RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEU
BILTHOVEN

Report nr. 284550002

The dose-response relation in human volunteers for gastro–intestinal pathogens

P.F.M. Teunis, O.G. van der Heijden,
J.W.B. van der Giessen, and A.H. Havelaar

May 1996

This report was written as part of project nr 284550 on behalf of the Veterinary Inspectorate (VHI) and the Public Health Inspectorate (HIGB), Ministry of Public Health, Welfare, and Sports (VWS).

National Institute of Public Health and the Environment (RIVM), Anthonie van Leeuwenhoeklaan 9, PO Box 1, NL-3720 BA Bilthoven, The Netherlands.
Tel. ..31 30 274 9111, Fax. ..31 30 274 2971

Mailing list

1		Hoofdinspectie Gezondheidsbescherming
2		Veterinaire Hoofdinspectie
3		B. Sangster
4		D.G. Groothuis
5		J. Jansen
6		J. Goebbels
7		F. van Knapen, Universiteit Utrecht
8		F.M. Rombouts, Landbouw Universiteit Wageningen
9		J.H.J. Huis in 't Veld, Universiteit Utrecht
10		M.H. Zwietering, Landbouw Universiteit Wageningen
11		S. van Gerwen, Landbouw Universiteit Wageningen
12		J.J.M. Bedaux, Vrije Universiteit Amsterdam
13		C.N. Haas, Drexel University, Philadelphia Pennsylvania
14		S. Regli, EPA Washington, D.C.
15		W. Jakubowski, EPA Cincinnatti, Ohio
16		C. Hurst, EPA Cincinnatti, Ohio
17		M.V. Yates, University of California at Riverside
18		C.P. Gerba, University of Arizona, Tucson
19		J.B. Rose, University of South Florida, St. Petersburg
20		N.F. Lightfoot, PHLS, Newcastle
21		H. Tillett, PHLS, Colindale, London
22	– 23	Depot Nederlandse publicaties en Nederlandse bibliografie
24		Directie Rijksinstituut voor Volksgezondheid en Milieu
25		Directeur Sector 2
26		M.J.W. Sprenger
27		J. Seidell
28		J.G. Loeber
29		A.M. Henken
30		T.G. Kimman
31		J. Meulenbelt
32		J.D.A. van Embden
33		W. Slob
34		J.F.M. Versteegh
35		M. Kretzschmar
36	– 45	Leden projectgroep Gastro-Enteritis
46		H. van Loveren
47		P.A. Steerenberg
48		J. Garssen

Contents

Summary

Published data on infection of human hosts with various protozoa, bacteria, and viruses causing gastro-enteritis are used to establish a quantitative relationship between ingested dose and the risk of infection. For all data sets analyzed, this relationship is determined by fitting either an exponential curve or a beta-poisson curve. These relationships can e.g. be applied to assess the risk of infection associated with drinking water or consumption of food contaminated with a low dose of an infectious pathogen.

Samenvatting

Gepubliceerde gegevens omtrent infectie van humane proefpersonen met pathogene micro–organismen die gastro-enteritis veroorzaken (protozoa, bacteriën en virussen), worden gebruikt om een kwantitatieve relatie vast te stellen tussen de ingenomen dosis en het risico op infectie. Voor alle bestudeerde datasets wordt deze relatie bepaald door het fitten van een exponentiële curve dan wel een beta–poisson curve. Deze relaties kunnen b.v. worden toegepast bij het vaststellen van het risico op infectie bij inname van drinkwater of voedsel dat is verontreinigd met een kleine hoeveelheid van een infectieus pathogeen.

Chapter 1

Introduction

Gastroenteritis is one of the main causes of disease and mortality worldwide, mainly in young children in developing countries (Benenson, 1990). Although in the western world, the mortality rate is decreased dramatically because of the high standards of hygiene, the incidence of gastroenteritis can still be very high. In the Netherlands there is an estimated incidence of 6.75 million cases a year of infectious and non-infectious gastroenteritis with a main concern in the high loss of productivity and the caretaking load (Hoogenboom-Verdegaal *et al.*, 1992; Hoogenboom-Verdegaal, 1993).

Infectious gastro-enteritis can be caused by a variety of bacteria, viruses and parasites, but also intoxications can result in gastro-enteritis. Diarrhea is one of the main symptoms of gastro-enteritis. Fever can be an indication for infectious gastro-enteritis but in case of enteritis caused by certain bacteria, this symptom is absent. In general, enteric pathogenic microorganisms have the ability to cause infection and eventually symptoms of gastro-enteritis by two different mechanisms, production of an enterotoxin or invasion of gastro-intestinal tissue.

Enterotoxin producing microorganisms like certain enterotoxigenic *E. coli* strains and *Vibrio cholerae* typically colonize the upper small bowel (duodenum and jejunum) and clinical symptoms are limited to the effect of the toxin on the intestinal mucosa, leading to watery diarrhea without fever or other systemic symptoms. This in contrast to the effect of invasive microorganisms, like *Salmonella*, *Campylobacter*, or rotaviruses, which in general colonize, invade and damage the enteric mucosa resulting in enteritis. Microorganisms, like *Shigella* species, colonizing the large bowel (colon) may cause a febrile colitis with symptoms of unformed stool with blood and mucus (dysentery). In this respect, *Shigella* is different in that this species possesses the ability of both producing an enterotoxin and invade the intestinal mucosa.

The interaction between a (pathogenic) microorganism and the host may be treated as a chain of conditional events, whereby the completion of any stage is required for the realization of the next stage. For microorganisms which can cause gastro-enteritis this means that in general they first have to enter the host with for example

ingested (contaminated) food or water, or via any other route of faecal-oral transmission. If a microorganism fails to enter the host, it cannot possibly cause infection and thus disease. On the other hand, if a microorganism enters the gastro-intestinal tract, it may eventually reach a location suitable for colonization, which may be followed by toxin production or invasion. This is not an inevitable outcome, but when it occurs, infection may follow. Within this context, infection is defined as colonization, multiplication and possibly invasion of the gastro-intestinal tract by the pathogenic microorganism. In this respect, infection may be confirmed by the microbiological examination of stool specimens and in some cases also the determination of an immune response.

Infection does not have to be accompanied by symptoms. During an asymptomatic infection the host may be carrying and transmitting pathogenic microorganisms, without having any notion whatsoever. Often, however, infection will lead to symptoms of illness. Generally, gastroenteritis is diagnosed when two or more of the following symptoms occur simultaneously: vomiting, fever, watery diarrhea (Benenson, 1990). A more precise definition is the following: diarrhea or vomiting, and at least two of the following symptoms: diarrhea, fever, vomiting, nausea, abdominal pain, abdominal cramps, and blood or mucus in stool, within a period of 7 days. Acute gastroenteritis may be life-threatening, with dehydration as a severe problem. Such a situation may occur in young, elderly or immunocompromised persons: those infected with another organism, especially the AIDS–virus, but also patients using immune suppressive drugs after organs transplants, or certain forms of chemotherapy. In such cases, the probability of dying as a result of gastroenteric infection may be considerable.

Finally, an infection may become chronic. In such cases long–term effects may occur, like endocarditis after infection by the Coxsackievirus (type B), Guillain-Barré syndrome after infection by *Campylobacter*, hemorrhagic uremic syndrome after a shigalike toxin producing *E. coli* (VTEC) infection and reactive arthritis after *Salmonella* infection. A complicating factor here is the verification of the causal relation between the presence of a pathogenic microorganism and the occurrence of symptoms in the host. For instance, Crohn's disease and *Mycobacterium paratuberculosis*.

A quantitative description of the dose response relations of pathogenic microorganisms in humans may give an insight into the risk of becoming infected after the ingestion of a certain amount of microorganisms.

In this report, dose response relations have been determined for microorganisms which can cause gastroenteritis in humans. For this purpose, available literature has been selected, where human volunteers were experimentally exposed to different doses of microorganisms. In some cases, also those microorganisms have been included, which are not primary causative agents of gastro–enteritis, but which seemed relevant to include because they colonize the intestinal tract, like poliovirus, for instance.

Chapter 2

The dose-response relation

The relation between ingesting a certain amount of pathogenic micro-organisms and the (possible) results may be described in a number of ways. The amount of organisms entering the digestive tract of a (potential) host per exposure event (eating a contaminated meal, drinking a glass of contaminated water) is expressed in a *number* of functional particles of the pathogenic organism: colony-forming particles, plaque-forming particles, spores, (oo)cysts, etc. This is called the *dose*, a quantitative measure for the intensity of exposure of the host to the pathogenic organism.

At a certain dose, certain effects will occur. These may be described as a series of effects increasing in severity with increasing doses: a dose-effect relation. Generally, there will not be a one–to–one relationship between the size of the dose and the kind of effect this dose produces. At increasing doses, there will probably be a gradual (or not) change in the probability of one effect occurring, and a differently changing variation in the probability of occurrence of another effect. Instead of a single dose-effect relation, giving a quantitative description of the events occurring subsequently with increasing doses, there is rather a number of different dose-response relations, describing the relation between various effects and the magnitude of the dose. Hence, for a certain, well defined effect, the dose-response relation describes the quantitative relation between the *intensity* of exposure (the dose) and the *frequency* of the occurrence of this effect within the exposed population of hosts (the response).

In principle, a dose-response relation may be determined experimentally. Volunteers are fed various doses of a pathogenic organism, a group of hosts at every dose, and the results are scored. For organisms causing gastro-enteritis, such an experiment may often be performed without serious risks for the volunteers involved. Often however, the final goal of such an experiment is prediction of probabilities of infection or disease at the doses occurring in normal, real life situations. These doses are often so low that the probability of infection is quite small. This in turn means, that for reasonably accurate determination of the probability of infection at such low doses, large numbers of volunteers would be needed. Such an experiment would soon be impossible to complete, not in the least because of the high needs

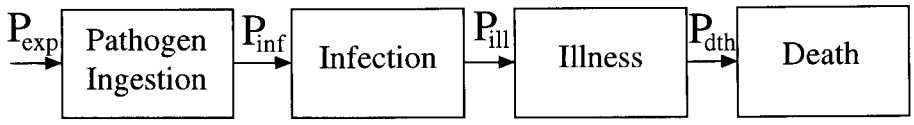


Figure 2.1: Subsequently occurring events after exposure to pathogenic micro-organisms (modified, after Haas *et al.* (1993)): at a certain concentration of pathogenic micro-organisms the probability of ingesting a number j is $P_{\text{exp}}(j)$. These may cause infection (determined by a criterium like: excretion of newly grown organisms, or seroconversion, or colonization of an organ in the host), with probability P_{inf}. Infection may result in symptoms, the host develops illness, with probability P_{ill}, and when symptoms are severe, they may eventually result in death of the host (P_{dth}).

for clinical resources to provide adequate treatment to all the subjects involved.
A suitable model description of the dose-response relation offers a way out of this dilemma: experimental data at high doses are used to calibrate the model (*i.e.* determination of best parameter values), after which extrapolation to low doses enables the estimation of risk under the circumstances occurring in real-life situations.

2.1 Conceptual basis for the dose-response relation

Under a few, not very restrictive assumptions a quantitative description of the dose-response relation may be formulated, with enough flexibility to be fitted to the majority of the available experimental data. The following model description has been derived originally to describe the numbers of infected cells on the leaves of the tobacco plant, after exposure to the tobacco-mosaicvirus (Furumoto and Mickey, 1967a,b). The same relation appeared to perform satisfactory for describing the interferon dose-response relation (Gifford and Koch, 1969). Haas first used this model description for the dose-response relation for infection of human volunteers with organisms causing gastro-enteritis (Haas, 1983; Haas *et al.*, 1993; Haas and Rose, 1994). The model is based upon the following causal chain of subsequently occurring events (figure 2.1).
Consumption of foods or (unboiled) water may bring a person into contact with pathogenic micro-organisms. The ingestion of a certain amount of food may imply swallowing a number of pathogenic organisms. Any organism entering the host has a certain probability of surviving to reach a location within the digestive system which is suitable for colonization, and multiply itself, thereby achieving infection. If this happens, infection may be asymptomatic,so that the vital functions of the host are not affected. The host may also fall ill, however, when the symptoms are severe enough it is even possible that the probability of dying may not be neglected. In figure 2.1 these probabilities are listed:

$P_{\text{exp}}(j \mid \textbf{dose})$ Probability of having j pathogenic organisms within the ingested portion.

P_{inf}(**infection** | j) Conditional probability of infection provided that j pathogenic organisms have been swallowed.

P_{ill}(**illness** | **infection**) Conditional probability of illness provided infection of the host.

P_{dth}(**death** | **illness**) Conditional probability of dying after the host has fallen ill.

2.2 Dose-response model

The conceptual model described above characterizes the events leading from exposure to infection, disease, and death as transition probabilities. This is a stochastic representation of the occurrence of infection, illness and death. Now the building blocks of figure 2.1 may be implemented.

2.2.1 Exposure

Assuming that the pathogenic organisms are distributed randomly within the consumed medium (food, drinking water), with mean number per portion μ, the probability of swallowing j organisms is Poisson distributed:

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

2.2.2 Infection

When, for infection to succeed, at least one organism has to survive and reach a target site within the host, and when any organism ingested has a probability r of surviving and causing infection, the probability of infection after exposure to j organisms is:

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

with $P_{\text{sur}}(k \mid j)$ the probability of k organisms surviving of j having been ingested. The probability of infection after exposure to at least one pathogenic organism is:

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

Exponential model When organisms are distributed randomly (Poisson) and the probability of infection for any organism equals r, then:

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

Beta-Poisson model When the probability r is not constant, but instead has a certain probability distribution as well, a Beta distribution, with density function:

$$f(r \mid \alpha, \beta) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} r^{\alpha-1}(1-r)^{\beta-1}, 0 \leq p \leq 1$$

Then the probability of infection may be expressed as:

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

provided $\beta \gg \alpha$ (Furumoto and Mickey, 1967a).

2.2.3 Illness

The probability of developing illness symptoms is:

$$P^*_{\text{ill}} = P^*_{\text{inf}} P_{\text{ill}}$$

With P_{ill} the conditional probability of illness after infection.

2.2.4 Death

The probability of dying is:

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

With P_{ill} the conditional probability of dying after developing illness.

2.3 Properties of the Beta-Poisson model

Assuming Poisson distributed organisms in the consumed medium, and a Beta distributed probability of infection after entrance into the host, the Beta Poisson model provides a flexible description of the dose-response relation for infection. The exponential model may be regarded a limiting case of the more general Beta Poisson model.
Properties of the Beta-Poisson model:

- Fits well with many dose response datasets. Variation of the two parameters α and β allows adjustment of the model curve to as good as any of the dose response datasets studied so far.

- Adds plausibility to the assumption that ingestion of a single pathogenic organism is sufficient to cause infection. This follows from the stochastic nature of the model.

- Conservative when extrapolating to low doses: the estimated risk of infection exceeds that of alternative (deterministic) models. One alternative for the Beta Poisson model follows from the assumption of a lognormally distributed threshold for infection. The dose response relation then assumes the shape of the cumulative lognormal distribution function. Upon fitting of the Beta Poisson model and the lognormal model to the same dataset, extrapolation to low doses leads to the highest risk of infection in the Beta Poisson model. Thus, for use in risk assessment, the Beta Poisson model is conservative.

Chapter 3

Fitting the model to experimental data

Suppose, there are k doses, and just as many groups of experimental subjects, with numbers T_i ($i = 1, 2, \ldots, k$), of which I_i are infected. This means that in group i, $T_i - I_i$ subjects are not infected. The log-likelihood function:

$$\ell(\alpha, \beta) = -2 \sum_{i=1}^{k} \{I_i \log f(\alpha, \beta \mid \mu_i) + (T_i - I_i) \log[1 - f(\alpha, \beta \mid \mu_i)]\}$$

With the dose response relation:

$$f(\alpha, \beta \mid \mu_i) = 1 - \left(1 + \frac{\mu_i}{\beta}\right)^{-\alpha}$$

The parameter values that maximize this function are called the maximum likelihood estimates ($\hat{\alpha}, \hat{\beta}$), where the dose response relation fits best to the given dataset. The degree to which the model function fits to the data, the "goodness of fit", may be evaluated by using the difference (the deviation) between the maximum likelihood as defined above, and the maximum possible likelihood, without any constraints. The latter value may be calculated by equating the probability of infection to the observed fraction, at every applied dose (McCullagh and Nelder, 1989; Bedaux and Kooijman, 1995).

$$\ell_{\text{sup}} = -2 \sum_{i=1}^{k} \left\{ I_i \log \left(\frac{I_i}{T_i} \right) + (T_i - Ii) \log \left(\frac{T_i - Ii}{T_i} \right) \right\}$$

In which terms with $I_i = 0$ or $T_i - I_i = 0$ give a contribution 0 to this sum. The difference between both likelihoods, also called the Deviance D (McCullagh and Nelder, 1989), may be tested:

$$D = \ell(\alpha, \beta) - \ell_{\text{sup}} \sim \chi^2_{k-2}$$

As in a normal likelihood ratio test, by comparison to a χ^2 distribution with $k-2$ degrees of freedom (Haas *et al.*, 1993).
Analogous to previous reasoning, a likelihood ratio based confidence interval for the parameter pair (α, β) may be calculated.

3.1 Confidence interval

Since we have here a model with two parameters, a confidence interval about the dose response curve cannot be calculated directly from that of the parameters. By means of resampling from the original dataset (bootstrapping), a collection of new datasets may be constructed. Each of these reconstructed datasets is now used to fit a dose response curve as described previously, thereby providing a collection of parameter pairs, $(\hat{\alpha}_i, \hat{\beta}_i)$, corresponding to an equal number of dose response relations. If, for instance, a confidence interval for the probability of infection at a certain dose is required, this may be calculated as the position of two percentiles of the set of function values at this dose. Examples are provided for several actual datasets.

3.2 Pooling datasets

Analogous to the "goodness" of fit test based on the likelihood ratio, a criterium for the acceptability of merging two datasets may be formulated.
Suppose, we have two datasets we want to merge (for the same organism, or two closely related strains, in the same host, for example). Each of these may be subjected to the procedure described in 3. This produces two log-likelihood values $\hat{\ell}_1$ and $\hat{\ell}_2$, with parameters $(\hat{\alpha}_1, \hat{\beta}_1)$ en $(\hat{\alpha}_2, \hat{\beta}_2)$. Then, the same procedure is applied to the merged dataset, resulting in a new log-likelihood $\hat{\ell}_{1+2}$ and a new set of parameters $(\hat{\alpha}_{1+2}, \hat{\beta}_{1+2})$.
If the difference $\hat{\ell}_1 + \hat{\ell}_2 - \hat{\ell}_{1+2}$ is less than $\chi^2_{4-2,0.95}$, the hypothesis that both datasets can be discriminated under the used model, must be rejected.

Chapter 4

Pathogenic protozoans

4.1 *Giardia lamblia*

4.1.1 Experimental data

Reference: Rendtorff (1954a).

Strain *Giardia lamblia* cysts (clinical isolate).

Volunteers Male inmates, physically healthy and tested for absence of cysts before the experiment by stool specimen examination.

Inoculum Cysts were isolated from human faecal material, washed several times in saline and concentrated. Small numbers of cysts were counted directly (using a micromanipulator) in a microscopic specimen. Doses of 10,000 cysts and above were estimated by using appropriate dilutions of a concentrate enumerated in a hemocytometer. For ingestion, *Giardia* cysts were placed with some saline in gelatin capsules.

Method Gelatin capsules were given orally to volunteers with 4 to 6 ounces of water. Smears of stool specimens were examined daily for presence of cysts. Trophozoites were rarely seen, and only in the presence of cysts.

Results Results are summarized in table 4.1. No clinical illness occurred which could be attributed to *Giardia* infections. Only a few cysts will establish infection in adult men which means that only a few transferred cysts may be of importance. Prepatent periods ranged from 6 to 15 days. Infections disappeared spontaneously after 5 to 41 days, but in 2 cases, infections persisted for as long as 129 and 132 days.

4.1.2 The dose response curve

The best fitting dose response curve appears to be that of the exponential model, a special case of the Beta Poisson model, with both $\hat{\alpha}$ and $\hat{\beta}$ very large. Based on

Dose	Total	Infected	Ill
1	5	0	0
10	2	2	0
25	20	6	0
100	2	2	0
10^4	3	3	0
10^5	3	3	0
3.10^5	3	3	0
10^6	2	2	0

Table 4.1: Results of the dose response experiment (Rendtorff, 1954a) for *Giardia lamblia* in healthy volunteers. Dose: ingested number of *G. lamblia* cysts. Total: number of experimental subjects at a certain dose. Infected: number of subjects infected (excreting *G. lamblia*). Ill: number of persons with symptoms of gastro-enteritis (fever, vomiting, diarrhea).

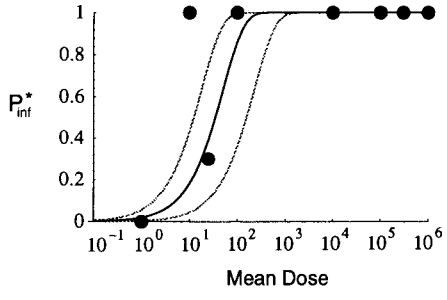


Figure 4.1: Dose response relation for infection of healthy volunteers by *Giardia lamblia*. Shown are the experimentally determined fractions infected, the dose response curve of the best fitting (exponential) model, and the limits of a 95% confidence interval.

Exponential				
$\hat{r}$	D	df	sg	95% ci for r
1.99×10^{-2}	8.37	7	-	0.44×10^{-2} – 5.66×10^{-2}

Table 4.2: Summary of calculated characteristics for *Giardia lamblia*. Shown are: maximum likelihood values for the dose response parameter, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: likelihood-based 95% confidence interval for the dose response parameter.

