Abstract

Dose-response models were developed for nineteen gastroenteritis-causing microbes. Three dose-response modeling equations were used: linear, linear two-population, and beta-Poisson. Models were fitted to experimental data from a variety of literature sources using a least sum of squares method to derive equation parameters. The deviances of each microbe's equations were calculated to identify those equations that were statistically valid. Of the fitted models, the beta-Poisson equation most frequently provided the best fit to the experimental data and was the only equation that could be fitted to data for 18 of the 19 microbes.

Acknowledgements

Special Thanks to Dr. Crawford-Brown for his guidance, Dr. Sobsey, Dr. Ball, Dr. Koren, The National Science Foundation's Ecology of Infectious Diseases Program, and the Wallace Genetic Foundation for the funding that supported this study.

Introduction

Gastroenteritis, a gastro-intestinal (GI) illness, is responsible for an estimated 3,000,000 deaths each year (37). Children in developing countries are among the most frequent fatalities of such illnesses. Many types of microbes, such as E. coli or Salmonella, are responsible for GI infections. These microbes are often found in human or animal fecal matter and can be found in most water systems. The discharge of untreated wastewater effluent or run-off of fecal matter into water systems can introduce higher concentrations of microbes capable of producing GI illnesses. In 1993, Milwaukee, WI experienced a dramatic increase in gastroenteritis cases after consuming contaminated drinking water. Approximately 400,000 people fell ill with gastroenteritis related to Cryptosporidium parvum during this event (30). Pathogenic microbes pose a threat to human health worldwide. The further study of these organisms to identify factors that increase infection rates and to identify points of health intervention is critical for improving public health.

Swimming in contaminated water and drinking from such sites are two of the most common sources of microbe ingestion. The Neuse River is an important source of drinking water in North Carolina. It also serves as a recreational site for many youth summer camps in the area. Children at these camps may experience an increased incidence of gastroenteritis from exposure to the water. It is expected that an increase in the number of GI cases could be observed during summer months because a high number of children attending these camps will be swimming in and ingesting water, incidentally while swimming, from the river. This increase would be due to both an increase in the

concentration of relevant microbes during summer months and an increase in the number of people swimming in the river.

A risk assessment of the GI health impacts associated with swimming in the Neuse River would include several steps: hazard identification, exposure assessment, dose-response assessment, and risk characterization. This process will be supported through the dose-response modeling conducted in this research. If the initial concentration of pathogenic microbes in the water and the size of the exposed population are known, it should be possible to estimate the number of GI cases that will result from these exposures. In this study, dose-response models were developed that may be used to estimate the probability of developing a GI illness given the ingestion rates that may be estimated through an exposure assessment. Three mathematical modeling equations were used and compared to observational data in order to select the best fitting model for use in a risk assessment.

Literature Review

The 5 pathogenic microbe genera included in this study are: Salmonella, Cryptosporidium parvum, E. coli, Giardia lamblia, and Campylobacter. These microbes are found in water supplies and fecal matter throughout the world and the Neuse River. Ingestion of these microbes can lead to the development of acute gastroenteritis and outbreaks of this illness occur worldwide in both industrialized and developing nations (16, 17, 20, 26). An understanding of the illness, its transmission and source organisms, and dose-response characteristics is vital to developing an effective response model.

Gastroenteritis

Gastroenteritis is an infection of the intestinal system that can be caused by a broad range of organisms. Exposure to certain viruses, bacteria, and protozoa can all lead to the development of this illness. Victims usually exhibit the same general symptoms: diarrhea, stomach pains, fever, nausea, and vomiting are typical. Intestinal cramping is common as is inflammation of the intestines (6). The secretion symptoms, such as vomiting or diarrhea, dehydrate the victim. In severe cases, especially those involving young children, the elderly, and the immuno-compromised, this can lead to death. The illness is infectious and is especially transmissible through fecal-oral routes. Many of the illness-causing organisms can also be transmitted through food, water, and contaminated equipment (6). Secondary infections frequently occur in health care workers and family members of victims (6). Different specific treatment measures may apply to cases caused by different organisms but, in general, re-hydration of the victim is a key component. Salmonella gastroenteritis, for example, appears to be treatable with ciprofloxacin, but not with amoxicillin (39). Erythromycin, on the other hand, may be an effective treatment for Campylobacter jejuni enteritis (39).

The economic and mortality effects of gastroenteritis make it a very important illness to study and control. The World Health Organization estimates that diarrhoeal diseases accounted for 13%, or approximately 1.4 million, of the deaths of children under the age of 5 worldwide (36). Overall, diarrhoeal diseases account for approximately 3,000,000 deaths each year worldwide and were the fourth leading cause of death in 1990 (37). Economic losses related to the illness are also high. In England alone between 2002 and 2003 it is estimated that healthcare related cases cost the country more than

$180 million (USD) (29). Liddle, et al. estimated that the cost of treating a child in Sydney, Australia with a case of rotavirus acute gastroenteritis was approximately $1700 (28). Hundreds of millions of dollars more are lost globally due to lost worker hours, lowered productivity, and occupation of hospital bed space.

Recreational Water Sources of Infection

Recreational swimming has been found to be a major source of infection by these microbes. According to Fayer, et al., recreational water sources of cryptosporidium infections alone have affected 10000 people in the U.S. over a 12 year period (11). Between 1999 and 2000, 36 outbreaks of gastroenteritis related to recreational water were documented in the U.S. (27). The months June through August accounted for approximately 86% of these outbreaks. The majority of these outbreaks occurred at swimming pools, but several occurred at lakes and ponds. A single outbreak related to a Minnesota lake resulted in 220 gastroenteritis cases (27). The combination of contamination by fecal matter and frequency of exposure make recreational water use a major source of gastroenteritis infection.

The EPA currently regulates the quality of recreational freshwater and local health departments regulate pool water quality. Pool water quality standards therefore differ by state. The federal standard for recreational freshwater, however, is <126 colony forming units (CFU) per 100 mL of water for E. coli and < 33 CFU per 100 mL of water for enterococci (46). Pools are the most commonly studied sources of recreational infection. Mathieu, et al. found that oral ingestion of contaminated pool water presented the highest risk of infection compared to other swimming-related exposure routes (31). Contamination with fecal matter appears to be a major source of these microbes. The

existence of fecal matter in recreational water increases both the concentration of these microbes and the difficulty of treating the water. For example, the amount of chlorine administered to sanitize contaminated water from cryptosporidium increases if fecal matter is present (4). A positive correlation between water quality, related to indicator bacteria concentrations, and gastroenteritis cases has been found by Pruss (40). Rivers are especially prone to having high fecal matter concentrations due to non-point sources of pollution. Animal farms, for example, have been identified as potential contributors to increased pathogenic bacteria levels in nearby surface waters (24). Kramer, et al. described the first known U.S. outbreak caused by cryptosporidium in a recreational lake in 1994. The researchers identified contaminated rain run-off as one of the chief contributors to pathogenic microbe concentrations in the lake (25). Factors such as these make recreational water a common source of infection by the organisms mentioned. As such it is important to develop methods of estimating, controlling, and mitigating the risk of illnesses developed as a result of interaction with recreational water.

Escherichia coli

E. coli is a well-known genus of gram-negative bacteria that is generally classified into four groups: enterotoxigenic, enteropathogenic, enteroinvasive, and enteroaggregative E. coli (39). Enterotoxigenic forms have been identified as the probable cause of most diarrhoeal illnesses associated with E. coli (5). Travelers' diarrhea is commonly associated with E. coli infections (7). Typical symptoms include diarrhea, cramps, and fever. Transmission usually occurs through the consumption of improperly prepared food or other fecal oral routes. Person to person transmission is rare,

but possible (8). Re-hydration is the most common treatment method but ciprofloxacin has been found to be effective in treating enterotoxigenic infections (39).

Campylobacter

Campylobacter is a genus of gram-negative bacteria that is spiral shaped. These organisms have one or two flagella that allow them some mobility. The intestinal illness associated with this organism is called Campylobacteriosis, a form of gastroenteritis. It is estimated that 99% of infections by a campylobacter organism are caused by the campylobacter jejuni species (12). Symptoms generally last for a week, but longer lasting cases have been identified (1, 23). As with the other organisms presented in this paper, the typical symptoms of infection are diarrhea, cramps, and fever. However, severe cases have been linked to the development of illnesses such as pancreatitis, gastrointestinal hemorrhaging, and Guillain-Barré syndrome (1). Blaser, et al., have suggested that campylobacter is more common than salmonella or shigella as a cause of gastrointestinal illnesses (3). The unsafe handling and consumption of chicken has been identified as the dominant transmission pathway for this species (21). However, transmission through liquid consumption, including water, has also been identified (35). Person to person transmission is rare, but can occur through fecal oral routes, such as improper hand washing by food handlers. Re-hydration is the most common treatment for campylobacter-related gastroenteritis.

Giardia lamblia

Giardia lamblia is one of the two protozoa included in this study. This flagellate exists in trophozoite and cyst forms. According to the review by Furness, et al. of U.S. data from the CDC, Giardia is the most commonly laboratory-identified parasite in the

country (13). Giardiasis is the form of gastroenteritis associated with Giardia lamblia, also known as Giardia intestinalis. The cyst form is the more environmentally resistant form and can survive in the environment for months. Symptoms, if they occur, are usually noticeable after a one week incubation period and may last for several weeks (22). The typical symptoms are diarrhea, cramps, fever, nausea, and vomiting (22). Ingestion of Giardia cysts is the main form of transmission and usually occurs through fecal oral routes involving improperly handled food. Giardia has been found to be transmissible through ingestion of contaminated water during recreational swimming (46). The infectious dose of this organism is estimated to be as low as 10 cysts (13, 38, 44). In addition to re-hydration, treatment with tinidazole and quinacrine has proven to be effective (39).

Cryptosporidium parvum

The second parasite involved in this study is cryptosporidium parvum, a parasitic protozoan. It also exists in several life cycle stages, but the oocyst stage is the only form known to exist outside of a host. The form of gastroenteritis associated with this protozoan is Cryptosporidiosis, but should not be confused with Cryptococcosis, a pulmonary infection caused by Cryptococcus neoformans. According to CDC surveillance data, cryptosporidium is the most commonly identified cause of gastroenteritis associated with swimming pools due to its strong resistance to chlorine treatment (46). However it has also been identified in cases associated with fresh-water swimming (46). Typical symptoms include diarrhea and vomiting as well as occasional anorexia (6). Transmission usually occurs through fecal oral routes and transmission through both contaminated food and water has been identified (6). As with Giardia, this

protozoan is highly infectious and the infectious dose may be as low as 30 oocysts (18). Antibiotic treatment of cryptosporidium associated illnesses has mostly been unsuccessful, but new data suggest nitazoxanide may be effective (43, 46).

Salmonella

Salmonella is a genus of gram-negative rod-shaped bacteria. The form of gastroenteritis commonly associated with these bacteria is salmonellosis. Salmonella typhi, the source of typhoid fever, is not included as one of the gastroenteritis-causing organisms in this study. Nearly 2000 serotypes of this bacterium have been identified as causing human illnesses (42). The illness usually becomes apparent after a short incubation period of a few days and lasts for approximately a week (39). Diarrhea, fever, and cramps are common symptoms. Some patients, especially young children and immuno-compromised victims, have an increased risk for illnesses such as meningitis and osteomyelitis (39). Transmission commonly occurs through fecal oral routes and includes consumption of contaminated food or water. Consumption and handling of contaminated eggs and poultry are widely known as sources of salmonella-related illnesses. However, illnesses caused by ingesting contaminated water appear rare. From 1999 to 2000, for example, the U.S. only experienced one documented outbreak of salmonella-related gastroenteritis (27). This outbreak was associated with contaminated drinking water. Re-hydration remains an important component of treatment. The use of ciprofloxacin appears to be an effective antibiotic treatment option (39).

Dose-Response Assessment

Once average doses are determined, a mathematical dose-response assessment can be conducted. Dose-response assessments are necessary to extrapolate experimental

response data to responses from lower doses and likely environmental concentrations. The assessment in this study will attempt to establish the relationship between the number of organisms or CFU that a person is exposed to and the probability of development of an illness or infection. Due to lack of complete response data for each of the relevant microbes, this study will mainly focus on infection as the response. For the purposes of this study infection is defined as the lab-detectable presence of the relevant microbe in a study participant after exposure. Illness is marked by the development of clinical symptoms of gastroenteritis.

There are several mathematical models that can be used in dose-response assessments; the inability to select from amongst them introduces uncertainty into a dose-response assessment. The equations used in the current assessment are: linear one-population, linear two-population, and beta-Poisson. These equations all differ in their strengths and weaknesses. A more in-depth discussion of these attributes can be found in the Methods section of this study. All three equations will be used to attempt to find the best fit to the data referenced in this study and to characterize the uncertainty in model choice.

