International Congress on Environmental Modelling and Software

7th International Congress on Environmental Modelling and Software - San Diego, California, USA - June 2014

Jun 16th, 2:00 PM - 3:20 PM

Norovirus dose-response in sewage-related QMRA: The importance of virus aggregation

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Abstract: Norovirus infection and illness are increasingly used as end-points in sewage-related health risk assessments, especially as data from clinical trials outbreak studies have recently become available. The authors of a very recent clinical trial have inferred that the human median infectious dose is higher than previous estimates and similar to that of other RNA viruses. This finding has the potential to cause a revision of previous risk assessments. However, in reaching this conclusion, the potential role of aggregation of these viruses seems not to have been accounted for, although it was accounted for in an earlier trial. In fitting (hypergeometric) dose-response curves to data from Norovirus clinical trials, the potential for the laboratory-stored stock of this non-culturable virus to have become aggregated onto storage matrix substances, as observed in a clinical trial, must be addressed. That is because standard dilution series calculations for doses are based on the assumption of uniform mixing of disaggregated particles. "Low" doses derived in this manner may therefore actually be "No" doses and lack of an infection response in trial participants at such doses may reflect absence of the virus in inocula, rather than reflecting its infectivity status. The possible consequences of including aggregation in a re-analysis of such data is explored. Research into the potential for sewage treatment processes to promote aggregation would be of benefit to sewage-related QMRAs.

Keywords: Norovirus; dose-response; aggregation; clinical trial; outbreak

1   INTRODUCTION

The aetiological agent in diluted sewage-contaminated water causing excess illnesses among swimmers and other recreational water users is increasingly identified as Norovirus (e.g., Sinclair 2009). Until recently, information on dose-response characteristics of this pathogen has been lacking. But in recent years three studies have emerged that have shed light on this gap—so much so that Norovirus infection and illness impacts have gained an increasing role in quantitative microbial risk assessments (QMRA) (e.g., Soller et al. 2010). These studies are:

1.   Analysis of Norwalk (genogroup GI.1) virus clinical trial data, reported by Teunis et al. (2008);
2.   Outbreak studies for a range of Noroviruses (GI, GI.1, GI.4, GII, GII.4, GII.8, GII.9) for consumers of raw oysters in southern France (Thebault et al. 2013);
3.   An independent Norwalk virus (GI.1) clinical trial study, reported by Atmar et al. 2013.

We will focus herein on differences between infection (cf. illness) dose-response for Norwalk (GI.1) viruses between these three studies. There are also differences in predicted illness responses, but to some extent at least, they are driven by differences in infection dose-response. We also focus only on the "susceptible" individuals in the trials and outbreak study (~70% in the first study). For studies 1 and 2 these are defined as "Se+". Virus binding to intestinal epithelial cells depends on a secretor phenotype: non-secretors (Se−) lack the receptor and so the virus cannot bind to cause infection. In the third study two susceptible groups were defined: (i) secretor-positive blood group O or A; (ii) all secretor-positive persons. For consistency we will use only the latter. In this way we can assume that the distribution of susceptible individuals in the three studies are made a similar as possible, though there is insufficient information to further quantify the relative consistency and ages of susceptible individuals between the three studies.

A key feature of this analysis is that the aggregation of viruses in doses given to individuals in a clinical trial must be accounted for when calibrating a suitable dose-response curve. However, when applying the dose-response curves to non-aggregated virions the aggregation parameter is set to nought *whilst the other parameters remain unchanged*.

