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ORIGINAL PAPER

Virological Quality of Irrigation Water in Leafy Green Vegetables and Berry Fruits Production Chains

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Abstract This study condenses data acquired during investigations of the virological quality of irrigation water used in production of fresh produce. One hundred and eight samples of irrigation water were collected from five berry fruit farms in Finland (1), the Czech Republic (1), Serbia (2), and Poland (1), and sixty-one samples were collected from three leafy green vegetable farms in Poland, Serbia, and Greece. Samples were analyzed for index viruses of human or animal fecal contamination (human and porcine adenoviruses, and bovine polyoma viruses), and human pathogenic viruses (hepatitis A virus, hepatitis E virus, and noroviruses GI/GII). Both index and pathogenic viruses were found in irrigation water samples from the leafy green vegetables production chain. The data on the presence of index viruses indicated that the highest percentage of fecal contamination was of human origin (28.1 %, 18/64), followed by that of porcine (15.4 %, 6/39) and bovine (5.1 %, 2/39) origins. Hepatitis E virus (5 %, 1/20) and noroviruses GII (14.3 %, 4/28) were also detected. Samples from berry fruit production were also positive for both index and pathogenic viruses. The highest percentage of fecal contamination was of human origin (8.3 %, 9/108), followed by that of porcine, 4.5 % (4/89) and bovine, 1.1 % (1/89) origins. Norovirus GII (3.6 %, 2/56) was also detected. These data demonstrate that irrigation water used in primary production is an important vehicle of viral contamination for fresh produce, and thus is a critical control point which should be integrated into food safety management systems for viruses. The recommendations of Codex Alimentarius, as well as regulations on the use of water of

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appropriate quality for irrigation purposes, should be followed.

Keywords Virological quality · Irrigation water · Produce · Molecular detection · Food safety

Introduction

Raw and minimally processed fruits and vegetables are typically sold to the consumer in a ready-to-use or ready-to-eat form. These products do not generally contain preservatives or antimicrobial agents and rarely undergo any heat processing prior to consumption (Seymour and Appleton 2001). The consumption of fecally contaminated vegetables and fruits is now recognized as a predominant mode for the transmission of human enteric viruses, which are increasingly recognized as a significant public health concern (Wei and Kniel 2010). According to the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), 11.6 % of cases of viral infections were caused by consumption of vegetables, fruit, berries, juices, and mixed food (EFSA/ECDC 2015; Carducci et al. 2015). A review on the Microbial Risk Assessment (MRA) studies on water and safety of fresh produce revealed that viruses resulted in the highest risk estimates compared to other microbial agents; additionally, leafy greens were identified as the commodity of greatest concern, compared to different foodstuffs (De Keuckelaere et al. 2015).

Several outbreaks implicated to consumption of fresh produce have been known or suspected to have arisen from contamination in the field, suggesting irrigation water was a route of contamination (Gerba and Choi 2006; Hirneisen and Kniel 2013; El-Senousy et al. 2013). Water has always played a key role as a vehicle for the transmission of pathogens transmitted by the fecal–oral route (Gerba and Choi 2006). Fresh waters in the environment offer excellent conditions for the survival of enteric viruses (Rzeżutka and Cook 2004). Human enteric viruses are frequently isolated from fresh water, while approximately 70 % of fresh water is being used for irrigation worldwide (Wei et al. 2011). In the UK, 71 % of irrigation water is obtained from surface waters, which receive treated sewage effluent, while a survey of salad vegetable producers showed that over 50 % of growers will harvest baby leaf crops within 24 h of the last irrigation (Tyrell et al. 2006; Rajwar et al. 2015).