Knowledge Gaps

There are only a few studies in which exposure and dose-response data from these microbes have been successfully collected for use with these models, especially related to recreational water exposure. However, a 1996 report for the Netherlands compiled the dose response data for different microbes that attack the gastro-intestinal system (45). This report referenced information from other studies from several nations in order to develop a model for each microbe. The beta-Poisson and exponential models were used

in its modeling so the report did not provide parameters of the other models considered here.

Several of the microbes have limited data sets. Other studies only included fewer than 20 total people exposed. In these cases, a dose level would often only have two exposed individuals to study, thereby decreasing the statistical validity of any results (45). A wide range of doses has not been studied in several cases, for example E. coli CFA+/-. In the reports included in this current study, only three dose levels were examined for these two strains and all three doses were sufficiently high to cause infection in every patient. Larger, more complete data sets are needed in order to provide more accurate predictive models. These limitations should be kept in mind while reviewing the data and results presented below.

Data

The data collected for this study were found in a variety of literature sources. Many of these sources were also cited in a report prepared by the Netherlands' Ministry of Public Health, Welfare, and Sports (45). That report focused on dose-response relationships for gastroenteritis-causing pathogens and attempted to derive beta-Poisson parameters from the data found in each study. The majority of the beta-Poisson parameters used at the beginning of this present study were gathered from the Netherlands' report (45). It is important to note that due to the limited nature of the data available, the dose-response relationships developed later in this study focus on infection, not illness as the response.

Cryptosporidium parvum

Data on the dose-response relationship for Cryptosporidium parvum were gathered from DuPont, et al (9). Subjects were given an intended dose ranging from 30 to 1 million oocysts. Table 1.1 summarizes the data extracted from the DuPont study. Quantal infection outcomes occurred in at least one volunteer at each dose level and clearly increased with dose in this experiment. However, symptoms, the key indicators of illness, were not observed at all levels nor developed in all of the volunteers exposed to higher dose levels (9). No beta-Poisson parameters were extracted from the Netherlands' study, which derived exponential parameters instead (45).

Giardia lamblia

Giardia lamblia data were collected from Rendtorff's study of the species' cysts in humans (41). Doses ranging from 1 to 1 million cysts were delivered to a total of 40 volunteers. Rendtorff's data indicate that the infectious dose may be very low for this species. Every volunteer who consumed 100 or more cysts became infected. However, none of the volunteers became ill. Table 1.2 summarizes the data collected from Rendtorff's study. As with Cryptosporidium parvum, no beta-Poisson parameters were available from the Netherlands' study (45).

Table 1.1 Experimental Dose-Response Data for C. parvum (9)		
Dose (oocysts)	**# Subjects Exposed**	**# Subjects Infected**
34	5	1
108	8	3
313	3	2
504	6	5
1129	2	2
11460	3	3
113900	1	1
1139000	1	1
Results of the DuPont, et al. C. parvum dose response experiment (9). Dose: ingested # of oocysts. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.		

Table 1.2 Experimental Dose-Response Data for Giardia lamblia (41)		
Dose (cysts)	**# Subjects Exposed**	**# Subjects Infected**
1	5	0
10	2	2
25	20	6
100	2	2
10000	3	3
100000	3	3
310000	3	3
1000000	2	2
Results of Rendtorff's G. lamblia dose response experiment (41). Dose: ingested # of cysts. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.		

Campylobacter jejuni

Campylobacter jejuni data for both the A3249 and 81-176 strains were found in the same study by Black, et al. (2). Black et al. examined 72 volunteers exposed to the A3249 strain and 39 volunteers exposed to the 81-176 strain. Doses ranged from 810 to 2.1 billion organisms (2). The A3249 strain was less infectious than 81-176 and allowed for a better fitting derivation of exposure-response equation parameters. The 81-176 strain caused an infection to develop for all volunteers at each dose level. Therefore, illness data were used to derive the exposure-response parameters in this study. This makes it difficult to compare the 81-176 strain results to other microbes' results. However, since all the data used in the equation parameter derivations were illness data, these results are valid for modeling risk of illness for the 81-176 strain. Tables 1.3 and 1.4 display the data collected from Black, et al. and the Netherlands report used in this study. Only beta-Poisson parameters for the A3249 strain were extracted from the Netherlands' study (45).

Table 1.3 Experimental Dose-Response Data for Campylobacter jejuni A3249 (2)		
Dose (organisms)	**# Subjects Exposed**	**# Subjects Infected**
810	10	5
8100	10	6
91000	13	11
810000	11	8
1100000	19	15
110000000	5	5
Results of the Black, et al. C. Jejuni A3249 dose response experiment (2). Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.		

Table 1.4 Experimental Dose-Response Data for Campylobacter jejuni 81-176 (2)		
Dose (organisms)	**# Subjects Exposed**	**# Subjects Infected**
1100000	7	3
210000000	10	6
2100000000	22	9
Results of the Black, et al. C. Jejuni 81-176 dose response experiment (2). Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.		

Escherichia coli

Two strains of Escherichia coli, H10407 (O78:H11) CFA+ and H10407 P CFA-, were examined in this study. Both of these strains were studied by Evans et al. in 1978 (10). Doses ranged from 1 million to 1.2 billion organisms. Data was available on 24 volunteers exposed to CFA+ and 23 volunteers exposed to CFA- volunteers. Tables 1.5 and 1.6 list the data collected from the Evans, et al. experiment. As with the 81-176 Campylobacter strain, all volunteers at all dose levels became infected. Therefore, data for the development of illness were used in this study with the exception of CFA-. In this case, the illness data could also not be used because either all or none of the volunteers became ill at each dose level. No beta-Poisson parameters were collected from the Netherlands' study (45).

Table 1.5 Experimental Dose-Response Data for E. coli CFA+ (10)		
Dose (organisms)	**# Subjects Exposed**	**# Subjects Ill**
1000000	7	1
100000000	7	6
1200000000	10	9
Results of the Evans, et al. E. Coli CFA+ dose response experiment (10). Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be ill after exposure to the corresponding dose.		

Table 1.6 Experimental Dose-Response Data for E. coli CFA- (10)		
Dose (organisms)	**# Subjects Exposed**	**# Subjects Infected**
1000000	7	7
100000000	6	6
1200000000	10	10
Results of the Evans, et al. E. Coli CFA- dose response experiment (10). Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.		

Salmonella meleagridis

Data from thirteen Salmonella species or strains were researched for this study. Three strains of Salmonella meleagridis were examined: I, II, and III. A 1951 study by McCullough, et al. exposed more than 64 people to the three species (32). Doses ranged from 12,000 to 50 million organisms (32). The experiment used healthy male prisoners as the test subjects and exposed them to bacterial organisms extracted from dried eggs. Dose-response relationships could be determined for strains I and III based on infection data. Strain II, however, required the use of illness data because all subjects became infected at all dose levels. Beta-Poisson parameters were available from the Netherlands study for strains I and III. The parameters for strain II were used from the Netherlands' estimates of the parameters for all three strains combined (45). These three parameter sets were used as starting values in the parameter derivation process in this present report. Table 1.7 lists the data used from the McCullough experiment and the Netherlands report.

Table 1.7 Experimental Dose-Response Data for S. meleagridis I, II, and III (32)			
Strain	**Dose (organisms)**	**# Exposed**	**# Infected**
I	12000	6	3
	24000	6	3
	52000	6	3
	96000	6	3
	155000	6	5
	300000	6	6
	720000	5	4
	1145000	6	6
	5500000	6	5
	24000000	5	5
	50000000	6	6
II	1000000	6	0
	5500000	6	0
	10000000	6	1
	20000000	6	2
	41000000	6	5
III	158000	6	1
	1500000	6	5
	7675000	6	6
	10000000	6	5

Results of the McCullough, et al. S. meleagridis dose response experiment (32). Strain: tested strain #; I, II, or III. Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.

Salmonella anatum

McCullough and his research group also examined the dose-response relationship of three Salmonella anatum strains (32). This experiment was published in the same paper as the meleagridis study and also used healthy male prisoners as the test subjects. Doses again ranged from 12,000 to 67 million organisms (32). Adequate infection data existed for use in the modeling process for each strain. Beta-Poisson parameters were available for all three strains from the Netherlands report (45). A summary of the anatum data used from the McCullough experiment and the Netherlands report is available in Table 1.8.

Table 1.8 Experimental Dose-Response Data for S. anatum I, II, and III (32)			
Strain	**Dose (organisms)**	**# Exposed**	**# Infected**
I	12000	5	2
	24000	6	3
	66000	6	4
	93000	6	1
	141000	6	3
	256000	6	5
	587000	5	4
	8600000	6	6
II	89000	6	5
	448000	6	4
	1000000	6	6
	3900000	6	4
	10000000	6	6
	23900000	6	6
	44500000	6	6
	67200000	8	8
III	159000	6	2
	1250000	6	6
	4670000	6	6

Results of the McCullough, et al. S. anatum dose response experiment (32). Strain: tested strain #; I, II, or III. Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.

Salmonella pullorum

Salmonella pullorum has four strains that were studied in 1951 by McCullough, et al. in a separate report from the meleagridis and anatum report (34). This experiment was structured in the same manner as the two mentioned above and used the same subject pool. Doses ranged from 10,000 to 16 billion organisms. Infections and illnesses were only observed at the higher dose levels of at least 1 billion organisms ingested (34). This made it difficult to determine parameters for the various dose-response models. Strain II required the use of illness data in the equation fitting process. No beta-Poisson parameters were used from the Netherlands report (45). Table 1.9 summarizes the data used from McCullough experiment.

Table 1.9 Experimental Dose-Response Data for S. pullorum I, II, III, and IV (34)

Strain	Dose (organisms)	# Subjects Exposed	# Subjects Infected (II III)
I	10000	6	0
	1790000000	6	0
	10000000000	6	6
	16000000000	6	3
II	1380000	6	0
	163000000	6	0
	6750000000	5	4
III	2300000	6	0
	93000000	6	0
	1200000000	6	0
	7600000000	6	6
IV	188000	6	0
	13900000	6	0
	110000000	6	0
	1280000000	6	1
	3975000000	6	6

Results of the McCullough, et al. S. pullorum dose response experiment (34). Strain: tested strain #; I, II, III, or IV. Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.

Salmonella newport, derby, and bareilly

McCullough's group studied another three species of Salmonella in 1951 (33). These three species are Salmonella newport, S. derby, and S. bareilly. This experiment was conducted in the same manner as his other 1951 studies. Doses ranged from 125,000 to 15 million organisms (33). The newport and bareilly species caused infection in nearly all subjects at each dose level, but still allowed the equations to be fitted. The Netherlands report did not provide any beta-Poisson parameters (45). Table 1.10 summarizes the McCullough data.

Table 1.10 Experimental Dose Response Data for S. Newport, derby, and bareilly (33)

Microbe	Dose (organisms)	# Subjects Exposed	# Subjects Infected
S. newport	152000	6	3
	385000	8	6
	1350000	6	6
S. bareilly	125000	6	5
	695000	6	6
	1700000	6	5
S. derby	138000	6	3
	700000	6	4
	1600000	6	4
	6400000	6	3
	15000000	6	4

Results of the McCullough, et al. S. newport, S. bareilly, and S. derby dose response experiment (33). Microbe: tested species; newport, bareilly, or derby. Dose: ingested # of organisms. Exposed: # of study subjects exposed to the corresponding dose. Infected: # of study subjects determined to be infected after exposure to the corresponding dose.

Methods

All exposure-response modeling was performed using Microsoft Excel. Each microbe species was modeled on separate Excel spreadsheets, but variations of the same species, such as Salmonella Anatum I and II, were modeled on the same page, but with separate data. Each microbe was examined using three different dose-response equations. The parameters of each equation were derived through the macro Excel Solver using a relative least squares procedure. The tested parameters were used in the relevant equation to produce a predicted probability of the development of an infection. This predicted probability was compared to the experimental probability found in referenced literature. The best fitting parameters were selected using the relative least squared method. This value is represented as S in this study. In addition, the deviance between the maximum likelihood for a model and the maximum possible likelihood estimates was used to determine the accuracy of the fit of each derived-parameter model. This deviance value is listed as D in this study. The lower the relevant S and D values, the more accurate and statistically valid an equation is considered. In the cases of several microbes, some equation parameters could not be statistically accepted and, therefore, these microbes may only have one or two working models in the spreadsheet as opposed to three.

