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A reconsideration of the *Campylobacter* dose–response relation

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SUMMARY

As a major foodborne pathogen, *Campylobacter jejuni* receives much attention in quantitative risk assessment. To date, all dose–response assessments have been based on a single human feeding study which unfortunately provides incomplete and possibly biased information on the dose–response relation. An incident at a dairy farm, where several children from a school class became ill as a result of drinking raw milk contaminated with *C. jejuni*, appeared to show a very clear dose–response relation between the amount of milk consumed and the attack rate. This relation was very nearly exponentially shaped and, therefore, seemed to conflict with the rather slowly rising dose–response relation established in the feeding study. Here we show that both datasets can be reconciled when illness and infection are considered separately. This not only provides new information on the illness dose–response relation for *Campylobacter*, but also amends the infection dose–response relation because of their conditional dependence.

INTRODUCTION

Campylobacter jejuni is a very common human pathogen causing substantial health and economic losses to society and as a result this food- and waterborne bacterium prominently features in microbial risk assessment.

Somewhat in contrast with its significance to public health, dose–response information on *Campylobacter* infection in humans is scarce. Results from a single human feeding study are available [1, 2] but unfortunately only high doses, resulting in high attack rates were applied. Therefore, the low-dose behaviour of *Campylobacter* especially is not well known [3, 4], but its success as a parasite suggests high infectivity.

Dose–response information on the probability of acute illness resulting from *Campylobacter* infection is also not well known. Results from the same human feeding study mentioned above show a decreasing probability of illness with increasing dose [1, 5]. Although this has been established in a statistical sense, many risk scientists are not at ease with such a

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dose–response relation and new observational data are very much needed.

A few years ago, a small outbreak of campylobacteriosis was reported in the United Kingdom [6]. Illness appeared to be linked to the consumption of raw milk, and a very clear dose–response relation was found. Recently a similar incident took place in The Netherlands: during a farm visit children consumed raw milk and subsequently became ill [7–9]. Here too, a clear relation of the attack rate with the amount of milk consumed could be established. Unfortunately, in both case studies the investigating scientists were denied a sample of the contaminated milk and the concentration of bacteria in the contaminated milk could not be determined. As a result we only have relative dose information.

Our goal here is to show that this outbreak information can be used to obtain improved insight in the dose–response relationship for *Campylobacter*, both for illness and for infection. We will approach this problem by investigating whether and how the epidemiological outbreak data are consistent with the published dose–response models based on the data of Black et al. [1].

DATA

We assume that bacteria are suspended homogeneously in the raw milk storage, so that their occurrence may be described by a Poisson distribution with a fixed concentration. The quantity of milk consumed was expressed as numbers of cups; in the Dutch study [8] consumption of a small quantity (one draught) was also recorded. It is assumed that one cup contains the equivalent amount of six draughts: children who had one cup of contaminated milk were exposed to six times as many bacteria as those who only had one draught. One cup of milk is assumed to correspond to a volume of 0·188 l.

A case was defined as a person with diarrhoea or vomiting combined with two or more other symptoms, e.g. fever (>40 °C), abdominal pains, nausea. Median period between exposure and appearance of symptoms was 3 days [8]. Three adult teachers also drank unpasteurized milk, but none of them became ill. The children were aged between 8 and 13 years.

In the English study cases suffered from abdominal pain, diarrhoea, fever and/or vomiting. Median incubation period was 4 days [6]. Child cases were 3 or 4 years old, there were also three adult cases.

METHODS

Likelihood supremum

The recorded responses are binary (ill or not ill) so that the likelihood is binomial and models can be tested against a binomial likelihood supremum.

The binomial likelihood supremum gives the maximum value of the (log)-likelihood for the observed responses, without any constraints, by setting the probabilities at all observed doses equal to the observed fractions [10]

$$l_{\text{sup}} = -2\log\left[\prod_{\forall i}\left(\frac{k_i}{n_i}\right)^{k_i}\left(1-\frac{k_i}{n_i}\right)^{n_i-k_i}\right] \tag{1}$$

when in dose group i, k_i of n_i subjects are infected.

Illness cases who were not exposed to milk

To account for the cases who had not been exposed to raw milk, we assume an alternative route of transmission causing illness with an equal probability p_0 to all children who visited the farm.