Generally, bacterial indicators such as fecal coliforms are used for the assessment of the quality of irrigation water. However, bacteria have limited value as indicators of enteric viruses, because the survival rate of viruses in water and on food, plant, and soil surfaces is higher than that of bacteria in these environments (Cheong et al. 2009; Wyn-Jones et al. 2011). The virological quality of irrigation water largely depends on the source of the water (Cheong et al. 2009). Groundwater, surface water, and human wastewater are commonly used for irrigation. The risk of disease transmission from pathogenic microorganisms present in irrigation water is influenced by the level of contamination—the persistence of pathogens in water, in soil, on crops, and the route of exposure (Steele and Odumeru 2004). Groundwater, which is usually microbiologically safer than other irrigation water sources such as surface water and reclaimed water, may be exposed to contamination by enteric viruses from surrounding aquifers. This is because enteric viruses have the potential to move deep into the subsurface environment, penetrate an aquitard, and reach a confined aquifer due to their extremely small size (27–75 nm), which enables them to readily pass through sediment pores (Borchardt et al. 2003; Cheong et al. 2009). Private household wells, used for irrigation purposes, may be more vulnerable to viral contamination because they may be maintained less carefully and tested less frequently for water sanitary quality (Borchardt et al. 2003). Surface water is of variable microbial quality (Steele and Odumeru 2004). Fecal material may be introduced into agricultural land through a variety of means, which includes contamination of soil or irrigation water with wild animal feces, flood and runoff from nearby farms, leakage of septic tanks or sewage pipes, and overflow of animal lagoons (Wei et al. 2010, 2011). Human wastewater is usually of very poor microbial quality and requires extensive treatment before it can be used safely to irrigate crops (Steele and Odumeru 2004).

Irrigation water is delivered from surface or subsurface sources to fields via pipe-based or canal-based delivery systems. Water delivery systems can be diverse, and application of transported water to crops can include furrows, drip methods, and sprinkler systems (Pachepsky et al. 2012). Many factors, such as water availability and cost, soil type, slope, depth of water table, economics, and cropping rotations, determine the mode of irrigation rather than food safety issues. Flood and spray irrigation represent the greatest risk as any contamination within the water is directly deposited onto the edible leaves of crops (FDA 1998). Although the percentage of pathogen transfer from contaminated water to produce by some types of irrigation methods (e.g., drip irrigation) may be low, risks can still be considered significant because of the low numbers of some enteric pathogens, such as viruses, necessary to cause infection (Gerba and Choi 2006).

Since irrigation water is an important 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

and Duizer 2004). According to the recommendations of Codex Alimentarius Commission (FAO/WHO 1998), water of suitable quality should be used for irrigation. In Europe, regulations (Regulation (EC) No 852/2004 of The European Parliament and of the Council on the Hygiene of Foodstuffs, and Article 5 (c) of Annex 1 of the General Hygiene Provisions for Primary Production and Associated Operations) state that potable water or clean water should be used whenever necessary to prevent contamination (Maunula et al. 2013).

During the course of two studies, one on the presence of enteric virus contamination in the berry fruit supply chain (Maunula et al. 2013) and the other on leafy green vegetables supply chain (Kokkinos et al. 2012), samples were taken from irrigation water used at primary production sites, and analyzed for the presence of index viruses of human or animal fecal origin: human adenoviruses (hAdV), porcine adenoviruses (pAdV), and bovine polyoma viruses (bPyV), as well as human pathogenic viruses: hepatitis A virus (HAV), hepatitis E virus (HEV), and noroviruses GI/GII (NoV GI/GII). The aim of this present study is to condense data acquired during these investigations to demonstrate the importance of assessing the virological quality of irrigation water used in fresh produce production.

Materials and Methods

Sampling

The sampling and analysis strategy is according to previously published literature (Kokkinos et al. 2012; Maunula et al. 2013). The sampling plans were developed using background information questionnaires, based on HACCP (Hazard Analysis and Critical Control Point) audit principles, which were completed for each premises. Food safety fact-finding visits were made to the premises during which, through direct observation of conditions and practices, more points were identified where contamination with viruses could potentially occur and more irrigation water samples were collected.

Description of Enterprises

A summarized table with irrigation data for the enterprises involved, per produce chain, and country (A, B, C, D, E), is presented in Table 1.

For the berry fruit production chain, irrigation water samples were collected from four enterprises in four countries (the Czech Republic, Serbia, Finland, and Poland). Surface water (two cases), ground water (two cases), and a combination of surface and ground water (one case) were used for irrigation. Spraying or dripping was the mode of irrigation applied. Water was directly pumped for irrigation without previous storage in all cases except one, where it was stored in open basins. No waterborne outbreaks were reported in any of the studied sampling areas during the sampling periods (Table 1).

