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Infectivity of GI and GII noroviruses established from oyster related outbreaks

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Abstract:

Noroviruses (NoVs) are the major cause of acute epidemic gastroenteritis in industrialized countries. Outbreak strains are predominantly genogroup II (GII) NoV, but genogroup I (GI) strains are regularly found in oyster related outbreaks. The prototype Norwalk virus (GI), has been shown to have high infectivity in a human challenge study. Whether other NoVs are equally infectious via natural exposure remains to be established. Human susceptibility to NoV is partly determined by the secretor status (Se+/−). Data from five published oyster related outbreaks were analyzed in a Bayesian framework. Infectivity estimates where high and consistent with NV(GI) infectivity, for both GII and GI strains. The median and CI95 probability of infection and illness, in Se+ subjects, associated with exposure to a mean of one single NoV genome copy were around 0.29[0.015–0.61] for GI and 0.4[0.04–0.61] for GII, and for illness 0.13[0.007–0.39] for GI and 0.18[0.017–0.42] for GII. Se− subjects were strongly protected against infection. The high infectivity estimates for Norwalk virus GI and GII, makes NoVs critical target for food safety regulations.

Highlights

► From five oyster related outbreaks data, infectivity of Norovirus was evaluated. ► Bayesian analysis was done conditionally from the ingested dose (genome copies). ► Infectivity for secretor positive (Se+) and negative (Se−) individuals is different. ► Difference of infectivity between genogroups (GI and GII) is not detected. ► High infectivity estimates was confirmed for NoV GI and GII in Se+ individuals.

Keywords: Norovirus ; Shellfish ; Bayesian analysis ; Dose–response relationship ; Fucosyltransferases

Noroviruses (NoVs) contribute significantly to morbidity worldwide [1, 2]. NoV infection occurs primarily through person-to-person transmission but also through contaminated food or water, and in particular by exposure to fomites [3]. NoVs are the cause of approximately 90% of epidemic non-bacterial gastroenteritis outbreaks. The majority of outbreaks occurs during winter months but sporadic cases do occur throughout the year [4, 5].

Outbreaks occur in semi closed communities such as hospitals, schools, cruise ships, nursing homes and military settings [3]. Severity is higher for risk groups, such as immune-compromised individuals and the elderly [3]. NoVs are highly diverse genetically and antigenically, and among 5 genogroups, two are frequently associated with human outbreaks : genogroup I, and more frequently genogroup II [6]. Genogroup II (GII) NoVs, in particular the GII.4 cluster, have become predominant in human transmission of infection over the last two decades, but genogroup I (GI) strains co-circulate in the human population and are regularly involved in food and in particular oyster outbreaks [7, 8]. Like influenza, large outbreaks occur periodically with people of all ages infected [3]. One explanation is that immunity seems to be short-lived and incomplete [3], although continuing replacement of strains of the dominant GII.4 cluster suggests immune-driven selection to facilitate escape from protective (herd) immunity [9].

Differential genetic host susceptibility has also been identified. Since NoV strains bind to carbohydrates of the histo-blood group antigen family, pleiotropic interactions of alleles at three loci (*FUT3*, *FUT2* and *ABO*) determining the Lewis, Secretor and ABO phenotypes also contribute to explaining differences in occurrence of strains and genogroups in the human population [10,11].

The attributable fraction of NoV gastroenteritis linked to food consumption is estimated at around 26% for the US [2]. Nevertheless, this estimate varies a lot between studies and the data are sparse. Hygiene recommendations are required to limit the spread of outbreaks [12], in particular in closed settings. Limiting the contamination of food could be crucial, to prevent primary cases and curb outbreaks at the origin, as direct person-to-person transmission is likely and can be initiated by foodborne cases. Contaminated drinking water or food such as vegetables or molluscs has been shown to cause outbreaks [13-15]. To protect consumers and compare the effects of different management strategies, microbial risk assessment provides a comprehensive and reliable scientific prospective tool [16]. In order to evaluate the potential consequences of intervention measures in food contamination, the probability of primary cases of gastroenteritis should be predicted in an exposed population [16].

A human trial has been conducted to estimate infectivity and morbidity for a range of doses of Norwalk virus (GI.1) using a watery inoculum [17]. Genetic factors determining

histo-blood group secretor status were taken into account and Se- status appeared to confer a complete protection against Norwalk virus (GI.1), in agreement with epidemiological studies [17]. The study highlighted the high infectivity of Norwalk virus with an average probability of infection of 0.5 for a single virus genome [17]. As the human trial was limited to a single virus isolate of a strain (G1.1), little is known yet on whether this may be generalized to other strains in food related outbreaks.

For such purpose, outbreaks are an important source of information, as a complement to human challenge studies [18]. In the present study we have used information on the genogroup of the virus and the secretor status of the human hosts in oyster outbreaks to investigate infectivity of NoV GI and GII in conditions of natural exposure.

Methods

Outbreak data

From a database of oyster related outbreaks in France, outbreaks were selected if the exposed population and the attack rate were known, as well as the numbers of oysters consumed, and if the concentration of NoVs was known from analysis of a sample of oysters from the same batch. Further conditions were that the same strain of NoV should have been detected in human stools and oysters linked with the outbreak, and symptoms had to be consistent with NoV gastroenteritis. In some of the outbreaks other enteric viruses (enterovirus, rotavirus) were detected in stool and oysters, but it was concluded retrospectively that the main cause was NoV [19]. A case was defined by the sudden onset of vomiting or diarrhoea or both with maximum incubation period of 48h [20], exposed subjects were included if they ate from the same contaminated meal. For the last outbreak, in 2008, the secretor phenotype for 33 individuals out of 34 was determined from saliva [21].

The outbreak data are summarized in **Table 10** and further details have been published separately [19, 21-23]. The numbers of exposed individuals ranged from 2 to 36. Individual data about consumption and host status (secretor phenotype and blood group), were not always present as summarized in **Table 10**. The contamination levels as numbers of RNA copies by oyster s and the genotype of strains found in the oyster samples for each outbreak are given in **Table 10**. In some outbreaks there was co-contamination by both GI and GII strains.

For all outbreaks, oyster analyses were performed by the same laboratory using the same method for NoV quantification [24]; thus all viral doses were measured on the same scale.

The dose was calculated as numbers of genome copies per oyster, without correction for extraction and amplification efficiency, by extrapolating from the weight of the digestive gland (where the contamination is 90 to 99% concentrated depending on the strain [25]) to the weight of the whole oyster (based on the weight of total meat). In all following analyses the dose is expressed in number of genome (RNA) copies.

Table 1 **Available information for each outbreak**.

Year of outbreak	Number exposed	Number ill	Individual status Secretor	Individual status ABO	Individual Consumption *	Range values **	Norovirus strain	contamination ***
2008	34	23	Yes	Yes	Yes	2-6	GII.4	18-955-37-0
2006 a	27	11	No	No	No	4-6	GII GI	1100 2300
2006 b	2	2	No	No	No	4-6	GI	275-6783
2002	36	21	No	No	No	1-6	GII.4+GII.8 GII.4+GII.9 GI.4	25 125 25
2000	4	4	No	No	Yes	7-18	GI.1	85-237

***Individual Consumption in number of oysters **Range values of number of oysters consumed**

***The level of contamination, (results of pool analysis of digestive gland of several oysters), are given by the number of genomes by oyster**

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Dose-response model

The dose response models most commonly used for microbial pathogens are based on the conditional relation between exposure, infection and (acute) illness [26]. Exposure is equivalent to ingestion of one or more organisms (dose ingested). If p_m is the probability that any single ingested pathogen successfully passes all (m) defensive barriers in the host, this parameter summarizes the effects of host-pathogen interactions for infection [26].