If P_{milk} (CV_{ing}) is the probability of campylobacteriosis due to ingestion of V_{ing} l milk with a concentration C c.f.u./l (dose CV_{ing} c.f.u.), the total probability of illness is

$$P_{\text{ill}} = 1-(1-p_0)(1-P_{\text{milk}}(CV_{\text{ing}})). \tag{2}$$

Exponential model for illness dose response

The exponential dose–response relation

$$f(CV_{\text{ing}}|p_m) = 1-\mathrm{e}^{-p_m CV_{\text{ing}}} \tag{3}$$

can be used to model the illness dose–response relation. Because this is a scalable relation, any change in dose can be compensated for by a change in the infectivity parameter p_m [4], the binomial likelihood can be calculated without knowing the pathogen concentration. The likelihood function becomes

$$l_{\text{exp}}(C_i p_m) = -2\log \times\left[\prod_{\forall i} f(CV_{\text{ing},i}|p_m)^{k_i}(1-f(CV_{\text{ing},i}|p_m))^{n_i-k_i}\right] \tag{4}$$

using the same notation as above for exposed and infected subjects.

Beta Poisson model for infection dose response

The exponential relation can also be used to model the infection dose–response relation. It can be considered a special case of the 'single-hit' model for microbial infection, in the absence of heterogeneity [4]. In general, the beta Poisson dose–response model† can be used

$$f(CV_{\text{ing}}|\alpha,\beta)=\int_{p_m=0}^{1}\frac{p_m^{\alpha-1}(1-p_m)^{\beta-1}}{B(\alpha,\beta)}(1-\mathrm{e}^{-p_m CV_{\text{ing}}})\mathrm{d}p_m$$
$$=1-{}_1F_1(\alpha,\alpha+\beta;-CV_{\text{ing}}). \quad (6)$$

This model incorporates heterogeneity into host–pathogen interaction by using a beta distribution for the infectivity parameter p_{m} [4]. Parameters can be estimated using a binomial likelihood function, as given above for the exponential case.

Conditional illness dose response

Whilst infection may be asymptomatic, the occurrence of illness symptoms can be considered conditional on infection: without infection, we assume illness does not occur. The (unconditional) probability of illness can be calculated as the product of the probability of infection and the conditional probability of illness given infection.

Usually, this conditional illness probability is treated as a fixed quantity. For some pathogens, however, there appears to be evidence indicating a dose-dependent response for illness (among infected subjects). We have previously described such conditional dose–response relations with a model based on illness hazard during infection [5]. Both sets of observations in Table 1 indicate increasing probability of illness with dose, saturating at levels close to 1. This means that, conditional on infection, the dose–response relation for illness can only increase with dose: a constant probability would merely scale down the infection dose–response relation to a level below 1, and a decreasing relation would lead to decreasing numbers of illness cases at high doses. Both alternatives do not seem to occur here. We, therefore, choose a conditional illness dose–response model which increases with applied dose. Teunis et al. [5] argued that the conditional probability of illness depends on two properties: the duration of infection (i.e. the time period colonization exists) and the hazard of becoming ill when colonized. The longer infection persists, the higher the probability of becoming ill. Under mild assumptions (gamma-distributed duration of infection and linearly increasing illness hazard with dose) the conditional illness dose–response model simply becomes

$$h(CV_{\text{ing}}|r,\eta)=1-(1+\eta CV_{\text{ing}})^{-r}, \quad (7)$$

with parameters η and r.

Consequently, a higher dose not only leads to an increased probability of infection, but also to an increase in the probability of becoming ill after infection has occurred. As argued before [5], a possible explanation for such a somewhat surprising observation is that a higher initial dose allows the pathogen numbers to quickly reach levels that are damaging to host tissues, before host defences can slow down growth sufficiently to prevent tissue damage.

The (unconditional) dose–response relation for illness can be written as the product of the infection and illness dose–response functions

$$f(CV_{\text{ing}}|\alpha,\beta)h(CV_{\text{ing}}|r,\eta)=$$
$$[1-{}_1F_1(\alpha,\alpha+\beta_i-CV_{\text{ing}})][1-(1+\eta CV_{\text{ing}})^{-r}], \quad (8)$$

parameters can be estimated again with a binomial likelihood function.

Table 1. *Data of van der Brondhof et al.* [8] *and Evans et al.* [6]

Amount consumed	Ill	Exposed	Percentage ill
van den Brandhof et al.			
None	2	35	6
1 Draught	2	12	17
$\frac{1}{2}$ Cup	7	18	39
1 Cup	13	21	62
2 Cups	6	6	100
Evans et al.			
None	2	17	12
$\frac{1}{2}$ Cup	3	7	43
1 Cup	14	21	67
2 Cups	4	5	80

† Here we give the exact equation, involving a Kummer confluent hypergeometric function [4]; an approximation

$$f(CV_{\text{ing}}|\alpha,\beta)=1-{}_1F_1(\alpha,\alpha+\beta;-CV_{\text{ing}})$$
$$\approx 1-\left(1+\frac{CV_{\text{ing}}}{\beta}\right)^{-\alpha} \quad (5)$$

has become more widely known [11, 12]

Combining information from volunteer and outbreak studies

In the outbreak studies we have observations of illness cases. As these ill subjects must also have been infected, these observations provide us with information on infection, as well as illness probabilities.

