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Enteric virus presence in green vegetables and associated irrigation waters in a rural area from Argentina. A quantitative microbial risk assessment

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ABSTRACT

The aim of this study was to assess the presence of norovirus, rotavirus and infective enterovirus in leafy green vegetables and irrigation waters collected from a farm located at the province of Córdoba, Argentina, and to estimate the quantitative risk of infection by consuming these vegetables. During June 2014–July 2015, vegetables (n = 101) and their corresponding irrigation waters (n = 24) were collected. Viruses were concentrated in both matrices by polyethylene glycol precipitation and then were subjected to RT-PCR to assess the presence of norovirus and rotavirus. The concentrates were also inoculated in CaCo-2 cells to monitor the occurrence of infective enterovirus. The frequency of detection of norovirus, rotavirus and infective enterovirus in irrigation waters was 37.5%, 20.8% and 37.5% and in crops 60.4%, 22.7% and 35.6% respectively. Similar profiles of norovirus genogroups and rotavirus G-types distribution were observed in green vegetables and irrigation waters. The estimated risk of rotavirus infection associated with raw consumption of the vegetables harvested in that rural farm was 0.2 per person per day. This study demonstrates a wide distribution of human pathogenic viruses in irrigation waters and green leafy vegetables, which is of concern when, as in this case, the vegetables are eaten raw.

1. Introduction

Fresh produce is clearly considered as part of a healthy diet, however foodborne illnesses related to its consumption are widely reported (Fuzawa et al., 2020; Painter et al., 2013). In fact fresh vegetables have been acknowledged as important transmission sources of enteric viruses, since they are usually eaten raw and receive minimal processing to reduce or eliminate viral pathogens (Goyal and Goyal, 2006). Among fresh produce, leafy greens have been identified as the commodity of highest microbiological risk estimates compared to different foodstuffs (De Keuckelaere et al., 2015).

Environmentally transmitted enteric viruses include major etiological agents of gastroenteritis, meningitis and hepatitis. These viruses are non-enveloped, which give them great stability in the environment, and are of great concern because they can remain as infectious particles on food surfaces for long periods of time, increasing the possibility of transmission to humans (Bosch et al., 2011; Marti & Barardi, 2016).

Norovirus (NoV) is the leading cause of foodborne illness worldwide, causing gastroenteritis with high morbidity and mortality (Maunula & von Bonsdorff, 2016). Other prevalent foodborne viral pathogen is group A rotavirus (RVA), which is recognized as a major cause of infectious gastroenteritis in children worldwide, but has also been reported to be a pathogen in adults (Parashar et al., 2013). Besides, infective enterovirus (iEV) is useful as a potential indicator of the presence of viable viral particles in environmental samples due to its predominance in fecally contaminated environments and its capacity to replicate in cell culture (Prez et al., 2015).

Enteric viruses can contaminate fresh produce at different stages from the farm-to-table continuum, during growth, harvest, transportation and further processing and handling. Several viral outbreaks re-

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lated to the consumption of fresh produce have been known or suspected to have arisen from contamination in the field, suggesting that irrigation water was a route of contamination (El-Senousy et al., 2013; Gerba et al., 2006; Prez et al., 2018). In this sense, the link between the fecally contaminated irrigation water and vegetable crops establishes an environmental contaminated scenario that could result in a risk to human health via foodborne human enteric viruses. Quantitative microbial risk assessment (QMRA) is a modelling technique that is widely used in assessing health risks, particularly infection, resulting from human exposures to waterborne and foodborne pathogens (Barker et al., 2014; Jones & Su, 2015).

