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Short Communication

Tracking enteric viruses in green vegetables from central Argentina: potential association with viral contamination of irrigation waters

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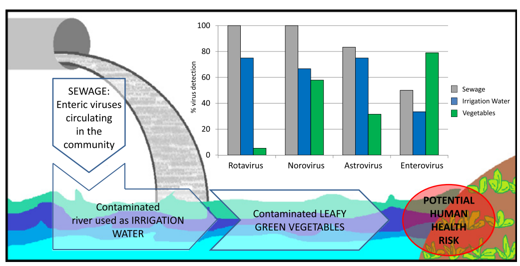
HIGHLIGHTS

- Enteric virus was frequently detected in vegetables, irrigation waters and sewage.
- Irrigation water is a possible source of viral contamination in raw vegetables.
- Viral variants in the community were similar in vegetables and irrigation waters.

GRAPHICAL ABSTRACT



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ABSTRACT

Consumption of green vegetable products is commonly viewed as a potential risk factor for infection with enteric viruses. The link between vegetable crops and fecally contaminated irrigation water establishes an environmental scenario that can result in a risk to human health. The aim of this work was to analyze the enteric viral quality in leafy green vegetables from Córdoba (Argentina) and its potential association with viral contamination of irrigation waters. During July–December 2012, vegetables were collected from peri—urban green farms ($n = 19$) and its corresponding urban river irrigation waters ($n = 12$). Also, urban sewage samples ($n = 6$) were collected to analyze the viral variants circulating in the community. Viruses were eluted and concentrated by polyethylene glycol precipitation and then were subject to Reverse Transcription Polymerase Chain Reaction to assess the genome presence of norovirus, rotavirus and human astrovirus. The concentrates were also inoculated in HEp—2 (Human Epidermoid carcinoma strain #2) cells to monitor the occurrence of infective enterovirus. The frequency of detection of the viral groups in sewage, irrigation water and crops was: norovirus 100%, 67% and 58%, rotavirus 100%, 75% and 5%, astrovirus 83%, 75% and 32% and infective enterovirus 50%, 33% and 79%, respectively. A similar profile in sewage, irrigation water and green vegetables was observed for norovirus genogroups (I and II) distribution as well as for rotavirus and astrovirus G—types. These results provide the first data for Argentina pointing out that green leafy vegetables are contaminated with a broad range of enteric viruses and that the irrigation water would be a source of contamination. The presence of viral genomes and infective particles in food that in general suffer minimal treatment before consumption underlines that green crops can act as potential sources of enteric virus transmission. Public intervention in the use of the river waters as irrigation source is needed.

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1. Introduction

Leafy green vegetables are important components of the current human diets but are one of the main foods involved in the transmission of human enteric viruses since they are eaten raw and usually without any further washing/decontamination procedures (Little and Gillespie, 2008).

Enteric viruses can be accidentally introduced at different steps in vegetable chain production, being crop irrigation with fecally contaminated water the main critical vehicle of contamination (Beuchat, 2002; Hirneisen and Kniel, 2013; Miura et al., 2018). It should be noted that viruses can enter into environmental waters through the discharge of treated, insufficiently treated or untreated sewage. In recent years it has been reported a clear epidemiological evidence of the link between consumption of wastewater irrigated crops and viral community illness (Pavione et al., 2013; Mok et al., 2014).

In Argentina, a high occurrence of enteric viruses, such as norovirus (NoV), rotavirus (RV), human astrovirus (HAstV), hepatitis A (HAV) and E viruses (HEV) and human enterovirus (EV) has recently been reported in a variety of water environments, some of which are used for irrigation purpose (Blanco Fernandez et al., 2012; Fernandez et al., 2012; Martinez Wassaf et al., 2014; Yanez et al., 2014; Barril et al., 2015; Ferreyra et al., 2015; Prez et al., 2015; Giordano et al., 2016; Farías et al., 2018). This underlines that raw vegetables could potentially be contaminated with these viruses through the irrigation water, but at present no such studies have been conducted in our country.

Over 100 types of viruses, collectively known as enteric viruses and many of them recognized as pathogenic agents, are excreted in high concentration in the feces of infected humans and non−humans. They are highly stable in the environment because they lack the lipid envelope, being able to persist for long time in waters. Despite the relatively low concentration of viruses in fecal impacted waters, its presence carry health risks since they have very low infectious doses (10–100 virions) and therefore even a few viral particles in water can infect a person (La Rosa et al., 2012).

Among the enteric viruses, RV and NoV are the most common etiological agents of gastroenteritis in humans. RV is widely known as one of the most important childhood diarrheal pathogens worldwide and it is estimated that causes approximately >600,000 children deaths annually all over the world. In Argentina, it is responsible for 40% of hospital admissions of acute diarrhea, and it is estimated that may cause between 30 and 50 deaths annually in the country (Bok et al., 2001). In January 2015 the monovalent rotavirus vaccine (G1[P8], GSK®) was introduced in the Argentine National Immunization Program. It has been reported that this vaccine has an estimated efficiency of 94.5% against severe disease and death (Ruiz−Palacios et al., 2006), but it does not prevent mild symptomatic infections in secondary contacts with the virus nor viral transmission to susceptible hosts. It must also be noted that RV has been implicated in waterborne and food−borne outbreaks worldwide (CDC, 2000; Mizukoshi et al., 2014).

