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A review of foodborne viruses in fresh produce on the island of Ireland

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Foreword and acknowledgements

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Glossary of terms

AdV	adenovirus
CoV	coronavirus
EC	European Commission
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EU	European Union
exposure assessment	qualitative or quantitative evaluation of the likely intake of biological, chemical, and physical agents through food, as well as exposures from other sources if relevant
FAO	Food and Agriculture Organization
FBO	food business operator
FCV	feline calicivirus
fresh produce	fresh fruit and vegetables grown in the field (with or without cover) or in protected facilities
FSA	Food Standards Agency
FSAI	Food Safety Authority of Ireland
FV	flavivirus
GAP	Good Agricultural Practice
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
HAV	hepatitis A virus
hazard characterisation	qualitative or quantitative evaluation of the nature of the adverse health effects associated with a hazard; for the purpose of microbiological risk assessment, the concerns relate to microorganisms or their toxins (poisonous by-products)
hazard identification	identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods
HBGA	histo-blood group antigen
HEV	hepatitis E virus
HPAI	highly pathogenic avian influenza virus (“bird flu”)

HPP	high pressure processing
HPSC	Health Protection Surveillance Centre
ID_{50}	dose of an infectious organism required to produce infection in 50 per cent of the experimental subjects
IOI	island of Ireland (including the Republic of Ireland and Northern Ireland)
ISO	International Organization for Standardization
LOD	limit of detection
MAP	modified atmosphere packaging
MNV	murine norovirus
Mpa	megapascals
NI	Northern Ireland
NiV	Nipah virus
nm	nanometer
NoV	norovirus
PAA	peroxyacetic acid
PCR	polymerase chain reaction, a method of increasing the volume of DNA, for example that of a virus, to make it easier to quantify (detect and count)
RA	risk assessment
RASFF	EU Rapid Alert System for Food and Feed
risk characterisation	process of determining the qualitative or quantitative estimation, including attendant uncertainties, of the likelihood and severity of known or potential adverse health effects, based on hazard identification, hazard characterisation and exposure assessment
RNA	ribonucleic acid
RT–PCR	reverse transcription–polymerase chain reaction, a test that detects a single strand of RNA, for example from a virus, and converts it into corresponding DNA (by reverse transcription); this is then increased in volume in the laboratory (by polymerase chain reaction, or PCR), which makes it easier to quantify or count
RV	rotavirus
SARS	severe acute respiratory syndrome
SARS–CoV	severe acute respiratory syndrome–causing coronavirus
TV	Tulane virus

TS	technical specification(s)
UK	United Kingdom
VITAL	A European Commission (EC) Seventh Framework Programme: Integrated monitoring and control of foodborne viruses in European food supply chains (VITAL)
WHO	World Health Organization

Executive summary

Viruses are a leading cause of foodborne disease in the European Union. Norovirus and hepatitis A virus are recognised as the main viruses of public health concern. Transmission of these viruses typically occurs through foods that are consumed raw such as shellfish and fresh produce, with outbreaks involving these foods becoming increasingly common.

Consistent with this, in 2013 there was an hepatitis A virus outbreak in Ireland that was related to the consumption of imported frozen berries. This outbreak, and the increasing implication of fresh produce in virus outbreaks globally, has prompted requests for a review of current knowledge about viral contamination of fresh produce on the island of Ireland. Particular focus on current methods of detection for viruses in produce, and epidemiology and prevalence studies, is called for.

In 2012, a standard method for the detection of Norovirus and hepatitis A virus in salad vegetables and soft fruits was published by the International Organization for Standardization (ISO/TS 15216-1 and ISO/TS 15216-2). This method has led to enhanced surveillance of foods following virus outbreaks in many countries. It has also enabled some countries to evaluate viral occurrence in different produce types through the supply chain.

Surveillance studies undertaken following outbreaks overseas show that the main fresh produce commodities implicated in the transmission of viruses are berries, lettuces, tomatoes, melons and scallions (spring or green onions). Commodities such as grapes, pineapples, mangoes and pomegranates have only been implicated occasionally. Norovirus and hepatitis A virus have also been detected in a wide variety of produce types that have not yet been linked to illness outbreaks. These include dates, leeks, radishes and parsley. This suggests that a broader array of foods may be responsible for viral illness outbreaks than previously considered.

Currently on the island of Ireland there is no laboratory testing capability for detection of viruses in produce and there are no data on viral occurrence in fresh produce. In prevalence surveys conducted overseas, detection rates varied widely, with some studies showing very high rates of contamination and others very low rates: norovirus detection rates ranged between zero and 62.5% for vegetables and between zero and 50% for fruits.

Comparing detection rates across studies is challenging because methods of analysis vary, as do the origins of the foods sampled. This also hampers predictions of viral prevalence on the island of Ireland. Increasing global adoption of International Organization for Standardization/TS as the standard method for virus detection should improve comparability of studies in the future.

The lack of viral occurrence data limits attempts to quantitatively evaluate the risk of viral illness relating to fresh produce consumption on the island of Ireland. Additionally, the absence of a testing

capability means that it is difficult to confirm the involvement of foods in viral illness outbreaks. Thus, an important consideration for the future is the implementation of the ISO/TS method for detecting viruses in produce on the Island of Ireland. The development of this capability would enhance surveillance of suspected foods and assist in identifying sources of contamination.