2   COMPARING THE TEUNIS AND THEBAULT STUDIES

As shown in Figure 1, the infection dose-response for studies 1 and 2 are rather similar, rising steeply at the origin and flattening out before the median Human Infectious Dose (HID$_{50}$) is reached: 26 virions for the clinical trial and 7 virions for the outbreak study. The outbreak study analysis indicates the stronger infectivity, particularly at higher doses (higher than commonly predicted for sewage-related QMRA exercises).
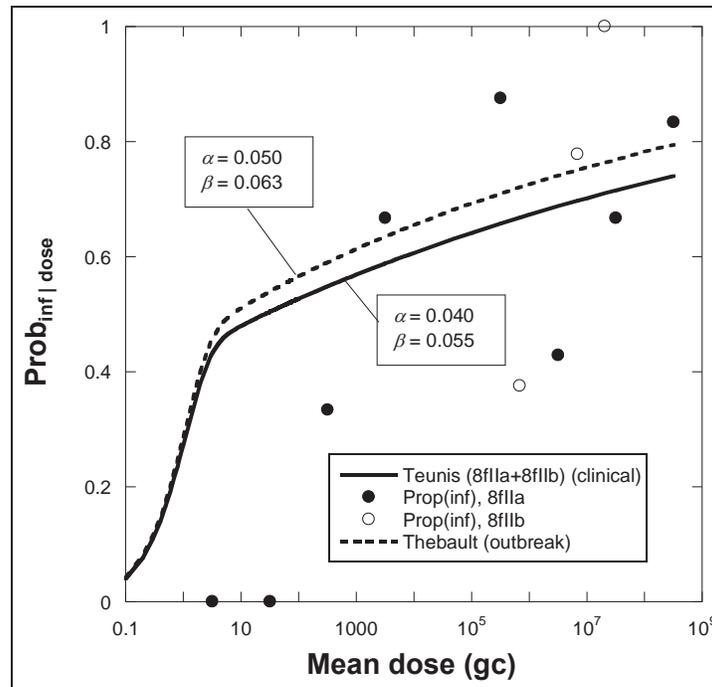

Figure 1. Non-aggregation infection dose-response curves from Teunis et al. (2008) and Thebault et al. (2013). Both curves have been calculated using the formula Pr(infection | d) = 1 − $_1F_1(\alpha, \alpha + \beta, -d)$, where $_1F_1$ is the Kummer confluent hypergeometric function, d is the mean dose received by a group of trial participants (in genome copies) and α and β are shape and location parameters. The Teunis (α, β) set is taken from their Table III with aggregation parameter (a) set to zero. The Thebault (α, β) set was obtained by fitting to their published median curve, using the "FindFit" procedure in Mathematica®. (Note that the first four doses reported in Teunis et al. 2008 are all a factor of 10 too high—*pers. comm*. Dr. Peter Teunis, RIVM.)

2.1   Accounting for aggregation
At first sight the continuous curve shown in Figure 1 is a poor fit with the low dose data. The explanation for that is provided by the observation in Teunis et al. (2008) that the first part of their trial (8fIIa) used laboratory-stored virions that had become strongly aggregated, as judged by electron microscopy (the second part, 8fIIb, used fresh non-aggregated inocula).[1] Accounting for that aggregation gives rise to a different functional form of the ingestion dose-response equation, the Gauss hypergeometric function: Pr(infection | d) = 1 − $_2F_1[\alpha, d(1-a)/a, \alpha + \beta, -a/(1-a)]$, results for which are shown in Figure 2. Note that the value of a in that Figure (a = 0.9997) corresponds to a

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[1] Electron microscopy alone may not be sufficient to fully characterise aggregation, because samples must first be stained and dried (*pers. comm*. Dr Jeremie Langlet, ESR, Porirua, New Zealand).

