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Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks

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Abstract

A human dose response model for *Escherichia coli* O157 would enable prediction of risk of infection to humans following exposure from either foodborne or environmental pathways. However, due to the severe nature of the disease, volunteer human dose response studies cannot be carried out. Surrogate models from *Shigella* fed to humans and *E. coli* O157 to rabbits have been utilised but are significantly different to one another. In addition data obtained by animal exposure may not be representative for human beings. An alternative approach to generating and validating a dose response model is to use quantitative data obtained from actual human outbreaks. This work collates outbreak data obtained from global sources and these are fitted using exponential and beta-Poisson models. The best fitting model was found to be the beta-Poisson model using a beta-binomial likelihood and the authors favour the exact version of this model. The confidence levels in this model encompass a previously published *Shigella* dose response model. The potential incorporation of this model into QMRAs is discussed together with applications of the model to help explain foodborne outbreaks.

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1. Introduction

Escherichia coli O157 is a widespread pathogen causing severe human infection which can be either foodborne (e.g., cooked and raw meats, dairy products, vegetables etc.), waterborne (e.g., drinking or

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swimming water), environmental (e.g., direct contact with farm animals or contaminated pasture) or by human to human transmission. The infectious dose for this organism is estimated to be low (<10 viable cells (Griffin and Tauxe, 1991) and <a few hundred (Doyle et al., 2001)) and the sequelae can be severe, particularly among children. For example it has been reported (Locking et al., 2001) that in a study of 183 *E. coli* O157 cases in Scotland, 44% were in children under 10 years of age, 77% of cases reported bloody diarrhoea, 57% were admitted to hospital and 8% developed haemolytic uraemic syndrome (HUS). This high reporting rate among children may be influenced by under reporting of adult cases. A number of large outbreaks have occurred, e.g., the Central Scotland outbreak in 1996 where the consumption of contaminated meat led to the direct death of 17 elderly people and more than 500 falling ill (Cowden et al., 2001), a hamburger outbreak in Washington State 1992–1993 where 501 cases were reported, including 151 hospitalisations, 45 cases of HUS and 3 deaths (Bell et al., 1994), and an outbreak in school-age children in Osaka, Japan, in 1996 which eventually resulted in 7966 reported cases including 3 deaths (Michino et al., 1999).

A number of dose response models have been used in quantitative microbiology to describe the relationship between the level of microbial exposure (i.e., the dose or number of organisms ingested) and the likelihood of occurrence of an adverse consequence (i.e., illness (Holcomb et al., 1999)). The most commonly used models are single hit models, where only one organism ingested is required to cause infection even though the probability of this occurring may be very small. The simplest form of this model is the exponential model (Haas et al., 1999) which assumes that the number of organisms ingested takes the form of a Poisson distribution and that each micro-organism has an equal and independent survival probability of causing infection to the host which can be calculated from the binomial distribution. However, each individual host may respond differently to a given pathogen and this variation can be incorporated into the dose response model by describing the survival probability of the pathogen by a probability distribution. The most commonly used distribution used to describe this variability is a beta distribution, though it must be noted that any

unimodal distribution could potentially be used (Johnson et al., 1995). This beta-Poisson dose response model can be approximated by a simple equation (see Eq. (4)) and in most cases provides a statistically significant improvement in fit over the exponential model (Crockett et al., 1996).

Fitting of these models to data is usually performed using a maximum likelihood technique with the likelihood derived from the binomial distribution. However, overdispersion can occur when the variation between replicate individuals is greater than expected. This is likely to happen in outbreaks where individuals of a wide range of susceptibilities (e.g., from relatively low susceptibility healthy adults to higher susceptibility in the elderly and infants) may be exposed to the pathogen. Haas et al., (1999) demonstrates that overdispersion can be described using a beta-binomial likelihood.

Quantitative microbiological risk assessments (QMRA) have been performed to determine the risk of *E. coli* O157 infection both from foods (Cassin et al., 1998) and the environment (Strachan et al., 2002). These types of risk assessment are particularly useful in proposing mitigation strategies for reducing risk of infection. However, microbiological risk assessments require validated dose response models to ensure accuracy and assess uncertainty. Validation is ideally performed using data obtained from outbreaks (e.g., as developed for *Salmonella* (Fazil et al., 2001)). Surrogate dose response models have been used in *E. coli* O157 QMRAs but have yet to be fully validated with outbreak data. These include a surrogate *Shigella* beta-Poisson model based on feeding studies in humans formulated by Crockett et al. (1996) which was chosen because *Shigella* infections can be foodborne and the toxins produced are similar to those of *E. coli* O157 (Doyle et al., 2001). This model pooled experimental data from *Shigella flexneri* and *dysenteriae* strains and was shown to be statistically indistinguishable from separate dose response models of each species, suggesting its potential to represent the *Shigella* species. Haas et al. (2000) proposed a dose response model for *E. coli* O157 from rabbits inoculated through an oral catheter. The two models are considerably different with the Haas version requiring approximately 500 times as many organisms to infect 50% of animals exposed compared to the Crockett model. Strachan et al. (2001) demonstrated

that the surrogate *Shigella* model gave the closest fit to data obtained from an environmental outbreak. Powell et al. (2000) proposed a dose response envelope for *E. coli* O157 with bounding values of the dose response defined by two separate beta-Poisson dose response curves fitted to human clinical trial data for two surrogate pathogens (*Shigella dysenteriae* and enteropathogenic *E. coli* (EPEC)).