Heterogeneity in this host-pathogen relationship can be modeled as a Beta distribution, with two parameters α and β [27]. Contamination in food products, in real world situations, can be described as a sample from a suspension with varying concentration. A Poisson-gamma mixture, equivalent to negative binomial distribution of number of genome copies, leads to a hypergeometric (2F1) dose response relationship [17]. Conditional on the ingested numbers of pathogens, this relationship can be described with a Beta-binomial distribution (**Table in appendix**).

The host (secretor status) and pathogen (genogroup) effects were incorporated as follows. The parameters of the infection dose response model were transformed as (Eq.1):

u = α /(α +β) and v = α + β (Eq.1):

The parameter u, the expectation of the Beta distributed probability p_m , depends on secretor status (Se) and genogroup (g) as (Eq. 2):

logit(u) = μ0 + λ × Se + γ × g . (Eq. 2)

Hence, for each combination of genogroup, and secretor status, parameters α and β can be defined, leading to 4 different dose response relationships.

For the probability of illness among infected subjects an existing dose response model based on the concept of illness hazard during infection was used, with key parameters r and η [26]. Under mild assumptions (gamma distributed duration of infection and linearly increasing illness hazard with dose) the conditional probability of illness (P_{ill}/ dose, inf) knowing dose (dose) and infection (inf) response can be described by the Eq3:

$$P(ill/dose,\eta,r,\inf) = 1-(1+\eta\times dose)^{-r} \quad \text{(Eq.3)}$$

For exposure to GI or GII the probability of infection becomes (Eq4.):

Pinf2= 1-(1-pinf(α_{GI}, β_{GI},dose $_{GI}$)) × (1-pinf(α_{GII}, β_{GII},dose $_{GII}$)) (Eq.4)

With pinf the probability to be infected by GI or GII, knowing specific parameters α, β (linked to GI, GII and secretor status) and ingested doses for GI or GII virus (see Source code in appendix)

For simplicity, as host and pathogen factors were assumed to act on early stages of infection (the virus entering host intestinal cells), they were assumed to only affect infection dose response: The risk of illness is considering the dose as the sum of dose by GI and GII and the parameters of the illness dose response model, η and r were assumed independent of NoV genogroup (GI or GII) or secretor status.

Bayesian framework

A Bayesian framework was used to estimate parameters and predict the probabilities of primary interest. The directed acyclic graph outlining the parameters and their relationship in the model is shown in **Figure 16**. Details of chosen distributions for all parameters are given in the **appendix**.

We followed an approach similar to a published proposal [28]. In a first step, a core model describing the functional dose response relationship was defined. Prior distributions were allocated to all parameters in order to produce a very flexible prior dose response, in order to accommodate any possible variation in infectivity and morbidity. Then, in a second step, the core model was extended by incorporating all available data (**Figure 16**) to produce posterior estimates.

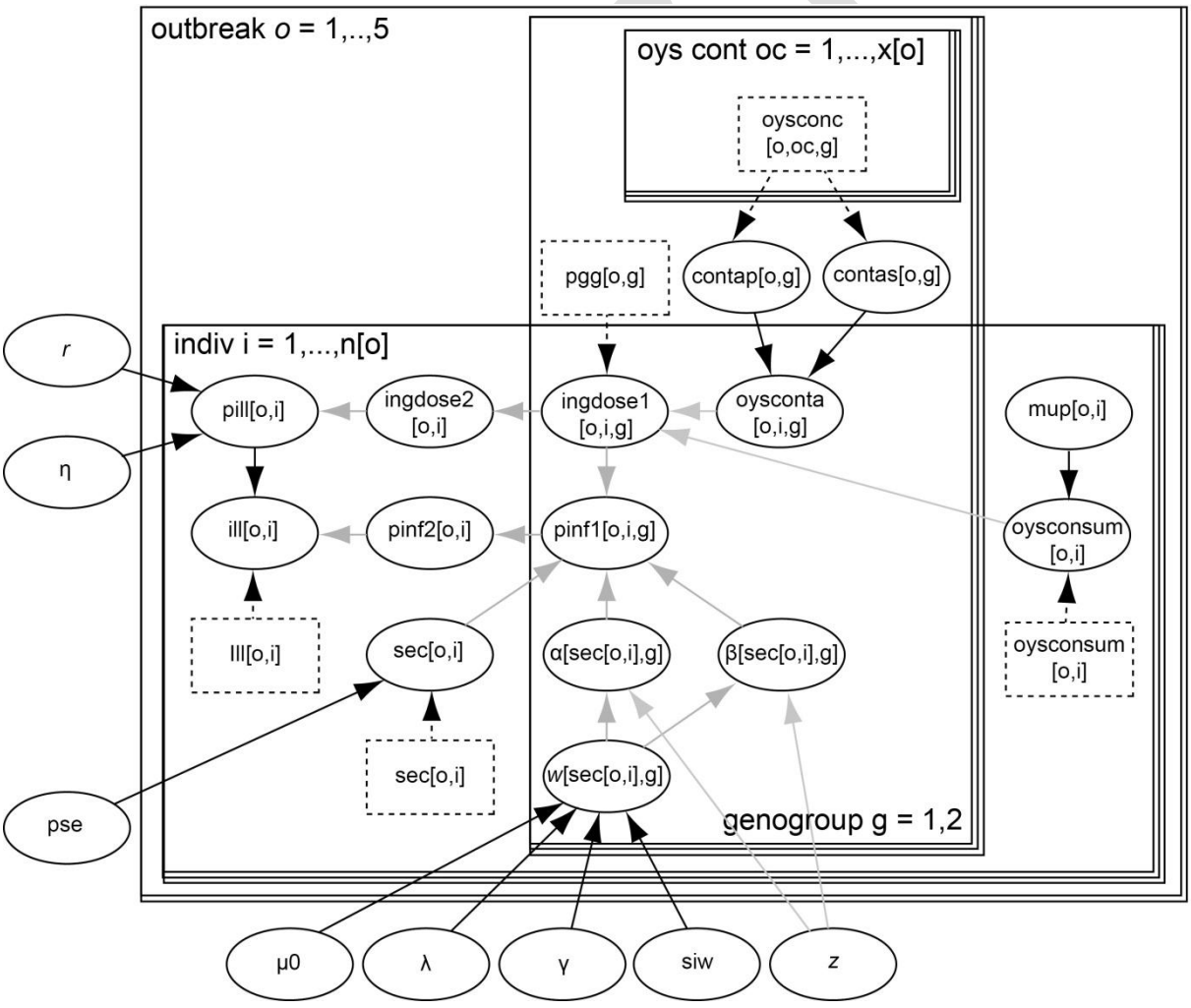

FIGURE 1: DIRECTED ACYCLIC GRAPH OF NOROVIRUS DOSE-RESPONSE MODEL.

Legend: All model quantities are presented as nodes. Data are denoted by dashed rectangle. Logical links are gray arrows and stochastic links black arrows. Solid

rectangles describe the loops with reference to an index indicated in the corner of the rectangle (o for loop inside each outbreak, for example).

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Oysconc in dashed rectangle: observed data of contamination in oysters; oc number of observation in oyster;Contap: parameter p (success probability) of Negative Binomial distribution;
contas: parameter s of Negative Binomial distribution;
oysconta: sampled value of oyster contamination from Negative Binomial distribution;
pgg in rectangle: data about the genotype involve in the outbreak
oysconsum in dashed rectangle individual consumption data;
oysconsum in oval sampled from truncated Poisson distribution with mean mup (prior);
ingdose1: Ingested dose for each individual and each genogroup;
ingdose2: Sum of doses for GI and GII; pinf1: the probability of infection by one genogroup; pinf2 probability of infection by GI, GII or both;
pill: the probability of illness;
ill(in oval): illness knowing probability of infection and illness;
ill(in rectangle): illness data in outbreak
pse: probability to be Secretor(=1) in general population;
sec in rectangle : data of individual secretor status
r, η, μ0, λ, γ, siw, z: parameters of dose-response
See appendix Table 1 for other legend explanation.

