Food Control 51 (2015) 333–339

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Virological fit-for-purpose risk assessment in a leafy green production enterprise

P. Kokkinos [a, *], M. Bouwknegt [b], K. Verhaelen [b, c], K. Willems [d, e], R. Moloney [f], A.M. de Roda Husman [b], M. D'Agostino [g], N. Cook [g], A. Vantarakis [a, *]

[a] Environmental Microbiology Unit, Department of Public Health, School of Medicine, University of Patras, University Campus, GR 26500 Rion, Patras, Greece
[b] Centre for Infectious Disease Control, National Institute for Public Health and the Environment, RIVM, Netherlands
[c] Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands
[d] Laboratory for Process Microbial Ecology and Bioinspirational Management, Consortium for Industrial Microbiology and Biotechnology, Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium
[e] Scientia Terrae Research Institute, Sint-Katelijne-Waver, Belgium
[f] Environmental Health Service, Health Service Executive, Sandfield Centre, Ennis, Co., Clare, Ireland
[g] Food and Environment Research Agency (FERA), Sand Hutton, York, United Kingdom

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form
19 November 2014
Accepted 25 November 2014
Available online 3 December 2014

Keywords:
Leafy green vegetables
Enteric viruses
Guidance sheet
Decontamination
Risk assessment

ABSTRACT

The purpose of the study was to provide an example of integrated monitoring and control of foodborne viruses using the assessment of one vertical production enterprise and the recommendations given to it. A risk assessment for virological hazards in lettuce was carried out based on the Codex Alimentarius framework, modified to consider viral hazards associated with fresh (whole) lettuce. The fit-for-purpose model was constructed by a complex process of: 1) identification of premises-selection of sampling sites, through the analysis of background information questionnaires based on HACCP audit principles and food safety fact-finding visits, 2) development of sampling guidance documents, 3) a half-year sampling campaign, 4) molecular analysis of the presence of index, human adenoviruses (hAdV) and human pathogenic viruses, noroviruses (NoVGI, NoVGII), and hepatitis A virus (HAV), 5) fit-for-purpose risk assessment, 6) development of a fit-for-purpose guidance sheet for the enterprise food safety manager for the prevention of contamination of leafy greens by viruses. Molecular virological analysis resulted in the detection of hAdV (55.65%, 123/221), and NoVGII (16.66%, 4/24), while NoVGI (0/55) and HAV (0/60) were not detected in the analyzed samples. HAdVs were detected in samples of all three phases (production, processing, and point of sale). The elevated prevalence of hAdVs supports the existence of routes of produce viral contamination. NoVs GI were detected at the point of sale in fresh lettuce heads, supporting the previous finding that pathogenic viruses can follow the routes of index viruses. NoVs GII were detected in irrigation water, harvesters' hands and manure samples, indicating sewage contamination of water, and unsatisfactory levels of hygiene concerning hands hygiene and use of toilet facilities. As a result of the study a fit-for-purpose guidance sheet was finally produced for the prevention of contamination of leafy green vegetables by viruses. The integrated monitoring and control process of the study can be applied to all leafy green vegetables production sites.

1. Introduction

Increased consumption, larger scale production and more efficient distribution of fresh produce over the past two decades have contributed to an increase in the number of illness outbreaks caused by this commodity (Olaimat & Holley, 2012; Soon, Manning, Davies, & Baines, 2012; Warriner, Huber, Namvar, Fan, & Dunfield, 2009). In Europe, it is now recognised by stakeholders (Van

* Corresponding authors. Tel./fax: +30 2610 997935.
E-mail address: pkokkin@med.upatras.gr (P. Kokkinos).

http://dx.doi.org/10.1016/j.foodcont.2014.11.041
0956-7135/

Boxstael et al., 2013) that the fresh produce supply chain has become increasingly characterized by global sourcing and international trade resulting in a more complex food chain which greatly increases the challenges for food safety. Contamination with enteric viruses is now recognised as one of the most important issues for the European fresh produce market (Van Boxstael et al., 2013).

The World Health Organisation (WHO) identified Norovirus and hepatitis A virus in fresh produce as a priority virus/commodity combination for which control measures should be considered (FAO/WHO, 2006). There is currently limited knowledge about where in the supply chain contamination occurs or about the mechanism by which human pathogens colonize and survive on or in fruits and vegetables (Berger et al., 2010). Human enteric viruses can be introduced into the food supply chain during different stages of food production, but there is no strict evidence on which stage of the production process is the most vulnerable for virus contamination. However, it is likely that in the majority of contamination events fresh produce has become contaminated on the farm during growing or harvesting, and therefore primary production is the supply chain phase most at risk of viral contamination (Heaton & Jones, 2007). Routes of contamination are varied and include contamination with faecal material of waters used for irrigation and pesticide application, contamination by infected food handlers, and application of organic wastes to agricultural land as fertilizer (Heaton & Jones, 2007). As agriculture becomes more intensive, produce fields may be next to animal production zones, and the ecological connections between wild animals, farm animals, and produce may be closer. Moreover, due to changes in processing, more cutting and coring may be performed in the field at the time of harvest. Once contamination occurs there are at present no points at which microbiological hazards can be effectively abated (Lynch, Tauxe, & Hedberg, 2009). At present, the food industry relies on postharvest interventions to limit the number of enteropathogens present on fresh produce at point of sale (Heaton & Jones, 2007).