For the leafy green vegetables production chain, irrigation water samples were collected from three enterprises in three countries (Greece, Poland, and Serbia). Ground water was used in all three cases. Spraying and drip irrigation were applied in combination. Water was directly used for irrigation without previous storage in only one case, while it was stored in open basins in the other two cases. Similarly, no waterborne outbreaks were reported in any of the studied sampling areas during the sampling periods (Table 1).

The analytical methods concerning the sample process control virus (SPCV), the treatment of irrigation waters, nucleic acids extraction, the molecular assays for index and pathogenic viral targets (HAV, HEV, NoVGI, NoVGII, hAdV, pAdV, bPyV), and their quantitation, have been previously described in detail (Kokkinos et al. 2012; Maunula et al. 2013).

Results

Both index and pathogenic viruses were found in irrigation water samples from the leafy green vegetables production chain. The data on the presence of index viruses indicated that the highest percentage of fecal contamination was of human origin (28.1 %, 18/64), followed by that of porcine (15.4 %, 6/39) and bovine (5.1 %, 2/39) origins. HEV (5 %, 1/20) and NoV GII (14.3 %, 4/28) were also detected.

Samples from berry fruit production were also positive for both index and pathogenic viruses. The highest percentage of fecal contamination was of human origin (8.3 %, 9/108), followed by that of porcine, (4.5 %, 4/89) and bovine (1.1 %, 1/89) origins. NoV GII (3.6 %, 2/56) was also detected.

The virological results of the irrigation water samples are summarized in Table 2.

Discussion

Enteric diseases linked to consumption of fresh produce have dramatically increased in the last several decades, and contaminated water used in irrigation has been considered a major vehicle for crop contamination (Hirneisen and Kniel 2013). Since bacterial indicators may not be suitable for predicting some potential viral contamination and

Table 1 Irrigation data for the enterprises involved in the present study, per produce chain, and country (A, B, C, D, E)

	Country ID	Type of irrigation water	Mode of irrigation	Types of irrigated cultures	Storage of water before irrigation
Berry fruits	A	Superficial water (river, pond)	Spraying	Strawberries	Direct pumping from the river or storage to the pond
	B	Ground water (well water)	Dripping	Strawberries	Direct pumping from the river or maximum storage for 24 h
		Superficial water (river)			
	C	Ground water (well water)	Dripping	Raspberries, and blackberries	Storage in open basins
	C	Superficial water (river water)	Spraying	Raspberries, tomato	No storage
	D	Ground water (well water)	Dripping	Raspberries	No storage
Leafy green vegetables	C	Ground water (well water)	Spraying and dripping	Lettuce, tomato, cucumber	Storage in open basins
	D	Ground water (well water)	Spraying after planting and dripping afterwards	Butterhead lettuce	No storage
	E	Ground water (well water)	Spraying and dripping	Butterhead lettuce	Storage in open basins

Table 2 Virological analysis data of irrigation water samples used for the production of vegetables and berry fruits

Type of produce	Country ID	Viruses tested						
		hAdV	pAdV	bPyV	HAV	HEV	NoVGI	NoVGII
Vegetables	D	1/22	0/22	0/22	0/20	1/20	0/20	0/20
	C	0/17	6/17	2/17	nd	nd	nd	nd
	E	17/25	nd	nd	0/15	nd	0/15	4/8
Berry fruits	A	0/19	nd	nd	nd	nd	nd	nd
	B	8/36	1/36	0/36	0/36	0/36	0/36	2/36
	C	0/25	0/25	0/25	nd	nd	nd	nd
	C	1/9	1/9	0/9	0/1	0/1	0/1	0/1
	D	0/19	2/19	1/19	0/19	0/19	0/19	0/19

Number of positive samples/number of total samples

nd no data, *hAdV* human adenovirus, *pAdV* porcine adenovirus, *bPyV* bovine polyomavirus, *HAV* hepatitis A virus, *HEV* hepatitis E virus, *NoVGI* norovirus GI, *NoVGII* norovirus GII

water is not regularly monitored for viruses, water that has passed the indicator test may still contain viruses. Moreover, there is also a general perception that the hygienic quality of irrigation water is less important than that of drinking water (Maunula et al. 2013).