If we assume that the dose–response relations for infection (including asymptomatic cases) for the outbreak data and the volunteer data are the same, we can combine dose–response models for infection and illness into a single likelihood function:

$$\begin{aligned} l_{\text{inf, ill}}(C,\, p_0, \alpha, \beta, r, \eta) = \\ -2\log\left[\prod_{\forall i} f(D_i|\alpha,\beta)^{k_i}(1-f(D_i|\alpha,\beta))^{n_i-k_i}\right] \\ -2\log\left[\prod_{\forall j} g(CV_{\text{ing},j}|p_0,\alpha,\beta,r,\eta)^{k_j}\right. \\ \left.\times(1-g(CV_{\text{ing},j}|p_0,\alpha,\beta,r,\eta))^{n_j-k_j}\right]. \end{aligned} \tag{9}$$

The first part of this likelihood function uses the information from the volunteer data [1], with known dose (D_i) and only the infection dose–response model as infection has been observed directly. The second part describes the information from the outbreak data, using the same infection dose–response model and the conditional illness dose–response model for the observed illness cases. A fraction (p_0) again takes care of illness cases attributed to other causes than exposure to contaminated milk. The dose–response model for the outbreak data then is

$$\begin{aligned} g(CV_{\text{ing}}|p_0,\alpha,\beta,r,\eta) = \\ 1-(1-p_0)[1-f(CV_{\text{ing}}|\alpha,\beta)h(CV_{\text{ing}}|r,\eta)]. \end{aligned} \tag{10}$$

Parameter estimation and uncertainty analysis

Parameter estimation for the hypergeometric model is improved by transformation to

$$\left.\begin{aligned} u &= \alpha/(\alpha+\beta) \\ v &= \log(\alpha+\beta) \end{aligned}\right\} \tag{11}$$

so that we are estimating the mean value (u) of the beta distribution for p_m and a quantity that is inversely related to its variance (for very large positive values of v the variance tends to zero).

Similarly, parameter estimation for the illness model is improved by the transformation

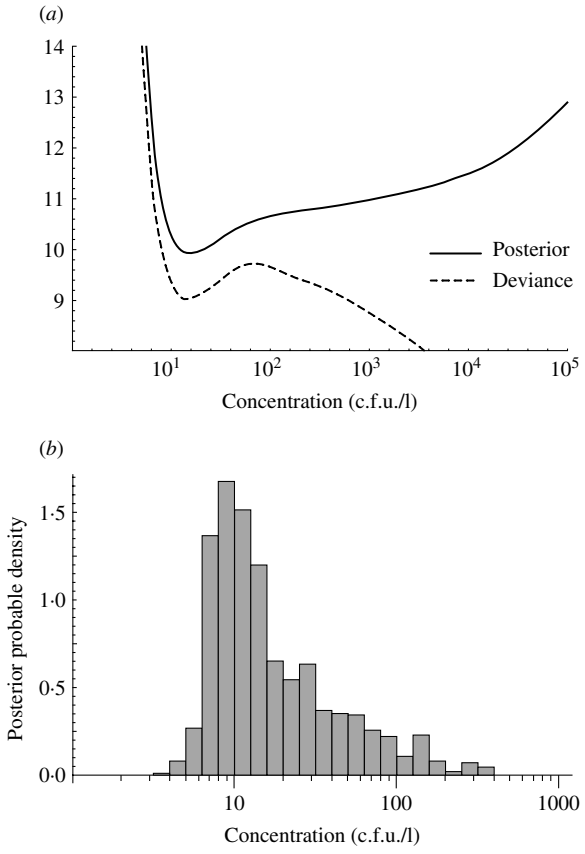

Fig. 1. Combined model for infection and illness dose response. (*a*) Shape of the deviance function (keeping all other parameters at their optimum values) to show the local optimum near a concentration of 15·2 c.f.u./l. Also shown (−2 times the log of) the posterior density, shifted arbitrarily to align with the deviance function. (*b*) Uncertainly in estimated concentration.

$$\left.\begin{aligned} w &= \log(r\eta) \\ z &= \log(r/\eta). \end{aligned}\right\} \tag{12}$$

In addition to this, both u and p_0 are logit-transformed so that they can be estimated on an interval $(-\infty, \infty)$.