Over the past years, significant improvements of the analytical techniques for the virological analysis of food and environmental samples have been made (Felix-Valenzuela, Resendiz-Sandoval, Burgara-Estrella, Hernandez, & Mata-Haro, 2012; Le Guyader et al., 2004; Papafragkou et al., 2008). Although several studies have been reported in the literature on the estimation of enteric virus contamination of growing vegetables irrigated with reclaimed water, and the consequent infection risk associated with consuming raw vegetables (Barker et al., 2013; Hamilton, Stagnitti, Premier, Boland, & Hale, 2006; Petterson, Ashbolt, & Sharma, 2001; Stine, Song, Choi, & Gerba, 2005) very few studies on the "virological quality" of vegetable production enterprises have been reported (Kokkinos et al., 2012). Sampling for microbiology, usually refers to the statistical representativeness of the samples, and economic and logistical considerations usually limit the number, type and location of samples to be taken. Sampling for virological analyses of food does not necessarily follow the bacterial approach since the low level of contamination, and the complexity and cost of assays are much greater (Bosch et al., 2011).

Adenoviruses (hAdVs) have been shown to be excreted by the populations of all geographical areas and to be the most abundant viruses detected in urban sewage without significant seasonal variation, and for these reasons have been proposed as indicators of human fecal contamination in water and food (Formiga-Cruz et al., 2002; Pina, Puig, Lucena, Jofre, & Girones, 1998). The feasibility of using hAdVs as indicators of human enteric viruses in environmental and shellfish samples was suggested by Pina et al. (1998) who reported that these viruses were easily detected and seemed to be more abundant and stable in environmental samples.

A complete description of the characteristics of deterministic and probabilistic QMRA is available in the FAO/WHO Guidelines on Risk Characterization of Microbiological Hazards in Food. Current thinking on the validity of risk assessments suggests that the dimensions of validity i.e., whether the risk assessment is "fit-for-purpose", should be based on five attributes: 1) Quality and transparency of evidence, 2) Quality of inference, 3) Transparency of inference (strict and real), 4) Timeliness, and 5) Resource requirements (FAO/WHO, 2006). The development of a step-wise approach for a transparent prioritization and fit-for-purpose risk assessment is proposed by EFSA (2012).

In the present study, hAdVs were used as index viruses to demonstrate that a route of contamination existed from the enteric tracts of humans to those points in the supply chain at which they were detected, while NoV GI, GII and HAV were selected as pathogenic target viruses, during a fit-for-purpose virological analysis study of a vertical production vegetables enterprise in Greece, to estimate the infection risk for humans through consumption of the leafy vegetables by using quantitative viral risk assessment (QVRA). The study was part of the European FP7 project VITAL (Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains) (http://www.eurovital.org/) which aimed to gather data on virus contamination to provide a basis for subsequent quantitative viral risk assessment and recommendation of control measures.

The present study aimed to provide an example of integrated monitoring and control of foodborne viruses using the assessment of one vertical production enterprise and the recommendations given to it. While it focuses on lettuce, it was designed to easily be adapted to consider other leafy green vegetables, and be applied to all leafy green vegetables production sites.

2. Materials and methods

2.1. Strategy for the development of a fit-for-purpose model

The study focused on human enteric viruses in fresh lettuce. 'Fresh lettuce' was defined as 'perishable vegetable that has not been frozen or manufactured into articles of food of a different kind or character'. The output of the risk assessment was a fit-for-purpose model for viruses that constitute a significant risk (considering both likelihood and consequence) associated with the consumption of lettuce.

The fit-for-purpose model was constructed by a complex process of: 1) identification of premises-selection of sampling sites, through the analysis of background information questionnaires based on HACCP audit principles and food safety fact-finding visits, 2) development of sampling guidance documents, 3) a half-year sampling campaign, 4) molecular analysis of the presence of index, human adenoviruses (hAdV) and human pathogenic viruses, noroviruses (NoVGI, NoVGII), and hepatitis A virus (HAV), 5) fit-for-purpose risk assessment, 6) development of a fit-for-purpose guidance sheet for the enterprise food safety manager for the prevention of contamination of leafy greens by viruses. A diagrammatic representation of this approach is presented in Fig. 1.

2.2. Questionnaires

Three background information questionnaires were produced to collect data on all three study phases (production, processing, and point of sale) of the food chain. A structured questionnaire was used to collect data regarding the production phase of vegetables, which was divided into the following main areas of interest: 1) Enterprise (farm) review, 2) Quality management systems, 3) Physical location and lay-out, 4) Production processes, 5) Post-production processes,

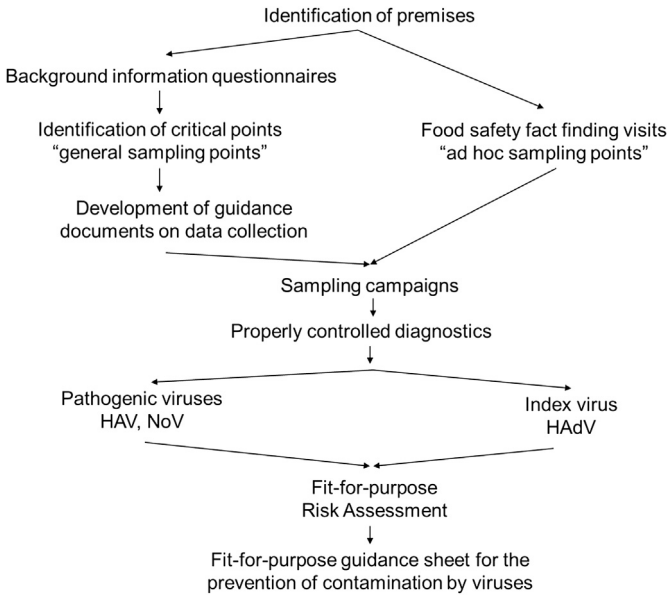

Fig. 1. Diagrammatic representation of the sampling, and analytical strategies of the study, as well as the study's outcomes, which are summarized in the fit-for-purpose guidance sheet, for the prevention of contamination of leafy greens by viruses.

