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Impact of Acquired Immunity and Dose-Dependent Probability of Illness on Quantitative Microbial Risk Assessment

A. H. Havelaar[1,2,*] and A. N. Swart[1]

Dose-response models in microbial risk assessment consider two steps in the process ultimately leading to illness: from exposure to (asymptomatic) infection, and from infection to (symptomatic) illness. Most data and theoretical approaches are available for the exposure-infection step; the infection-illness step has received less attention. Furthermore, current microbial risk assessment models do not account for acquired immunity. These limitations may lead to biased risk estimates. We consider effects of both dose dependency of the conditional probability of illness given infection, and acquired immunity to risk estimates, and demonstrate their effects in a case study on exposure to *Campylobacter jejuni*. To account for acquired immunity in risk estimates, an inflation factor is proposed. The inflation factor depends on the relative rates of loss of protection over exposure. The conditional probability of illness given infection is based on a previously published model, accounting for the within-host dynamics of illness. We find that at low (average) doses, the infection-illness model has the greatest impact on risk estimates, whereas at higher (average) doses and/or increased exposure frequencies, the acquired immunity model has the greatest impact. The proposed models are strongly nonlinear, and reducing exposure is not expected to lead to a proportional decrease in risk and, under certain conditions, may even lead to an increase in risk. The impact of different dose-response models on risk estimates is particularly pronounced when introducing heterogeneity in the population exposure distribution.

KEY WORDS: Campylobacter; dose response; immunity; QMRA

1. INTRODUCTION

Quantitative microbial risk assessment comprises several steps. Exposure assessment aims to estimate the (distribution of) exposure of a human population to a specified pathogenic microorganism, for example, by food. Dose-response (DR) modeling aims to quantify the probability of infection and illness as a function of the ingested dose. Risk characterization combines the results of exposure assessment and DR modeling to arrive at an estimate of the risk of illness in the population, expressed per year, per consumption, or otherwise. The standard approach to microbial DR modeling is to consider the processes ultimately leading to illness as a sequence of events (exposure → infection → illness) and to estimate the conditional probabilities for an exposed individual to progress through this sequence.[1–4] Both probabilities are assumed to depend on the ingested dose and parameters are estimated from sparse data sets (volunteer experiments) where subjects were exposed to known (average) doses.

[1]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands.
[2]Division Veterinary Public Health, Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands.
*Address correspondence to Arie Havelaar, Centre for Infectious Disease Control, National Institute for Public Health and the Environment, PO Box 1, Bilthoven 3720 BA, the Netherlands; arie.havelaar@rivm.nl.

Available data often do not allow conclusions on dose dependence of the conditional probability of illness given infection and models with constant probability (i.e., independent of the ingested dose) are suggested as a reasonable default.[3] Alternatively, DR parameters may be fitted using outbreak data.[5–7] Fitting DR models to outbreak data implies direct estimation of the exposure→illness probability. If, as typically done, the same models are used as for fitting exposure→infection data, then implicit assumptions about the conditional infection→illness probability are being made. In risk characterization, it is typically assumed that the outcomes of subsequent exposures are statistically independent. Our work on modeling the effects of acquired immunity on the dynamics of enteric infections has suggested that this standard approach overestimates the risk of illness, in particular at a high force of infection.[8] Furthermore, by comparing model results for campylobacteriosis with observational data on the incidence of illness in the Dutch population, it was suggested that the probability of illness given infection is overestimated when data from volunteer experiments exposed to high ($>10^5$ cfu[3]) doses are used, and that it may be (much) lower at more realistic low ($<10^3$ cfu) ingested doses. We present a novel approach to risk characterization, taking into account both the impact of acquired immunity and dose dependence of the conditional probability of illness given infection and illustrate our approach by example of risk estimates for *Campylobacter jejuni*.

2. DOSE-RESPONSE MODELS

2.1. Current Approach in Microbial Risk Assessment, Independent Exposures

In the simplest case, the exponential DR model is used:

$$p_{\text{inf}} = 1 - e^{-r\,D}, \tag{1}$$

where p_{inf} is the probability of infection for an individual, given a single exposure to a sample of food from a batch in which bacteria are Poisson distributed with arithmetic mean dose D (cfu), and r (cfu^{-1}) is the DR parameter, or single-hit probability: the probability for a single organism to survive and initiate infection.[1–4] In this context, infection is defined as a state in which at least one of the organisms in the inoculum has survived all barriers to reach a site suitable for colonization and actively multiplies in the body of the host.

If there is variability in the host-pathogens interaction, r can be described by a Beta distribution at the cost of an extra parameter, and

$$p_{\text{inf}}(D) = 1 - {}_1F_1(a, a+b, -D). \tag{2}$$

Here ${}_1F_1$ denotes the Kummer confluent hypergeometric function.[9] If $\beta >> 1$ and $\alpha << \beta$, the simplified Beta-Poisson model holds:

$$p_{\text{inf}} = 1 - \left(1 + \frac{D}{b}\right)^{-a}. \tag{3}$$

We will not pursue this further and use the hypergeometric model in our calculations.

As suggested previously,[4] we introduce the probability of illness conditional on infection as $p_{\text{ill|inf}}$, and note that $p_{\text{ill}} = p_{\text{ill|inf}}\, p_{\text{inf}}$.

The risk of illness at exposure event j may be dependent on the history of exposures. However, if we assume illness outcome is independent of previous exposures, then the probability of illness at exposure j is $p_{\text{ill}}(D_j)$ and, the illness event ($X_j = 1$) is Bernoulli distributed:

$$X_j \sim \text{Ber}(p_{\text{ill}}(D_j)). \tag{4}$$

If we assume a fixed number of exposures (E) in a specified period of time (e.g., a year), then:

$$\begin{aligned} i &= \sum_{j=1}^{E} \text{E}(x_j) = \sum_{j=1}^{E} p_{\text{ill}}(D_j) \\ &= \sum_{j=1}^{E} p_{\text{ill|inf}}(D_j)\, p_{\text{inf}}(D_j). \end{aligned} \tag{5}$$

For a population of size N, the number of illnesses (I) is:

$$I = \sum_{k=1}^{N} \sum_{j=1}^{E} p_{\text{ill|inf}}(D_{j,k})\, p_{\text{ill}}(D_{j,k}). \tag{6}$$

In the special case where the probability of illness given infection is independent of the exposure dose, we write $p_{\text{ill|inf}} = \pi$. Then, the unconditional probability of illness given a single exposure is:

$$p_{\text{ill}} = \pi\, p_{\text{inf}}(D) \tag{7}$$

and, for constant average dose D for each individual at each exposure event:

$$I = NE\pi\, p_{\text{inf}}(D). \tag{8}$$

[3]cfu: colony forming unit.