Dose				Total	Infected	Symptoms	Crypto-
Intended	Actual						sporidiosis
30	3.4	$\pm$ 0.3	$\times 10^1$	5	1	0	0
100	1.08	$\pm$ 0.22	$\times 10^2$	8	3	3	3
300	3.13	$\pm$ 0.24	$\times 10^2$	3	2	0	0
500	5.04	$\pm$ 0.19	$\times 10^2$	6	5	3	2
10^3	1.129	$\pm$ 0.16	$\times 10^3$	2	2		0
10^4	1.146	$\pm$ 0.105	$\times 10^4$	3	3	(4)	1
10^5	1.139	$\pm$ 0.039	$\times 10^5$	1	1		0
10^6	1.139	$\pm$ 0.004	$\times 10^6$	1	1	1	1

Table 4.3: Results of the dose response experiment (DuPont *et al.*, 1995) for *Cryptosporidium parvum* in healthy volunteers. Dose: ingested number of *C. parvum* oocysts. Total: numbers of experimental subjects at a certain dose. Infected: numbers of subjects infected (excretion of *C. parvum* in faeces). Symptoms: numbers of subjects with symptoms (infection and fever, vomiting, diarrhea). Cryptosporidiosis: subjects with enteritis excreting oocysts. At doses above 1000 oocysts only the total number of cases of enteritis was reported. The dose for both cases of cryptosporidiosis were obtained via dr. C.N. Haas (personal communication).

the likelihood criterium for goodness of fit given in chapter 3, the deviance between the likelihood of the exponential model and the maximum possible likelihood is not statistically significant ($D = \hat{\ell} - \ell_{\text{sup}} = 8.37$, $\chi^2_{8-1,0.95} = 14.07$), so that this model is accepted as fitting the data. The best value for the parameter $\hat{r}$ is 0.0199, with 95% confidence range 0.0044 – 0.0566; in figure 4.1 the corresponding limiting values for the dose response curve are shown. A summary of the calculated results is given in table 4.2.

4.2 *Cryptosporidium parvum*

4.2.1 Experimental data

Reference: DuPont *et al.* (1995).

Strain isolate from a calf

Volunteers Physically healthy students and staff of the Texas Medical Center in Houston. Only subjects who were seronegative (IgG and IgM) for *C. parvum* on ELISA (no further information) were eligible. Totally 29 persons, 12 men and 17 women. Age range 20–45 years.

Inoculum The oocysts were multiplied in one–day–old Holstein calves (dose 2. 10^8). Oocysts were purified from faeces by repeated filtering and centrifugation on density gradients, and stored in potassium dichromate. One lot of inoculum was cultured in aerobic and anaerobic conditions for bacteria and another lot was tested in cell culture lines for adventitious viral agents (HIV, enteroviruses, myxoviruses). The viability was tested by means of *in vitro* excystation (84 $\pm$ 4%). In addition to this,the infectivity was assayed in mice. Prior to administration, the oocyst suspension was rinsed in buffered saline,

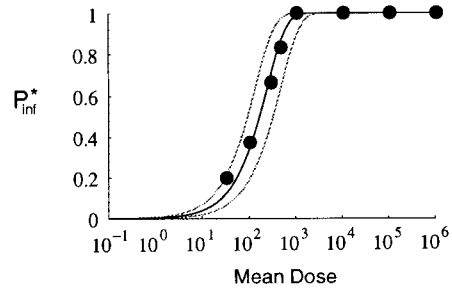


Figure 4.2: Dose response relation for infection of healthy volunteers by *Cryptosporidium parvum.* Shown are: experimentally determined fractions infected, the best fitting dose response curve (the exponential model), and the limits of a 95% confidence interval.

diluted, and counted in a haemocytometer (three- or sixfold) and confirmed by IFT with a monoclonal antibody. According to the mean count the desired doses were allotted, and placed in gelatin capsules. Various concentrations of oocysts were placed in gelatin capsules.

Method capsules were given orally with 250 ml of PBS. No other food or beverages were given for 90 minutes before or after ingestion. Stool specimens were collected daily for the first 2 weeks, followed by 2 days a week for the next 2 months. All samples were tested for the presence of *Cryptosporidium* oocysts by means of immunofluorescence assay. Stools were categorized according to their substance: *formed* (retains shape), *soft* (assumes the shape of the container), or *watery* (pourable). The latter two categories were considered unformed. Infection was defined as detecting oocysts in the faeces more than 36 hrs after oral administration. Diarrheal illness was defined as the passage of 3 unformed stools in 8 hours or more than 3 unformed stools in 24 hours in addition to the presence of 1 or more signs/symptoms including fever, nausea, vomiting, abdominal pain, cramps, and gas-related intestinal symptoms. Cryptosporidiosis: subjects with diarrheal illness excreting oocysts.

Results results are summarized in table 4.3. At doses above 1000 oocysts only the total number of cases of enteritis was reported. Infection, defined as a positive stool for oocysts, occurred at all dose levels. The ID50 of this strain in mice was 60 oocysts. Excretion of oocysts was associated with the development of clinical enteric symptoms, but not all (61%) subjects excreting oocysts became ill. With the higher dose of oocysts, infection tended to occur sooner and lasted longer.

4.2.2 Dose response curve

As in *Giardia*, the best fitting dose response curve appears to be that of the exponential model, a special case of the Beta Poisson relation, with both α and β very large,

Exponential				
$\hat{r}$	D	df	sg	95% ci
4.005×10^{-3}	0.360	7	-	2.05×10^{-3} – 7.23×10^{-3}

Table 4.4: Summary of calculated characteristics for *Cryptosporidium parvum*. Shown are: maximum likelihood values for the dose response parameter, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: likelihood-based 95% confidence interval for the dose response parameter.

and a fixed probability of infection for every oocyst that has entered the host. Based on the likelihood test in chapter 3, the deviance between the exponential model and the maximum possible likelihood is not significant ($D = \hat{\ell} - \ell_{\text{sup}} = 0.360$, $\chi^2_{8-1,0.95} = 14.07$), so that this model is accepted as fitting the data. The parameter value $\hat{r}$ is 0.004005, with 95% confidence range 0.00205 – 0.00723; in figure 4.2 the corresponding limits of the dose response curve are shown. A summary of the calculated results is given in table 4.4.

4.3 *Entamoeba coli*

4.3.1 Experimental data

Reference: Rendtorff (1954b).

Strain clinical human isolate.

Volunteers Male prisoners, physically healthy and tested for parasites before the experiment by examination of 10 daily collected stool specimens per individual.

Inoculum *Entamoeba* cysts were obtained by means of the zinc-sulfate flotation method, washed by repeated transfer to sterile saline and afterwards the cysts were isolated and counted by the micromanipulator. The cysts were ejected from the micromanipulator needle into sterile saline in a gelatin capsule. Appropriate dilutions of known amounts of cysts were made. The number of cysts were recounted and different diluted volumes containing cysts were injected in capsules.

Method Capsules were given with 120-180 ml of tap water orally. Men were followed for the development of clinical symptoms and stool specimens were taken and examined by the direct smear technique (stained and unstained). The number of cysts and trophozoites were estimated.

Results None of the volunteers developed clinical symptoms. The results of the parasitological examinations are summarized in Table 4.5. Three different experiments were carried out, data of the 3 experiments are given separated in the table. For dose response relations, there is no reason not to combine these data.

Dose	Total	Infected
0	2	0
10^2	2	1
10^3	2	0
10^4	2	2
10^0	2	1
10^1	2	0
10^2	2	1
10^0	6	0
10^1	6	3

Table 4.5: Results of the dose response experiment (Rendtorff, 1954b) for *Entamoeba coli* in healthy volunteers. Dose: ingested numbers of cysts. Total: number of experimental subjects at a certain dose. Infected: number of subjects with infection (excretion of cysts).

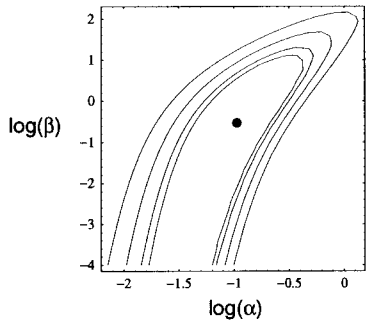


Figure 4.3: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by *Entamoeba coli* (Rendtorff, 1954b), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively. Also shown is the set of parameter pairs, obtained by maximum likelihood fit of the Beta Poisson curve to resampled data sets (see 3.1).

4.3.2 Dose response curve

Contrary to the other intestinal protozoan parasites, the best fitting dose response curve appears to be that of the Beta Poisson model. Based on the likelihood test in chapter 3, the deviance between the Beta Poisson curve and the maximum possible likelihood is not significant ($\hat{\ell} - \ell_{\text{sup}} = 5.24$, $\chi^2_{5-2,0.95} = 7.815$), so that this model is accepted as fitting the data. The parameter values are $\hat{\alpha}$=0.106 and $\hat{\beta}$=0.295. Confidence intervals for (α, β) are shown in figure 4.3. Numerical results are summarized in table 4.6.

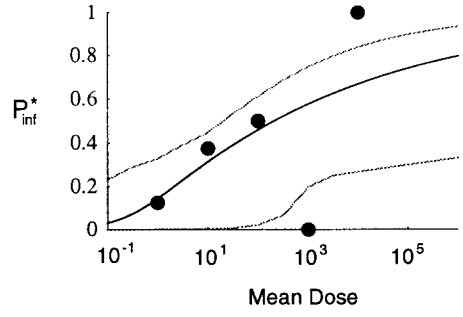


Figure 4.4: Dose response relation for infection of healthy volunteers by *Entamoeba coli*. Shown are: experimentally determined fractions infected, the best fitting dose response curve (Beta Poisson model), and the limits of a 95% confidence interval.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
2.53×10^{-3}	29.4	4	+	0.106	0.295	5.24	3	-	24.1	+

Table 4.6: Summary of calculated characteristics for *Entamoeba coli*. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{sup}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

Chapter 5

Viruses

5.1 Rotavirus

5.1.1 Experimental data

Reference: Ward *et al.* (1986).

Strain strain CJN (clinical isolate, not passed prior to administration), was originally isolated from the faeces of a sick child (8 years old), admitted to hospital with acute gastro–enteritis (Children's Hospital Medical Center, Cincinnatti, may 1982). For enumeration, adaptation to growth in cell culture took place via two passages through primary kidney cells from the African green monkey, thereafter the virus was grown in MA-104 cells. Typing of adapted CJN–virus was performed by means of gel electrophoresis and serotyping.

Volunteers 62 adult males, 18-45 years old. Preserum was tested for rotavirus by serum neutralization test (SNT) against a reassortant of the tissue culture adapted CJN strain. For this study, volunteers were selected by low SNT titer (< 30), because it was assumed that these people are more susceptible to the

Dose	Total	Infected	Ill
9.10^{-3}	5	0	0
9.10^{-2}	7	0	0
9.10^{-1}	7	1	2
9	11	8	6
9.10^{1}	7	6	2
9.10^{2}	8	7	5
9.10^{3}	7	5	3
9.10^{4}	3	3	2

Table 5.1: Results of the dose response experiment (Ward *et al.*, 1986) for rotavirus (CJN) in healthy volunteers. Dose: ingested numbers of ffu (focus forming units). Total: number of experimental subjects at a certain dose. Infected: number of subjects with infection (excretion of rotavirus, or seroconversion, or both). Ill: number of subjects with symptoms of gastro–enteritis (fever, vomiting, diarrhea).

homologous virus strain. According to Ward *et al.* (1986), clinical illness in adults is possible and susceptibility may be correlated with either loss of immunity or reinfection with another serotype.

Inoculum 5% suspension of stool in Earle's balanced salt solution was blended for 2 minutes followed by centrifugation of 30 minutes 8000×g. Supernatant was filtered (0.2 μm), screened for hepatitis A virus, stored in 2 ml aliquots at -70°C. The mean concentration of virus particles was 1.4×10^9/ml, the concentration of "focus – forming units" (ffu) was $9\pm3\times10^4$/ml. Hence, the ration particle/ffu's amounted to 1.56×10^4.

Method Volunteers were given 50 ml 4% $NaHCO_3$ prior to the administration of 50 ml virus suspension in Earle's balanced salt solution. During 4 hours before and after administration of the virus, subjects were not allowed to drink or eat, except for clean water. Until 2 days after administration of the virus suspension, subjects were not allowed to drink milk, in order to prevent interference by milk immunoglobulins. Volunteers remained 6 days in isolation and were physically examined, and stool and blood were taken daily. Stool was checked by an antigen detection test (ELISA) for rotavirus shedding. Infection was defined as either shedding virus in stool or a multiple serum antibody rise by a serum neutralization test (SNT). Symptoms were subdivided into 4 categories: 0 (no symptoms), 1 (mild symptoms), 2 (moderate symptoms), and 3 (severe symptoms). Stools were sampled at the day of administration (day 0), and 6 subsequent days, thereafter on days 10–11, 13–14, and 26–28. Faecal detection of virus was performed by means of ELISA (detection limit 10^6 virus particles per ml faecal suspension. In addition to this, seroconversion for rotavirus was determined.

Results The results are given in table 5.1. Not all volunteers who became infected developed clinical illness, but the incidence of illness seemed not to be related to the size of the dose. Any low dose of virus may cause infection, and may also lead to illness. The dose required to cause infection in adults appeared to be similar to the dose needed for infection in a cell culture. Also remarkable was the fact that adults can develop clinical illness and not shed sufficient rotavirus to be detected by ELISA.

5.1.2 Dose response curve

Based on the likelihood test in chapter 3 the difference between the Beta Poisson model and the maximum possible likelihood is not significant ($D = \hat{\ell} - \ell_{sup} = 6.18$, $\chi^2_{8-2,0.95} = 12.59$), so that the model is accepted. Corresponding parameter values are: $\hat{\alpha}$=0.253, $\hat{\beta}$=0.422. The 95% confidence range in the parameter plane is shown in figure 5.1, in figure 5.2 the corresponding limiting values of the dose response relation are shown. Numerical results are summarized in table 5.2.

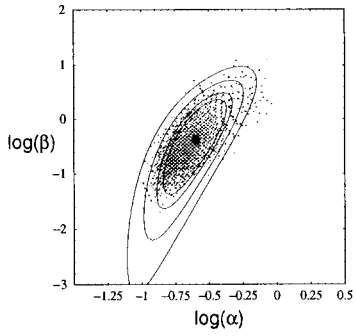


Figure 5.1: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by rotavirus (Ward *et al.*, 1986), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively. Also shown is the set of parameter pairs, obtained by maximum likelihood fit of the Beta Poisson curve to resampled data sets (see 3.1).

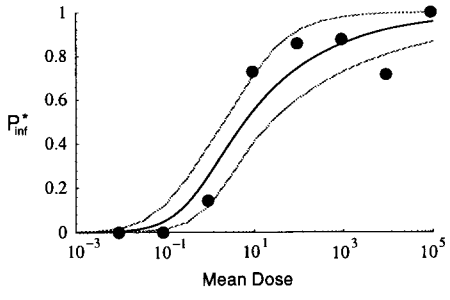


Figure 5.2: Dose response relation for infection of healthy human volunteers by rotavirus. Shown are: fractions infected subjects in the experiment, dose response curve of the best fitting (Beta Poisson) model, and the limits of a 95% confidence range about the best fitting curve.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
1.00×10^{-3}	125	7	+	0.253	0.422	6.18	6	-	119	+

Table 5.2: Summary of calculated characteristics for rotavirus. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

Dose	Total	Intestinal Shedding	Sero-conversion	Infection
0	34	0	0	0
3.3×10^2	50	14	7	15
1.0×10^3	20	8	3	9
3.3×10^3	26	18	11	19
1.0×10^4	12	11	3	12
3.3×10^4	4	2	1	2
3.3×10^5	3	2	1	2

Table 5.3: Results of the dose response experiment (Schiff *et al.*, 1984) for echovirus (12) in healthy volunteers. Dose: ingested numbers of pfu (plaque forming units). Total: number of experimental subjects at a certain dose. Intestinal shedding: number of subjects with positive faecal samples. Seroconversion: number of subjects with at least a fourfold increase in antibody titer. Infection: number of subjects with infection (excretion of echovirus, or seroconversion, or both).

5.2 Echovirus 12

5.2.1 Experimental data

Reference: Schiff *et al.* (1984).

Strain Echovirus 12, clinical isolate.

Volunteers 149 persons, physically healthy, 18-45 years. HAI (Haemagglutinating Inhibition) antibody to Echovirus 12 negative.

Viral inoculum virus was originally isolated from a child with erythema infectiosum. Virus had been passed twice in primary rhesus monkey kidney cells and safety tested. Stock virus was further purified on sucrose gradients. Peak fractions of virus were pooled, diluted in PBS and stored in aliquots of 1 ml at -70°C. Virus titer was determined on human rhabdomyosarcoma cells 5.2×10^7 pfu/ml.

Method Viral suspension was prepared by dilution of stock Echovirus 12 into chilled PBS and was kept on ice just before administration. One ml of viral aliquots were diluted in 100 ml of chilled drinking water and ingested. Aliquots of each dilution were titrated within 24 hours by plaque assay. Rectal swabs of the volunteers were cultured onto LLC-MK2 cells containing streptomycin, penicillin and amphotericin B. Tissue cultures were inspected for CPE daily for 14 days. Positive isolates were harvested and frozen at -70°C. Tissue cultures with no CPE were subcultured into fresh LLC-MK2 cells and observed for 7 days. Positive isolates were identified by Neutralization test. Antibodies were detected by HAI assay and micro neutralization test.

Result Most viral shedding occurred the first week after inoculation, regardless of the viral dose. Duration of shedding was independent of the dose. No volunteer developed significant illness.

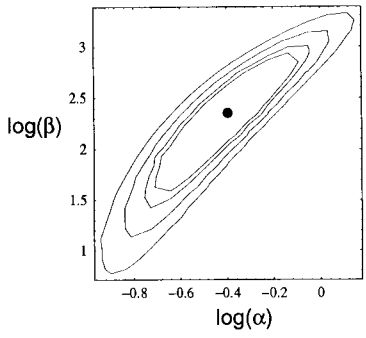


Figure 5.3: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by echovirus (Schiff *et al.*, 1984), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively. Also shown is the set of parameter pairs, obtained by maximum likelihood fit of the Beta Poisson curve to resampled data sets (see 3.1).

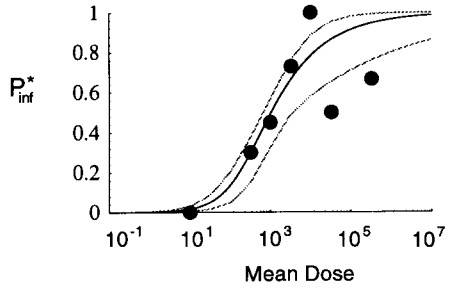


Figure 5.4: Dose response relation for infection of healthy human volunteers by echovirus. Shown are: fractions infected subjects in the experiment, dose response curve of the best fitting (Beta Poisson) model, and the limits of a 95% confidence range about the best fitting curve.

5.2.2 Dose response curve

Based on the likelihood test in chapter 3 the difference between the Beta Poisson model and the maximum possible likelihood is significant at the 95% level ($D = \hat{\ell} - \ell_{\text{sup}} = 13.0$, $\chi^2_{7-2,0.95} = 11.07$), so that the model is rejected. By the same procedure, the maximum likelihood value of the Beta Poisson model is within a 99% confidence range about the maximum possible likelihood.
Maximum likelihood values for the Beta Poisson parameters are: $\hat{\alpha} = 0.401$, $\hat{\beta} = 227.2$. The 95% confidence range in the parameter plane is shown in figure 5.3, in figure 5.4 the corresponding limiting values of the dose response relation are shown. Numerical results are summarized in table 5.4.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
1.05×10^{-4}	165	6	+	0.401	227.2	13.0	5	+	151	+

Table 5.4: Summary of calculated characteristics for echovirus. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{sup}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

Dose	Total	Infected
2.10^{-1}	2	0
2	3	2
2.10^{1}	4	4
2.10^{2}	4	4

Table 5.5: Results of the dose response experiment (Koprowski, 1956) for poliovirus 1 (SM) in adults. Dose: ingested multiples of PFP. Total: number of experimental subjects at a certain dose. Infected: number of subjects with infection (excretion of virus).

5.3 Poliovirus

5.3.1 Poliovirus 1 SM in adults

Experimental data

Reference: Koprowski (1956).

Strain poliovirus Type I SM strain (attenuated by rodent adaptation followed by passages in chick embryo and monkey kidney tissue cultures).

Volunteers 13 adult human subjects (no type I poliovirus antibody titer)

Inoculum and method Various virus dilutions in 0.5 ml polyethylene glycol within a hard gelatin capsule were swallowed together with 8 ml of milk. Dosages were determined by virus titration in tenfold dilutions by a plaque assay (PFP) using monkey kidney cells. Infection was defined as virus shedding.

Results are summarized in table 5.5. It was noted that the calculated dose of 2 PFP was sufficient to produce virus shedding.

Dose response relation

Both for the Exponential and the Beta Poisson model, the differences between likelihood of the model and the maximum possible likelihood are not significant at the 95% level, so that both models must be accepted. The shape of the confidence regions (figure 5.5) clearly indicates that in this case, the Beta Poisson model approximates the Exponential case (where $r \leftarrow \alpha/\beta$). The experimental data are distributed evenly over the response range, leading to limited uncertainty in dose response (figure 5.6). Therefore, the Exponential curve gives a satisfactory description for risk analysis. Numerical results are summarized in table 5.6.

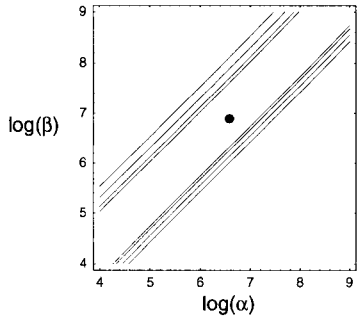
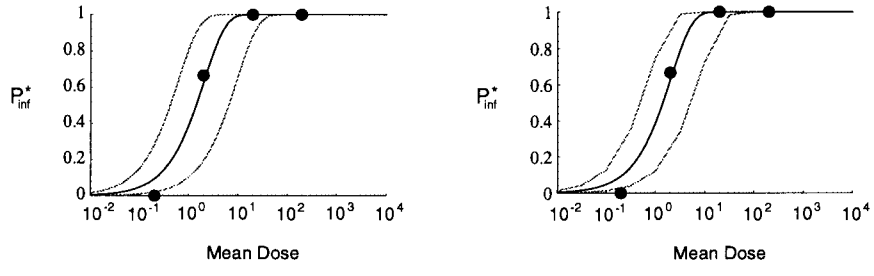


Figure 5.5: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by Poliovirus type 1 SM (Koprowski, 1956), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the contours of the 90, 95, 99, 99.9% range are shown, respectively.



