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Health risks derived from consumption of lettuces irrigated with tertiary effluent containing norovirus

Helena Sales-Ortells [a,b,*], Xavier Fernandez-Cassi [c], Natàlia Timoneda [c,d], Wiebke Dürig [a], Rosina Girones [c], Gertjan Medema [a,b]

[a] KWR Watercycle Research Institute, P.O. Box 1072, 3430 BB Nieuwegein, The Netherlands
[b] Section Sanitary Engineering, Department of Water Management, Faculty of Civil Engineering and Geosciences, Delft University of Technology, P.O. Box 5048, 2600 GA Delft, The Netherlands
[c] Department of Microbiology, Faculty of Biology, University of Barcelona, Av. Diagonal 643, Barcelona 08028, Spain
[d] Computational Genomics Laboratory, Department of Genetics, Institute of Biomedicine, University of Barcelona, Av. Diagonal 643, Barcelona 08028, Spain

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ABSTRACT

Wastewater is a valuable resource for water-scarce regions, and is becoming increasingly important due to the rising frequency of droughts as a result of climate change. The health risks derived from ingestion of lettuce that has been irrigated with effluent from a wastewater treatment plant (WWTP) in Catalonia (Spain) were estimated following a quantitative microbial risk assessment (QMRA) approach using site-specific data. Norovirus (NoV) was selected for this analysis, since it is the most common cause of acute gastroenteritis outbreaks in Catalonia. Two scenarios, irrigation with secondary and with tertiary effluent, were analysed. An uncertainty analysis was conducted to determine the impact of possible internalization of NoV into edible parts of the lettuce. The mean disease burden for ingestion of lettuce irrigated with secondary and tertiary effluent was 7.8×10^{-4} Disability Adjusted Life Years (DALYs) per person per year (pppy) and 3.9×10^{-4} DALYs pppy, respectively. A sensitivity analysis revealed that the model parameters with higher influence on the probability of disease are the concentration of NoV in the effluent and the consumption of lettuce. In order to decrease the disease burden to the guidance level of 10^{-6} DALYs pppy, the tertiary treatment should be able to achieve a 4.3 log reduction of the concentration of NoV. If internalization of NoV into lettuces occurs, this would require a reduction of 7.6 log. This is the first time that site specific data and virus internalization in crops are incorporated in a QMRA of irrigation of lettuce and its impact is quantified.

1. Introduction

Wastewater has been widely used in the past for irrigation purposes. It is still in use in developing countries due to water scarcity, the associated nutrient value of these waters for crop growth, and economic limitations. In developed countries, the use of treated wastewater is increasingly seen as a way to deal with water scarcity (exacerbated by climate change), as a more economical alternative to inter-basin transfers, and as an environmentally sustainable practice (Drechsel, Scott, Raschid-Sally, Redwood, & Bahri, 2010).

Uses of reclaimed water include irrigation of landscapes, recreational fields, plants' nurseries, or agricultural lands for food crops, amongst others. In Spain, 362.2 Hm^3 of reclaimed water (42.39 Hm^3 in Catalonia) are used annually, corresponding to 10.6% of the total volume of treated wastewater. 71% of it is used for agricultural irrigation (Iglesias, Ortega, Batanero, & Quintas, 2010).

Although domestic wastewater is treated by secondary or tertiary wastewater treatment, reclaimed water can contain infectious pathogens, posing a risk for public health. Wastewater treatment methodologies are used to reduce concentrations of faecal indicators, e.g. faecal coliforms (FC) or *Escherichia coli* (EC), to below certain standards (BOE, 2007). However, wastewater treatment can be considerably less effective in the elimination of enteric pathogens, such as enteric viruses (EV) and protozoa (Montemayor et al., 2008). Whilst concentrations of FC and EC are usually monitored at the wastewater treatment plants (WWTP), EV, which are relatively resistant to treatment technologies, are not (BOE, 2007), and concentrations of faecal indicators below the standards do not imply absence of EV hazards.