Specification of prior distributions

The prior probability distribution ("prior") for the estimated fraction of Se+ among exposed individuals was defined by a Beta distribution with parameters estimated from published literature [11]. In case of co-contamination (both genogroups present) contamination with GI is assumed to be independent of contamination with GII.

Numbers of NoV in oysters may be clustered: numbers of NoV in oysters were modeled as a Poisson-gamma mixture (Negative-Binomial) distribution.

Because, in all the outbreaks we studied, each individual ate from the same common meal, it was feasible to estimate a range for the numbers of oysters consumed, in case that information was missing. The dose ingested by each individual could be calculated as the product of a random sample from the Negative Binomial distribution of numbers of NoV per oyster, and a random sample from the numbers of oysters consumed, calculated separately for GI or GII NoV. When individual secretory status is unknown, the information is kept at the level of the choosen (informative) prior, that is the probability of secretor positive status in the general population, estimated in a separate and published analysis [11].

Vague priors of all parameters of infection dose-response were chosen from a Normal (or Log Normal) (μ0, λ, γ, z) with mean zero. Priors of the log transformed parameters η and r are described by a non-informative Normal distribution. All prior

distributions are given in **the appendix**. The priors of parameters for Se+ Se-/GI GII are set identical.

Model implementation

Models were run with Jags (Jags 3.2) [29] with R. 2.14.0 [30]. Parameter estimates were obtained with 3 chains of 15,000,000 iterations of the Gibbs sampler, thinning every 5000 iterations (to avoid autocorrelations), with a burn-in phase of 200,000 iterations. Source code of the extended core model is given in the **appendix**.

Model assessment

Convergence was assessed using the Gelman-Rubin diagnosis with three parallel chains [31]. A partial sensitivity analysis was performed, changing the standard deviation of key prior parameters (μ0, λ, γ) from 1 to 3. Posterior distributions from the eight resulting models were graphically compared. Median values and 95% credibility intervals of posterior distribution of each key parameter (μ0, λ, γ, η, r, α, β) were evaluated with 9,000 posterior samples, with the model of increased flexibility (standard deviation of 3 for each parameter, μ0, λ, γ) . In order to characterize differences between dose-response relationships, the two parameters of the Beta (relation for infection) are given for each combination of covariates (Se+/Se-, G I and II). Other metrics include mean and variance in p_m characterizing the heterogeneity of the dose response [32, 33]. For a Poisson inoculum (fully dispersed virus, homogenously mixed), with mean dose (μdose) and heterogeneous of pm, represented by a beta distribution(α, β) the probability of infection can be integrated to yield the confluent hypergeometric function($_1F_1$). For each mean dose, median and 95th percentile of probability of infection are calculated and plotted, for sampled values of α and β, using the relation below (Eq.5):

$$p\inf(\mu_{dose}/\alpha,\beta) = 1 - {}_1F_1(\alpha,\alpha+\beta,-\mu_{\text{dose}}) \quad \text{(Eq.5)}$$

The (unconditional) dose-response relation curves for illness were also plotted. The dose-response probability for illness can be written as the product of the infection and illness dose response probabilities (Eq.5):

$$Pill(\mu_{dose}/\eta,r,p\inf) = (1-(1+\eta\times\mu_{\text{dose}})^{-r})\times p\inf \quad \text{(Eq.6)}$$

With Poisson distribution of doses (mean λ), and with the maximum of possible value for pm (equal one), the maximum marginal risk of infection is Eq.7 [27]:

$Pinf_{max}=1-e^{-\lambda}$ (Eq.7)

This curve is the maximum infectivity limitation curve, plotted with dose-response for infection and disease.

Further characteristics of those curves are the median infectious dose and the dose causing acute enteric illness with 50% probability (ID 50), and the quantiles 95% of the probability of infection and disease for a mean dose of one genome copy (as quantified by RT-PCR method). the difference between Pinf(1) and pm can be described by Eq. 8 and Eq. 9:

For Poisson exposure:

Pinf(Dose) = 1 - exp(-$p_m \times$ Dose) (Eq.8)

(with pm beta distributed) therefore:

Pinf(1) = 1- exp(-p_m) (Eq.9)

which is different from p_m (Pinf(1) approaches p_m only for p_m<<1).

Then, p_m is a conditional probability of infection (given ingested dose or exposure to 1 genome copy). Calculation of p_m separates the infection probability from the distribution of exposure. The distribution of probability of infection per virus (p_m) (exact single genome copy ingested), on a logistic scale [34], is plotted. Infectivity of the virus is characterized by p_m, knowing that it separates the infection probability from the distribution of exposure.

In order to investigate the adequacy of the model with the observed data, we calculated the posterior caracteristics (quantiles) of the expected numbers of cases in groups of individuals with the same known exposure for the same outbreak. The contamination level was taken from posterior mean dose. Whenever the consumption and/or the secretor status were unknown, they were sampled from the model posterior distribution. Samples of size 9000 were simulated for this purpose.

Results

Boxplots of key parameters ($\mu 0$, λ, γ) for each model are given in **figure 17.**