We use non-informative normal (0, 10) priors for all transformed parameters. Priors for u, v, w, z, and p_0 are assumed to be uncorrelated. For the log-transformed concentration of bacteria in milk a normal prior was used, located at 10 times the local optimum (152 c.f.u./l), with 4 S.D. to curb the increase in likelihood at concentrations >100 c.f.u./l (see Fig. 1*a*).

The product of the likelihood and the joint prior parameter distributions is the joint posterior distribution for (u, v, w, z, p_0). Posterior mode (maximum posterior probability) parameter values can be found by direct numerical optimization of the posterior probability.

Table 2. *Likelihood ratio test for difference in dose response of the two outbreak studies used*

Study	l_{sup}
van den Brandhof et al. [8]	78·113
Evans et al. [6]	53·614
Sum ($l_{\text{sup},1}+l_{\text{sup},2}$)	131·727
Pooled ($l_{\text{sup},1+2}$)	134·117
Difference ($\triangle l_{\text{sup}}$)	2·390

The difference $\triangle l_{\text{sup}}=l_{\text{sup},1+2}-l_{\text{sup},1}-l_{\text{sup},2}$ may be considered (asymptotically) a χ^2 deviate with $5+4-5=4$ (difference in numbers of dose groups) degrees of freedom, and is not significant [$\chi^2_{0\cdot95}(4)=9\cdot49$].

Bayesian uncertainty estimates were calculated using a Markov chain Monte Carlo [4]. Markov chains were constructed with the algorithm of Metropolis and Hastings [13], implemented in Mathematica® [14]. To improve the speed of calculations, values for the confluent hypergeometric function were calculated by means of a series approximation (adapted from NetLib; Source code available from the corresponding author). This was implemented as an external application, via MathLink® [15].

RESULTS

Agreement of the two outbreak studies

The outcomes of both outbreak studies [6, 8] can be compared by looking at their respective binomial likelihood supremum (l_{sup}) values, and comparing these with the same quantity for the pooled data: adding all observations with equal ingested volumes of milk.

We see that when the fractions from the pooled data are used, this only leads to an insignificant decrease in (log)-likelihood (Table 2). We conclude that the shapes of the two observed dose–response relations are quite similar.

Obviously this tacitly assumes that similar ingested amounts imply similar doses: the concentrations of bacteria in milk are assumed equal (at least close) for both outbreaks. The similarity of these two dose–response relations offers some support for this assumption. Not only are the two observed dose–response relations very similar in shape, they also involve virtually the same amounts of contaminated milk.

Table 3. *Exponential dose–response model fitted to the outbreak data*

Study	Dev	$\hat{p}_0$	$\chi^2_{0\cdot95}$ (D.F.)
van den Brandhof [8]	1·905	0·053	9·488
Evans et al. [6]	0·168	0·119	7·815
Pooled	0·408	0·072	9·488

Deviance (Dev) difference in -2 log-likelihood from a (unconstrained binomial) likelihood supremum ($l-l_{\text{sup}}$).

Shape of the illness dose–response relation

Likelihood analysis indicates that the observed dose–response relation for campylobacteriosis is not significantly different from an exponential model (deviances in Table 3) are smaller than $\chi^2_{0\cdot95}$ with the appropriate number of degrees of freedom). This is true for both outbreaks, and when we pool the two datasets by simply grouping all cases that consumed the same amounts of milk, the dose–response relation for the combined data also appears exponential in shape.

The concentration of bacteria in the milk is not known. However, since the exponential dose–response relation always has the same shape, irrespective of the dose scale – the probability of becoming ill depends only on the product of dose and a single parameter – we can study their agreement with this dose–response model without knowing the exact dose.

Illness model: reconciliation of with the volunteer data

Naturally we would like to know what could have been the most likely dose in these two outbreaks. Since we do know the amounts of milk consumed by the exposed subjects, we need to attempt to infer the concentration of the bacteria, to determine the position of the exponential relation on the dose axis. The dose–response relation from the volunteer study might be useful, but this relation gives the probability of infection as a function of the dose, including asymptomatic cases. To employ this model we need to translate infection-to-illness probabilities. The conditional (hazard) model for dose response of illness given infection can be used here to augment the analysis.

First, we applied the conditional illness dose–response model to the separate outbreak data, using the (hypergeometric) beta Poisson model on the Black et al. [1] data to calculate the probability of infection.

Table 4. *Infection and illness dose–response models fitted jointly to outbreak data and volunteer data* [*1*], *assuming a concentration of 100 c.f.u./l in the contaminated milk which caused the two outbreaks of compylobacteriosis*. [*Also shown* (*bottom rows of pooled data*): '*optimum*' *concentration* (*15·22 c.f.u./l*) *and equivalent concentration* (*537·9 c.f.u./l*) *with corresponding parameters*.]