Linear One-Population Model

The linear one population equation is based on the assumption that the likelihood of a result, in this case infection, increases linearly with dose at low doses, measured here in number of organisms. This equation is written as:

$$1.\ P(\text{in}) = 1 - e^{(-K * D)}$$

where K is the slope factor of the dose-response relationship and D is the dose received. The linear one population equation only has one parameter to derive, K. The equation to derive the value of K from one dose-response level is:

$$2.\ K = \ln(1 - P(in)) / D$$

However, this does not necessarily produce the correct value of K, but only produces the value of K that is supported by the data for that particular dose-response level. A more accurate value of K was determined through a comparison between the predicted and experimental probabilities over the full dose range. The differences between these predicted probabilities and their respective experimental probabilities were squared, summed, and divided by their respective experimental probabilities. The equation for this process is:

$$3.\ \text{Sum of relative squares (S)} = \sum ((\text{Experimental} - \text{Calculated})^2 / \text{Experimental})$$

This produced a sum of the relative squares of the differences between the calculated and experimental probabilities. In theory, the value of K that produces the lowest sum of these relative squares should be the most accurate value that can be derived given the experimental data. Excel's Solver function was used with the sum of the relative squares set as the target, or outcome, cell and the value of K set as the changing cell. A K value was derived by determining the minimum value of the target cell. In some instances, however, the sum of the relative squares could not be used to derive K. Many of the exposure-response data levels included experimental probabilities of 0. When this value is used in the relative squares equation in Excel it produces an error. This is because of the mathematical nature of any number divided by 0:

$$4.\ \lim_{(x \to 0)} (Y / X) = \infty$$

Therefore, in instances where experimental probabilities of 0 were present, a least sum of squares, as opposed to a relative least sum of squares, was used to determine the value of K. This formula is written as:

5. Sum of squares (S) = $\sum$ (Experimental – Calculated)^2

By doing so, the problem of approaching a limit of infinity was avoided. Appendix A lists the microbes whose K values were determined using least squared and relative least squared methods, respectively, along with the K values and sum of squares for each microbe.

Linear Two-Population Model

The linear two-population model is based on the same formula as the linear one-population model. However, it accounts for some differences in susceptibility in the population. For example, assume that one half of the population is highly susceptible to infection by cryptosporidium parvum (K = 100 for this example), but the other half of the population is nearly completely immune (K = 0.0000000001). The linear one-population model would not properly fit a K value to any experimental data. Instead, it would roughly average the two real K values in the population. The linear two-population equation takes the sum of the two probabilities of infection based on the two possible K values to find the total probability of infection in a population. This formula is:

6. $P(in) = F_1 * (1 - e^{(-K1* D)}) + F_2 (1 - e^{(-K2* D)})$

where K_1 and K_2 are the two different possible K values. F_1 and F_2 are the fractions of the population that are associated with the two K values, respectively.

As with the linear one-population models, the K value used in each two-population model was derived using either the least sum of squares or relative least sum

of squares. The same microbes that required the use of the non-relative least sum of squares in the one-population models also required the same technique in the two-population model.

beta-Poisson Model

The beta-Poisson is the most mathematically flexible model used in this study. According to Haas, the beta-Poisson model was originally used for human-microbial assessments by Furumoto and Mickey in 1967 (14, 15, 19). The flexibility of the model often allows it to provide a better fit to data. In human health assessments, it also allows for variability in responses and "diversity in pathogen competence" (19). The linear models are not able to allow for this variability to as great a degree. The beta-Poisson equation, as used in this study, is:

$$7.\ P(in) = 1 - (1 + (dose / beta))^{-alpha}$$

Beta can also be described through the median infectious dose, also known as the N_{50} dose, where 50% of subjects exposed become infected. When modified in this manner, the equation can be re-written as:

$$8.\ P(in) = 1 - (1 + (dose / N_{50}) * (2^{1/alpha} - 1))^{-alpha}$$

The first version, equation 7, of the beta-Poisson equation was used in the Netherlands report and was also used in this present study. This provided an easy basis for comparison between the parameters derived here and those derived in other studies. It was important to perform this comparison in order to provide some validity to the procedures used in this report. Appendix A lists the parameters derived for each microbe.

Deviation

Whereas the least squares criteria was used to determine the best fitting parameter values for each microbe's equations, a measure of the deviance was used to determine the statistical validity of each fitted equation. In other words, the least squares method was used to minimize the difference between predicted and experimental values while the deviance method was used to determine if the remaining difference was small enough to be considered statistically insignificant. The "goodness of fit" of each equation with derived parameters was measured by the deviance between the maximum likelihood and maximum possible likelihood estimates. The method used here, also used in the Netherlands report, was based on the method reported in Haas, et al (19, 45). The difference between the two likelihood estimates was compared to the corresponding X^2 value at the relevant level of degrees of freedom. All Deviances (D) were compared at the 95% confidence level ($p = 0.05$). If D was greater than the corresponding X^2 value, then the model was considered inaccurate and rejected. This means that a model was retained only if the researcher could be 95% confident that the difference between the predicted probabilities and the experimental results is statistically insignificant. The formula for the maximum likelihood estimate is (38):

$$9.\ l = -2^* \sum[p_i * \log (P_{in}) + (n_i - p_i) * \log (1 - P_{in})]$$

where p_i is the number of subjects infected at dosage i, n_i is the number of subjects exposed at dosage i, and P_{in} is the calculated probability of infection at dosage i. The formula for the maximum possible likelihood estimate is (38):

$$10.\ l_{pos} = -2^* \sum[p_i * \log (p_i / n_i) + (n_i - p_i) * \log ((n_i - p_i)/n_i)]$$

where p_i is the number of subjects infected at dosage I and n_i is the number of subjects exposed at dosage i. Any case in which n_i or p_i equaled 0 or where a log (0) was produced, that portion of the equation was assigned a value of 0. The Deviance, D, is the difference between l and l_{pos}:

$$11.\ D = l - l_{pos}$$

DF, the degrees of freedom, associated with each case was dependent on the type of equation being compared and the number of experimental dose levels, i, for each microbe. The linear equation has 1 unknown parameter, the two-population equation has 3 unknown parameters, and the beta-Poisson equation has 2 unknown parameters. The DF equation is then:

$$12.\ DF = i - x$$

where i is the number of experimental dose levels and x is the number of unknown parameters in the relevant equation. The X^2 value at the corresponding level of DF can be determined by using a statistical table. If $D > X^2$, which indicated that statistically significant differences existed between the likelihood values, then the equation was considered inaccurate and rejected.

Results

Equation parameters, sum of squared differences, and equation fit results are summarized below for each microbe. These results are also available in Appendix A. Parameters were derived for each equation for every microbe with the exception of one case noted below where parameters from related strains were used. In some cases, certain equation models could not be accepted as accurate based on the deviance criteria. Most often this occurred with the linear two-population model, which always had the

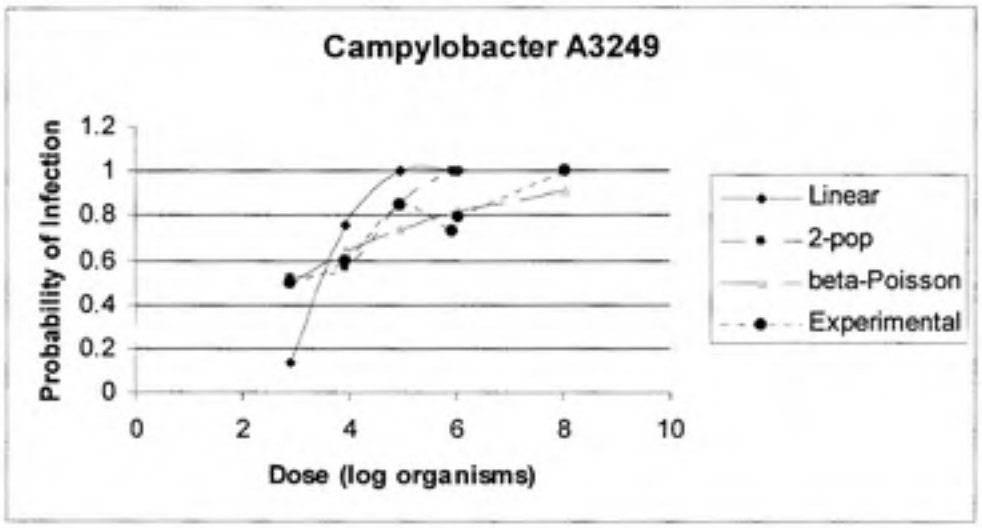
lowest DF value. A lower DF value requires a lower relevant D value in order for a model to be accepted. It is possible for the linear two-population model to reduce down to the linear model. In instances where this occurred, only the linear model was reported as accepted. However, every microbe has at least one accepted equation fitted to the data. At the beginning of this study it was expected that the beta-Poisson equation would produce the most accurate fit to the data. This proved to be the case for 10 of the 19 microbes studied.

Campylobacter

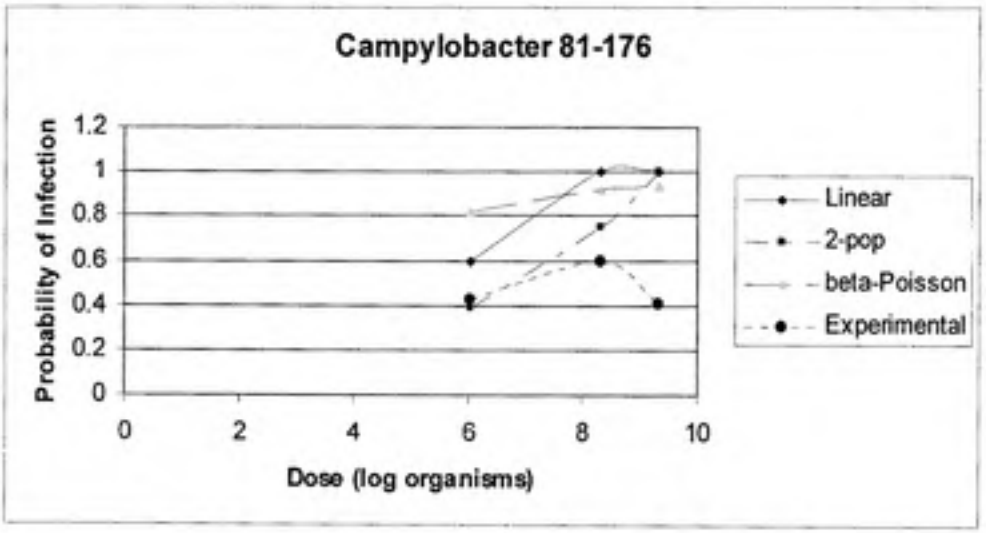
Only one equation could be accepted as fitted to the Campylobacter A3249 data. It was difficult for any of the equations to be very accurately fitted to the experimental data because the probability of infection did not always appear to rise monotonically with dose in the experimental data (2). The beta-Poisson equation offered the lowest sum of squares (S) of 0.035 and was followed by the linear two-population equation with an S of 0.161. A better fitting of the beta-Poisson equation (S = 0.035) was achieved using the previously described least squared method of this study. The alpha value in this case was still 0.145, as found in the Netherlands report (45). The beta value changed to 7.759, a slight increase over the Netherlands' value. The beta-Poisson equation was the only one statistically acceptable with D = 1.05. Campylobacter 81-176 had the lowest sum of squares with an S of 0.897 with the linear two-population model. As with the A3249 strain, none of the parameters could be fitted better because probability of infection did not rise with dose in the experimental data. Beta-Poisson parameters could not be derived from the data so the parameters from the A3249 strain were used. This resulted in the worst parameter fit of the three models with an S of 1.22, but was not drastically

worse than the linear model, with an S of 1.19. However, the linear equation was the only one found to be statistically acceptable with D = -18.41. With the exception of the first strain's beta-Poisson results, the parameters derived for both Campylobacter strains were among the least accurate in this study and had S values that were often several magnitudes greater than those associated with the other microbes. The D values of the rejected equations were also quite large.