mean aggregate size of $\mu_a \approx 400$, using the formula presented in Supplementary Information for the Teunis et al. (2008) paper: $\mu_a = -a[(1-a)\ln(1-a)]^{-1}$ virions.
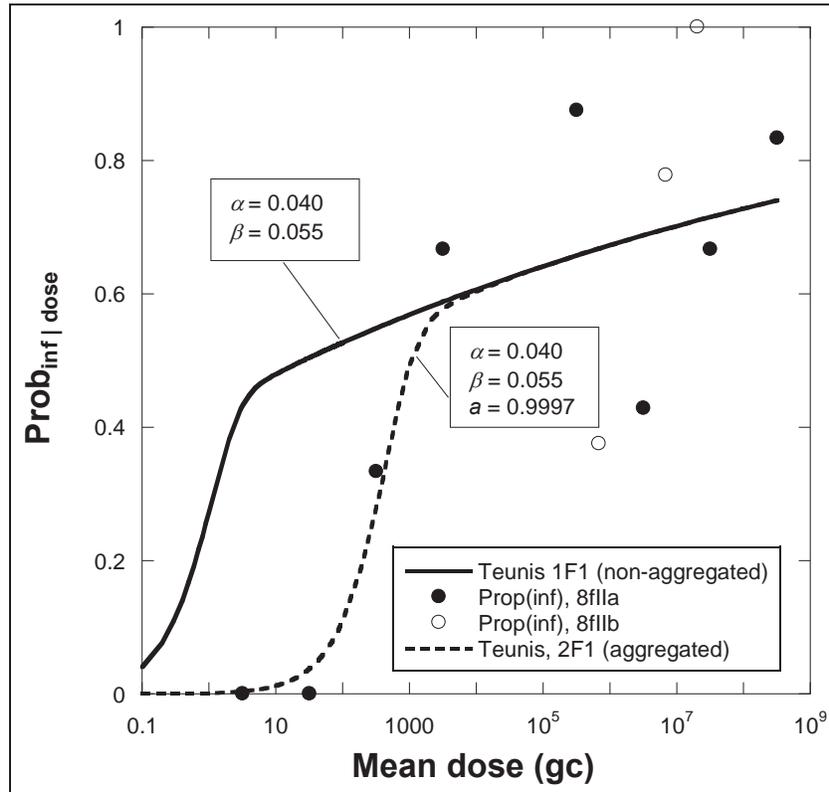

$\alpha = 0.040$
$\beta = 0.055$

$\alpha = 0.040$
$\beta = 0.055$
$a = 0.9997$

Legend:
— Teunis 1F1 (non-aggregated)
● Prop(inf), 8fIIa
○ Prop(inf), 8fIIb
---- Teunis, 2F1 (aggregated)

Figure 2. Accounting for virus aggregation in the clinical trial data analysed by Teunis et al. (2008).

Inspection of Figure 2 shows that the fit of this equation (the dashed line) with the low dose data is close, and that the HID_{50} is now 1058 virions. Consequently, if a QMRA were to be based on fresh non-aggregated virions (such as the 8fIIb data) the solid curve in Figure 2 (or Figure 1) is the appropriate choice—even though the dashed curve in Figure 2 offers the best fit. The reason for this at-first-puzzling feature lies in the discrete nature of these virions: Standard dilution series calculations for doses are based on the assumption of uniform mixing of disaggregated particles. Effectively that assumes that there will always be *some* viruses present in diluted inocula. However, for aggregated conditions "Low" doses derived in this manner will usually actually be "No" doses and lack of an infection response in trial participants at such doses may reflect absence of the virus for aggregated inocula, rather than reflecting its infectivity status. This possibility is strongly suggested by the two lowest doses in Figures 1 and 2, for which the infection response is completely absent.

3   THE ATMAR STUDY

This study reports significantly lower infectivity than studies 1 and 2, with a HID_{50} of about 2800 for the Se[+] individuals, based on a logistic regression approach. The authors concluded that this result is similar to that of other RNA viruses. While acknowledging the examination of aggregation made by Teunis et al. (2008), this issue was not addressed in the paper. However, a supporting publication (Atmar et al. 2011) notes that "The study vaccine contained … a mucoadhesive agent…", which is suggestive of some aggregation.