Study	Conc. (c.f.u./l)	Dev	$\chi^2_{0\cdot95}$ (D.F.)	
van den Brandhof et al. [8]	100	10·554	12·592	
Evans et al. [6]	100	5·423	11·071	
Pooled	100	9·547	12·592	
	15·22	9·055	12·592	
	537·9	9·055	12·592	
Study	Conc. (c.f.u./l)	$\hat{p}_0$	$(\hat{\alpha}, \hat{\beta})$	$(\hat{r}, \hat{\eta})$
van den Brandhof et al. [8]	100	0·058	(0·050, 0·038)	$(2\cdot13\times10^{-7}, 5\cdot02\times10^{5})$
Evans et al. [6]	100	0·136	(0·068, 0·081)	$(6\cdot27\times10^{-5}, 2\cdot78\times10^{3})$
Pooled	100	0·077	(0·038, 0·022)	$(8\cdot13\times10^{-7}, 1\cdot23\times10^{5})$
	15·22	0·081	(0·024, 0·011)	$(3\cdot63\times10^{-9}, 2\cdot44\times10^{8})$
	537·9	0·077	(0·057, 0·047)	$(1\cdot84\times10^{-7}, 1\cdot06\times10^{5})$

However, we did not fix the infection dose–response parameters at the values optimized for the volunteer data. Instead, we let the outbreak data help in determining the infection dose–response relation since illness, which can be observed, is conditional on infection. Hence the dose–response relation for infection is determined by both the outbreak data and the data from the Black et al. study [1], whereas the illness dose–response relation (conditional on infection) is determined by the outbreak data alone. If we choose a concentration (of bacteria in milk) we can again determine all parameters in the model in a straightforward manner.

We can see that at a (arbitrarily chosen) concentration of 100 c.f.u./l the infection dose–response parameters $(\hat{\alpha}, \hat{\beta}) = (0{\cdot}038,\ 0{\cdot}022)$, are quite different from those originally found for just the volunteer data of Black et al. [1]: (0·145, 7·59) [4]. The presence of a (small) fraction of cases who cannot be directly attributed to consumption of contaminated milk might influence model fitting by allowing for a dose-related illness risk <1. This can be investigated by removing the unexposed cases and setting the parameter $p_0 = 0$. When the combined infection–illness model is then fitted again at a concentration of 100 c.f.u./l, the minimum deviance is (again from an unconstrained supremum) 9·83, which is still not significant $[\chi^2_{0\cdot95}(4) = 12{\cdot}592]$. Therefore, the agreement between the outbreak data and the volunteer data is not dependent on incorporating this fraction p_0.

If we use the combined likelihood function to calculate maximum likelihood (minimum deviance) values as a function of the concentration we see an interesting profile shown in (see Fig. 1*a*). At high concentrations, the probability of infection is always near 1, and the shape of the outbreak dose–response relation is attributed completely to the illness dose–response relation. This is increasingly true at higher concentrations, and the deviance decreases steadily with increasing concentration (the likelihood increases).

At very low concentrations the unconditional probability of illness (the product of the infection and conditional illness dose–response models) cannot increase beyond the probability of infection. The latter probability is limited by the probability of exposure [4] and cannot reach high levels at concentrations <1 c.f.u./cup: at least a few (one or more) pathogens must be ingested to have a non-zero response. Therefore, the deviance must increase steeply below this concentration.

There appears to be a local minimum in deviance between these two extreme regions. Concentrations of $\sim$15 c.f.u./l are apparently superior to intermediate concentrations of 100 c.f.u./l or higher. Here the information from the outbreak 'fills in' the data gap in the dose–response relation from the volunteer study, and it is worthwhile noting that here the deviance remains below the appropriate $\chi^2_{0\cdot95}$ level (compare left- and rightmost columns in Table 4).

Concentrations higher than this 'local' optimum cause an increase in deviance, however, above concentrations of 537·9 c.f.u./l the deviance decreases below that of the local optimum near 15·2 c.f.u./l. We deemed concentrations in this range to be implausible (see Discussion below) and an appropriate prior was

applied to remove this region of ever-increasing likelihood values (posterior density in Fig. 1*a*).

DISCUSSION

By combining data from a human feeding study and outbreak data from two similar incidents we determined an updated dose–response relation for infection by *C. jejuni*. The updated relation shows increased infectivity at low doses and a steeper increase with dose than the one previously reported, which was based only on the human feeding study. At low doses the probability of infection is directly proportional with dose, with average slope $\hat{\alpha}/(\hat{\alpha}+\hat{\beta})=0{\cdot}686$ in the updated relation, $\sim$36 times more infectious than previously estimated (0·019) [4]. The outbreak data have also provided us with information on illness dose response which is also different from the clinical study, as discussed below.