The city of Córdoba, in Argentina, obtains fresh produce derived from the horticulture of the green belt of the central area of the province of Córdoba. The local green belt is made up of small productive units of 2–15 ha, which are intensively exploited and are located inside or on the periphery of the urban ejido of cities and close to water courses (AProduCo, 1995). In this work we studied one of those production units located in the city of Colonia Caroya, in the north center of the province of Córdoba, which has 20,821 inhabitants and a population density of 298.7 habitants/km^2 (INDEC, 2010). Colonia Caroya takes water from two channels that are supplied by underground water and provide water to the city for irrigation. Although the first sections of both channels are piped, then they pass through open-air channels, crossing slums towards the producing units. The availability of water used for each of the producing units is distributed in water shifts and, in general, these shifts occur every fifteen days.

In this context, the aim of the present study was to assess the presence of NoV, RVA and iEV in leafy green vegetables and irrigation waters collected from Colonia Caroya, Argentina. In addition, a QMRA model was applied to estimate the risk of rotavirus infection arising from the consumption of raw vegetables irrigated with the studied water.

The results from this study allow knowing the state of the situation of the irrigation water and leafy vegetables in a locality with a population density lower than that already known in the province of Córdoba (Prez et al. 2015, 2018bib_Prez_et_al_2015bib_Prez_et_al_2018). As the vegetables produced in this city are marketed in the Capital city of the province, the knowledge of their virological quality will allow establishing measures and recommendations for primary producers and also hygiene practices for consumers.

2. Materials and methods

2.1. Raw material

A total of 101 vegetable samples that can be consumed uncooked like chicory (n = 25 *Cichorium intybus*) and lettuce (n = 76 *Lactuca sativa, Lactuca sativa* var. *crispa* and *Lactuca sativa* var. *capitata*) were monthly collected during the period June 2014–July 2015 from a farm located in Colonia Caroya. The complete information of the harvested samples analyzed in this study is accessible in the Electronic Supplementary Material Table S1. In parallel, 2 L of water samples used for irrigation of the vegetables were collected twice a month, with exception to the period January–April where the rains were intensive and the irrigation water sampling was carried out only once during each of those month (n = 24).

2.2. Bacteriological analysis of water samples

The membrane filtration technique was used to enumerate total and fecal coliform (Clesceri, 1998, ISO7899, 2016) and *Enterococcus faecalis* testing (ISO7899, 2016). For these techniques, 100 mL of water samples were used.

2.3. Concentration of water samples

The concentration of viruses in irrigation water samples was performed using the method of polyethylene glycol (PEG) precipitation (Lewis & Metcalf, 1988; Prez et al., 2018).

2.4. Elution and concentration of viruses from vegetables

The viral elution and concentration in vegetables was carried out according to Prez et al., 2018. The viral concentrates were stored at −80 °C until further processing.

2.5. Infective enterovirus detection

Enterovirus infectivity was evaluated by infecting CaCo-2 cell culture with the viral concentrates. Prior to spiking the concentrated into the culture flasks, the samples were quickly thawed at 37 °C, treated twice with chloroform (1:1), and antibiotics and antimycotics were added (penicillin 100,000 IU/mL; streptomycin 2.5% and amphotericin B 250 μg/mL). Then, each viral concentrate (1 mL/flask) was analyzed twice using standard operating procedures (Prez et al., 2018; WHO, 2004).

2.6. Nucleic acid extraction and cDNA synthesis

Viral RNA was extracted from the viral concentrates using the commercial QIAamp viral RNA kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. Extracted RNA was reverse-transcribed into cDNA using random hexamer primers and M-MLV reverse transcriptase (Invitrogen, CA, USA).

2.7. Virus genome amplification and characterization

Norovirus and rotavirus detection was performed by heminested PCR using primers and conditions described in Prez et al., 2015 and 2018. Positive and negative controls were included in all PCR runs.

2.8. Gel electrophoresis

All the PCR products were resolved by 10% polyacrylamide gel electrophoresis (Laemmli, 1970) followed by silver staining (Herring et al., 1982).