The aim of this paper is to collate *E. coli* O157 outbreak data that have been obtained by the authors and from published studies and from these generate a dose response model. We determine which of the surrogate models most closely resembles the outbreak model and apply this to understanding outbreaks where either the dose or the attack rate is unknown. In addition, using the dose response model together with national disease incidence data we estimate the average dose of *E. coli* O157 ingested on a country by country basis.

2. Materials and methods

Table 1 presents feeding study data for *Shigella* spp. in healthy male human adults, *E. coli* O157 in infant New Zealand white rabbits and EPEC strains fed at different doses to adult male human volunteers. Human outbreak data detailing *E. coli* O157 numbers are given in Table 2. Listed below are summaries of these outbreaks for the global locations investigated. Each is associated with a degree of error (e.g., dependent on the number of samples analysed (not always reported) and that bacteria are unlikely to be evenly distributed in food) that is discussed individually where appropriate.

2.1. UK, New Deer

In May 2000, a scout camp was held at the New Deer agricultural showground, UK. Twenty people (from 228 attendees) aged between 8 and 20 were later confirmed with *E. coli* O157 with dates of onset suggestive of a point source outbreak. Investigations showed that the field had been grazed by sheep prior to the camp and subsequent analysis revealed 17 of 28 animals tested were shedding *E. coli* O157. Samples taken from the field for microbiological analysis showed *E. coli* O157 present in soil, sheep faeces,

Table 1

Dose response data sets from human and animal feeding studies

<i>Shigella</i> in humans		<i>E. coli</i> O157 in rabbits ^a		EPEC in humans ^a	
Dose ^b	+/Total ^c	Dose	+/Total	Dose	+/Total
^d 10	1/10	Control	0/7	^e 10 ⁶	0/4
^d 200	2/4	10 ⁵	1/3	^e 10 ¹⁰	3/5
^d 2000	7/10	10 ⁶	2/5	^f 10 ⁸	0/5
^d 10 ⁴	5/6	10 ⁷	5/5	^g 10 ⁶	1/5
^h 10 ⁴	1/4	10 ⁸	12/13	^g 10 ⁸	1/5
^h 10 ⁵	3/4	10 ⁹	5/5	^g 10 ¹⁰	5/5
^h 10 ⁶	7/8	3×10 ⁹	2/2	ⁱ 5×10 ⁸	3/5
^h 10 ⁷	13/19	10 ¹⁰	6/6	ⁱ 2.5×10 ⁹	6/6
^h 10 ⁸	7/8			ⁱ 2×10 ¹⁰	2/2
^j 180	6/36				
^j 5000	33/49				
^j 10 ⁴	66/87				
^j 10 ⁵	15/24				

^a Rabbit data (Pai et al., 1986) and EPEC strains in humans (Levine et al., 1978).

^b Dose is the number of colony forming units ingested.

^c +/Total is the number of subjects infected with symptoms of disease divided by the total number exposed (attack rate).

^d *S. dysenteriae* data (Levine et al., 1973).

^e O127.

^f O128.

^g O142.

^h *S. flexneri* data (Dupont et al., 1969).

ⁱ B-171-8.

^j *S. flexneri* data (Dupont et al., 1972).

standing water and a climbing frame. Drinking water at the site and remaining food from the camp showed no presence of the pathogen. Isolates of *E. coli* O157 from animal, environmental and human sources were indistinguishable by pulsed field gel electrophoresis (PFGE). Heavy rainfall during the camping period caused localised flooding resulting in mud and faecal material being widespread. The likely route of *E. coli* O157 transmission was via hands contaminated with mud. Strachan et al. (2001) modelled the transfer of *E. coli* O157 from sheep to the soil and subsequently to humans and calculated the dose ingested to be between 4 and 24 organisms. This figure is based on the assumption that the number of organisms shed by individual sheep is averaged for the whole flock and that faeces (and hence *E. coli* O157) is evenly distributed in the upper soil layer. Furthermore, numbers shed by sheep were estimated some 3 weeks after the outbreak and were assumed to be unchanged over this period. However, this assumption was supported by subsequent calculations of surviving

Table 2
E. coli O157 outbreak data

Outbreak number and reference	Outbreak site	Vehicle	Estimated dose	Total number of subjects	Number of subjects infected ^a
1 (Strachan et al., 2001)	UK, New Deer	Sheep faeces/soil	14	228	20
2 (Nauta et al., 2001; Shinagawa et al., 1997)	Japan, Morioka	Salad/seafood sauce	31	871	215
3 (Keene and Sazie, 1997)	USA, Oregon	Deer jerky	10000	12	10
4 (Uchimura et al., 1997)	Japan, Kashiwa	Melon	1100	71	32
5 (Bell et al., 1994; Tuttle et al., 1999; The National Academy of Sciences 2002)	USA, Washington	Hamburger	23	5634	398
6 (Tilden et al., 1996)	USA, California/ Washington	Salami	23	2778	17
7 (Warmer et al., 1995)	USA, Illinois	Water	75 ^b	2350	12
8 (Anon. 1997)	UK, Wyre	Cheese	380	360	2

^a Secondary cases have been removed.