The health risks derived from irrigation of fresh produce with reclaimed water have been previously studied for EV (Hamilton, Stagnitti, Premier, Boland, & Hale, 2006; Petterson, Ashbolt, & Sharma, 2001, 2002; Seidu et al., 2008; Shuval, Lampert, & Fattal, 1997; Stine, Song, Choi, & Gerba, 2005). Few studies focused on the norovirus

* Corresponding author at: KWR Watercycle Research Institute, P.O. Box 1072, 3430 BB Nieuwegein, The Netherlands. Tel.: +31 30 6069 649.
E-mail addresses: hsalesortells@gmail.com (H. Sales-Ortells), xaviako@gmail.com (X. Fernandez-Cassi), Natalia.timoneda@gmail.com (N. Timoneda), wiebkeduerig@gmail.com (W. Dürig), rgirones@ub.edu (R. Girones), gertjan.medema@kwrwater.nl (G. Medema).

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(NoV) risks (Mara & Sleigh, 2010a,b; Mok, Barker, & Hamilton, 2014). Mara and Sleigh (2010a,b) found infection risks of NoV to range between 10^{-5} and 1 per person per year (pppy), depending on the initial concentration, and concluded that additional reduction of the NoV concentration in wastewater is needed, but easily achievable by water treatment. Mok et al. (2014) found, for an estimated concentration of 6.0×10^7 virus/L in raw sewage, a 90% Confidence Interval (CI) of 4.7×10^{-4} to 4.4×10^{-3} Disability Adjusted Life Years (DALY) pppy in lettuce irrigated with wastewater treated by stabilization ponds. Other wastewater treatment methods (Actiflo, chlorination, ozone or UV) did not reduce the disease burden below the WHO recommendation of 10^{-6} DALY pppy (WHO, 2006), but this reduction could be achieved by a combination of the stabilization pond with any of the other treatment technologies. Those studies, however, did not use site-specific data on NoV concentrations in reclaimed water, and only considered the viruses deposited on lettuce surface (and not internalization of viruses through the roots). Furthermore, all QMRA studies have used a model derived from *Bacteroides fragilis* bacteriophage B40-8 (Petterson et al., 2001, 2002) to estimate the NoV field-decay, whilst recent studies (Carratalà et al., 2013; Hirneisen & Kniel, 2013) have provided more specific data to estimate the inactivation of NoV, not only in-field, but also during crops transport and storage.

The objective of this study was to quantify the health risks of lettuce irrigation with treated domestic wastewater in Catalonia (Spain) and the effect of secondary versus tertiary wastewater treatment on these health risks. This study followed a Quantitative Microbial Risk Assessment (QMRA) approach and NoV was selected as reference pathogen, since it is the most common cause of acute gastroenteritis outbreaks in Catalonia (Martínez et al., 2013). Recent literature indicates the ability and extent of lettuces to internalize virus particles (Dicaprio, Ma, Purgianto, Hughes, & Li, 2012; Esseili, Wang, Zhang, & Saif, 2012; Wei, Jin, Sims, & Kniel, 2011). This is an important element that influences the outcome of the risk assessment and has not been considered in previous QMRA studies. We introduced this as an alternative scenario in the QMRA model and quantified the effect on the health risks.

2. Methods

2.1. Study-site description

The WWTP is located on the North-East coast of Spain and is designed to treat wastewater from 175,000 inhabitants with a flow capacity of 35,000 m^3/day. The conventional secondary treatment consists of sedimentation and activated sludge. The tertiary treatment, with a design capacity of 600 m^3/h, consists of flocculation by addition of iron chloride, followed by filtration (pulsed-bed sand filters), UV treatment (2 banks with 4 medium pressure lamps each, with a UV dose of 25–30 mJ/cm^2, according to the UV supplier) and chlorination (dosing of 3 to 6 mg/L of sodium hypochlorite with a contact time of 30 to 90 min). This tertiary effluent is used for irrigation and its production depends on the demand of the users, being higher from April to October, with a peak in July–August. Characteristics of the secondary and tertiary effluent measured by the WWTP system can be found in the supplementary material (S1).

The tertiary effluent is intended to irrigate several vegetable farms located in the vicinity of the WWTP. At the farms, different vegetables are irrigated through sprinkler, furrow, or drip irrigation. Most of them, however, with lettuce in particular, are irrigated with a sprinkler system every other day, in the evening. Lettuce is harvested, transported to the local market, and sold to the customers twice per week.

