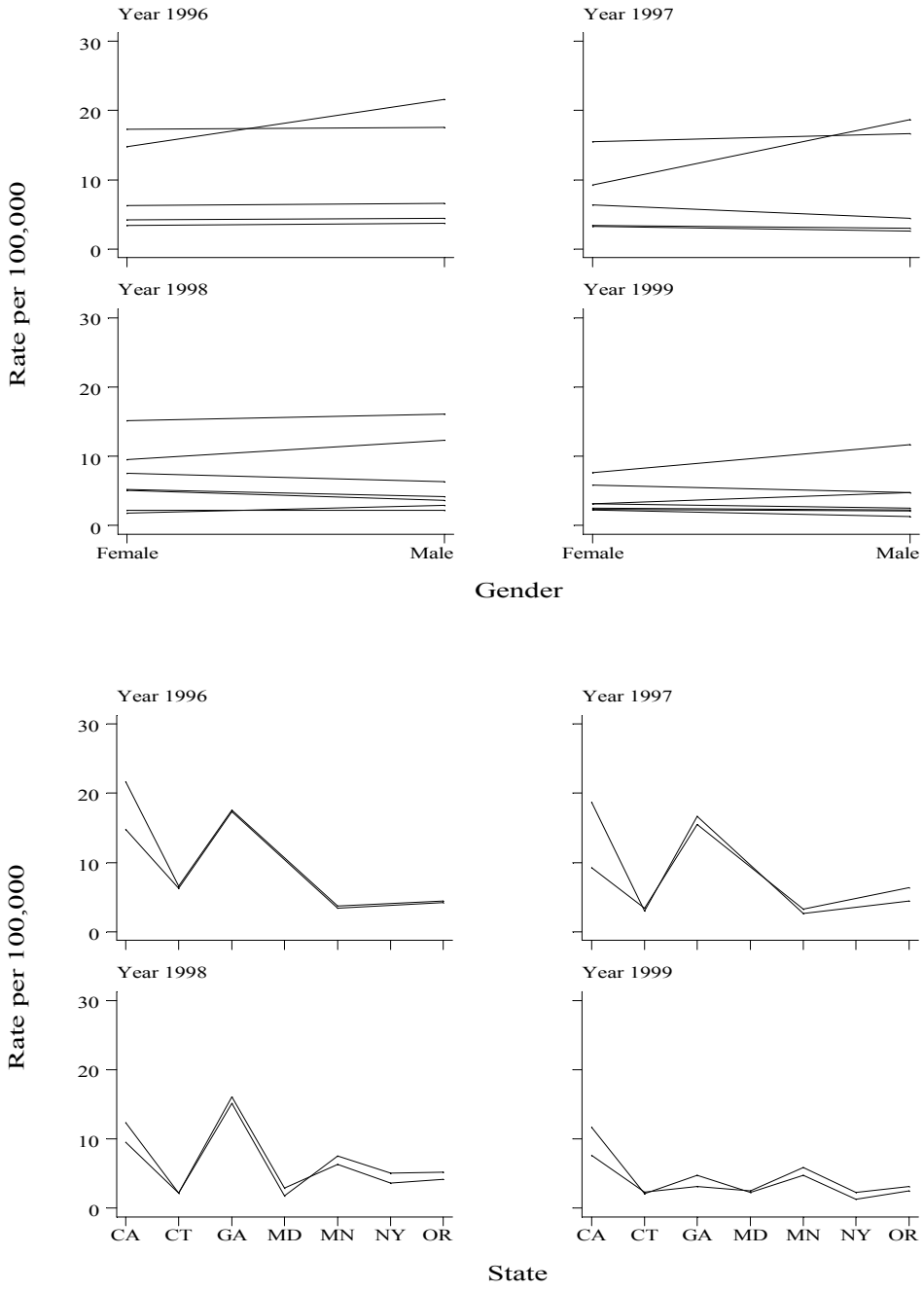
Note: each line connects the rates of a specific state (upper set of graphs) or gender (lower set of graphs).

Figure 3.15. Gender and state effects on *Salmonella* rates



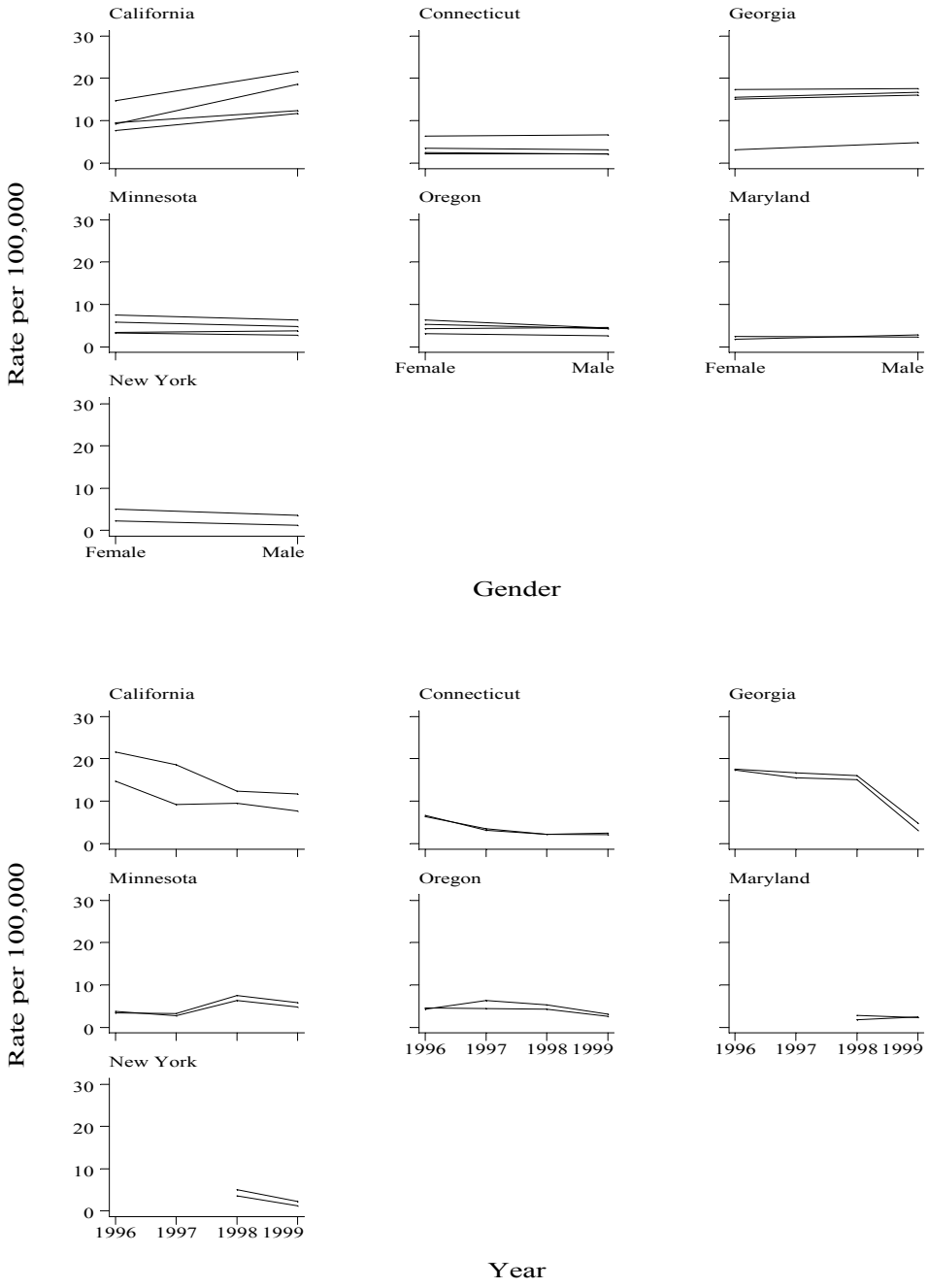
Note: each line connects the rates of a year (upper set of graphs) or gender (lower set of graphs).

Figure 3.16. Gender and year effects on *Salmonella* rates



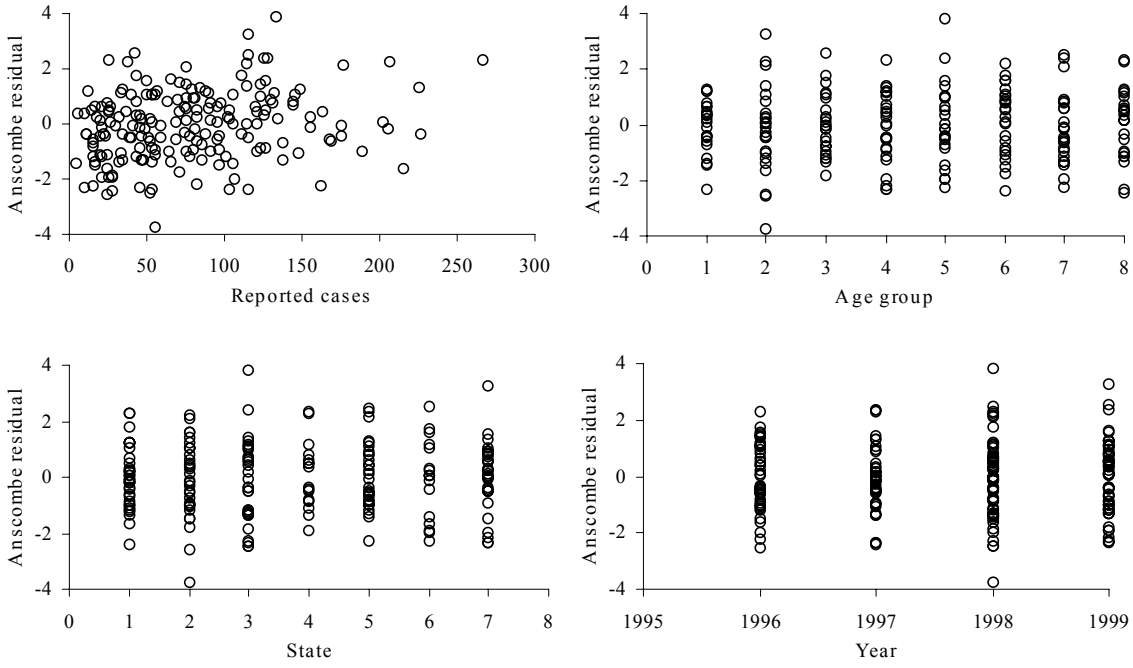
Note: each line connects the rates of a specific state (upper set of graphs) or gender (lower set of graphs).

Figure 3.17. Gender and state effects on *Shigella* rates



Note: each line connects the rates of a specific year (upper set of graphs) or gender (lower set of graphs).

Figure 3.18. Gender and year effects on *Shigella* rates



Notes: Age groups, 1=less than 1 year, 2=1-9 years, 3=10-19 years, 4=20-29 years, 5=30-39 years, 6=40-49 years, 7=50-59 years, 8=equal to/greater than 60 years; State, 1=California, 2=Connecticut, 3=Georgia, 4=Maryland, 5=Minnesota, 6=New York, 7=Oregon.