2.9. Sequence analysis

Heminested PCR amplicons for norovirus genogroup GI or GII were purified and sequenced in both directions with the primers G1SKF/G1SKR and G2SKF/G2SKR, respectively, using the dideoxy-nucleotide chain terminator method with Big Dye TM terminator version 3.1 cycling conditions on an automated sequencer (model 3730XL; Applied Biosystems, Foster City, CA, USA) by Macrogen Inc. (Seoul, South Korea). The editing, alignment and comparison of the nucleotide sequences obtained were performed using the MEGA-X program (Molecular Evolutionary Genetic Analysis) version 10.1 (Kumar et al., 2018). The consensus sequences obtained were compared with the sequences of standard strains published in the GenBank, using the BLAST program. Multiple sequence alignment was performed with the Clustal W program. Phylogenetic relationships were calculated using the Kimura-2-parameter method (Kimura, 1980) as a model of substitution. The statistical significance of the inferred phylogenies was estimated using the Neighbor-Joining method to construct the phylogenetic tree with a bootstrap of 1000 pseudo-replicate data sets. The GenBank accession numbers for the sequences obtained in this study are MT048671 to MT048678 and MT076466.

2.10. Rotavirus quantification

cDNA from concentrated samples that showed rotavirus-positive results by conventional RT-PCR were quantified by quantitative PCR (qPCR) following the conditions and primers described in Prez et al., 2015.

2.11. Quantitative microbial risk assessment

Haas et al. (2014) identified four formal procedures of determining QMRA, namely:

2.11.1. Hazard identification

Rotavirus was chosen as the model microbial hazard based on its occurrence and persistence in water (Abad et al., 1994), possibilities for detection and quantification (Zeng et al., 2008), availability of published works (Parashar et al., 2013; Sanborn & Takaro, 2013), representability of an important group of pathogenic agents (Health-Canada, 2019), the occurrence of diseases such as diarrhea and gastroenteritis in the population (Degiuseppe & Stupka, 2020; Estes et al., 2013; Mandile et al., 2020) and finally because it was found in treated wastewater (He et al., 2012; Lodder & de Roda Husman, 2005; Ueki et al., 2005). Also, the World Health Organization recommends the use of rotavirus as a reference viral pathogen in the absence of specific information on causative agents (WHO, 2004).

2.11.2. Exposure assessment

The exposure analysis was based on the following principles: the average genomic concentration of rotavirus in vegetable samples, the consumption of vegetables per individual per day in Argentina, the loss of viral load due to washing of vegetables and the viability of viruses (Table 1). To calculate the exposure dose per day, the concentration of rotavirus in a sample was multiplied by the daily amount of vegetable consumption, the fraction of viral recovery after washing and the fraction of particles detected capable of causing an infection.

Genomic concentrations of rotavirus were determined by RT-qPCR. A major limitation of this technique is its inability to determine the viability and infectivity of the detected viruses, since the presence of viral nucleic acids does not necessarily indicate the presence of infectious viruses (Bofill-Mas et al., 2010; Hamza et al., 2009). To address this limitation, the relationship previously estimated by Chigor et al. (2014) of the relationship between genomic copies (gc) and infective rotavirus particles was used in this work. However, the relationship between infectious viruses and viral genomic copies probably varies with the analyzed matrix as well as with the different combinations of primers and probes used for viral detection (Chigor et al., 2014; Rodriguez et al., 2009; Rutjes et al., 2009; Ward et al., 1984). Therefore, the use of this relationship carries an unknown level of uncertainty. Vegetable consumption data per person per day in Argentina was obtained through the review of multiple national surveys (Giacobone et al., 2018).

Table 1
Parameters used for modeling the QMRA.