Table I. Parameter and Symbol List for Sources of Parameter Estimates for *C. jejuni* (See Text).

Parameter	Description	Estimate	Unit	Source
p_{inf}	Probability of infection	Calculated	–	–
r	Probability of 1 cfu causing infection	0.018	cfu^{-1}	(Ref. (1))
D	Arithmetic mean dose per exposure occasion	Variable	cfu	–
a, b	Parameters of the (β) variability distribution of r	0.145,8.007	–	(Ref. (1))
p_{ill}	Probability of illness	Calculated	–	–
$p_{ill\|inf}$	Conditional probability of illness given infection	Calculated	–	–
π	Fixed probability of illness given infection	0.33	–	(Ref. (27))
E	Number of exposures	Variable	$year^{-1}$	–
i	Number of illnesses in an exposed person	Calculated	$year^{-1}$	–
N	Population size	16.5 million	–	CBS[a]
I	Number of illnesses in the population	Calculated	$year^{-1}$	–
λ	Force of infection ($S \rightarrow P$ transition)	Calculated	$year^{-1}$	–
α	Loss of full immunity ($P \rightarrow Q$ transition)	13	$year^{-1}$	(Ref. (8))
γ	Loss of partial immunity ($Q \rightarrow S$ transition)	1	$year^{-1}$	(Ref. (8))
A	Life span of an individual	80	year	(Ref. (8))
R	Number of $S \rightarrow P$ transitions per lifespan	Calculated	–	–
R_n	Naive estimate for R (not accounting for immunity)	Calculated	–	–
τ	Inflation factor (ratio between R and R_n)	Calculated	–	–
η, ρ	Dose-response parameters for illness given infection	5.15×10^{-4}, 1.67×10^{-1}	cfu^{-1}	(Ref. (16))[b]

[a]Statistics Netherlands, www.cbs.nl/en
[b]Fit to data as published.

Table II. Probability of Illness, and Incidence of Illness in the Dutch Population for Several Models, Evaluated at $E = 52$ $year^{-1}$ and $D = 1$ cfu

	P_{ill}	Incidence per Person per Year	Incidence in the Population per Year
Naive	5.3×10^{-3}	2.7×10^{-1}	4.5×10^{6}
Binomial	5.3×10^{-1}	2.4×10^{-1}	4.0×10^{6}
Immunity	5.3×10^{-3}	1.4×10^{-1}	2.3×10^{6}
Dose	1.4×10^{-6}	7.1×10^{-5}	1.2×10^{3}
Dose-immunity	1.4×10^{-6}	3.7×10^{-5}	6.0×10^{2}

Note that in this case the size of the exposed group and the number of exposures can be used interchangeably. N persons having E exposures is equivalent to E persons having N exposures. We describe the above as the *naive model* for risk characterization.

A numerical example of the naive model uses parameter values typical for *C. jejuni* as shown in Table I. Data were obtained from References 1, 10, and 11. Results were calculated in R version 3.0.1.[12] For a single exposure to an average dose $D = 1$ cfu, $p_{inf} = 1.6 \times 10^{-2}$ and $p_{ill} = 5.3 \times 10^{-3}$. For repeated exposures ($E = 52$ $year^{-1}$), $i = 2.7 \times 10^{-1}$ $year^{-1}$. The number of illnesses in the Dutch population ($N = 16.5$ million) would be approximately $I = 4.5 \times 10^{6}$ $year^{-1}$, orders of magnitude higher than the observed 9.2×10^{4} $year^{-1}$ (95% CI 1.3×10^{4} to 2.5×10^{4}) from epidemiological studies.[13] Table II gives an overview of the results of this model, and the following models.

2.2. Binomial Model for Multiple Exposures

A slightly more involved approach assumes that illness can only occur once in an individual over a specified unit of time (e.g., a year). This approach can be interpreted as a simplified approach to account for acquired immunity. Note that the period of one year is arbitrary and could be chosen to match the (average) duration of protection by acquired immunity for a specific pathogen. Again assuming that the outcomes of subsequent exposures are independent, we estimate the probability of X cases of illness occurring in an individual by a *binomial model*:[4,14,15]

$$P(X = 0) = \prod_{j=1}^{E} (1 - p_{ill}(D_j));$$
$$P(X = 1) = 1 - \prod_{j=1}^{E} (1 - p_{ill}(D_j)). \qquad (9)$$

Note that this distribution is obtained by collapsing all probability mass of the binomial distribution

on $i \geq 1$ onto $i = 1$, thereby artificially maximizing the infection events to one. If all D_j are equal, then:

$$P(X = 1) = 1 - (1 - p_{\text{ill}})^E \approx E p_{\text{ill}} \text{ for small} p_{\text{ill}}. \quad (10)$$

The expected number of illnesses in a population is simply obtained by multiplying $p(X = 1)$ with the population size. If p_{ill} is not small, the size of the exposed group and the number of exposures can no longer be used interchangeably. In our example, we find $i = E(X) = P(X = 1) = 2.4 \times 10^{-1}$ year^{-1}, close to the naive estimate because the binomial function is approximately linear at a low probability of illness. If the dose per exposure event were higher, say 100 per exposure occasion, the naive approach to risk characterization would predict that $i = 4.6$ year^{-1}, whereas the binomial approach would predict that i is at its maximum value of 1 year^{-1}.

2.3. Incorporating the Effects of Acquired Immunity

Swart *et al.*[8] have introduced a simple compartmental model to account for the effects of acquired immunity under an imposed force of infection, such as from an animal reservoir, food, or the environment. In this model, it is assumed that:

- all individuals are born susceptible (S);
- individuals may become (asymptomatically) infected with force of infection λ; incorporating both the intensity of exposure and the DR function;
- when infected, there is a fixed probability π of developing symptomatic illness;
- an infected individual is immediately fully protected (P) against subsequent infection;
- waning of immunity is represented by transitions from P to a state of partial protection (Q) with rate α and then back to S with rate γ;
- when a partly protected individual is exposed (with force of infection λ), the immune status is boosted to full protection ($Q \rightarrow P$) without clinical illness.