Figure 3.19. Anscombe residuals of final model for *Campylobacter*

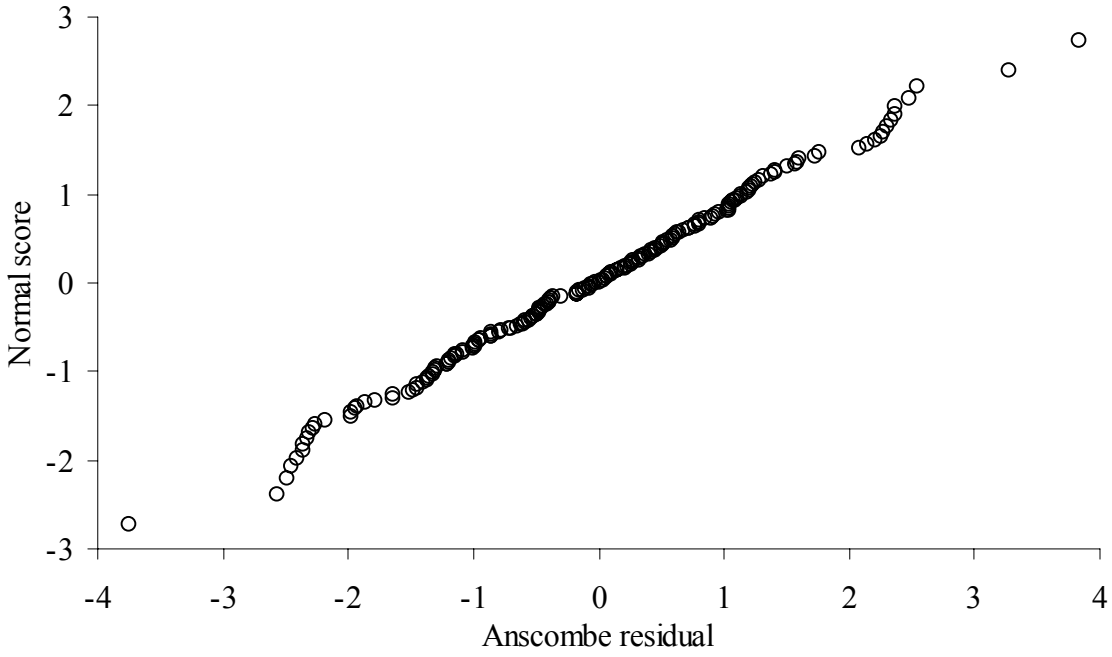
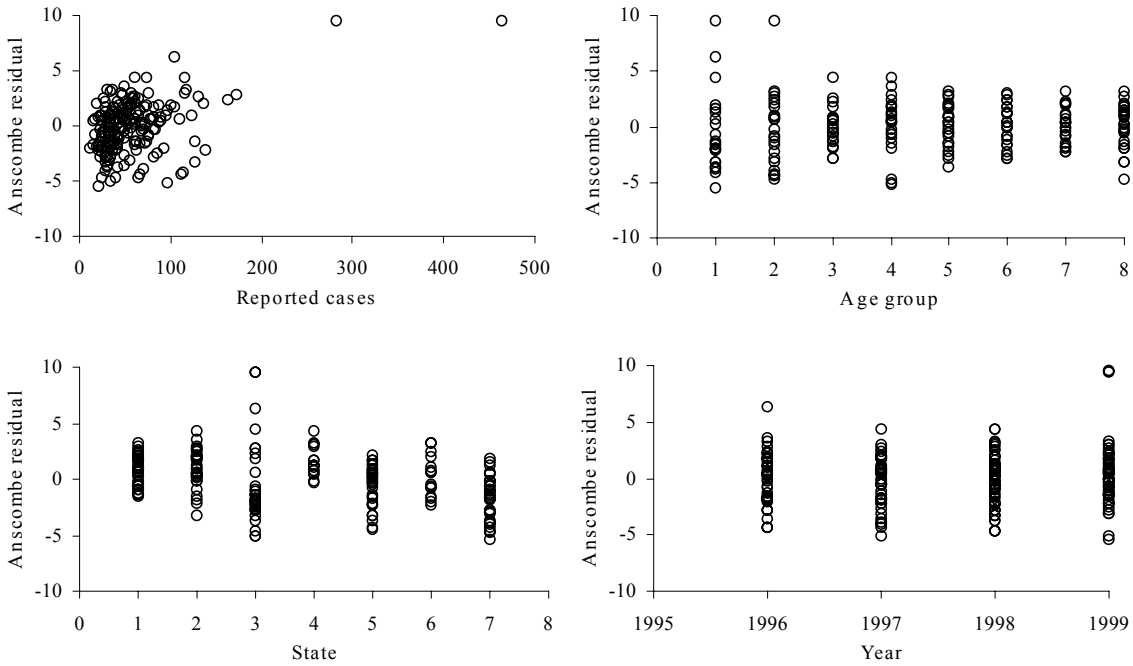


Figure 3.20. Normal probability plot of Anscombe residuals in the final model for *Campylobacter*



Notes: Age groups, 1=less than 1 year, 2=1-9 years, 3=10-19 years, 4=20-29 years, 5=30-39 years, 6=40-49 years, 7=50-59 years, 8=equal to/greater than 60 years; State, 1=California, 2=Connecticut, 3=Georgia, 4=Maryland, 5=Minnesota, 6=New York, 7=Oregon.

Figure 3.21. Anscombe residuals of final model for *Salmonella*

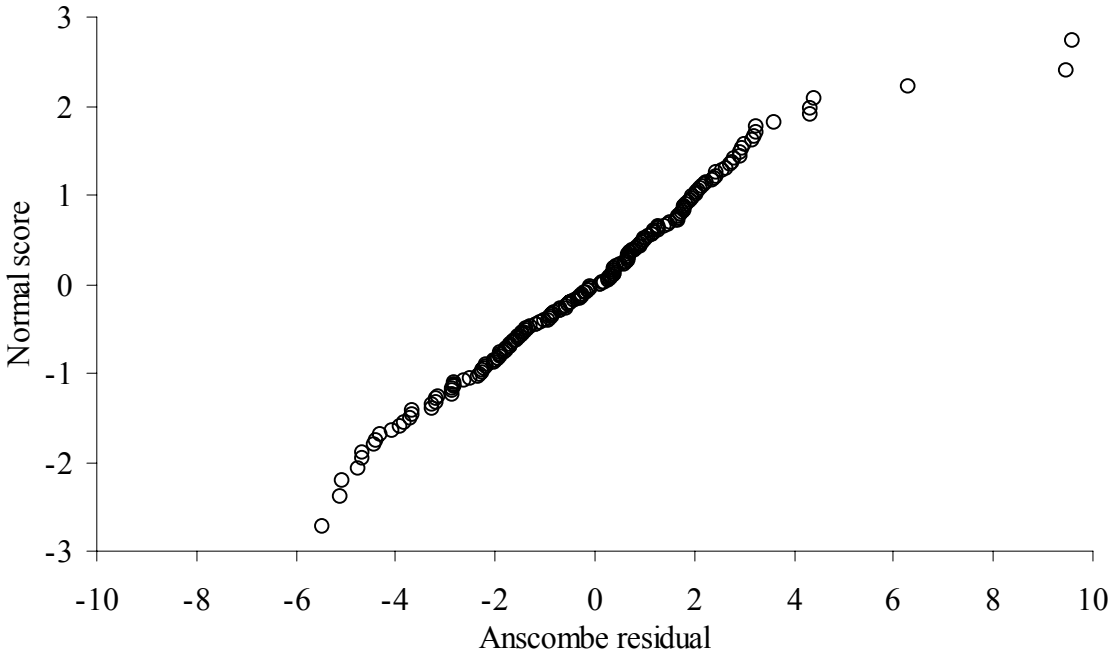
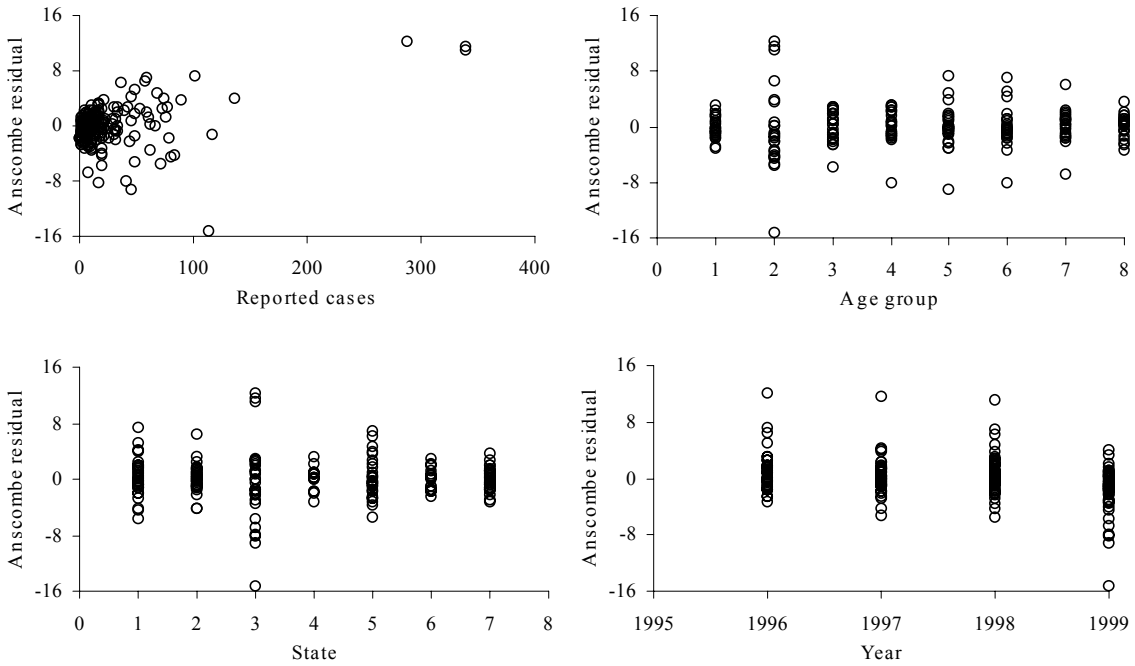


Figure 3.22. Normal probability plot of Anscombe residuals in the final model for *Salmonella*



Notes: Age groups, 1=less than 1 year, 2=1-9 years, 3=10-19 years, 4=20-29 years, 5=30-39 years, 6=40-49 years, 7=50-59 years, 8=equal to/greater than 60 years; State, 1=California, 2=Connecticut, 3=Georgia, 4=Maryland, 5=Minnesota, 6=New York, 7=Oregon.

Figure 3.23. Anscombe residuals of final model for *Shigella*

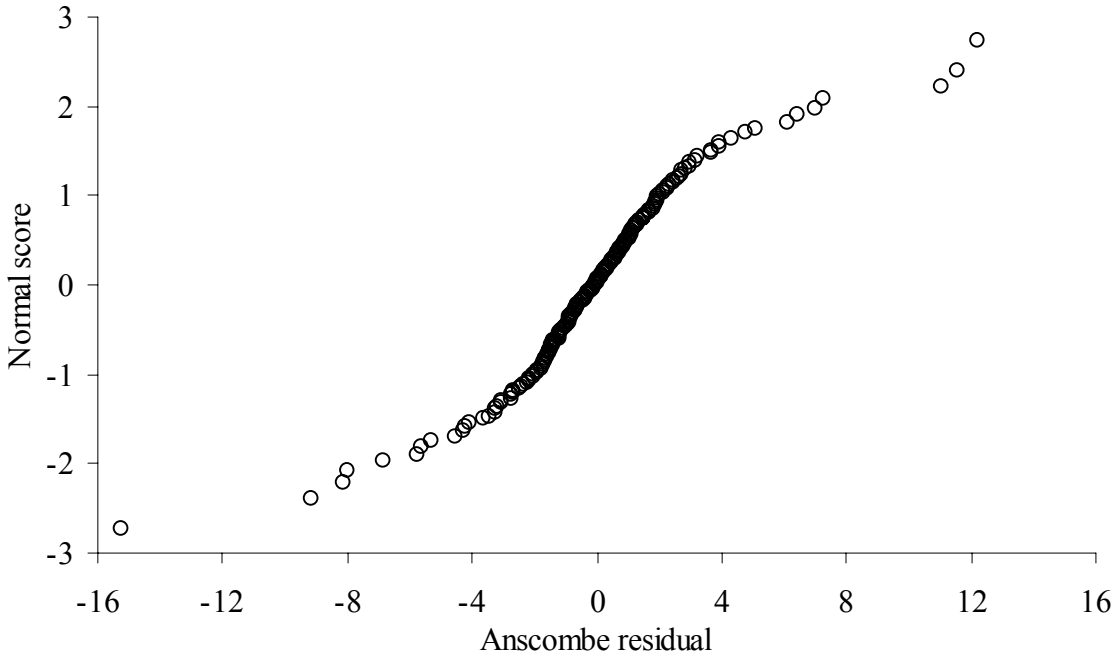


Figure 3.24. Normal probability plot of Anscombe residuals in the final model for *Shigella*

4 SECOND-ORDER MODELING OF UNCERTAINTY AND VARIABILITY IN MICROBIAL HAZARD CHARACTERIZATION

4.1 Problem Definition, Motivation, and Objectives

Over the last decade, heightened awareness about the consequences of foodborne illnesses has fomented the application of quantitative risk assessment to food safety problems (Jaykus, 1996; Lammerding & Paoli, 1997; Buchanan et al., 1998). A four-step paradigm – hazard identification, exposure assessment, hazard characterization, and risk characterization – is commonly followed (CAC, 1999). Practically, hazard characterization has taken the form of a dose-response assessment, in which a mathematical function links an exposure dose to an infection/illness probability. In some instances, a constant has been used to adjust for increased susceptibility in a population subgroup or for animal-to-human extrapolation (USDA/FSIS, 1998; FDA/CFSAN, 2001). A more thorough consideration of the multifaceted interaction between pathogen, host, and food medium is commonly advocated (CAC, 1999). For example, the development of methods that can incorporate the impact of host factors, such as age and immune status, on susceptibility is deemed necessary (ILSI/RSI, 2000).

Surprisingly, the reasons for such a proposition are nowhere stated explicitly. There may be the desire to better address risk managers' concerns about specific population subgroups. Also, as most microbial risk assessors are life-science professionals by training, the assumption is perhaps that biological plausibility invariably translates into quantitatively relevant risk differences. Generally speaking, the unstated conviction seems to be that more detailed models are necessarily more accurate ones. This view is, however, too simplistic, as a trade-off of involved uncertainties is likely to occur (Cullen & Frey, 1998). On the one hand, the larger number of inputs in the more complex model may undoubtedly reduce the uncertainty associated with model structure. On the other hand, uncertainty due to more inputs, which may each have their own estimation error, can become larger. It is thus critical that hazard characterization methods are developed under careful consideration of attendant uncertainties. This

includes separation of variability (interindividual, spatial, and temporal heterogeneity) and uncertainty (lack of knowledge).