(a) Exponential

(b) Beta Poisson

Figure 5.6: Dose response relation for infection caused by Poliovirus 1 (SM) in children. Shown are the fractions of infected subjects at each dose, the curve of the best fitting model, and the limits of a 95% confidence range. Both Exponential and Beta Poisson curves are given. Compare confidence intervals with those in figure 5.11.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
0.491	0.415	3	-	3.80×10^{6}	7.74×10^{6}	0.415	2	-	0	-

Table 5.6: Summary of calculated characteristics for Poliovirus type 1 (SM). Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

Dose	Total	Infected
$10^{3.5}$	97	28
$10^{4.5}$	91	42
$10^{5.5}$	84	48

Table 5.7: Results of the dose response experiment (Lepow *et al.*, 1962) for poliovirus (Sabin type 1 LSc2ab) in newborn infants. Dose: multiples of TCD50 (50% tissue culture infective dose). Total: number of experimental subjects. Infected: number of subjects with infection.

5.3.2 Poliovirus 1 (LSc2ab) in newborn infants

Experimental data

Reference: Lepow *et al.* (1962).

Strain Sabin type I (LSc2ab)

Volunteers 272 newborn infants weight >5 lb. Infants were less then 5 days old before they received a dose of virus. The cohort included breast-fed and formula fed babies and the maternal titer was measured by a serum neutralization test. The majority of children were from groups of low socio-economic status.

Viral inoculum virus stock solution contained $10^{7.5}$ TCD50 (not TCID50!) per ml. (TCD50 was defined as 50% end-point of infectivity in tissue culture). This stock solution was further diluted (TCD50 $10^{4.5}$ and $10^{3.5}$) in Hank's solution and the concentration was verified by virus titration on monkey kidney tissue cultures.

Method Virus suspension was given in 1 ml Hank's solution. Half of the babies in each dosage group received the vaccine by sucking from a 2 ml syringe, the other half received the vaccine via naso-gastric intubation. Faecal specimens were taken on day 6 and 7 and virus was isolated with routine methods. Viral isolates were identified by neutralization with hyperimmune Type I poliomyelitis antiserum.

Results are summarized in table 5.7. It was noted in this paper that there was no difference in the response to vaccination between the ingested and intubated group of babies. However, babies with a high passive immune titer (>512) and breast-fed babies appeared to have a lower rate of virus excretion.

Dose response relation

The difference between the Beta Poisson model and the maximum possible likelihood is not significant at the 95% level ($D = \hat{\ell} - \ell_{\text{sup}} = 0.052$, $\chi^2_{3-2,0.95} = 3.841$), so that the model is accepted. Maximum likelihood values for the Beta Poisson parameters are: $\hat{\alpha} = 0.114$, $\hat{\beta} = 159$. The 95% confidence range in the parameter plane

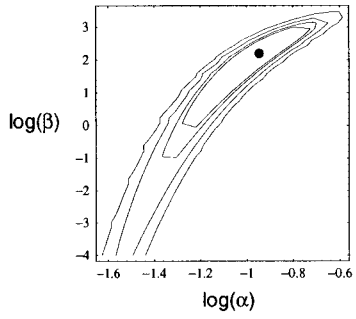


Figure 5.7: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by Poliovirus Sabin type 1 (LSc2ab) (Lepow *et al.*, 1962), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively.

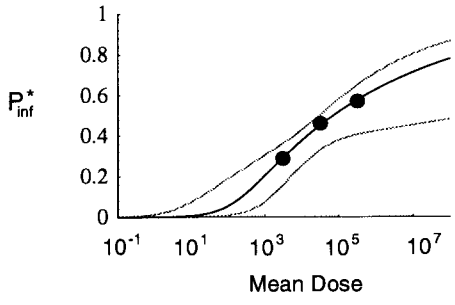


Figure 5.8: Dose response relation for infection caused by Poliovirus Sabin type 1 (LSc2ab) in newborn infants. Shown are the fractions of infected subjects at each dose, the curve of the best fitting Beta Poisson model, and the limits of a 95% confidence range.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
6.18×10^{-6}	186	2	+	0.114	159.0	0.052	1	-	186	+

Table 5.8: Summary of calculated characteristics for Poliovirus Sabin type 1 (LSc2ab). Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

Dose	Total	Infected
7	1	0
16	2	0
27	2	0
42	1	0
50	6	3
55	3	1
65	6	0
80	1	1
90	4	3
160	3	3
210	2	2
280	1	1

Table 5.9: Results of the dose response experiment (Minor *et al.*, 1981) for poliovirus 1 in infants. Dose: ingested multiples of TCID50 (50% tissue culture infective dose) . Total: number of experimental subjects. Infected: number of subjects with infection.

is shown in figure 5.7, in figure 5.8 the corresponding limiting values of the dose response relation are shown. Numerical results are summarized in table 5.8.

5.3.3 Poliovirus type 1 in infants

Experimental data

Reference: Minor *et al.* (1981).

Strain poliovirus type 1 (attenuated live oral vaccine strain, Pfizer Ltd, Sandwich, Kent, UK)

Volunteers 2–month–old infants

Inoculum oral, dose expressed as multiples of TCID50.

Method A volume of 0.5 ml of final vaccine was given in the oral cavity by 1 ml syringe. Stool samples or rectal swabs were collected daily for 10 days. Specimens were cultured onto Wisl cell cultures and incubated for two weeks at 37°C. Suspected isolates were passed once and tested for neutralization by a rabbit hyperimmune antiserum.

Result Summarized in table 5.9. According to this study, an estimated TCID50 of 20 is equivalent to a human infective dose (HID50) of 1.

Dose response relation

The difference between the likelihood of the Exponential model and the maximum possible likelihood is not significant ($-2(\hat{\ell} - \ell_{\text{sup}}) = 14.4$, $\chi^2_{12-1,0.95} = 19.7$), so that the model is accepted. Parameter value: $\hat{r}$=0.0091 . The 95% confidence range for the parameters is shown in table 5.10, in figure 5.9 corresponding limits for the dose response curve are shown.

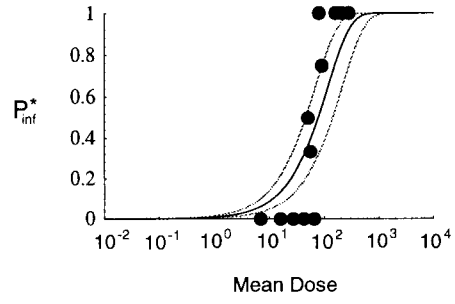


Figure 5.9: Dose response relation for infection caused by Poliovirus 1 in healthy volunteers. Shown are the fractions of infected subjects at each dose, the curve of the best fitting Exponential model, and the limits of a 95% confidence range.

Exponential				
$\hat{r}$	D	df	sg	95% ci
9.10×10^{-3}	14.4	11	-	4.98×10^{-3} – 15.3×10^{-3}

Table 5.10: Summary of calculated characteristics for Poliovirus 1. Shown are: maximum likelihood value for the dose response parameter, deviance ($D = \hat{\ell} - \ell_{sup}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: limits of a likelihood-based 95% confidence interval for the dose response parameter.

5.3.4 Poliovirus 3 Fox in premature infants

Experimental data

Reference: Plotkin *et al.* (1959).

Strain poliovirus 3 Fox (attenuated)

Volunteers clinically healthy premature infants, weighing between 1740 and 2160 grams. No age given.

Inoculum and method virus dosages were stored at -20°C and thawed immediately before administration. Virus was titrated in Vervet Kidney tissue cultures. Calculated dosages of virus were aspirated into a tuberculin syringe and infused into a nasogastric tube. The infusion of virus was followed by infusion of 2-3 ml of saline. Stool specimens were collected before and after the administration of virus at a 3 day interval, inoculated into Vervet Kidney cells, CPE agents harvested and identified by neutralization with type-specific antiserum.

Result Summarized in table 5.11, numbers of infants infected after exposure to three different doses of the attenuated viruses. The two highest dosages were reported as a range, rather than a single value. For our calculations we have used the arithmetic means of the upper and lower limits, 65 (30 – 100) and 150 (100 – 200) TCID50.

Dose	Total	Infected
10	3	2
30 – 100	9	7
100 – 200	4	4

Table 5.11: Results of the dose response experiment (Plotkin *et al.*, 1959) for poliovirus 3 (Fox) in healthy volunteers. Dose: ingested numbers of TCID50 (50% tissue culture infective dose). Total: number of experimental subjects at a certain dose. Infected: number of subjects with infection (excretion of virus).

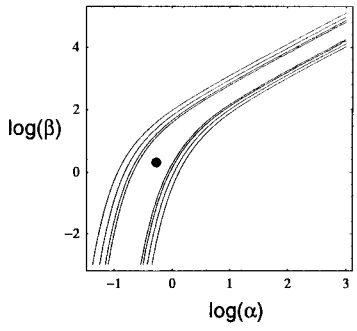


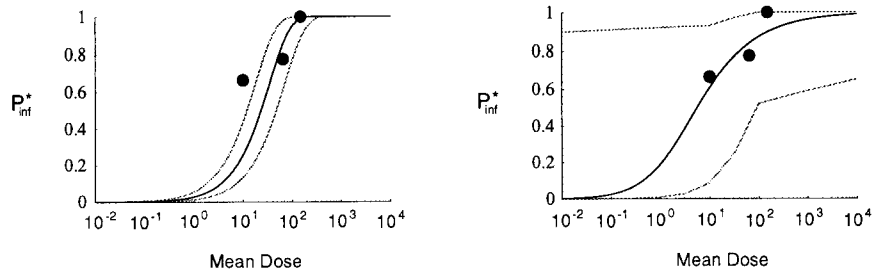
Figure 5.10: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by Poliovirus type 3 Fox (Plotkin *et al.*, 1959), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the contours of the 90, 95, 99, 99.9% range are shown, respectively.

Dose response relation

Both for the Exponential and the Beta Poisson model, the differences between likelihood of the model and the maximum possible likelihood are not significant at the 95% level, so that both models must be accepted. However, the Beta Poisson model appears to allow for both a much less steep dose response curve, and a much larger confidence range (figure 5.11). Therefore, this model provides the most pessimistic extrapolations, which may be preferred for risk analysis. Comparison of figure 5.11 with figure 5.6 shows the profound influence a balanced distribution of data points may have on the uncertainty range. The 95% confidence range in the parameter plane is shown in figure 5.10. Numerical results are summarized in table 5.12.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
2.992×10^{-2}	2.69	2	-	0.533	2.064	1.16	1	-	1.52	-

Table 5.12: Summary of calculated characteristics for Poliovirus 3 (Fox). Shown are: maximum likelihood values for the dose response parameters of the Exponential and Beta Poisson models, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: difference in deviances of both models, and significance according to a $\chi^2_{df,0.95}$ level.



(a) Exponential

(b) Beta Poisson

Figure 5.11: Dose response relation for infection caused by Poliovirus type 3 Fox in infants. Shown are the fractions of infected subjects at each dose, the curve of the best fitting model, and the limits of a 95% confidence range. Both Exponential and Beta Poisson curves are given, to show that, although there is no statistically significant difference in goodness of fit, the Beta Poisson model allows for a much less steep curve, and a much more generous estimate of the uncertainty.

Dose	Total	Infected
1	10	3
2.5	9	3
10	3	2

Table 5.13: Results of the dose response experiment (Katz and Plotkin, 1967) for poliovirus 3 (Fox) in premature newborn infants. Dose: ingested numbers of TCD50 (50% tissue culture infective dose). Total: number of experimental subjects at a certain dose. Infected: number of subjects with infection (excretion of virus?).

5.3.5 Poliovirus 3 Fox in premature infants

Experimental data

Reference: Katz and Plotkin (1967).

Strain poliovirus 3 Fox (attenuated)

Volunteers 22 premature infants (weight 1500-2200 grams)

Method Fox strain was given within the first 48 hours of life. Virus was given in 5 ml of Hanks' solution using an oro-gastric tube. Stool specimens were collected for tissue culture using 4 primary Green monkey kidney tissue. 0.1 ml of a 10% stool specimens in Hanks suspension were inoculated onto the tissue cultures and incubated for 30 minutes prior to washing in PBS. Tubes were then incubated for 1 week in Eagle's medium plus 2% FCS. These tubes were then examined for CPE. Positive tubes were subcultured, virus harvested and used in SN test. The dilutions were retitrated before administration..

Result Summarized in table 5.13.

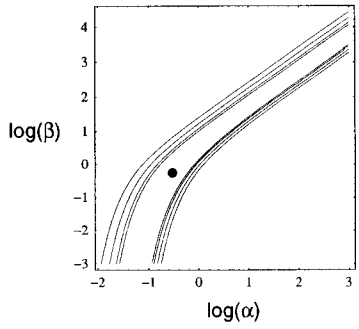
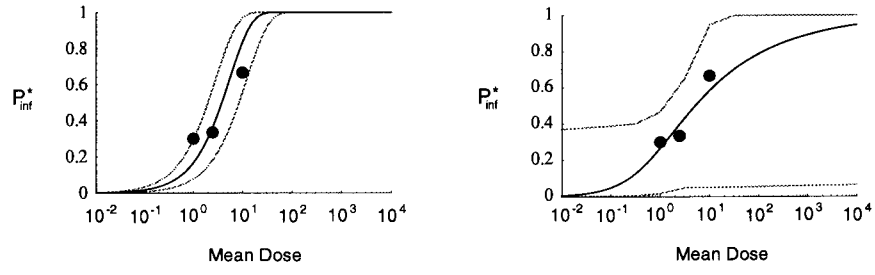


Figure 5.12: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by Poliovirus type 3 Fox (Katz and Plotkin, 1967), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the contours of the 90, 95, 99, 99.9% range are shown, respectively.



(a) Exponential (b) Beta Poisson

Figure 5.13: Dose response relation for infection caused by Poliovirus type 3 Fox in premature newborn infants. Shown are the fractions of infected subjects at each dose, the curve of the best fitting model, and the limits of a 95% confidence range. Both Exponential and Beta Poisson curves are given.

Dose response relation

Both for the Exponential and the Beta Poisson model, the differences between likelihood of the model and the maximum possible likelihood are not significant at the 95% level, so that both models must be accepted. However, the Beta Poisson model appears to allow for both a much less steep dose response curve, and a much larger confidence range (figure 5.13). Therefore, like with the previous data set, the Beta Poisson model is better suited for conservative estimates of the risk of infection. The 95% confidence range in the parameter plane is shown in figure 5.12. Numerical results are summarized in table 5.14.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
0.182	1.66	2	-	0.299	0.552	0.31	1	-	1.348	-

Table 5.14: Summary of calculated characteristics for Poliovirus 3 (Fox). Shown are: maximum likelihood values for the dose response parameters of the Exponential and Beta Poisson models, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: difference in deviances of both models, and significance according to a $\chi^2_{df,0.95}$ level.

5.4 Norwalk virus

Reference: Graham *et al.* (1994).

Strain clinical strain (outbreak of acute gastro-enteritis in Norwalk).

Volunteers 21 females, 30 males; 19-39 years medical students.

Method Unknown amount of $NaHCO_3$ was given 2 minutes before and 5 minutes after the ingestion of the viral inoculum (2 ml of a 1/100 dilution of the 8FIIa inoculum in sterile TRIS-buffered saline plus 80 ml of sterile dd water). The frequency, weight and consistency of stool and the occurrence of clinical symptoms (abdominal discomfort, chills,body-and headache, nausea, vomiting, and fever) was recorded. Stool specimens and serum were collected to detect antigen and antibody using an ELISA. Virus antigen was also detected by RIA and RT-PCR.

Result Impossible to determine dose response relation because no description of the dose. All 50 (?) volunteers received the same dose. Of these 36 became infected. The reference is included because it is the only experimental study on infection by SRSV we could find.

Chapter 6

Bacteria

6.1 *Campylobacter jejuni*

6.1.1 Experimental data

Reference: Black *et al.* (1988).

Strain For this study two different strains of *Campylobacter jejuni* have been used. Strain A3249 (Penner serotype 27) has been isolated from a 16 year old boy, who had been ill for two days, with diarrhea, headaches, nausea, and fever. Strain 81-176 (Penner serotype 23/26) was isolated from an ill nine-year old girl during an outbreak in Minnesota. Symptoms included diarrhea, abdominal cramps, and fever, but none of the patients had grossly bloody stools. Strain A3249 appeared to form two different types of colonies, spreading and non–spreading, probably reflecting flagellated and aflagellate variants. In the administered doses equal amounts of each of these two variants were mixed.

Volunteers Volunteers were young adults (from Baltimore, MD, USA). Persons with HLA allotype B27 were excluded from this study, because for this group infection by *C. jejuni* has been reported to increase the risk of developing arthritis.

Bacterial inoculum Stock solutions, stored at -70°C in glycerol, were subcultured on blood agar or Mueller Hinton and incubated at 42°C in 6% oxygen and 10% CO_2 by using an anaerobic jar containing a Campy Pack II (BBL, Cockesville) overnight. Of this, 40-50 colonies were subcultured in the same way and then harvested with 5 ml of PBS, diluted to the number of organisms required for challenge and standardized spectrophotometrically. Replicate pour plates before and after challenge were made to determine the exact number of organisms given. Each strain was passed 5 – 10 times on laboratory media before administration to the experimental subjects.

Method The organisms were administered suspended in 150 ml of milk (from sterile spray dried milk). The influence of the pH in the stomach was studied

Strain	Dose	Total	Infected	Ill
A3249	8.10^2	10	5	1
	8.10^3	10	6	1
	9.10^4	13	11	6
	8.10^5	11	8	1
	1.10^6	19	15	2
	1.10^8	5	5	0
A3249	1.10^8	4	4	2
81-176	1.10^6	7	7	3
	2.10^8	10	10	6
	2.10^9	22	22	9

Table 6.1: Results of the dose response experiment (Black *et al.*, 1988) for *Campylobacter jejuni* in healthy volunteers. Dose: ingested number of *C. jejuni* A3249 or 81-176. Total: number of subjects exposed to a given dose. Infected: number of subjects infected (excretion of *C. jejuni*). Ill: number of subjects with gastro-enteric symptoms (fever, vomiting, diarrhea). the 4 extra subjects receiving 10^8 *C. jejuni* A3249, took these in a bicarbonate solution, instead of milk.

by giving 4 persons a dose of *C. jejuni* suspended in a solution containing 2 g of $NaHCO_3$, instead of milk. These have not been included into the dose response analysis (see table 6.1). After exposure, subjects were monitored: serology for *C. jejuni*–specific antibodies (IgG, IgA, IgM) at post–challenge days 11, 21, and 28. During the first 12 days after challenge, all stools were sampled, categorized according to formedness, and analysed for *C. jejuni*. Illness was scored as developing diarrhea or fever, or both.

Results Six studies were performed to establish the relationship between ingested dose of *C. jejuni* A3249 and infection/illness. The results are summarized in table 6.1. At the lowest dose (800 organisms) 1 person became ill and 50% were shedding the organisms. The attack rates for persons given the highest dose was 100%, but none of these persons became ill. One of the 12 ill subjects (out of a total of 72 volunteers) developed only fever without diarrhea. This person had been given 10^6 organisms. The incubation period ranged from 68 hours with the onset of fever to 88 hours with the onset of diarrhea.

It was noted that the dose of strain A3249 contained equal amounts of spreading and non–spreading colonies. Flagellated bacteria may be better equipped to colonize and invade and thus cause infection. Therefore, with the spreading type *C. jejuni*, the probability of infection may be higher than with the non–spreading type. However, Caldwell *et al.* (1985) has demonstrated that the spontaneous occurrence of aflagellate colonies is a reversible process; the switch from aflagellate to flagellate colonies appears to occur not as frequently ($4–8\times10^{-7}$ per cell / generation) as vice versa ($3–5\times10^{-3}$ per cell / generation) *in vitro*, but much more in a rabbit model. Therefore, the real infectious dose may not be simply halved in this respect.

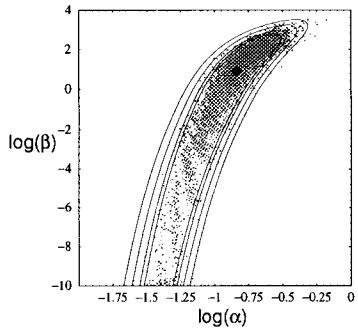


Figure 6.1: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by *Campylobacter jejuni* A3249 (Black *et al.*, 1988), in the parameter space (α, β). From the inside outwards about the point $(\hat{\alpha}, \hat{\beta})$ the contours of a 90, 95, 99 , and 99.9% confidence range are shown. Also shown is the collection of parameters pairs, obtained by means of maximum likelihood fitting of the Beta Poisson model to datasets generated by resampling (see 3.1).

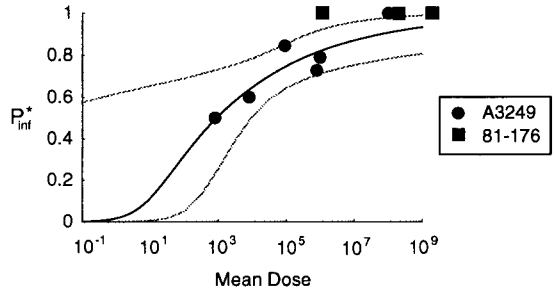


Figure 6.2: Dose response relation for infection of human volunteers by *Campylobacter jejuni* A3249 (Black *et al.*, 1988) (points representing fractions calculated from table 6.1). Best fitting curve with the Beta Poisson model, and 95% confidence range.