Variable	Units	Distribution parameters and/or values	References
RVA concentration	gc/g	Pert (Min = 74.7; Media = 460; Max = 1150)	Data collected in the present study
RVA prevalence		Beta (α_1 = 24; α_2 = 79)	Data collected in the present study
Virus reduction by post-harvest washing	%	Pert (Min = 0.07; Media = 0.23; Max = 0.32)	Bae et al. (2011)
Infectious virus: Viral genome		1:10	Chigor et al. (2014)
Vegetable consumption in Argentina	g/day	Pert (Min = 100; Media = 135; Max = 150)	Giacobone et al. (2018)
Dose-response		β-Poisson (α = 0.2531, N_{50} = 6.17)	Haas et al. (2014)

2.11.3. Dose–response model

The β-Poisson dose-response model was used to estimate the probability of rotavirus infection (Haas et al., 2014). The following model equation was used:

$$P_I(d) = 1 - \left[1 + \left(\frac{d}{N_{50}}\right)\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha}$$

where $P_I(d)$ is the probability or risk of an individual to be exposed to a single pathogen dose (d) through ingestion; α is a parameter that characterizes the dose-response relationships referred to as pathogen infectivity constants; d is the pathogen dose and N_{50} is the median infective dose or the number of pathogens required to cause an infection in 50% of the exposed population. Then the $P_I(d)$ was multiplied by the prevalence of the virus in the vegetables to estimate the probability of infection.

2.11.4. Variability and uncertainty in the data

The uncertainty was introduced through the analysis of data distribution by the sampling source (raw leafy green vegetables). Monte Carlo simulations were made for 10,000 iterations using the @Risk software 6.3 (Palisade Corporation, Newfield, New York). In each iteration, samples were taken from the data distribution function. The output of the analysis was the mean of the risk of the infection as well as the frequency distribution of the probabilities of infection.

2.12. Statistical analysis

Rotavirus concentration was transformed into Log_{10}. χ^2 test was used to compare categorical variables. The descriptive statistics for the virological quality variables were used. Means and confidence intervals of 95% were calculated. *P*-values lower than 0.05 indicated significant differences for the variables measured. Statistical analyses were performed with InfoStat version 2018 (Di Rienzo et al., 2018).

3. Results and discussion

The virological quality of the irrigation water, mainly that used to irrigate leafy green vegetables that are consumed raw, is closely linked to the probability of infection of the consumers (Kokkinos et al., 2012). Among the wide range of enteric viruses that can contaminate vegetables, NoV and RVA have epidemiological significance as foodborne pathogens (EFSA, 2011; Gastañaduy et al., 2013, pp. 303–311).

3.1. Levels of indicator bacteria in irrigation water

Many researchers have demonstrated that there is no correlation between the detection of viral particles and the level of indicator bacteria (Fujioka et al., 2015; Masachessi et al., 2021; Muniesa et al., 2018). However, the determination of fecal bacteria loads in irrigation waters is an easy and cheap analysis, which can prevent using the waters for the irrigation of green leaves when the bacteriological parameters exceed the recommended levels. In this sense, in the present study we analyzed the levels of indicator bacteria in the irrigation waters from Colonia Caroya. All the irrigation water samples were positive for total coliforms, fecal coliforms and *Enterococcus faecalis*. During the study period high levels of indicator bacteria were always found in the irrigation waters. The mean counts of indicator bacteria in waters were as follows: total coliforms 7×10^3 CFU/100 mL (4×10^2 - 7x10^4); fecal coliforms 2×10^3 CFU/100 mL (6×10^1 - 1x10^4) and *E. faecalis* 7×10^2 CFU/100 mL (1×10^1 - 5x10^4).

There is a general perception that the hygienic quality of irrigation water is less important than that of drinking water (Maunula et al., 2013), and therefore many times its microbiological quality is not evaluated. The analysis of bacterial indicators in the irrigation waters from Colonia Caroya revealed a high degree of faecal contamination during the whole studied period. The results obtained here revealed that previous analysis of indicator bacteria would warn on the use of these waters to irrigate the leafy green vegetables.