This study develops an analytical framework that permits quantitative consideration of variability and uncertainty in microbial hazard characterization. Second-order modeling employing two-dimensional Monte Carlo simulation and stratification into homogeneous population subgroups is applied to integrate select uncertainty and variability elements that were discussed in previous chapters of this dissertation. Specifically, the bootstrap method studied in Chapter 2 is here applied to simulate sampling error of *Campylobacter jejuni* dose-response models. Results from the analysis of FoodNet surveillance data (Chapter 3) are further used to reflect both variability and uncertainty in *Campylobacter* susceptibility due to the effect of age.

4.2 Treatment of Uncertainty and Variability

4.2.1 Justification for Separating Uncertainty and Variability

Models in microbial risk assessment are commonly the objects of a probabilistic analysis (Lammerding & Fazil, 2000). Within this context, one ought to recognize that two distinct sources of variation – uncertainty and variability – are often concurrent. Variability relates to interindividual, temporal, or spatial heterogeneity of the population under study, while uncertainty refers to lack of knowledge about specific factors, parameters, or models (Bogen & Spear, 1987). For any given input that is stochastically expressed, variability describes the distribution of possible input values across individuals in the population. Instead, uncertainty characterizes the level of confidence (degree of belief) regarding the value for a given individual. Environmental risk assessors have pointed out that, when interpreting the results of probabilistic risk assessment, variability and uncertainty have different implications (Bogen & Spear, 1987; Hattis & Burmaster, 1994; Hoffman & Hammonds, 1994; Anderson et al., 1999). The distinction is particularly relevant for policy reasons. An understanding of variability permits a characterization of risks that are specific to those population subgroups of potential concern to a decision-maker (e.g. children, pregnant women,

immunocompromised individuals). Knowledge of uncertainty can both qualify the certitude of the reached conclusions and prioritize further research. Separation of uncertainty and variability throughout a risk assessment model is consequently advocated.

4.2.2 Second-Order Modeling of Uncertainty and Variability

Nauta (2000) introduced the terminology of "second-order modeling" to label probabilistic risk assessment models that separate uncertainty and variability. The term "second-order" was originally proposed to refer to those inputs that are expressed through two (or more) probability distributions – one for uncertainty, the other for variability (Frey & Burmaster, 1999). Specification of these second-order random variables is done on the basis of expert judgment and/or statistical analysis. Maximum likelihood methods and the bootstrap method are examples of the latter (Burmaster & Thompson, 1998; Frey & Rhodes, 1996). Once the second-order inputs are defined, a variety of approaches has been proposed to propagate both uncertainty and variability throughout the risk assessment model into the final results. These techniques include methods based on mathematical derivation or approximation of the input variables, propagation of moments using Taylor series expansion, and two-dimensional Monte Carlo simulation (Bogen & Spear, 1987; Bogen, 1995; Rai et al., 1996; Frey, 1992; Hoffman & Hammonds, 1994).

The framework of the two-dimensional Monte Carlo simulation is briefly described. The approach originally assumed independence between the uncertainty and variability (Frey, 1992), but further development has led to a generalization that handles different levels of correlation (Frey & Rhodes, 1996). For second-order inputs, two separate sets of samples are generated through Monte Carlo simulation. The "two-dimensional" character of the simulation derives from the fact that uncertainty and variability are modeled along two different dimensions of the model. This can best be pictured as a matrix in which each row represents a given individual, and each column is one uncertainty fractile. (With regard to the empirical cumulative distribution of a random variable, a fractile is the fraction of values that are less than or equal to a given value.) Consequently, the distribution of values within a row depicts the uncertainty for a

given member of the population, while the distribution of the values within a column is the variability for a given realization of uncertainties. The risk assessment model is calculated for each uncertainty and variability level of the different input variables (i.e. across the same cell of different matrices).

Separation of uncertainty and variability only makes sense if the purpose is to assess risk at a population level (Frey & Rhodes, 1996). When one is rather interested in the risk faced by an individual randomly selected from the population, an analysis that confounds uncertainty and variability can be justified. Clearly, second-order modeling represents a high degree of sophistication in risk assessment. While it leads to risk estimates that have an added value in terms of risk management, it poses specific challenges to a risk assessor. In the case of a Monte Carlo simulation for instance, the two-dimensional data structure implies that, depending on the chosen sample size, computation can be intensive (Frey, 1992). If 500 samples are obtained for both variability and uncertainty, a data set of 250,000 combinations results. Further, risk assessors have to specify separate uncertainty and variability distributions of the input variables and to accommodate potential correlations among these distributions (Frey & Rhodes, 1996). When data are not readily available, this implies coherent elicitation of expert judgment. Last but not least, particular care needs to be reserved to the communication of the applied methodology and of the risk assessment outcome. When uncertainty analysis is conveyed simply and clearly using familiar examples and languages, risk managers are able to appreciate its relevance .

When variability is expressed in terms of a nominal variable (e.g. gender, race, age category), performing an analysis which accounts separately for each homogeneous population subgroup seems to be an efficient approach. Nonetheless, available literature appears to overlook stratification as a mean to deal with variability.

4.2.3 Applications in Microbial Risk Assessment

Conceptualization of uncertainty and variability in microbial risk assessment has largely mimicked work done in the field of environmental risk assessment. The utility of

probabilistic analysis has long been recognized (Jaykus, 1996), and this approach nowadays prevails (Lammerding & Fazil, 2000). Within this context, the different implications of uncertainty and variability have likewise been noted (McNab, 1997). Their separation has often been advocated (CAC, 2000a; ILSI/RSI, 2000), and this is increasingly being carried out.

Nauta (2000) recently illustrated the relevance of second-order modeling in microbial risk assessment. In particular, the predictions of a growth model for *Bacillus cereus* in pasteurized milk were contrasted for the cases in which uncertainty and variability are confounded and separated, respectively. Only when separation is respected, is a risk for a large outbreak identified. The author concludes that, since second-order modeling is relevant in the relatively simple model under consideration, it should likewise be in a more complex model such as a complete risk assessment. The use of second-order modeling of time and temperature effects on the growth of *Salmonella* Enteritidis in eggs has similarly been advanced (CAC, 2000b). A British risk assessment model of *Campylobacter* infection deriving from poultry consumption also applies second-order modeling (Emma Hartnett, pers. comm.). As of May 2002, available referenced literature on this study does not highlight the details of the application (Hartnett et al., 2001).

Two-dimensional Monte Carlo simulation is extensively employed in the risk assessment for *Listeria monocytogenes* in selected ready-to-eat foods that the U.S. Food and Drug Administration is currently completing (FDA/CFSAN, 2001). Specifically, it is used to integrate the inputs of the exposure assessment step as well as the variability and uncertainty relating to pathogen virulence and host susceptibility. However, the two-dimensional framework could not be maintained throughout the risk assessment. The two-dimensional exposure assessment is actually converted into a one-dimensional simulation (viewed to represent uncertainty only) in order to make dose-response modeling possible.

It is perhaps not coincidental that dose-response assessment represents such an impediment to the realization of second-order modeling. While microbial risk assessors

have often modeled exposure in great detail, hazard characterization has essentially relied on the predictive capability of dose-response functions. Quantitative approaches have been proposed to deal with model uncertainty (Kang et al., 2000; Latimer et al., 2001). However, although confidence bands of specific microbial dose-response functions have been examined (Teunis et al., 1996), there has never been an attempt to incorporate parameter uncertainty into a risk assessment. Chapter 2 of this dissertation formally investigates the application of the bootstrap method to address the sampling error associated with microbial dose-response functions. A main conclusion is that such a source of uncertainty is considerable. Its recognition and treatment thus appears due in microbial hazard characterization.

4.2.4 Dose-Response Curve for Heterogeneous Populations

Hewlett and Plackett (1979) discuss the interpretation of quantal responses in bioassay. While this discussion refers to pharmacology, translation to the microbial dose-response framework is plain. Dose-response experiments often lead to the observation of a sigmoid curve that can be interpreted as the cumulative distribution of interindividual susceptibility to a pathogen. When a population is composed of subgroups that are heterogeneous in relation to susceptibility, a mixture distribution arises. If one knows the fraction of each group in the whole population and the specific response (e.g. infection probability) of each group (for instance, through experimentation), a dose-response curve for the heterogeneous population can nonetheless be obtained as the sum of group-specific responses, each weighted for the relative population fraction. (Section 1.4.1 and Figure 1.8 review the concept in detail).

Unfortunately, the implementation of such an approach is not straightforward within the context of microbial risk assessment. Children, the elderly, pregnant women, and immunocompromised persons are usually considered to be particularly susceptible to foodborne pathogens (Gerba et al., 1996). While census data are available to determine the proportions of specific groups within the population, there is little to no experimental data concerning the dose-response relation in particular subpopulations. In fact, microbial risk assessors have often lamented that volunteers in human feeding studies

with foodborne pathogens were mainly young, healthy males (Buchanan et al., 2000). The counterpart of such a remark is that the obtained dose-response curve ought to be very representative of the census stratum that the volunteers represent. Quite in conformity to good experimental design, human feeding studies select patently similar volunteers in order to control for interindividual variability, and thus isolate the effect of a varying dose.

While often limited in their ability to quantify exposure, epidemiological studies often target the understanding of demographic and behavioral factors involved in illness events. Specifically, case-control studies are routinely applied to foodborne outbreaks to identify a contaminated food vehicle and ascertain other risk factors. A popular analytical tool is the logistic model because its parameter coefficients can readily be interpreted as odds ratios, a measure of relative risk. Other epidemiological study designs and methods attempt to achieve the same objective. In Chapter 3, the Poisson loglinear model was used to analyze FoodNet surveillance data: the estimated incidence rate ratios are a type of relative risk. For surveillance of relatively rare events, calculation of relative risk and odds ratios leads to equivalent figures (Gordis, 1996). It can be shown that that would also be the case for the FoodNet data. (With yearly rates of less than 1 per 4,000 persons, foodborne bacterial infections are rare events in statistical terms.)

Quite coincidentally, the logistic function has also been a rather popular dose-response function in toxicology (Hartung, 1987). Microbial risk assessors have in large part dismissed it based on theoretical considerations linked to low-dose extrapolation (Haas, 1983). However, as shown in Chapter 2, when one considers the sampling error involved in microbial dose-response modeling, differences between functions are relative, at best. In terms of microbial risk assessment, the logistic function actually offers the distinct opportunity to blend dose-response modeling and epidemiological information. It has been pointed out that, of all age categories, data from human feeding studies should be especially representative of young adults. A dose-response model fitted to those data would thus estimate the infection/illness probability for the young adult age

category. The location parameter of this function reflects the median susceptibility across young adults (since dose is generally log-transformed), while the scale parameter reflects the dispersion of susceptibility. One could formulate the hypothesis that age categories mainly differ in their median susceptibility while susceptibility spread remains constant. An age-specific dose-response curve would then be obtained by summing the coefficient of the logistic model from the epidemiological analysis to the location parameter coefficient of the logistic dose-response function. This can best be depicted considering that the logistic function in a dose-response graph with log-transformed dose and logit-transformed response draws a linear curve. The summation essentially translates into vertically shifting the intercept of the dose-response curve. The slope itself remains unchanged.

4.3 Data and Methods

All computations of this study were carried out with the SAS/STAT software version 8.01 (SAS Institute, Cary, NC). Information on exposure dose was obtained from a concurrent study that models cross-contamination of ready-to-eat food due to *Campylobacter jejuni* (Heejeong Latimer, pers. comm.). A lognormal function was satisfactorily fitted by maximum likelihood estimation to the provided data (PROC NLIN; location parameter 1.87; shape parameter 1.68; Kolmogorov-Smirnov goodness-of-fit test, p=0.09). This distribution is assumed to represent the average dose to which the population is exposed at different occasions. The actual dose contained in a serving is expected to follow a Poisson distribution, whose parameter is the mean dose given by the lognormal model.

Dose-response modeling was conducted as described in Chapter 2. Data from a volunteer feeding trial with *Campylobacter jejuni* were used (Black et al., 1988). Parameters of the dose-response function – either the log-logistic model or the beta-Poisson model – were estimated through maximum likelihood estimation (PROC NLIN). Specifically, the estimates for the parameters β_0 and β_1 of the log-logistic model were -1.385 and 0.484, respectively. When uncertainty was simulated, bootstrapped parameter

pairs were estimated based upon either binomial or beta-binomial resampling of the experimental data.

The risk of *Campylobacter* infection of seven distinct age groups (less than 1 year of age, 1 to 9, 10 to 19, 30 to 39, 40 to 49, 50 to 59, greater than 60) relative to individuals of 20 to 29 years of age was calculated with Poisson regression (see Chapter 3). Reported counts of *Campylobacter* infections for the four-year period between 1996 and 1999 were extracted from annual FoodNet reports (CDC, 2000), while denominators for the population at risk were derived from online data of the U.S. Census Bureau (U.S. Census Bureau, 2002). The Poisson loglinear model was fitted by means of Generalized Estimating Equations (PROC GENMOD). The final model contained the main effects age, site, and year as well as the interaction between site and year. The coefficients of the seven age groups are considered to follow a normal distribution in which the mean is the estimate of the age parameter from the Poisson regression and the standard deviation is the standard error of such a parameter. These means and standard errors are reported in Table 3.6 (column 2 and 3).