The estimated concentration of 15·2 c.f.u./l implies that a milk tanker carrying $\sim$40 000 l would only contain 608 000 bacteria, possibly equivalent to $<$1 mg of faeces from an infected cow (0·6 mg, assuming 10^9 c.f.u./g in faeces), a very minute contamination indeed.

Similarity of the two raw milk outbreaks

The outbreak dose–response data used here show remarkable similarity. Both are close to an exponential shape, and even the observed fractions at approximately the same intake of contaminated milk are quite close. Since both outbreaks occurred in similar circumstances, on both occasions involving children of primary school age, presumably with similar susceptibilities, the concentrations of bacteria may have been similar in both outbreaks (Fig. 2). See 'Estimation of the concentration of bacteria in raw milk' section below for a more formal argument. For this reason we pooled data from the two outbreaks and treated them as a single dataset.

Reconciliation with volunteer data

At first glance, the steepness of the outbreak dose–response relation appears incompatible with the published model, based on the volunteer study of Black et al. [1]. The latter study differentiated between infection and illness, and it is important to keep in mind that the published models [3, 4] describe the probability of infection, including both symptomatic and asymptomatic cases. It has been argued before that illness is different from infection and may have a different dose–response relation [5, 16]. Noting that illness is conditional on infection (only infected subjects may become ill) we can write the illness dose–response relation as the product of two functions: the dose–response relation for infection and a function describing the (dose–dependent) probability of illness in infected subjects. The observed outbreak dose–response relation is the result of this product function. We have demonstrated that with such a separate illness dose–response model, the outbreak data can be easily reconciled with the published infection dose–response model.

The updated infection dose–response relation for *C. jejuni* indicates higher probabilities of infection at low doses than the relation published previously [3, 4]. Many published dose–response relations for infection appear to approach the theoretical limit to infectivity (i.e. $p_m=1$). This could be an adaptation to survival as a parasite: for pathogens who depend on environmental transmission the probability of encountering a host is not high and whenever this happens, it is an advantage if the probability of successful infection is as high as possible. For this reason it is not surprising to find that the most infectious organisms in a sample are often close to the theoretical upper limit of infectivity [4].

The updated infection dose–response parameters are both quite small, and not very different from each other (Table 4). These parameters determine the (beta) distribution of p_m, the 'single-hit' probability (i.e. the probability of infection per single ingested pathogen. At the values found here this distribution is strongly concave with high probabilities near 0 and 1: bacteria have either a very small or a very high probability of causing infection. The corresponding dose–response relation rises steeply ('exponentially') at low doses, but then flattens and only reaches levels near 100% at very high doses. This can be seen in Figure 1*b*. Apparently a small fraction of the exposed cases is at low risk of becoming infected, even at high doses.

Estimation of the concentration of bacteria in raw milk

The use of a separate model for illness given infection, with increasing illness probability with dose, implies that the higher the concentration of bacteria in milk, the better the fit of the combined infection–illness

model, (see Fig. 1*a*). If the illness risk starts to rise only at high doses the shape of the infection dose–response relation has less bearing on the combined dose–response relation, because at doses where the illness risk starts increasing, the infection risk is already high and changes little when the dose increases further. But is this plausible? We have seen that a concentration of $\sim$250 c.f.u./l is less likely than 15 c.f.u./l, and it is only near 550 c.f.u./l that the likelihood increases again above the local optimum near 15 c.f.u./l. Below such a high concentration infection could still occur with high probability, but it would be asymptomatic. Only if the concentration increases further, would symptoms start appearing, with probability rapidly increasing with dose. Note that such a dose dependency would have to exist in an infected host, with high numbers of bacteria already present. As argued previously [5] dose dependence of the illness risk possibly results from events that occurred during or shortly after exposure: exposure to a high dose increases the probability that many bacteria initiate infection and this might shorten the time needed to reach a certain critical number (for the appearance of symptoms) within an infected host.

If the concentration of bacteria in the contaminated milk is low ($<$500 c.f.u./l) the observed steep dose illness response relation can be easily reconciled with the results from the human feeding study. If the concentration is high this is not so straightforward. We would also need to explain how the two outbreaks could show such similar dose dependence, which is also more plausible at low concentrations. High concentrations would either have to be equal or the susceptibilities of the children involved would need to have exactly the inverse ratio of the two concentrations. At sufficiently low concentrations of the bacteria the infection dose response would have a strong influence on the illness dose response and it is again determined to a large extent by the probability of exposure at low doses, which does not depend on the host susceptibility nor the infectivity or pathogenicity of the microorganism.