6) Product quality and traceability. Also a structured questionnaire was used to collect data regarding the processing phase of vegetables, which was divided into the following main areas of interest: 1) Enterprise (company) review 2) Quality management systems, 3) Physical location and lay-out, 4) Production processes, and 5) Product quality and traceability. Finally, a structured questionnaire was used to collect data from the point of sale phase, which was divided into the following main areas of interest: 1) Food business review, 2) Quality management systems, 3) Physical location and lay-out, 4) Process flow and control, 5) Product quality, labelling and traceability.

2.3. Sampling strategy

Samples were taken at "general" and "ad hoc" sampling points which were perceived as important critical points for virus contamination through the analysis of background information questionnaires based on HACCP audit principles and food safety fact-finding visits, respectively. Sampling was based on a previously developed fit-for-purpose sampling guidance document and was performed during a 6 months period. Samples were collected from the production farm, processing plant and the point of sale premise. General samples comprised irrigation water, toilets/latrines, toilet door handles, harvesters' hands, manure (production), rinsing water (processing), fresh lettuce heads (point of sale). Six (6) ad hoc samples were collected in total and comprised of: 1) a swab of three (3) empty plastic crates which were reused by supermarkets, 2) a swab of three (3) plastic crates with lettuce heads to be sent to supermarkets, 3) a swab from the inside walls of a track used for the transportation of lettuce crates, 4) a swab of a worker's knife used to cut lettuce heads from the fields, 5) a piece of sponge which was used to clean the bottom of the fresh cut lettuce heads.

To be able to estimate the infection risks for humans through consumption of salad vegetables by using quantitative viral risk assessment (QVRA), and considering that the counts of target pathogenic viruses such as NoV and HAV at the sampling points were expected to be very low, estimates of the index virus human adenovirus (HAdV) were determined at several points in the food supply chain by PCR.

2.4. Virus analysis

Virus concentration, nucleic acids extraction and real-time (RT-) PCR were performed according to standardised VITAL protocols. The VITAL Standard Operating Procedures (SOPs) are available in the public domain of the project's web site (http://www.eurovital.org/), providing validated tools for viral detection in food and the environment. The analytical methods incorporated a sample process control (murine norovirus, MuNoV) and an internal amplification control. Each sample was spiked with 10 μl of a culture of MuNoV before the lysis step of the extraction. Detection of MuNoV RNA by RT-PCR was used to demonstrate extraction of amplifiable nucleic acid. To control for inhibition of the Real-time (RT-) PCR, a target-specific IAC was included in all reactions (Diez-Valcarce, Cook, Hernández, & Rodríguez-Lázaro, 2011; Diez-Valcarce, Kovač, Cook, Hernández, & Rodríguez-Lázaro, 2011). Synthetic multiple-target RNA and DNA oligonucleotides were constructed for use as quantification standards for nucleic acid amplification assays, overcoming the problems related to the difficulty of obtaining practical quantities of viral RNA and DNA from the target viruses (D'Agostino, Cook, Rodriguez-Lazaro, & Rutjes, 2011; Martínez-Martínez, Diez-Valcarce, Hernández, & Rodríguez-Lázaro, 2011). Murine Norovirus (MuNoV), human Norovirus GI and GII, hepatitis A virus (HAV), and human adenovirus (hAdV) molecular detection was performed as described elsewhere (Kokkinos et al., 2012). The efficiency and the robustness of the developed methods have been tested through collaborative trials involving 11 European laboratories (D'Agostino et al., 2012).

2.5. Risk assessment (RA)

The data on virus prevalence were analyzed using risk modelling. A risk model consists of a series of interrelated parameters which are each described by a probability distribution. These distributions reflect the statistical parameter uncertainty given the observations made along the food chain. The eventual risk estimates are obtained by taking 50,000 Monte Carlo samplings from these uncertainty distributions of parameters and by subsequently calculating for each sampling the probability of an adverse health event.

2.6. Fit-for-purpose guidance sheet for the prevention of viral contamination

To facilitate the development of new measures to prevent virus contamination of leafy green vegetables and for virus reduction and control in case of virus contamination, the results of sample analysis obtained by the data gathering laboratory and the area of concern (AOCs), i.e. non-compliance with good practices, e.g. Global GAP, etc., identified by the fact-finding mission were integrated by cluster analysis of the different sampling sites and correlation analysis of the identified clusters and positive samples. This was done in order to identify links between positive samples and AOCs, with the aim of determining what if any non-compliances with prerequisite programs such as good agricultural practice and good hygiene practice could open vulnerabilities in the food supply chain to virus contamination.

