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Development of a dose-response relationship for *Escherichia coli* O157:H7

Charles N. Haas[a,*], Aadithya Thayyar-Madabusi[a], Joan B. Rose[b], Charles P. Gerba[c]

[a]*School of Environmental Science, Engineering and Policy, Drexel University, Philadelphia, PA 19104, USA*
[b]*Department of Marine Science, University of South Florida, St. Petersburg, FL, USA*
[c]*Department of Soil and Environmental Science, University of Arizona, Tucson, AZ, USA*

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Abstract

E. coli O157:H7 is an emerging food and waterborne pathogen. The development of acceptable guidelines for exposure to this organism based on quantitative microbial risk assessment requires a dose response curve. In this study, a prior animal study was used to develop a dose response relationship. The data was adequately fit by the beta-Poisson dose response relationship. This relationship was validated with reference to two outbreaks of this organism. It was found that the low dose extrapolation of the animal data using the beta-Poisson relationship provided estimates of risk concordant with those noted in the outbreaks. The fitted dose response relationship in conjunction with population estimates of the prevalence of *E. coli* O157:H7 illness indicates that the overall exposure is quite low in the US. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Escherichia coli* O157:H7; Risk assessment; Dose-response; Epidemiology

1. Introduction

Escherichia coli strains are ubiquitously present in the intestinal tracts of humans and other warm-blooded animals. While many strains are harmless some are pathogenic pathogenic. *E. coli* O157:H7 was identified as a pathogen in 1982 during two outbreaks that occurred in Oregon and Michigan, USA (Riley, 1983). Illness was characterized by abdominal cramping, watery diarrhea followed by bloody diarrhea with little or no fever. The outbreak was associated with eating at a single fast-food chain, and the implicated items included beef, pickles and onions. The only previously known isolation from humans was from a California woman in 1975.

Through 1994, 68 outbreaks or clusters have been reported with a total of 2,334 cases (Doyle et al., 1997). Some reports suggest that there may be as many as 20,000 (Berkelman et al., 1994) to 25,000 (Todd, 1989) cases annually. A few population based studies have been performed. MacDonald

*Corresponding author. Tel.: +1-215-895-2283; fax: +1-215-895-2267.
E-mail address: haas@drexel.edu (C.N. Haas)

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reported (MacDonald et al., 1988) the incidence rate for laboratory confirmed infection to be 8 per 100,000 persons per year. Ostroff et al. (1989) reported the incidence rate to be 2.1 per 100,000 persons per year. (Griffin and Tauxe, 1991) reported the incidence rate in Canada to be 5.2 per 100,000 persons per year. The most recent estimate in the US, based on the CDC Foodnet surveillance program, is 2.7 cases per 100,000 persons per year (Centers for Disease Control and Prevention, 1998). The true incidence is likely higher than these estimates, due to incomplete diagnosis and reporting.

Persons at extreme ages, i.e. the very young and the elderly, are at greater risk of *E. coli* O157:H7 infection. The case fatality rate ranges from 3 to 36% (Su and Brandt, 1995). The spectrum of severity from an initial infection is complex, however is fairly well characterized based on studies from several outbreaks (Council for Agricultural Science and Technology, 1994).

E. coli O157:H7 infection has most often been associated with beef products. However waterborne (Swerdlow et al., 1989; Warrner et al., 1996) and foodborne outbreaks via acidic food items such as salami and apple cider (Steele and Murphy, 1982; Lett and Besser, 1993) have also been documented. These organisms have been found to produce shiga-like toxins similar to *Shigella dysenteriae* (Doyle et al., 1997). The serious nature of *E. coli* O157:H7 infection has led to increased vigilance and surveillance. Using quantitative risk analysis techniques, a risk assessment model can be determined for *E. coli* O157:H7, and this model can be used to assess and manage the risks due to exposure to this pathogen via different modes.