^b Estimated from total *E. coli* counts.

numbers in soil using accepted environmental decay rates (Wang et al., 1996). The mass of soil likely to be ingested during camping is based on a dry weight estimation (van Wijnen et al., 1990). Our figures are based on wet weight and may therefore slightly overestimate the bacterial load ingested.

2.2. Japan, Morioka

In September 1996, an outbreak of *E. coli* O157 occurred after a school lunch which infected 208 children and 7 adults (Nauta et al., 2001; Shinagawa et al., 1997). The exposed population was determined as 828 pupils and 43 teachers. Among 153 frozen-stored samples of raw food materials and cooked dishes, *E. coli* O157 was isolated from pumpkin salad and seafood sauce by immunomagnetic separation. No *E. coli* O157 was detected in the raw ingredients of the salad and sauce which suggests that contamination occurred during the preparation of the meal. Pathogen numbers were estimated by the most probable number method at 4–18 *E. coli* O157/100 g, a relatively narrow range which might suggest an even distribution throughout the food. The average dose ingested was estimated to be approximately 31 organisms per person.

2.3. USA, Oregon

In November 1995, 11 cases (6 confirmed and 5 presumptive) of gastroenteritis among members of

three households and two friends (12 in total) in an Oregon community were associated with consumption of contaminated homemade venison jerky (Keene and Sazie, 1997). All but one of the cases (an infant that was presumably infected by person-to-person transmission) had eaten the jerky with some individuals consuming >500 g over a period of several days. The deer used to prepare the jerky was shot and eviscerated in the field, hung outdoors at ambient temperatures (1 °C to 16 °C) for 5 days, and dismembered on the family's band saw. Meat (approximately 10 kg) was cut into thin strips and marinated in a refrigerator, then dried in several batches in a food dehydrator at 51.7 °C to 57.2 °C for 12–14 h per batch. *E. coli* O157 was isolated from two leftover pieces of jerky and were indistinguishable by PFGE from human isolates. Counts of *E. coli* O157 in the jerky specimens were 3–93 cfu/g (mean of approximately 50/g) and we estimated an average of 200 g consumed resulting in a dose of 10,000 organisms.

2.4. Japan, Kashiwa

During the summer of 1997, an outbreak of *E. coli* O157 occurred at a daycare centre where a total of 71 people ate contaminated melon and 28 children and 4 adults were identified as infected (Uchimura et al., 1997). Frozen foods eaten on the premises and microbiological swabs taken from the kitchen were analysed. Melon served at lunch was found to contain

E. coli O157 and isolates from both patients and melon were all VT1 and VT2 positive and had identical RAPD-PCR patterns. The implicated food was found to contain 43 cfu/g *E. coli* O157 and it was estimated that approximately 1.1×10^3 organisms were ingested by each person in a 25-g melon piece served per child.

2.5. USA, Washington

Between November 1992 and February 1993, an outbreak of *E. coli* O157 involving more than 700 cases occurred in the western USA and was associated with eating undercooked ground beef patties at restaurants of a major fast-food chain (Bell et al., 1994; Tuttle et al., 1999). Bell et al. (1994) designated 398 primary cases in Washington. Isolates of *E. coli* O157 obtained from recalled ground beef patties epidemiologically associated with the outbreak were indistinguishable by PFGE from those isolated from patients. Seventy-six of the ground beef patties were assayed quantitatively for *E. coli* O157:H7 using the most probable number method. Contamination of uncooked patties ranged from <0.3 to 15 *E. coli* O157/g (median 1.5 organisms/g). Each patty weighed 45 g, hence the number of *E. coli* O157 per patty ranged from <13.5 to 675 (median 67.5 organisms/patty). It was calculated (Powell et al., 2000) that the median number of *E. coli* O157 in the served undercooked burgers was approximately 23 organisms. Errors in bacterial numbers in this case would also include those associated with inactivation due to cooking. The USDA risk assessment of *E. coli* O157 in ground beef (The National Academy of Sciences, 2002) when considering this outbreak estimated the number of contaminated patties to be 5634 when taking into account under-reporting.

2.6. USA, California/Washington

In November 1994, an outbreak of 17 cases of *E. coli* O157:H7 infection in California and the state of Washington was associated epidemiologically with consumption of pre-sliced, dry, fermented salami (Tilden et al., 1996). The salami was produced at a single facility in California in 310-lb (approximately 141 kg) batches. *E. coli* O157:H7

was isolated from intact packages of the implicated salami obtained at retail stores, with isolates from patients and the salami having indistinguishable PFGE profiles. Enumeration of *E. coli* O157:H7 in implicated salami samples by most probable number determination revealed uniformly low-level contamination, ranging from 0.3 to 0.4 cfu/g. The estimated quantity of salami consumed by four case patients ranged from 6 to 113 g (we assumed 50-g portions in the model which equates to an exposure of 2778 people), with the calculated number of *E. coli* O157:H7 organisms consumed ranging from 2 to 45 bacteria (with an average of 23).