For any given dose, the infection probability P in the heterogeneous population is the sum of age-specific infection probabilities P_i each weighted for the fraction of the relative age group δ_i in the population, that is

$$P = \sum_i \delta_i P_i$$

The age-specific fraction δ_i for the estimated population of the United States in July 2002 is: less than 1 year of age, 0.014; 1 to 9, 0.123; 10 to 19, 0.145; 20 to 29, 0.131; 30 to 39, 0.145; 40 to 49, 0.158; 50 to 59, 0.118; greater than 60, 0.166 (U.S. Census Bureau, 2002). The infection probability P_i is estimated through a dose-response function. The human feeding trial with *Campylobacter jejuni* was conducted with young adults (Black et al., 1988), and a dose-response model fitted to the relative data is thus assumed to reflect the dose-response relationship in 20 to 29 year-old individuals.

When a log-logistic model is applied, P_i for this age group can thus be expressed as

$$P_{20\text{ to }29} = f(\beta_0, \beta_1; d) = 1 / 1 + e^{-[\beta_0 + \beta_1 * \log_{10}(d)]} \qquad \text{eq. 4.1}$$

where β_0 and β_1 are the parameters of the log-logistic dose-response model, and *d* is dose. Equation 4.1 is the baseline model used in our analyses. It can be extended to model dose-response for the remaining seven age groups. Specifically, the Poisson regression coefficients mentioned in the previous paragraph are defined as β'_{0i}, and are summed to the parameter β_0. The age-specific infection probability P_i is thus

$$P_i = f(\beta_0, \beta'_{0i}, \beta_1; d) = 1 / 1 + e^{-[(\beta_0 + \beta'_{0i}) + \beta_1 * \log_{10}(d)]} \qquad \text{eq. 4.2}$$

This implies two assumptions. Firstly, risk differences among age groups resulting from the FoodNet surveillance data are viewed to arise solely based on differences in susceptibility. In other words, exposure is assumed to be comparable for all age groups. Secondly, susceptibility distributions differ from one age group to another only in terms of the median, while the dispersion is similar for all age groups (i.e. the parameter β_1 is correct independently of age). To account for varying serving size, and thus dose size, in different age groups, a factor γ_i that proportionally adjusts dose can be introduced. P_i is then

$$P_i = f(\beta_0, \beta'_{0i}, \beta_1, \gamma_i; d) = 1 / 1 + e^{-[(\beta_0 + \beta'_{0i}) + \beta_1 * \log_{10}(\gamma_i * d)]} \qquad \text{eq. 4.3}$$

As calculated from the "Continuing Survey of Food Intake by Individuals", the mean serving size for poultry in select age groups is: 32.8 g for individuals of less than 1 year of age; 103.5 g for the 20 to 29 age group; 85.3 g of people older than 60 years (Roberta Morales, pers. comm.). When compared to young adults, the dose adjustment factor γ_i was thus assumed to be 0.3 and 0.8 for infants and the elderly, respectively.

The performed analyses considered the three models described by equations 4.1 to 4.3. A first set of computations ignored the effect of age, and thus corresponds to the

implementation of the model described in Equation 4.1 (or the corresponding beta-Poisson formulation when dose-response functions were contrasted). This baseline model is equivalent to using the log-logistic dose-response function based on the experimental *Campylobacter* data. (As mentioned in the previous paragraph, this baseline is assumed to represent the 20 to 29-year-old age group.) By employing Equation 4.2, the relative effect of age was then considered. Finally, the influence of adjusting for varying serving size was assessed for infants and the elderly. With reference to young adults, these two age groups represent an increased and a decreased relative risk for *Campylobacter* infection, respectively.

Either "first-order analysis" or "second-order analysis" was conducted on the proposed models. Specifically, the term first-order is used for an analysis that, while probabilistic, confounds uncertainty and variability. In contrast, the two elements are kept separated in a second-order analysis. The effect of age was separated by stratifying the analysis, while the other sources of uncertainty and variability were propagated using Monte Carlo simulation. The latter was practically done through the coding of iterative DO loops within the DATA step and the use of random number functions. First-order analysis drew 500 samples from the lognormal distribution of dose described earlier. The other parameters were the point estimates of the dose-response function or the Poisson regression. The combination of two-dimensional Monte Carlo simulation with stratification achieved second-order analysis. Dose was generated through a two-dimensional simulation that nested one iterative loop into another. The first loop simulated variability by creating 500 samples of the variable dose from the mentioned lognormal distribution. Each of those samples was uniquely labeled with a value between 1 and 500 contained in the index variable *V* (basically, the iteration number). Five-hundred Poisson random samples were generated starting from those first 500 samples. This second loop represented uncertainty, and the index variable *U* contained the iteration number. The resulting data set of 250,000 observations can be thought as a virtual matrix of size 500x500, in which one dimension represents variability and the

other uncertainty. Together, the index variables *V* and *U* uniquely identify each cell of the matrix.

Parameter uncertainty of the dose-response function was simulated with bootstrap method. Each one of the 500 pairs of parameter coefficients was progressively labeled through the index variable *U*, and copied 500 times (each number receiving a progressive number for the index variable *V*). Uncertainty in the age effect was simulated by drawing 500 samples from seven normal distributions (one for each age groups excluding the 20 to 29-year-old baseline) whose parameters were defined based on the results of the Poisson regression. Five-hundred copies in the variability dimension were also created. The three data sets were merged by the index variables *V* and *U*, and either one of the equations 4.1 to 4.3 was resolved. Each one of 250,000 observations thus contained a value for dose and the corresponding infection probability for each of the eight age groups.

The data set resulting from the simulation was further used in two ways. On one hand, it was reformatted so that each line would only contain the response for one age group and the corresponding input variables (250,000 x 8 = 2,000,000 observations). The Spearman rank correlation between response and model variables was then calculated for a random sample of 200,000 observations (PROC SURVEYSELECT, PROC CORR). On the other hand, the frequency of iterations for a given combination of rounded dose and response was used to estimate an empirical joint probability mass (PROC FREQ). Such a probability reflects the likelihood of the specific joint realization of variability and uncertainty. After smoothing the data (PROC G3GRID) and calculating the fractiles of the empirical distribution (PROC RANKS), this grid was used to generate contour plots and 3-dimensional plots.

4.4 Results

The first set of results focuses on the combined effect of variability in dose and parameter uncertainty of the dose-response function (Figure 4.1 to Figure 4.3). The baseline model employs the log-logistic dose-response function, and bootstrapping is

performed through a binomial resampling scheme. Age is not considered. Figure 4.1 shows the results of first-order analysis. From these graphs, it is apparent that the dose-response function creates a perfect correlation between dose and response: given a dose, the response is defined with certitude. In contrast, second-order analysis accommodates the sampling error arising from dose-response modeling, and the dose-response relation becomes a surface (Figure 4.2). While the shape of the dose-response surface is still driven by the model, the Spearman rank correlation is now 0.72. Fractiles of the empirical cumulative distribution of the joint probability are drawn in the contour plot. These contours can be interpreted as confidence regions, in that the confidence level is equal to 1 minus the fractile, multiplied by 100. In particular, the 0.05 fractile describes the 95% confidence region, the 0.25 fractile the 75% confidence region, and so on. Figure 4.3 directly contrasts the results from three analytical approaches that represent an increasing level of sophistication. The deterministic analysis only considers the median of the dose distribution (i.e. the mean in a $\log_{10}$ scale, 1.87), and the response (0.382) directly results from the dose-response function. Stochastic simulation in a single dimension generates a similar pair of most likely values ($\log_{10}$ dose 1.97, infection probability 0.394). However, it is now possible to make a statement of confidence regarding the range of these estimates. Specifically, the 90% confidence intervals for dose goes from -0.74 to 4.49, and that for response from 0.149 to 0.687. The distinct advantage of the two-dimensional probabilistic simulation is that it allows confidence statements for any given value of dose and response. The most likely pairs ($\log_{10}$ dose 2.43, infection probability 0.46 – as estimated from higher resolution of the 0.96 and 0.97 fractile contours) are notably shifted towards higher values of both dose and response. Prior to data smoothing (Table 4.2), one would have concluded that the most likely estimates were the pair of 3.2 $\log_{10}$ dose and 0.6 response (this combination has the highest joint probability out of the 121 cells). Such pair would sit on the right-hand vertex of the 5% confidence region of Figure 4.3.

Figure 4.4 and Figure 4.5 contrast the influence of different combinations of dose-response function and bootstrap resampling scheme. Generally, once one considers the

sampling error involved in microbial dose-response modeling, the choice of model or bootstrap algorithm appears to have little relevance. The log-logistic model produces more conservative responses at low doses. This is particularly evident in Figure 4.4, but can also be noticed in the 0.75 fractile contour of the second-order analysis (Figure 4.5). The beta-binomial resampling generates somewhat broader confidence regions, especially at low doses (upper, left-hand sector of the graphs, Figure 4.5). Given the small difference, further analysis is limited to the log-logistic dose-response function and, when second-order analysis is carried out, to binomial resampling.

From Figure 4.6 on, the influence of age is illustrated. As shown in Table 3.6, the epidemiological analysis suggests that infants (less than 1 year of age) have about a two-fold greater risk than the reference group of young adults. On the other extreme, an age between 10 and 19 years or greater than 60 years is associated with a nearly halved relative risk. The ordering of the curves in Figure 4.6 closely reflects the relative risks of Table 3.6. However, the vertical shift with respect to the reference age group (20 to 29 years of age, blue curve) increased, the larger/smaller the relative risk is. The distances between curves remain proportionally constant throughout dose levels. Figure 4.7 shows that a logit transformation of the response axis actually leads to parallel curves. The exception is the curve obtained by the weighted sum of the age-specific responses (designated as “combined”, black line), which is meant to reflect the dose-response relation for the heterogeneous population. As it is especially evident from the 95% confidence interval graphs, this line is somewhat less steep than the age-specific ones are. The vertical shift between age groups is maintained in the second-order analysis (Figure 4.8 and Figure 4.9), and the shape of the confidence regions appears very similar. In Figure 4.9, when comparing the results for infants (red line) with those of the 20-29 age groups (blue line), it would appear that, for any given dose, the relative risk of 2 roughly translates into an 0.10-0.15 increase in infection probability independently of the considered dose or confidence level. Conversely, a 0.10-0.20 decrease in infection probability seems to be associated with a relative risk of 0.5, as judged from the 10 to 19 age group (yellow line). In contrast, a similar pattern cannot be inferred starting from the

y-axis (infection probability) because the vertical shift of the confidence regions is not linked to a proportional horizontal change (25% confidence region graph of Figure 4.8). For any given infection probability, the associated confidence interval of dose varies from one age group to another.

Adjusting for a smaller serving size for infants and the elderly has a limited impact on the results (Figure 4.10). For infants, the shape of the confidence regions for infants is unchanged, and there is a limited decrease in infection probability. Overall, the difference with the reference age group diminishes. In contrast, the results for the individuals older than 60 years of age are unaffected by the dose-adjustment.

The following Spearman rank correlations resulted between age-specific responses and model inputs: $\log_{10}$ dose, 0.67; dose-response model parameter β_0, 0.56; dose-response model parameter, β_1 -0.51; age effect parameter β'_{0i}, 0.33. The two parameters of the dose-response model are highly inversely correlated (-0.96). With dose adjustment, correlations between age-specific response and the mentioned inputs were similar, and that of the dose adjustment factor γ_i was 0.01.

4.5 Discussion and Conclusions

The methodology presented in this study achieves two advancements in microbial risk assessment. On the one hand, integration of an important source of uncertainty (sampling error) linked to dose-response modeling more openly reflects the degree of reliability inherent in the risk assessment and its outcomes. This has relevant implications in terms of risk management. Secondly, the potential role of a specific host factor and, by reflection, the need for a more detailed characterization of the multifaceted process of foodborne infection, is evaluated. As no similar work relating to microbial hazard characterization has been published yet, there is a limited opportunity to put our results into the perspective of other researchers' findings.

In Chapter 2, it is shown that there is a relevant amount of sampling error associated with the estimation of a microbial dose-response function. The results of the

present chapter confirm that such a circumstance carries over into the probabilistic framework of a microbial risk assessment model. When Figure 4.5 is compared to Figure 2.25, even though the stochastic simulation of the variable dose causes the 95% confidence region to broaden, the underlying influence of the parameter uncertainty of the dose-response function is still manifest. This is also clear when the results of first-order analyses are contrasted with those of second-order analyses (e.g. Figure 4.1 versus Figure 4.2). Although other sources of variation are compounded, the blurring of the previously exact relationship between dose and infection probability is largely due to the consideration of the uncertainty associated with the dose-response function.