3.2. Enteric viruses in leafy green vegetables and irrigation waters

The prevalence of NoV, RVA and iEV in irrigation waters and leafy green vegetables are summarized in Table 2. At least one virus type was detected in 58% of the irrigation waters (14/24) and in 76% of the vegetable samples (77/101). Overall, in both matrices (n = 125), NoV showed a relatively higher positive rate (56%, 70/125), followed by iEV (36%, 45/125 samples), meanwhile RVA was the less detected viral pathogen (22%, 28/125 samples). No statistically significant differences in the frequency of enteric viruses detection nor in RVA concentration were noted in terms of the leafy green vegetable type (chicory or lettuce) ($P > 0.05$) (Table 2).

Different prevalence rates of NoV detection were reported in leafy greens from other countries; 1.1% in three European countries (Kokkinos et al., 2012), 2.9% in Italy (Purpari et al., 2019), 5.3% in United Kingdom (Cook et al., 2019), 12.4–50% in France (Baert et al., 2011; Loutreul et al., 2014), 33.3% in Belgium (Baert et al., 2011), and 28.2–54% in Canada (Baert et al., 2011; Mattison et al., 2010). In this study, the prevalence of NoV in leafy green vegetables (60%) was similar to that reported in France and Canada and also similar to that described by our research group in leafy greens from Córdoba city, Argentina (58%) (Prez et al., 2018). RVA detection in raw vegetables from Colonia Caroya was lower than NoV prevalence. This tendency seems to be common all over the world. Published studies reported 0% RVA detection in vegetables from Italy and South Korea (Cheong et al., 2009; Purpari et al., 2019; Shin et al., 2019), 0.4% in Canada (Mattison et al., 2010), 1.7% in three regions of southern Africa (van Zyl et al., 2006), 5% in leafy greens from Córdoba city, Argentina (Prez et al., 2018) and 8–21.2% in Mexico (Parada-Fabian et al., 2016; Quiroz-Santiago et al., 2014). This could be the reflection of rotavirus vaccination at least in countries where the rotavirus vaccine was introduced.

Although during the period January–April 2015 there was viral detection (NoV and iEV) in the irrigation waters analyzed, RVA was not detected. Previous studies carried out in the province of Córdoba revealed a higher detection rate of RVA in environmental waters in the dry and cold months (May–September) (Barril et al., 2015). This viral distribution could be another explanation of the lack of detection of RVA particles in the irrigation waters of Colonia Caroya during the warmest and wettest months of the year.

Table 2
Enteric viruses in irrigation waters and leafy green vegetables.

Samples	NoV detection (%)	RVA detection (%)	Mean RVA load (gc/L) (Min-Max)	iEV detection (%)
Waters	9/24 (37)	5/24 (21)	6.3×10^5 (5×10^5-8.8×10^5)	9/24 (37)
Vegetables	61/101 (60)	23/101 (22)	4.6×10^2 (7.5×10^1-1.2×10^3)	36/101 (35)
Chicory	18/25 (72)	6/25 (24)	5.1×10^2 (7.5×10^1-1.2×10^3)	12/25 (48)
Lettuce	43/76 (56)	17/76 (22)	4.4×10^2 (7.5×10^1-1.1×10^3)	24/76 (31)
P-value (chicory vs. lettuce)	**0.3**	**0.9**	**0.6**	**0.1**

A major drawback of the molecular biology for the detection of viruses is its inability to determine the viability and infectivity of the viruses detected. Therefore, the molecular detection of enteric viruses cannot confirm the role of vegetables as transmitting vehicles of infectious enteric viruses (Hamza et al., 2009). In this study, the detection of iEV provided a measure of viral viability, indicating adequate conditions of the vegetable matrix to maintain the infectivity of the other viruses analyzed by genomic detection. In addition, the detection of iEV reflects that fecal contamination is of human origin.

3.3. Norovirus and rotavirus characterization

Many vegetable samples (19%, 20/101) showed multiple NoV genogroups or RVA genotypes in a single sample as a mixture of viral strains in the sample analyzed, while not many water samples revealed multiple genogroups/genotypes (8%, 2/24).