Graph 3.1 Campylobacter A3249 Dose-Response Curves



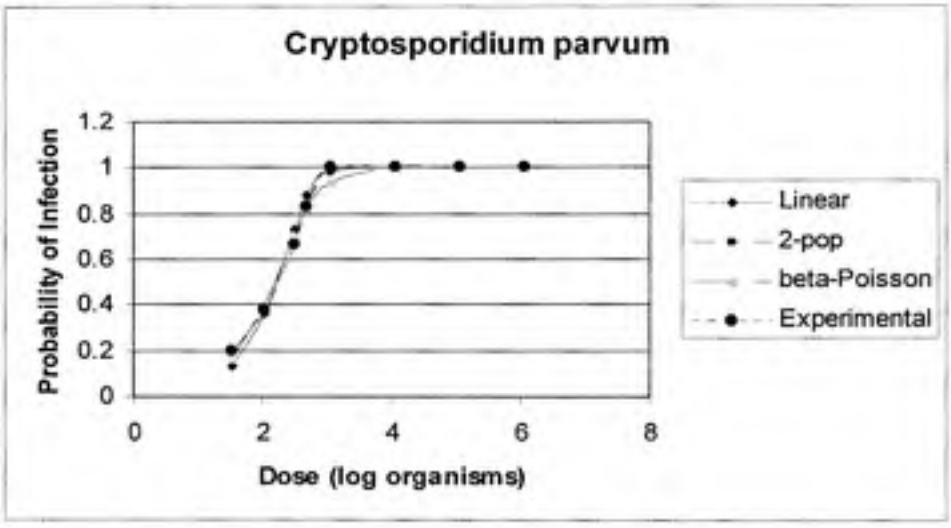
Graph 3.2 Campylobacter 81-176 Dose-Response Curves



Cryptosporidium parvum

Cryptosporidium parvum's experimental data allowed for an accurate fitting of the equations. Visual comparisons of Graphs 3.1, 3.2, and 3.3 show that the equations in Graph 3.3 are far more accurate than those in the other two graphs. The linear two-population equation was the best fitting with an S value of 7.6 E-4 and had D = 0.042. This was one of the lowest S values calculated for any of the equations for any of the microbes in this study. The two-population equation's S value was more than 200 times smaller, a large difference, than the S value of Campylobacter A3249. The beta-Poisson equation was more accurate than the linear equation, but both had S and D values on the same order of magnitude, 0.0155 and 0.163 versus 0.0316 and 0.167 respectively.

Graph 3.3 Cryptosporidium parvum Dose-Response Curves

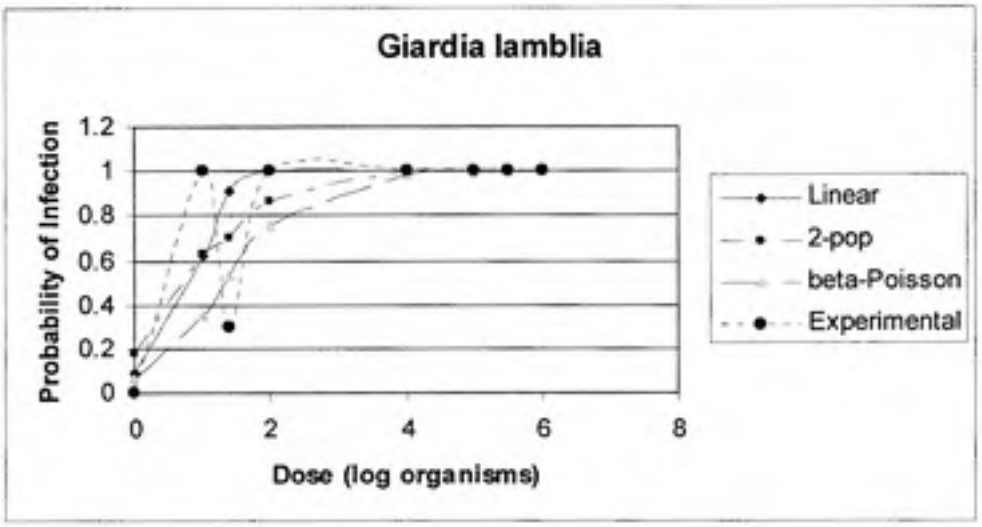


Giardia lamblia

Probability of infection did not increase monotonically with dose in the experimental data for Giardia lamblia and included a drop from P=1 to P=0.3 followed by an increase back to P=1 (41). Therefore the S and D values for all three equations were closer in magnitude to those of the Campylobacter strains. Again, the linear two-

population equation had the best fit, S = 0.35 and D = 8.02. The linear equation's S value was 0.52 and was very closely followed by the beta-Poisson equation, S=0.54. However, the linear equation was rejected based on deviance, D = 20.88. Based on comparisons to the experimental data, the three equations model probabilities associated with higher dose levels more effectively than at lower dose levels.

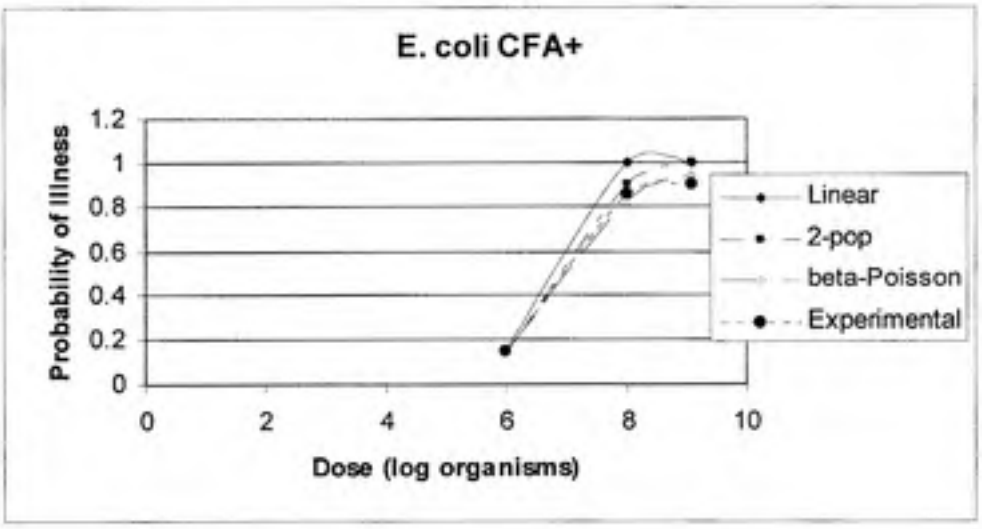
Graph 3.4 Giardia lamblia Dose-Response Curves



Escherichia coli

Beta-Poisson equations could not be fitted to either E. coli strain in the Netherlands report (45). However, the parameters for this equation were derived for CFA+ in this study. The CFA+ strain's only acceptable fit was with the beta-Poisson equation with an S of 0.0035 and a D of 0.18. CFA-'s data could not be used to fit any equations because so little data were available. All three S values from the CFA+ strain were less than 0.04, but only the beta-Poisson equations was found to be statistically acceptable.

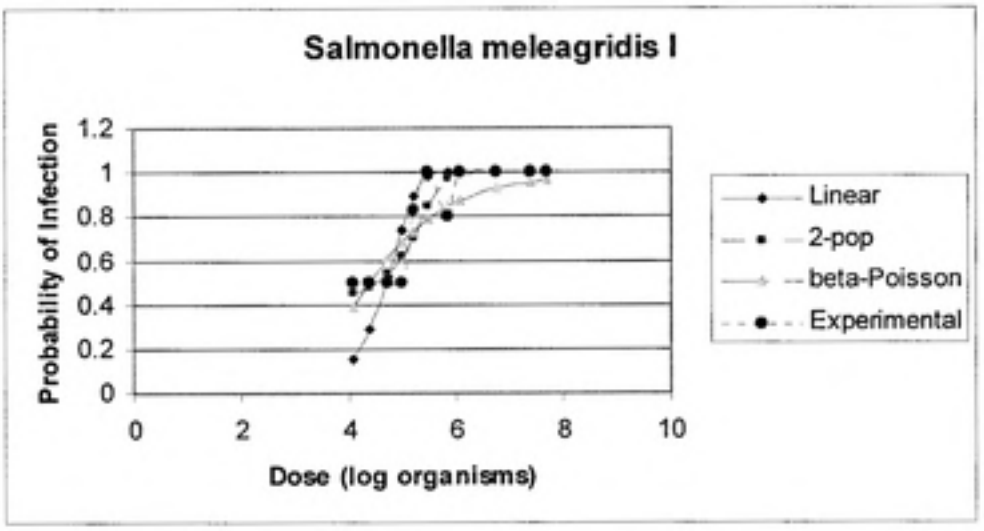
Graph 3.5 E. coli CFA+ Dose-Response Curves



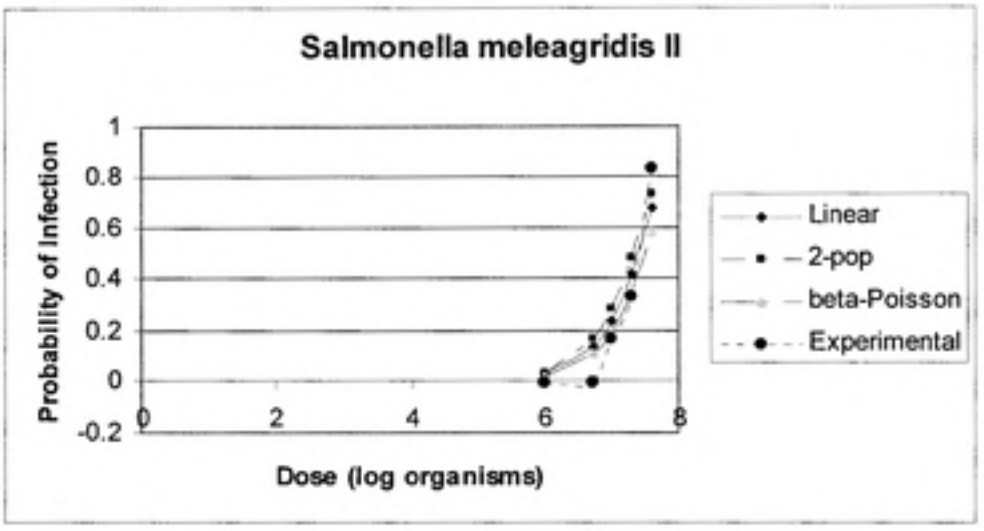
Salmonella meleagridis

Parameters could be derived for each equation for strains I, II, and III of Salmonella meleagridis, but the two-population equation was not statistically valid for strains I and III. The linear model was also rejected for strain III. For strains I and III, the beta-Poisson equation provided the most accurate fit (S = 0.2, D = 3.38 and S = 0.06 D = 1.04), but the linear equation provided the best fit for strain II. Originally a pooled beta-Poisson estimate was used from the Netherlands report because it was reported that no parameters were derived for this strain (45). However, the parameters were derived here and appear to produce a reasonably accurate fit. These two parameters are much larger than the corresponding parameters for the other strains, so a more in-depth dose-response study may be needed to determine their accuracy. The best fitting models for each strain were among the most accurate in this study. Much of the remaining inaccuracy is due to the fact that the probability of infection did not always increase monotonically with dose in the experimental data (32).

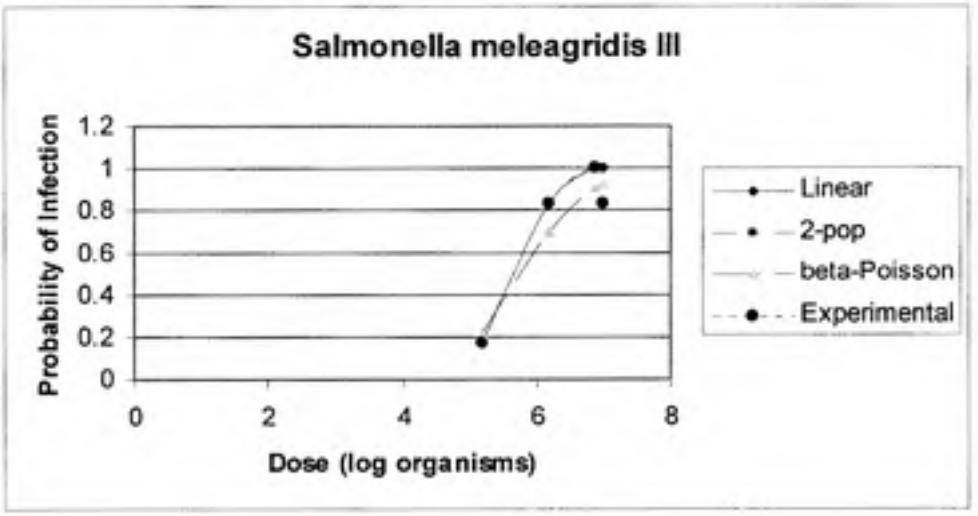
Graph 3.6 Salmonella meleagridis I Dose-Response Curves