Against the general expectation that a risk assessment ought to provide specific guidance in terms of risk management, the amount of uncertainty that is revealed by the second-order analysis might appear at first sight disconcerting. As a matter of fact, the increasingly sophisticated methodology does not affect one's ability to identify most likely outcomes. As shown in Figure 4.3, when one moves from a deterministic to a stochastic analysis and from first-order to second-order modeling, most likely values of dose and response can always be identified. The location of these pairs varies due to the random nature of the Monte Carlo simulation (deterministic versus first-order stochastic analysis) and to an increased complexity of the applied models (second-order stochastic analysis). The notable improvement is the ability to make statements on the degree of confidence/belief in the results. While the first-order analysis characterizes the confidence of the most likely estimates, a similar statement is possible for any point in the dose-response plan when one applies second-order analysis.

The capability of making confidence statements has far reaching implications in terms of risk management. This was originally pointed out in environmental risk assessment (Bogen & Spear, 1987; Hattis & Burmaster, 1994; Hoffman & Hammonds, 1994; Anderson et al., 1999), but has since been recognized in the food safety arena (Lammerding & Fazil, 2000). The emphasis given in this study to a graphical representation of the results is not casual. On the one hand, it is meant to ease potential risk communication challenges posed by the relative complexity of second-order

analysis. More important, there was an overt desire to represent the final results while still displaying exposure. Figure 4.11 shows the possible interpretation of second-order analysis for policy making. Confidence statements are possible irrespective on whether a risk manager is interested in establishing the infection probability for a given dose or the dose for a given infection probability. As a strategy based on post-exposure mitigation is obviously untenable in food safety, the exposure process (whether in terms of dose modulation or avoiding the exposure altogether) clearly is pivotal to risk management options. Thus, a representation of the final risk that still conveys the level of exposure offers an unparalleled insight for a decision-maker. For illustration sake, the exposure scenario considered in this study is simple. At best, it represents a single exposure to a food item contaminated with *Campylobacter jejuni*. However, one could imagine replacing dose and infection probability with other relevant variables from the steps of exposure assessment or hazard characterization, respectively. For instance, the x-axis could represent dose before preparation, and the y-axis the number of exposed persons. The gained insight would be equivalent.

While the focus of this study was on developing a second-order analytical framework for microbial hazard characterization, the methods used to link the simulation output to the graphical representation warrant further refinement. Joint probability of dose and response was obtained empirically by cross-tabulating the frequencies of the two rounded variables. This grid was smoothed and expanded before being graphed as contour or 3-dimensional plots. While the most likely results of the second-order analysis appears in good agreement with those of the deterministic analysis and first-order analysis (Figure 4.3), there is discrepancy between the joint probabilities prior and subsequent to smoothing (Table 4.2 and Figure 4.3). More important, judging the accuracy of the confidence regions – undoubtedly the key feature of a second-order analysis – is hardly possible. This is a major concern because inaccuracy would mostly affect high-level confidence regions (i.e. 90% and 95%). Overall, the critical step in implementation of the proposed analytical approach is the definition of a joint probability. In addition to an empirical approach, parametric method should be

investigated. Once a joint probability distribution is defined, the statistical field of likelihood estimation offers an adequate cadre of methods for developing reliable graphical inference (Owen, 2001).

A related issue concerns the number of iterations in the Monte Carlo simulation. In this study, a relatively small sample size of 500 iterations was chosen. Morgan and Henrion (1990) discuss formal ways of determining the number of iterations that would fulfill a desired level of accuracy. Given the two-dimensional structure of a second-order analysis, an increase in sample size implies exponentiation of the observation number. Theoretically, computation capability is the limiting factor in achieving a better accuracy through an increase in iterations. For the baseline model, we contrasted a simulation with 500 iterations to one with 1,000 simulations (250,000 and 1,000,000 observations, respectively; results not shown). Graphical appearance of the results was similar for the two cases. The larger simulation required a longer, but still manageable computation time (essentially because of the increased data management demand, such as sorting and merging of data files). However, it was at the level of outcome analysis that the added burden became manifest. Imposing a more limited number of iterations for a comparable level of accuracy, Latin Hypercube Sampling should be considered as an alternative to the Monte Carlo method (Morgan & Henrion, 1990).

For the specific case under consideration, i.e. *Campylobacter* infection, consideration of the age effect causes changes in the estimated risk (Figure 4.6 and Figure 4.8). While not as dramatic as those linked to the sampling error of the dose-response function, those differences are nonetheless perceptible. Based on the analysis of Chapter 3, a relative risk of 1.87 for *Campylobacter* infection was established for infants (less than 1 years of age) in comparison to young adults (20 to 29 years of age, reference group). In this chapter, such a relative risk is associated with a 0.1-0.15 increase in infection probability. Similarly, the 0.43 relative risk for the 10 to 19 age group translates into a 0.1-0.2 decrease in response. The fact that relative risks are ratios explains why the differences for the other age groups are more limited than those at extreme relative risks. Also, since differences in response between age groups remain

constant over the observed dose range, their relevance diminishes with increasing dose. From the graphical results, one wonders whether consideration of the age effect matters in terms of the risk assessment. In contrast, the Spearman rank correlation between age-specific responses and age effect parameter is 0.33. While lower than those associated with dose and parameters of the dose-response function, such a correlation is still regarded as high. Overall, the incertitude is perhaps indicative that relative risks of 0.5 and 2 may represent pragmatic thresholds, and that only host factors associated with a relative risk lower than 0.5 or greater than 2.0 ought to be pondered for inclusion into a microbial hazard characterization. In Chapter 3, the incidence rate ratio for *Salmonella* infections in infants was 9.2 (95% confidence interval, 5.8-14.3). There is little doubt that a relative risk of such a magnitude would have a great impact on the risk assessment. Finally, it is noteworthy that higher probabilities for a *Campylobacter* infection are estimated for the reference group (20 to 29 years of age) than for the whole population (blue lines versus black lines in Figure 4.6 and Figure 4.8). As pointed out, the case of the reference group arises from the plain fitting of the dose-response function to the data of the human feeding trial. Since this is a common practice in microbial risk assessment, it would appear that, owing to being ignorant of the age effect, there has been an implicit tendency to overestimate the risk.

Of course, the conclusions regarding the influence of age rely on the soundness of the assumptions made in formulating Equation 4.2. A first assumption is that the relative risks estimated from the FoodNet surveillance data mainly reflect susceptibility differences. As reviewed in Chapter 3, age effects have also been explained with differences in exposure. However, site was also included in the Poisson regression model from which the age effect estimates originated, and it is advanced that differences in exposure would by and large be accounted for by the site variable. Furthermore, it is assumed that only the mean of susceptibility changes across population subgroups. The concept of susceptibility is already so challenging in qualitative terms, that creating speculative quantitative statements is hardly warranted. However, while we cannot justify the assumption, there appears to be no evidence to doubt it either. Also, short of

ignoring the age effect, the proposed approach is the simplest that we can advance, and thus the one that carries the least of assumptions.

A constant to adjust for smaller serving sizes in specific age groups was introduced in Equation 4.3. This correction turns out to have a modest impact in practical terms (Figure 4.10). The very small Spearman rank correlation between age-specific responses and the dose adjustment factor (0.01) supports such a conclusion. Nonetheless, Equation 4.3 makes explicit that factors associated with exposure can potentially have a major impact in hazard characterization. In fact, owing to their position in the considered model, such elements would modify the steepness of the dose-response relation. A change in the shape as well as in the location of the confidence regions would result.

In conclusion, the present study proposes an analytical framework that, with regard to the risk of *Campylobacter* infection, integrates uncertainty associated with dose-response modeling as well as variability due to age. The former has an overbearing influence on the analytical outcome. In contrast, inclusion of the age factor has a questionable relevance. This indicates that biological plausibility and epidemiological evidence do not necessarily translate into risk assessment relevance. Consequently, the advocacy of more closely modeling variability in microbial risk assessments may reflect a misplaced emphasis: the characterization of key sources of uncertainties and their consistent propagation throughout a microbial risk assessment actually appear of greater importance.

4.6 References

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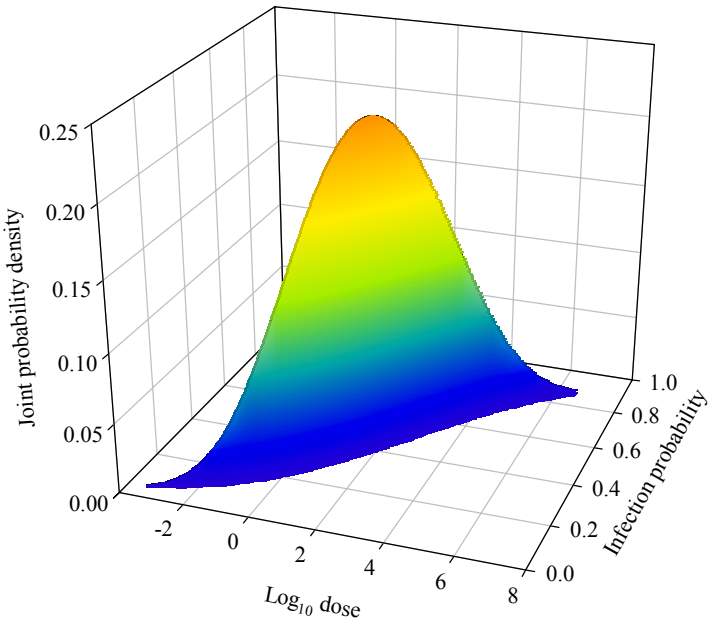
Table 4.1. Age effect on *Campylobacter* infection rates as estimated from Poisson regression of 1996-1999 FoodNet surveillance data

Parameter - Level	Estimate	Standard error	Incidence rate ratio (IRR)	IRR 95% confidence interval
Age				
- <1	0.6262	0.0439	1.87	1.72-2.04
- 1-9	-0.1587	0.0499	0.85	0.77-0.94
- 10-19	-0.8534	0.0397	0.43	0.39-0.46
- 20-29	0.0000	--	1.00	--
- 30-39	-0.1463	0.0455	0.86	0.79-0.94
- 40-49	-0.3302	0.0408	0.72	0.66-0.78
- 50-59	-0.4263	0.0572	0.65	0.58-0.73
- >=60	-0.6657	0.0423	0.51	0.47-0.56

Table 4.2. Joint probability of dose and infection probability resulting from second-order analysis of baseline model

		Log_{10} dose										
		-0.8	0	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2
Infection probability	1.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.004	0.001
	0.8	0.001	0.001	0.001	0.003	0.003	0.005	0.008	0.009	0.011	0.004	0.000
	0.7	0.002	0.002	0.004	0.008	0.012	0.026	0.034	0.017	0.003	0.000	0.000
	0.6	0.002	0.004	0.009	0.018	0.024	0.050	0.032	0.006	0.000	0.000	0.000
	0.5	0.005	0.005	0.011	0.026	0.040	0.048	0.013	0.000	0.000	0.000	0.000
	0.4	0.006	0.007	0.018	0.038	0.041	0.031	0.001	0.000	0.000	0.000	0.000
	0.3	0.008	0.012	0.025	0.045	0.035	0.010	0.000	0.000	0.000	0.000	0.000
	0.2	0.017	0.016	0.036	0.038	0.016	0.001	0.000	0.000	0.000	0.000	0.000
	0.1	0.038	0.026	0.029	0.016	0.001	0.000	0.000	0.000	0.000	0.000	0.000
	0.0	0.022	0.006	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000