A prerequisite to the use of risk assessment methods is the existence of a suitable dose-response relationship for the organism under investigation (National Academy of Sciences, 1983; Haas et al., 1999). The objective of this study was to develop a dose-response relationship for *E. coli* O157:H7.

2. Data

There are no known human dose response studies for *E. coli* O157:H7, hence it was necessary to ascertain whether dose-response from animal studies would produce relevant information. The dose-response data (Table 1) for *E. coli* O157:H7 was obtained from a study done by Pai and co-workers (Pai et al., 1986). These workers studied the pathogenesis of diarrheal disease due to *E. coli* O157:H7 in New Zealand white infant rabbits (2–3 days old). The rabbits were inoculated with 1 ml of bacterial suspension through an oral catheter. After inoculation, the animals were observed daily for diarrhea. After disease-induced mortality, or sacrifice, post-mortem sections of intestine were weighed carefully and dissected. Infection was confirmed from a bacterial count in the intestines.

Table 1
Dose-response data for *E. coli* O157:H7 (Pai et al., 1986).

Dose	Number of subjects	Positive (infection)
Control	7	0
100,000	3	0
1,000,000	5	2
10,000,000	5	5
100,000,000	13	12
1,000,000,000	5	5
3,000,000,000	2	2
10,000,000,000	6	6

3. Analysis

Based on the success of prior work (Regli et al., 1991; Haas et al., 1993, 1999), the dose-response data were analyzed using the method of maximum likelihood. The data was fit to a dose-response relationship that describes the observed infectivity as a function of dose. Prior work has shown that the exponential (Eq. (1)) and the beta-Poisson model (Eq. (2)) provide good fits for microbial dose-response data.

In both models it is assumed that the dose is Poisson distributed and that one organism is sufficient to cause infection. The exponential model is the simplest dose-response model. If ''*j*'' organisms are ingested from a dose ''*d*'', and ''*k*'' organisms survive for at least one to cause infection, then the probability of infection (P_i) can be expressed as:

$$\pi_i = 1 - \exp\left(-\frac{d}{k}\right) \quad (1)$$

where d is the dose and k represents the number of

organisms that are ingested for one to survive and cause infection. The exponential model assumes that pathogen–host interactions can describe the pathogen–host survival probability by a discrete value.

The beta-Poisson model takes into account the variations that exist in pathogen–host interactions. The pathogen–host survival probability can be described by a probability distribution (Furumoto and Mickey, 1967a,b; Haas, 1983a). The probability of infection, P_i, can be expressed as:

$$\pi_i = 1 - \left[1 + \frac{d}{N_{50}}(2^{1/\alpha} - 1)\right] \quad (2)$$

where d represents the dose, α is the slope parameter and N_{50} is the dose that would infect half the exposed population. The magnitude of the parameter alpha indicates the model's proximity to the exponential model. As alpha approaches infinity, the beta-Poisson model approaches the exponential model.

The parameters of the models are determined by the maximum likelihood method (MLE). The values of the parameters that minimize the deviance, Y (Eq. (3)), are the maximum likelihood estimates.

$$\min Y = -2\sum_{i=1}^{k}\left[P_i \ln\frac{\pi_i}{\pi_i^0} + (T_i - P_i)\ln\left(\frac{1-\pi_i}{1-\pi_i^0}\right)\right] \quad (3)$$

where P_i is the predicted probability of infection determined by Eqs. (1) or (2), and π_i^0 is the observed probability of infection ($\pi_i^0 = P_i/T_i$). The number of positive or infected subjects at a particular dose is P_i and T_i is the total number of subjects at that dose. If the optimum value of the deviance, also termed the residual deviance, is less than the tabulated Chi-squared value at $j-q$ degrees of freedom (j is the number of doses and q the number of parameters in the model-one for exponential and two for beta-Poisson), then the fit is considered acceptable. Table 2 shows the results of the dose-response.

