

## Estimation of norovirus infection risks to consumers of wastewater-irrigated food crops eaten raw

Duncan Mara and Andrew Sleigh

### ABSTRACT

A quantitative microbial risk analysis—Monte Carlo method was used to estimate norovirus infection risks to consumers of wastewater-irrigated lettuce. Using the same assumptions as used in the 2006 WHO guidelines for the safe use of wastewater in agriculture, a norovirus reduction of 6 log units was required to achieve a norovirus infection risk of  $\sim 10^{-3}$  per person per year (ppy), but for a lower consumption of lettuce (40–48 g per week vs. 350 g per week) the required reduction was 5 log units. If the tolerable additional disease burden is increased from a DALY (disability-adjusted life year) loss of  $10^{-6}$  ppy (the value used in the WHO guidelines) to  $10^{-5}$  ppy, the required pathogen reduction is one order of magnitude lower. Reductions of 4–6 log units can be achieved by very simple partial treatment (principally settling to achieve a 1-log unit reduction) supplemented by very reliable post-treatment health-protection control measures such as pathogen die-off (1 – 2 log units), produce washing in cold water (1 log unit) and produce disinfection (3 log units).

**Key words** | agriculture, norovirus, quantitative microbial risk analysis, reuse, wastewater

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### INTRODUCTION

The third edition of the World Health Organization's guidelines for the safe use of wastewater in agriculture (WHO 2006) represents a radical departure from the first two editions (WHO 1973, 1989). The latter specified required qualities of treated wastewater that were deemed safe for crop irrigation, whereas the current edition is based solely on risk analysis, at least for viral, bacterial and protozoan pathogens; no recommendations are made for effluent quality, except for helminthic pathogens. For viral, bacterial and protozoan pathogens the approach is 'from field to fork' and the guidelines describe a risk-based procedure for determining what reductions of these pathogens are required to protect consumer health.

The starting point for any risk analysis is the setting of a tolerable level of risk. In the 2006 WHO guidelines this is taken as an additional burden of disease of  $\leq 10^{-6}$  DALY loss per person per year (ppy), where DALY is a disability-adjusted life year, which is used as a metric to compare the

disease burden of different diseases and disabilities (DCPP 2008). This tolerable DALY loss of  $\leq 10^{-6}$  ppy is the same as that used in the third edition of the WHO guidelines on drinking-water quality (WHO 2004) and reflects the approach of the Stockholm Framework which recommends applying the same level of tolerable disease risk to all water exposures, whether these are, for example, drinking fully treated drinking water or consuming food crops irrigated with treated wastewater (Fewtrell & Bartram 2001).

The 'index' viral, bacterial and protozoan pathogens used in the 2006 guidelines were rotavirus, *Campylobacter* and *Cryptosporidium*, respectively. In this paper we report the results of our risk analyses for norovirus (NV), which is a very common, if not the commonest, cause of gastroenteritis affecting all age groups (Widdowson *et al.* 2005) and certainly the commonest viral cause of gastroenteritis (rotavirus mainly affects children under the age of 3 years), for which dose–response data are now available (Teunis *et al.* 2008).

## QUANTITATIVE MICROBIAL RISK ANALYSES

The quantitative microbial risk analysis–Monte Carlo (QMRA-MC) methodology used to estimate NV infection risks as a result of consuming wastewater-irrigated lettuce was based on the work of Shuval *et al.* (1997), Haas *et al.* (1999), Mara *et al.* (2007) and Benke & Hamilton (2008). The Benke & Hamilton method for calculating the annual risk of infection firstly determines an annual risk of infection by performing a Monte Carlo simulation with the number of simulations set equal to the number of days of exposure per year (rounded down to an integral value); it then repeats this any specified number of times and determines the resulting 50- and 95-percentile annual infection risks.