Graph 3.7 Salmonella meleagridis II Dose-Response Curves



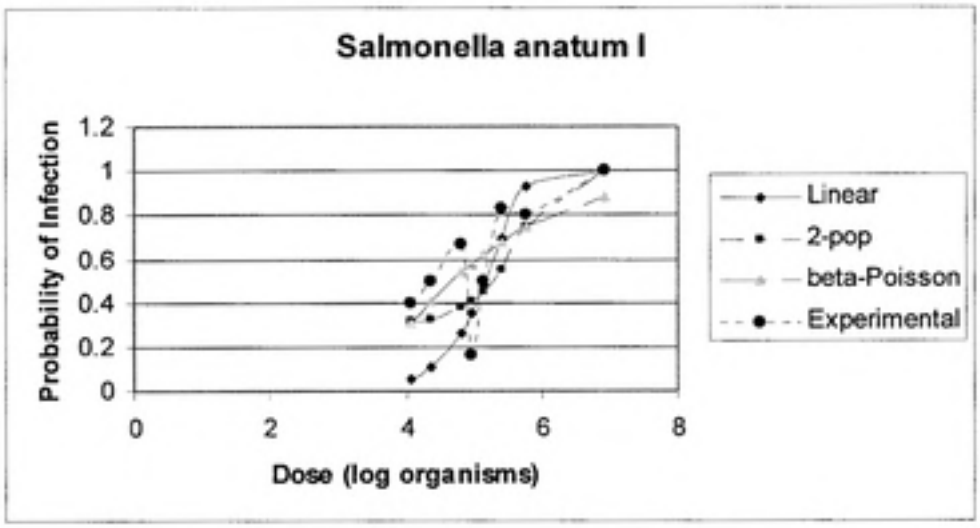
Graph 3.8 Salmonella meleagridis III Dose-Response Curves



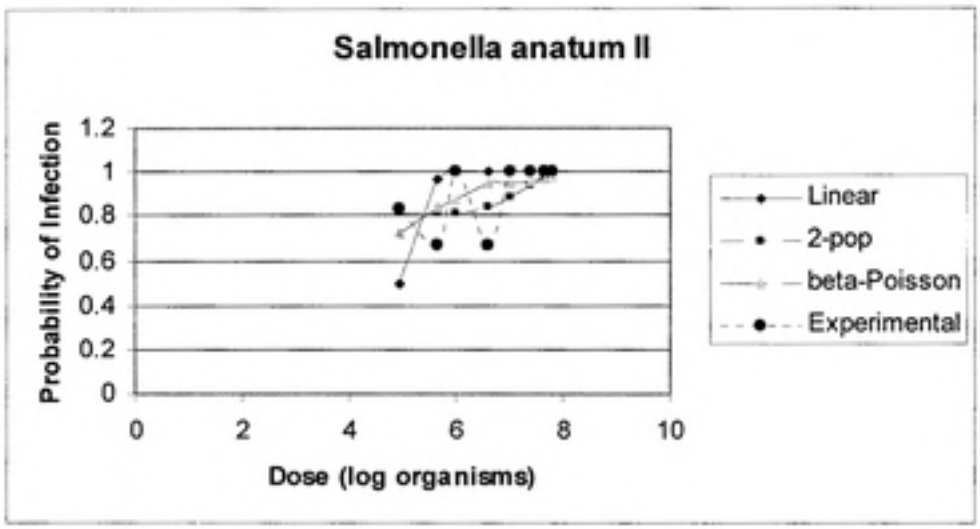
Salmonella anatum

Two of Salmonella anatum's three strains, I and II, have experimental data in which probability does not increase monotonically with dose (32). This decreased the accuracy of the equations fit to these microbes. However, equations fit to strain III had some of the lowest S values in this study. The linear model had the lowest S value, 0.00158, for strain III and was accepted based on its deviance, D = 0.21. The two-population equation also had the lowest S and D values for the other two strains, 0.65 and 2.98, respectively, for strain I and 0.13 and 3.02, respectively, for strain II. The beta-Poisson equation had the second best fit for strains II and III, but had one of the largest S values in this study for strain I, 1.56. These parameters for this beta-Poisson equation were found in the Netherlands report (45). The beta-Poisson equation was the only equation found to be statistically acceptable for all three strains.

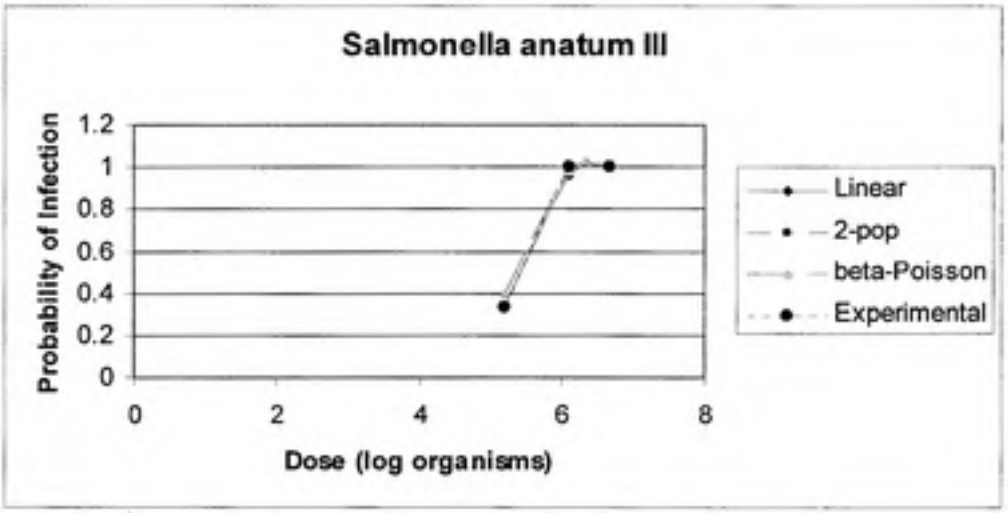
Graph 3.9 Salmonella anatum I Dose-Response Curves



Graph 3.10 Salmonella anatum II Dose-Response Curves



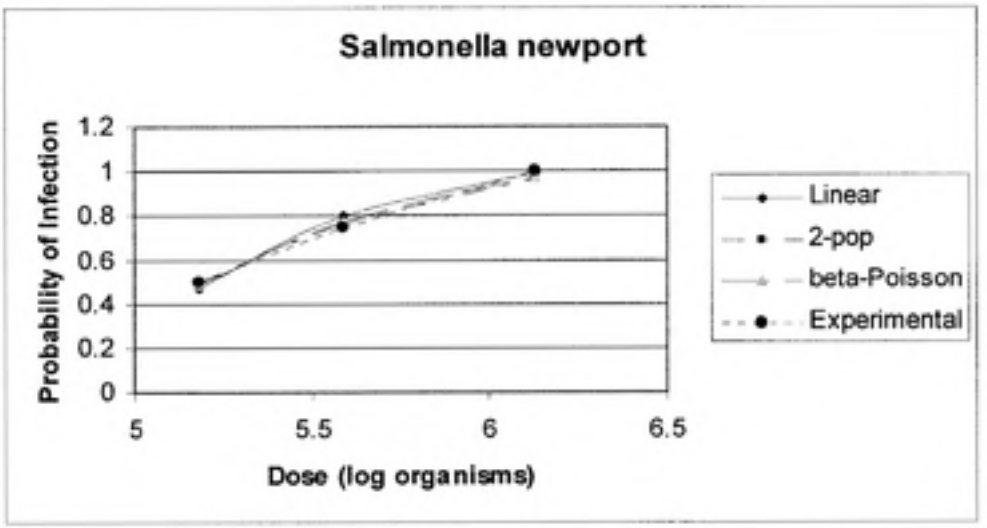
Graph 3.11 Salmonella anatum III Dose-Response Curves



Salmonella newport

Salmonella newport's experimental data allowed for parameter fittings of the equations, but the two-population model was rejected based on D. All three S values were less than 0.006 with the lowest being 0.0015 for the two-population equation. The beta-Poisson S was 0.0018, a slight increase over the two-population model. The linear equation was considered slightly more accurate than the beta-Poisson equation. This is because the linear D value (0.08) was approximately half that of beta-Poisson value (0.15) while the linear acceptable X^2 value was larger than beta-Poisson's value. The accuracy of these fittings is most likely due to the fact that only three dose levels were employed in the McCullough study (33).

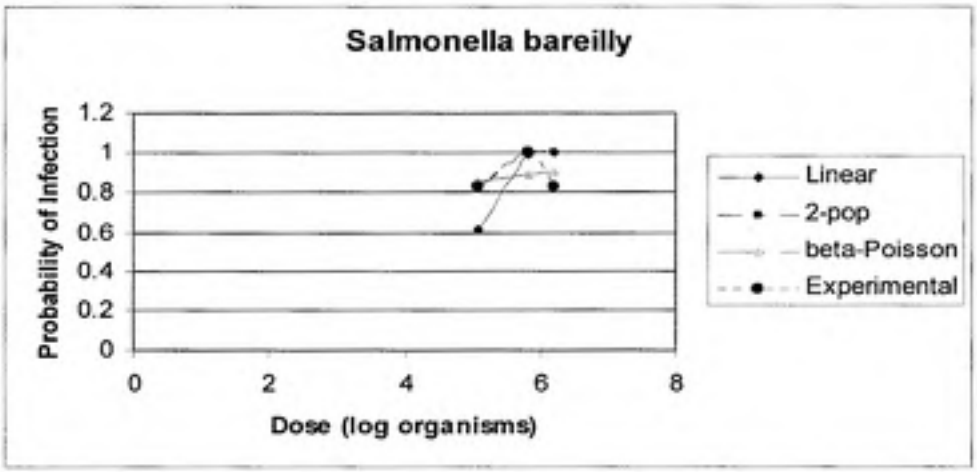
Graph 3.12 Salmonella newport Dose-Response Curves



Salmonella bareilly

The S values for all three equations for Salmonella bareilly were also very small, but were approximately an order of magnitude greater than those for S. newport. The beta-Poisson equation offered the best fit in this case with S = 0.019 and D = -11.7, but was followed closely by the linear equation. The two-population equation was rejected based on D. As with S. newport, only three dose levels were found in the McCullough study so the equations were easily fitted to the experimental data (33).

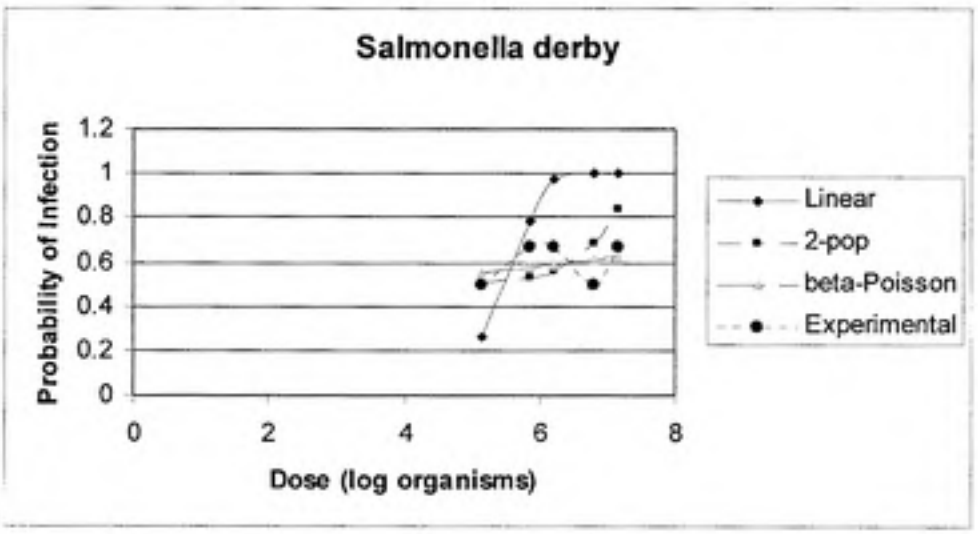
Graph 3.13 Salmonella bareilly Dose-Response Curves



Salmonella derby

Salmonella derby had more dose levels represented in the experimental data than the previous two species (33). However, the beta-Poisson equation could still be fitted to the data with the lowest S value of 0.053. The beta-Poisson D value was also the lowest and was equal to 0.33. The linear equation could not fit this data well and was rejected based on D.