NoV GI was by far the most frequent genogroup identified both in vegetable (66%) and irrigation water samples (70%). A total of nine norovirus-positive samples (13%) could be sequenced, one GI corresponding to a vegetable sample, and 8 GII corresponding to 5 vegetable and 3 irrigation water samples. A single genotype was detected for NoV GII (GII.4 variant Sydney 2012) in vegetable and water samples and NoV GI.7 was detected in the sequenced GI vegetable sample (Fig. 1).

The genetic heterogeneity of the viral strains detected both in vegetable and water samples highlighted that NoV GI strains were significantly more likely to contaminate both matrices. This finding is consistent with previous researches in vegetables (Cook et al., 2019; Loutreul et al., 2014; Prez et al., 2018) and waters (de Deus et al., 2019; Lopez-Galvez et al., 2016; Shaheen et al., 2019). Despite its high prevalence, the viral genotype could only be identified in one NoV GI strain, corresponding to a vegetable sample. Difficulties in NoV GI sequencing could be due to low number of genome copies in the positive samples or the deterioration of the viral genome due to physicochemical environmental conditions. This is the first report of NoV GI.7 detection in vegetable samples worldwide. However, it has already been detected in environmental matrices such as groundwater in Korea (Lee et al., 2018), surface waters in Belem, Brazil (Teixeira et al., 2017), waterborne outbreaks (Arvelo et al., 2012; Sekwadi et al., 2018) and frozen oysters from Ireland (Rajko-Nenow et al., 2013).

Phylogenetic analysis of NoV GII strains showed the circulation of genotype GII.4 variant Sydney 2012, both in irrigation waters and their associated vegetables from Colonia Caroya. GII.4 is the most prevalent genotype worldwide, and particularly in our region. Previous environmental studies revealed the frequent detection of NoV GII.4 in surface waters from Córdoba, Argentina (Blanco Fernandez et al., 2011) and in sewage from Uruguay (Victoria et al., 2016).

RVA VP7-gene was successfully characterized in all rotavirus positive samples. It was observed a similar pattern of G-type rotavirus distribution in vegetables and irrigation waters. G3 was the most common genotype in both matrices (36% in vegetables; 50% in water), followed by G2 (30% in vegetables; 16% in water) and G9 (15% in vegetables; 33% in water). In vegetable samples, G4 (12%) and G1 (6%) were also detected. RVA VP4-gene could be determined in 18/22 (81%) vegetable samples and in 1/5 (20%) irrigation waters. In vegetables, P[8] and P[10] were the most frequent P-types (34% each), followed by P[4] (15%). Also P[6] and P[9] were detected (7% each). In the irrigation waters, P[8] was the only genotype identified. These results agree with the VP7 and VP4 genotypes in circulation in environmental matrices and clinical cases detected in the province of Córdoba since the year 1979 (Barril et al. 2010, 2015bib_Barril_et_al_2010bib_Barril_et_al_2015; Prez et al. 2015, 2018bib_Prez_et_al_2015bib_Prez_et_al_2018).

Overall, the detection of the same NoV strains both in irrigation waters and associated vegetables suggests that irrigation water is a source of vegetable contamination. Also the identification of a similar pattern of G-type RVA distribution in both matrices, reinforce this theory.

3.4. Quantitative microbial risk assessment

QMRA is widely used to estimate the risk of infection by contact with various microbiological quality indicator organisms (bacteria and parasites) in food and waters of different uses (Maffei et al., 2017; Meester et al., 2019; Soderqvist et al., 2019). However, only few works include risk analysis by contact with viral pathogens in food and aqueous matrices. In Valencia, Spain, a risk analysis was performed on outbreaks of hepatitis A virus transmitted by shellfish exported from Peru, which showed values of probability of infection >0.8 (Pinto et al., 2009); in Brazil, the risk of infection from consuming irrigated vegetable crops with effluents from sewage treatment plants was <0.1 (Pavione et al., 2013); and in Kumasi, Ghana, a study related to the consumption of street food salads revealed annual values of 1 in the probability of RVA infection (Barker et al., 2014). In general, the use of models to estimate the risks to human health associated with raw consumption of vegetables is specific to the site and the conditions present at each local scenario (Pavione et al., 2013).