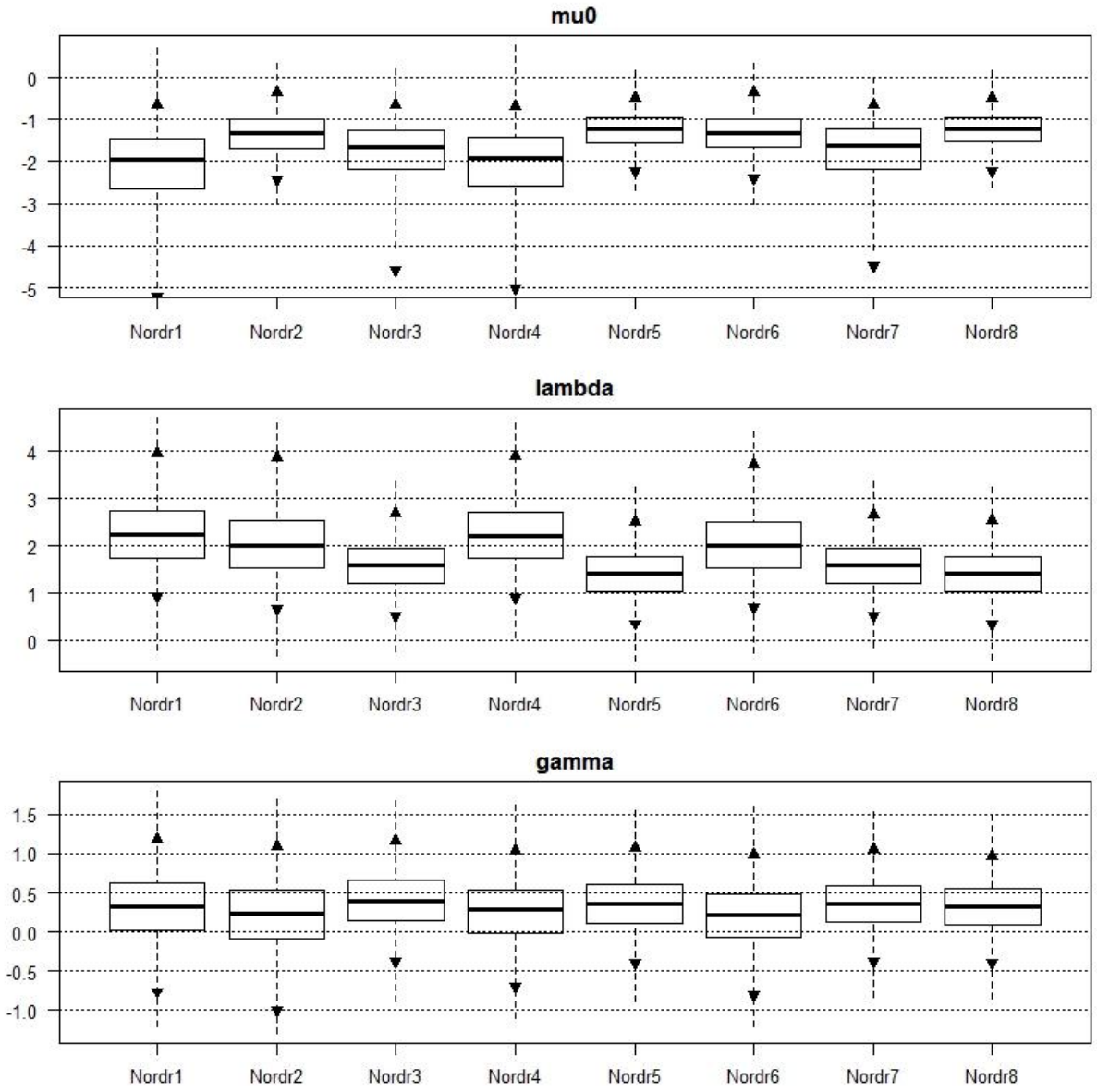

FIGURE 2: BOXPLOT OF POSTERIOR DISTRIBUTION.

Legend: median and 95% CI of the posterior distribution parameters μ0, λ, γ. Respective standard deviation of priors for each model: Nordr1($sd_{\mu0}$=3,sd_{λ}=3,sd_{γ}=3), Nordr2($sd_{\mu0}$=1,sd_{λ}=3,sd_{γ}=3), Nordr3($sd_{\mu0}$=3,sd_{λ}=1,sd_{γ}=3), Nordr4($sd_{\mu0}$=3,sd_{λ}=3,sd_{γ}=1), Nordr5($sd_{\mu0}$=1,sd_{λ}=1,sd_{γ}=3), Nordr6($sd_{\mu0}$=1,sd_{λ}=3,sd_{γ}=1), Nordr7 ($sd_{\mu0}$=3, sdλ=1, sdγ=1), Nordr8 ($sd_{\mu0}$=1, sdλ=1, sdγ=1)

Category	Parameter	Posteriors		
		Median	2.5th percentile CI	97.5th percentile CI
All	μ0	-1.96	-5.22	-0.66
	λ	2.23	0.93	3.95
	γ	0.32	-0.76	1.18
	r	0.99	0.59	1.63
	η	0.99	0.37	2.67
for Se+/GI	α	$1.2*10^{-2}$	$3.13*10^{-5}$	0.59
	β	$1.13*10^{-2}$	$2.01*10^{-5}$	5.1
	mean(p_m)	0.45	0.025	0.96
	var(p_m)	0.17	0.004	0.25
for Se-/GI	α	$3.04*10^{-4}$	$5.3*10^{-7}$	$1.36*10^{-2}$
	β	$2.88*10^{-2}$	$8.14*10^{-5}$	5.57
	mean(p_m)	$9.4*10^{-3}$	$1.5*10^{-4}$	0.19
	var(p_m)	$8.49*10^{-3}$	$4.02*10^{-5}$	0.15
for Se+/GII	α	$1.72*10^{-2}$	$5.2*10^{-5}$	0.61
	β	$8.24*10^{-3}$	$1.6*10^{-5}$	5.19
	mean(p_m)	0.62	0.05	0.96
	var(p_m)	0.17	0.006	0.25
for Se-/GII	α	$5.5*10^{-4}$	$1.12*10^{-6}$	$2.16*10^{-2}$
	β	$2.79*10^{-2}$	$8.13*10^{-5}$	5.59
	mean(p_m)	0.018	$2*10^{-4}$	0.30
	var(p_m)	0.016	$5*10^{-5}$	0.20

TABLE 2: STATISTICS OF POSTERIOR DISTRIBUTIONS OF THE MAIN PARAMETERS

Priors for (μ0, λ, γ) were symmetric around 0 are given in appendix Table 2, by monte-carlo simulation to give an idea about the precision of the simulation. For all these models, posteriors show that for μ0, the posterior distribution is shifted to negative values. The positive posterior values of λ represent the strong protective effect of Se- status, as is also apparent for all these models. The effect of genogroup is described by γ. The fraction of posterior sample of gamma greater than zero, as shown in Figure 2, is not large or small (between 69.3 and 85%, for different variance of gamma distribution priors) indicating that we do not have strong evidence that GI and GII have different infectivities. Posterior 95% CI of key parameters (μ0, λ, γ, η, r, α, β), for the model with increased flexibility (standard deviation of μ0, 3, λ, 3, γ, 3 respectively), are given in **Table 11,** stratified by genogroup and secretor status. Priors of this model are detailed in the **appendix**. The estimated risk of infection per ingested virus particle p_m is high, with posterior median values for the mean around 0.5 for Se+ subjects, 2.5th percentile around 0.03, 97.5th percentile around 0.96. For non secretor, this value is much lower, around 1/40 for the mean of p_m (**Table 11**).

Dose-response graphs of predicted probabilities (median and 95% credible interval) of infection and illness as a function of doses are shown respectively in **Figures18** and 19.