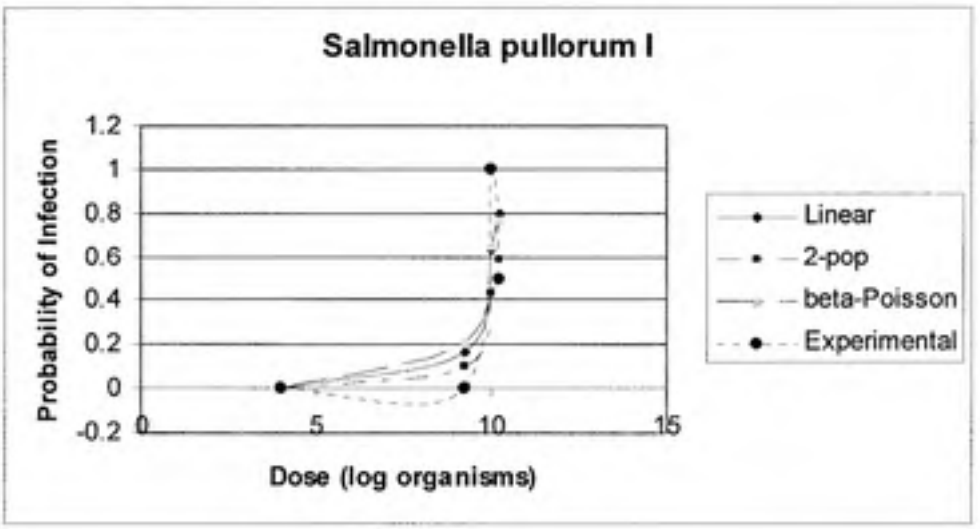
Graph 3.14 Salmonella derby Dose-Response Curves



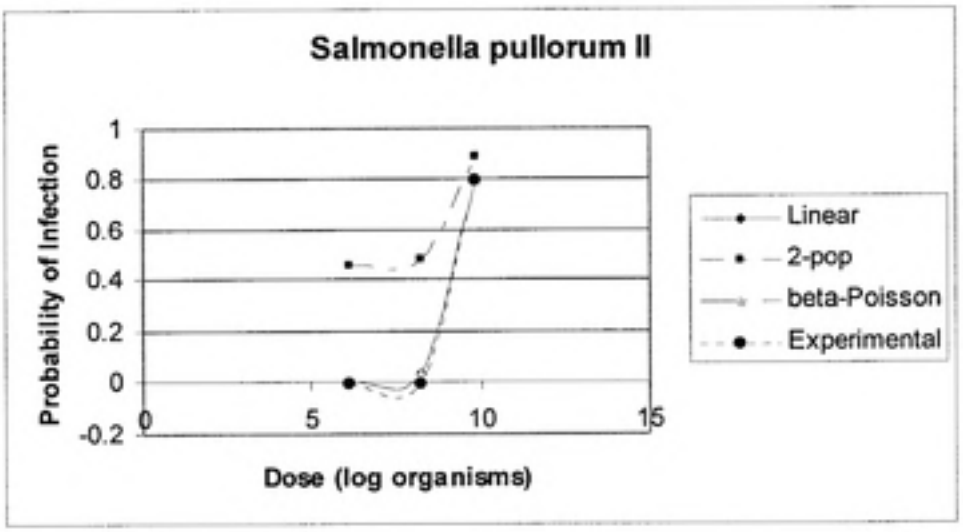
Salmonella pullorum

Four strains of S. pullorum were included in this study. The linear two-population equation was rejected in each case because there were too few dose levels represented for each microbe for the fitted equations to be considered statistically acceptable. The linear and beta-Poisson equations were accepted for each strain. Most of the probabilities in the experimental data of all four strains were either 0 or 1, so establishing the nature of the middle portion of the curves was difficult. The beta-Poisson equation allowed for the most accurate fit for all strains, I, II, III, and IV. The beta-Poisson S values were 0.24, 0.00143, 0.089, and 0.098, respectively. The beta-Poisson D values were 4.38, 0.202, 2.53, and 2.13, respectively. Strain II's linear equation's S, 0.00145, and D, 0.204, were very close to the beta-Poisson equation's values and should be considered as approximately as accurate as the beta-Poisson equation.

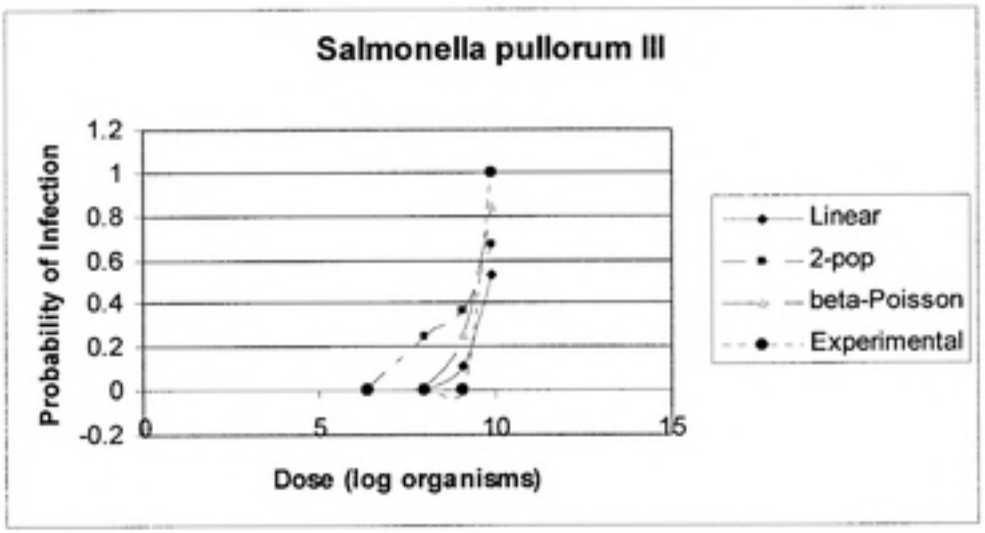
Graph 3.15 S. pullorum I Dose-Response Curves



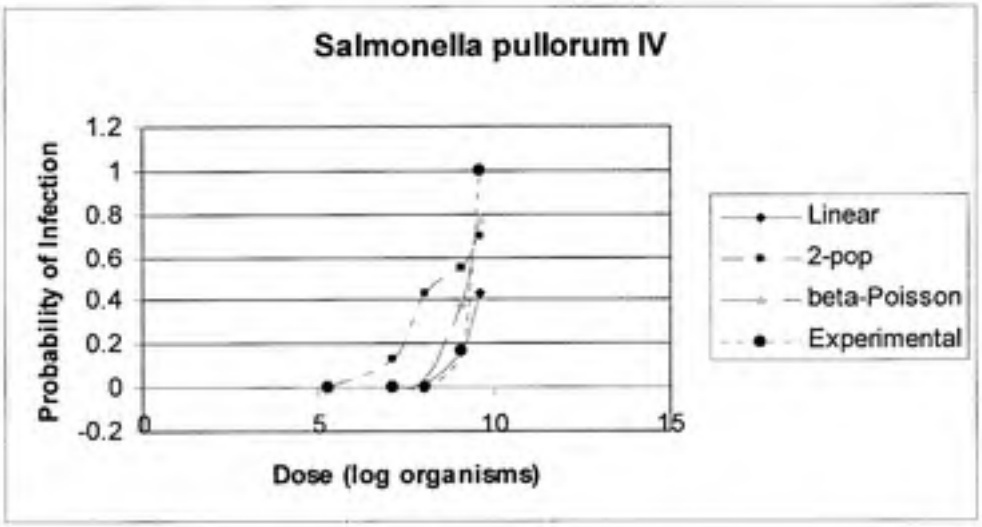
Graph 3.16 S. pullorum II Dose-Response Curves



Graph 3.17 S. pullorum III Dose-Response Curves



Graph 3.18 S. pullorum IV Dose-Response Curves



Discussion

Model Fits to Literature Values

Many of the beta-Poisson parameters derived in this study were comparable to those derived in other studies, particularly the Netherlands report (45). This appears to validate the method of parameter derivation used in this study. Parameters derived here for Campylobacter A3249 were very close to those derived in the Netherlands report. This study's alpha parameter differed from the Netherlands alpha by less than 0.2% (45). The beta parameters differed by 2.24%. As in the Netherlands report, no parameters could be derived for Campylobacter 81-176 (45). S. meleagridis I's derived parameters differed by 16% (alpha) and 30% (beta) from those found in the literature (45). These differences were among the largest found in this study and may have been due to differences in the parameter derivation methods used here and in the Netherlands report. The difference in beta values, while large, will not lead to greatly different results between this current study's model and the Netherlands' model. Changes in the alpha parameter produce more significant changes in outcomes than changes in the beta

parameter. Strain III's parameters more closely matched those found in the literature with alpha differing by 9% and beta differing by 0% (45). Parameters were derived in this study for the S. anatum strains, but were nearly equivalent to the parameters found in the Netherlands report. Therefore, the parameters from the Netherlands report were used in this current study (45). Several microbes were modeled using the exponential equation in the Netherlands report because their derived alpha and beta values were so large (45). Many of the same microbes listed in the literature as having large parameter values also had large parameter values derived here. Cryptosporidium parvum, S. Newport, and all four S. pullorum strains have large alpha and beta values. It is not known how well these values match those found in the Netherlands report because the values are not listed in the latter, but are mentioned to have large magnitudes. The derivation of large parameter values here in the same cases as in the Netherlands report suggests that the derivation method used here is working in a similar manner to the method used in the Netherlands report.

The beta-Poisson parameters derived here for some microbes do not match those found in the literature. In the case of the E. coli CFA+ strain, this can be explained by this study's use of experimental illness data, as opposed to infection data, to model the dose response curve. This was not done in the Netherlands report, which derived no parameters using infection data for either E. coli strain (45). The derived parameters for Giardia lamblia, S. meleagridis II, S. bareilly, and S. derby all differ greatly from those found in literature (45). In all of these cases, except S. meleagridis II, the Netherlands report used the exponential model because it derived large beta-Poisson parameters, but relatively small parameters were derived in this current study. In the case of S.

meleagridis II, beta-Poisson parameters were derived here and appeared to have a better fit to the data than the pooled parameters found in the Netherlands report (45).

Public Health Implications

An in-depth study of dose-response relationships can be useful for understanding how public health is affected by these microbes. Combined with exposure assessments and studies on concentrations in various media, dose-response models can be used to identify the major pathways of diseases. Water-quality planning and public health intervention strategies can be developed using these models. For example, a study of the concentration of Cryptosporidium parvum in a local recreational lake can be used in conjunction with the microbe's exposure assessment and dose-response model to estimate the health risk faced by users of the lake. If the risk appeared to be acceptable, then nothing need be done to address the microbe's concentration in the lake. If the risk appeared to be above some arbitrary acceptable level, then local officials could plan a strategy to mitigate this risk. This plan may include restricting the activities that can take place at the lake, regulating the flow of pollution into the water, or chemically treating the water directly to decrease the C. parvum concentration.

Dose-response models can also be used to identify the relative importance of addressing different microbe concentrations in water. For example, suppose a lake has high concentrations of two microbes, Giardia lamblia and Salmonella meleagridis I, that come from two different sources. If half of exposed people received an average dose of 1000 microbes of either species, 50% Giardia and 50% S. meleagridis I, then approximately 96% of those exposed to Giardia and 29% of those exposed to S. meleagridis I would become infected. If the local authority responsible for addressing

these issues had limited resources for doing so, it would be more beneficial, assuming the severity and treatment costs of each disease were similar, to design policies to reduce the risk from Giardia lamblia rather than to reduce the risk from S. meleagridis I.

Further Study

The most important aspect of improving the modeling of the dose-response relationships for these microbes is conducting more in-depth human studies. Some microbes had only been studied at 3 or 5 dose levels. All of them only had 2-8 people studied at each dose level. In most cases, these smaller studies do not provide enough data to accurately fit any model equations. The sampling size is so small that the dose-response relationship can be skewed by a few study subjects' results. For example, the data for S. pullorum I suggests that at a dosage of 1 E10 organisms, every exposed subject will become infected. However, increasing the dosage to 1.6 E10 organisms appears to decrease the probability of infection from 1 to 0.50. Since the model equations are all based on the assumption that the likelihood of a response increases with dose, this increases the difficulty and decreases the accuracy of fitting an equation to the data. More complete data sets will allow for more accurate modeling.

The method for determining the accuracy of each equation could also be modified to improve these results. A least sum of squares approach does produce an approximation of the variance between the predicted and experimental curves, but minimizing this value may not be the most effective way to fit an equation. The comparison of deviance to relevant X^2 values allows for the statistical significance of each equation to be examined, but does not necessarily identify the most accurate equation. More reliable programs than Microsoft Excel should also be used in the

calculation of the minimum sum of squares. Microsoft Excel's Solver function is an add-in analysis program designed to determine the maximum, minimum, or target value of a designated target cell. The target cell's value must be subject to the values in at least one other reference cell. The Solver function changes the values in these corresponding reference cells, subject to user-provided constraints, to determine the values necessary to produce the desired target cell outcome. On multiple occasions, Microsoft Excel's Solver function would produce a sum of squares that was greater than the starting value. On other occasions, the program would simply not change one or any of the assigned variables. Changing these variables by hand showed that lower sum of squares values were possible. Therefore, despite the overall small values of the sum of squares found in this study, some may not necessarily be the true minimum values because of errors in the Solver function. The use of ParamFit, a program that uses Excel for population-level modeling, would help to increase the accuracy of this study.