2.7. USA, Illinois

During the summer of 1995 there was an outbreak of *E. coli* O157 in Rockford, Illinois, where 12 people were infected (Warrner et al., 1996). Epidemiological investigations revealed no common food source, but it was established that those ill had visited a lake swimming beach on June 24–25th. Between 2200 and 2500 people were estimated to have visited the beach area on these days but subsequent analysis of water samples, taken after the lake had been closed to the public, failed to find *E. coli* O157. However, routine *E. coli* tests from two water samples collected 4 days before the outbreak were found to contain levels of 600 and 900 per 100 ml respectively (mean 750 per 100 ml). Retrospective testing (July 10th) showed *E. coli* levels at >500 per 100 ml. No *E. coli* O157 were isolated from the faeces of waterfowl roosting on the lake and there were no cattle farms or sewage outlets nearby suggesting the source of *E. coli* O157 could be bathing humans themselves. It is our experience that humans rarely excrete pure cultures of *E. coli* O157 and faeces usually include other coliforms and non-pathogenic *E. coli*. We estimate for this outbreak that a proportion (10%) of the commensal *E. coli* in the lake were serotype O157, based on personal observations from clinical samples of human *E. coli* O157 infections. We also assume that the quantity of water ingested whilst swimming was of the order of 100 ml per person bathing (Haas et al., 2000). Hence, estimated number of *E. coli* O157 ingested per person is 75 (0.1×750). Our estimates are quite clearly stated as such, and are backed by personal experience within medical microbiology.

Table 3
Beta-Poisson model parameterisation for previous dose response studies

Model	α	β	Y	Points
<i>Shigella</i> human	0.162	15.86	24.0	13
<i>E. coli</i> O157 rabbit	0.487	1.81×10^5	3.1	8
EPEC human	0.221	3.11×10^6	11.2	9

2.8. UK, Wyre

During October/November 1997, five cases of gastroenteritis in N.W. England were linked with the consumption of cheese made from unpasteurised milk. Two cases had consumed the same brand of cheese manufactured by a local producer. Microbiological testing revealed both had *E. coli* O157, with the same genetic profile (PFGE) as strains isolated from the cheese. In addition, the same strain was found in a rectal swab from an animal housed on one farm supplying milk for this particular type of cheese. The organism was isolated from 10 out of 11 samples taken from two truckles (9-kg cheeses) produced in late August 1997. Numbers estimated by MPN were 5–10 *E. coli* O157/g (Anonymous, 1997). Although three more human cases in N.W. England were infected with *E. coli* O157 at this time, there was no proven link to buying or eating the suspect cheese. The number of people who consumed the contaminated cheese was 360 (18 kg (two 9 kg cheeses)/50 g eaten) and for this study it was estimated that 25–50 g portions are consumed per person per meal and that the two 9 kg cheeses were evenly contaminated.

In order to fit the outbreak data to the dose response models these data were normalised by giving 1/8 th weight to each of the 8 outbreaks. This ensures that the large outbreaks do not dominate the likelihood function and hence fitting of the dose response parameters. This approach is the same as that used for a *Salmonella* outbreak dose response model (Fazil et al., 2000).

2.9. Dose response modelling

The exponential dose response model is a “single hit” model where only one organism is required to cause infection and all organisms are independent. Given a dose of D organisms with a Poisson

distribution and given that each organism has a probability p_m of surviving to cause infection then the probability (π_1) of the host becoming infected can be calculated from:

$$\pi_1 = 1 - e^{-p_m D} \quad (1)$$

Heterogeneity between the pathogen and the host can be incorporated using a beta distribution $B(\alpha, \beta)$

$$\pi_1 = 1 - \int_0^1 e^{-p_m D} B(\alpha, \beta) dp_m \quad (2)$$

Integrating out p_m in the above equation results in a confluent hypergeometric function. This model is the exact beta-Poisson model:

$$\pi_1(D, \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta, -D) \quad (3)$$

An approximation to this model holds when $\beta \gg 1$ and $\alpha \ll \beta$ and this is known as the approximate beta-Poisson model.

$$\pi_1 = 1 - \left[1 + \frac{D}{\beta} \right]^{-\alpha} \quad (4)$$

The maximum likelihood method is used to fit dose response data to the models described above by minimising the deviance (Y) (see Appendix 1). Appropriate confidence intervals can be generated by Markov Chain Monte Carlo methods (see Appendix 1). All calculations were performed using Mathematica (Version 5.0, Wolfram Research, Canada).

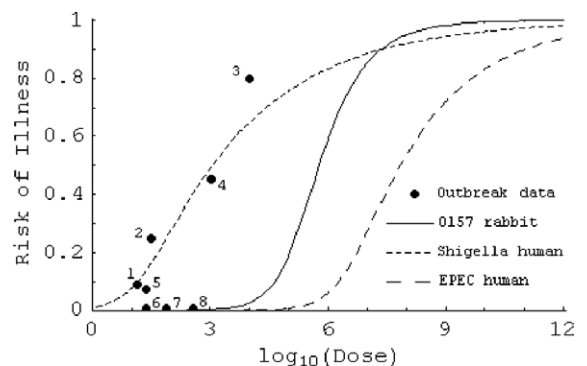


Fig. 1. Beta-Poisson dose response models for animal/human-feeding studies with outbreak data superimposed ([1] UK, New Deer, [2] Japan, Morioka, [3] USA, Oregon, [4] Japan, Kashiwa, [5] USA, Washington, [6] USA, California/Washington, [7] USA, Illinois and [8] UK, Wyre).