These authors introduce R, the number of times that an individual makes the $S \rightarrow P$ transition in a lifespan of A years. The number of illness episodes during this lifespan is πR. When A is large compared to the average residence time in any of the compartments S, P, and Q, then R can be approximated by:

$$R = \lambda A \frac{\alpha\gamma}{(\alpha + \lambda)(\gamma + \lambda)} \equiv \lambda A \tau. \quad (11)$$

The force of infection may be expressed as:

$$\lambda = E p_{\text{inf}}(D). \quad (12)$$

The factor τ is obtained from Equations (9) and (10),

$$\tau = \frac{\alpha\gamma}{(\alpha + \lambda)(\gamma + \lambda)} = \frac{\alpha\gamma}{(\alpha + E p_{\text{inf}}(D))(\gamma + E p_{\text{inf}}(D))}. \quad (13)$$

In the naive approach to risk characterization (i.e., not accounting for acquired immunity), R would be estimated as λA. Hence, the impact of acquired immunity can be characterized by the inflation factor τ appearing in Equation (12). Note that this is also the fraction of susceptibles in the population as $A \rightarrow \infty$.

The inflation factor can be used as a multiplier for p_{inf}, to scale the naive risk estimate to an estimate that takes immunity into account. We describe this as the *immunity model*:

$$I = \tau N E \pi p_{\text{inf}}(D). \quad (14)$$

Note that τ is a nonlinear function of p_{inf} (Equation (13)), hence I is dependent on dose. Furthermore, note that N and E are not interchangeable, since τ is a function of E via λ.

Swart *et al.*[8] have estimated the immune-related parameters from a volunteer challenge-rechallenge experiment;[16] see Table I. Using these parameter estimates, Fig. 1 shows τ as a function of D and E. At low values of both D and E, there is little impact of acquired immunity on the risk estimate. At increasing doses, the impact of acquired immunity becomes more pronounced, in particular for $E > 14$. Note that the protective effect of acquired immunity in the population is of particular importance at high average doses. More precisely,

$$\tau \rightarrow \frac{\alpha\gamma}{(\alpha + E)(\gamma + E)}, \quad \text{for} D \rightarrow \infty \quad (15)$$

Since $p_{\text{inf}}(D)$ tends to one for $D \rightarrow \infty$, the maximum number of cases is limited to $I = \tau N E \pi$. When $E \rightarrow \infty$, we have $\tau \rightarrow 0$ and $I \rightarrow 0$ for all values of D.

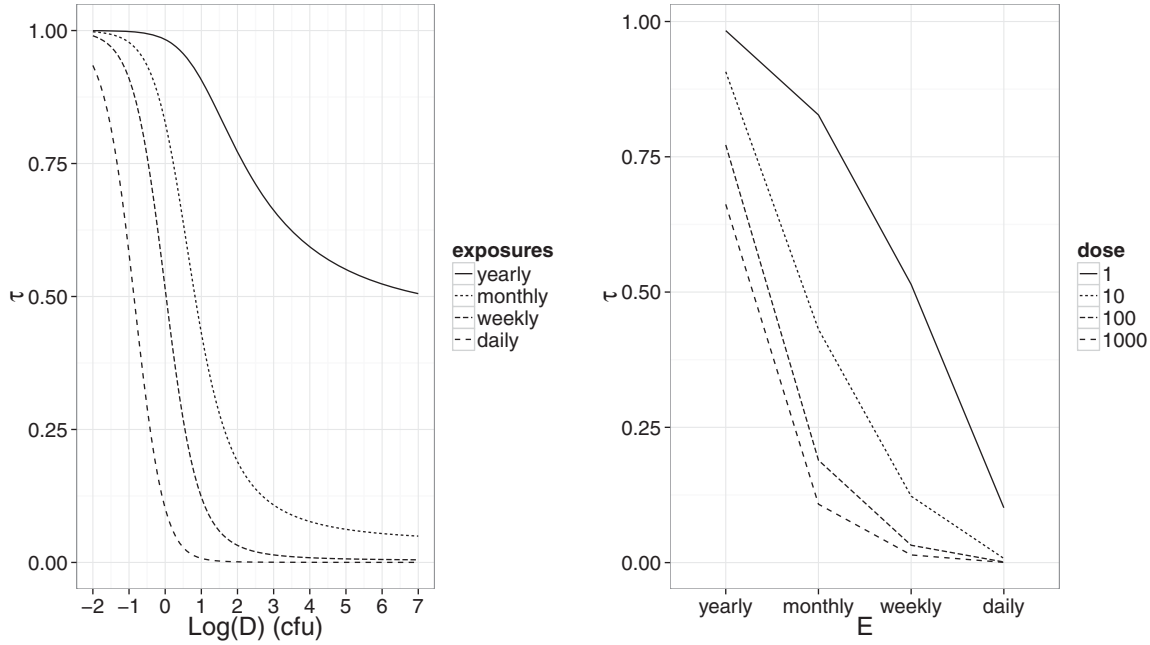

Fig. 1. Reduction of the risk of illness by accounting for acquired immunity compared to a naive approach to microbial risk assessment. Left panel: Inflation factor (τ) as a function of average dose (D) for different intensities of exposure (E). Right panel: Inflation factor (τ) as a function of intensity of exposure (E) for different average doses (D).

Furthermore, note that the expression for τ can be rearranged to the form

$$\tau = \frac{\tilde{\alpha}\tilde{\gamma}}{(\tilde{\alpha} + p_{\text{inf}})(\tilde{\gamma} + p_{\text{inf}})} \tag{16}$$

by introducing the dimensionless quantities $\tilde{\alpha} = \alpha/E$, $\tilde{\gamma} = \gamma/E$. Thus, the relevant parameters for the inflation factor are the quotients of the rates of loss of protection over exposure.

For our example, we use $\lambda = 8.3 \times 10^{-1}$ year^{-1} and $\tau = 5.1 \times 10^{-1}$.[8] Hence, the risk of illness while accounting for acquired immunity is $i = 1.4 \times 10^{-1}$ year^{-1}. The predicted number of cases in the Dutch population would decrease to 2.3×10^{6} year^{-1}, still far higher than the observed number.