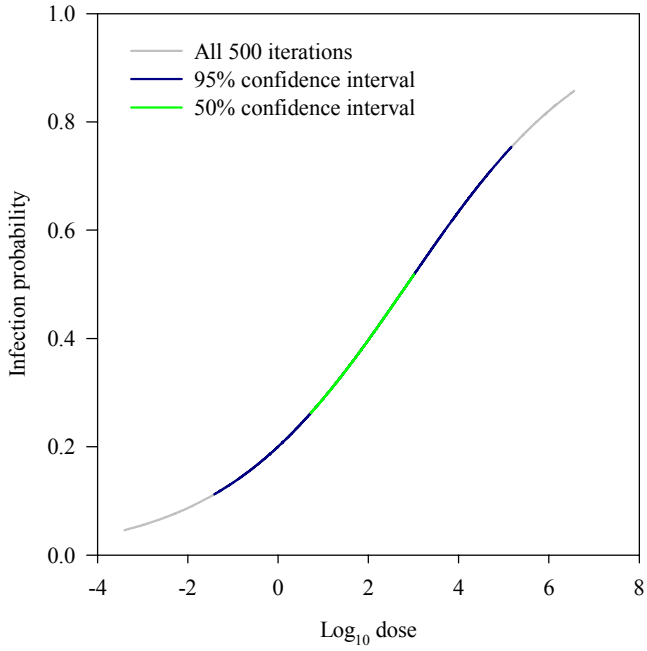


Figure 4.1. First-order analysis of baseline model

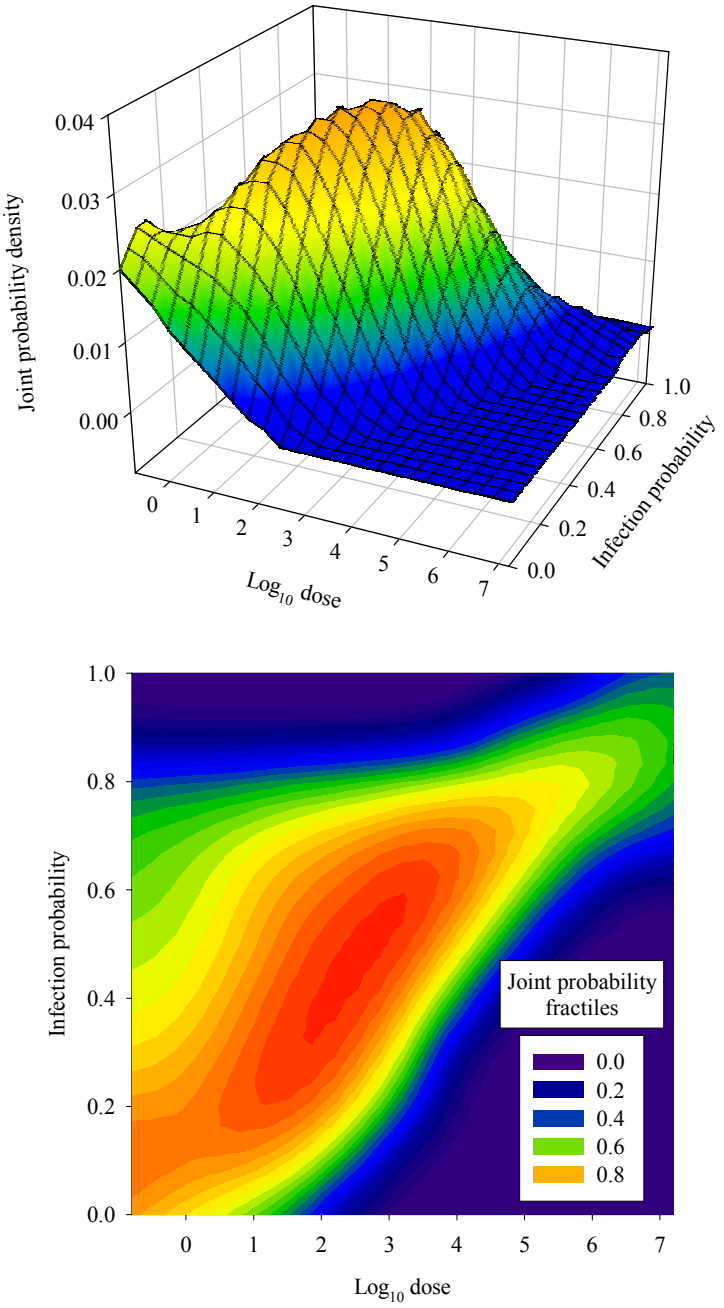


Figure 4.2. Second-order analysis of baseline model

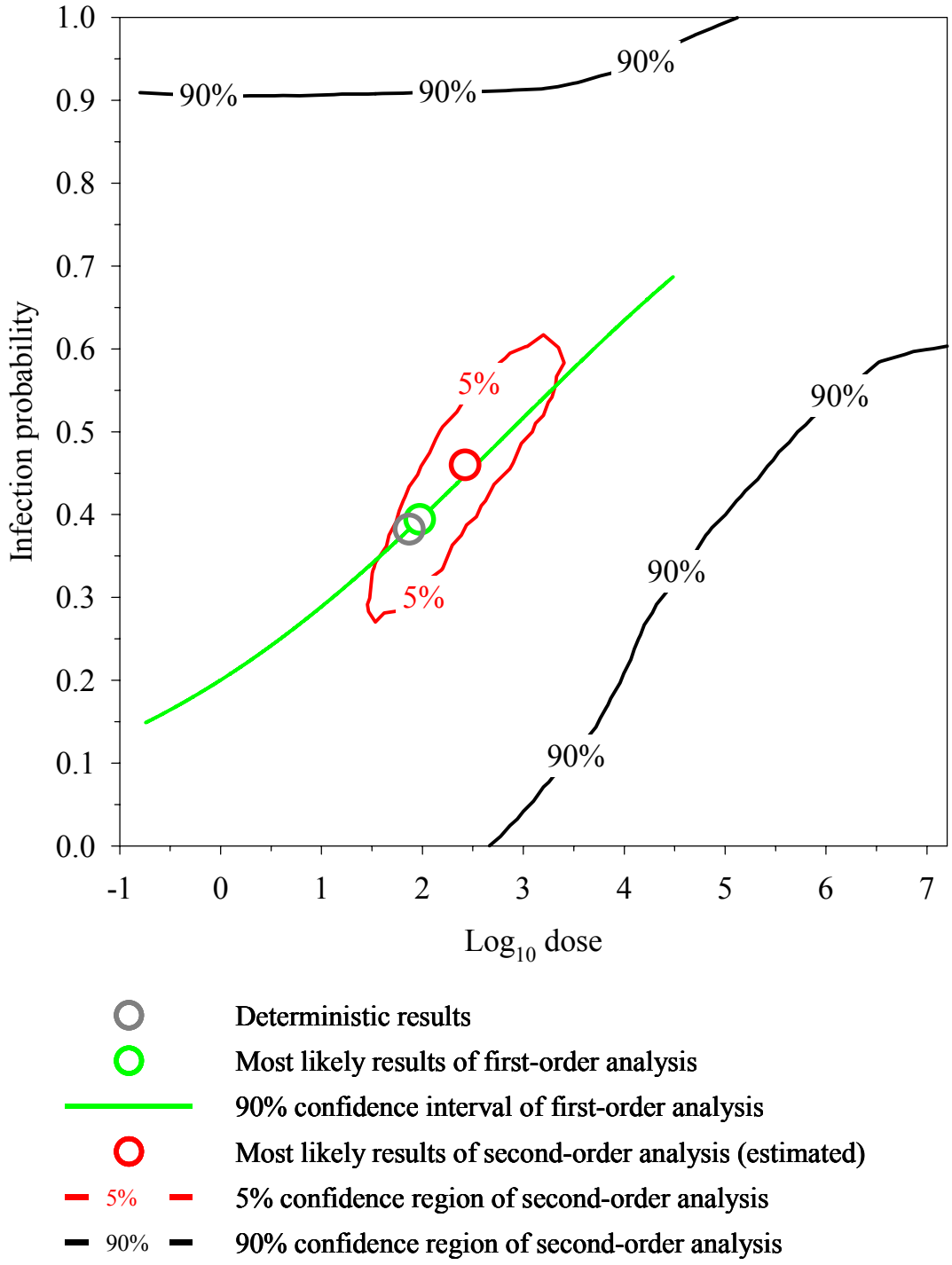


Figure 4.3. Relationship between deterministic and probabilistic results

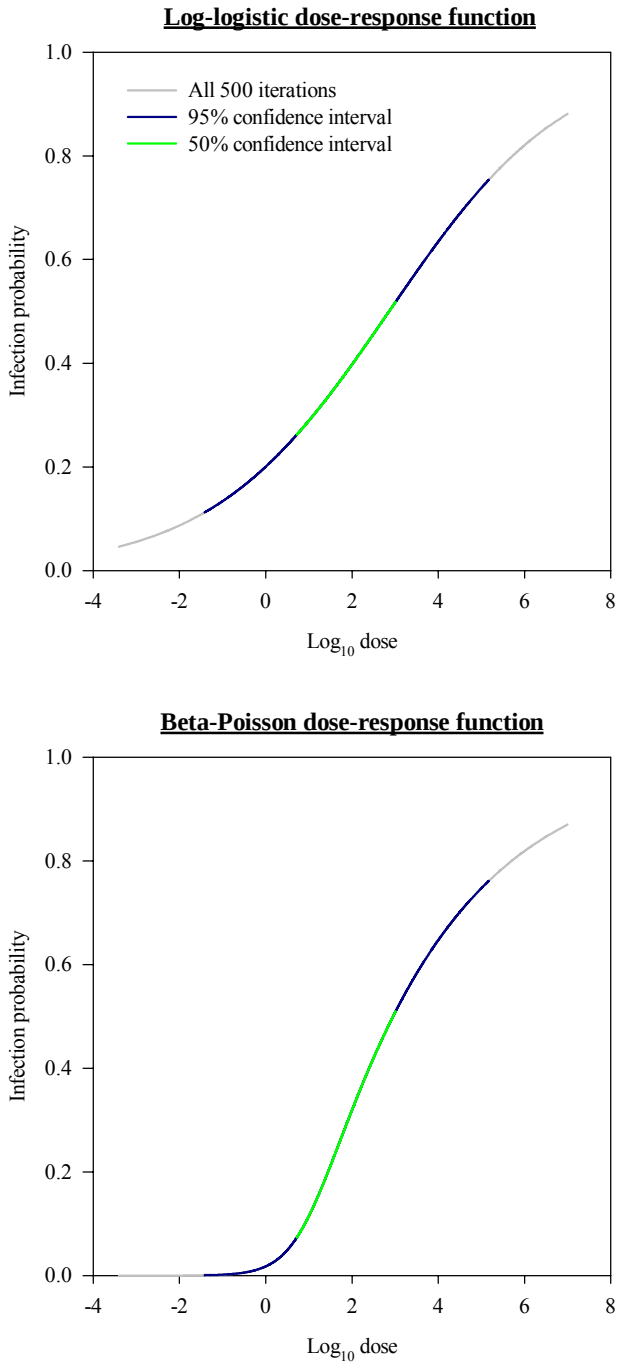


Figure 4.4. First-order analysis with either the log-logistic or the beta-Poisson dose-response function

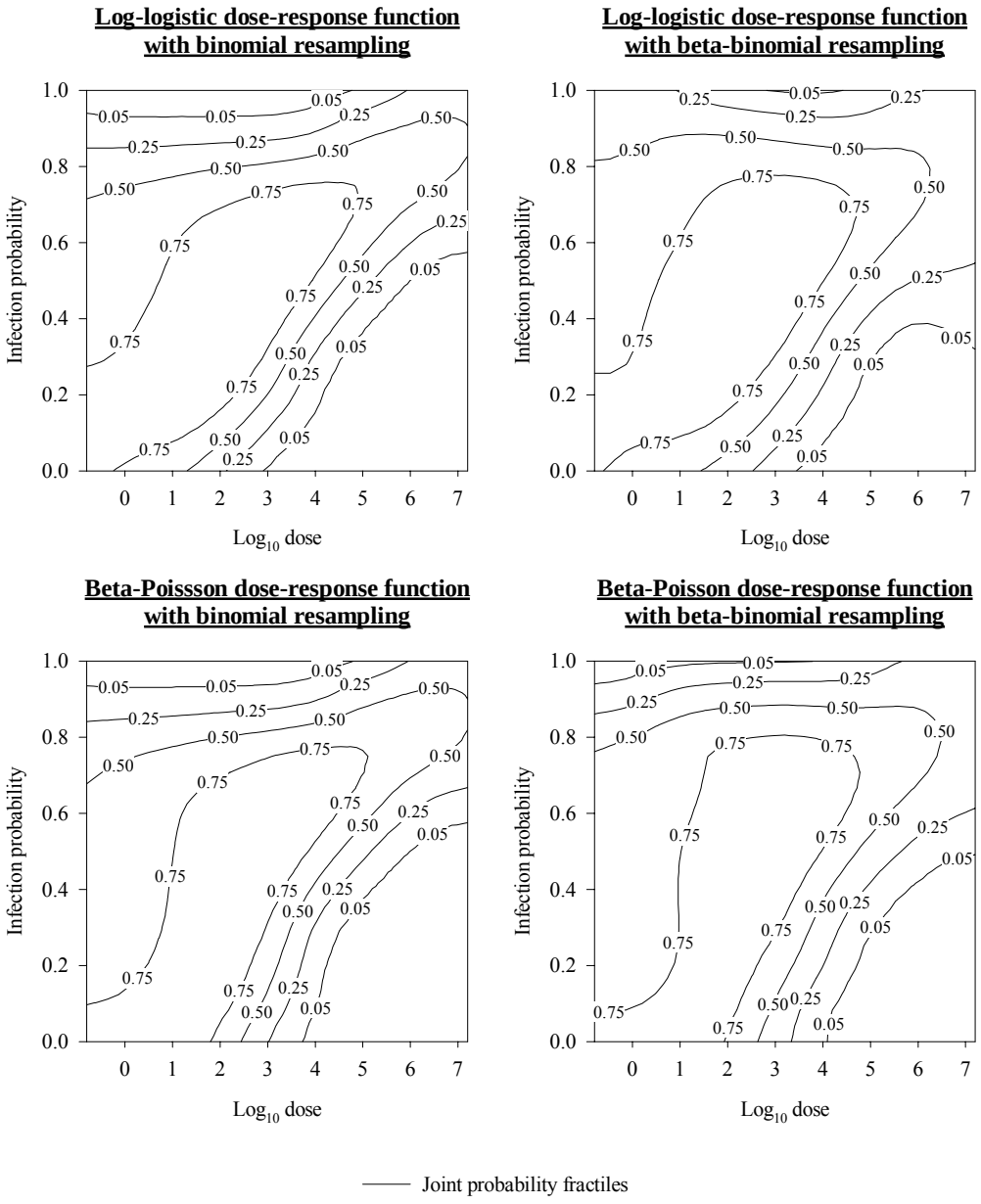


Figure 4.5. Second-order analysis for different combinations of dose-response function and bootstrap resampling scheme