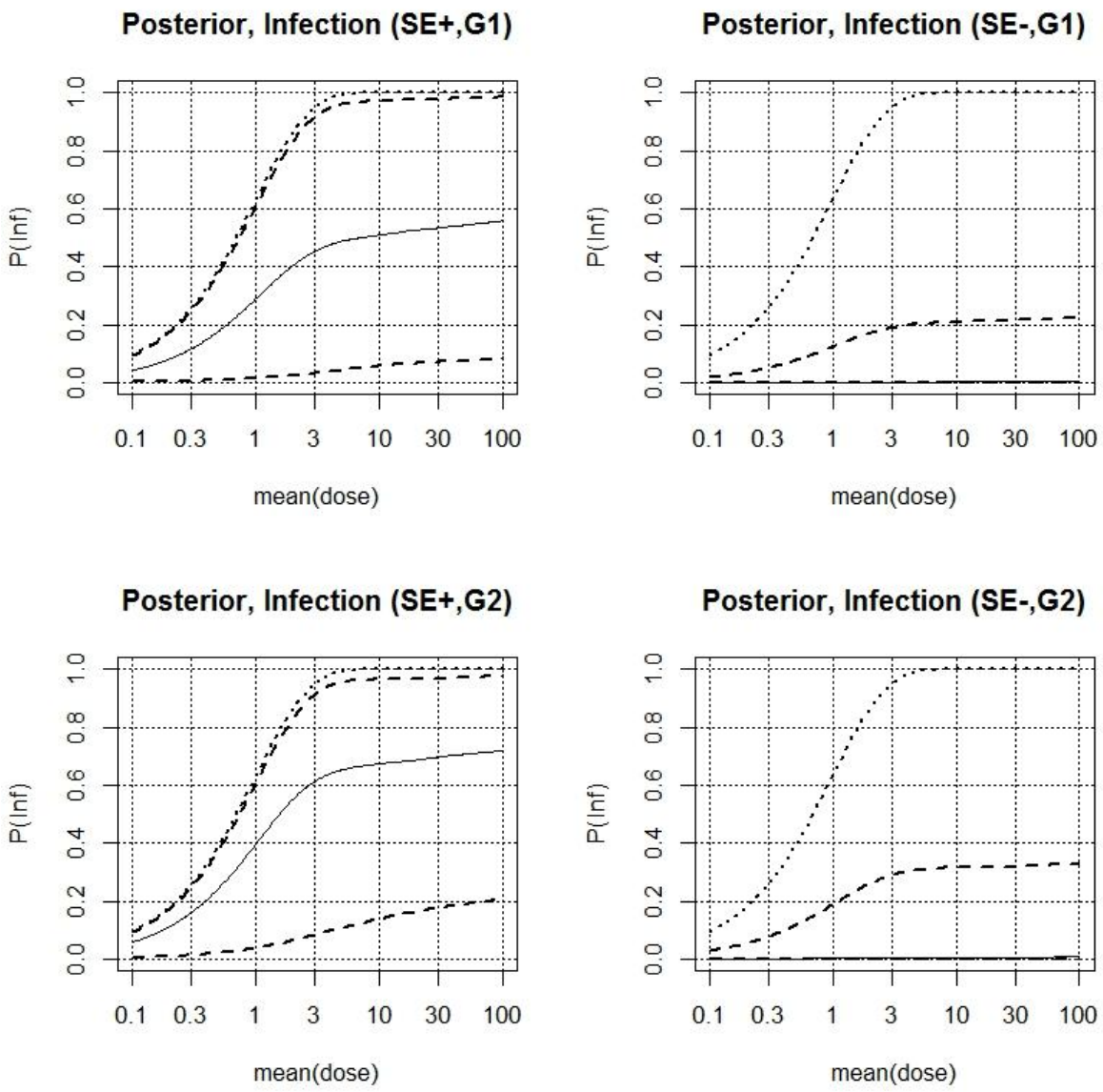

Figure 3: Posterior dose-infection relationships.

Legend: Solid line: median of dose-response curves; dashed line: credible interval 95%; dot dash line: maximum infectivity limitation curve.

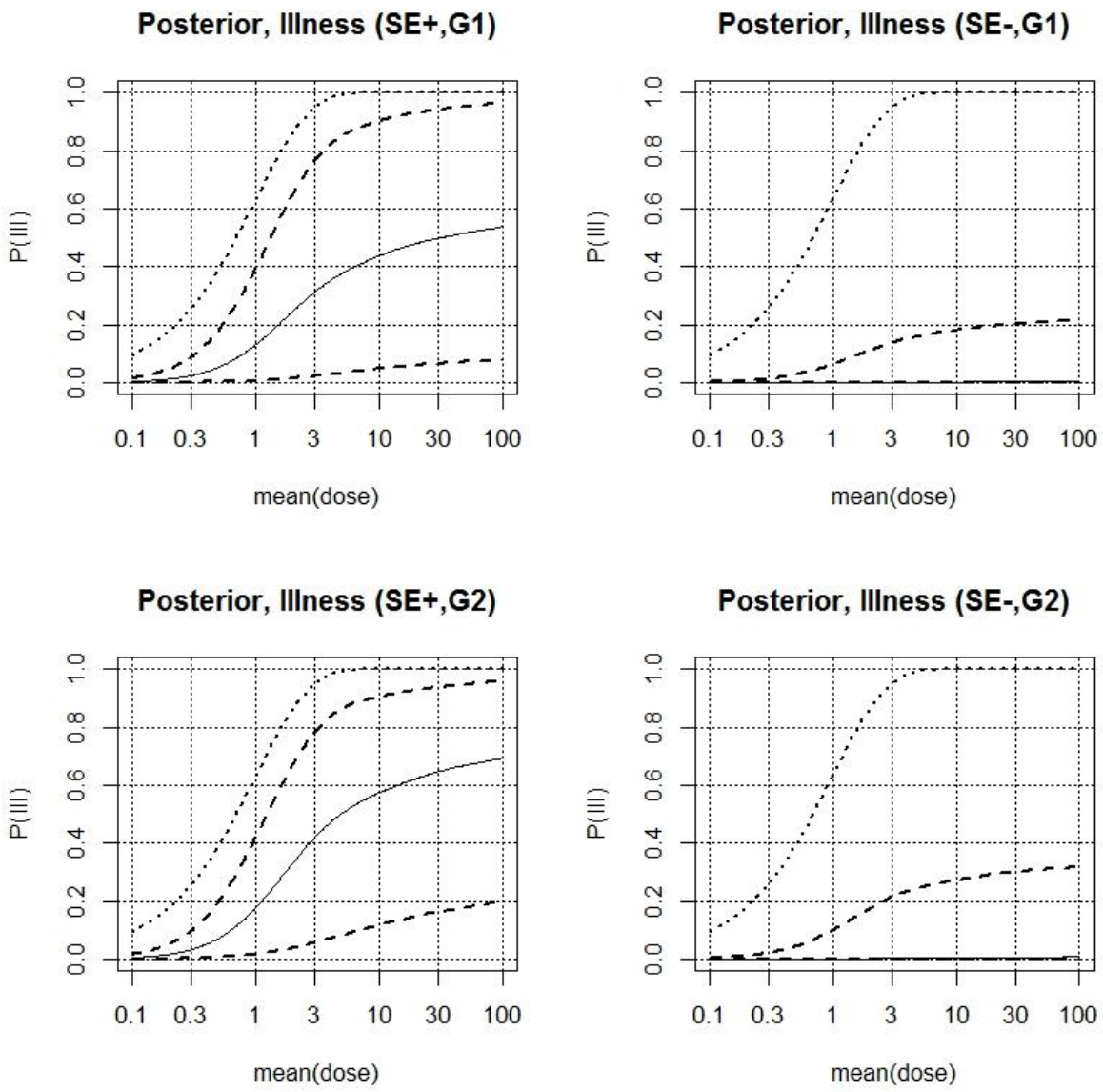

Figure 4: Posterior dose-illness relationships.

Legend: Solid line: median of dose-response curves; dashed line: credible interval 95%; dot dash line: maximum infectivity limitation curve.

Characteristics such as median infectious dose (ID_{50}) and probability of infection or illness at an average dose of a single virus are given in **Table 12**.

Category	Infection/disease	statistics	Median	2.5th percentile CI	97.5th percentile CI
Se+/GI	**Infection risk curve**	ID 50	7.1	0.73	$>10^6$
		prob inf with mean 1	0.29	0.015	0.61
	Disease risk curve	ID 50	32	1.32	$>10^6$
		prob dis with mean 1	0.13	0.007	0.39
Se-/GI	**Infection risk curve**	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob inf with mean 1	$9*10^{-4}$	4.4*10-6	0.12
	Disease risk curve	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob dis with mean 1	$4.25*10^{-4}$	$2.1*10^{-6}$	$6.19*10^{-2}$
Se+/GII	**Infection risk curve**	ID 50	1.6	0.74	$>10^6$
		prob inf with mean 1	0.4	0.04	0.61
	Disease risk curve	ID 50	4.86	1.24	$>10^6$
		prob dis with mean 1	0.18	0.017	0.42
Se-/GII	**Infection risk curve**	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob inf with mean 1	$2.12*10^{-3}$	$0.96*10^{-5}$	0.19
	Disease risk curve	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob dis with mean 1	$1.03*10^{-3}$	$4.2*10^{-6}$	0.1

TABLE 3: STATISTICS OF POSTERIOR DOSE-INFECTION (AS PLOTTED IN FIGURE 1) AND DOSE-DISEASE CURVES (AS PLOTTED IN FIGURE 2)

Characteristics such as median infectious dose (ID_{50}) and probability of infection or illness at an average dose of a single virus are given in **Table 12**. Median ID_{50} estimates ranging between 1.6 and 7.1 genome copies per oyster consumed (**Table 12**), probability of infection with a mean dose of a single NoV genome (Poisson distribution) are 0.29 [0.015-0.61] for GI and 0.4 [0.04-0.61] for GII in Se+ subjects (**Table 12**). For Se- subjects the probability of infection and disease with a mean dose of a single NoV genome (Poisson distribution) are lower, 9.10^{-4}[$4.4.10^{-6}$-0.12] for GI and $2.12.10^{-3}$[$0.96.10^{-5}$-0.19]for GII.