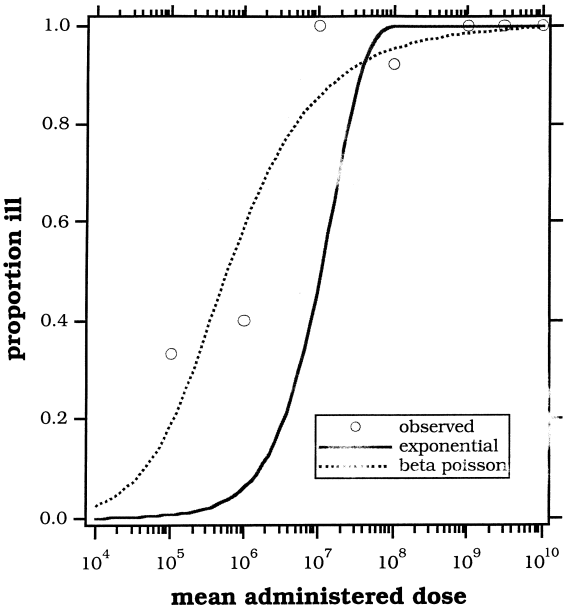

Fig. 1. Comparison of exponential and beta-Poisson fits to experimental data.

From this table, it is seen that the fit to the exponential dose-response relationship fails a goodness of fit test ($P<0.05$), while the fit to the beta-Poisson relationship is acceptable. As measured by a likelihood ratio test (the difference in deviances for the two models), the improvement in fit provided by the beta-Poisson model is statistically significant.

A graphical comparison of the fit to the exponential and beta-Poisson model is given in Fig. 1. It is

Table 2
Results of dose-response analysis for illness based on Pai et al. (1986) data

Model	Best fit		Residual deviance (Y)	P[a] (goodness of fit)
	Parameter	Value		
Exponential	k[b]	1.6×10^7	24.4	0.001
Beta Poisson	α[c]	0.49	3.1	0.79
	N_{50}[d]	5.96×10^5	3.1	0.79

[a] P value for improvement in fit $=4\times10^{-6}$ ($\Delta Y=21.3$, 1 df).
[b] k = The number of organisms surviving for at least one to cause infection.
[c] α = The slope parameter of Eq. (2).
[d] N_{50} = The dose that would infect half of the population.

evident that the exponential dose response relationship is far steeper than the data evidence. From the quantitative comparisons in Table 2, and the qualitative comparison in Fig. 1, it is concluded that the beta-Poisson model provides an acceptable fit to the dose-response data (but that the exponential model does not).

In this figure, 95% confidence limits are shown. These were obtained by bootstrapping the dose-response experiment, and depict the symmetrical limits.

4. Discussion

Once the dose-response models have been established, it is desirable to validate the models with human epidemiological information. This is particularly necessary since the underlying data here are based on animal studies, and there is as yet little experience with interspecies dose-response extrapolation from animals to humans. Furthermore, dose-response experiments are frequently, and of necessity, conducted at higher doses and levels of risk than may be encountered in an actual exposure situation. A convenient and simple method to determine the plausibility of the dose-response models, is to compare the model estimates with human outbreak information. The comparison entails the use of attack rates and duration of exposure to determine the likely dose of the pathogen in the incriminated source. This is then compared with the levels observed during the outbreak.

4.1. Validation

It is necessary to obtain outbreak information with the following well-documented characteristics: (i) vehicles of infection, (ii) attack rates and (iii) measurements of bacteria levels in the incriminated sources. It is difficult to obtain outbreak data that has these characteristics accurately determined. Two outbreaks were chosen for evaluation because the vehicles of infection, attack rates and bacterial levels were known to be fairly accurate.

On the fifth of July a county health department in Illinois started to receive reports of *E. coli* O157:H7 infection among children (Warrner et al., 1996), Subsequent interviews determined no common food source but they all had visited a state park with a lake swimming beach. There were a total of 12 cases identified. On June 24 and 25 an estimated 2200 and 2500 people visited the beach area. On June 21, two samples were collected and analyzed and these had levels of *E. coli* of 660–900 per 100 ml. No specific enumeration of *E. coli* O157:H7 was reported.