2.4. Incorporating the Effects of Dose-Dependent Conditional Probability of Illness

The above approach does not include the possibility that the probability of illness given infection $p_{\text{ill|inf}}$ is dependent on the ingested dose. There is very little literature on infection-illness modeling. Teunis *et al.*[17] propose a model, in which $p_{\text{ill|inf}}$ can either be constant, increase, or decrease with dose. Using scarce literature data, they show that examples of each of these results can be found in existing data sets. For the data set on volunteer experiments with *C. jejuni* published by Black *et al.*,[18] a decrease of $p_{\text{ill|inf}}$ with dose was found. However, this data set may be subject to randomization bias due to a small number of volunteers per dose group and the possible inclusion of volunteers with preexisting immunity to *C. jejuni*. It is likely that, by chance, volunteers with preexisting immunity were included in the high-dose groups as not all volunteers were infected even by a dose of 10^6 cfu. Subsequent experiments by Tribble *et al.*,[16] who preselected volunteers based on serological testing for IgA, showed that all immunologically naive volunteers exposed to *C. jejuni* became infected and ill at doses of 10^5 cfu and above, and that the probability of illness given infection increased with dose.

We adopt the model as proposed by Teunis *et al.*[17] for $p_{\text{ill|inf}}$ increasing with dose:

$$p_{\text{ill|inf}} = 1 - (1 + \eta D)^{-\rho}, \tag{17}$$

where ρ and η D are the shape and scale parameters of an underlying Gamma distribution for the duration of infection, respectively.

Using the data supplied by Tribble *et al.*[16] for illness in infected individuals (100% infection rate), we estimate $\hat{\eta} = 5.15 \times 10^{-4}$ and $\hat{\rho} = 1.67 \times 10^{-1}$

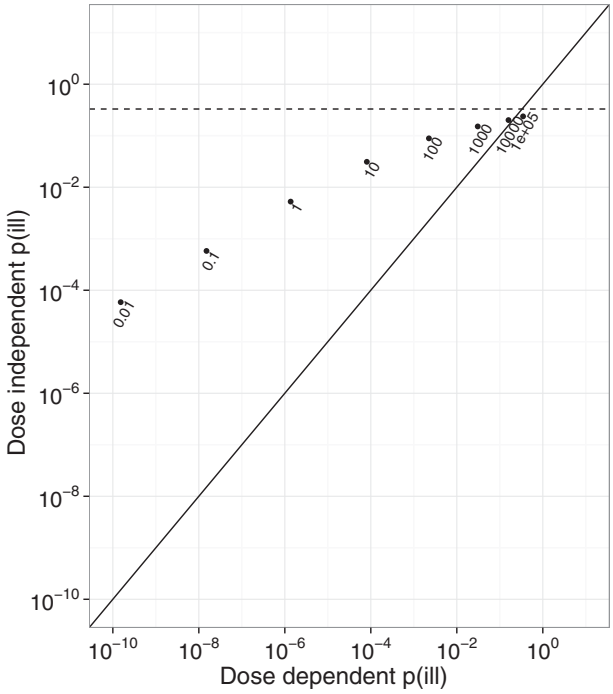

Fig. 2. Unconditional probability of illness as a function of dose (indicated by dots and corresponding values) for dose-response models with conditional probability of illness given infection either constant (horizontal axis) or dose dependent (vertical axis). The dashed horizontal line indicates the limiting value $p_{\text{ill}} = \pi$ for high dose. The line of equality is added for comparative purposes. Note that p_{inf} is dose dependent in both cases.

using maximum likelihood estimation. We confirmed that neither the constant nor the decreasing model performed better than the increasing model by the likelihood ratio test.

By substituting Equation (17) into Equation (5), and again assuming a constant average dose D for each individual at each exposure event, we find the *dose model*:

$$I = NE(1 - (1 + \eta D)^{-\rho})p_{\text{inf}}(D). \qquad (18)$$

Note that N and E can still be used interchangeably.

Fig. 2 shows the impact of introducing dose dependency of $p_{\text{ill|inf}}$ by comparison of the unconditional probability of illness for the dose model and the naive model at single exposure to average doses ranging between 10^{-2} and 10^{5} cfu. The naive model predicts illness risks that are 3–6 orders of magnitude higher than the dose model for doses below 1 cfu. The risk predicted by the naive model is lower than the prediction of the dose model at doses greater than approximately 10^{3} cfu because the naive model asymptotically reaches its maximum value π. In contrast, the maximum value of the dose model is 1, which is in accord with observed data sets from outbreaks.

For the dose model, we find $i = 7.1 \times 10^{-5}$ illnesses per individual per year, amounting to $I = 1.2 \times 10^{3}$ illnesses in the general population, well below the epidemiological estimate.

2.5. Combined Model with Immunity and Dose Dependence

The *dose-immunity model* combines the effects of acquired immunity and dose-dependent conditional probability of illness:

$$I = \tau NE(1 - (1 + \eta D)^{-\rho})p_{\text{inf}}(D). \qquad (19)$$

We now find $i = 3.7 \times 10^{-5}$ and $I = 6.0 \times 10^{2}$ at $D = 1$, reducing the number of illness cases further below the prediction from the dose model.

3. COMPARING THE DIFFERENT MODELS

The expected incidence of disease I as a function of exposure D depends very strongly on the model used, as is illustrated in Fig. 3. In the models that do not account for immunity, I increases steeply with D. The increase is monotonous; hence, any decrease in exposure leads to a predicted decrease in the predicted incidence of illness. In models that do take immunity into account, I levels off at higher doses. The binomial model reaches a constant value, whereas the immunity model reaches a maximum, with decreasing values of I at higher doses. This would imply that there are situations in which decreasing exposure would lead to increasing disease incidence. Introducing dose dependency of the probability of illness given infection shifts the illness-dose curve to the right but beyond a certain dose, a steep increase of the predicted incidence of illness is still observed. In the models that account for immunity, the disease incidence is bounded. Comparing the different curves, it is clear that at low doses, disease incidence estimates are mainly affected by the choice for dose dependency of the probability of illness, whereas at higher doses, the impact of immunity dominates.