The results of the 3 studies with *C. jejuni* strain 81–176 are also summarized in table 6.1. Eighteen of the 39 volunteers developed clinical illness and the attack rates seemed higher than for strain A3249. The incubation periods for both strains were similar. It was noted that after rechallenge of 2 persons who developed illness receiving 10^6 CFU strain A3249 with 10^9 CFU of the same strain 28 days later, none developed illness, indicating that short term protective immunity for the homologous strain can occur.

6.1.2 Dose response curve

Based on the likelihood criterium given in chapter 3 the difference between the likelihood of the fitted Beta Poisson model and the maximum possible likelihood is not significant ($D = \hat{\ell} - \ell_{\text{sup}} = 2.422$, $\chi^2_{5-2,0.95} = 9.488$), leading to acceptance of the model. Corresponding parameter values are: $\hat{\alpha}$=0.145, $\hat{\beta}$=7.589 . The 95% confidence range in the parameter plane is shown in figure 6.1, in figure 6.2 the corresponding limits for the dose response relation are given. Summarized results of

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
3.52×10^{-6}	108	4	+	0.145	7.589	2.422	3	-	106	+

Table 6.2: Summary of calculated characteristics for *Campylobacter jejuni* A 3249. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

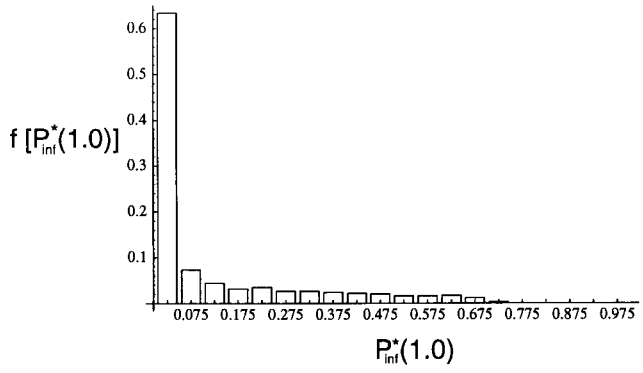


Figure 6.3: Estimated probability distribution for the probability of infection at a mean dose of 1.0 *C. jejuni*.

the fitting procedures are given in table 6.2.
There is a remarkably high uncertainty at low doses. This is not a property of the used model, but rather results from the lack of data at these doses. This does not make this does response relation worthless for risk assessment, since the probability of high risks at low doses remains limited, as may be appreciated by inspecting the estimated probability distribution for P^*_{inf} at a mean dose of 1.0 organisms, as shown in figure 6.3

6.2 *Salmonella*

6.2.1 *S. meleagridis, S. anatum*

Experimental data

Reference: McCullough and Wesley Eisele (1951a).

Strains *Salmonella*

- *meleagridis* 3 different strains
- *anatum* 3 different strains

All strains were isolated from market samples of high moisture spray-dried whole egg powder.

Volunteers healthy males/prisoners (three weekly stools negative for *Salmonella*)

Bacterial inoculum lyophilized strains were subcultured on TSA overnight, then suspended in saline and this suspension was standardized turbidimetrically. Doses were administered in a glass of eggnog. Suspension which was given was plated on TSA for final determination of the bacterial count.

Method No $NaHCO_3$ was given before oral uptake of the bacteria. Faecal samples were taken rectally daily for culturing. Blood samples were taken for agglutination tests. Faeces were cultured directly on *Shigella–Salmonella* agar and Bismuth sulphite agar and after enrichment into selenite F and Tetrathionate broth containing brilliant green. *Salmonella* were identified using a group specific agglutination serum and biochemically. Serum was tested using agglutination tests with standardized formalinized antigen made from the homologous strains used in this study. Clinical illness was defined as having diarrhea (at least).

Results Three strains of *S. meleagridis* were used initially. Results are given in table 6.3. The number of bacteria needed to develop illness differed for these 3 strains. Strain 3 was the most virulent. Results of the experiments done with 3 different strains of *S. anatum* are given in table 6.4. Again, the dose needed to develop illness differed between the 3 different strains of this *Salmonella* species. One of the conclusions in this paper is as follows: "there is wide variation in the infective dose occurring from strain to strain of the same species".

Strain	Dose	Total	Infected	Ill
S. meleagridis I	1.2×10^4	6	3	0
	2.4×10^4	6	3	0
	5.2×10^4	6	3	0
	9.6×10^4	6	3	0
	1.55×10^5	6	5	0
	3.0×10^5	6	6	6
	7.2×10^5	5	4	0
	1.145×10^6	6	6	0
	5.5×10^6	6	5	0
	2.4×10^7	5	5	1
	5.0×10^7	6	6	5
S. meleagridis II	1.0×10^6	6	6	0
	5.5×10^6	6	6	0
	1.0×10^7	6	6	1
	2.0×10^7	6	6	2
	4.1×10^7	6	6	5
S. meleagridis III	1.58×10^5	6	1	0
	1.5×10^6	6	5	0
	7.675×10^6	6	6	1
	1.0×10^7	6	5	2

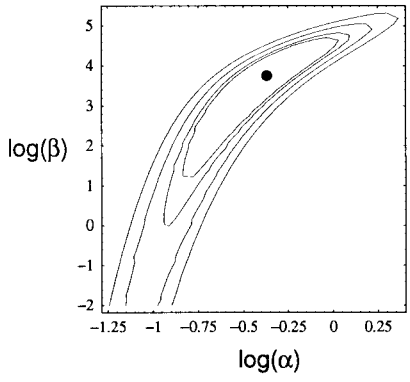
Table 6.3: Results of the dose response experiment (McCullough and Wesley Eisele, 1951a) for *Salmonella meleagridis* in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers of subjects infected at a given dose. Ill: number of subjects with gastro-enteric symptoms (diarrhea).

Strain	Dose	Total	Infected	Ill
S. anatum I	1.2×10^4	5	2	0
	2.4×10^4	6	3	0
	6.6×10^4	6	4	0
	9.3×10^4	6	1	0
	1.41×10^5	6	3	0
	2.56×10^5	6	5	0
	5.87×10^5	5	4	2
	8.60×10^6	6	6	3
S. anatum II	8.9×10^4	6	5	0
	4.48×10^5	6	4	0
	1.0×10^6	6	6	0
	3.9×10^6	6	4	0
	1.0×10^7	6	6	0
	2.39×10^7	6	6	0
	4.45×10^7	6	6	1
	6.72×10^7	8	8	4
S. anatum III	1.59×10^5	6	2	0
	1.25×10^6	6	6	2
	4.67×10^6	6	6	4

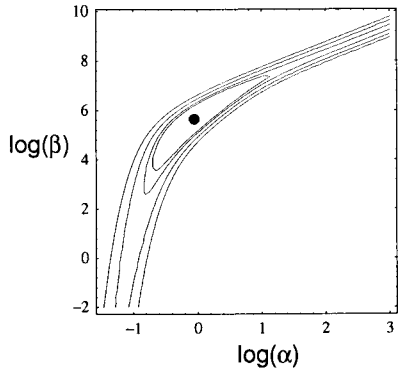
Table 6.4: Results of the dose response experiment (McCullough and Wesley Eisele, 1951a) for *Salmonella anatum* in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers of subjects infected at a given dose. Ill: number of subjects with gastro-enteric symptoms (diarrhea).

Dose response relation of *Salmonella meleagridis*

Numerical results are summarized in table 6.5.
The deviance of the Beta Poisson model fitted to the pooled data set may be compared to the sum of the deviances of the separate data sets (Beta Poisson curves for *S. meleagridis* I and III. Although the exponential curve provides satisfactory goodness of fit (deviance from maximum possible likelihood not significant), the improvement with the Beta Poisson model is significant by the likelihood criterium (ΔD for *S. meleagridis* 3 in table 6.5). Therefore, the Beta Poisson model is preferred here. The goodness of fit of the pooled model is not significantly different from the goodness of fit of the two separate models ($D = D_{1+3} - (D_1 + D_3) = 7.73$, $\chi^2_{2+2-2,0.95} = 5.991$). Hence, under the assumption that the Beta Poisson model is valid, the data for *Salmonella meleagridis* I and III may not be treated as describing a single dose response relation. The data for *S. meleagridis* II do not allow fitting of a dose response model, but addition to the pooled data has some influence on the shape of the confidence interval (figure 6.5).
Based on the likelihood criterium given in chapter 3 the difference between the likelihood of the Beta Poisson model fitted to the pooled complete data set (I+II+III), and the maximum possible likelihood for this pooled set, is not significant ($D = \hat{\ell} - \ell_{\text{sup}} = 18.4$, $\chi^2_{18-2,0.95} = 26.30$), leading to acceptance of the model. Corresponding parameter values are: $\hat{\alpha}$=0.428, $\hat{\beta}$=8524 . The 95% confidence range in

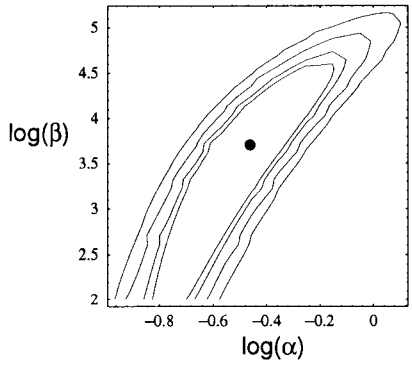


(a) *S. meleagridis* I

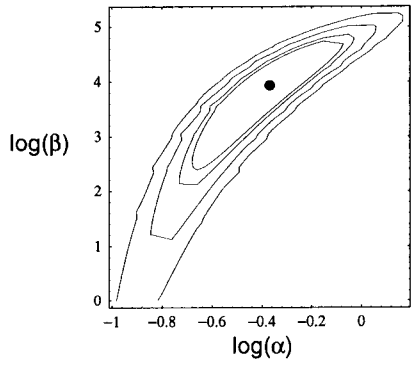


(b) *S. meleagridis* III

Figure 6.4: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by *Salmonella meleagridis* I and III (McCullough and Wesley Eisele, 1951a), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively.



(a) *S. meleagridis* I+III



(b) *S. meleagridis* I+II+III

Figure 6.5: Confidence range for the dose response parameters of the Beta Poisson model fitted to the pooled data for infection by *Salmonella meleagridis* I, II, and III (McCullough and Wesley Eisele, 1951a), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively. At the left, the positions of the 95% contours of the models for the separate data sets are given, together with the 95% contour of the pooled model.

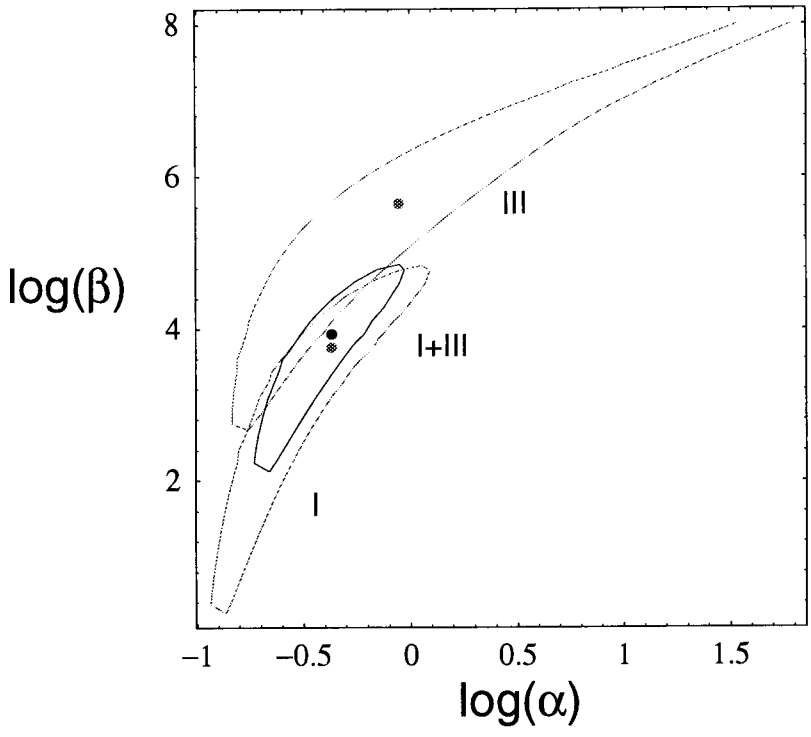
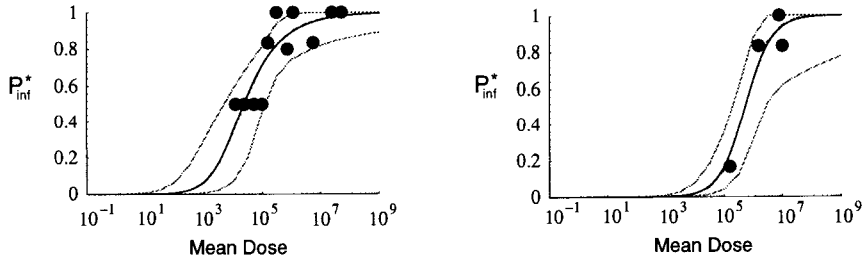


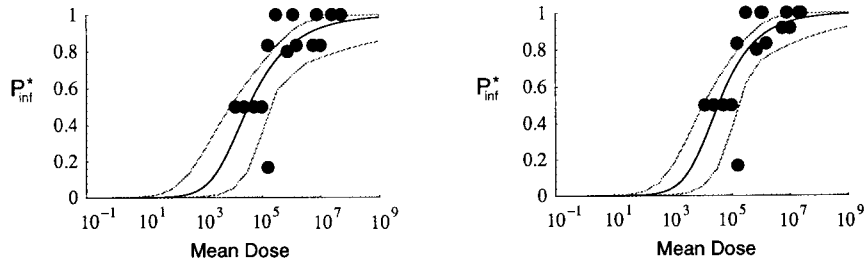
Figure 6.6: 95% confidence ranges for the dose response parameters of the Beta Poisson model fitted to the pooled data for infection by *Salmonella meleagridis* I and III, combined with the 95% confidence range for the pooled model.



(a) *S. meleagridis* I: Beta Poisson

(b) *S. meleagridis* III: Beta Poisson

Figure 6.7: Dose response curves fitted to the data for infection by *Salmonella meleagridis* I and III (McCullough and Wesley Eisele, 1951a), with observed fractions and 95% confidence limits. The data set for *Salmonella meleagridis* does not allow fitting of a separate curve.



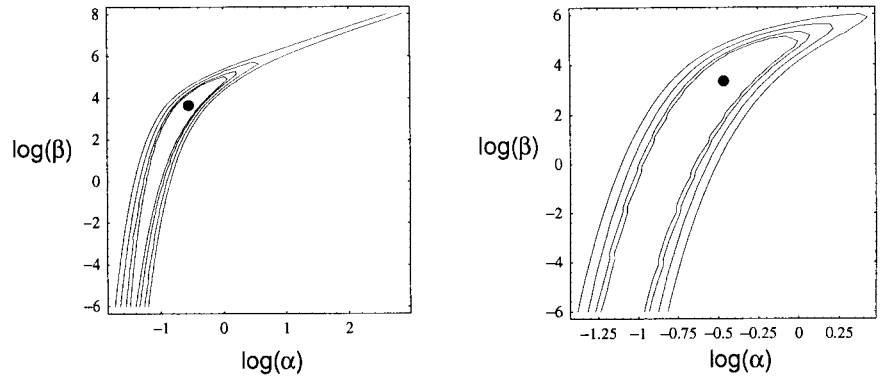
(a) *S. meleagridis* I+III

(b) *S. meleagridis* I+II+III

Figure 6.8: Dose response curve fitted to the pooled data for *Salmonella meleagridis* I and III, and for *Salmonella meleagridis* I, II, and II (McCullough and Wesley Eisele, 1951a).

	Exponential				Beta-Poisson						
	$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
1	2.98×10^{-6}	66.0	10	+	0.429	5682	7.57	9	-	58.4	+
3	4.44×10^{-7}	7.8	3	-	0.885	441332	2.30	2	-	5.51	+
1+3	1.45×10^{-6}	101.9	14	+	0.344	5090	17.6	13	-	84.3	+
1+2+3	1.55×10^{-6}	102.0	17	+	0.428	8524	18.4	16	-	83.7	+

Table 6.5: Summary of calculated characteristics for *Salmonella meleagridis* I (1) and III (3), and the pooled data set (1+3 and 1+2+3). Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{sup}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.



(a) *S. anatum* I (b) *S. anatum*II

Figure 6.9: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by *Salmonella anatum* I and II (McCullough and Wesley Eisele, 1951a), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively.

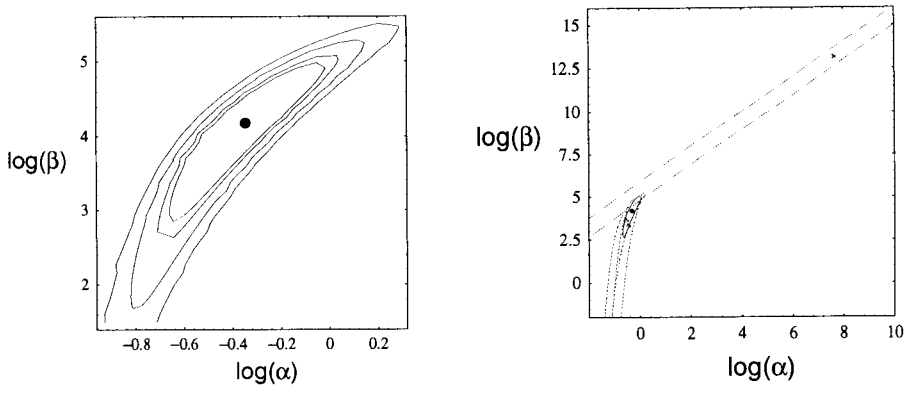
	Exponential				Beta-Poisson						
	$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
1	5.37×10^{-6}	20.4	7	+	0.281	4550	7.98	6	-	12.4	+
2	1.24×10^{-6}	34.7	7	+	0.342	2270	8.39	6	-	26.3	+
3	3.16×10^{-6}	0.33	2	-	4.01×10^{7}	1.27×10^{13}	0.33	2	-	0	-
1+2+3	2.36×10^{-6}	73.5	18	+	0.451	15177	23.1	17	-	50.4	+

Table 6.6: Summary of calculated characteristics for *Salmonella anatum* I (1), II (2), and III (3), and the pooled data set (1+2+3). Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

the parameter plane is shown in figure 6.6, in figure 6.8 the corresponding limits for the dose response relation are given.

Dose response relation of *Salmonella anatum*

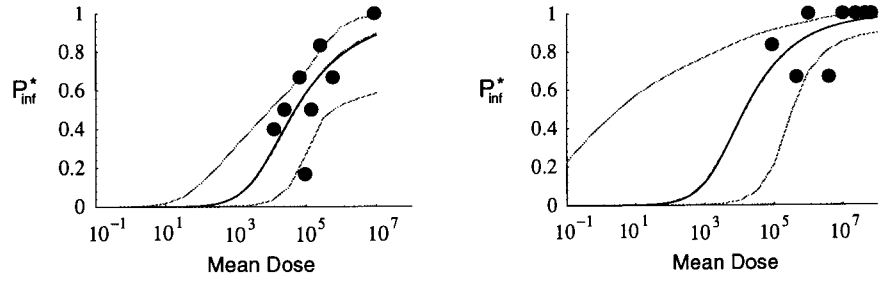
Numerical results are summarized in table 6.6. The likelihood-based confidence interval for the exponential dose response parameter r, for data from *S. anatum* III, is: $1.17\times10^{-6} - 8.31\times10^{-6}$.
The deviance of the Beta Poisson model fitted to the pooled data set may be compared to the sum of the deviances of the separate data sets (Beta Poisson curve for *S. anatum* I and II, and Exponential curve for *S. anatum* III). The difference between these two measures may be evaluated using a χ^2-criterium, as given in section 3.2. By this token, the goodness of fit of the pooled model is not significantly different from the goodness of fit of the three separate models ($D = D_{1+2+3} - (D_1 + D_2 +$



(a) *S. anatum* I+II+III

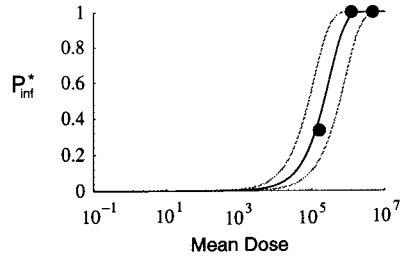
(b) *S. anatum* combined

Figure 6.10: Confidence range for the dose response parameters of the Beta Poisson model fitted to the pooled data for infection by *Salmonella anatum* I, II, and II (McCullough and Wesley Eisele, 1951a), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively. At the left, the positions of the 95% contours of the models for the separate data sets are given, together with the 95% contour of the pooled model. Note the oblique band for the exponential model fitted to the data for *S. anatum* III.



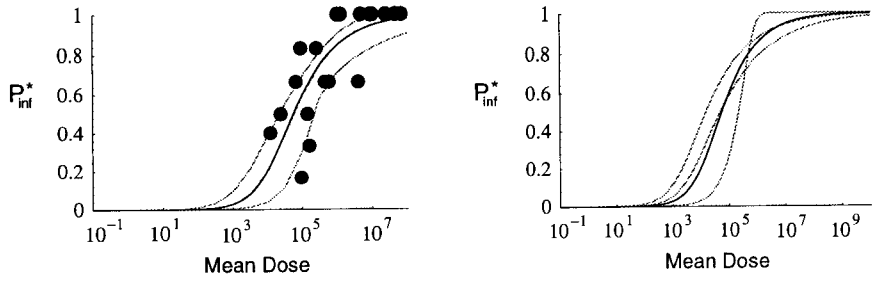
(a) *S. anatum* I: Beta

(b) *S. anatum* II: Beta



(c) *S. anatum*III: Exp

Figure 6.11: Dose response curves fitted to the data for infection by *Salmonella anatum* I, II, and III (McCullough and Wesley Eisele, 1951a), with observed fractions and 95% confidence limits.



(a) *S. anatum* I+II+III

(b) *S. anatum* combined

Figure 6.12: Dose response curve fitted to the pooled data for *Salmonella anatum* I, II, and III and combined plot of separately fitted curves and the dose response curve fitted to the pooled data (*Salmonella anatum* I, II, and III and I+II+III) (McCullough and Wesley Eisele, 1951a).