In the present study the probability of RVA infection by consuming green leafy vegetables was estimated. The average genomic concentration of RVA in vegetables was 4.6×10^2 gc/g (range 7.5×10^1 – 1.2×10^3 gc/g).

Taking into account the loss of virus particles due to the washing of vegetables, the ratio of infectious virus to viral genome and the consumption per person of vegetables in Argentina, the average dose of rotavirus exposure per day was $3.5\times10^3 \pm 1.6\times10^3$ gc/day, resulting in a probability of infection of 0.20 ± 0.04 per person per day.

The sensitivity of the results of the reference model to the values and input parameters of the model, determined by the Spearman correlation, revealed that the probability of rotavirus infection due to vegetable consumption was more sensitive to the viral prevalence and concentration in the vegetables, while the size of the portion of vegetables consumed and the viral reduction by washing did not show a significant impact on the risk of foodborne infection (Fig. 2).

Numerous sources of uncertainty can provide different data to estimate risk. Published studies on virus reduction using tap water to wash vegetables, by immersion for 2 min or by rinsing with a contact time of less than 1 min, revealed reduction values of viral load of approximately 1 log (Bae et al., 2011). Assuming this reduction in the value of the virus concentration in vegetables could lead to underestimating or overestimating the probability of viral infection. However, it is noteworthy that in the results obtained in the farm of Colonia Caroya this modeling parameter had a low impact on the risk estimate.

There is few data in Argentina regarding the spectrum of health conditions of the population infected with rotavirus (that is, the distribution of the asymptomatic and symptomatic population after rotavirus infection, the population that requires medical attention and that dies). This limits the analysis of the risk characterization to estimate the magnitude of the public health problem of rotavirus infection, making it impossible to extrapolate the risk of individual infection to the impact of rotavirus disease in the community.

4. Conclusions

The detection of enteric viruses both in irrigation waters and associated vegetables from Colonia Caroya, and also the identification of similar viral genotypic profiles in both matrices, suggest that viral contamination of the vegetables could originate, at least partially, in the farm during the phase of production. The presence of viral genomes and infectious particles in these raw vegetables represents a risk for the consumers considering that salads do not undergo any treatment able to guarantee the viral inactivation. Since irrigation water is a vehicle of microbial contamination for fresh produce, primary products must be produced only in areas where water used for irrigation purposes is of appropriate quality (Koopmans & Duizer, 2004). In this instance, sustained surveillance of enteric viruses in aquatic environments used for watering fresh produce should periodically be carried out.

In case of not having control measures in the quality of irrigation water, several studies have analyzed the most effective methods to eliminate viruses during washing of vegetables prior to consumption. Examples of these are the immersion of the vegetables in drinking water, rinsing with running water, adding additives such as sodium hypochlorite, vinegar, detergents and/or disinfectants, among others (Bae et al., 2011; Bosch et al., 2018; Samadi et al., 2009). However, some of these washing additives are controversial because they can leave chemical residues on the leaves surfaces, or are not effective at all