Fig. 4 shows, for parameter estimates as discussed before, how the epidemiological estimate of human disease incidence can be reconstructed, using the dose-immunity model. The figure shows that the estimated incidence is mainly sensitive to

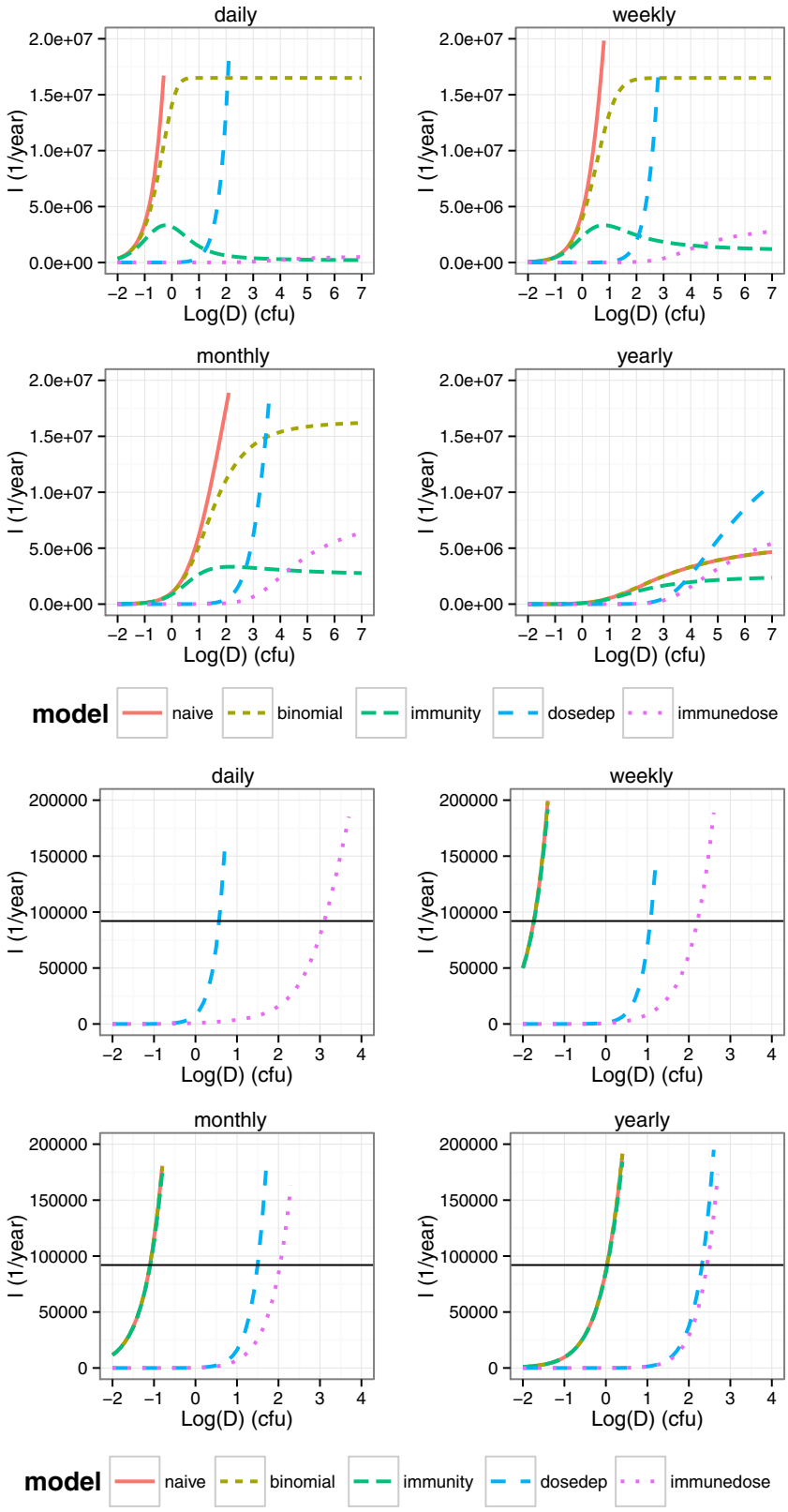

Fig. 3. Incidence of illness I as a function of the mean dose D, for different assumptions about immunity and dose dependence of the the probability of illness given infection. Parameter estimates are described in the text. The horizontal line indicates the epidemiological estimate. The right-most panel zooms in on the region where the models predicts outcomes close to this estimate.

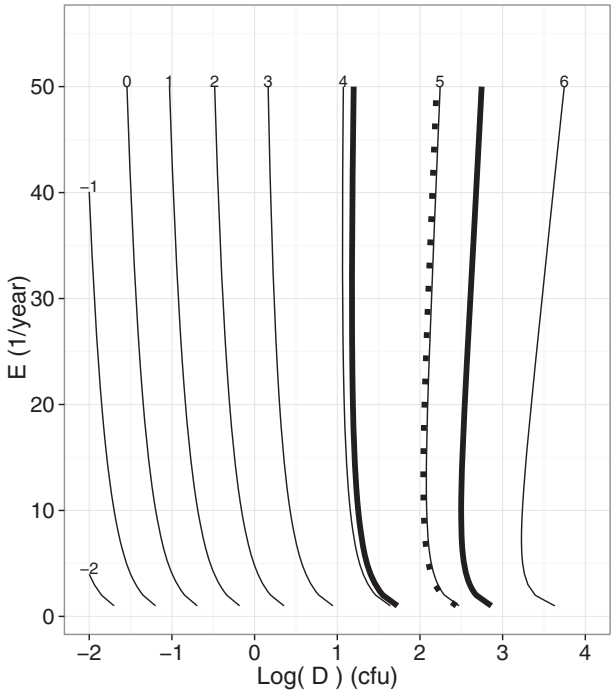

Fig. 4. Contour plot of selected values of $\log_{10}(I)$ (values indicated at the top of the curves) as a function of D and E for the dose-immunity model. The two thick curves are the 2.5% and 97.5% confidence limits for the epidemiological estimate of disease incidence. The dotted curve is the most likely epidemiological estimate.