$D_3 = 6.42$, $\chi^2_{2+2+1-2,0.95} = 7.815$). Hence, under the assumption that the Exponential/Beta Poisson model is valid, the data for *Salmonella anatum* may be treated as describing a single dose response relation.
Based on the likelihood criterium given in chapter 3 the difference between the likelihood of the Beta Poisson model fitted to the pooled data set, and the maximum possible likelihood for this pooled set, is not significant ($D = \hat{\ell} - \ell_{\text{sup}} = 23.1$, $\chi^2_{19-2,0.95} = 27.59$), leading to acceptance of the model. Corresponding parameter values are: $\hat{\alpha}$=0.451, $\hat{\beta}$=15177 . The 95% confidence range in the parameter plane is shown in figure 6.10, in figure 6.12 the corresponding limits for the dose response relation are given.

6.2.2 *S. newport*, *S. derby*, and *S. bareilly*

Experimental data

Reference: McCullough and Wesley Eisele (1951b).

Strains *Salmonella*

- *newport*
- *derby*
- *bareilly*

Method All strains were isolated from market samples of spray-dried whole egg. Volunteers, bacterial inoculum and methods are similar compared to the previous paper.

Results In this study, only one strain of each species was used in the experiments. The results of the 3 Salmonella strains are summarized in Table 6.7. In both papers infected means faecal culture positive.

Strain	Dose	Total	Infected	Ill
S. newport	1.52×10^5	6	3	1
	3.85×10^5	8	6	1
	1.35×10^6	6	6	3
S. derby	1.38×10^5	6	3	0
	7.0×10^5	6	4	0
	1.6×10^6	6	4	0
	6.4×10^6	6	3	0
	1.5×10^7	6	4	3
S. bareilly	1.25×10^5	6	5	1
	6.95×10^5	6	6	2
	1.7×10^6	6	5	4

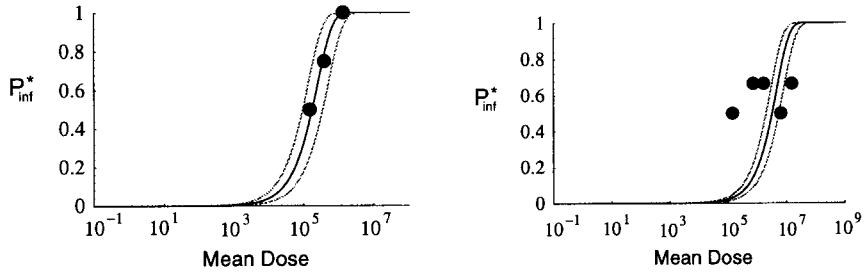
Table 6.7: Results of the dose response experiment (McCullough and Wesley Eisele, 1951b) for *Salmonella newport*, *S. derby*, and *S. bareilly* in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers of subjects infected at a given dose. Ill: number of subjects with gastro-enteric symptoms (diarrhea).

	Exponential						
Species	$\hat{r}$	D	df	sg	95% ci		
S. newport	3.97×10^{-6}	0.16	2	-	1.98×10^{-6}	–	7.46×10^{-6}
S. bareilly	3.19×10^{-6}	13.4	2	+	1.65×10^{-6}	–	5.81×10^{-6}
S. derby	2.19×10^{-7}	32.6	4	+	1.28×10^{-7}	–	3.51×10^{-7}

Table 6.8: Summary of calculated characteristics for *Salmonella newport*, *Salmonella derby*, and *Salmonella bareilly*. Shown are: maximum likelihood values for the exponential dose response parameter, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: likelihood based (95%) confidence limits for the dose response parameter.

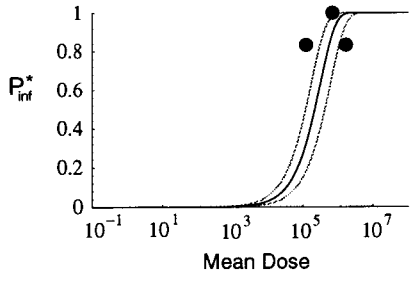
Dose response curves

Numerical results are summarized in table 6.8. The deviances of the Exponential model fitted to the three data sets may be compared to the χ^2 at the 95% level with the appropriate number of degrees of freedom (table 6.8). In figure 6.13 the corresponding limits for the dose response relation are given. The Beta Poisson model failed to produce satisfying fits for all three data sets.



(a) *S. newport*

(b) *S. derby*



(c) *S. bareilly*

Figure 6.13: Dose response curves fitted to the data for infection by *Salmonella newport*, *Salmonella derby*, and *Salmonella bareilly* (McCullough and Wesley Eisele, 1951b), with observed fractions and 95% confidence limits.

Strain	Dose	Total	Infected	Ill
S. pullorum I	1.0×10^{4}	6	0	0
	1.79×10^{9}	6	0	0
	1.0×10^{10}	6	6	6
	1.6×10^{10}	6	3*	6
*S. pullorum*II	1.38×10^{6}	6	0	0
	1.63×10^{8}	6	0	0
	6.75×10^{9}	5	5	4
S. pullorum III	2.3×10^{6}	6	0	0
	9.3×10^{7}	6	0	0
	1.2×10^{9}	6	0	0
	7.6×10^{9}	6	6	6
*S. pullorum*IV	1.88×10^{6}	6	0	0
	1.39×10^{7}	6	0	0
	1.1×10^{8}	6	0	0
	1.28×10^{9}	6	1*	3
	3.975×10^{9}	6	6	2

Table 6.9: Results of the dose response experiment (McCullough and Wesley Eisele, 1951c) for *Salmonella pullorum* in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers of subjects infected at a given dose. Ill: number of subjects with gastro-enteric symptoms (diarrhea). *: faeces cultured after 48 hours pi.

6.2.3 *S. pullorum*

Experimental data

Reference: McCullough and Wesley Eisele (1951c).

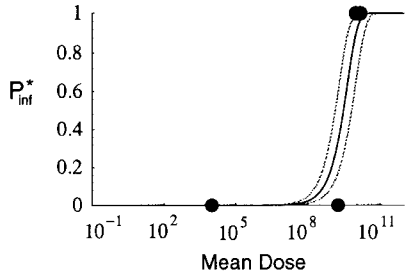
Strains *S. pullorum* strains I,II and III, all isolated from market samples of spray-dried whole egg. Strain IV was obtained from a human salmonellosis case.

Methods Volunteers, bacterial inoculum and methods are the same as in the previous papers.

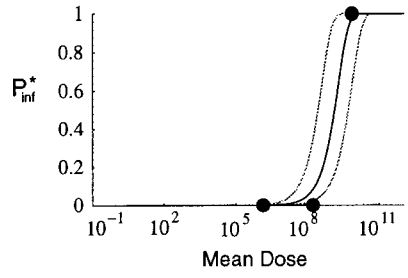
Results The results of feeding the 4 strains of *S. pullorum* are summarized in Table 6.9. It was noted that bacteria were recovered better when the first faecal culture was 24 hours pi instead of 48 hours pi, which was the case in some of the experiments. Infected in this case was only recorded as faecal culture positive.

Dose response relation of *Salmonella pullorum*

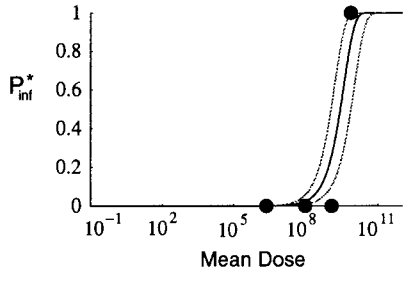
Numerical results are summarized in table 6.10. The deviances of the Exponential model fitted to the four separate data sets may be compared to the χ^2 at the 95% level with the appropriate number of degrees of freedom (table 6.10). In figure 6.14



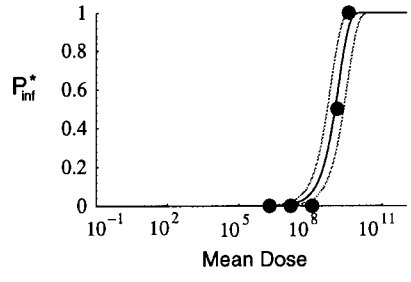
(a) *S. pullorum* I



(b) *S. pullorum* II

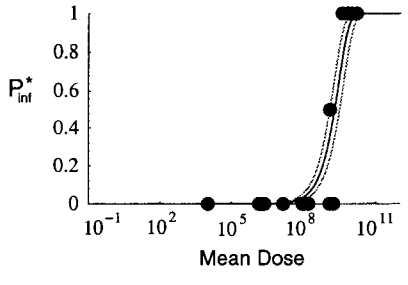


(c) *S. pullorum* III

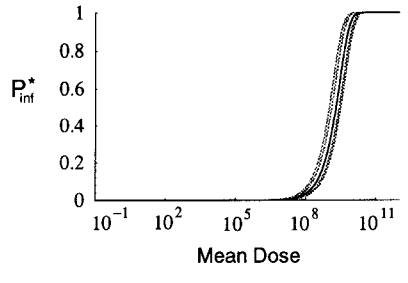


(d) *S. pullorum* IV

Figure 6.14: Dose response curves fitted to the data for infection by *Salmonella pullorum* I through IV (McCullough and Wesley Eisele, 1951c), with observed fractions and 95% confidence limits.



(a) *S. pullorum* I+II+III+IV pooled data



(b) *S. pullorum* I+II+III+IV combined

Figure 6.15: Dose response curve fitted to the pooled data for *Salmonella pullorum* I, II, III, and IV (McCullough and Wesley Eisele, 1951c), and the combined set of separate dose response curves, to show their similarity.

	Exponential						
	$\hat{r}$	D	df	sg	95% ci		
1	2.17×10^{-10}	6.49	3	-	1.00×10^{-10}	–	4.76×10^{-10}
2	5.28×10^{-10}	1.33	2	-	1.40×10^{-10}	–	26.21×10^{-10}
3	2.54×10^{-10}	5.83	3	-	0.92×10^{-10}	–	6.15×10^{-10}
4	6.72×10^{-10}	2.02	4	-	2.92×10^{-10}	–	14.23×10^{-10}
1+2+3+4	3.48×10^{-10}	20.4	15	-	2.18×10^{-10}	–	5.50×10^{-10}

Table 6.10: Summary of calculated characteristics for *Salmonella pullorum* I (1), II(2), III(3), and IV (4), and the pooled data set (1+2+3+4). Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: likelihood based (95%) confidence limits for the dose response parameter.

the corresponding limits for the dose response relation are given. The Beta Poisson model failed to produce satisfying fits for all four data sets.
The exponential dose response model also appeared to fit well to the pooled data set. The deviance between the four separate models and the pooled model is: ΔD=4.69, with df=1+1+1+1-1=3, and $\chi^2_{3,0.95}$=7.815, not significant. The curve fitted to the pooled data thus may be used to represent the dose response relation of all four separate experiments.

Strain	Dose	Total	Infected	Ill
S. typhi Quailes	10^3	14	-	0
	10^5	116	-	32
	10^7	32	-	16
	10^8	9	-	8
	10^9	42	-	40

Table 6.11: Results of the dose response experiment (Hornick *et al.*, 1970) for *Salmonella typhi* Quailes in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers infected, not reported in this study. Ill: number of subjects with symptoms of typhoid fever.

6.2.4 *S. typhi* (typhoid fever)

Experimental data

Reference: Hornick *et al.* (1970).

Strain *S. typhi* Quailes (pathogenic strain, isolated from a carrier)

Volunteers healthy adult males, not exposed previously.

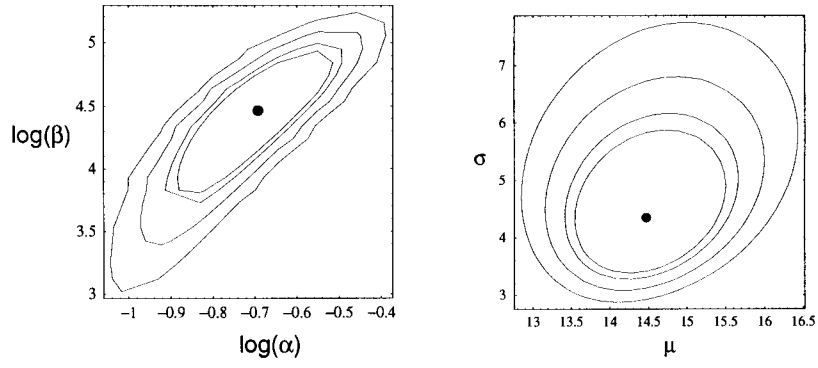
Bacterial inoculum This strain isolated from a carrier containing the Vi (envelope) antigen was propagated onto solid and then liquid media and harvested after 6 hours at 37°C.

Method bacteria were suspended in 30 ml of milk (exact amount of viable organisms) and administered by gargling and swallowing. Bacterial examination was mentioned in the results, but no method has been described, nor numbers of positive stools at different given doses.

Results Infection was not reported separately, only illness (table 6.11). Illness was described as developing fever (higher than 103 °F) followed by headaches and abdominal pain. Subsequently anorexia, myalgia and fatigue occurred. In some volunteers given 10^5 and 10^7 bacteria, positive stool specimens were reported, the question whether all volunteers in these groups became infected, remains unclear, however.

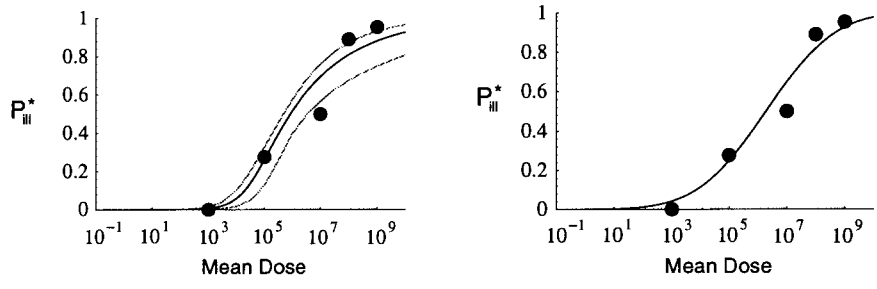
Dose response relation of *Salmonella typhi*

Numerical results are summarized in table 6.12. Both the Exponential and the Beta Poisson model appear to fail the likelihood-based test for goodness of fit. Therefore, we tried an alternative relation: the Lognormal model. Here, the dose response curve assumes the shape of the cumulative distribution function of the lognormal distribution. Such a relation may result from a threshold for infection which is distributed lognormally among the exposed population, which is why this model was classified as deterministic by Haas (1983). The Lognormal curve appeared to pass



(a) *S. typhi*: Beta Poisson relation (b) *S. typhi*: Lognormal relation

Figure 6.16: Confidence range for the dose response parameters of the Beta Poisson model and the Lognormal model fitted to the data for infection by *Salmonella typhi* (Hornick *et al.*, 1970), in the parameter plane (α, β), or (μ, σ), respectively. From the inside around the maximum likelihood estimates, the concentric contours of the 90, 95, 99, and 99.9% range are shown.



(a) *S. typhi*: Beta Poisson relation (b) *S. typhi*: Lognormal relation

Figure 6.17: Dose response curves fitted to the data for infection by *Salmonella typhi* (Hornick *et al.*, 1970), for both the Beta Poisson model (with 95% confidence limits) and the Lognormal model.

model	parameter values	D	df	sg
exponential	$\hat{r}$=2.14×10^{-8}	342	4	+
beta-poisson	$\hat{\alpha}$=0.203 $\hat{\beta}$=29173	8.63	3	+
lognormal	$\hat{\mu}$=14.47 $\hat{\sigma}$=4.35	5.42	3	-

Table 6.12: Summary of calculated characteristics for *Salmonella typhi*. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$, for three dose response models: Exponential, Beta Poisson, and Lognormal.

the test for goodness of fit, based on the deviance from the maximum possible likelihood (dropping all constraints). Deviance based confidence intervals for both the Beta Poisson and the Lognormal model may be compared by inspecting figure 6.16. The dose response curves in figure 6.17, however, show that the difference in shape between the two models is not very large. This may also be appreciated from the small amount by which the Beta Poisson model exceeds $\chi^2_{3,0.95}(= 7.815$, compared to 8.63, see table 6.12). We therefore propose to nevertheless accept the fitted Beta Poisson curve for use in risk analysis, so as to keep the risk of underestimating the probability of infection at low doses as small as possible.

6.3 *Plesiomonas shigelloides*

6.3.1 Experimental data

Reference: Herrington *et al.* (1987).

Strain clinical isolates from stool specimens, strains P009–P013.

Volunteers n=33 age: males 18-31 years, physically healthy (Baltimore community). Before bacterial challenge, stool specimens were taken for bacterial and parasitic examination. Some volunteers were pretreated with ampicillin 500 mg 4dd for 9 doses ending 9 h before challenge (see table 6.13).

Bacterial inoculum stock cultures -70°C were plated onto Trypticase soy agar o/n 37°C, 20-30 agglutinated confirmed colonies were suspended in TSB. Samples of this suspension were plated on TSA o/n 37°C. Then, bacteria were harvested in 3 ml 0.85% sterile saline and the concentration was standardized spectrophotometrically. Dilutions of the suspensions were made in sterile saline. The identity of the inoculum was confirmed by Gram stain and agglutination with specific antiserum.

Method Two grams of $NaHCO_3$ dissolved in 150 ml dd water and of this 120 ml were given to each volunteer. One minute later the bacterial inoculum, suspended in the remaining 30 ml $NaHCO_3$ was ingested. Afterwards all stool specimens were cultured by inoculation onto salmonella-shigella agar plates with and without streptomycin (10 mg/ml). Colonies were confirmed by oxidase testing and by agglutination with specific rabbit antiserum. Blood samples were taken daily and cultured onto TSA for *Plesiomonas*. Volunteers were examined daily and interviewed for complaints of nausea, appetite loss, abdominal discomfort, headache, malaise, fever (37.8°C or higher) or other symptoms, also diarrhea was scored.

Results None of the volunteers became ill or developed diarrhea. Bacteriological examination of stool specimens is shown in table 6.13.

6.3.2 Dose response curve

The best fitting dose response curve appears to be that of the Beta Poisson model. Based on the likelihood test in chapter 3, the deviance between the Beta Poisson curve and the maximum possible likelihood is not significant ($\hat{\ell} - \ell_{\text{sup}} = 2.68$, $\chi^2_{5-2,0.95} = 7.815$), so that this model is accepted as fitting the data. The parameter values are $\hat{\alpha}$=0.057 and $\hat{\beta}$=1171 . Confidence intervals for (α, β) are shown in figure 6.18. Numerical results are summarized in table 6.14.

Strain	Dose	Total	Infected	Ill
P. shigelloides	10^3	4	0	0
	10^5	4	1	0
	10^6	3	2	0
	10^8	4	1	0
	4×10^9	7	4	0
Pretreated with ampicillin(500 mg 4dd 9 doses) before inoculation				
P. shigelloides	10^5	4	1	0
	10^8	7	3	0

Table 6.13: Results of the dose response experiment (Herrington *et al.*, 1987) for *Plesiomonas shigelloides* in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers of subjects infected at a given dose. Ill: number of subjects with gastro-enteric symptoms (none reported in this experiment).

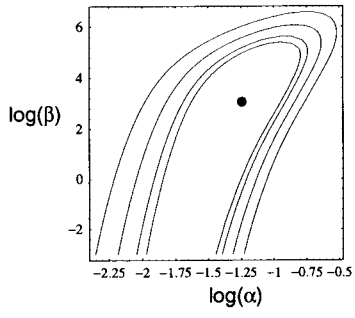


Figure 6.18: Confidence range for the dose response parameters of the Beta Poisson model fitted to the data for infection by *Plesiomonas shigelloides* (Herrington *et al.*, 1987), in the parameter plane (α, β). From the inside around the point $(\hat{\alpha}, \hat{\beta})$, the concentric contours of the 90, 95, 99, 99.9% range are shown, respectively. Also shown is the set of parameter pairs, obtained by maximum likelihood fit of the Beta Poisson curve to resampled data sets (see 3.1).

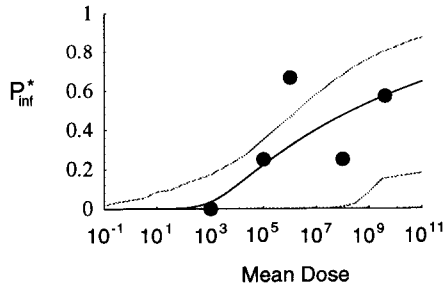


Figure 6.19: Dose response relation for infection of healthy volunteers by *Plesiomonas shigelloides*. Shown are: experimentally determined fractions infected, the best fitting dose response curve (Beta Poisson model), and the limits of a 95% confidence interval.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
4.42×10^{-10}	47.2	4	+	0.057	1171	2.68	3	-	44.5	+

Table 6.14: Summary of calculated characteristics for *Plesiomonas shigelloides*. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{sup}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

6.4 *Shigella*

6.4.1 *Shigella flexneri* 2a##

Experimental data

Reference: DuPont *et al.* (1969).

Strain strain 2457T originally came from Lt. Col. Oscar Felsenfeld, Tokyo, Japan. Sent to Dr. S. Formal in the autumn of 1954, and freeze dried. Passed through a starved, opium treated guinea pig in 1959, and freeze dried again. Passed through a monkey in 1963 and freeze dried. Stored at -60°C, suspended in skimmed melk.

Volunteers Subjects were well informed inmates (Maryland House of Correction, Jessup, Maryland, USA). Male sex, age 21 – 44 years (mean 28 years), no recent episodes of diarrhea, no past history of shigellosis, to their own knowledge.