3. Results

3.1. Enterprise characterization

3.1.1. Production phase

The enterprise was founded more than twenty (20) years ago. The vegetables production area was 50 Ha and more than 800 ton

of vegetables were exported to EU countries, annually. The quality manager had 6 years of experience and was not a member of a professional consortium/organisation. It employed full time personnel (15 persons) as well as seasonal labour (30 persons, 32 weeks annually, for 7–8 h per day, on average), mostly for manual harvesting. The enterprise worked under specific contract specifications. Good Agricultural Practice (Global GAP) was practiced for ten years. Internal and external auditing was in place. A formal quality system, i.e. ISO 22000, was under development throughout the food supply chain. At primary production, domestic animals were found to have access and/or were present on the premises. Raw manure was stored on-site. No topographical features (e.g. slopes) were found near the fields, which could encourage run-off. Moreover, there was not any industrial, and/or farming activity adjacent to the fields. Similarly, no private or commercial sewage treatment facility or waste material landfill was located adjacent to the fields. Field sanitary accommodation which was reasonably accessible to field workers was provided, including WC, washing hand basin, and antimicrobial hand soap. Wash hand basins were provided with a constant supply of hot and cold water, which was of potable quality, but hand-free taps were not provided, and unsuitable cloth towels were used for hand drying, while disposable paper towels were not available. Produce was directly packed on-site in the final packing containers which were properly handled in order to prevent cross-contamination and were kept covered. Damaged containers were disposed of. The enterprise used its own transport vehicles, which were all suitable and solely designated for transporting foodstuffs and specifically for the finished product. Transport vehicles were cleaned and/or sanitised in a scheduled basis, were refrigerated and were equipped with temperature data loggers. Field/staff sanitary accommodation, the transport vehicles, as well as the harvesting equipment that come in direct contact with the product were cleaned and/or sanitised in a scheduled basis. Shallow untreated wells were used as primary water sources. Shallow wells were sampled with satisfactory results complied with EU regulations, but water was pumped to a shallow, untreated open water basin which was used as a reservoir. During production, workers received hygiene training commensurate with their work duties. They were instructed in hand washing where there is risk of contamination (e.g. before starting work, after using the bathroom, etc). Workers with any notifiable infectious disease were excluded from work. Harvesting equipment (knifes) were cleaned and/or sanitised daily. No suitable protective clothing was worn by food workers, except for disposable gloves. Primary water source and the product were tested microbiologically and chemically in an accredited laboratory. A labelling and traceability system as well as a recall system was in place. Moreover, in-house traceability software was under development by the enterprise.

3.2. Processing phase

The enterprise was the same described above for the production phase. Its main activities included the pre-preparation of raw foodstuffs for further processing, seedling and grafted vegetable plants and packing for agricultural products. The products were restricted to minimally processed products aiming the market of distributors and transporters, retailers (retail trade), and the service sector (restaurants, canteens, caterings, public houses, etc). The enterprise did not work under specific contract specifications. Good Agricultural Practice (Global GAP) was practiced. Internal and external auditing was in place. A formal quality system, i.e. ISO 22000, was under development throughout the food supply chain. Two buildings were used by the food enterprise. The general layout (e.g. zoning & process flow) minimised the risk of cross contamination. The external perimeter was designed to prevent harbourage of rodents. Sanitary accommodation was provided in the buildings solely for food workers. The accommodation was independently ventilated to external air, and there was an independently ventilated lobby between the sanitary accommodation and the food processing areas. The sanitary accommodation included a WC, washing hand basins supplied with hot and cold water, as well as with antimicrobial soap, and hand drying facilities. The water supplied for hand washing was compliant with EU drinking water regulations. The method for sewage disposal was private (septic tank), and this location complied with legal requirements (e.g. distance, slope, etc).

Shallow untreated private wells were used as water sources. Wells were properly constructed in order to protect the water from run-off and flooding, as well as from animal contamination. Wells were regularly maintained and repaired. The quality of water was satisfactory and it complied with EU regulations as has been assessed by municipal authorities and accredited private laboratory analysis. The level of lighting allowed the safe handling of food and enabled cleaning to be carried out. The premises were ventilated. Cleaning equipment designated solely for the cleaning of sanitary accommodation was not colour coded. The equipment installation allowed effective cleaning and complied with ISO standards of hygiene design. Contact surfaces were non-impervious, smooth, durable, and easily cleanable. Food processing areas were cleaned and sanitised according to a cleaning schedule which was in place. Cleaning chemicals were separately stored in a well-ventilated and secure area and workers were trained in how to use them. Food workers were medically screened prior to being employed. They were instructed in personal hygiene, and specifically regarding hand washing. Workers with symptoms of infectious diseases were excluded from work. Protective clothing was provided, but was not colour coded for the different processes. There was a provision for the storage of outer clothing, and it was laundered in-house. A HACCP team was in place, product standards were defined and critical steps controlled. The company carried out its own supplier audit and there was a control system for the receipt of goods (temperature of chilled foods, internal temperature of transport vehicles, date coding, practices during delivery, hygienic condition of transport vehicle). Different types of food were adequately stored (storage rooms zoned separately) and control measures were in place to prevent cross-contamination during storage. Temperature was monitored and recorded and humidity controlled. Disposable material was used for packaging. Equipment/utensils that come in direct contact with food were cleaned and/or sanitised. Batch samples were not retained. Microbial and chemical testing of products was carried out in accredited laboratories on a scheduled base and the results were generally satisfactory. A labelling and traceability system as well as a recall system was in place. Moreover, in-house traceability software was under development by the enterprise. The business had not experienced any product recall.