Depending on factors such as rainfall, temperature, soil structure, organic carbon content, soil pore water pH, cation concentrations, ionic strength, and virus taxon-specific factors such as capsid diameter and isoelectric point, viruses can move considerable distances in the subsurface environment. Penetration to depths as great as 67 m and horizontal migration as far as 408 m in glacial till and 1600 m in fractured limestone have been reported (Borchardt et al. 2003). Viruses can persist for several months in soils and groundwater when temperatures are low and soils are moist (Borchardt et al. 2003). In South Korea, Cheong et al. (2009) found adenoviruses in 4/29 and 3/30 irrigation water and vegetable samples, respectively (Cheong et al. 2009). In a survey of irrigation waters in Arizona, noroviruses were isolated from 18.2 and 20.7 % of canal water samples in central and western Arizona, respectively, which originated from dammed reservoirs or the Colorado River (Wei and Kniel 2010). Khan and colleagues analyzed fresh vegetables irrigated with fecally contaminated water for the detection of HAV, and identified an area where all grown vegetables were

HAV contaminated by irrigation water (Khan et al. 2014). HAV was also detected in irrigation water from a dam on a commercial fresh produce farm in South Africa by Rachida et al. (2016), while other studies on the detection of HAV in surface water used for the irrigation of fresh produce and other surface water sources in South Africa were previously reported (Taylor et al. 2001; Britz et al. 2012; Said et al. 2014; Rachida et al. 2016).

In the farms examined during the studies of Kokkinos et al. (2012) and Maunula et al. (2013), ground water was used for the irrigation of leafy green vegetables. Both index and pathogenic viruses were detected. HAdVs (28.1 %, 18/64), pAdVs (15.4 %, 6/39), and bPyVs (5.1 %, 2/39) were identified, showing fecal contamination of both human and animal origin. Pathogenic viruses, HEV (5 %, 1/20), and NoVGII (14.3 %, 4/28), were also detected, while HAV and NoVGI were not identified in the analyzed samples. Both surface water and ground water were used for the irrigation of berry fruits. Similarly, both index and pathogenic viruses were detected. HAdVs (8.3 %, 9/108), pAdVs (4.5 %, 4/89) as well as NoVGII (3.6 %, 2/56) were identified. Table 2 summarizes virological analysis data of all irrigation water samples (collected from both "general" and "ad hoc" sampling sites), used for the production of vegetables and berry fruits, and this explains any difference in comparison to the previous reports of Kokkinos et al. (2012) and Maunula et al. (2013). Microbial source tracking (MST) can differentiate or identify sources of fecal contamination (Warriner et al. 2009). The selected index viruses of the study have been successfully applied as MST tools.

A higher prevalence of pathogenic enteric viruses was found in the leafy green production chain compared to that of berry fruit. However, the number of samples tested for viral contamination was relatively small. Future monitoring efforts for human pathogenic viruses along food production chains would benefit from even larger sample sizes, combined with highly sensitive detection methods.

No wastewater was used for irrigation in the primary production enterprises of the study, but an estimate is that worldwide more than 20 million hectares (ha) of irrigated agriculture uses raw, treated, and/or partially diluted wastewater (Hamilton et al. 2006).

The most frequently reported foodborne viral infections associated with the consumption of fresh fruit or vegetables are viral gastroenteritis and hepatitis A (Seymour and Appleton 2001). However, an important emerging viral pathogen such as HEV, detected in an irrigation water sample of the study, should also be considered.