Bacterial inoculum Plated on blood agar, two days before administration. After overnight incubation at 37°C, approximately 20-30 colonies were harvested and suspended in trypticase soy broth. This suspension was inoculated on trypticase soy agar plates and incubated overnight at 37°C. Immediately prior to the experiment colonies were harvested in about 3 ml standard sterile saline per plate and the suspension was standardized roughly by means of turbidimetry. Serial dilutions in saline were inoculated fourfold onto trypticase soy agar plates, before and after administration, to determine numbers of viable *S. flexneri.*

Method The pathogens were suspended in 30 ml of fat milk, and administered at the end of the morning on an empty stomach. After that, all men were admitted to a special hospital ward, and observed continually. During the acute phase of the illness complete blood analysis was performed, serum was taken weekly to test for antibodies to *Shigella.* Stools were examined daily.

Results Shown in table 6.15. Infection was not reported in this study, illness was defined as fever (orally 100°F or higher), severe abdominal cramping, diarrhea (more than twice unformed stools within 24 hours.), or excretion of blood and mucus.

Dose	Total	Infected	Ill
10^4	4	-	1
10^5	4	-	3
10^6	8	-	7
10^7	19	-	13
10^8	8	-	7

Table 6.15: Results of a dose response experiment (DuPont *et al.*, 1969) for *Shigella flexneri* in healthy human volunteers. Dose: number of ingested organisms. Total: number of subjects per dose. Infected: not given. Ill: number of subjects with symptoms of dysentery (abdominal pain, fever, diarrhea, excretion of mucus and/or blood).

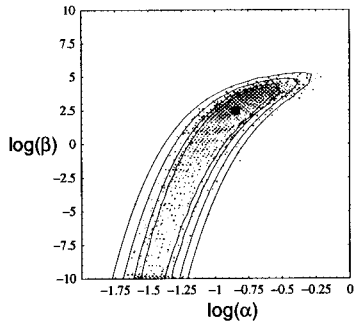


Figure 6.20: Confidence range for the dose response parameters of the Beta Poisson model fitted to the illness data for *Shigella flexneri* (DuPont *et al.*, 1969), in the parameter space (α, β). Concentrically outward from the point $(\hat{\alpha}, \hat{\beta})$ the contours for 90, 95, 99, en 99.9% significance levels. Also shown: set of parameter pairs, obtained by maximum likelihood fit of the Beta Poisson curve to resampled data sets (see 3.1).

Dose response curve

Although the Beta Poisson model has been used for infection so far, and not to describe the probability of the occurrence of symptoms, the data of this experiment appear to allow such an approach. This may be caused by the high virulence of this organism, as a result of which infection leads to illness in as good as 100% of the cases. In spite of the success of this approach, in a purely phenomenological sense, the analysis of illness data usually does not produce such simple results[1]

[1] Often a decreased fraction of illness cases is found at high doses, so that the dose response relation for illness (gastro-enteric symptoms) appears to have an optimum (maximum), see e.g. sections 4.2 and 6.1. The Beta Poisson model does not provide any clue to the causes of such effects.

Exponential				Beta-Poisson						
$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
1.101×10^{-7}	73.6	4	+	0.143	284.3	3.439	3	-	70.2	+

Table 6.16: Summary of calculated characteristics for *Shigella flexneri*. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

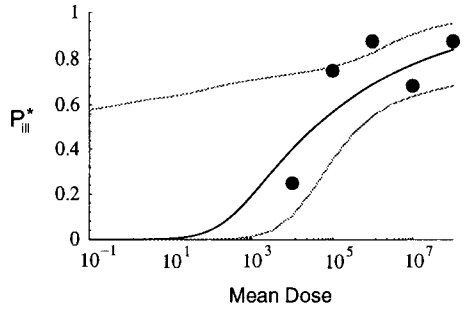


Figure 6.21: Dose response relation for illness caused by *Shigella flexneri* in healthy volunteers. Shown are the fractions of ill subjects at each dose, the curve of the best fitting Beta Poisson model, and the limits of a 95% confidence range.

Based on the criterium in chapter 3, the difference between the likelihood of the Beta Poisson model and the maximum possible likelihood is not significant ($-2(\hat{\ell}-\ell_{sup}) = 3.439$, $\chi^2_{5-2,0.95} = 7.815$), so that the model is accepted. Parameter values are: $\hat{\alpha}$=0.143, $\hat{\beta}$=284.3 . The 95% confidence range for the parameters is shown in figure 6.20, in figure 6.21 corresponding limits for the dose response curve are shown.

Strain	Dose	Total	Infected	Ill
S. paradysenteriae	10^8	4	1	-
	10^9	4	4	-
	10^{10}	8	8	-

Table 6.17: Results of the dose response experiment (Shaughnessy *et al.*, 1946) for *Shigella paradysenteriae* (Flexner W FWI) in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: number of subjects infected. Ill: number of subjects with dysenteric symptoms (no numbers reported).

6.4.2 *Shigella paradysenteriae* (*S. flexneri*)

Experimental data

Reference: Shaughnessy *et al.* (1946).

Strain *Shigella paradysenteriae* (Flexner W FWI) in the original publication, now *S. flexneri.*

Volunteers Study carried out to determine the effect of vaccination. Data only obtained from the control group (no vaccination, only challenge). Prisoners were checked for absence of shedding *Shigella* by bacteriological examination before challenge, and also for absence of any recent history of diarrheal disease.

Bacterial inoculum Bacteria grown on veal infusion slant, washed off with dd water and the inoculum size determined by a nephelometer.

Method Two grams of $NaHCO_3$ in 30 ml water was given to each volunteer, 5 minutes later followed by ingestion of the bacteria in 275 ml of milk. Administration of bacteria in milk was later discontinued, because it was thought that milk might interfere with the uptake of bacteria. Therefore, the bacteria were suspended in water. Stool specimens were cultured and colonies identified biochemically and immunologically (method not described).

Results Volunteers were also examined for clinical signs, but unfortunately enough no quantitative data were described. The results of the dose-infection experiment are shown in table 6.17.

Dose response curve

Based on the criterium in chapter 3, the difference between the likelihood of the Exponential model and the maximum possible likelihood is not significant ($-2(\hat{\ell} - \ell_{sup}) = 0.27, \chi^2_{3-1,0.95} = 5.991$), so that the model is accepted. Parameter value: $\hat{r} = 3.85 \times 10^{-9}$. The 95% confidence range for the parameters is shown in table 6.18, in figure 6.22 corresponding limits for the dose response curve are shown.

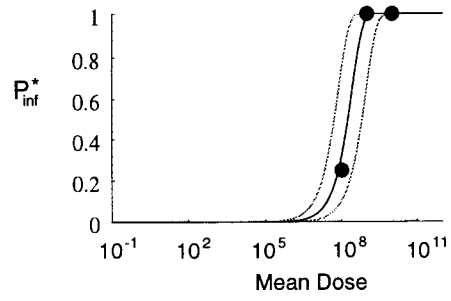


Figure 6.22: Dose response relation for infection caused by *Shigella paradysenteriae* in healthy volunteers. Shown are the fractions of infected subjects at each dose, the curve of the best fitting Exponential model, and the limits of a 95% confidence range.

Exponential				
$\hat{r}$	D	df	sg	95% ci
3.85×10^{-9}	0.27	2	-	1.07×10^{-9} – 13.3×10^{-9}

Table 6.18: Summary of calculated characteristics for *Shigella paradysenteriae*. Shown are: maximum likelihood value for the dose response parameter, deviance ($D = \hat{\ell} - \ell_{sup}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: limits of a likelihood-based 95% confidence interval for the dose response parameter.

6.4.3 *Shigella dysenteriae* 1

Experimental data

Reference: Levine *et al.* (1973).

Strain Strain A-1 is sensitive to antibiotics, and was isolated from a patient with mild symptoms, at the beginning of the Guatemala pandemic. M 131 is a strain with multiple resistance to antibiotics, isolated in 1970 from a patient in Guatemala with severe dysenteria.

Volunteers Experimental subjects were inmates (male sex, young adults from the Maryland House of Correction, Jessup, Maryland, USA). They were inform-

Strain	Dose	Total	Infected	Ill
M 131	10^1	10	-	1
	2.10^2	4	-	2
	2.10^3	10	-	7
	10^4	6	-	5
A-1	2.10^2	4	-	1
	10^4	6	-	2

Table 6.19: Results of the dose response experiment (Levine *et al.*, 1973) for *Shigella dysenteriae* 1 in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: not reported in this study. Ill: number of subjects with dysenteric symptoms (abdominal pain, fever, diarrhea, excretion of mucus and/or blood).

ed thoroughly of the risks involved with the experiment. Volunteers were admitted to a closed ward (University of Maryland) and put under constant observation by medical staff. Prior to the study, subjects were examined (anamnesis, full physical examination, urinalysis and haematological analysis, X-ray chest study, ECG). Subjects with allergy to penicillin were excluded from the experiment.

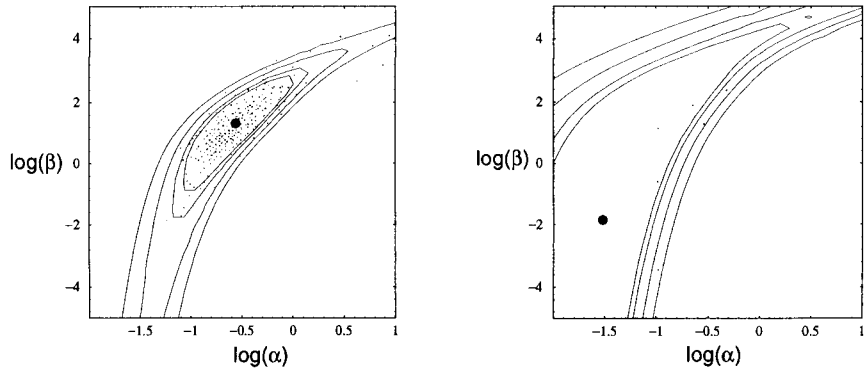
Bacterial inoculum Cultures were incubated on agar plates during 24 hours, harvested and diluted if necessary. Pour plates were prepared before and after administration, to verify the dose.

Method All doses were given in 45 ml of milk. Subjects given the endemic virulent strain A-1 and the pandemic virulent strain M 131, were not given bicarbonate. If subjects developed high fever (above 103°F) or severe diarrhea (more than 10 loose stools within 24 hours), the experiment was ended prematurely. Finally, all subjects were given ampicillin (oral or im.), 2–6 g/day, during 5 days. Eight ill subjects were intubated to collect specimens of intestinal fluid during episodes of clinical illness. These samples were inoculated immediately into enriched medium: trypticase-soy broth containing 200μg/ml bacitracine (no inhibition *S. dysenteriae*). The presence of toxins in intestinal fluid was tested in a (HeLa-) cell culture. Rectal swabs were inoculated daily onto agar plates. Faecal samples were plated and serial dilutions of homogenate were incubated in trypticase-soy broth.

Results Objective criteria for illness that were used: fever (orally 100°F or higher), diarrhea (three or more loose stools within 24 hours), dysenteria (blood and mucus in stools) and vomiting. In this study, infection is equated to illness. The following remarks are made, however: ileal and jejunal samples were taken from six subjects at an advanced stage of incubation. From a seventh person only the jejunum was sampled. In only one of the samples of jejunal fluid the Shiga bacterium was found. Two of the six ileal samples were positive; concentrations of 10^4 and 10^1 organisms/ml were found, respectively. In eight subjects a fluid sample was taken from the small intestine during the phase of clinical illness. Nine samples of jejunal fluid, and seven samples of ileal fluid have been analyzed. Of these sixteen samples, only one, from the ileum, appeared to contain the organism, at a concentration of 20 /ml. A stool sample taken simultaneously produced 10^6 organisms/g faeces. The pathogenic organism was recovered from 14 out of 15 ill subjects. The maximal concentration of excreted Shiga organisms was 10^6-10^{10} /g faeces.

Dose response curves

The dose response data used here do not describe the incidence of infection, but that of symptoms (fever, diarrhea, dysenteria). The same remarks as given in 6.4.1 are



(a) *S. dysenteriae* 1: strain M 131

(b) *S. dysenteriae* 1: strain A-1

Figure 6.23: Confidence range for the dose response parameters of the Beta Poisson model fitted to data for illness by *Shigella dysenteriae* 1, strains M 131 and A-1 (Levine *et al.*, 1973), in the parameter plane (α, β). Outward about the point $(\hat{\alpha}, \hat{\beta})$ the contours for a 90, 95, 99, 99.9% confidence range.

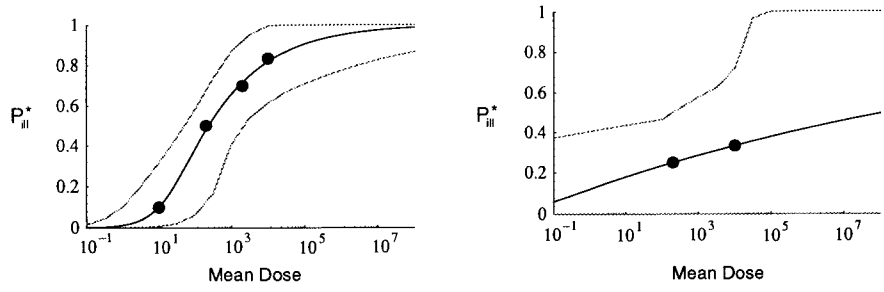
	Exponential				Beta-Poisson						
	$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
M 131	4.52×10^{-4}	13.21	3	+	0.277	21.16	0.032	2	-	13.17	+
A-1	6.04×10^{-5}	4.78	1	+	0.030	0.014	1.68×10^{-7}			4.78	+
both	2.05×10^{-4}	26.7	3	+	0.157	9.16	1.08	2	-	25.6	+

Table 6.20: Summary of calculated characteristics for *Shigella dysenteriae* 1. Shown are: maximum likelihood values for the dose response parameters, deviance ($D = \hat{\ell} - \ell_{\text{sup}}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.

holding here. The Beta Poisson curve appears to fit well to the data, in general this need not be the case for end points beyond infection.

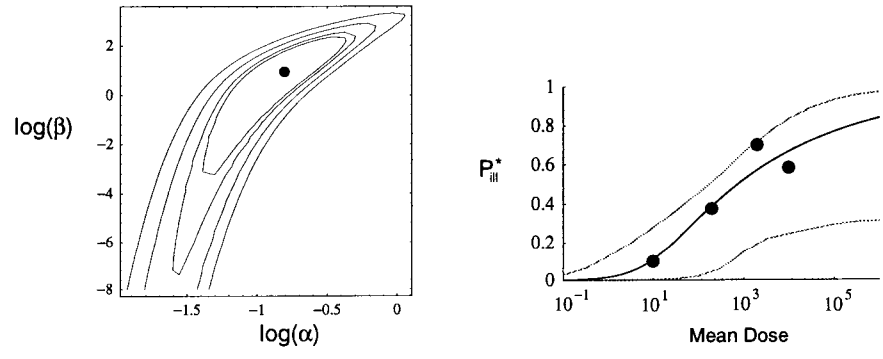
Based on the criterium in chapter 3, for strain M 131 the difference between the likelihood of the best fitting Beta Poisson model and the maximum possible likelihood is not significant ($-2(\hat{\ell}-\ell_{\text{sup}}) = 0.032$, $\chi^2_{4-2,0.95} = 5.991$), so that the model is accepted. Corresponding parameter values are: $\hat{\alpha}$=0.277, $\hat{\beta}$=21.16 . Maximum likelihood values for the parameters of the Beta Poisson relation for data from strain A-1 are: $\hat{\alpha}$=0.301, $\hat{\beta}$=0.0142 . These results are summarized in tabel 6.20. Also shown are the results for fitting of the same models to the pooled data set. The difference between the sum of the separate deviances and the deviance of the pooled model is: ΔD=1.08-0.032=1.05, with 2+2-2=2 degrees of freedom, leading to acceptance of the hypothesis that the fit of the pooled model is not worse than that of the two separate models.

For the separate models, 95% confidence intervals in the parameter plane are shown in figure 6.23, in figure 6.24 the corresponding limits for the dose response relation are shown. For the pooled model, this is shown in figure 6.25.



(a) *S. dysenteriae* 1: strain M 131 (b) *S. dysenteriae* 1: strain A-1

Figure 6.24: Dose response relation for illness caused by *Shigella dysenteriae* 1, strains M 131 and A-1 in healthy volunteers. Shown are fractions of ill subjects, dose response curve of the best fitting (Beta Poisson) model, and the limits of a 95% confidence range about the best fitting curve.



(a) confidence ranges (b) dose response

Figure 6.25: The Beta Poisson model fitted to pooled data for *Shigella dysenteriae* 1 (M 131 and A-1) (Levine *et al.*, 1973).

6.5 *Vibrio cholerae*

6.5.1 Inaba 569B and Ogawa 395

Experimental data

Reference: Cash *et al.* (1974).

Strain *Vibrio cholerae* two different serotypes, classical Inaba 569B and classical Ogawa 395, both isolated in India from patients with cholera.

Volunteers 111 healthy persons. Selection based on bacteriological examination of stool, which should be negative for *V. cholerae*. Patients were under constant surveillance during the experiment.

Bacterial inoculum Stock cultures were stored in skimmed milk at -70°C. Bacteria were cultured in BHI agar overnight at 37°C. 20-30 colonies (identity determined by group- and type specific antisera) were suspended in BHI and this suspension was inoculated on BHI agar. After 5-6 h, colonies were harvested in 5 ml sterile saline. Bacteria were washed and afterwards the bacterial suspension was standardized spectrophotometrically and diluted to approximate the number of organisms required for inoculation.

Method *Vibrio cholerae* were suspended in 1 ml buffered saline (pH 7.2) and were given with water with or without base (2 gram $NaHCO_3$ in dd 60 ml water, half before and half of the solution drunk after ingestion of *V. cholerae*) orally. To determine the accuracy of the dosage, plate counts were made. Infections were noted on clinical and bacteriological criteria. Stool specimens of patients were cultured directly in thiosulfate citrate bile salt sucrose agar (TSBS), MacConkey agar, gelatin agar, NGAP agar and NGP agar. Suspected colonies were inoculated on TSI agar and tested with antiserum. Gastric samples were taken by a nasogastric tube to determine the pH of the stomach fluid acidity.

Results The results of challenge of volunteers by inoculation of Inaba 569B without $NaHCO_3$ are shown in table 6.21 and the results of the same experiment with $NaHCO_3$ are given in table 6.22. In this experiment not only strain Inaba 569 was tested, but also strain Ogawa 395. These results are included in table 6.22. Infection is scored as having a positive stool for *Vibrio cholerae*. However 1 subject did develop diarrhea without having vibrios in his stool at any time. In general, the probability of infection was dependent on the administration of $NaHCO_3$. The buffering effect was studied by analyzing gastric contents and pH measurements. Stomach contents were assumed to be buffered at pH above 5.0 . Volunteers who overcame the buffering effect at 30 minutes after ingestion (return to baseline acid levels), had a lower attack rate than those whose stomach contents remained buffered. However, these

Strain	Dose	Total	Infected	Ill
V. cholerae	10^4	2	0	-
Inaba 569 B	10^6	4	0	-
	10^7	4	0	-
	10^8	4	2	-
	10^9	2	1	-
	10^{10}	1	1	-
	10^{11}	2	2	-

Table 6.21: Results of the dose response experiment (Cash *et al.*, 1974) for *Vibrio cholerae* Inaba 569B administered without pH buffer in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers of subjects infected (excretion of *V. cholerae*. Ill: numbers not reported.

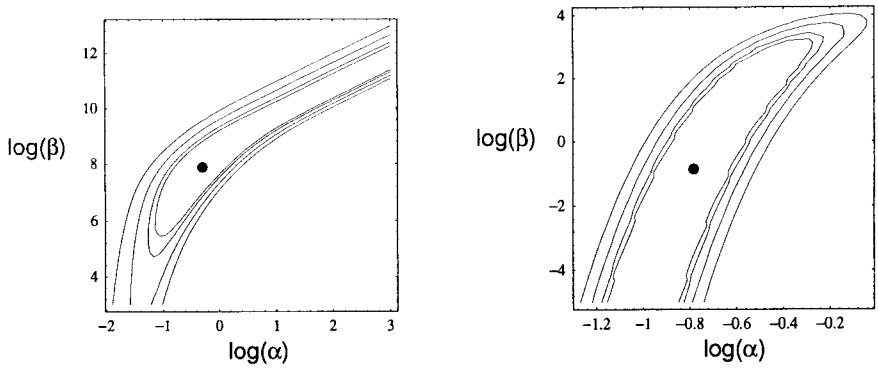
Strain	Dose	Total	Infected	Ill
V. cholerae	10^4	13	11	-
Inaba 569 B	10^6	52	48	-
	10^8	2	2	-
Ogawa 395	10^6	25	22	-

Table 6.22: Results of the dose response experiment (Cash *et al.*, 1974) for *Vibrio cholerae* Inaba 569B and *Vibrio cholerae* Ogawa 395, both administered with pH buffer in healthy human subjects. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: numbers of subjects infected (excretion of *V. cholerae*. Ill: numbers not reported.

differences in pH-related attack rates were only suggestive, not significant (Cash *et al.*, 1974). A dose of 10^6 bacteria of either classical 569B or classical Ogawa 395 appeared to produce illness similar to naturally acquired cholera, when given with $NaHCO_3$. No severe cases of diarrhea were observed at an infecting inoculum of 10^4 bacteria. Because the symptoms of illness were the same for both strains at a dose of 10^6 bacteria, it was concluded that the clinical observations were for all individuals infected with this dose (numbers of illness cases not reported). Further it was noted that the more severe the illness, the shorter the incubation period.