In Se+ subjects the probability of acute enteric disease was also very high: with a mean dose of one genome copy, the median probability of illness is, for GI, 0.13 [0.007-0.39], for GII, 0.18 [0.017-0.42], and much lower for Se-individuals (**Table 12**)

A density graph of the probability density of p_m (transformed to logit scale) can be constructed using a posterior sample of the infectivity parameters (α, β) showing strong heterogeneity in infectivity for Se+ subjects and smaller heterogeneity in Se- subjects (**Figure 20**) for the 2.5th percentile.

Results of the prediction are given in the **Table 13**. The posterior predictive distributions look plausible with respect to the observed data, the observed numbers of cases are always within the predicted the 95% credible interval.

year of outbreak	group	SE+(1)/ SE-(0)	number of oysters eaten	observed contamination /oyster	number exposed	observed illness cases	posterior quantile estimate of number of illness cases				
							2.5%	25%	50%	75%	97.5%
2008	1	0	3	GII: 118-955-37-0	3	0	0	0	0	0	2
2008	2	0	4		2	0	0	0	0	0	1
2008	3	0	6		1	1	0	0	0	0	1
2008	4	1	2		3	3	1	2	3	3	3
2008	5	1	3		17	12	6	13	15	16	17
2008	6	1	4		2	2	0	1	2	2	2
2008	7	1	6		4	3	1	3	4	4	4
2008	8	NA	3		1	1	0	1	1	1	1
2008	9	1	2-6		1	1	0	1	1	1	1
2006a	10	NA	4-6	GII: 1100 GI: 2300	27	11	10	12	14	16	20
2006b	11	NA	4-6	GI: 275-683	2	2	0	1	2	2	2
2002	12	NA	1-6	GII: 25-125 GI: 25	36	21	17	21	22	24	27
2000	13	NA	7	GI: 85-237	1	1	0	1	1	1	1
2000	14	NA	9		2	1	0	1	2	2	2
2000	15	NA	18		1	1	0	1	1	1	1

Table 13. observed numbers of cases in some identically exposed individuals and related simulated predictions from the posterior distribution model

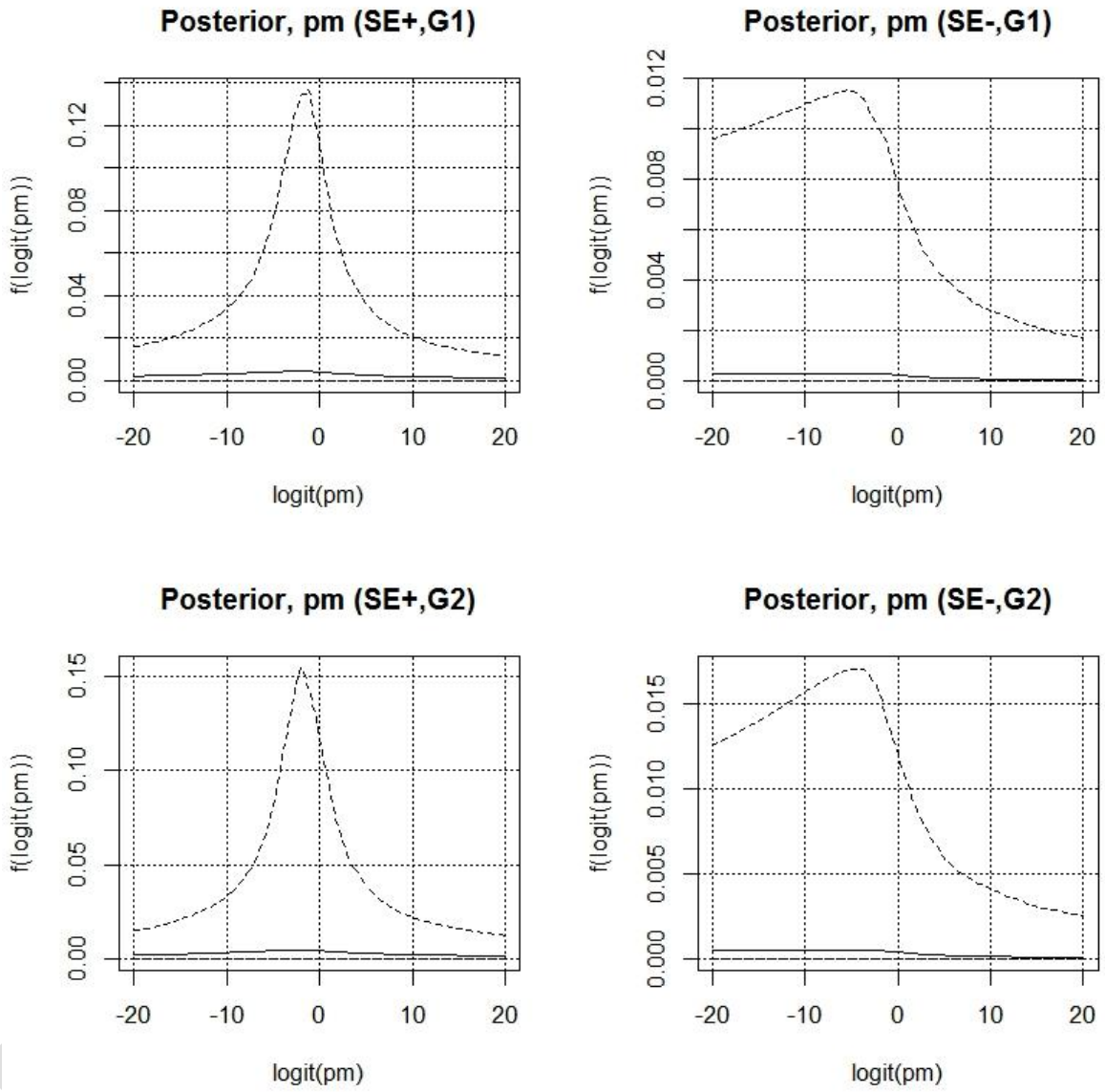

Figure 5. Density graph of the posterior single-hit probability of infection P_M, transformed to logit scale.

Legend: Solid line: contour of median density of probability; dashed line: contour of a 95% credible interval.

Discussion

Strong differences were found between secretor and non secretor phenotypes (Tables 2 and 3). For secretor positives, infection probability and disease probability at low dose were high. In a human challenge study, the median infectious dose for Norwalk virus (GI.1) in Se+ subjects, was found to be around 18 genome copies [17], and probability of infection for a single Norwalk genome copy was close to 0.5. For Se+ subjects, our results are very similar to these clinical estimates, for both GI and GII NoV, with median ID_{50} estimates ranging between 1.6 and 7.51 genome copies per oyster consumed (**Table 12**), and probability of

infection(p_m) for a single NoV genome copy near 0.5(Table 11). In Se+ subjects the probability of acute enteric disease was high, tand this is in agreement with high attack rates reported in NoV outbreaks, keeping in mind that around 20% of the population is less susceptible (secretor negative)[10, 11]. This high apparent infectivity (low ID_{50}) in PCR units suggests that there cannot be large fraction of uninfectious (defective) genome copies.