3.3. Point of sale phase

The food business at point-of-sale was a supermarket founded more than 50 years ago. The food business operator (FBO) had twenty two years of experience, was the responsible manager, and was not a member of a professional consortium/organisation. A formal food safety system was implemented at point-of-sale for three years. Internal and external auditing was in place. The system was not accredited. No quality system was implemented. The layout minimised the risk of cross contamination (e.g. zoning and process flow). Structurally suitable designated staff sanitary accommodation was provided including, WC, wash hand basins with a constant supply of hot and cold water, foot-operated taps, antimicrobial soap, and hand drying facilities. The water supplied for

hand washing was of potable quality and compliant with EU drinking water regulations. A sanitary/hygiene room was provided and sanitizers and disinfectants were used but cleaning equipment was not solely for the cleaning of sanitary accommodation. Paper towels were provided in all of the in-process hand drying facilities and antimicrobial soap in all of them. Refrigerated vehicles were used and temperature was monitored using data loggers. The premises were cleaned according to a documented cleaning plan. All areas were identified and included in the plan, and the maintenance frequency of each area documented. A verification system was in place. The equipment was maintained in good condition, and the cleaning frequency was documented and verified. Pest control measures were in place at point-of-sale. Pest control plans included rodents, flying insects, and crawling insects. Public potable water, which was compliant with EU drinking water regulations, was available for all activities, at point-of-sale. Water source was sampled and analysed on a regular basis. A combination of hairnets or hats, suits or aprons and boots or shoes were worn. Evidence of good practice regarding the wearing of protective clothing and hand washing was found. Documentation on hygiene and sanitation policies and/or practices was provided which included a training schedule. A labelling and traceability system was in place. A product recall system was also in place. The business experienced product recalls and customer complaints, especially during the hot summer period.

3.4. Prevalence of index and pathogenic target viruses

3.4.1. General sampling points

Summarized results of index and pathogenic target viruses at the general sampling points, per phase, matrix and virus type are presented in Table 1.

HAdVs were detected at 55.65%, in all types of samples, representing all three studied phases. NoVGII were detected at 16.66%. HAV and NoVGI were not detected in the analysed samples.

3.5. Ad hoc sampling points

HAdVs were detected in swabs of a) a food worker's knife used to cut lettuce heads from the fields, and b) a swab of three empty plastic crates which were reused by supermarkets.

3.6. Risk assessment (RA)

Hepatitis A virus and Noroviruses GI were not found in the lettuce head production chain of the study, while Noroviruses GII were detected at 16.66%, in 4 out of 24 analyzed samples. The risks of infection from consumption of lettuce heads were 3×10^{-4} (6×10^{-6}–5×10^{-3}) for NoV and 3×10^{-8} (7×10^{-10}–3×10^{-6}) for hepatitis A (Bouwknegt et al., submitted for publication). The production chain contained different identified potential contamination points: irrigation, contact between lettuce heads and harvesters' hands, and use of manure. Noroviruses were found in irrigation water, on harvesters' hands, and interestingly in bovine manure samples, supporting the finding of high prevalence of index viruses of human faecal contamination (hAdVs) throughout the whole chain, until the point of sale. Positive bovine manure samples for hAdVs most probably derived from workers defecating on the manure dump in the mistaken belief that it would not pose a hazard. The aforementioned findings highlight the existence of contamination routes which pathogenic viruses could follow. Pathogenic viruses were not detected on lettuce heads collected at point of sale, while a significantly high percentage of samples was found positive for hAdVs 64/89 (71.91%).

HAdVs were found in a large number of samples, providing more robust estimates of the virus concentrations, the exposure levels and the larger contribution of hand hygiene compared to irrigation water to the virus contamination. Hand transfer was found to be a more likely contamination source for lettuce than irrigation water, based on the monitoring data and subsequent modelling. For targeted risk-management, full compliance with strict hand-hygiene measures by food handlers will improve virus-safe production of fresh produce most as compared with the other examined sources. This effect will be further aided by compliance with other hygiene and water quality regulations in production and processing facilities (Bouwknegt et al., submitted for publication).

Table 1
Summarized results, of index and pathogenic target viruses, at the general sampling points, per phase, matrix and virus type (Kokkinos et al., 2012).

Point of interest	HAdV	HAV	NoVGI	NoVGII
Production				
Irrigation water	17/22	0/15	0/15	1/5
Toilets/latrines	2/5	0/2	0/2	0/1
Toilet doorhandles	2/4	0/2	0/2	n.d.
Harvesters hands	31/87	0/8	0/5	1/12
Manure	3/5	0/2	n.d.	2/2
Processing				
Rinsing water	2/6	0/1	0/1	n.d.
Point of sale				
Fresh lettuce	64/89	0/27	0/27	0/4

Number of positives/number of tested; n.d.: no data.

3.7. Fit-for-purpose guidance sheet for the prevention of viral contamination

Analysis of the AOCs (medical screening, environment, premises and structure, design and lay-out – zoning, distribution and transport, cleaning and sanitation, maintenance, pest control, waste management, personnel hygiene, process control: HACCP, monitoring: product/water quality, traceability/recall/labelling) in the salad vegetable supply chain of the study has revealed that relatively more areas of concern were identified in the production phase than processing and point-of-sale. In addition, among these areas of concern, there was a higher ratio of significant areas of concern over minor areas of concern for production phase. Notably, in primary production of salad vegetables the analysis of AOC clusters and virus contamination data has revealed correlation between key non-compliances (poor quality irrigation water, poor sanitation, and poor hand hygiene) and contamination of produce (personal communications by Willems K. and Moloney R.). The Guidance Sheets contain recommendations based on accepted good practices and augmented by findings from the analysis of critical points performed during the fact-finding missions. The Guidance Sheets have been placed on the public pages of VITAL project website (www.eurovital.org). The guidance complements that given by the Codex Alimentatrius Commission (CAC, 2012).