Citations

1. Allos BM. Campylobacter jejuni Infections: update on emerging issues and trends. Clin Infect Dis. 2001; 32(8):1201-6.

2. 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.

3. Blaser MJ, Wells JG, Feldman RA, Pollard RA, Allen JR. Campylobacter enteritis in the United States. A multicenter study. Ann Intern Med. 1983;98(3):360-5.

4. Carpenter C, Fayer R, Trout J, Beach M J. Chlorine disinfection of recreational water for Cryptosporidium parvum. Emerg Infect Dis. 1999; 5(4):579-84.

5. Cohen MB, Giannella RA. Enterotoxigenic Escherichia coli. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, editors. Infections of the gastrointestinal tract. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2002: 579–94

6. Couturier, Alain J. Editor. Occupational and Environmental Diseases. OEM Press, Beverly Farms, Mass. 2000: 60, 367-368, 374.

7. Daniels N, Neimann J, Karpati A, et al. Traveler's diarrhea at sea: three outbreaks of waterborne enterotoxigenic Escherichia coli on cruise ships. JID. 2000;181:1491-5.

8. Diarrheagenic Escherichia coli (non-Shiga toxin-producing E. coli) http://www.cdc.gov/ncidod/dbmd/diseaseinfo/diarrecoli_t.htm

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

10. 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.

11. Fayer R, Morgan U, Upton SJ. Epidemiology of Cryptosporidium: transmission, detection and identification. International Journal for Parasitology 2000; 30 (12-13): 1305-1322

12. Friedman CR, Neimann J, Wegener HC, Tauxe RV. Epidemiology of Campylobacter jejuni infections in the United States and other industrialized nations. In: Nachamkin I, Blaser MJ, eds. Campylobacter. 2d ed. Washington, DC: ASM Press; 2000:121–38

13. Furness BW, Beach MJ, Roberts JM. Giardiasis surveillance--United States, 1992-1997. MMWR CDC Surv. Summ. 2000;49(7):1-13.

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

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

16. Gallimore CI, Green J, Richards AF, Cotterill H, Curry A, Brown DW, Gray JJ. Methods for the detection and characterisation of noroviruses associated with outbreaks of gastroenteritis: outbreaks occurring in the north-west of England during two norovirus seasons. J Med Virol. 2004;73(2):280-8.

17. Grotto I, Huerta M, Balicer RD, Halperin T, Cohen D, Orr N, Gdalevich M. An outbreak of norovirus gastroenteritis on an Israeli military base. Infection. 2004;32(6):339-43.

18. Guerrant RL. Cryptosporidiosis: an emerging, highly infectious threat. Emerg Infect Dis. 1997;3(1):51-7.

19. Haas, Charles N, Rose Joan B, Gerba Charles P. Quantitative Microbial Risk Assessment. John Wiley & Sons, Inc. New York. 1999.

20. Hansman GS, Doan LT, Kguyen TA, Okitsu S, Katayama K, Ogawa S, Natori K, Takeda N, Kato Y, Nishio O, Noda M, Ushijima H. Detection of norovirus and sapovirus infection among children with gastroenteritis in Ho Chi Minh City, Vietnam. Arch Virol. 2004;149(9):1673-88.

21. Harris NV, Weiss NS, Nolan CM. The role of poultry and meats in the etiology of Campylobacter jejuni/coli enteritis. Am J Public Health. 1986;76(4):407-11.

22. Heresi G, Cleary TG. Giardia. Pediatr Rev. 1997;18(7):243-7.

23. Kapperud G, Lassen J, Ostroff SM, Aasen S. Clinical features of sporadic Campylobacter infections in Norway. Scand J Infect Dis 1992; 24:741–9.

24. Khaleel R, Reddy KR, and Overcash MR. Transport of potential pollutants in runoff water from land areas receiving animal wastes: a review. Water Res. 1980;14: 421.436.

25. Kramer MH, Sorhage FE, Goldstein ST, Dalley E, Wahlquist SP, Herwaldt BL. First reported outbreak in the United States of cryptosporidiosis associated with a recreational lake. Clin Infect Dis. 1998 Jan;26(1):27-33.

26. Lau CS, Wong DA, Tong LK, Lo JY, Ma AM, Cheng PK, Lim WW. High rate and changing molecular epidemiology pattern of norovirus infections in sporadic cases and outbreaks of gastroenteritis in Hong Kong. J Med Virol. 2004;73(1):113-7.

27. Lee SH, Levy DA, Craun GF, Beach MJ, Calderon RL. Surveillance for waterborne-disease outbreaks--United States, 1999-2000. MMWR Surv. Summ. 2002;51(8):1-47

28. Liddle JL, Burgess MA, Gilbert GL, Hanson RM, McIntyre PB, Bishop RF, Ferson MJ. Rotavirus gastroenteritis: impact on young children, their families and the health care system. Med J Aust. 1997;167(6):304-7

29. Lopman BA, Reacher MH, Vipond IB, Hill D, Perry C, Halladay T, Brown DW, Edmunds WJ, Sarangi J. Epidemiology and cost of nosocomial gastroenteritis, Avon, England, 2002-2003. Emerg Infect Dis. 2004;10(10):1827-34.

30. MacKenzie WR, Schell WL, Blair KA, et al. Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. Clin Infect Dis 1995; 21:57-62.

31. Mathieu E, Levy DA, Veverka F, Parrish MK, Sarisky J, Shapiro N, Johnston S, Handzel T, Hightower A, Xiao L, Lee YM, York S, Arrowood M, Lee R, Jones JL. Epidemiologic and environmental investigation of a recreational water outbreak caused by two genotypes of Cryptosporidium parvum in Ohio in 2000. Am J Trop Med Hyg. 2000;71(5):582-9.

32. 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.

33. 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.

34. 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.

35. Michaud S, Menard S, Arbeit RD. Campylobacteriosis, Eastern Townships, Quebec. Emerg Infect Dis. 2004;10(10):1844-7.

36. Murray CJL, Lopez AD, Mathers CD, Stein C. The global burden of disease 2000 project: aims, methods, and data sources. Geneva: World Heath Organization; 2001.

37. Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet. 1997;349(9061):1269-76.

38. Ortega YR, Adam RD. *Giardia*: overview and update. Clin Infect Dis 1997;25:545-50

39. Owen R, Surawicz C. Gastrointestinal and Hepatic Infections. W.B. Saunders Company, Philadelphia. 1995. p495, 519-524, 527.

40. Pruss A. Review of epidemiological studies on health effects from exposure to recreational water. Int J Epidemiol. 1998;27(1):1-9.

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

42. Salmonellosis http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_g.htm

43. Smith HV, Corcoran GD. New drugs and treatment for cryptosporidiosis. Curr Opin Infect Dis. 2004;17(6):557-64.

44. Steiner TS, Thielman NM, Guerrant RL. Protozoal agents: what are the dangers for the public water supply? Annu Rev Med 1997;48:329-40.

45. Teunis PFM, can der Heijden OG, van der Giessen JWB, Havelaar AH. The dose-response relation in human volunteers for gastro-intestinal pathogens. National Institute of Public Health and the Environment, The Netherlands. 1996.

46. Yoder JS, Blackburn BG, Craun GF, Hill V, Levy DA, Chen N, Lee SH, Calderon RL, Beach MJ. Surveillance for Waterborne-Disease Outbreaks Associated with Recreational Water --- United States, 2001—2002. MMWR Surv Summ. 2004;53(8):1-22.

Appendix A

Equation Parameters, S and D Values, and Method of Derivation for Each Microbe

Microbe	Model Equation	Parameters K units = 1/organisms, (or cysts, or oocysts) Beta units = organisms, cysts, or oocysts	Sum of Squares (S)	Deviance (D)/ Accepted?/DF	Method of S Derivation
Campylobacter A3249	Linear	K = 0.000175363	0.49843325	12.6898838 No 5	Relative
	Two-population	F1 = 0.482 F2 = 0.518 K1 = 1.338E-05 K2 = 6215.66	0.161389418	69.77913039 No 3	Relative
	Beta-Poisson	Alpha = 0.1447 Beta = 7.759	0.034554152	1.054503105 Yes 4	Relative
Campylobacter 81-176	Linear	K = 8.30869E-07	1.188027609	-18.41501058 Yes 2	Relative
	Two-population	F1 = 0.618 F2 = 0.382 K1 = 4.363E-09 K2 = 22838.31	0.896699101	96.47960439 No 0	Relative
	Beta-Poisson	Alpha = 0.145 Beta = 7.589	1.217178276	24.96767864 No 1	Relative
C. parvum	Linear	K = 0.00421	0.031610159	0.166963277 Yes 7	Relative
	Two-population	F1 = 0.8902 F2 = 0.1098 K1 = 0.003245 K2 = 0.10828	0.000766981	0.041709222 Yes 5	Relative

	Beta-Poisson	Alpha = 1.895045014 Beta = 345.0550297	0.015537531	0.16308279 Yes 6	Relative
Giardia lamblia	Linear	K = 0.09794	0.526208412	20.87865197 No 7	Non-relative
	Two-population	F1 = 0.379 F2 = 0.621 K1 = 0.0104 K2 = 0.3324	0.348773967	8.024865242 Yes 5	Non-relative
	Beta-Poisson	Alpha = 0.5467 Beta = 8.3662	0.541231807	4.555778731 Yes 6	Non-relative
E. coli CFA+	Linear	K = 1.616E-07	0.035203912	8.720228172 No 2	Relative
	Two-population	F1 = 0.73 F2 = 0.27 K1 = 2.073E-07 K2 = 1.032E-08	0.01373887	9.146285236 No 0	Relative
	Beta-Poisson	Alpha = 0.4828 Beta = 2622927.06	0.003465221	0.179736807 Yes 1	Relative
S. meleagridis I	Linear	K = 1.418E-05	0.499106091	7.387224062 Yes 10	Relative
	Two-population	F1 = 0.574 F2 = 0.426 K1 = 4.350E-06 K2 = 0.00067	0.119426939	21.36155691 No 8	Relative
	Beta-Poisson	Alpha = 0.369911196 Beta = 4384.612412	0.201900681	3.383617467 Yes 9	Relative

S. meleagridis II	Linear	K = 2.74E-08	0.058598059	1.432498207 Yes 4	Non-relative
	Two-population	F1 = 0.61 F2 = 0.39 K1 = 2.74E-08 K2 = 4.37E-08	0.075620232	1.717828565 Yes 2	Non-relative
	Beta-Poisson	Alpha = 32500376.27 Beta = 1.508E+15	0.074748235	1.480952899 Yes 3	Non-relative
S. meleagridis III	Linear	K = 1.1714E-06	0.033403181	7.827957767 No 3	Relative
	Two-population	F1 = 0.55 F2 = 0.45 K1 = 1.1714E-06 K2 = 1.1945E-06	0.033423265	7.912245993 No 1	Relative
	Beta-Poisson	Alpha = 0.8051 Beta = 441332	0.057051704	1.045378227 Yes 1	Relative
S. anatum I	Linear	K = 4.614E-06	1.1028963	7.831854166 Yes 7	Relative
	Two-population	F1 = 0.700 F2 = 0.300 K1 = 1.7243E-06 K2 = 0.999	0.646633596	2.980424892 Yes 5	Relative
	Beta-Poisson	Alpha = 0.281 Beta = 4550	1.155582491	3.4212006 Yes 6	Relative
S. anatum II	Linear	K = 7.622E-06	0.441427157	52.37140782 No 7	Relative

	Two-population	F1 = 0.193 F2 = 0.807 K1 = 4.768E-08 K2 = 290.71	0.129666654	3.019951199 Yes 5	Relative
	Beta-Poisson	Alpha = 0.342 Beta = 2270	0.196591862	4.121023598 Yes 6	Relative
S. anatum III	Linear	K = 2.61E-06	0.001585472	0.20501104 Yes 2	Relative
	Two-population	F1 = 1 F2 = 0 K1 = 2.575E-06 K2 = 1.94E-06	0.001621561	0.212922719 No 0	Relative
	Beta-Poisson	Alpha = 40100000 Beta = 1.27E+13	0.011672538	0.143607766 Yes 1	Relative
S. newport	Linear	K = 4.163E-06	0.005106386	0.077516769 Yes 2	Relative
	Two-population	F1 = 0.607 F2 = 0.393 K1 = 6.93E-06 K2 = 1.896E-06	0.001464063	0.167963393 No 0	Relative
	Beta-Poisson	Alpha = 3.519 Beta = 728299.86	0.001832924	0.146145592 Yes 1	Relative
S. bareilly	Linear	K = 7.694E-06	0.089112173	-2.833717443 Yes 2	Relative
	Two-population	F1 = 0.740 F2 = 0.260 K1 = 1.667E-05 K2 = 9.977E-06	0.033333396	1.106492035 No 0	Relative