The first step was to determine the tolerable NV disease and infection risks corresponding to a tolerable DALY loss of  $10^{-6}$  pppy, using a DALY loss of  $9 \times 10^{-4}$  per case of NV disease (Kemmeren *et al.* 2006) and an NV disease/infection ratio of 0.8 (Moe 2009). Thus:

$$\begin{aligned} \text{Tolerable NV disease risk} &= \frac{\text{Tolerable DALY loss pppy}}{\text{DALY loss per case of NV disease}} \\ &= \frac{10^{-6}}{9 \times 10^{-4}} = 1.1 \times 10^{-3} \text{ pppy} \end{aligned}$$

$$\begin{aligned} \text{Tolerable NV infection risk} &= \frac{\text{Tolerable NV disease risk pppy}}{\text{NV disease/infection ratio}} \\ &= \frac{1.1 \times 10^{-3}}{0.8} = 1.4 \times 10^{-3} \text{ pppy} \end{aligned}$$

Following the recommendations in Teunis & Havelaar (2000), the NV dose–response dataset of Teunis *et al.* (2008) was used in place of the  $\beta$ -Poisson equation in the QMRA-MC computer program developed to determine median NV infection risks pppy (the program is available at [www.personal.leeds.ac.uk/~cen6ddm/QMRA.html](http://www.personal.leeds.ac.uk/~cen6ddm/QMRA.html)). A series of 10,000-trial QMRA-MC risk simulations was run and the resulting estimates of median risk obtained and the assumptions on which they are based (which are the same as those used in the 2006 guidelines but without pathogen die-off) are given in Table 1. This shows that an

**Table 1** | Median norovirus infection risks from the consumption of 100 g of wastewater-irrigated lettuce every two days estimated by 10,000-trial Monte Carlo simulations \*

Wastewater quality ( <i>E. coli</i> per 100 ml)	Median norovirus infection risk pppy
$10^7$ – $10^8$	1
$10^6$ – $10^7$	1
$10^5$ – $10^6$	1
$10^4$ – $10^5$	0.94
$10^3$ – $10^4$	0.25
100–1,000	$2.9 \times 10^{-2}$
10–100	$2.9 \times 10^{-3}$
1–10	$2.9 \times 10^{-4}$

\*Assumptions: 10–15 ml wastewater remaining on 100 g lettuce after irrigation; 0.1–1 norovirus per  $10^5$  *E. coli*; no die-off between harvest and consumption.

*E. coli* reduction of 6 log units (from  $10^7$ – $10^8$  per 100 ml to 10–100 per 100 ml) results in a norovirus infection risk of  $2.9 \times 10^{-3}$  pppy, which is only marginally higher than the tolerable norovirus infection risk of  $1.4 \times 10^{-3}$  pppy determined above. This required 6-log unit reduction can be achieved by a combination of wastewater treatment and the post-treatment health-protection control measures detailed in the 2006 guidelines (modified by the produce washing and disinfection results reported by Amoah *et al.* 2007), the most important of which are shown in Table 2. These are extremely reliable and in effect they always occur. The required 6-log unit reduction could be achieved, for example, by a 1-log unit reduction by wastewater treatment, a 2-log unit reduction through die-off and a 3-log unit reduction by produce disinfection.

In many developing countries, especially in Africa and Asia, lettuce is not consumed at the rate of 100 g every two days, the value used by Shuval *et al.* (1997), Mara *et al.* (2007) and in the WHO guidelines. Seidu *et al.* (2008) reported consumption in urban Ghana of 10–12 g lettuce in ‘fast food’ on each of four days a week. A second series of 10,000-trial QMRA-MC risk simulations was therefore run for this lettuce consumption pattern and the resulting estimates of median risk are given in Table 3, which shows that an *E. coli* reduction of 5 log units (from  $10^7$ – $10^8$  per 100 ml to 100–1,000 per 100 ml) results in a norovirus infection risk of  $3.6 \times 10^{-3}$  pppy, which again is only marginally higher than the tolerable norovirus

**Table 2** | Selected post-treatment health-protection control measures and associated pathogen reductions

Control measure	Pathogen reduction (log units)	Notes
Pathogen die-off	0.5–2 per day	Die-off after last irrigation before harvest (value depends on climate, crop type, etc.)
Produce washing	1	Dipping salad crops, vegetables and fruit in clean cold water for ~ 5 seconds
Produce disinfection	3	Soaking salad crops, vegetables and fruit in a disinfectant solution for ~ 5 minutes and rinsing with clean water
Produce peeling	2	Fruits, root crops

Sources: WHO (2006) and Amoah *et al.* (2007).

infection risk determined above. This required 5-log unit reduction could be achieved by, for example, a 1-log unit reduction by wastewater treatment, a 1-log unit reduction through die-off and a 3-log unit reduction by produce disinfection.