However, credible intervals are wide and the lower limit of the credible interval should be examined carefully. The 2.5 th percentile represents the lowest plausible infectivity, and for secretor positives, those values are still high, with the 2.5^{th} percentile of E(p_m) (Expectation) around 0.05 (Table 11), compared to the lower limit of the prior near 10^{-5} (see Table in appendix).

At low doses, there are few symptomatic cases, so that the chance of reporting is low (endemic cases), while at high doses there are many illnesses among the infected cases and the cluster, or outbreak, is easily detected, as suggested recently in a study comparing shellfish implicated in outbreaks compared to background environmental levels [35]. However, exposure is different from place to place [40], and data of French outbreaks, show that there were identified outbreaks with values of contamination relatively low (see **Table 10**).

We did not detect any difference in infectivity between GI and GII strains among the five outbreaks analyzed here. However, the variances of estimated infectivities (p_m) are high, and inclusion of additional outbreaks might reduce the uncertainty and reveal a difference in infectivity.

It may be surprising to find GI NoV so frequently involved considering the large dominance of GII in human outbreaks [9]. Different factors such as distinct resistance to waste water treatment [36] or selective mechanisms in bio-accumulation of NoV strains have to be considered [25, 37].

The genogroup effect as two distinct classes of susceptibility is a simplification. Heterogeneity of responses can be found between strains within genogroups, possibly linked with the ABO blood group phenotype [21, 38, 39]. Because the ABO blood group was only known in one of the studied outbreaks and its effect could vary between strains within genogroups, this mechanism of genetic susceptibility was neglected as well as any pre-existing acquired immunity. However the use of a Beta-Poisson (Hypergeometric 2F1 in this case) dose-response takes into account any variability of response of the host, and we may assume that it is incorporated into the dose-response estimates reported here.

Analysis of the saliva of consumers in outbreaks suggested that the effect of secretor status may not always be all or none [21]. Susceptibility of secretor negative individuals requires the existence of other ligands with weaker binding or the occurrence of rare strains that can infect non-secretors.

The dose method used for oyster analysis includes quality control such as extraction efficiency and absence of RT-PCR inhibitors. Only samples complying with these controls (over 10% extraction efficiency and absence of inhibitors) were quantified [24] knowing that

the extraction efficiency ranged between 13% and 38% in these shellfish analyses. As no method is currently available to evaluate NoV viability, we assumed that the fraction virus viable for infection was identical between the different oyster related outbreaks. All these factors have to be considered for future quantitative risk assessment studies.

For this first approach, we considered effects of genogroup or secretor status on infection and not on the illness dose-response relationship. It has been shown that secretor negative subjects are protected against infection, and thus their risk of becoming ill is also decreased [10]. For other enteric pathogens, variation in infectivity among strains has been demonstrated [18]. Since illness is conditional on infection, any effect acting on the probability of infection also modifies the marginal probability of illness. As in these outbreaks the data do not provide infection status, we have chosen the simplest way to take these covariates into account.

Contamination by multiple infectious agents is frequent in oyster-related outbreaks because of the fecal origin of contamination, by sewage contaminated water [19]. Since no information is available regarding mechanisms of cooperation or antagonism between infectivity or morbidity of NoV genogroups, we assumed there was no interaction. We consider also in this study only outbreaks with undetectable bacterial contamination and with identical NoV sequences in stool and shellfish samples of the same outbreak.

The actual scarcity of information is reflected by wide credible intervals. When additional outbreak data become available, with at least information on host secretor status, ABO blood type, size of exposed population, food intake and level of NoV in the contaminated food, the proposed dose response model may be improved, including ABO type as a covariate, and/or enable users to make more specific assumptions about effects on infection or illness. The generic model used here is described into the appendix, can also be used for the study of other outbreaks.

Conclusions

In conclusion, this study uses outbreaks to establish a human dose-response model for GI and GII, confirming that these viruses are highly infectious to humans with the secretor positive phenotype. Se- subjects have a strongly decreased susceptibility to NoV infection from either genogroup, as previously demonstrated with human challenge studies using G1.1. This is remarkable because the present results are based on outbreaks induced by consumption of contaminated oyster with a natural mix of strains and genogroups. For several years now, the increased recognition of the role of food, especially oysters, in gastroenteritis outbreaks has raised questions for safety regulations. Current processes (depuration, relaying, high pressure treatment or home cooking) as commonly performed are not effective to eliminate NoVs from oysters. Improving microbiological criteria for shellfish or food items by including NoVs surveillance measures will help to improve the safety of food introduced on the market [40]. Oyster producers must avoid harvesting from fecally contaminated areas and food business operators need such information to consider their safety limits [40]. This work will be useful for risk assessors and risk managers to

establish acceptable limit for NoV in oysters to be harvested and placed on the market, and may also be helpful for other risky food such as raspberries [41]. The present study provides new insights that will need to be considered for future regulation.

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Appendix

Parameter	status	Components	Value / Distribution	Rationale
o		Index of outbreak	1 to 5	
g		Index of Genogroup	1 or 2	
i, o		Index of individual i in outbreak o	1 to n [o],…see Table 1	see Table 1
pse	*marginal*	Probability to be Secretor(=1) in general population	Beta(79, 19)	[11]
Sec[i,o]	*conditional*	Secretor status of individual	Bernoulli(pse):(0, 1)	Informed by individual data or by prior pse
Contap[o,g]	*marginal*	Parameter p of Negative Binomiale distribution	$10^{uniform[-4,0]}$	Informed by observed data in oyster samples [o, g]
Contas[o,g]	*marginal*	Parameter s of Negative Binomiale	Round ($10^{Uniform(1, 1000)}$)	Informed by observed data in oyster samples [o, g]
oysconta [o, i, g]	*conditional*	Number of virus /oyster	Negbin (contap[o,g],contas[o,g])	
Ran[o, 1]	*fixed*	Minimum value of consumption (number of oyster)	Respective values	Data from outbreaks
Ran[o, 2]	*fixed*	Maximum value of consumption (number of oyster)	Respective values	Data from outbreaks

Mup[o]	fixed	Parameter of Poisson distribution	$\sqrt{ran[o,1] \times ran[o,2]}$	Geometric Mean value calculation
Oysconsum[o,i]	*marginal*	Oyster Consumption	Poisson (Mup[o]) Truncate (ran[o, 1]*ran[o, 2])	Informed by individual data or rank
Pgg[o,g]	*fixed*	Presence of genogroup in outbreak	0 or 1	Data from outbreak
ingdose1[o,i,g]	*intermediate*	Ingested dose for each individual and each genogroup	oysconsum[o,i]*Pgg[o,g]*oysconta[o,i,g]	
μ0	*marginal*	Intercept muw	Normal(mean=0,std=3)	Low informative
λ	*marginal*	Parameter of the Secretor effect	Normal(mean=0,std=3)	Low informative
γ	*marginal*	Parameter of the Genogroup effect	Normal(mean=0,std=3)	Low informative
Muw[sec[i,o],g]	*intermediate*	Expectation of beta distribution	μ0+λ*(sec[i,o]*2-1)+γ*(g*2-3)	
siw	*fixed*	Std of w	1	Low informative
w	conditional	Logit of the mean of beta distribution	Normal(mean=Muw,std=siw)	Low informative
z	marginal	Log (of quantity inversely related with variance)	Normal(mean=0,std=4)	Low informative
α[sec[i,o],g]	*intermediate*	First Parameter of dose-infection relationship	$\frac{\exp(w[i,g])}{1+\exp(w[i,g])} \times \exp(z)$	
β[sec[i,o],g]	*intermediate*	Second parameter	$\left(1-\frac{\exp(w[i,g])}{1+\exp(w[i,g])}\right)\times \exp(z)$	
pinf1[i,o,g]	*intermediate*	Probability of infection knowing exact dose	$\left(1-\frac{\Gamma(a[i,g]+b[i,g])\times\Gamma(b[i,g]+Ingdose1[o,i,}{\Gamma(b[i,g])\times\Gamma(a[i,g]+b[i,g]+Ingdose1[o,i,}\right.$	Beta-Binomial
pinf2[i, o, g]	*intermediate*	Probability of infection with both genogroups	1-(1-pinf1[i, 1])*(1-pinf1[i, 2])	independence of action
ingdose2[i,o]	*intermediate*	Sum of doses for GI and GII	ingdose1[i,o, 1]+ingdose1[i,o, 2]	
η	*marginal*	1st parameter	$\exp(Normale(mean=0, std=0.5))$	Low informative