	Beta-Poisson	Alpha = 0.1462 Beta = 0.2033	0.018771815	-11.70583389 Yes 1	Relative
S. derby	Linear	K = 2.214E-06	0.939705283	91.50102759 No 4	Relative
	Two-population	F1 = 0.504 F2 = 0.496 K1 = 442451.51 K2 = 7.324E-08	0.160353096	1.172491687 Yes 2	Relative
	Beta-Poisson	Alpha = 0.0394 Beta = 0.000252	0.053370535	0.333027415 Yes 3	Relative
S. pullorum I	Linear	K = 1E-10	0.251062245	4.467782619 Yes 3	Non-relative
	Two-population	F1 = 0.7 F2 = 0.3 K1 = 4.332E-11 K2 = 1E-10	0.336666396	4.971168852 No 1	Non-relative
	Beta-Poisson	Alpha = 1.8537 Beta = 13278122421	0.240849772	4.383877081 Yes 2	Non-relative
S. pullorum II	Linear	K = 2.384E-10	0.001453196	0.204260781 Yes 2	Non-relative
	Two-population	F1 = 0.54 F2 = 0.46 K1 = 2.384E-10 K2 = 0.00003	0.451188907	6.783824087 No 0	Non-relative
	Beta-Poisson	Alpha = 36080926.39 Beta = 1.52779E+17	0.001435713	0.202443324 Yes 1	Non-relative

S. pullorum III	Linear	K = 1E-10	0.231584617	3.960843688 Yes 3	Non-relative
	Two-population	F1 = 0.71 F2 = 0.29 K1 = 1E-10 K2 = 0.00000002	0.310754718	6.091751641 No 1	Non-relative
	Beta-Poisson	Alpha = 160920809.9 Beta = 6.50732E+17	0.089759135	2.532767455 Yes 2	Non-relative
S. pullorum IV	Linear	K = 1.424E-10	0.322508435	4.462473562 Yes 4	Non-relative
	Two-population	F1 = 0.54 F2 = 0.46 K1 = 1.424E-10 K2 = 2.384E-08	0.447254794	7.263720867 No 2	Non-relative
	Beta-Poisson	Alpha = 24895725.38 eta = 6.49995E+16	0.098109761	2.134777139 Yes 3	Non-relative

Appendix B Sample Calculations

Sample Calculations for Cryptosporidium parvum

1. Linear Equation Parameter Fitting

$P_{in} = 1 - e^{-k * d}$

Using data from Table 1.1:

Dose (oocysts)	Linear P_{in}	Experimental P_{in}
34	0.133392852	0.2
108	0.365408487	0.375
313	0.732330367	0.666666667
504	0.880241446	0.833333333
1129	0.991383448	1
11460	1	1
113900	1	1
1139000	1	1

(final Solver derived k and S values used in the next steps)

$k = 0.00421$

Least Relative Sum of Squares

$S = \sum ((\text{Derived } P_{in} - \text{Experimental } P_{in})^2 / \text{Experimental } P_{in})$

$S = (((0.133392852 - 0.2)^2 / 0.2) + ((0.365408487 - 0.375)^2 / 0.375) + ((0.732330367 - 0.667)^2 / 0.667) + ((0.880241446 - 0.833)^2 / 0.833) + ((0.991383448 - 1)^2 / 1) + ((1 - 1)^2 / 1) + ((1 - 1)^2 / 1) + ((1 - 1)^2 / 1))$

$S = 0.0316$

Using Microsoft Excel Solver function to find the minimum possible S by changing k:

$S = 0.0316$

$k = 0.00421$

Deviance

$D = l - l_{pos}$

$l = -2 * \sum[p_i * \log(P_{in}) + (n_i - p_i) * \log(1 - P_{in})]$

$l = -2 * ((1 * \log(0.1334) + (5 - 1) * \log(1 - 0.1334)) + (3 * \log(0.365) + (8 - 3) * \log(1 - 0.365)) + (2 * \log(0.732) + (3 - 2) * \log(1 - 0.732)) + (5 * \log(0.8802) + (6 - 5) * \log(1 - 0.8802)) + (2 * \log(0.991) + (2 - 2) * \log(1 - 0.991)) + (3 * \log(1) + (3 - 3) * \log(1 - 1)) + (1 * \log(1) + (1 - 1) * \log(1 - 1)) + (1 * \log(1) + (1 - 1) * \log(1 - 1)))$

$l = 10.94$ (for cases of log(0) = DNE, a contribution of 0 is used)

$l_{pos} = -2 * \sum[p_i * \log(p_i / n_i) + (n_i - p_i) * \log((n_i - p_i)/n_i)]$

$l_{pos} = -2 * ((1 * \log(1/5) + (5 - 1) * \log((5 - 1) / 5)) + (3 * \log(3/8) + (8 - 3) * \log((8 - 3) / 8)) + (2 * \log(2/3) + (3 - 2) * \log((3 - 2) / 3)) + (5 * \log(5/6) + (6 - 5) * \log((6 - 5) / 6)) + (2 * \log(2/2) + (2 - 2) * \log((2 - 2) / 2)) + (3 * \log(3/3) + (3 - 3) * \log((3 - 3) / 3)) + (1 * \log(1/1) + (1 - 1) * \log((1 - 1) / 1)) + (1 * \log(1/1) + (1 - 1) * \log((1 - 1) / 1)))$

$l_{pos} = 10.78$ $\quad D = 10.94 - 10.78 = 0.16$

DF = # dose levels – unknown equation parameters = 8 – 1 = 7

At DF = 7, p = 0.05 X^2 value is 14.07. Since $D < X^2$, the equation is accepted.

2. Linear Two-population Equation Parameter Fitting

$P_{in} = f1 * (1 - e^{-k1 * d}) + f2 * (1 - e^{-k2 * d})$

Using data from Table 1.1:

Dose (oocysts)	2-pop P_{in}	Experimental P_{in}
34	0.200002777	0.2
108	0.372973498	0.375
313	0.67763692	0.666666667
504	0.826564572	0.833333333
1129	0.977184852	1
11460	1	1
113900	1	1
1139000	1	1

(final Solver derived f, k, and S values used in the next steps)

$f_1 = 0.89$ $k_1 = 0.00325$

$f_2 = 0.11$ $k_2 = 0.108$

Least Relative Sum of Squares

$S = \sum ((\text{Derived } P_{in} - \text{Experimental } P_{in})^2 / \text{Experimental } P_{in})$

$S = (((0.200002777 - 0.2)^2 / 0.2) + ((0.372973498 - 0.375)^2 / 0.375) + ((0.67763692 - 0.667)^2 / 0.667) + ((0.826564572 - 0.833)^2 / 0.833) + ((0.977184852 - 1)^2 / 1) + ((1 - 1)^2 / 1) + ((1 - 1)^2 / 1) + ((1 - 1)^2 / 1))$

$S = 0.00077$

Using Microsoft Excel Solver function to find the minimum possible S by changing f_1, k_1, and k_2:

$S = 0.00077$

$f_1 = 0.89$ $k_1 = 0.00325$

$f_2 = 0.11$ $k_2 = 0.108$

Deviance

$D = l - l_{pos}$

$l = -2 * \sum[p_i * \log(P_{in}) + (n_i - p_i) * \log(1 - P_{in})]$

$l = -2 * ((1 * \log(0.200) + (5 - 1) * \log(1 - 0.200)) + (3 * \log(0.373) + (8 - 3) * \log(1 - 0.373)) + (2 * \log(0.678) + (3 - 2) * \log(1 - 0.678)) + (5 * \log(0.827) + (6 - 5) * \log(1 - 0.827)) + (2 * \log(0.977) + (2 - 2) * \log(1 - 0.977)) + (3 * \log(1) + (3 - 3) * \log(1 - 1)) + (1 * \log(1) + (1 - 1) * \log(1 - 1)) + (1 * \log(1) + (1 - 1) * \log(1 - 1)))$

$l = 10.82$ (for cases of log(0) = DNE, a contribution of 0 is used)

$l_{pos} = -2 * \sum[p_i * \log(p_i / n_i) + (n_i - p_i) * \log((n_i - p_i)/n_i)]$

$l_{pos} = -2 * ((1 * \log(1/5) + (5 - 1) * \log((5 - 1) / 5)) + (3 * \log(3/8) + (8 - 3) * \log((8 - 3) / 8)) + (2 * \log(2/3) + (3 - 2) * \log((3 - 2) / 3)) + (5 * \log(5/6) + (6 - 5) * \log((6 - 5) / 6)) + (2 * \log(2/2) + (2 - 2) * \log((2 - 2) / 2)) + (3 * \log(3/3) + (3 - 3) * \log((3 - 3) / 3)) + (1 * \log(1/1) + (1 - 1) * \log((1 - 1) / 1)) + (1 * \log(1/1) + (1 - 1) * \log((1 - 1) / 1)))$

$l_{pos} = 10.78$ $\quad D = 10.82 - 10.78 = 0.04$

DF = # dose levels – unknown equation parameters = 8 – 3 = 5

At DF = 5, p = 0.05 X^2 value is 11.07. Since $D < X^2$, the equation is accepted.

3. beta-Poisson Equation Parameter Fitting

$P(in) = 1 - (1 + (dose / beta))^{-alpha}$

Using data from Table 1.1:

Dose (oocysts)	beta-Poisson P_{in}	Experimental P_{in}
34	0.163134201	0.2
108	0.403119953	0.375
313	0.705775433	0.666666667
504	0.818470409	0.833333333
1129	0.936182968	1
11460	0.998762171	1
113900	0.999983228	1
1139000	0.999999785	1

(final Solver derived alpha, beta, and S values used in the next steps)

alpha = 1.895 beta = 345.06

Least Relative Sum of Squares

$S = \sum ((\text{Derived } P_{in} - \text{Experimental } P_{in})^2 / \text{Experimental } P_{in})$

$S = (((0.163134201 - 0.2)^2 / 0.2) + ((0.403119953 - 0.375)^2 / 0.375) + ((0.705775433 - 0.667)^2 / 0.667) + ((0.818470409 - 0.833)^2 / 0.833) + ((0.936182968 - 1)^2 / 1) + ((0.998762171 - 1)^2 / 1) + ((0.999983228 - 1)^2 / 1) + ((0.999999785 - 1)^2 / 1))$

S = 0.0155

Using Microsoft Excel Solver function to find the minimum possible S by changing f_1, k_1, and k_2:

S = 0.0155

alpha = 1.895 beta = 345.06

Deviance

$D = l - l_{pos}$

$l = -2 * \sum[p_i * \log(P_{in}) + (n_i - p_i) * \log(1 - P_{in})]$

$l = -2 * ((1 * \log(0.163) + (5 - 1) * \log(1 - 0.163)) + (3 * \log(0.403) + (8 - 3) * \log(1 - 0.403)) + (2 * \log(0.706) + (3 - 2) * \log(1 - 0.706)) + (5 * \log(0.818) + (6 - 5) * \log(1 - 0.818)) + (2 * \log(0.936) + (2 - 2) * \log(1 - 0.936)) + (3 * \log(0.9987) + (3 - 3) * \log(1 - 0.9987)) + (1 * \log(0.999) + (1 - 1) * \log(1 - 0.999)) + (1 * \log(0.999) + (1 - 1) * \log(1 - 0.999)))$

$l = 10.94$ (for cases of log(0) = DNE, a contribution of 0 is used)

$l_{pos} = -2 * \sum[p_i * \log(p_i / n_i) + (n_i - p_i) * \log((n_i - p_i)/n_i)]$

$l_{pos} = -2 * ((1 * \log(1/5) + (5 - 1) * \log((5 - 1) / 5)) + (3 * \log(3/8) + (8 - 3) * \log((8 - 3) / 8)) + (2 * \log(2/3) + (3 - 2) * \log((3 - 2) / 3)) + (5 * \log(5/6) + (6 - 5) * \log((6 - 5) / 6)) + (2 * \log(2/2) + (2 - 2) * \log((2 - 2) / 2)) + (3 * \log(3/3) + (3 - 3) * \log((3 - 3) / 3)) + (1 * \log(1/1) + (1 - 1) * \log((1 - 1) / 1)) + (1 * \log(1/1) + (1 - 1) * \log((1 - 1) / 1)))$

$l_{pos} = 10.78$ $\quad D = 10.94 - 10.78 = 0.16$

DF = # dose levels – unknown equation parameters = 8 – 2 = 6

At DF = 6, p = 0.05 X^2 value is 12.59. Since $D < X^2$, the equation is accepted.