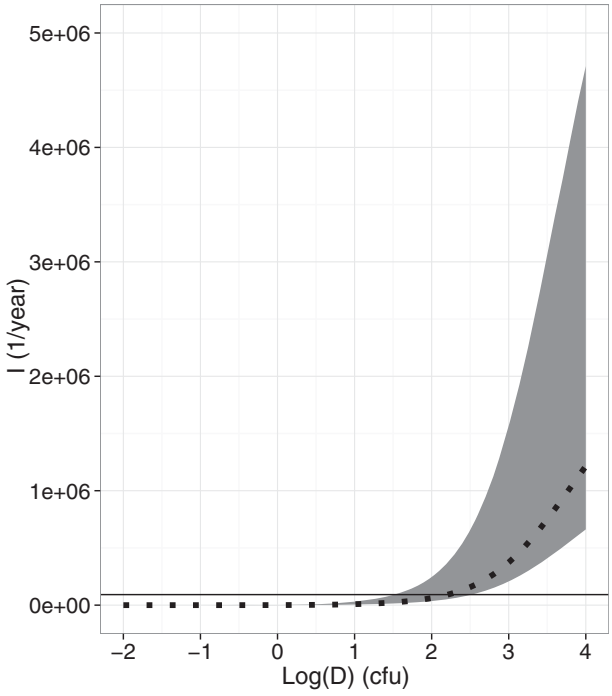

Fig. 5. Sensitivity analysis for the number of illness cases as a function of dose at an exposure of $E = 52$ year^{-1}. The dotted line represents the curve at the maximum likelihood estimate of parameters. The shaded area represents the 95% confidence area under variation of parameters as detailed in the main text.

the dose per exposure event. The epidemiological estimate would be reconstructed with doses of approximately 1.5 to 3.0 log cfu per event, with little impact of the exposure frequency above five events per year. However, such average doses are considerably higher than previously estimated.[10] As the parameters for both immunity and dose dependency of the conditional probability of illness are based on few data, such discrepancies may well be related to parameter uncertainty. We therefore performed a sensitivity analysis, in which both parameters of the immunity model (α and γ) and one parameter of the dose-dependent model (ρ) were varied. We did not vary the η parameter in the dose-dependent model, as the results are not very sensitive to changes in this parameter (results not shown). Since there is little guidance in choosing appropriate distributions and ranges for the uncertainty analysis, we chose to vary the parameters by uniformly sampling from the range spanned by half the default estimate and double the default estimate. We find (see Fig. 5) that the uncertainty region rapidly grows with increasing dose. The asymmetry of the uncertainty band around the maximum likelihood estimate suggests that at the default values of parameters, we are more likely to underestimate incidence rather than overestimate.

4. HETEROGENEITY IN POPULATION EXPOSURE

In the preceding discussion, we ignored any kind of heterogeneity: heterogeneity between individuals and heterogeneity for individuals over time. This is true for both the time between exposures, and the magnitude of the dose. We acknowledge that all these heterogeneities are of importance; however, some of them are hard to model within our current framework.

Considering heterogeneity for a single individual, having variation in the time between exposures and the magnitudes of doses (e.g., sporadic high doses, interspersed with frequent low doses), the history of infection becomes important. The immune system responds based on previous exposures, and bestows a measure of protection on the individual.

Table III. Exposure Scenarios for *C. Jejuni* in Subgroups of the Dutch Population

Scenario	Exposure Pathway	Population Fraction (f)	E (Year^{-1})	D (cfu)
L/L	Recreational water	0.31	0.2	1
L/H	Raw chicken liver	0.01	2	149
H/L	Sheep and goats	0.004	183	6.5
H/H	Petting zoos	0.02	52	16
AVG	Average exposure	1.00	24	1

This implies that the basic parameter r, the infectivity of a single organism, becomes dependent on the history of infection in some (yet) unclear fashion. This takes us out of the scope of established DR theory.

The other kind of heterogeneity, that is, between individuals in the population, is more straightforward to model. In the simplest case of groups of individuals with clear exposure profiles, we may simply run the models for each group.

We evaluate the impact of different DR models for illness in four exposure scenarios, based on quantitative estimates of the Dutch population to *C. jejuni* by different pathways as presented by Evers *et al.*[10,19] Four exposure scenarios are analyzed; see Table III:

- *Low frequency of exposure, low dose (L/L)—recreational water.*
  On average, Dutch adults (84% of the population) visit surface water recreational sites 0.61 year^{-1}. There is no information on the distribution of visits around this average. We assume that adults who visit recreational surface water do so twice a year, hence $E = 2$ year^{-1} and the fraction of adults who do so is 36%, which is 30% of the total population ($f = 0.3$). The average dose per exposure is defined by ingestion of 0.01 l per exposure occasion with a mean concentration of 10 cfu/L, hence $D = 0.1$ cfu. As in models accounting for immunity, N and E are not interchangeable, we need to account for the fact that exposure can only take place to discrete organisms, hence we modify this scenario to $E = 0.2$ and $D = 1$ cfu (keeping the product ED, the total dose, constant). We ignore the (very small) probability that the Poisson average $D = 0.1$ implies a realized exposure greater than 1 cfu.
- *Low frequency of exposure, high dose (L/H)—consumption of raw chicken liver.*
  In a two-day period, 2/6,250 respondents in the Dutch Food Consumption Survey indicated to have eaten raw or undercooked chicken livers. We assume that consumers who eat chicken livers do so once per two months. The prevalence of Campylobacter on chicken livers is 33%, hence $E = 2$ year^{-1} and $f = 0.01$. The average portion size per eating occasion is 85 g and the concentration of Campylobacter is 1.75 cfu/g; hence $D = 150$ cfu.
- *High frequency of exposure, low dose (H/L)—direct animal contact with sheep and goats (i.e. farmers).*
  About 0.4% of the Dutch population has regular contacts with sheep and goats. On average, 50 contacts with animals are assumed per day, with 1% probability of exposure to fecal material per contact; thus $E \approx 180$ year^{-1}. The amount of feces transferred per contact is 0.001 g with a Campylobacter concentration of 1×10^4 cfu/g and prevalence of 65%, hence $D = 6.5$ cfu.
- *High frequency of exposure, high dose (H/H)—petting zoos.*
  On average, 0.3% of the Dutch population visits a petting zoo on any given day. If we assume that those who do regularly visit petting zoos do so once per week, then $E = 52$ year^{-1} and $f = 0.02$. The average dose per visit can be estimated from the prevalence and concentration of Campylobacter in petting zoo animals (9.5% and 2.8×10^5 cfu/g, respectively) and the number of animals contacts per day (20), the probability of fecal transfer per contact (0.01) and the amount of feces transferred (0.003 gram); hence $D = 16$ cfu.
- *The average exposed person.*
  To evaluate the impact of heterogeneity in exposure patterns, we also calculate a hypothetical exposure scenario, in which every individual in the population would be exposed weekly to an average dose, based on the four exposure scenarios described above, that is, $f = 1.00$; $E = 52$ year^{-1}, resulting in $D = 0.48$ cfu. As discussed for recreational water, we modify this scenario to $E = 24$ year^{-1} and $D = 1$ cfu.