NoV is a major cause of acute viral gastroenteritis, affecting people of all age groups worldwide. Outbreaks of NoV gastroenteritis can be seasonal or sporadic cases that occur throughout the year (Glass et al., 2009). CDC estimates that each year NoV causes 19 to 21 million illnesses, 56,000 to 71,000 hospitalizations and 570 to 800 deaths. They are associated with outbreaks due to contact with infected persons or ingestion of contaminated food or water (Moore et al., 2015). Therefore, studying this viral group and its potential sources of infection in a region with scarce data, acquires great relevance.

HAstV has been associated to endemic diarrheal episodes and outbreaks of gastroenteritis in industrialized and non−industrialized countries and has been detected in sewage and surface waters worldwide (Nadan et al., 2003; Jones et al., 2017).

Human EVs comprise a large genus within the *Picornaviridae* family. They affect millions of people worldwide each year and are often found in respiratory secretions and in stool of infected persons. EV usually cause subclinical infections, but sometimes they are associated with serious diseases, such as acute flaccid paralysis, aseptic meningitis and encephalitis, acute myocarditis, acute haemorrhagic conjunctivitis, and hand, foot, and mouth disease (Pallansch and Roos, 2007). Because most of the human EV can replicate in cell cultures, they are good indicators to confirm the presence of viable and infectious viruses in environmental samples. Moreover, EV infections have been linked to outbreaks of food−borne and waterborne diseases.

The aim of this work was to determine the presence of NoV, RV, HAstV and infective EV (iEV) in fresh leafy green vegetables and to establish whether river water used for irrigation is a possible source of viral contamination in fresh vegetables. This study also focuses in the viral analysis of urban sewage waters as a mirror of the viral agents circulating in the community (van Zyl et al., 2006; Barril et al., 2010; Ruggeri et al., 2015). The findings of this work provide the first data for Argentina of the viral quality of green leafy vegetables and its link with the viral quality of the irrigation water.

2. Materials and methods

2.1. Background

Córdoba city is the capital of the province of Córdoba, located in the central region of Argentina and has approximately 1,317,298 inhabitants with a population density of 2308 habitants/km^2 (INDEC, 2010) (Fig. 1A). The Suquía River traverses Córdoba city from west to east. Its water flow is 10 m^3/s, subject to a seasonal fluctuation. In its path through the city, the Suquía River receives the discharge of untreated or poorly treated industrial effluents and sewage and after leaving the city it receives the treated discharges of the main wastewater treatment plant (WWTP) named “Bajo Grande” (Fig. 1B). This sewerage system covers 61% of the population and no industrial wastewater is treated in this facility. The treatment at the WWTP involves the following steps: 1) grates that filter and extract solid waste, 2) grit chambers with buckets to extract finer trash from the liquid, 3) primary sedimentation tanks with surface sweeper and pumps that absorb sediment and pump it to a mud concentrator, 4) primary percolators, with air inlet through basal orifices and filled with ridge rolled (aerobic process), 5) secondary percolators, 6) secondary sedimentation tanks and 7) labyrinth of disinfection of the liquid with sodium hypochlorite.

A system of irrigation canals is derived from the Suquía River, which is used to irrigate the vegetables of the farms located in a green belt area called “Chacra La Merced”. The vegetables harvested in this area are sold in a wholesale market. One of the main irrigation canal rises at the San Jose Bridge of the Suquía River, located 200 m downstream from the WWTP “Bajo Grande” (Fig. 1B).

2.2. Sample collection

A total of 37 samples were collected during July to December 2012 in the city of Córdoba corresponding to waters or vegetables from three different points: raw sewage from the WWTP Bajo Grande ($n = 6$), superficial water from the San Jose Bridge ($n = 12$), and green leafy vegetables ($n = 19$) from a farm located at the green area Chacra La Merced (Fig. 1B). Raw sewage samples (1.5 L) were monthly collected from the inlet channel of the municipal WWTP. Irrigation waters (1.5 L) were collected twice a month at the San Jose Bridge, located approximately at the entrance of Chacra La Merced area (Fig. 1B). Water samples were taken on weekday mornings in sterile bottles. The vegetable sampling was conducted three times a month, collecting five types of green leafy vegetables that can be consumed uncooked, like spinach (*Spinacia oleracea*), lettuce (*Lactuca sutiva*), arugula (*Eruca sativa*), chicory (*Cichorium intybus*) and silver beet (*Beta vulgaris* var. *cicla*). The green vegetables were acquired according to the availability of recently green leafy vegetables harvested (approximately 700 g each). All sewage, irrigation water and vegetable samples were kept in individual