As mentioned earlier we have expressed our belief that high concentrations are less plausible as a lognormal prior distribution for the concentration, centred around 10 times the local optimum (152 c.f.u./l) with log-standard deviation 4. The posterior dose–response relations in Figures 3 and 4 appear not to depend strongly on such prior assumptions (a 10-fold increase in the prior geometric mean concentration changes the posterior mode concentration $\sim$10%).

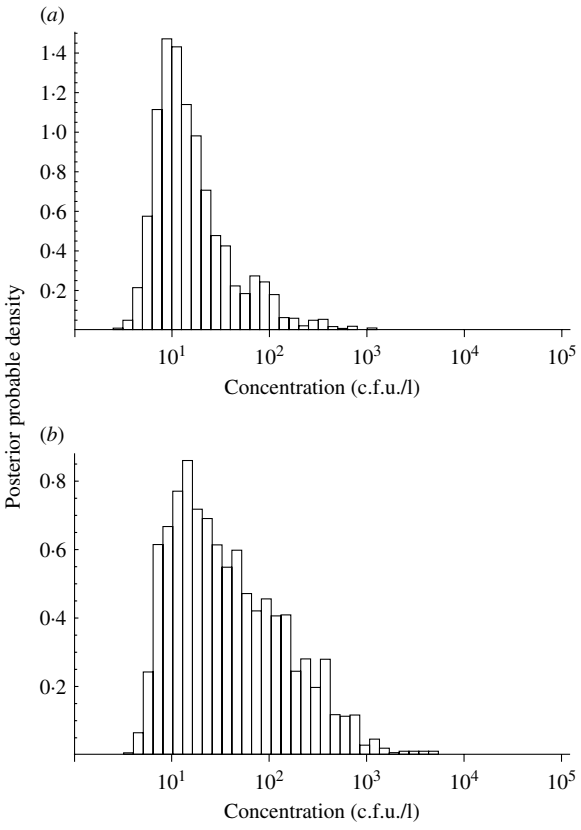

Fig. 2. Separate estimation of concentrations of bacteria in contaminated milk. Combined model for infection and illness dose response. Posterior uncertainty in (*a*) concentration in the milk in the Dutch outbreak [8] and (*b*) the UK outbreak [6].

Several studies report the presence of *Campylobacter* spp. in raw milk, but few provide concentration estimates. Humphrey & Beckett [17] report 16 ± 30 c.f.u./100 ml, slightly higher than we have inferred. Use of real-time PCR appears to produce less negative results than culture methods [18] and results in high concentration estimates: $6{\cdot}4 \times 10^7 \pm 5{\cdot}3 \times 10^5$ c.f.u./ml [19], but the fraction of infectious units remains to be established.

Earlier we assumed that both outbreaks involved milk contaminated with the same concentration of *Campylobacter*. Thus far we have treated both outbreaks as a having the same concentration, and all dose–response parameter estimates were based on that assumption. We can formally test the validity of this assumption by applying the combined infection–illness model but treating both outbreaks as completely separate events, with separate concentrations of bacteria in the contaminated milk. The

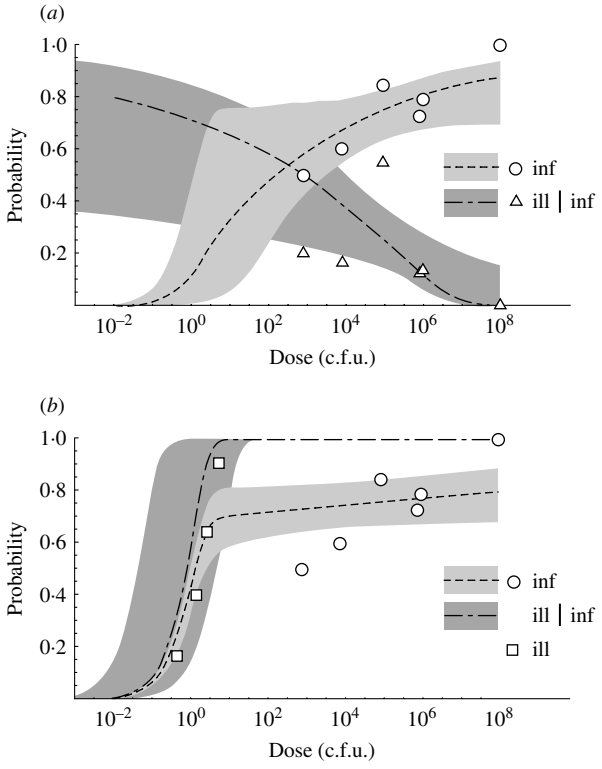

Fig. 3. Infection and conditional illness dose response (illness among infected subjects): posterior mode curves and predictive intervals. (*a*) Human feeding study [1]; (*b*) combined model based on human feeding study and the two milk outbreaks.