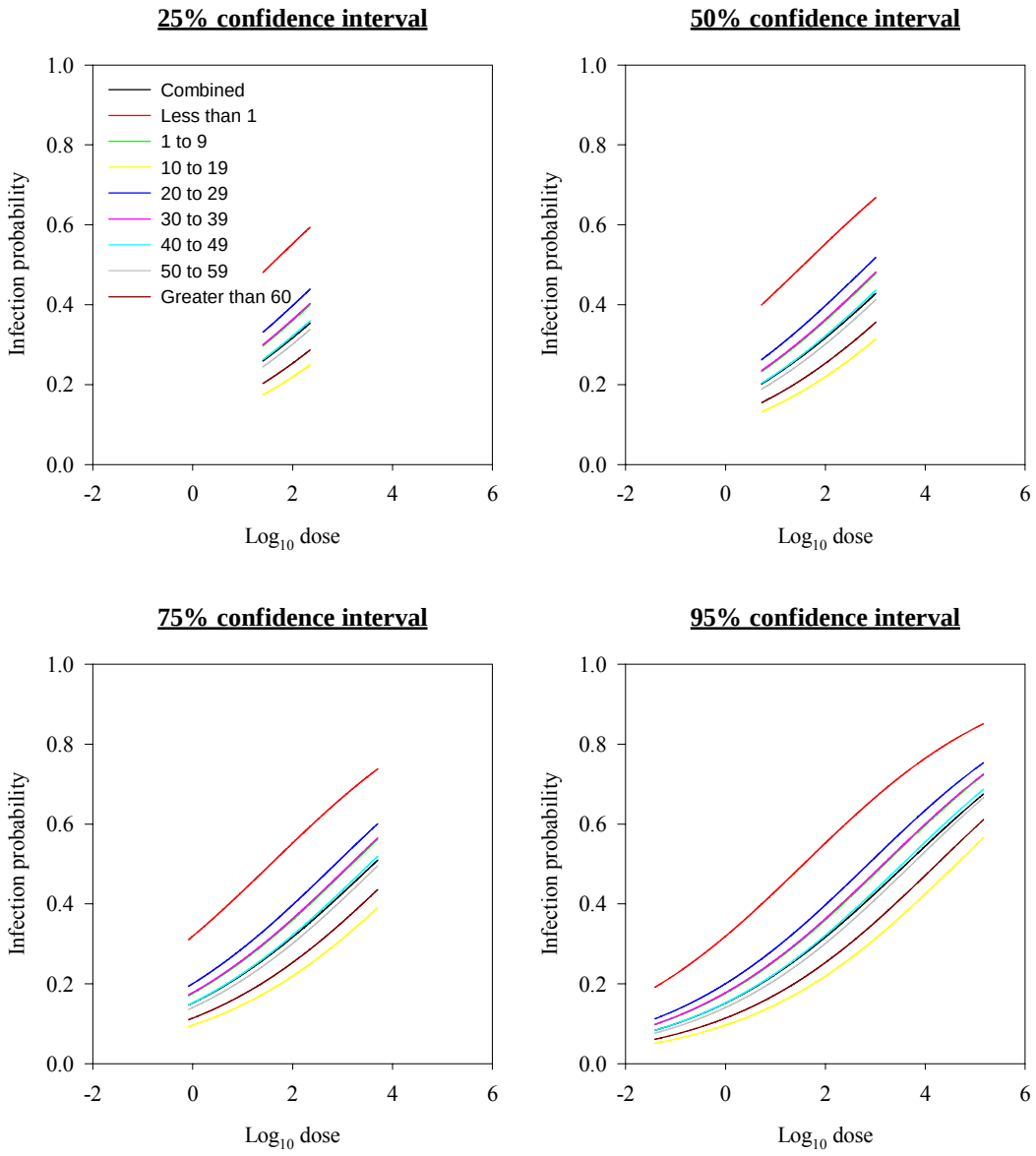


Figure 4.6. First-order analysis for different age groups

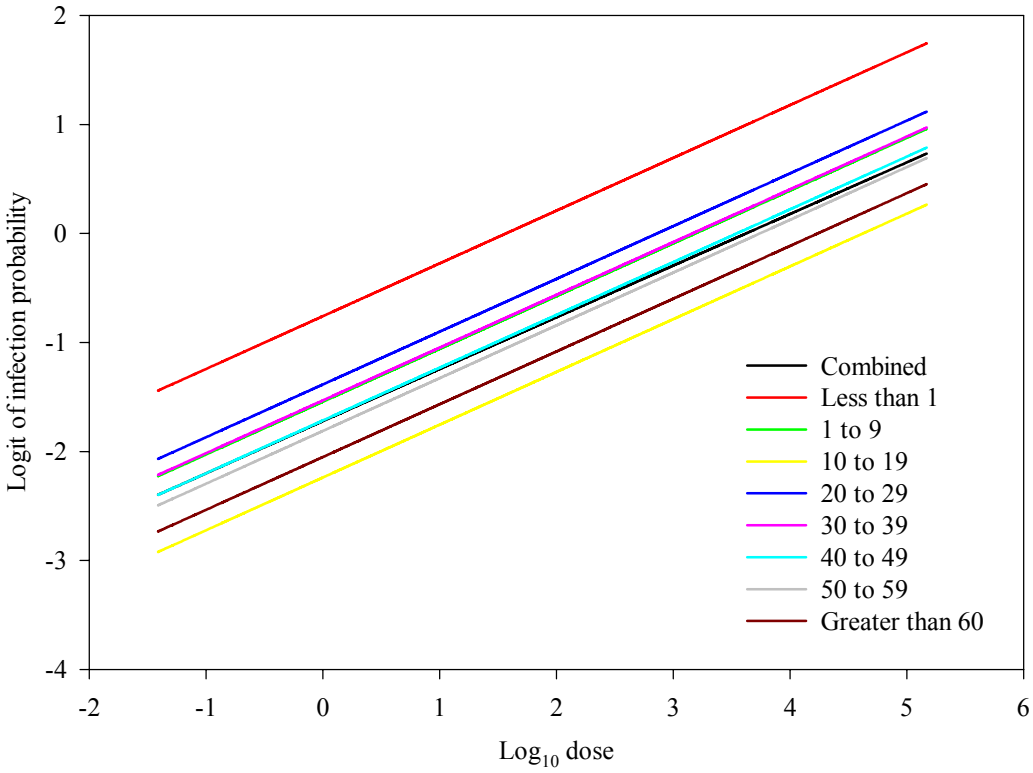


Figure 4.7. First-order analysis for different age groups with logit-transformed response (95% confidence interval)