Prior work (Crockett et al., 1996; Haas et al., 2000; Powell et al., 2000), using a binomial likelihood function have demonstrated that the approximate beta-Poisson dose response model provides a suitable fit to the human- and animal-based feeding study data presented in Table 3 and Fig. 1.

Here, we determine the fit of the exponential, exact and approximate beta-Poisson dose response models to the *E. coli* O157 outbreak data using both binomial and beta-binomial likelihood functions.

3. Results and discussion

Figs. 2 and 3 and Table 4 give the results of fitting the dose response models to these outbreak data. A best fit (i.e., minimum deviance) model was not found for the approximate and exact beta-Poisson using the binomial likelihood. Similar problems in fitting the approximate beta-Poisson model using a binomial likelihood to foot and mouth disease data have been reported by French et al. (2002).

Best fit models were obtained for the exponential model for both formats of the likelihood. These models are similar but with the beta-binomial likelihood having slightly broader confidence intervals. Although the best fits were not significant, when comparing the deviance with the critical values of the χ^2 distribution, the application of the beta-binomial likelihood considerably reduced the deviance (from 1604 to 56) compared with using a binomial likelihood. Similarly, both the exact and approximate beta-Poisson models had considerable reductions in the best fit deviance values for the beta-binomial compared with the binomial likelihood.

The overall best fit is the exact beta-Poisson with beta-binomial likelihood. Figs. 2 and 3 demonstrate that the approximate version of this model is virtually identical to it. This exact model has a significant reduction in deviance compared with its exponential counterpart but is still not a statistically significant fit (best fit $Y > \text{critical } \chi^2$). However this must be reconciled with the following: the variation in microbial data obtained from outbreaks will certainly be larger than in controlled feeding studies due to the use of differing enumeration methods, each with different sensitivities; uneven distribution of the pathogen within the food; the actual numbers of *E. coli* O157

in a sample may change prior to consumption due, for example, improper storage at high temperatures or to partial cooking and numbers of *E. coli* O157 in food and the exact quantity of food ingested can usually not be definitively calculated.

Comparing the exact beta-Poisson beta-binomial outbreak model with the previously used surrogate dose response models for *E. coli* O157, it is apparent that the *Shigella* model is contained within the confidence intervals. In fact the *Shigella* model is very similar to the median model. This is in contrast with the *E. coli* O157 rabbit model which underestimates the risk of illness for doses $<10^5$ and the EPEC model which underestimates the risk for doses $<10^7$. The dose response envelope proposed by Powell et al. (2000) with the lower band limit represented by the *Shigella* model and the upper band by EPEC appears to underestimate the lower band (at doses $<10^3$) and overestimates the upper band. However, further outbreak data points are required to verify this. The current data points represent those outbreaks where data have been collected and made available. The authors see no reason why these data may be biased (e.g., to more virulent strains of the pathogen). This can only be assessed when future data become available.

A number of outbreaks have occurred where either the attack rate or pathogen numbers are unknown and it is useful for epidemiological purposes to calculate the missing co-ordinate using the outbreak dose response model. For example, an unusually large number of cases of *E. coli* O157 in a NE Scotland primary school arose in June 1999 (Grampian Health Board, 2000). Cheese from unpasteurised goats' milk was suspected, made for a school project where small amounts (<30 g) were consumed in the classroom. As all the cheese was eaten, none remained for pathogen detection or enumeration although the attack rate was estimated with some accuracy in the confined community. Laboratory investigations isolated strains of *E. coli* O157 from a number of animals at the farm including the goat. Isolates had the same PFGE pattern as the patients. Of the 28 people who ate the cheese (restricted to one school class), only one was not infected and of the five who refused the cheese, none became unwell. Six children were asymptomatic which gave an attack rate of 0.75. The exact beta-Poisson model predicts a median dose with order of