4. Discussion

The importance of fresh produce in the transmission of foodborne disease is being increasingly recognized (Gandhi, Mandrell, & Tian, 2010; Heaton & Jones, 2007; Kokkinos et al., 2012). The present study aimed to provide an example of integrated monitoring and control of foodborne viruses using an assessment of one lettuce vertical production enterprise and the recommendations given to it, using a fit-for-purpose approach. Fit-for-purpose "risk

assessment" is the right tool to adequately answer the risk manager's questions on the risks for humans because of microbiological hazards, for a specific food enterprise.

The fit-for-purpose model was constructed by a complex multi-step process described previously. HAdVs have been used as index viruses of human faecal contamination. HAdVs have been detected in all types of samples representing all three studied phases (production, processing and point of sale). This finding indicates the existence of contamination routes which pathogenic viruses could follow, until the point of sale. HAdVs have been detected in irrigation and rinsing water samples, indicating sewage contamination. Moreover, they have been detected in swabs from toilets/latrines, toilet door-handles and harvesters hands, which strongly indicates poor hygienic conditions. Both these findings are contradictory to the data collected initially from the questionnaires, which stated that irrigation and rinsing water of high microbiological standards was used, and that the workers received extensive training on good hygiene practices. Interestingly, bovine manure samples have been found positive for hAdVs, indicating improper use of toilets by field workers. Industrial type toilets were present in different parts of the lettuce growing fields. The prevalence of hAdVs at the point of sale was high, since 64 out of the 89 analysed samples were found positive. HAV and NoV GI were not detected at all. On the contrary, NoV GII have been detected in irrigation water, harvesters' hands and manure, supporting the findings of hAdVs. Viral prevalence results may have been biased by the sampling scheme. The numbers of samples tested for viral contamination were relatively small in this study, especially considering the expected low prevalence. Therefore, the presented results should be interpreted as indicative and for greater confidence in the results a greater number of samples would have to be tested (Berto, Martelli, Grierson, & Banks, 2012). Furthermore, the monitoring, as applied in the current study, was more likely to detect structural contamination events rather than episodic contamination events. Therefore, sampling points that tested negative throughout the monitoring might be important for episodic viral contamination nevertheless. However, viruses were found in the studied food production chain of which food products are consumed raw by consumers, and infections were likely to occur. The relevance of studies to reduce viral contamination in these food production chains is therefore shown to be eminent.

Because of the increasing incidence of food-borne viral infections, the Codex Alimentarius Committee on Food Hygiene issued an international draft on a Code of Hygienic Practice for the control of viruses in foods (Ambrozic, Bozic, Jevsnik, Cook, & Raspor, 2011). These guidelines follow the format of the Codex *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969), and contain the sections of that document which are relevant to different supply chains including that of salad vegetables.

Controlling viruses in fresh produce requires a preventive food chain approach with a focus on avoiding viral contamination rather than reducing the presence of persistent viruses (EFSA, 2012). This is especially true for perishable foods, such as fresh produce, because their nature makes it difficult to apply mitigation measures while maintaining the organoleptic properties.

For the determination of suitable water sources in the primary production, e.g., the Codex guidelines on hygienic practice for fresh leafy vegetables (CAC, 2010) provide guidance for on-site sanitary surveys to assess the microbial contamination potential of water sources. Additionally, treatments to reduce pathogen loads are advisable when water sources pose a high risk of contamination e.g., surface water, reclaimed wastewater, and shallow, unprotected groundwater (CAC, 2010), especially when produce is eaten raw or minimally processed. The WHO guidelines on the use of wastewater in agriculture (WHO, 2006) provide further detailed recommendation on the suitability of water treatment processes applicable in agriculture, such as water stabilization ponds (Mara & Sleigh, 2010a; da Silva et al., 2008), and additionally on application techniques for irrigation to minimize water contact with produce, such as drip irrigation. It is estimated that combining various pre- and post-harvest measures such as water treatments, drip irrigation, withholding periods (time between irrigation and harvest), or produce washing, may result in up to a 6 $\log_{10}$-unit reduction of pathogens on produce (WHO, 2006). However, data on the actual reduction of norovirus particles on produce by e.g. drip irrigation or withholding periods are lacking (Mara & Sleigh, 2010b). Appropriate and continuous hand hygiene is probably the most relevant measures to prevent virus contamination of fresh produce by food handlers (Moe, 2009; Mokhtari & Jaykus, 2009). Currently, washing hands under running water with soap for 20 s and subsequent hand drying with paper towels is recommended (CAC, 2012; EFSA, 2011; Hall et al., 2011). Washing hands for 10 s with soap reportedly reduced about 1 $\log_{10}$-unit of norovirus genomes from hands (Liu, Yuen, Hsiao, Jaykus, & Moe, 2010), representing a conservative reduction efficacy of hand washing because the recommended procedure was not entirely followed. Nevertheless, already a 1 $\log_{10}$-unit reduction of noroviruses from hands may reduce the public health risk substantially dependent on the distribution of contamination levels on produce (Verhaelen et al., 2013). Not only the actual virus reduction on hands, but also the compliance to good hand hygiene practices, determines the successful prevention of norovirus introduction by infected food handlers. Yet food handlers reportedly wash their hands about one-third of the time after performing activities for which hand washing is endorsed (Green et al., 2006). It has been suggested that hand washing compliance amongst fresh produce farm workers can be enhanced by educational and training programs and easy access to hand washing facilities (Soon & Baines, 2012). In addition, the exclusion of food handlers showing clinical symptoms from the workplace is recommended (CAC, 2012). In the absence of a vaccine against norovirus infection, vaccination of food handlers as a prevention measure is not feasible (Richardson, Bargatze, Goodwin, & Mendelman, 2013).