### DALY LOSS OF $\leq 10^{-5}$ PER PERSON PER YEAR?

In *Levels of Protection*, one of the documents in the rolling revision of its drinking-water quality guidelines, WHO (2007) states that:

‘in locations or situations where the overall burden of disease from microbial, chemical or radiological exposures by all exposure routes is very high, setting a  $10^{-6}$  DALY [loss] per person per year annual risk from waterborne exposure will have little impact

**Table 3** | Median norovirus infection risks from the consumption of 10–12g of wastewater-irrigated lettuce on four occasions per week estimated by 10,000-trial Monte Carlo simulations \*

Wastewater quality ( <i>E. coli</i> per 100 ml)	Median norovirus infection risk pppy
$10^7$ – $10^8$	1
$10^6$ – $10^7$	1
$10^5$ – $10^6$	0.97
$10^4$ – $10^5$	0.30
$10^3$ – $10^4$	$3.6 \times 10^{-2}$
100–1,000	$3.6 \times 10^{-3}$
10–100	$3.6 \times 10^{-4}$
1–10	$3.6 \times 10^{-5}$

\*Assumptions: 10–15 ml wastewater remaining on 100 g lettuce after irrigation; 0.1–1 norovirus per  $10^5$  *E. coli*; no die-off between harvest and consumption.

on the overall disease burden. Therefore, setting a less stringent level of acceptable risk, such as  $10^{-5}$  or  $10^{-4}$  DALY [loss] per person per year, from waterborne exposure may be more realistic, yet still consistent with the goal of providing high-quality, safer water and encouraging incremental improvement of water quality’.

Following the principles of the Stockholm Framework (Fewtrell & Bartram 2000), this should be applied *mutatis mutandis* to wastewater use in agriculture.

If a tolerable additional burden of disease of  $10^{-5}$  DALY loss pppy is accepted, then the resulting NV disease infection risks are an order of magnitude higher (i.e.  $\sim 10^{-2}$  pppy, rather than the  $\sim 10^{-3}$  pppy calculated above), but still lower than the actual global annual incidence of diarrhoeal disease which, in order of magnitude terms, is 0.1–1 pppy (Mathers *et al.* 2002). Therefore the required pathogen reduction is one order of magnitude lower. In the Ghanaian case referred to above this means a reduction of 4 log units, which could be achieved by, for example, a 1-log unit reduction by wastewater treatment, a 2-log unit reduction through die-off and a 1-log unit reduction by produce washing in cold water.

### IMPLICATIONS FOR WASTEWATER TREATMENT

In the above three examples wastewater treatment is required to produce only a single log unit pathogen reduction. This can be readily achieved by very simple treatment processes, such as an anaerobic pond, a three-tank or three-pond system, and overnight settling.

The three-tank or three-pond system is operated as a sequential batch-fed process: on any one day one tank or pond is filled with wastewater, the contents of another are settling, and the contents of the third are used for irrigation; this is a very reliable, almost foolproof system. In small-scale urban agriculture, as opposed to large-farm agriculture, a single tank is generally sufficient (and more affordable): on any day in the morning the tank contents are used for crop watering, and the tank is then refilled and its contents allowed to settle until the following morning.

## CONCLUSIONS

1. Risk analysis shows that norovirus requires the same level of pathogen reduction as that determined in the 2006 WHO guidelines: 6 log units for a lettuce consumption of 100 g every second day. For a lower consumption of 10–12 g on each of four occasions per week the required reduction is 5 log units.
2. In most developing countries a tolerable DALY loss of  $10^{-6}$  pppy is unnecessarily restrictive; a tolerable DALY loss of  $10^{-5}$  pppy is more realistic, yet still protective of consumer health. The resulting required pathogen reductions are then an order of magnitude lower.
3. Only very simple wastewater treatment systems are needed to achieve a single-log unit pathogen reduction as the balance of the required total pathogen reduction (i.e. 3–5 log units in the exposure examples herein) can be easily achieved by very reliable post-treatment health-protection control measures (pathogen die-off and produce washing or disinfection).

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