From the case report, the attack rate is computed to be 2.55×10^{-3}/person–day. The 95% confidence region to this rate (computed via likelihood ratio) is $1.36–4.29\times10^{-3}$/person–day.

If it is assumed that the *E. coli* (all strains) measured on June 21 approximated the concentrations of strain O157:H7 at the time of exposure of the cases, then the dose-response relationship can be used to estimate the resultant risk. To do this, it is assumed that 100 ml of water was ingested per person per day of swimming exposure; this has been used previously in recreational water risk assessment work (Haas, 1983b). From the coliform concentrations, this leads to an estimated individual risk of $1.69–2.31\times10^{-3}$/person–day (using the maximum likelihood estimates from the beta-Poisson fit). This clearly overlaps the risk experienced as obtained from the epidemiological study, and hence the results of this outbreak serve to support the applicability of the dose-response relationship obtained from the Pai et al. data (in animals) to human risk assessment.

In the fall of 1995 there was an outbreak of *E. coli* O157:H7 infection in an Oregon community (Keene and Sazie, 1997). Jerky prepared from deer meat was implicated as the source of the outbreak resulting in six confirmed cases of illness. The attack rate was 0.23/person–day (confidence range computed as 0.10–0.43/person–day). The estimated consumption was 250 g of the jerky per person per day over a two-day period. Two pieces of leftover jerky were tested for *E. coli*. O157:H7. They tested positive and a quantitative enumeration revealed *E. coli* O157:H7 concentrations ranged from 3 to 93 CFU/g.

From the best estimates of the dose-response curve, the individual risk resulting from consumption of 250 g/day of the jerky with O157:H7 levels of 3–93 CFU/g would be 0.002–0.055/person–day, which is actually somewhat lower than the observed risk. The confidence limits to the estimated risk (obtained from the confidence regions of the dose response curve as in Fig. 2) would include the observed risk. It may be that the point estimates

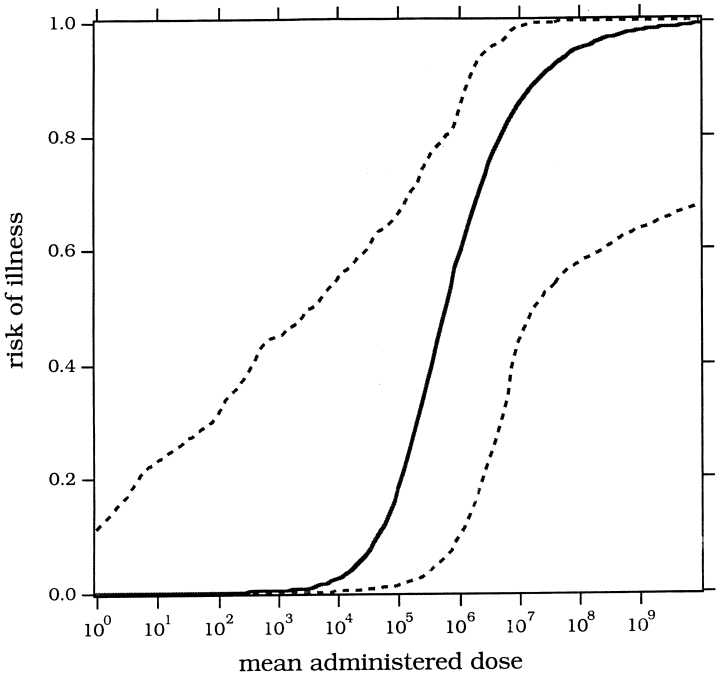

Fig. 2. Best fit (solid) and 95% confidence limits (dashed) for beta-Poisson dose response fit.

(using the best estimates of the dose response parameters) for the risk based on the concentrations of *E. coli* O157:H7 found in the jerky are lower than the observed risk due to either methodological deficiencies in the microbial analysis, or due to decreases of organism densities between the time of exposure and the time of enumeration (after the outbreak was identified).