Prevention and sanitation become the most important tools for maintaining the microbial quality and safety of fresh-cut commodities (El-Senousy et al. 2013). Reducing the contamination of food sources prior to harvest is critical to minimizing the risk of foodborne disease and illness (US FDA 2009). Water used in the culturing of food should be of drinking water quality, and guidelines specifically aimed at the reduction of viral contamination are needed, as it has become clear that current indicators for water quality are insufficient as predictors of viral contamination (Koopmans and Duizer 2004). However, it is not practical for all berry fruits and leafy green vegetables production farms to use potable water for irrigation. Preventing contamination of irrigation water is problematic due to the open nature of animal production and problems associated with manure management. Nevertheless, monitoring the microbiological quality of water is a key intervention to reduce the risk of transferring contamination to fresh produce. Furthermore, when contamination is detected in water, there is a need to rapidly identify the source and implement containment plans (Warriner et al. 2009). The Codex Committee on Food Hygiene guidelines for control of virus contamination of food (CAC 2012) recommend that efforts should be made to use only water which is clean during production and processing, and that corrective actions should be taken if sources of contamination are identified. Possible corrective actions include disinfection, e.g., by chlorine. The risk of virus contamination of leafy green vegetables via contaminated water may also be reduced using subsurface or drip irrigation rather than spray irrigation (Hamilton et al. 2006). Crops irrigated with sprinkler and furrow systems may have a higher chance of direct contact with viruses if irrigation water is contaminated (Gerba and Choi 2006). Fecal contamination of farmland and irrigation water is unlikely to be completely avoided and may occur due to the poor management of septic systems, animal lagoons, etc., and climate change-induced flooding and runoff. Research on risk assessment of the degree of viral contamination attributed to different irrigation methods, type of produce, and environment conditions affecting virus survival on produce in the field is also needed (Wei and Kniel 2010). A quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit supply chains was developed, by taking into account the data of irrigation water virological quality of the present study (Bouwknegt et al. 2015).

Recently, it was recommended that systems for monitoring water used in primary production systems should be evaluated (EFSA 2014). It is clear from the collated data from the studies of Kokkinos et al. (2012) and Maunula et al. (2013) that water used in primary production can be considered a critical point where virus contamination could enter the food supply chain. Thus, the establishment of criteria for virus contamination of water, used in primary production of berry fruit and leafy green vegetables, should be considered by the regulatory authorities.

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References

Borchardt, M. A., Bertz, P. D., Spencer, S. K., & Battigelli, D. A. (2003). Incidence of enteric viruses in groundwater from household wells in Wisconsin. *Appliled and Environmental Microbiology, 69*(2), 1172–1180.

Bouwknegt, M., Verhaelen, K., Rzeżutka, A., Kozyra, I., Maunula, L., von Bonsdorff, C. H., et al. (2015). Quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit chains. *International Journal of Food Microbiology, 198*, 50–58.

Britz, T.J., Sigge, G.O., Buys, E.M., Schmidt, S., Potgieter, N., Taylor, M.B. (2012). Extent of contamination in irrigation water and links to irrigated produce at-harvest: With further exploratory study of contamination of produce at point-of-retail. In: T. J. Britz, G. O. Sigge (Eds.), *Quantitative investigation into the link between irrigation water quality and food safety*, vol. IV. Research Report Water Research Commission Report No. 1773/4/12 ISBN 978-1-4312-0377-2. South Africa: Water Research Commission.

CAC. (2012). *Guidelines on the application of general principles of food hygiene to the control of viruses in food*. CAC/GL, 79: Codex Alimentarius Commission.

Carducci, A., Caponi, E., Ciurli, A., & Verani, M. (2015). Possible internalization of an Enterovirus in hydroponically grown lettuce. *International Journal of Environmental Research and Public Health, 12*(7), 8214–8227.

Cheong, S., Lee, C., Song, S. W., Choi, W. C., Lee, C. H., & Kim, S. J. (2009). Enteric viruses in raw vegetables and groundwater used for irrigation in South Korea. *Applied and Environmental Microbiology, 75*(24), 7745–7751.

De Keuckelaere, A., Jacxsens, L., Amoah, P., Medema, G., McClure, P., Jaykus, L.-A., et al. (2015). Zero risk does not exist: Lessons learned from microbial risk assessment related to use of water and safety of fresh produce. *Comprehensive Reviews in Food Science and Food Safety, 14*(4), 387–410.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2014). Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (Salmonella and Norovirus in berries). *EFSA Journal*, *12*(6), 3706.

EFSA/ECDC European Food Safety Authority and European Centre for Disease Prevention and Control. (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2013. Scientific Report of EFSA and ECDC. *EFSA Journal*, *13*.

El-Senousy, W. M., Costafreda, M. I., Pintó, R. M., & Bosch, A. (2013). Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. *International Journal of Food Microbiology, 167*(1), 74–79.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Food and Drugs Administration (FDA)). (1998). Guide to minimize microbial food safety hazards for fresh fruits and vegetables. http://www.foodsafety.gov/_dms/prodguid.

Gerba, C., & Choi, C. Y. (2006). Role of irrigation water in crop contamination by viruses. In S. M. Goyal (Ed.), *Viruses in foods* (pp. 257–263). New York: Springer.