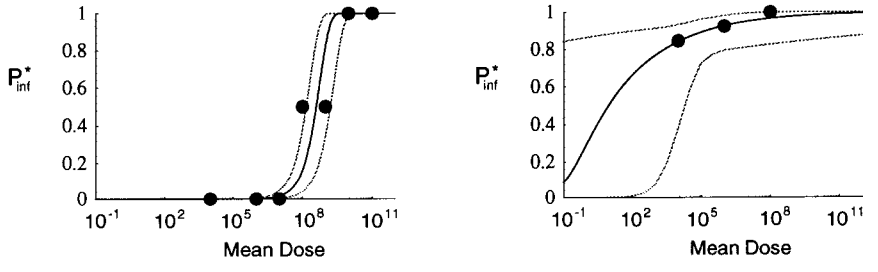
	Exponential				Beta-Poisson						
	$\hat{r}$	D	df	sg	$\hat{\alpha}$	$\hat{\beta}$	D	df	sg	ΔD	sg
a	1.76×10^{-9}	3.73	6	-	0.508	7.52×10^{7}	1.75	5	-	1.98	-
b	3.76×10^{-6}	65.7	2	+	0.164	0.136	0.149	1	-	65.6	+

Table 6.23: Summary of calculated characteristics for *Vibrio cholerae* 569b. Shown are: maximum likelihood value for the dose response parameter, deviance ($D = \hat{\ell} - \ell_{sup}$), number of degrees of freedom (df), and significance of the deviance compared to $\chi^2_{df,0.95}$. Also shown: the difference in deviances of both models (ΔD), and whether this difference is significant at the 95% level.



(a) without pH buffer (b) with pH buffer

Figure 6.26: Confidence range for the Beta Poisson dose response parameters for infection caused by *Vibrio cholerae* Inaba 569b in healthy volunteers, administered orally with and without pH buffer. Shown are the maximum likelihood estimates, and contours of a 90, 95, 99, and 99.9% confidence range.



(a) without pH buffer (b) with pH buffer

Figure 6.27: Dose response relation for infection caused by *Vibrio cholerae* Inaba 569b in healthy volunteers. Shown are the fractions of infected subjects at each dose, the curve of the best fitting model, and the limits of a 95% confidence range.

Strain	Pathogenic parent	cholera toxin genotype	El Tor hemolysin genotype	Shiga like toxin
JBK 70	El Tor Inaba N16961	A- B-	+	+
CVD 101	Classical Ogawa 395	A- B+	+	+
CVD 102	Classical Ogawa 395	A- B+	+	+
CVD 104	El Tor Inaba N16961	A- B-	-	+
CVD 105	Classical Ogawa 395	A- B-	-	+

Table 6.24: Summary of deletion mutant strains of *Vibrio cholerae* (Levine *et al.*, 1988).

Dose response curve

Based on the criterium in chapter 3, for *V. cholerae* given without pH buffer, the difference between the likelihood of the Exponential model and the maximum possible likelihood is not significant ($-2(\hat{\ell} - \ell_{\text{sup}}) = 3.73$, $\chi^2_{7-1,0.95} = 14.07$), so that the model is accepted. Parameter value: $\hat{r}$=1.76×10^{-9}. The 95% confidence range for r is: 4.28×10^{-10} — 4.84×10^{-9}. In figure 6.27 corresponding limits for the dose response curve are shown.

6.5.2 *V. cholerae* deletion mutants

Experimental data

Reference: Levine *et al.* (1988).

Strain *Vibrio cholerae*

- JBK 70 (deletion mutant of El tor Inaba N16961)
- CVD 101 (deletion mutant of Ogawa 395)

JBK 70 has deleted genes encoding the A and B subunits of cholera toxin by site directed mutagenesis (A-B-). CVD 101 has a deleted gene encoding the A subunit of choleratoxin (A-B+). All strains are summarized in table 6.24.

Volunteers Healthy young college students and other healthy young adults (Baltimore community). Cohorts of 5 to 10 volunteers were given a single dose of one of the vaccine strains , observed for 5 days. Afterwards they received oxytetracyclin 5 days.

Bacterial inoculum strains stored at -70°C, inocula were prepared (Levine *et al.*, 1984) and were given to volunteers with 2 grams of $NaHCO_3$.

Method Stool specimens were cultured on TCBS agar and inoculated in alkaline peptone water and Na- gelatin-phosphate broth. The enrichments were cultured overnight and subcultured onto TCBS agar. Suspicious colonies were confirmed as *V. cholerae* and serotyped. Diarrhea was defined as a passage of two or more loose stools within 48 hours with a volume of 200 ml or 1 loose stool at least 300 ml. Blood was taken of all volunteers before and 10, 21

Strain	Dose	Total	Infected	Ill
V. cholerae	10^6	4	3	1
JBK 70	10^8	5	5	2
	10^{10}	5	5	4
N16961	10^8	38	38	35

Table 6.25: Results of the dose response experiment (Levine *et al.*, 1988) for *Vibrio cholerae* JBK 70 and its parent strain El Tor Inaba N16961. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: number of subjects infected (excretion of *V. cholerae*. Ill: number of subjects developing gastro-enteric symptoms.

and 28 days after ingestion of the inoculum. Serum was tested with ELISA using IgG cholera antitoxin and vibriocidal antibody. Serum IgG and IgA antibodies were measured in ELISA's against Toxin coregulated (Tcp) pili, inactivated whole vibrio, a lysate of the homologous vibrio strain, LPS, and outer membrane protein antigen.

Results The JBK70 strain was ingested to volunteers in different doses and compared to the parent strain Inaba N16961, which was given at a dose of 10^6 bacteria to 38 volunteers. Of these 35 developed diarrhea and all persons had positive stools for V. cholerae. The results are summarized in table 6.25. Although JBK 70 colonized the intestines, the clinical symptoms of persons given JBK 70 were very mild. The same experiment carried out with CVD 101 and its parent strain Ogawa 395 are given in table 6.26. Unfortunately, data regarding bacteriological examinations were not reported. The data for the strain N16961 (dose of 10^8 bacteria) are very different from the rest and should probably not be used for dose response modelling. The results for the other deletion mutants are given in table 6.27. These strains were administered only at single doses, which may probably be used to fit with the CVD 101 dose response curve. CVD 102 is a thymidine-dependent auxotrophic mutant of CVD101. This is a live but nonproliferating *V. cholerae* strain.

No dose response relations were determined for the mutants. One main reason is that according to the data in tables 6.25, 6.26, and 6.27 almost all different doses which were given resulted into infection in all the exposed subjects, so that these data contain little information about the dose response relation.

Strain	Dose	Total	Infected	Ill
V. cholerae	10^4	6	6	4
CVD 101	10^5	5	5	2
	10^6	5	5	2
	10^7	5	5	3
	10^8	3	2	2
Ogawa 395	10^6	36	35	33

Table 6.26: Results of the dose response experiment (Levine *et al.*, 1988) for *Vibrio cholerae* CVD 101 and its parent strain Classical Ogawa 395. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: number of subjects infected (seroconversion, no data given about positive stool specimens). Ill: number of subjects developing gastro-enteric symptoms.

Strain	Dose	Total	Infected	Ill
V. cholerae				
CVD 102	10^7	5	2	0
CVD 104	10^7	6	6	2
CVD 105	10^5–10^8	9	9	3

Table 6.27: Results of the dose response experiment (Levine *et al.*, 1988) for *Vibrio cholerae* CVD 102, CVD 104, and CVD 105. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: number of subjects infected (excretion of *V. cholerae*). Ill: number of subjects developing gastro-enteric symptoms.

6.6 *Escherichia coli*

6.6.1 Enterotoxigenic *E. coli* (ETEC)

Experimental data

Reference: Evans *et al.* (1978).

Strain enterotoxigenic *E. coli*, strains H10407 (O78:H11) CFA+ and H10407 P CFA-.

Volunteers Students, 15 females and 12 males, age 18-27 years, physically healthy.

Bacterial inoculum Strains cultured in BHI O/N 37°C, followed by inoculation on Casamino acids yeast (CYE) medium for 4 h to obtain confluent growth. Inoculated again into BHI (overnight), harvested, adjusted to the appropriate OD (640 nm), and finally diluted to either 1×10^6 or 1×10^8 bacteria/50 ml in PBS.

Method Two grams of $NaHCO_3$ were given in 120 ml of water 3 minutes prior to bacterial ingestion. Inoculum size was confirmed by plate count techniques. Volunteers were monitored for clinical symptoms (diarrhea, nausea, vomiting, abdominal discomfort,fever). Stool specimens were taken for bacterial examination, volumes and weights were recorded.

Strain	Dose	Total	Infected	Ill
E. coli CFA+	10^6	7	7	1
	10^8	7	7	6
	1.2×10^9	10	10	9
E. coli CFA-	10^6	7	7	1
	10^8	6	6	0
	1.2×10^9	10	10	0

Table 6.28: Results of the dose response experiment (Evans *et al.*, 1978) for enterotoxigenic *E. coli*. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: number of subjects infected (excretion of *E. coli* ETEC). Ill: number of subjects developing gastro-enteric symptoms.

Results At a dose of 10^6 bacteria, there were no discernible differences between the two groups, only one volunteer in each group showed one watery stool. At a dose of 10^8, 6 out of 7 volunteers in the CFA+ group had watery diarrhea. In the group challenged with CFA- none had diarrhea. Number of volunteers infected and diseased are summarized in table 6.28.

Although the experimental results of this study using enterotoxigenic *E. coli* are given, no dose-response curves are determined. In this study, volunteers were given high doses resulting all into infection. To determine reliable dose response curves, an experimental study using lower doses of bacteria is needed.

6.6.2 Enteroadherent *Escherichia coli*

Experimental data

Reference: Mathewson *et al.* (1986).

Strain *Escherichia coli*, strains 189 and 221.

Volunteers 24 physically healthy adults.

Bacterial inoculum not mentioned

Method Two grams of $NaHCO_3$ in 130 ml sterile water were given 5 minutes before oral challenge. Volunteers were monitored 24 hours/day for developing clinical symptoms and all stool specimens were collected for bacteriological examination. Stools were plated on MacConkey agar with 150 μg ampicilline or 25 μg tetracyclin.

Result Strain 189 was associated with diarrhea in 1 of 4 volunteers at a dose of 7×10^8 bacteria. Three others in this group were complaining of abdominal pain and vomiting. Strain 221 at this dose was associated with diarrhea in 2 of 8 volunteers. only other 1 person in this group had enteric symptoms. At a dose of 10^{10}, strain 221 was associated with diarrhea in 3 of 8 volunteers. Five of the 8 volunteers had other enteric symptoms at this dose. Volunteers shedding bacteria are summarized in table 6.29.

Strain	Dose	Total	Infected	Ill
E. coli 189	7×10^8	4	4	1
	1×10^{10}	4	4	0
E. coli 221	7×10^8	7	7	2
	1×10^{10}	8	8	3

Table 6.29: Results of the dose response experiment (Mathewson *et al.*, 1986) for enteroadherent *E. coli*. Dose: number of organisms ingested. Total: number of subjects at a given dose. Infected: number of subjects infected (positive stools). Ill: number of subjects developing gastro-enteric symptoms.

Although the experimental results of this and the previous study using enterotoxigenic *E. coli* are given, no dose-response curves are determined. In these studies, volunteers were given high doses resulting all into infection in all the exposed subjects. To determine reliable dose response curves, an experimental study using lower doses of bacteria is needed.

Chapter 7

Discussion

In this report, the dose is defined as the amount of bacteria, viruses or parasites entering the digestive tract of a host and is expressed as a number of functional units. Under natural conditions of transmission (real life) it is often impossible to precisely determine the infective dose, since for example, the exact number of infectious salmonellae swallowed with a contaminated meal cannot be determined accurately. Some of these problems may be overcome in an experimental study. Experimental studies using human volunteers have been carried out for a number of enteric infections, especially in the 1950s and 1960s.
In the United States, volunteers were often recruited among male inmates from penitentiary institutions. In these studies, the relation between the number of subjects with a positive response (i.e. becoming infected, or ill) and the ingested dose is determined by feeding volunteers different doses of micro-organisms.

7.1 Determination of the dose

The reliability of the doses of microorganisms given to the volunteers is one of the critical points to determine dose response relationships. In the case of protozoan parasites given to volunteers, numbers were often defined as the amount of parasites counted in a direct smear or haemocytometer. In a recent study of *Cryptosporidium parvum* (DuPont *et al.*, 1995), not only the amount of parasites was determined, but also their viability by means of an excystation test. In that study, excystation rates appeared to range from 79.0% to 89.0% .
In volunteer studies using bacteria, all doses were determined using plate count tests and expressed as colony forming units (CFU): numbers of viable bacteria under *in vitro* conditions. Plate counts were determined before administration. After the doses were given, the remaining bacterial inoculum was tested again, except in the experiment with *Salmonella typhi.*
In case of the experiments done with viral pathogens, doses were expressed either as focus forming units (FFU), plaque forming units (PFU), or Tissue Culture Infectious Dose 50 (TCID50). Although the experimental designs to determine these

doses are different, in general the tests are all based on measuring viable counts *in vitro*. Therefore, dose response relations are also based on viable counts. These are not equal to the numbers of particles involved. For instance, the ratio numbers of FFU to numbers of physical virus particles for the rotavirus CJN strain as described in Ward *et al.* (1986) is 1/10,000 . This ratio is probably also different for numbers of *in vivo* infective particles. This is important to realize when the risk of infection is quantified, for instance by the probability of infection at a mean dose of one microorganism, as given in table 7.5.

7.2 Criteria for infection

The definition of infection and its detection are also important for the interpretation of dose response relations. Here infection is defined as shedding microorganisms in stool or in case of some viral infections, also developing a 2–4 fold rise in antibody titer.
Shedding of microorganisms is measured by microscopical examination of faeces in case of parasites, faecal culture in case of bacteria and tissue culture of faeces or antigen detection in faeces using an ELISA in case of viruses.
The sensitivity of these tests and the time after exposure are then important. An antigen detection ELISA could be less sensitive and thus may lead to wrong conclusions about the infection status. Also the fact that stool samples are not taken at the right time after infection may lead to false conclusions about infection occurring, yes or no.

7.3 Parameter range

Strictly speaking, the simplifications assumed by Furumoto and Mickey (1967a) in their derivation of the Beta Poisson dose response function require that the parameter β is large compared to the other parameter, α. For some of the data sets analyzed in this report, this condition is not fulfilled. However, their Beta Poisson function

$$P^*_{\text{inf}}(x) = 1 - (1 + \frac{x}{\beta})^{-\alpha}$$

remains a valid description of the dose response relation, for any nonnegative values of α and β. The approximations of Furumoto and Mickey may be avoided by means of numerical integration. The dose response then is not available in closed form, anymore. Maximum likelihood estimation of parameters then becomes a tedious procedure, but leads to only slightly different parameter values. This has been tested for the data for rotavirus (Ward *et al.*, 1986). Resulting parameter values were: (α, β)=(0.22, 0.45), instead of the values, found with the approximated formula: (α, β)=(0.26, 0.42) (Teunis *et al.*, 1994). Numerical integration also leads to a slightly

different shape of the dose response relation[1] (see figure 4.3 in (Teunis *et al.*, 1994)). Since the influence of using a more exact approach remains rather insignificant, we propose to use the approximated function in all cases, without the constraints imposed during its original derivation. This seems to be in agreement with existing practice (Rose and Gerba, 1991; Haas *et al.*, 1993).

7.4 Differences with published data

Information on the infectivity of pathogenic microorganisms is often reported in such a form that comparison with the present results is not very informative. For instance, Salyers and Whitt (1994) claim that *Shigella* species are highly infectious, with ID_{50} ranging between 100 and 200 organisms. Comparison with dose response curves in figures 6.21, 6.24, and 6.25 shows that this dose is well within the confidence area's for these experiments.
Some of the dose response relations fitted in this report are different from those published previously. In some of these cases, the differences are due to the use of a (partly) different data set. In addition to this, earlier work included fitting by means of a least squares procedure (χ^2-method, see *e.g.* (Haas, 1983)). The most important discrepancies are discussed below.

7.4.1 *Campylobacter*

In this report, data of Black *et al.* (1988) for strain A3249 of *Campylobacter jejuni* have been used. Rose and Gerba (1991) have published an overview of dose response parameter values for the Exponential and Beta Poisson models for various pathogenic microorganisms. These also include a not further specified *Campylobacter* species, presumably *C. jejuni.* No details are given about experimental and mathematical procedures. Therefore, nothing is known about the reliability of these parameter values, both in terms of significance levels, or suitable confidence interval, or in terms of strain or species specifications, experimental subjects, and their treatment.
Since the analysis given in this report leads to a much higher probability of infection, the present analysis seems to be a prudent choice for risk analysis, as long as more abundant data sets are not available.

7.4.2 Echovirus 12

For this virus we used experimental data reported in Schiff *et al.* (1984). Dose response results for echovirus were also reported by Haas (1983), but in this paper a

[1]Note that these small differences in each of the fitted parameter values still lead to a different low dose extrapolation α/β, since one parameter is increased, while the other is decreased in value relative to the values for the approximated function.

different data set from an older experiment was used. The results of the dose response analysis are different: Haas (1983) reports acceptable fit of all three models tested (the Lognormal, Exponential, and the Beta Poisson model). In a later paper (Regli *et al.*, 1991), an improved fitting procedure applied to the same data set we used (Schiff *et al.*, 1984) led to acceptance of the Beta Poisson model with parameter values slightly different from the values in the present report: $(\hat{\alpha}, \hat{\beta})$ = (0.374, 186.69). This leads to an approximated low dose proportionality constant of 2.0×10^{-3}, and $ID_{50}=1.004\times10^{3}$, quite close to the values calculated here ($(\hat{\alpha},\hat{\beta})$ = (0.401, 227.2), low dose appr. 1.76×10^{-3}, and $ID_{50} = 1.05\times10^{3}$). There is one remarkable discrepancy between our analysis and that reported in (Regli *et al.*, 1991), however: our failure to arrive at acceptance of the fitted Beta Poisson model to the data of Schiff *et al.* (1984).

7.4.3 Poliovirus

For the dose response of poliovirus, results from various experiments have been published. Haas (1983) used data from Lepow *et al.* (1962) for type 1 (Sabin), and from Katz and Price (1967) for type 3 (Fox). In a later study (Regli *et al.*, 1991), data were analyzed from Minor *et al.* (1981) and Lepow *et al.* (1962) for type 1, and from Katz and Plotkin (1967) for type 3.
With respect to the data reported in Regli *et al.* (1991) for poliovirus 3, the following remarks must be made: in our analysis, the Beta Poisson model fits better than the Exponential model, but the difference in deviance is not significant at the 95% level. In order to allow for maximum uncertainty in estimated probabilities of infection, the Beta Poisson model may still be regarded as the best model, so that there remains agreement with Regli *et al.* on the choice of model. The parameter values are different, however: $(\hat{\alpha}, \hat{\beta})$ = (0.409, 0.788), whereas in our case we have: $(\hat{\alpha}, \hat{\beta})$ = (0.299, 0.552). In Haas (1983) the used data have been explicitly reported, and inspection of these leads to an interesting discrepancy: the data are identical to those we have used in table 5.13, except for the second dose. In our case 2.5 TCD50, whereas Haas reports 1.5 TCD50. In Haas (1983) the data were taken from a paper referred to as Katz and Price (1967), with the same reference as Katz and Plotkin (1967). In Regli *et al.* (1991) data from Katz and Plotkin (1967) have been used in an improved analysis (maximum likelihood fitting, versus chi-squared method in the older paper) thereby producing the values mentioned above. In Katz and Plotkin (1967) a dose of 2.5 TCD50 has been used. Interestingly enough, changing the dose to 1.5 in our analysis procedure appears to exactly reproduce the parameter values of Regli *et al.* (1991).

7.5 Pooling data

In section 3.2 a likelihood ratio test is given to assess the differences between data sets given the Exponential/Beta Poisson model. Application to data sets for differ-

ent strains of *Salmonella* and two strains of *Shigella dysenteriae* has shown that in those cases the difference deviation between separate models and the pooled model is not significant. Therefore, these data may be considered as representing the same dose response relation, again given the models used here. In case of *Salmonella meleagridis* and *Salmonella anatum* (section 6.2.1) we therefore have to disagree with the authors of the original publication, who concluded that the infective doses varied widely between different strains of the same species (McCullough and Wesley Eisele, 1951a). Contrary to what might be expected, confidence intervals for the pooled models were not always markedly smaller that those of the separately fitted models. This is because of considerable scatter within and between data sets.

7.6 Interpretation

In this report, experimental data for a number of different pathogens are analyzed with the Exponential/Beta Poisson dose response model. For every data set, this leads to values for the parameters of the best fitting model, with an appropriate confidence range. This is the information that is needed for quantitative risk assessment, to translate an exposure estimate into a probability, or risk, that an effect occurs. The Beta–Poisson model is built upon the assumption that infection is a stochastic process. At low doses, when it is unlikely that more than one microorganism enters the host, the model predicts a nonzero probability of infection. The Beta–Poisson model is a "single–hit" model: any single pathogenic microorganism entering the host is capable of initiating infection. The assumption that two, three, or more organisms should enter simultaneously to start infection, leads to an increasingly steeper dose response function (Gifford and Koch, 1969). This is not found in dose response experiments. The steepest relations in human hosts are fitted well by the exponential model, which represents the steepest (limiting) case of the Beta–Poisson relation. Therefore, the ability to fit the Beta–Poisson model to many data sets adds plausibility to the absence of a threshold dose, below which infection would not possibly occur.

Now that we have analyzed various experimental data sets, the question arises whether the model parameters may have a meaning in a biological sense. In the original derivation of the Exponential/Beta Poisson model, (section 2.2), the parameter r represents the probability that any single organisms starts infection, given that it has successfully entered the host. In case of an exponential dose response relation, this probability is the same for any organism entering the host. A more realistic representation may be constructed when this probability r is allowed to vary between different organisms entering the host. When r is not constant, but has a probability distribution by itself, a Beta distribution, the dose response relation assumes the shape of the Beta Poisson function. Hence, the parameters (α ,β) may also be interpreted as describing a Beta probability distribution for the probability of infection by an organism, given that it has entered the host.