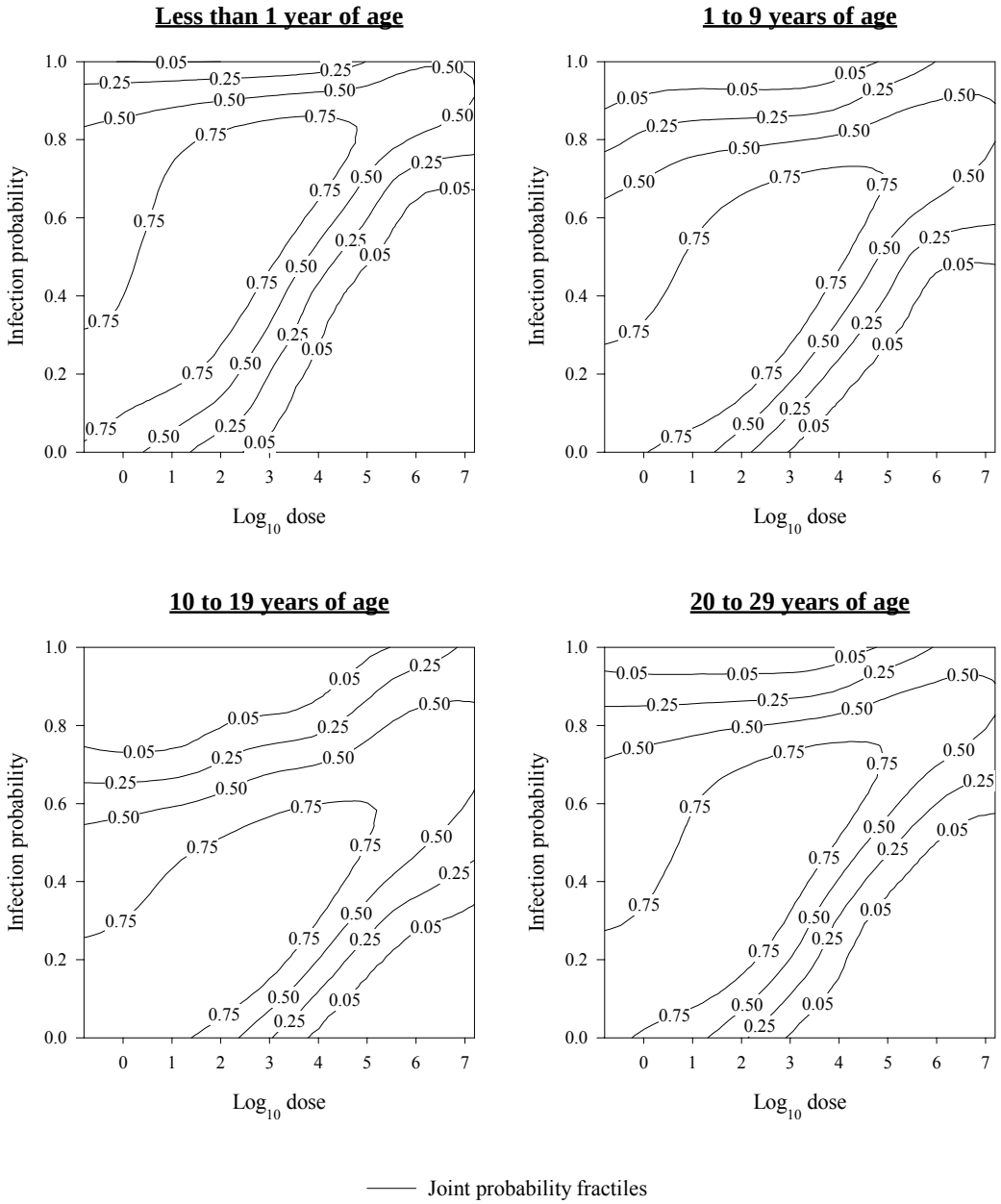


Figure 4.8. Second-order analysis for different age groups, by age group

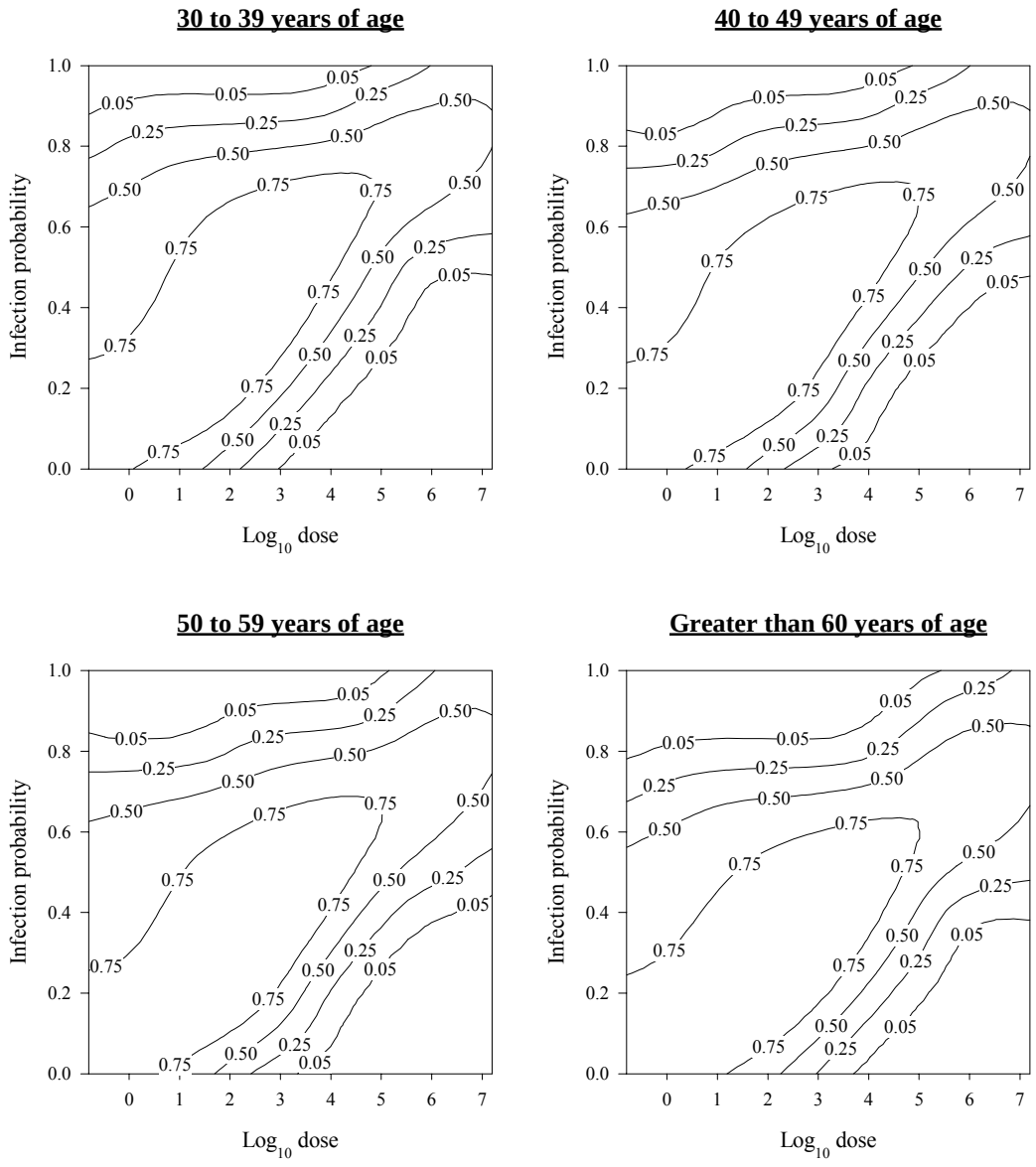


Figure 4.8 (cont.) Second-order analysis for different age groups, by age group

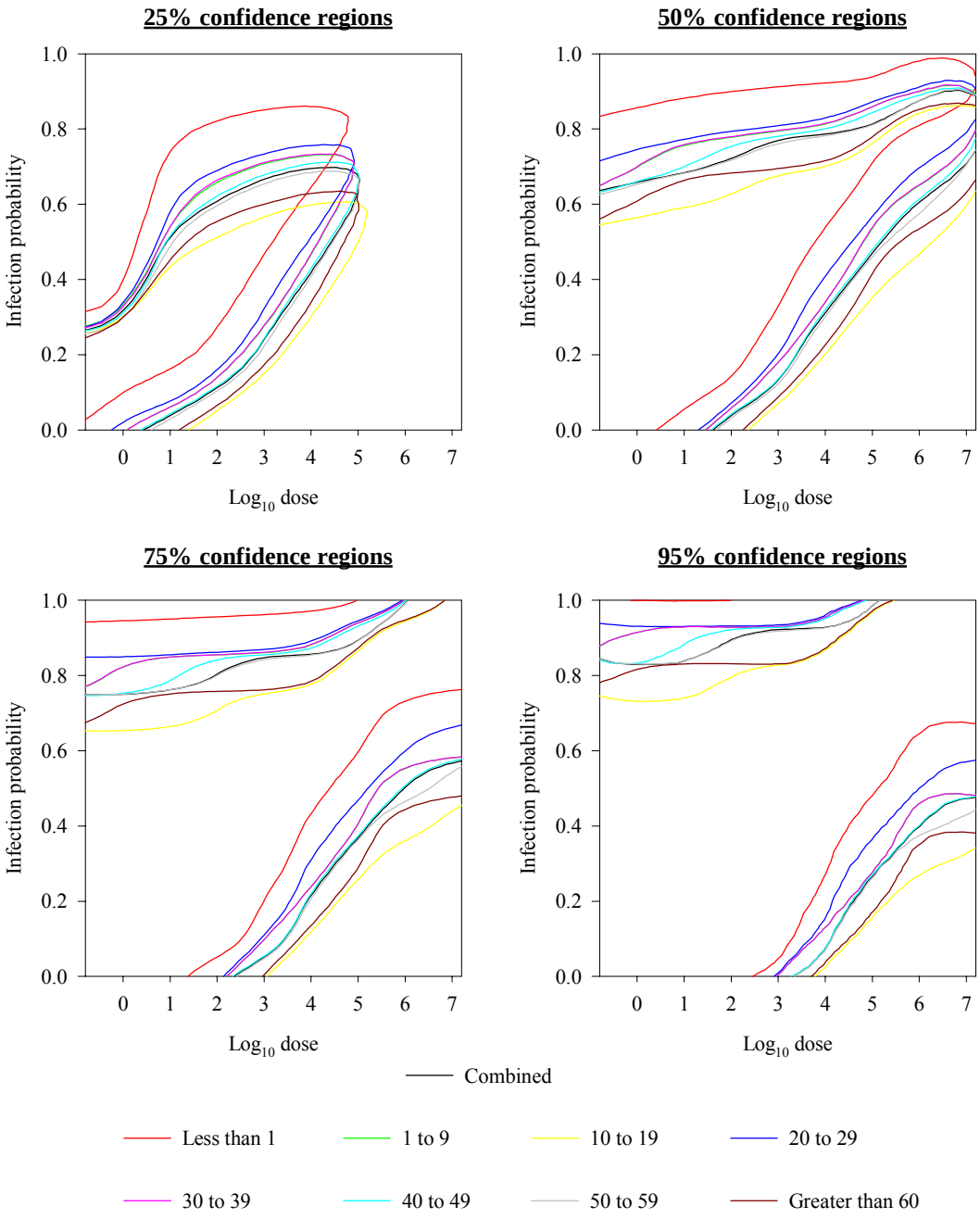


Figure 4.9. Second-order analysis for different age groups, by confidence level

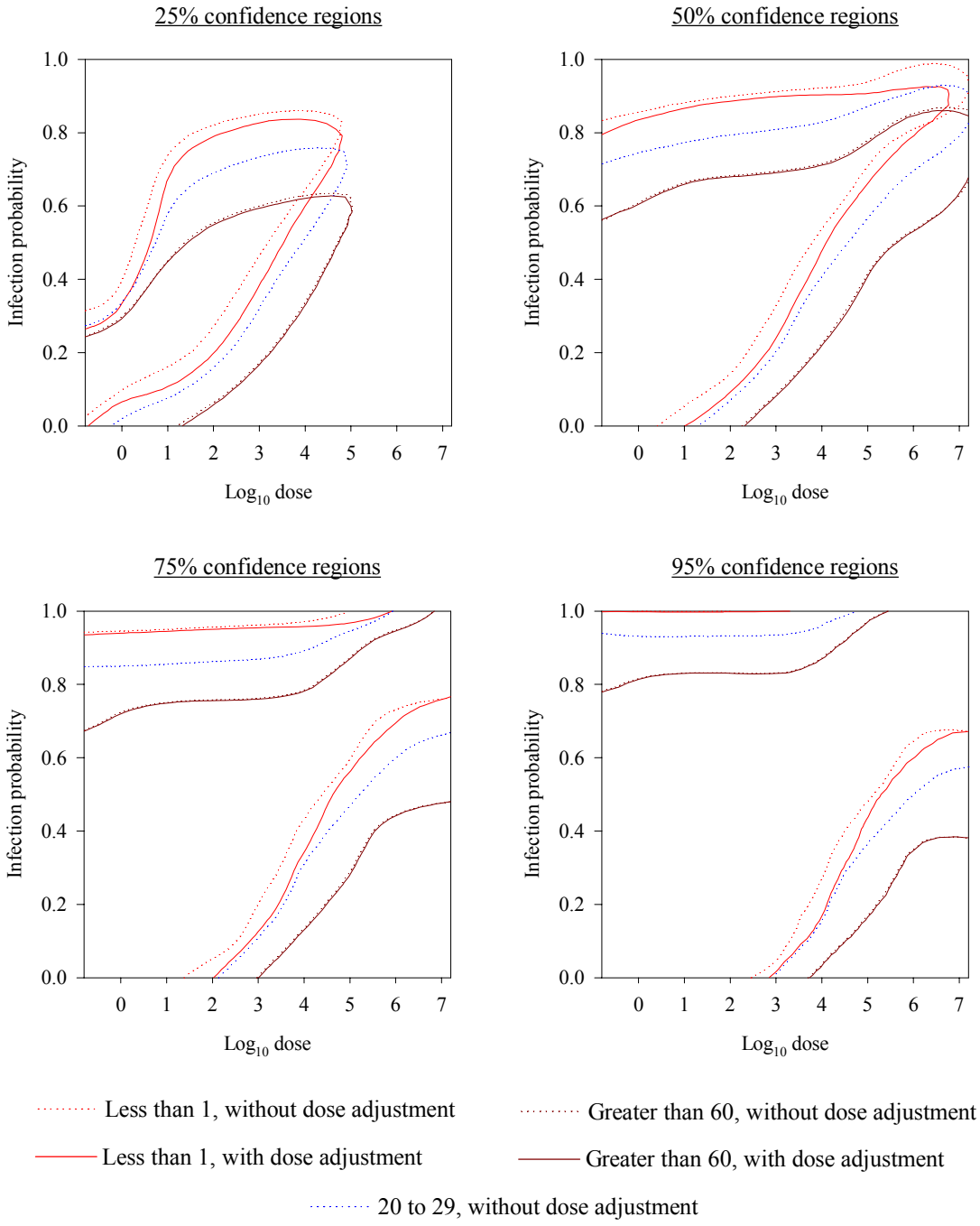


Figure 4.10. Second-order analysis for different age groups with dose adjustment

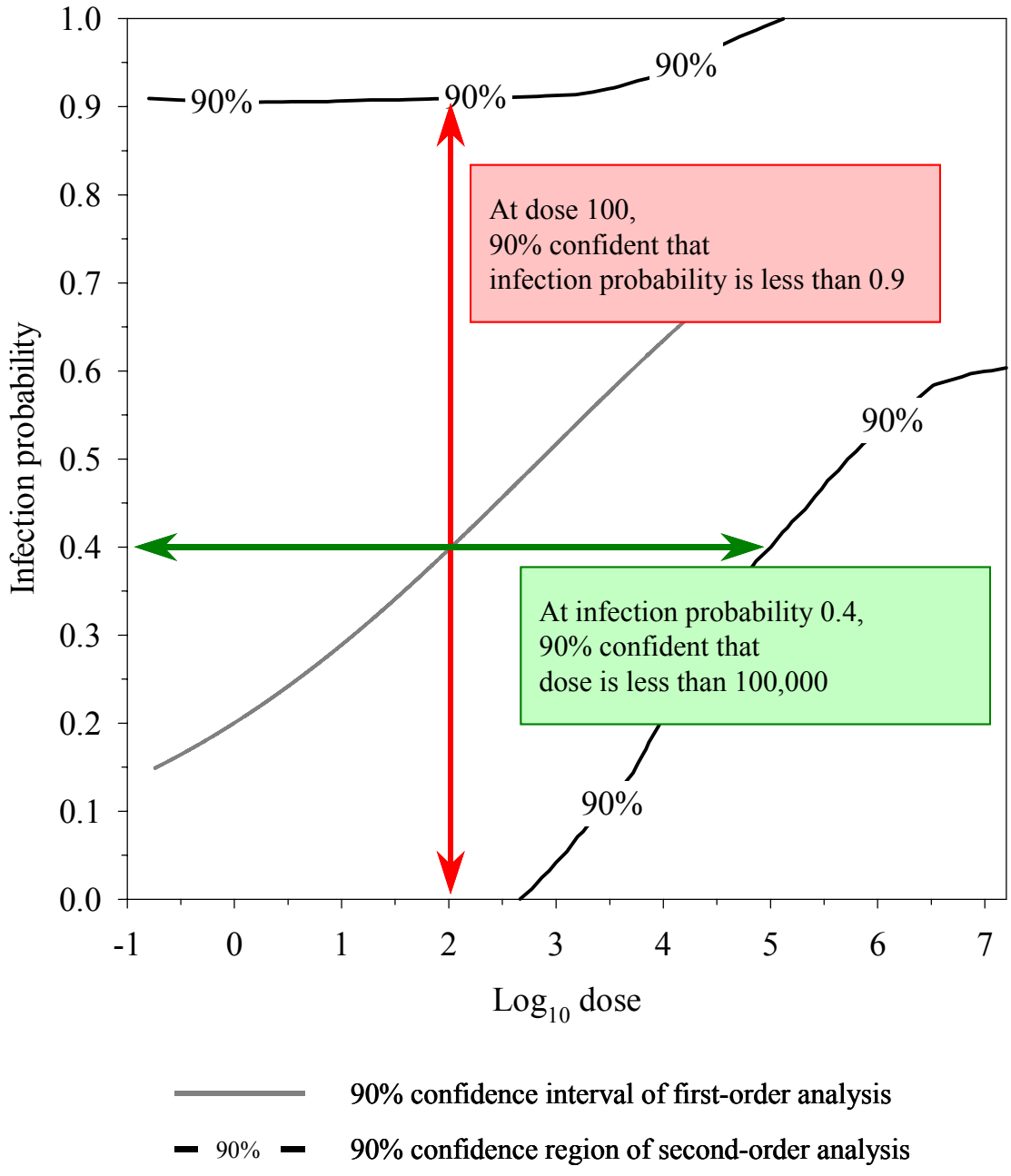


Figure 4.11. Interpretation of second-order analysis in policy-making