Due to the properties of noroviruses, their abundance in the environment and non-compliance to e.g., good hygiene practice, a consistent and complete prevention of norovirus contamination in food chains is yet illusionary. In addition to measures preventing norovirus contamination, appropriate measures reducing infectious norovirus loads on foods are thus required for adequate food safety. The efficacies of mitigation measures, e.g., washing, high pressure treatment, or irradiation, in reducing norovirus on fresh produces have been reviewed (Baert, Debevere, & Uyttendaele, 2009; Zuber, Butot, & Baert, 2013). To evaluate their effect on the reduction of a public health risk, quantitative risk assessments for specific food chains are required (FAO/WHO, 2008), allowing the implementation of performance criteria for mitigation measures in food safety regulation for fresh produce.

The study demonstrated overall the existence of virus contamination routes from human sources, by the detection of both pathogenic and indicator viruses, and specifically indicated the potential for virus contamination at primary production of leafy green vegetables. Conclusively, it provides an example of integrated monitoring and control of foodborne viruses using the assessment of one vertical production enterprise. While it focuses on lettuce, it was designed to easily be adapted to consider other leafy green vegetables, and be applied to all leafy green vegetables production sites in fit-for-purpose studies.

Acknowledgements

This work was supported by a European Community's Seventh Framework Programme (FP7/2007–2013) under Grant agreement no. KBBE-213178 (Integrated monitoring and control of food-borne viruses in European food supply chains, VITAL) led by the coordination team of Nigel Cook (FERA, UK), Martin D'Agostino (FERA, UK) and Franco M Ruggeri (ISS, Italy). The authors are grateful to the owners of the companies who took part in this study for the kind and skilful collaboration and assistance and to all VITAL members.

References

Ambrozic, M., Bozic, T., Jevsnik, M., Cook, N., & Raspor, P. (2011). Compliance of proposed Codex Alimentarius guidelines for virus management with principles of good practice. *Acta Alimentaria, 40*, 364–375.

Baert, L., Debevere, J., & Uyttendaele, M. (2009). The efficacy of preservation methods to inactivate foodborne viruses. *International Journal of Food Microbiology, 131*, 83–94.

Barker, F. S., O'Toole, J., Sinclair, M. I., Leder, K., Malawaraarachchi, M., & Hamilton, A. J. (2013). A probabilistic model of norovirus disease burden associated with greywater irrigation of home-produced lettuce in Melbourne, Australia. *Water Research, 47*(3), 1421–1432.

Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., et al. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology, 12*(9), 2385–2397.

Berto, A., Martelli, F., Grierson, S., & Banks, M. (2012). Hepatitis E virus in pork food chain, United Kingdom. *Emerging Infectious Diseases, 18*(8), 1358–1360.

Bosch, A., Sánchez, G., Abbaszadegan, M., Carducci, A., Guix, S., Le Guyader, F. S., et al. (2011). Analytical methods for virus detection in water and food. *Food Analytical Methods, 4*(1), 4–12.

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

CAC (Codex Alimentarius Commission). (2010). *Code of hygienic practice for fresh fruits and vegetables*. Adopted 2003. Revision 2010 (new Annex III for Fresh Leafy Vegetables). Codex Alimentarius Commission.

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

D'Agostino, M., Cook, N., Di Bartolo, I., Ruggeri, F. M., Berto, A., Martelli, F., et al. (2012). Multicenter collaborative trial evaluation of a method for detection of human adenoviruses in berry fruit. *Food Analytical Methods, 5*, 1–7.

D'Agostino, M., Cook, N., Rodriguez-Lazaro, D., & Rutjes, S. (2011). Nucleic acid amplification-based methods for detection of enteric viruses: definition of controls and interpretation of results. *Food and Environmental Virology, 2*, 55–60.

Diez-Valcarce, M., Cook, N., Hernández, M., & Rodríguez-Lázaro, D. (2011). Analytical application of a sample process control in detection of foodborne viruses. *Food Analytical Methods, 4*, 614–618.

Diez-Valcarce, M., Kovač, K., Cook, N., Hernández, M., & Rodríguez-Lázaro, D. (2011). Construction and analytical application of internal amplification controls (IAC) for detection of food supply chain relevant viruses by real-time PCR-based assays. *Food Analytical Methods, 4*, 437–445.

EFSA. (2011). Scientific opinion, an update on the present knowledge on the occurrence and control of foodborne viruses. *EFSA Journal, 9*, 2190–2286.

EFSA (European Food Safety Authority). (2012). *"Fit-for-purpose" risk assessment*. http://www.efsa.europa.eu/en/121107/docs/121107-p05.pdf.

FAO/WHO (Joint FAO/WHO (Food and Agricultural Organization/World Health Organization)). (2006). *The use of microbiological risk assessment outputs to develop practical risk management strategies: Metrics to improve food safety*. http://www.who.int/foodsafety/publications/micro/MRA_Outputs.pdf.

FAO/WHO. (2008). *Exposure assessment of microbiological hazards in food: Guidelines*.

Felix-Valenzuela, L., Resendiz-Sandoval, M., Burgara-Estrella, A., Hernandez, J., & Mata-Haro, V. (2012). Quantitative detection of hepatitis A, rotavirus and genogroup I norovirus by RT-qPCR in fresh produce from packinghouse facilities. *Journal of Food Safety, 32*, 467–473.

Formiga-Cruz, M., Tofiño-Quesada, G., Bofill-Mas, S., Lees, D. N., Henshilwood, K., Allard, A. K., et al. (2002). Distribution of human viral contamination in shellfish from different growing areas in Greece, Spain, Sweden and UK. *Applied and Environmental Microbiology, 68*, 5990–5998.