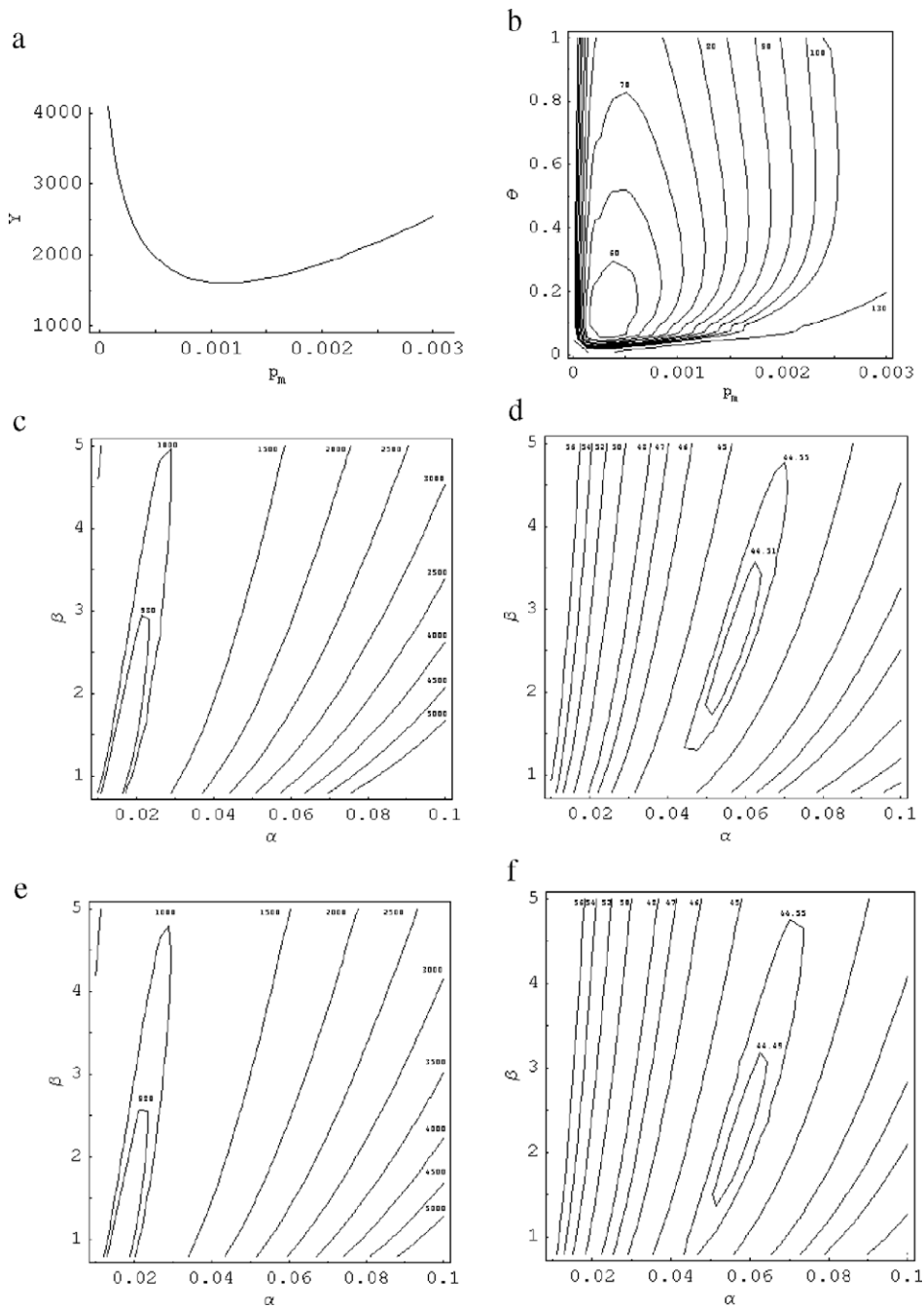


Fig. 2. Deviance plotted against dose response parameters for each of the following dose response models generated from the outbreak data. Exponential dose response model with (a) binomial likelihood (2-D graph with deviance Y plotted against the dose response parameter p_m) and (b) beta-binomial likelihood (contour graph of deviance Y plotted against dose response parameter p_m and likelihood overdispersion parameter θ). Exact beta-Poisson dose response model with (c) binomial likelihood (contour graph of deviance Y plotted against dose response parameters α and β) and (d) beta-binomial likelihood (contour graph of deviance Y plotted against dose response parameters (a) and (b) plotted at best fit value of θ). Approximate beta-Poisson dose response model with (e) binomial likelihood (contour graph of deviance Y plotted against dose response parameters α and β) and (f) beta-binomial likelihood (contour graph of deviance Y plotted against dose response parameters (a) and (b) and plotted at best fit value of θ).