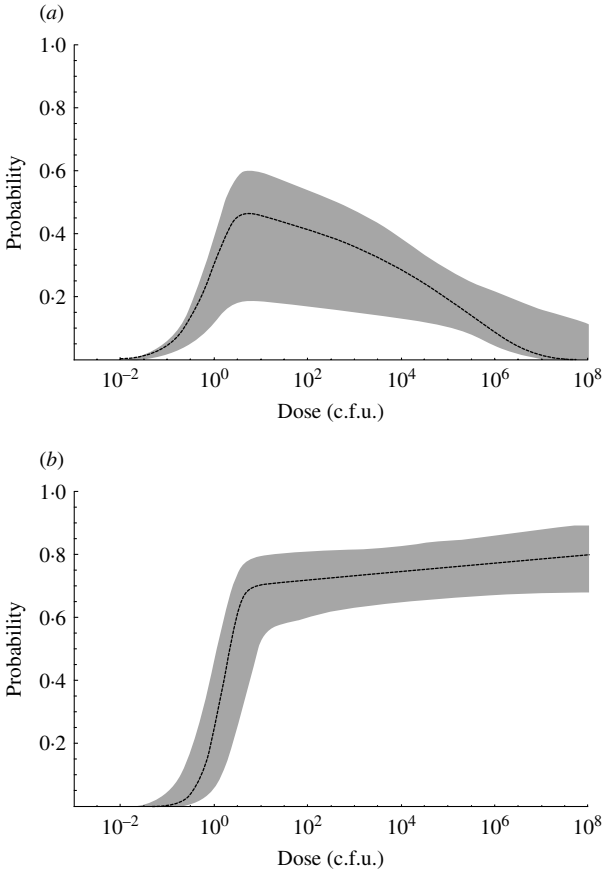

Fig. 4. Unconditional probability of illness (probability of becoming infected and ill) in adults. (a) Volunteer study of Black et al. [1] and (b) children (milk outbreaks).

optimum concentrations (cf. Fig. 1*a*) then are 14·14 and 16·42 c.f.u./l for the Dutch and UK outbreaks respectively, while the other parameters remain close to their previous estimates (Table 4). At optimum, the deviance between the models with shared and separate concentrations is 0·119, which is insignificant (against $\chi^2_{\text{D.F.}=1,0\cdot95}=3\cdot841$). Figure 2 shows (posterior) uncertainty distributions of the two separate concentrations, illustrating their similarities, as well as a slight difference: the Dutch data allow slightly more precise estimation of the concentration. Prior distributions for all parameters were the same as used earlier (see Parameter estimation section). We may conclude that a small difference in concentrations might have been present, but that our assumption of equal concentrations is valid.

Illness dose response

The human feeding study also resulted in some volunteers with symptoms and, interestingly, also with a dose-dependent probability. However, here the illness risk appeared to decrease with increasing doses [1, 5]. Even at the lowest dose applied (800 c.f.u.) one (out of five) subjects became ill, and dose–response analysis clearly suggested increasing risks at lower doses (Fig. 3*a*). Therefore, we have on the one hand the volunteer study showing, if anything, a high probability of illness in infected subjects at low doses, and on the other the observation of an illness risk increasing with dose, in children, so that higher doses are more likely to cause illness.

The two illness dose–response relations in Figure 4 are strikingly different. The relation derived from the volunteer study data predicts decreasing illness risks with increasing dose, while the relation derived from the outbreak data predicts a steeply increasing illness risk with increasing dose. An obvious difference is the host population for these two models: Figure 4*a* is for adult, immune competent volunteers, whereas Figure 4*b* concerns children. Black et al. [2] note that protective immunity can develop after infection with *C. jejuni*, by rechallenging some of the previously infected subjects. It is tempting to attribute the

differences in dose–response relations to acquired immunity, but we have no additional information to support this idea. If the dose–response relation of Figure 4*a* were valid for adults in general, exposure of adults to contaminated milk similar to the outbreaks studied here may often remain unnoticed because only few illness cases would occur.

CONCLUSION

C. jejuni is important as a foodborne pathogen, and it is, therefore, subject to many risk studies. The results from our analysis show that it may not be correct to assume that the infection risk is low at low doses, as suggested by the published infection model [3–5]. The used data [1] do not exclude a much steeper relation, mainly because of the absence of low dose observations. Another conclusion from the volunteer study, that the illness risk may be small, and possibly lowest at high doses, should also be put into perspective: while this may still be true for adult subjects with a history of campylobacteriosis, children may have a high risk of becoming ill, possibly due to a lack of protective immunity.

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