In both outbreaks the predicted concentrations come close to the observed concentrations lending credence to the dose-response model. This is particularly noteworthy in the first case (the swimming outbreak), where the observed microbial dose was several orders of magnitude below the lowest non-zero dose used in the underlying experimental dose-response work (Table 1). This suggests that the animal dose-response curve may be suitable for human risk assessment and that the beta-Poisson model is a suitable means for extrapolating this relationship to low doses. In separate work, we have shown that animal derived dose-response relationships for *Listeria monocytogenes* are suitable for assessing human risk (Haas et al., in press), and thus this work provides a second set of supporting evidence with respect to the utility of animal derived dose response relationships for quantitative microbial risk assessment.

In prior work, such as that of Cassin et al. (1998), risk assessment of *E. coli* O157:H7 has been conducted using human dose-response relationships for other organisms. Studies have shown *E. coli* O157:H7 to produce shiga-like toxins similar to *Shigella dysenteriae* (Doyle et al., 1997). And hence it has been suggested that *Shigella* can be used as a surrogate for *E. coli* O157:H7, since their mechanism of infection are quite similar. Table 3 compares the dose-response models of *Shigella* from prior studies and human dose-response models for strains of *E. coli* other than O157:H7 with the present study on *E. coli* O157:H7.

From a comparison of the median infectious dose (N_{50}) values, as well as the estimated risk at low dose (e.g., 100 organisms) it appears that the potency of *E. coli* O157:H7 in rabbits, which seems to be similar to the potency in humans, is closer to the potency of other pathogenic *E. coli* than to *Shigella*.

Table 3
Comparison of dose-response models of *Shigella*, *E. coli* (other than O157:H7) to *E.coli* O157:H7

Organism	Host	Reference	α[a]	N_{50}[b]	Risk from 100 organisms
Shigella (pooled strains)	Humans	(Crockett et al., 1996)	0.162	1127	0.275
E. coli O111 and O55	Humans	(Haas et al., 1999)	0.175	2.55×10^{6}	3.53×10^{-4}
E. coli O157:H7	Rabbits	Present study	0.49	5.9×10^{5}	2.59×10^{-4}

[a] α = The slope parameter of Eq. (2).
[b] N_{50} = The dose that would infect half of the population.

4.2. Implications

The estimated US incidence rate of *E. coli* O157:H7 illness is 2.7 cases per 100,000 persons per year (Centers for Disease Control and Prevention, 1998). This is equivalent to a daily risk of 7.4×10^{-8}/person–day. From the dose-response relationship (best estimates), the average daily intake of O157:H7 that would produce this risk is estimated to be 0.028 organisms per day. This suggests that the exposure to this organism (over the aggregate population average) is quite low (for example, one can also view this as an average of 35 days between ingestion of organisms). This analysis needs to be tempered by the possible underreporting of illness. Even with the enhanced surveillance system employed by CDC, individuals who either do not seek medical attention (i.e. sustain only mild cases of illness) or those who do seek medical attention, but who have illnesses which are improperly diagnosed, are not included within the incidence rate.

5. Conclusions

The beta-Poisson model based on animal studies adequately describes the human morbidity risk of *E. coli* O157:H7. The exponential model did not provide an acceptable fit to the animal data. The beta-Poisson model not only provided an acceptable fit, but also showed a statistically significant improvement in fit over the exponential model. The risk predicted by the animal dose-response model is concordant with illness rates evidenced in two documented outbreaks (one in water, one in food). Comparisons of real world situations with the model predictions are highly plausible. A comparison of the dose-response information of *Shigella* and *E. coli* O157:H7 has shown that it would be inappropriate to use *Shigella* as a surrogate for *E. coli* O157:H7. Based on the annual cases estimated in the US, the model predicts the average exposure to be 0.028 organisms.

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