Hamilton, A. J., Stagnitti, F. S., Premier, R., & Boland, A. M. (2006). Is the risk of illness through consuming vegetables irrigated with reclaimed wastewater different for different population groups? *Water Science and Technology, 54*(11–12), 379–386.

Hirneisen, K. A., & Kniel, K. E. (2013). Comparative uptake of enteric viruses into spinach and green onions. *Food and Environmental Virology, 5*(1), 24–34.

Khan, W., Irshad, M., Rehman, G., & Hussain, A. (2014). HAV in fresh vegetables: A hidden health risk in district Mardan, Pakistan. *Springerplus, 3*, 675.

Kokkinos, P., Kozyra, I., Lazic, S., Bouwknegt, M., Rutjes, S., Willems, K., et al. (2012). Harmonised investigation of the occurrence of human enteric viruses in the leafy green vegetable supply chain in three European countries. *Food and Environmental Virology, 4*(4), 179–191.

Koopmans, M., & Duizer, E. (2004). Foodborne viruses: An emerging problem. *International Journal of Food Microbiology, 90*(1), 23–41.

Maunula, L., Kaupke, A., Vasickova, P., Söderberg, K., Kozyra, I., Lazic, S., et al. (2013). Tracing enteric viruses in the European berry fruit supply chain. *International Journal of Food Microbiology, 167*(2), 177–185.

Pachepsky, Y., Morrow, J., Guber, A., Shelton, D., Rowland, R., & Davies, G. (2012). Effect of biofilm in irrigation pipes on microbial quality of irrigation water. *Letters in Applied Microbiology, 54*(3), 217–224.

Rachida, S., Matsapola, P. N., Wolfaardt, M., & Taylor, M. B. (2016). Genetic characterization of a novel hepatitis a virus strain in irrigation water in South Africa. *Journal of Medical Virology, 88*(4), 734–737.

Rajwar, A., Srivastava, P., Sahgal, M. (2015). Microbiology of fresh produce: Route of contamination, detection methods and remedy. *Critical Reviews in Food Science and Nutrition*, *1*, 0.

Rzezutka, A., & Cook, N. (2004). Survival of human enteric viruses in the environment and food. *FEMS Microbiology Reviews, 28*(4), 441–453.

Said, R., Wolfaardt, M., & Taylor, M. B. (2014). Molecular characterization of hepatitis A virus strains from water sources in South Africa. *Water Science and Technology, 69*, 923–933.

Seymour, I. J., & Appleton, H. (2001). Foodborne viruses and fresh produce. *Journal of Applied Microbiology, 91*(5), 759–773.

Steele, M., & Odumeru, J. (2004). Irrigation water as source of foodborne pathogens on fruit and vegetables. *Journal of Food Protection, 67*(12), 2839–2849.

Taylor, M. B., Cox, N., Very, M. A., & Grabow, W. O. K. (2001). The occurrence of hepatitis A and astroviruses in selected river and dam waters in South Africa. *Water Research, 35*, 2653–2660.

Tyrell, S. F., Knox, J. W., & Weatherhead, E. K. (2006). Microbiological water quality requiremenets for salad irrigation in the United Kingdom. *Journal of Food Protection, 69*, 2029–2035.

US FDA. (2009). Guidance for industry: Guide to minimize microbial food safety hazards of leafy greens; draft guidance. http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/FDAProduceSafetyActivities/.

Warriner, K., Huber, A., Namvar, A., Fan, W., & Dunfield, K. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. *Advances in Food and Nutrition Research, 57*, 155–208.

Wei, J., Jin, Y., Sims, T., & Kniel, K. E. (2011). Internalization of murine norovirus 1 by Lactuca sativa during irrigation. *Applied and Environmental Microbiology, 77*(7), 2508–2512.

Wei, J., & Kniel, K. E. (2010). Pre-harvest viral contamination of crops originating from fecal matter. *Food and Environmental Virology, 2*(4), 195–206.

Wyn-Jones, A. P., Carducci, A., Cook, N., D'Agostino, M., Divizia, M., Fleischer, J., et al. (2011). Surveillance of adenoviruses and noroviruses in European recreational waters. *Water Research, 45*(3), 1025–1038.