Note that these four scenarios should not be construed as a partitioning of the population into disjoint subgroups. An individual may be a member of

Table IV. Disease Incidence for Different Exposure Scenarios for *C. Jejuni* According to Five Dose-Response Models

(a) Individual Risk

Scenario (E/D)	Exposure Pathway	Annual Dose (cfu/person/year)	Incidence of Illness (person/year)				
			Naive	Binomial	Immunity	Dose	Dose-Immunity
L/L	Recreational water	0.2	1.1×10^{-3}	1.1×10^{-3}	1.0×10^{-3}	2.7×10^{-7}	2.7×10^{-7}
L/H	Raw chicken liver	300	2.0×10^{-1}	1.9×10^{-1}	1.2×10^{-1}	7.4×10^{-3}	4.4×10^{-3}
H/L	Sheep and goats	1,200	4.3×10^{0}	9.9×10^{-1}	1.5×10^{-1}	7.3×10^{-3}	2.6×10^{-4}
H/H	Petting zoos	830	2.1×10^{0}	8.9×10^{-1}	1.9×10^{-1}	8.8×10^{-3}	7.4×10^{-4}
AVG	Average exposure	24					
	1.3×10^{-1}	1.2×10^{-1}	8.9×10^{-2}	3.3×10^{-5}	2.3×10^{-5}		

(b) Population Risk

Scenario (E/D)	Exposure Pathway	Population Average Dose (cfu/person/year)	Incidence of Illness (population, year^{-1})				
			Naive	Binomial	Immunity	Dose	Dose-Immunity
L/L	Recreational water	0.06	5.4×10^{3}	5.4×10^{3}	5.4×10^{3}	1.4×10^{0}	1.4×10^{0}
L/H	Raw chicken liver	3.0	3.3×10^{4}	3.1×10^{4}	2.0×10^{4}	1.2×10^{3}	7.3×10^{2}
H/L	Sheep and goats	4.8	2.8×10^{5}	6.5×10^{4}	1.0×10^{4}	4.8×10^{2}	1.7×10^{1}
H/H	Petting zoos	17	7.0×10^{5}	2.9×10^{5}	6.3×10^{4}	2.9×10^{3}	2.6×10^{2}
SUM	Sum of four scenarios	25	1.0×10^{6}	3.9×10^{5}	9.8×10^{4}	4.6×10^{3}	1.0×10^{3}
AVG	Average exposure	24	2.1×10^{6}	2.0×10^{6}	1.5×10^{6}	5.4×10^{2}	3.8×10^{2}

none, one, or more of the defined groups. Still, it is interesting to compare the specific scenarios to the population average.

In Table IV, we see that the average dose for the total Dutch population varies between 0.2 cfu/person/year in the L/L scenario to 1,186 cfu/person/year for the H/L scenario. The average person in this population would be exposed to 24 cfu/year. Table IV also shows risk estimates at individual and population level. The latter are graphically shown in Fig. 6.

The different DR models have marked effects on the estimated individual and population risks, but the impact of the different models depends on the exposure scenario. Incorporating immunity in the DR model has an impact on scenarios with frequent exposures, as expected. The risk according to immunity models is estimated approximately 10× lower than for models that do not take immunity into account for both the H/L (sheep and goats) and H/H (petting zoos) scenarios. The impact of high doses (also a parameter in the inflation factor τ) is less pronounced, as can be seen from risk estimates for chicken liver. Dose dependence of $p_{\text{ill|inf}}$ has a very strong impact in scenarios with exposure to low doses, again as expected. In the L/L (recreational water) and AVG (average exposed person) scenarios, risk estimates are reduced more than 1,000-fold. In scenarios with exposure to higher doses, the effects are less strong (approximately 10-fold risk reduction).

The sum of the estimated population incidence for all four scenarios together is lower than the incidence estimated for the AVG scenario for the naive, binomial, and immunity models, whereas it is higher for the models including dose dependence of $p_{\text{ill|inf}}$. This is related to heterogeneity in exposure with some individuals being exposed to doses that are considerably higher than average. The sum of population incidence estimates of the immunity model is in the same order of magnitude as the epidemiological estimate. Note the contrast with the AVG scenario, indicating that simultaneously accounting for immunity and heterogeneity in the population exposure distribution has a profound impact on risk estimates.

Table V presents the impact of different DR models on attribution. This table also includes attribution based on exposure only, as suggested by Evers *et al.*,[10] who found that direct animal contact (as in petting zoos) was the most important source of exposure to *C. jejuni*. Most DR models also suggest that this exposure pathway is the main risk factor for illness with attributable fractions (AF) varying

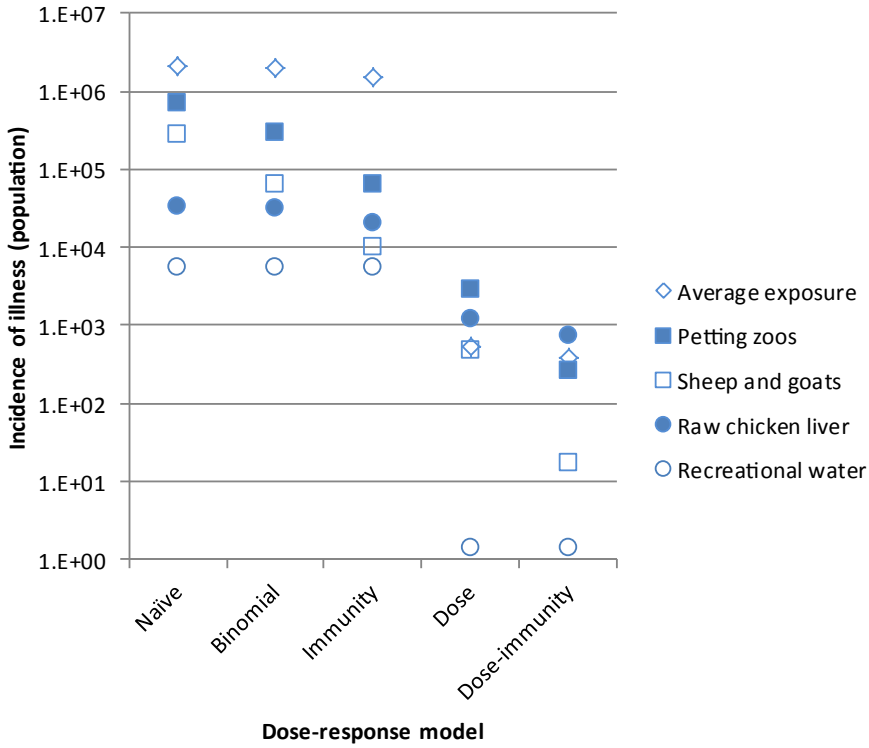

Fig. 6. Disease incidence for different exposure scenarios according to five dose-response models.