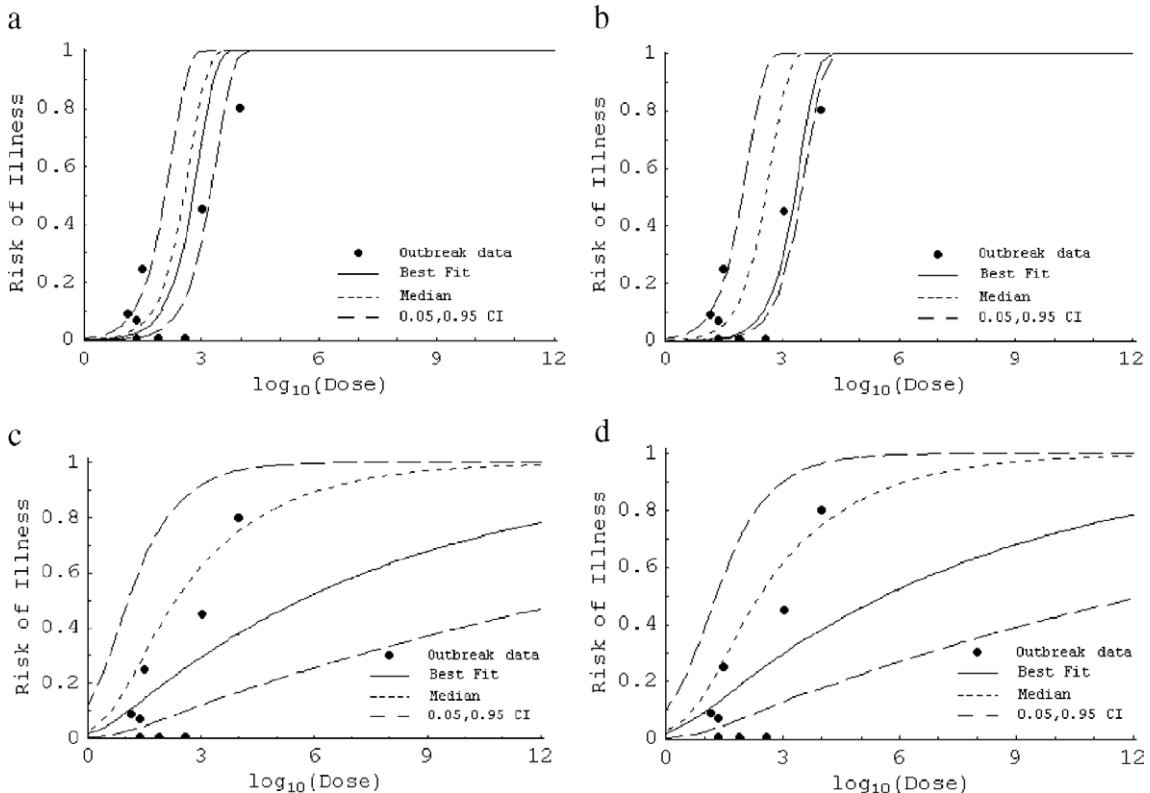


Fig. 3. Outbreak dose response models showing best fit and median models together with confidence intervals. (a) Exponential with binomial likelihood, (b) exponential with beta-binomial likelihood, (c) exact beta-Poisson with beta-binomial likelihood and (d) approximate beta-Poisson with beta-binomial likelihood.

magnitude 10^4 *E. coli* O157 or approximately 10^3 /g in the cheese which is plausible. Conversely, there may be occasions when the attack rate is unknown but pathogen numbers can be determined. If a food containing high numbers of *E. coli* O157 was distributed, a validated dose response relationship would help anticipate the size of the expected outbreak.

Scotland has approximately three times the rate of reported laboratory *E. coli* O157 infections compared with England and Wales, the USA or Japan (Table 5). The outbreak model can be used to predict the average dose ingested by each member of the population of a given country. These data show that on average a member of the population ingests only one organism every several hundred years. However, this must be reconciled with the fact that when infected food is eaten, tens of organisms are usually ingested and illness subsequently occurs. Recent surveillance reports show that although *E. coli* O157 can be found in the faeces of

approximately 1–5% of cattle (Paiba et al., 2003) with concentrations of up to as much as 10^6 /g (Omisakin et al. 2003), studies in uncooked red meat products show a lower prevalence of <1% (Chapman et al., 2001) and that the pathogen is eliminated if the food is cooked properly and hence it is not surprising that *E. coli* O157 is a rare disease in humans.

The plausibility of the dose response model could be further investigated by estimating the number of organisms ingested by different pathways of infection (e.g., via ground beef products) and determining the number of infections that should follow. This could then be compared with the number of cases reported by surveillance. Indeed the results of this type of analysis could be factored into the model as additional data points.

There is clearly the need for more outbreak data to validate our outbreak model further. The *Salmonella* dose response model formulated by outbreak data

Table 4
Dose response model fits to outbreak data

Model	Likelihood	Best fit parameters	Median fit parameters	Critical χ^2 , ^a	Deviance, Y (best fit)
Exponential	Binomial	$p_m=0.00113$	$p_m=0.00208$	14.07	1604.45
	Beta-	$p_m=0.000332$	$p_m=0.00155$	12.59	56.35
	Binomial	$\theta=0.119$	$\theta=0.479$		
Exact Beta	Binomial	No solution			
Poisson	Beta-	$\alpha=0.0565$	$\alpha=0.1635$	11.07	44.49
	Binomial	$\beta=2.5487$	$\beta=4.3682$		
		$\theta=0.3758$	$\theta=0.5890$		
Approximate Beta	Binomial	No solution			
Poisson	Beta-	$\alpha=0.0571$	$\alpha=0.2241$	11.07	44.47
	Binomial	$\beta=2.2183$	$\beta=4.8807$		
		$\theta=0.3750$	$\theta=0.5971$		

^a The deviance is required to be less than the critical χ^2 for a statistically significant fit.

(Fazil et al., 2001) has been greatly assisted by the directive in Japan which instructs large-scale cooking facilities to freeze 50-g portions of both raw food materials and cooked dishes for more than 2 weeks for possible future examination in the case of a food-poisoning incident. Moreover, it is interesting to note that kitchens with social responsibilities in Japan (e.g., schools and hospitals) have voluntarily adopted this scheme. This directive was released after the critical review of large outbreaks of *E. coli* O157 which occurred in Japan in 1996, when food samples were not stored resulting in the inability to detect and enumerate causative agents. If this directive was repeated worldwide, which may be feasible for large-scale suppliers of food, dose response outbreak data would become available not only for *E. coli* O157 but for the other

major foodborne pathogens as well. Of course, consideration would have to be made when storing these food samples to try and minimise the effects of the possible non-uniform distribution of the pathogen within a large batch of food.