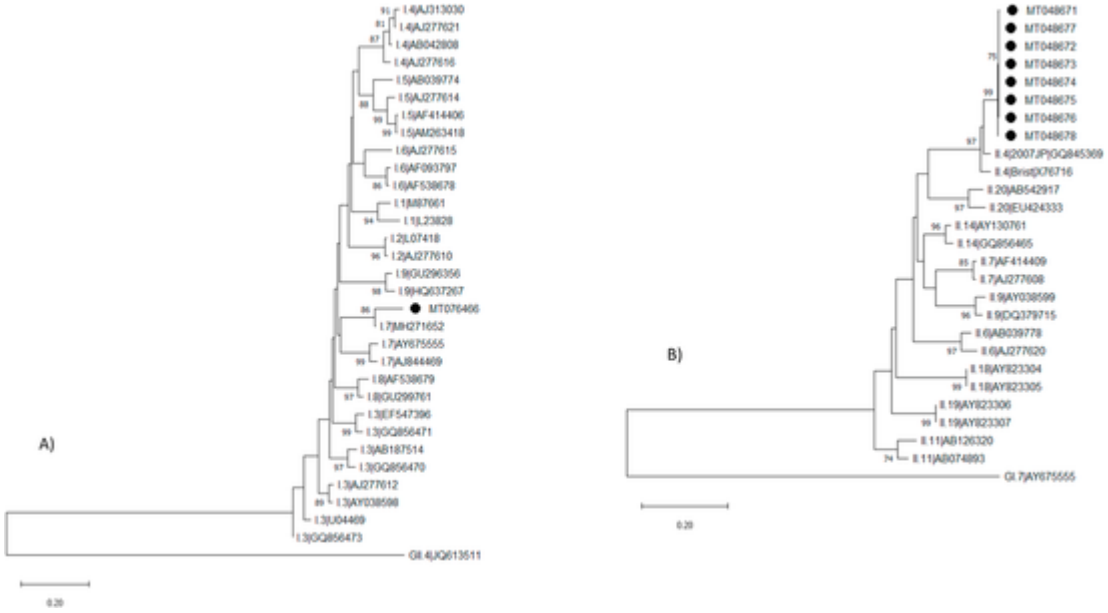

Fig. 1. Phylogenetic trees based on the capsid-gene nucleotide sequences of A) NoV GI (313 nt) and B) NoV GII (251 nt) strains. The trees were constructed by the Neighbor-Joining method and the Kimura two-parameter model. Bootstrap values above 70% are given at branch nodes. The scale bar represents 20% genetic distance. The strains isolated in Colonia Caroya, Córdoba - Argentina, are indicated by black circles (GenBank accession numbers MT048671 to MT048678 and MT076466).

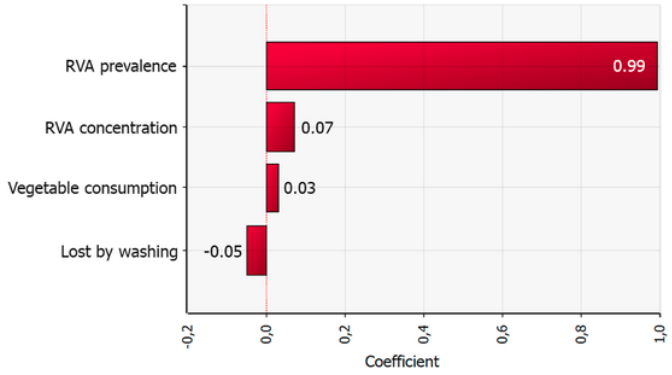

Fig. 2. Tornado graph that shows the parameters and variables that affect the probability of infection with RVA by consumption of raw vegetables from a farm located in Colonia Caroya, Córdoba - Argentina. The Spearman correlation coefficients were obtained through the sensitivity analysis of the @Risk program and are shown in each bar.

in reducing viral loads (Lin et al., 2017). For this reason, before consumption, it is suggested to wash the vegetables with safe water containing sodium hypochlorite with 15–20 ppm free chlorine levels for at least 1–2 min in order to improve the impact of washing in reducing viral loads on the surface of the vegetables (Bosch et al., 2018).

There is a great increase in the knowledge about viruses in food and in the number of reports of outbreaks produced by contaminated food, mainly in developed countries but also in developing countries. In Argentina, the lack of implementation of sanitary measures related to viruses in irrigation waters and leafy vegetables has slowed down the production of knowledge about viruses in these matrices. Therefore, the results obtained in this study highlight the importance of taking decisions to implement surveillance programs that guarantee the virological quality of food throughout the entire production chain in order to reduce the risk to public health from the consumption of these contaminated foods.

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Appendix A. Supplementary data

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