Gandhi, K. M., Mandrell, R. E., & Tian, P. (2010). Binding of virus like particles of Norwalk virus to romaine lettuce veins. *Applied and Environmental Microbiology, 76*(24), 7997–8003.

Green, L. R., Selman, C. A., Radke, V., Ripley, D., Mack, J. C., Reimann, D. W., et al. (2006). Food worker hand washing practices: an observation study. *Journal of Food Protection, 69*, 2417–2423.

Hall, A. J., Vinjé, J., Lopman, B., Park, G. W., Yen, C., Gregoricus, N., et al. (2011). Updated norovirus outbreak management and disease prevention guidelines. *Morbidity and Mortality Weekly Report, 60*, 1–18.

Hamilton, A. J., Stagnitti, F., Premier, R., Boland, A. M., & Hale, G. (2006). Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Applied and Environmental Microbiology, 72*(5), 3284–3290.

Heaton, J. C., & Jones, K. (2007). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *Journal of Applied Microbiology, 104*(3), 613–626.

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

Le Guyader, F. S., Schultz, A. C., Haugarreau, L., Croci, L., Maunula, L., Duizer, E., et al. (2004). Round-robin comparison of methods for the detection of human enteric viruses in lettuce. *Journal of Food Protection, 67*(10), 2315–2319.

Liu, P., Yuen, Y., Hsiao, H. M., Jaykus, L. A., & Moe, C. (2010). Effectiveness of liquid soap and hand sanitizer against Norwalk virus on contaminated hands. *Applied and Environmental Microbiology, 76*, 394–399.

Lynch, M. F., Tauxe, R. V., & Hedberg, C. W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection, 137*(3), 307–315.

Mara, D., & Sleigh, A. (2010a). Estimation of norovirus and *Ascaris* infection risks to urban farmers in developing countries using wastewater for crop irrigation. *Journal of Water and Health, 8*, 572–576.

Mara, D., & Sleigh, A. (2010b). Estimation of norovirus infection risks to consumers of wastewater-irrigated food crops eaten raw. *Journal of Water and Health, 8*, 39–43.

Martínez-Martínez, M., Diez-Valcarce, M., Hernández, M., & Rodríguez-Lázaro, D. (2011). Design and application of nucleic acid standards for quantitative detection of enteric viruses by real-time PCR. *Food and Environmental Virology, 3*(2), 92–98.

Moe, C. L. (2009). Preventing norovirus transmission: how should we handle food handlers? *Clinical Infection Diseases, 48*, 38–40.

Mokhtari, A., & Jaykus, L. A. (2009). Quantitative exposure model for the transmission of norovirus in retail food preparation. *International Journal of Food Microbiology, 133*, 38–47.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology, 32*(1), 1–19.

Papafragkou, E., Plante, M., Mattison, K., Bidawid, S., Karthikeyan, K., Farber, J. M., et al. (2008). Rapid and sensitive detection of hepatitis A virus in representative food matrices. *Journal of Virological Methods, 147*(1), 177–187.

Petterson, S. R., Ashbolt, N. J., & Sharma, A. (2001). Microbial risks from wastewater irrigation of salad crops: a screening-level risk assessment. *Water Environment Research, 73*(6), 667–672.

Pina, S., Puig, M., Lucena, F., Jofre, J., & Girones, R. (1998). Viral pollution in the environment and shellfish: human adenovirus detection by PCR as an index of human viruses. *Applied and Environmental Microbiology, 64*, 3376–3382.

Richardson, C., Bargatze, R. F., Goodwin, R., & Mendelman, P. M. (2013). Norovirus virus-like particle vaccines for the prevention of acute gastroenteritis. *Expert Review of Vaccines, 12*, 155–167.

da Silva, A. K., Le Guyader, F. S., Le Saux, J. C., Pommepuy, M., Montgomery, M. A., & Elimelech, M. (2008). Norovirus removal and particle association in a waste stabilization pond. *Environmental Science and Technology, 42*, 9151–9157.

Soon, J. M., & Baines, R. N. (2012). Food safety training and evaluation of hand-washing intention among fresh produce farm workers. *Food Control, 23*, 437–448.

Soon, J. M., Manning, L., Davies, W. P., & Baines, R. (2012). Fresh produce-associated outbreaks: a call for HACCP on farms? *British Food Journal, 114*(4), 553–597.

Stine, S. W., Song, I., Choi, C. Y., & Gerba, C. P. (2005). Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection, 68*(5), 913–918.

Van Boxstael, S., Habib, I., Jacxsens, L., De Vocht, M., Baert, L., Van De Perre, E., et al. (2013). Food safety issues in fresh produce: bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. *Food Control, 32*, 190–197.

Verhaelen, K., Bouwknegt, M., Carratala, A., Lodder-Verschoor, F., Diez-Valcarce, M., Rodriguez-Lazaro, D., et al. (2013). Virus transfer proportions between gloved fingertips, soft berries, and lettuce, and associated health risks. *International Journal of Food Microbiology, 166*, 419–425.

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.

WHO. (2006). Guidelines for the safe use of wastewater, excreta and greywater. *Wastewater Use in Agriculture, 2*. http://whqlibdoc.who.int/publications/2006/9241546832_eng.pdf.

Zuber, S., Butot, S., & Baert, L. (2013). Effects of treatments used in food processing on viruses. In F. J. M. Smulders, B. Norrung, & H. Budka (Eds.), *Foodborne viruses and prions and their significance for public health* (Vol. 1). The Netherlands: Wageningen Academic Publishers.