Figure 3 displays three hypergeometric functions fitted to this study's data (such fits to hypergeometric functions were not reported by Atmar et al. 2013).
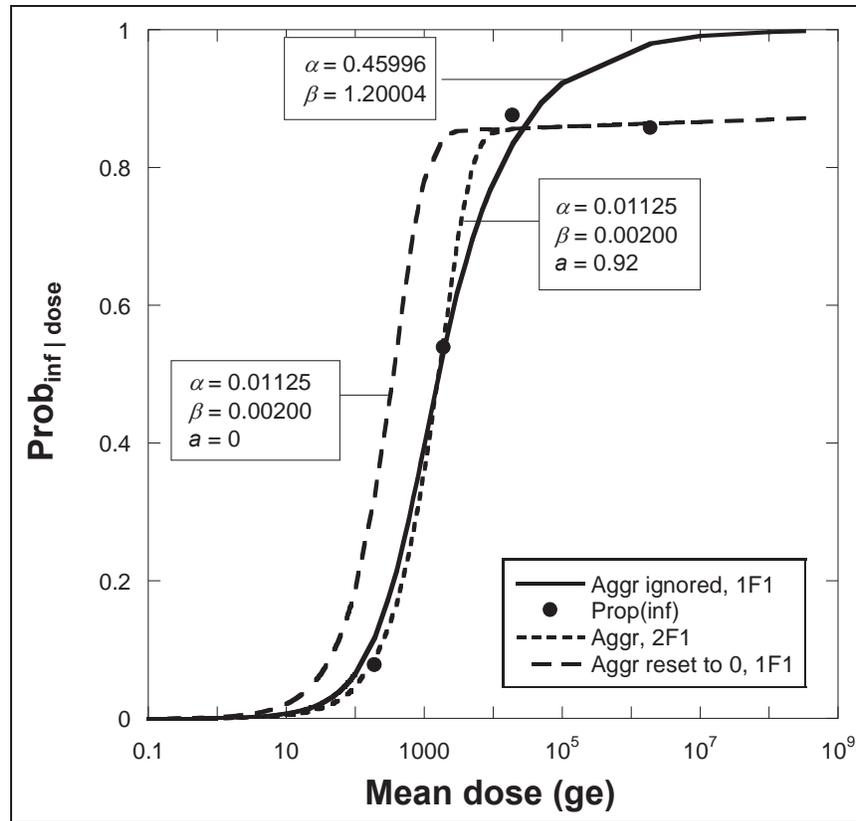
Figure 3. Accounting for virus aggregation in the clinical trial data analysed by Atmar et al. (2013). Doses in genome equivalents (ge) are defined as 400 times the trial's RT-PCR units. Note that the values of *α*, *β* and *a* in this Figure refer to doses measured in the trial's RT-PCR units, <u>not</u> to the ge values (the "FindFit" procedure in Mathematica® was not convergent for ge units). ge units are plotted here to facilitate comparison with Figures 1 and 2.

The first (solid line) is a best fit, ignoring any aggregation, giving rise to a HID_{50} of 1624. The second line (short dashes) fits a Gauss hypergeometric function with an arbitrary value of the aggregation parameter set to 0.92 (the Mathematica® "FindFit" procedure failed to converge at higher values of *a*, presumably because the sample size is small). This value of the aggregation parameter corresponds to a mean aggregate size of about 4.5 virions. This gives rise to a slightly lower HID_{50} (1610). The third line (long dashes) resets the aggregation parameter (*a*) to zero, but retains the second line's (α, β) set. The motivation for doing so is the same as for Figure 2: When analyzing a clinical trial with aggregated virions, that aggregation must be accounted for, but when applying the dose-response parameters to non-aggregated virions the parameter *a* is irrelevant. As expected, this resetting (of *a*), shifts the dose-response curve to the left; its HID_{50} is 355. Had the second curve used a higher value of *a*, an even greater reduction in apparent infectiousness would have arisen.

4   CONCLUSIONS AND RECOMMENDATIONS

The strident difference between the Norovirus HID_{50} values derived from data reported for the two independent clinical trials may be considerably lessened were the role of virus aggregation in the most recent trial quantified and accounted for.

In conducting QMRA for Noroviruses, the possibility of aggregation of the viruses needs to be taken into account; there can be substantial changes in indicated infectivity if this is not done. While fresh sewage can be expected to be not aggregated, we lack data and information on the degree of aggregation that may be accumulated during wastewater treatment processes (e.g., activated sludge processes deliberately seek some aggregation). That lack poses a challenge for risk analysts and infrastructure operators. Research to examine this question in some detail would appear to be fruitful.

ACKNOWLEDGMENTS

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