r	*marginal*	2nd parameter	$\exp(Normale(mean=0, std=0.25)$	Low informative
pill[o,i]	*intermediate*	Probability of illness	$(1-(1+\eta \times \text{Ingdose2}[o,i])^{-r}) \times pint$	[38]
Ill[o,i]	conditional	Illness	$Bern(pill[o,i])$	

TABLE 4. DEFINITION, DISTRIBUTIONS AND VALUES OF PARAMETERS USED IN THE MODEL.

Legend: Status, gives the situation in Bayesian framework: marginal status for root random nodes, conditional for the other random nodes, fixed for constant values, and intermediate for the other nodes.

Category	Parameter	Priors		
		Median	**2.5th percentile CI**	**97.5th percentile CI**
	μ0	0.005	-5.83	5.84
	λ	0.01	-5.89	5.87
	γ	-0.02	-5.97	5.87
	r	1	0.6	1.63
	η	1.005	0.38	2.64
	pse	0.81	0.74	0.87
	z	-0.02	-7.86	7.74
Se+/GI	α	0.15	$2.06*10^{-6}$	969
	β	0.14	$1.86*10^{-6}$	955
	mean(p_m)	0.51	$3.2*10^{-5}$	0.9999
	var(p_m)	0.004	$2.5*10^{-7}$	0.23
Se-/GI	α	0.15	$2.1*10^{-6}$	986
	β	0.14	$2*10^{-6}$	997
	mean(p_m)	0.51	$3*10^{-5}$	0.9999
	var(p_m)	0.004	$4*10^{-5}$	0.15
Se+/GII	α	0.15	$2.36*10^{-6}$	943
	β	0.15	$2.2*10^{-6}$	943
	mean(p_m)	0.5	$3.2*10^{-5}$	0.9999
	var(p_m)	0.004	$2.1*10^{-7}$	0.22
Se-/GII	α	0.13	$2.12*10^{-6}$	941
	β	0.15	$2.16*10^{-6}$	928
	mean(p_m)	0.49	$3.22*10^{-5}$	0.9999
	var(p_m)	0.004	$2.4*10^{-7}$	0.224

TABLE 5: STATISTICS OF PRIORS DISTRIBUTIONS OF THE MAIN PARAMETERS

Appendix program:
R code preparing data file for BUGS code:
given on request
BUGS code describing the extended core model.

```
model {

  # ANCESTOR NODES = HYPERPARAMETERS
  #common between outbreaks (otb)
  # proportion of positive secretors people
  pse ~ dbeta(79, 19);

   #hyperparameters for dose-response (infection risk)
   # central dose response
  simu0<-3;
  mu0 ~ dnorm(0, 1/simu0^2);
  # effect due to the secretary status of the individual
  silambda<-3;
  lambda ~ dnorm(0, 1/silambda^2);
  # effect due to the genotype of the virus
  sigamma<-3;
  gamma  ~ dnorm(0, 1/sigamma^2);

  # common shape for the dose response
  # irrespective the attributes
  muz<-0;
  siz<-4;
  z ~ dnorm(muz, 1/siz^2);
  siw<-1;

  # hyperparameters for disease risk

  logetadis ~ dnorm(0, 1/0.5^2);
  logrdis ~ dnorm(0, 1/0.25^2);
  eta <- exp(logetadis);
  r <- exp(logrdis);
  #

  ###   looping on outbreaks with the help of indexes matrices
  ###       over all individuals from any outbreaks
  ###       over all sampled oysters

  for (otb in 1:nbotb) {
    #
    ##############
    # modelling the level of contamination of
    # the oysters for the two genotype
    for (ge in 1:2) {
      #   the parameters
      #       ancestor
      conts[otb,ge] ~ dunif(-4,0);
      contmu[otb,ge] ~ dunif(1, 1000);
      #       parameter to use
      contap[otb,ge] <- pow(10,conts[otb,ge]);
      contas[otb,ge] <- round (contmu[otb,ge]);
    }
    # modelling the contamination of sampled oysters
    for (oys in oyind[otb, 1]:oyind[otb, 2]) {
      oys.con1[oys] ~ dnegbin (contap[otb, 1],contas[otb, 1]);
      oys.con2[oys] ~ dnegbin (contap[otb, 2],contas[otb, 2]);
    }
```

```
    #
    ##############
    # modelling the illness of individuals
        for (ind in indind[otb, 1]:indind[otb, 2]) {
      #
      # consumption of oysters
      mup[ind] <- sqrt (ran[otb, 1]*ran[otb, 2]);
      oysconsum[ind] ~ dpois(mup[ind]) T (ran[otb, 1],ran[otb, 2]);
      # secretory status
      sec[ind] ~ dbern (pse);
      # loop onto the two genogroupes I an II
      for (g in 1:2) {

        #
      # oyster contamination
      oysconta[ind,g] ~ dnegbin (contap[otb,g],contas[otb,g]);
      # ingested dose
      ingdose1[ind,g] <- oysconta[ind,g]*oysconsum[ind]*pgg[otb,g];
        #
        # modelling the dose-response for infection
        #
      # expectation
      muw[ind,g] <- mu0 +
                  (sec[ind]*2-1)*lambda +
                  (g*2-3)*gamma
                ;
      # variability around it
      w[ind,g] ~ dnorm(muw[ind,g], 1/siw^2);
      #
      u[ind,g] <- exp(w[ind,g]) / (1+exp(w[ind,g]));
      v[ind,g] <- exp(z);
      #
      a[ind,g] <- u[ind,g] * v[ind,g];
      b[ind,g] <- (1-u[ind,g]) * v[ind,g];
      #
      gammag1[ind,g] <- loggam(a[ind,g]+b[ind,g]) -
                    loggam(a[ind,g]+b[ind,g]+ingdose1[ind,g]) +
                    loggam(ingdose1[ind,g]+b[ind,g]) -
                    loggam(b[ind,g]);
      # proba of infection per genogroup
      pinf1[ind,g] <- (1-exp(gammag1[ind,g]));
      } # ending the loop over g
      #
      # probability of infection combining all genogroups
      pinf2[ind] <- 1-(1-pinf1[ind, 1])*(1-pinf1[ind, 2]);
      #
      # Looking for illness dose-response
      # common dose for conditional illness
      ingdose2[ind] <- sum(ingdose1[ind,])
      # probability to get ill for the assumed dose
      pill[ind] <- (1-pow(1+eta*ingdose2[ind],-r))*pinf2[ind];
      # modelling the illness
      ill[ind] ~ dbern (pill[ind]);
    } # ending loop over ind
    #
  } # ending loop over otb
  #
} # ending the model
```