The dose response models developed here will be of value in quantitative microbiological risk assessment and in particular those that utilise Monte Carlo simulation. For example the parameters given in Table 4 could be inserted into QMRA packages such as @RISK, Crystal Ball etc. If variation in the dose response data needs to be incorporated, it can be included by using a different set of the Metropolis generated dose response parameters for each Monte Carlo iteration. In addition the overdispersion (represented by θ) in the beta-binomial likelihood can also

Table 5
National incidence rates 2001 (except USA–2000) and predicted range of daily doses of *E. coli* O157 ingested per person calculated from the exact beta-Poisson outbreak model with beta-binomial likelihood using Markov Chain Monte Carlo samples of α , β and θ

Country	<i>E. coli</i> O157 infections per 100,000 persons per year	Daily risk per person per day	Outbreak dose response model predicted daily person intake Median with 95% confidence interval in brackets
USA	1.7 ^a	4.7×10^{-8}	1.1×10^{-6} (1.80×10^{-7} , 7.70×10^{-6})
Japan	1.3 ^b	3.6×10^{-8}	8.3×10^{-7} (1.35×10^{-7} , 5.90×10^{-6})
England and Wales	1.5 ^c	4.1×10^{-8}	9.4×10^{-7} (1.55×10^{-7} , 6.80×10^{-6})
Scotland	4.6 ^d	1.3×10^{-7}	3.0×10^{-6} (4.90×10^{-7} , 2.50×10^{-5})

^a (Anonymous, 2002a).

^b (Anonymous, 2002b).

^c (Anonymous, 2002c).

^d (Cowden et al., 2001).

be included so that outbreak situations can be simulated where, for example, a cohort of individuals have been exposed to a particular dose from a foodstuff. The dose response parameter sets, together with an example showing implementation of over-dispersion can be obtained from the authors by request.

4. Conclusion

This paper has demonstrated that dose response data from *E. coli* O157 outbreaks are most similar to the beta-Poisson dose response model developed for *Shigella* (Crockett et al., 1996). However, more outbreak data are required for further validation and the authors recommend that other countries follow the lead set by Japan in directing large-scale cooking establishments to store food portions for subsequent analysis in the event of food-poisoning outbreaks.

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Appendix A. Applying the maximum likelihood method to fit a model to dose response data

Given a set of t dose response data points where k_i of n_i subjects are infected after ingesting a dose d_i . We can define $\pi_i^0 = k_i/n_i$ and can calculate the predicted response of the model $\pi_i = \pi_i(d_i; \Theta)$, where Θ are the set of dose response parameters. Derived below are the likelihood and deviance functions assuming that the dose response data are (i) binomial and (ii) beta-binomial distributed.

A.1. Binomial

The binomial likelihood is

$$\ell = \prod_i \frac{n_i!}{k_i!(n_i - k_i)!} (\pi_i)^{k_i} (1 - \pi_i)^{n_i - k_i} \tag{5}$$

this can be compared with the binomial likelihood supremum

$$\ell_{\text{sup}} = \prod_i \frac{n_i!}{k_i!(n_i - k_i)!} (\pi_i^0)^{k_i} (1 - \pi_i^0)^{n_i - k_i} \tag{6}$$

The best fit is obtained by minimising the deviance

$$Y = -2 \ln \left(\frac{\ell}{\ell_{\text{sup}}} \right) \tag{7}$$

substituting Eqs. (5) and (6) into Eq. (7) yields

$$Y = -2 \sum_{i=1}^t \left[k_i \ln \left(\frac{\pi_i}{\pi_i^0} \right) + (k_i - n_i) \ln \left(\frac{1 - \pi_i}{1 - \pi_i^0} \right) \right] \tag{8}$$

A.2. Beta-binomial

The beta-binomial likelihood is

$$\ell = \prod_i \frac{n_i!}{k_i!(n_i - k_i)!} \frac{b \left(k_i + \frac{\pi_i}{\theta}, n_i - k_i + \frac{1 - \pi_i}{\theta} \right)}{b \left(\frac{\pi_i}{\theta}, \frac{1 - \pi_i}{\theta} \right)} \tag{9}$$

Where $b()$ is the mathematical beta function and θ is a parameter which represents the degree of overdispersion. The likelihood can be compared with the likelihood supremum and from this the deviance can be determined using Eq. (9).

$$Y = -2 \sum_{i=1}^t \left[\ln \frac{b \left(k_i + \frac{\pi_i}{\theta}, n_i - k_i + \frac{1 - \pi_i}{\theta} \right)}{b \left(\frac{\pi_i}{\theta}, \frac{1 - \pi_i}{\theta} \right)} - [k_i \ln(\pi_i^0) + (k_i - n_i) \ln(1 - \pi_i^0)] \right] \tag{10}$$

The minimum deviance for each model was determined using a Markov Chain Monte Carlo minimising model. Median dose response models and confidence intervals were generated using an algorithm based on the Metropolis Markov Chain Monte Carlo method (Metropolis et al., 1953).

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