Compared to the Exponential model, the Beta Poisson model allows for a less steep

dose response relation. The extra parameter also provides extra freedom in shape; therefore, the two parameter model also leads to the most generous estimate in confidence range, see *e.g.* section 5.3.4.
When very few experimental data are available, as is often the case, discrimination between the two models is not possible (no statistically significant difference in deviance D). The arguments given above then may still make the Beta Poisson model the best choice (less steep dose response relation, larger confidence range), especially when the results are to be used for risk assessment.
In a few cases, the Exponential model appears to provide acceptable fit to a data set that is not sparse, see for instance the pathogenic protozoa *Cryptosporidium parvum* (section 4.2) and *Giardia lamblia* (section 4.1). In these two cases, it is reasonable to assume that every (oo)cyst that enters the host has the same probability of causing infection.
Data sets like that for rotavirus (section 5.1), or *Salmonella typhi* (section 6.2.4) appear to have a very slow increase in probability of infection (illness) with the ingested dose. This may only be fitted with the Beta Poisson model. When insufficient data are available in only a certain range, as in the data set for *Campylobacter jejuni* (section 6.1), where small doses (with $P_{\text{inf}} < 0.5$) are lacking, the Beta Poisson model may still be fitted successfully, resulting in an appropriately shaped confidence interval for the dose response.
Quantitative differences between dose response relations for different experiments may indicate differences in biological factors of the infectious organism, like presence or absence of virulence factors, the rate at which the organism is able to multiply itself. Factors associated with the host may also vary: immune competence, stomach contents during exposure, general condition (feeding status), etcetera. Differences may also originate from different experimental conditions, like enumeration method, exposure conditions (administration in milk, bicarbonate solution, or suspended in plain water), or criteria used (infection merely judged by excretion, or by other criteria).
The first class of differences (differences in biological properties of the infectious microorganism) are most difficult to control. The results given in this report using the Beta–Poisson model indicate that differences between different strains of a species may sometimes be neglected, at least at the level of infection. Data of volunteer studies for different strains of the same *Salmonella* serotype were pooled (3 strains of *S. meleagridis*, 3 strains of *S. anatum* and 4 strains of *S. pullorum*) and analyzed. It can be concluded from these results that the differences in dose response relations of strains belonging to the same serotype are not statistically significant. When the results for two different serotypes of the same species, *S. pullorum* and *S. meleagridis*, are compared, it can be concluded that there are considerable differences in the dose response relations. Whether such generalizations may be made for other organisms is a question that needs a lot more investigation. Many systematic studies aimed at comparing the infectivity of different strains of pathogenic microorganisms will be needed before any generalization has a firm scientific basis. In the meantime, accepted practice is to try and find a reasonably worst case estimate

for any factor to be estimated.
The second class of differences (differences in biological properties of the host) is of great significance when risk assessment is to be extended to the population level. When a population is exposed to some pathogenic microorganism, should protection then be aimed at an adult, fully immunocompetent, healthy (male) person, the typical subject in a controlled dose response experiment? We would like to know whether the dose response relation for infection changes with age, or in other conditions affecting the immune status of a person. Or could it be that it is not the probability of infection, that changes, but rather the probability of symptoms leading to illness? Experiments with subpopulations at risk (YOPI: young, old, pregnant, immunocompromized) will remain impossible, so that such questions will have to be approached in other ways. Possibly experiments with animals in different immune stages may provide useful information.
The third class of differences (differences in experimental conditions) comprises problems that may, at least in principle, be controlled. Often (but not always) original experimenters may still be contacted, to clarify circumstances not reported in sufficient detail. Enumeration methods may be different to an extent that they cannot be compared anymore, especially in experiments dealing with viruses. When accurate enumeration of the microorganisms is not possible, modification of the Beta–Poisson model to account for uncertainty in the dose may be necessary. Thereby, the uncertainty in the estimated risks would at least reflect the uncertainty in the experimental conditions more accurately.
In this report, only the volunteer studies of *Salmonella* strains offered the opportunity to compare dose response relations for different strains of a single serotype, and between different serotypes of the same species. To combine results from different species, in order to cluster different microorganisms into groups with comparable infectivity, would even be more difficult. Since the benefits from such a procedure would be obvious, this approach needs more detailed study.

Definitions

Attack rate Proportion of those exposed to an infectious agent who become (clinically) ill (Salyers and Whitt, 1994).

Colonization Ability of a microorganism to remain at a particular site and multiply there (Salyers and Whitt, 1994).

Infection Illness process resulting from multiplication and spreading of a microbial agent within a host (Benenson, 1990).

Successful colonization of a microorganism capable of causing damage to its host (Salyers and Whitt, 1994).

Infectious Capable of causing disease (Salyers and Whitt, 1994).

Infectious dose (ID50) Number of microorganisms required to cause infection in 50% of experimentally infected animals; a measure of infectivity (Salyers and Whitt, 1994).

Clinical infection Manifest infection, with clinical symptoms (Benenson, 1990)

Subclinical infection Inapparent infection, without symptoms (Benenson, 1990)

Latent infection Subclinical infection of chronic nature, with infection and host resistance balancing each other (Benenson, 1990).

Invasive Capable of:

1. penetrating the host's defenses;
2. entering host cells or passing through mucous membranes (Salyers and Whitt, 1994).

Case fatality rate Cumulative incidence of death among those who develop an illness (strictly spoken not a rate, but a proportion)(Salyers and Whitt, 1994).

Lethality The ratio of the number of fatalities of a certain disease to the number of illness cases, or the probability of dying from a specific disease (expressed as a percentage or promillage) (Benenson, 1990).

Morbidity The ratio of the number of illness cases to the size of the exposed population, often expressed in multiples of 100,000 individuals per year (Benenson, 1990).

Pathogenesis The process of generation and development of a disease (Benenson, 1990).

Pathogenicity Potential to elicit illness (Benenson, 1990).

Prevalence The number of (illness) cases at a certain point in time (Benenson, 1990).

Incidence The increase in the number of cases within a certain period of time (Benenson, 1990).

Virulence The degree of illness potential, expressed as LD50 and ID50 (Benenson, 1990).

Ability of an organism to cause disease (Salyers and Whitt, 1994).

Virulence factors Contribute to the potency of a micro-organism to cause illness in a host (Benenson, 1990).

Contribute to the ability of a microorganism to cause infection (Salyers and Whitt, 1994).

Organism	Symptom scored	Parameter(s)	Low Dose Approximation
Giardia lamblia	excretion	$\hat{r}$=1.99×10^{-2}	1.99×10^{-2}
Cryptosporidium parvum	excretion	$\hat{r}$=4.00×10^{-3}	4.00×10^{-3}
Entamoeba coli	excretion	$\hat{\alpha}$=0.106 $\hat{\beta}$=0.295	0.359

Table 7.1: Summarized dose response data for protozoan parasites.

Organism	Symptom scored	Parameter(s)	Low Dose Approximation
Rotavirus[a]	excr/seroconv	$\hat{\alpha}$=0.253 $\hat{\beta}$=0.422	0.60
Echovirus 12[b]	excr/seroconv	$\hat{\alpha}$=0.401 $\hat{\beta}$=227.2	1.76×10^{-3}
Poliovirus			
1 sm	excretion	$\hat{r}$=0.491	4.91×10^{-1}
1 LSc2ab	excretion	$\hat{\alpha}$=0.114 $\hat{\beta}$=159.0	7.2×10^{-4}
1	excretion	$\hat{r}$=9.1×10^{-3}	9.1×10^{-3}
3 Fox (infants)	excretion	$\hat{\alpha}$=0.533 $\hat{\beta}$=2.064	0.258
3 Fox (premat.)	excretion	$\hat{\alpha}$=0.299 $\hat{\beta}$=0.552	0.542

[a]Administered in pH-buffered solution

[b]Rejected at the 95% level, within 99% confidence range for the deviance from maximum possible likelihood.

Table 7.2: Summarized dose response data for viruses.

Summarized results: parameters

The dose response relations shown above, may be used in risk assessment. At low doses, the Exponential dose response curve may be approximated by:

$$P^*_{\text{inf}}(\mu) \approx r\mu$$

At low doses, a linear approximation may also be used for the Beta Poisson model (Teunis *et al.*, 1994):

$$P^*_{\text{inf}}(\mu) \approx \frac{\alpha}{\beta}\mu$$

So that the fraction α/β represents the proportionality constant at low doses, like r in the Exponential model. The slope of the dose response relation at low doses is given in the following summary, as the low dose approximation.

Organism	Symptom scored	Parameter(s)	Low Dose Approximation
Campylobacter			
jejuni A3249	excretion	$\hat{\alpha}$=0.145 $\hat{\beta}$=7.589	1.91×10^{-2}
Plesiomonas			
shigelloides[a]	excretion	$\hat{\alpha}$= 0.057 $\hat{\beta}$=1171	4.87×10^{-5}
Salmonella			
anatum 1+2+3	excretion	$\hat{\alpha}$=0.451 $\hat{\beta}$=15177	2.97×10^{-5}
meleagridis 1+2+3	excretion	$\hat{\alpha}$= 0.428 $\hat{\beta}$=8524	5.02×10^{-5}
newport	excretion	$\hat{r}= 3.97\times10^{-6}$	3.97×10^{-6}
bareilly[b]	excretion	$\hat{r}= 3.19\times10^{-6}$	3.19×10^{-6}
derby[b]	excretion	$\hat{r}= 2.19\times10^{-7}$	2.19×10^{-7}
pullorum 1+2+3+4	excretion	$\hat{r}= 3.48\times10^{-10}$	3.48×10^{-10}
typhi[c]	illness	$\hat{\alpha}$= 0.203 $\hat{\beta}$=29173	6.96×10^{-6}
Shigella			
flexneri 2a# #	illn	$\hat{\alpha}$=0.143 $\hat{\beta}$=284.3	5.03×10^{-4}
paradysenteriae[a]	excretion	$\hat{r}=8.26\times10^{-10}$	8.26×10^{-10}
dysenteriae			
M 131 + A–1	illness	$\hat{\alpha}$=0.157 $\hat{\beta}$=9.16	1.71×10^{-2}
Vibrio			
cholerae 569b	excretion	$\hat{r}= 1.76\times10^{-9}$	1.76×10^{-9}
cholerae 569b[a]	excretion	$\hat{\alpha}$=0.164 $\hat{\beta}$=0.136	1.21

[a]Administered in pH-buffered solution

[b]Rejected at the 95% level.

[c]Rejected at the 95% level, within a 97.5% confidence range for the deviance from maximum possible likelihood. Although the lognormal CDF provides superior fit (within 95% confidence range for the deviance), the Beta Poisson model gives the highest low dose estimate, hence it is suited best for risk assessment.

Table 7.3: Summarized dose response data for bacteria.

Organism	Symptom scored	$P^*_{inf}(1.0)$	ID_{50}
Giardia lamblia	excretion	1.97×10^{-2}	34.8
Cryptosporidium parvum	excretion	4.00×10^{-3}	173.1
Entamoeba coli	excretion	1.45×10^{-1}	203.8

Table 7.4: Infectivity of protozoan parasites analyzed in this report.

Summarized results: $P^*_{inf}(1.0)$ and ID_{50}

The infectivity of a pathogenic microorganism may also be expressed as the probability of becoming infected (or ill, in some cases), after exposure to a mean dose of 1.0 organisms. With known parameter values, these probabilities may be calculated directly from the dose response function:

$$P^*_{inf}(1.0) = 1 - e^{-r}$$

And, for the Beta Poisson relation:

$$P^*_{inf}(1.0) = 1 - \left(1 + \frac{1}{\beta}\right)^{-\alpha}$$

Finally, the infectivity of an organism is often expressed as the dose at which half of the exposed population exhibits a response, or the I(nfective)D(ose)50. Given a dose response function, calculation of the ID50 is straightforward:

$$ID_{50} = \frac{\ln 2}{r}$$

And, for the Beta Poisson relation:

$$ID_{50} = \beta\left(2^{\frac{1}{\alpha}} - 1\right)$$

Organism	Symptom scored	$P^*_{inf}(1.0)$	ID_{50}
Rotavirus[a]	excretion	2.65×10^{-1}	6.11
Echovirus 12[b]	excretion	1.76×10^{-3}	1.05×10^{3}
Poliovirus			
1 sm	excretion	3.88×10^{-1}	1.411
1 LSc2ab	excretion	7.14×10^{-4}	6.93×10^{4}
1	excretion	9.10×10^{-3}	76.2
3 Fox (infants)	excretion	1.90×10^{-1}	5.513
3 Fox (premat.)	excretion	2.66×10^{-1}	5.05

[a]Administered in pH-buffered solution

[b]Rejected at the 95% level, within 99% confidence range for the deviance from maximum possible likelihood.

Table 7.5: Infectivity of viruses analyzed in this report.

Organism	Symptom scored	$P^*_{inf}(1.0)$	ID_{50}
Campylobacter jejuni A3249	excretion	1.78×10^{-2}	8.97×10^{2}
Plesiomonas shigelloides[a]	excretion	4.87×10^{-5}	2.24×10^{8}
Salmonella			
anatum 1+2+3	excretion	2.97×10^{-5}	5.54×10^{4}
meleagridis 1+2+3	excretion	5.02×10^{-5}	3.45×10^{4}
newport	excretion	3.97×10^{-6}	1.75×10^{5}
bareilly[b]	excretion	3.19×10^{-6}	2.17×10^{5}
derby[b]	excretion	2.19×10^{-7}	3.17×10^{6}
pullorum 1–4	excretion	3.48×10^{-10}	1.99×10^{9}
typhi[c]	illness	6.96×10^{-6}	8.58×10^{5}
Shigella			
flexneri 2a# #	illness	5.02×10^{-4}	3.59×10^{4}
paradysenteriae[a]	excretion	8.26×10^{-10}	8.39×10^{8}
dysenteriae M 131 + A–1	illness	1.61×10^{-2}	7.48×10^{2}
Vibrio			
cholerae 569b	excretion	1.76×10^{-9}	3.94×10^{8}
cholerae 569b[a]	excretion	2.94×10^{-1}	9.18

[a]Administered in pH-buffered solution

[b]Rejected at the 95% level.

[c]Rejected at the 95% level, within a 97.5% confidence range for the deviance from maximum possible likelihood. Although the lognormal CDF provides superior fit (within 95% confidence range for the deviance), the Beta Poisson model gives the highest low dose estimate, hence it is suited best for risk assessment.

Table 7.6: Infectivity of bacteria analyzed in this report.

Bibliography

Bedaux JJM, Kooijman SALM. Statistical analysis of bioassays, based on hazard modeling. to appear in: Journal of Environmental and Ecological Statistics.

Benenson AS, editor. Control of communicable diseases in man. 1015 Fifteenth Street SW, Washington, D.C. 20005: American Public Health Association, 1990.

Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ. Experimental *Campylobacter jejuni* infection in humans. Journal of Infectious Diseases 1988; 157(3):472–479.

Caldwell MBP, Guerry EC, Lee EC, Burrans JP, Walker RI. Reversible expression of flagella in *Campylobacter jejuni* enteritis. Infection and Immunity 1985; 42:1176–1182.

Cash RA, Music SI, Libonati JP, Snyder MJ, Wenzel RP, Hornick RB. Response of man to infection with *Vibrio cholerae*. i. Clinical, serologic, and bacteriologic responses to a known inoculum. The Journal of Infectious Diseases 1974; 129(1):45–52.

DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubow ski W. The infectivity of *Cryptosporidium parvum* in healthy volunteers. The New England Journal of Medicine 1995;332(13):855–859.

DuPont HL, Hornick RB, Dawkins AT, Snyder MJ, Formal SB. The response of man to virulent *Shigella flexneri* 2a. The Journal of Infectious Diseases 1969; 119:296–299.

Evans DG, Satterwhite TK, Evans jr DJ, DuPont HL. Differences in serological responses and excretion patterns of volunteers challenged with enterotoxigenic *Escherichia coli* with and without the colonization factor antigen. Infection and Immunity 1978;19(3):883–888.

Furumoto WA, Mickey R. A mathematical model for the infectivity-dilution curve of tobacco mosaic virus: theoretical considerations. Virology 1967a;32:216–223.

Furumoto WA, Mickey R. A mathematical model for the infectivity-dilution curve of tobacco mosaic virus: experimental tests. Virology 1967b;32:224–233.

Gifford GE, Koch AL. The interferon dose-response curve and its possible significance. Journal of Theoretical Biology 1969;22:271–283.

Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK. Norwalk virus infection of volunteers: new insights based on improved assays. The Journal of Infectious Diseases 1994;170:34–43.

Haas CN. Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. American Journal of Epidemiology 1983; 118(4):573–582.

Haas CN, Rose JB. Reconciliation of microbial risk models and outbreak epidemiology: the case of the Milwaukee outbreak. 1994. Presented at the annual conference of the AWWA, New York City, june 19-23.

Haas CN, Rose JB, Gerba C, Regli S. Risk assessment of virus in drinking water. Risk Analysis 1993;13(5):545–552.

Herrington DA, Tzipori S, Robins-Browne RM, Tall BD, Levine MM. In vitro and in vivo pathogenicity of *Plesiomonas shigelloides*. Infection and Immunity 1987; 55(4):979–985.

Hoogenboom-Verdegaal AMM. Infectieziekten van het maag-darmkanaal. In: Ruwaard D, Kramers PGN, editors, Volksgezondheid Toekomst Verkenning. Den Haag: Sdu Uitgeverij, 1993; 217–220.

Hoogenboom-Verdegaal AMM, During M, Engels GB, Hoogenveen RT, Hoekstra JA, vand den Bosch DA, Kuyvenhoven JV, Mertens PLJM, Smidt IR. Een bevolkingsonderzoek naar maag/darmklachten in vier regio's van Nederland uitgevoerd in 1991. Deel 1. Onderzoeksmethodiek en incidentieberekening gastro–enteritis. National Institute of Public Health and the Environment, 1992 RIVM report 149101001.

Hornick RB, Greisman SE, Woodward TE, DuPont HL, Dawkins AT, Snyder MJ. Typhoid fever: pathogenesis and immunologic control. The New England Journal of Medicine 1970;283(13):686–691.

Katz M, Plotkin SA. Minimal infective dose of attenuated polio virus for man. American Journal of Public Health 1967;57(10):1837–1840.

Koprowski H. Immunization against poliomyelitis with living attenuated virus. American Journal of Tropical Medicine and Hygiene 1956;5:446–452.

Lepow ML, Warren RJ, Ingram VG, Daugherty SC, Robbins FC. Sabin type 1 (LSc2ab) oral poliomyelitis vaccine: effect of dose upon response of newborn infants. American Journal of Disease in Children 1962;104:67–71.

Levine MM, Black RE, Clements ML, Lanata C, Sears S, Honda T, Young CR, Finkelstein RA. Evaluation in humans of attenuated *Vibrio cholerae* El Tor Ogawa strain Texas Star-SR as a live oral vaccine. Infection and Immunity 1984; 43(2):515–522.

Levine MM, DuPont HL, Formal SB, Hornick RB, Takeuchi A, Gangarosa EJ, Snyder MJ, Libonati JP. Pathogenesis of *Shigella dysenteriae* 1 (shiga) dysentery. The Journal of Infectious Diseases 1973;127(3):261–270.

Levine MM, Kaper JB, Herrington D, Losonsky G, Morris JG, Clements ML, Black RE, Tall B, Hall R. Volunteer studies of deletion mutants of *Vibrio cholerae* O1 prepared by recombinant techniques. Infection and Immunity 1988;56(1):161–167.

Mathewson JJ, Johnson PC, DuPont HL, Satterwhite TK, Winsor DK. Pathogenicity of enteroadherent *Escherichia coli* in adult volunteers. The Journal of Infectious Diseases 1986;154(3):524–527.

McCullagh P, Nelder JA. Generalized linear models, volume 37 of Monographs on statistics and applied probability. Chapman and Hall, London, 1989.

McCullough NB, Wesley Eisele C. Experimental human salmonellosis. I. Pathogenicity of strains of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray-dried whole egg. Journal of Infectious Diseases 1951a;88:278–289.

McCullough NB, Wesley Eisele C. Experimental human salmonellosis. III. Pathogenicity of strains of *Salmonella newport*, *Salmonella derby*, and *Salmonella bareilly* obtained from spray-dried whole egg. Journal of Infectious Diseases 1951b;89:209–213.

McCullough NB, Wesley Eisele C. Experimental human salmonellosis. IV. Pathogenicity of strains of *Salmonella pullorum* obtained from spray-dried whole egg. Journal of Infectious Diseases 1951c;89:259–266.

Minor TE, Allen CI, Tsiatis AA, Nelson DB, d'Alessio DJ. Human infective dose determinations for oral poliovirus type 1 vaccine in infants. Journal of Clinical Microbiology 1981;13(2):388–389.

Plotkin SA, Koprowski H, Stokes J. Clinical trials in infants of orally administered attenuated poliomyelitis viruses. Pediatrics 1959;23:1041.

Regli S, Rose JB, Haas CN, Gerba CP. Modeling the risk from *Giardia* and viruses in drinking water. Journal of the AWWA 1991;83:76–84.

Rendtorff RC. The experimental transmission of human intestinal protozoan parasites 1. *Giardia lamblia* cysts given in capsules. American Journal of Hygiene 1954a;59:209.

Rendtorff RC. The experimental transmission of human intestinal protozoan parasites. i. *Endamoeba coli* cysts given in capsules. American Journal of Hygiene 1954b;59:196–208.

Rose JB, Gerba CP. Use of risk assessment for development of microbial standards. Water Science and Technology 1991;24(2):29–34.

Salyers AA, Whitt DD, editors. Bacterial pathogenesis, a molecular approach. Washington, D.C.: ASM press, 1994.

Schiff GM, Stefanović GM, Young EC, Sander DS, Pennekamp JK, Ward RL. Studies of Echovirus-12 in volunteers: determination of minimal infectious dose and the effect of previous infection on infectious dose. Journal of Infectious Diseases 1984;150(6):858.

Shaughnessy HJ, Olsson RC, Bass K, Friewer F, Levinson SO. Experimental human bacillary dysentry - polyvalent dysentry vaccine in its prevention. Journal of the American Medical Association 1946;132(7):362–368.

Teunis PFM, Havelaar AH, Medema GJ. A literature survey on the assessment of microbiological risk for drinking water. National Institute of Public Health and the Environment, 1994. RIVM report 734301006.

Ward RL, Bernstein DI, Young EC, Sherwood JR, Knowlton DR, Schiff GM. Human Rotavirus studies in volunteers: determination of infectious dose and serological response to infection. Journal of Infectious Diseases 1986;154(5):871–880.