Table V. Attribution of Disease Incidence to Different Exposure Pathways According to Five Dose-Response Models

Scenario	Exposure Pathway	Exposure	Naive	Binomial	Immunity	Dose	Dose-Immunity
L/L	Recreational water	0.2%	0.5%	1.4%	5.5%	0.0%	0.1%
L/H	Raw chicken liver	11.4%	3.2%	7.9%	20.3%	26.2%	72.4%
H/L	Sheep and goats	18.9%	27.5%	16.6%	10.2%	10.5%	1.7%
H/H	Petting zoos	69.5%	68.8%	74.1%	64.0%	63.3%	25.8%

between 64% and 74%. In contrast, the dose-immunity model only estimates AF = 26% for petting zoos. According to this model, foodborne exposure (raw chicken liver) would be the main risk factor (AF = 72%). The AF for recreational water is low according to all DR models, even though it is 6% according to the immunity model.

5. DISCUSSION

By combining previously published modeling approaches, we propose an extension of current DR theory for microbial risk assessment to account for the impact of acquired immunity on the risk related to repeated exposures, and for the dose-dependent behavior of the conditional probability of illness given infection. We show that these modifications have a pronounced effect on the estimated incidence of human disease. Based on an existing exposure model, we recalculate the expected incidence of campylobacteriosis in the Netherlands, and compare the results with independent estimates from epidemiological research. The standard approach to risk characterization overestimates the incidence of campylobacteriosis by several orders of magnitude. Bouwknegt *et al.*[20] have systematically analyzed possible sources of uncertainty in both estimates and identified DR models including lack of consideration of acquired immunity as key factors. In this article, we propose methods to quantify the impact of such factors. Accounting for immunity reduces the risk estimate after repeated exposures by an inflation

factor equal to the (dose-dependent) fraction of susceptibles in the population. However, the incidence of campylobacteriosis is still overestimated. In this model, the probability of illness as a function of dose has a maximum value; hence decreasing the exposure may, under some circumstances, lead to increased disease risk. Introducing dose dependency of the probability of illness given infection strongly reduces the risk estimate. Applying this conditional probability (parameterized using very few data) leads to underestimation of the incidence of campylobacteriosis as compared to the epidemiological estimate. When combined with the impact of acquired immunity we find an even stronger underestimation. In this combined dose-immunity model, the incidence is bounded for $D \rightarrow \infty$.

Fig. 4 illustrates how dose and exposure frequencies determine disease incidence. We observe that in order to conform to epidemiological estimates, the dose range should be in a narrow band between approximately 1.5 and 3 log cfu, almost independently of the number of exposures. Such high exposure levels do not appear realistic when compared to existing exposure models.[10,11]

The impact of acquired immunity appears to limit the disease incidence at higher exposure frequencies, whereas dose dependency of the probability of illness given infection has a major impact at low (average) doses. Hence, depending on the scenarios to be evaluated, simpler model choices (and hence less demanding parameter estimations) might be chosen in future risk assessments. The naive approach, with constant probability of illness given infection and no impact of acquired immunity, appears to yield unrealistically high results under all circumstances. As this model has been applied in many published risk assessments, it is important to further evaluate the impact of our new approach to risk characterization on typical results of risk assessment studies, such as the public health risk of pathogen/food pairs. Because of the nonlinear nature of the models including immunity even the impact of interventions in the food chain aimed at reducing exposure dose and/or frequency may be wrongly estimated in current risk assessments.

The different impact of DR models on risk estimates is clearly illustrated by the scenario studies introducing heterogeneity in exposure in the population. The choice of DR model has a pronounced effect on both absolute risk estimates and attribution of illness to different exposure pathways. The observation that the risk estimate of the immunity model is very sensitive to heterogeneity in the population distribution is of particular interest. When applying the dose-immunity model, raw chicken liver, although eaten rarely and by a small fraction of the population only, is predicted to cause a large fraction of all cases in our hypothetical population. Also, in infectious disease surveillance outbreaks associated with undercooked chicken liver are increasingly recognized.[21–24] Furthermore, in Scotland 56% of *Campylobacter* strains in retail liver belonged to the top 10 genotypes in humans.[25]

The quantitative results presented in this article are illustrative, but must be interpreted with caution because there were very few data on which to base parameter estimates. Parameter estimates for the conditional probability of illness are based on a single study in the United States, with few subjects per dose category and exposure of volunteers to doses of 10^5 cfu or higher. Such experimental doses may not be representative of real exposures through food or environmental sources. Furthermore, variability between *Campylobacter* strains and hosts, or the physiological status of bacteria in response to changing conditions in the food chain, all may have an impact on the parameter values. The Beta-distribution for the single-hit parameter r, as used in the hypergeometric and Beta-Poisson model, can be interpreted as expressing variability between hosts, or between microorganisms, or both. Variability between hosts can be related to innate immunity and barriers such as stomach acid, bile salts, etc. Acquired immunity may also affect the single-hit probability, but very few data are available to quantify this effect. In our interpretation, acquired immunity primarily affects the probability of illness given infection, not the probability of infection given exposure. This is described in more detail in Ref. 8. To better understand the process of illness given infection, and provide better parameter estimates, more sophisticated models of pathogen-host interaction are needed. As more serosurveillance data, and models to reconstruct the incidence of (asymptomatic) infection from such data, become available,[26] this may offer additional probabilities to arrive at population-based parameter estimates.

In conclusion, we have shown that incorporating the effect of acquired immunity and/or dose dependence of the probability of illness give infection has a pronounced effect on absolute and relative risk estimates. We provide a theoretical basis for the incorporation of such effects in microbial risk

assessment studies. Further work is necessary to establish more realistic parameter estimates and take into account variability between *Campylobacter* strains and human hosts.

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