2.2. Hazard identification

NoV is a single stranded RNA virus that belongs to the Caliciviridae family (Lodder & de Roda Husman, 2005; Patel, Hall, Vinjé, & Parashar, 2009). They are found in water and food worldwide and are a leading cause of acute gastroenteritis (Mok et al., 2014), specially genogroups NoVGI and NoVGII (Rajko-Nenow et al., 2013). A study on epidemiological data from a 10-year period revealed that NoV was the most common cause of acute gastroenteritis outbreaks in Catalonia from 2004 to 2010 (Martínez et al., 2013).

Concentrations of NoV in wastewater range from 10^0 to 10^5 virus/L (Katayama et al., 2008; Lodder & de Roda Husman, 2005), with higher concentrations usually found in winter. In secondary effluents, NoV concentrations of the range of 10^1 to 10^3 have been found (Katayama et al., 2008). NoV has been linked with food outbreaks, including salad crops (Ethelberg et al., 2010; Gallimore et al., 2005; Makary et al., 2009; Rutjes, van den Berg, Lodder, & de Roda Husman, 2006; Wadl et al., 2010).

2.3. Exposure assessment

A conceptual exposure model was designed to describe the virus fate and transport from the secondary effluent to the consumers' fork (S2).

2.3.1. Norovirus concentration in effluent

Data on the concentration of NoV was obtained from the secondary ($n = 8$) and tertiary effluents ($n = 8$), and from a reservoir to store tertiary effluent ($n = 8$). Monthly samples were gathered for a period of 8 months. Detailed methodology is described in the supplementary material (S3). Briefly, viruses present in 10 L samples were concentrated using the skimmed milk organic flocculation method as described by Calgua, Fumian, et al. (2013). A negative control was included in each sampling event using tap water as matrix, and neutralizing the free chlorine by adding 100 mL of 10% sodium thiosulfate solution. Viral extraction of RNA from 140 μl of concentrates was done with the QIAamp® Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) employing the automated system QIACube (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Extracts were stored at −80 °C until analysed. A negative control of extraction was included in each extraction batch using free DNAse/RNAse molecular water. Samples were tested using specific real-time RT-qPCR for the viral pathogens NoVGI (Loisy et al., 2005) and NoVGII (Kageyama et al., 2003). Duplicate alliquots of undiluted and log10 diluted extracts were analysed. More than one non-template control (NTC) were included in the RT-qPCRs. MX3000Pro sequence detector system (Stratagene, La Jolla, CA, USA) was used to quantify the samples. Detection limits are 10 genome copies (gc) per reaction tube (Kageyama et al., 2003), equivalent to 570 gc/L. Plasmid DNA was used as a positive control and as a quantitative standard. RT-qPCR standards were generated as described by Calgua, Rodriguez-Manzano, et al. (2013). Recovery of the method can be found in Calgua, Fumian, et al. (2013).

Although most of the NoV outbreaks and clinical cases in Catalonia are related to NoVGII, both genogroups were added up since NoVGI is also a human pathogen. An ANOVA test was run to check for significant differences between the NoV combined concentrations in the three sampling points. Gamma and lognormal distributions were fitted to the data using the maximum likelihood estimation and the method of matching moments. These distributions were used because they have shown before to give a good fit to pathogens concentrations in water (Tanaka, Asano, Schroeder, & Tchobanoglous, 1998; Westrell et al., 2006). Goodness of fit was analysed graphically and by the Kormogorov–Smirnov test.

2.3.2. Norovirus removal by tertiary treatment

The absence of a statistical difference between the concentration of NoV in secondary and tertiary effluent (see Results) indicated little removal by the coagulation and sand filtration in the tertiary treatment. Also UV and chlorination did not reduce the concentration, but this could at least partly be due to the fact that RT-qPCR detects both active and inactive (i.e. non-infective) viruses (Sobsey, Battigelli, Shin, & Newland, 1998). Concentrations of *EC* in secondary and tertiary effluent