Generation of prevalence data for Norovirus and and hepatitis A virus would support quantitative risk assessment. Such data would also provide a scientific basis for future discussions regarding microbiological criteria for Norovirus in produce – which the European Food Safety Authority suggests should be considered in the future following the generation of further baseline data. The availability of virus testing may also be useful for primary food producers and importers to verify the efficacy (the effectiveness) of Good Hygienic Practice and Good Agricultural Practice and to identify potential high-risk points in the supply chain.

If viral monitoring is implemented on the island of Ireland in the future, the interpretation of reverse transcription–polymerase chain reaction (RT–PCR) testing results will need to be considered, along with appropriate sampling plans that minimise the risk of false negatives.

Virus persistence studies have demonstrated that Norovirus and hepatitis A virus can survive on the surface of produce for extended periods of time – longer than the shelf life of the products. Viruses can be accumulated through the roots of some plants, such as lettuce and onions, and are then sequestered, or stored, in tissues and cells. Recent data demonstrates the attachment of Norovirus to specific ligands (molecules that bind to other molecules) within lettuce tissues. This explains the resistance of these viruses to common processes such as washing. In addition, virus inactivation studies suggest that the use of chlorine or low heat may not be completely effective post-harvest treatments for reducing or eliminating viruses.

Further studies are needed on the infectivity of Norovirus and hepatitis A virus through common processes used by industry. This is becuase many studies to date have relied on culturable surrogate viruses – similar viruses that can be cultivated or grown more readily in a laboratory – which may be less stable than Norovirus and hepatitis A virus .

The fact that many produce types are consumed raw without any treatment means it is critical that viruses are not introduced during production and harvesting. This can be achieved by ensuring that water used at all stages of production is clean and that food handlers and pickers adhere to GHP. Current guidelines, including the recently produced FSAI Guidance Note No. 31 (*Fresh Produce Safety in Primary Production in Ireland*) and the Codex Guidelines on control of viruses in foods, provide adequate recommendations on these two key issues. It is very important that these are followed by food business operators. Training programmes for produce workers covering the role of foods in virus transmission, transmission pathways and details on the infectivity of Norovirus and hepatitis A virus, are also essential and should be prioritised.

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1 Key project recommendations

The following recommendations are made for the consideration of ***safe***food and Knowledge Network members, including regulatory agencies, food business operators (FBO's) and science providers on the island of Ireland (IOI).

1. Norovirus (NoV) and hepatitis A virus (HAV) are the main contributors to viral foodborne illness globally, and therefore should be the priority in future work programmes involving viruses on the IOI.

2. Emerging viruses, such as rotavirus, flavivirus, hepatitis E virus, severe acute respiratory syndrome and avian influenza (bird flu), have the potential to cause foodborne illness. Risk managers should be aware of issues relating to their presence in the food supply chain.

3. Emphasis should be placed on ensuring the safety of foods most commonly consumed on the IOI that have been firmly associated with viral outbreaks internationally. These include tomatoes, scallions (spring or green onions), lettuce, melons and berries.

4. There is increasing evidence of the presence of NoV and HAV in a wider array of fruits and vegetables, such as dates, leeks, parsley and others. Stakeholders should be aware that "non-typical" food types may be responsible for viral illness outbreaks in the future.
5. It is recommended that the ISO/TS method for detecting HAV and NoV in salad vegetables and soft fruits be implemented on the IOI.

6. Epidemiological investigations on the IOI to identify foods that may be responsible for viral outbreaks should be supported and reinforced.

7. Consideration should be given to the development of whole genome sequencing techniques for HAV and NoV on the IOI to accurately type strains involved in outbreaks and assist in confirming linkages between foods and patients.

8. It is recommended that a prevalence survey of NoV and HAV in high-risk produce types be conducted on the IOI, using the ISO/TS standard method of detection. Such a survey should involve a robust sampling plan in which samples are collected from vulnerable points in the supply chain of each produce type included, at which contamination could be introduced.

9. Following the collection of baseline viral occurrence data, it is recommended that a risk assessment of viruses in fresh produce on the IOI be conducted to evaluate the burden of illness (the impact disease has on people and the economy) and potential control options.

10. To enable modelling of viral reduction (as part of risk assessment) through commonly applied production and processing steps on the IOI, a detailed supply chain map is needed for each high-risk commodity, showing common practices applied (for example, irrigation, application of agrichemicals, washing and freezing).

11. Training programmes that cover good agricultural practice (GAP), good hygiene practice (GHP), the role of foods in virus transmission, transmission pathways and the infectivity of NoV and HAV should be targeted at workers involved in the production of tomatoes, scallions, berries and lettuce.

2 Introduction and background

Foodborne viruses are increasingly recognised as a significant food safety hazard and are now reported to be responsible for most illness outbreaks worldwide (Anonymous, 2008; Baert et al., 2011; Havelaar et al., 2015). Contamination of food with viruses can occur throughout the supply chain, including during primary production, processing and food preparation. Contamination principally occurs when foods come into contact with human faeces. Ready-to-eat (RTE) foods and foods that are consumed raw, such as bivalve shellfish and fresh produce, are frequently linked to foodborne viral outbreaks.

Recently there have been several significant illness outbreaks in the EU relating to foodborne viruses in fresh produce.

- In 2012 Germany experienced a foodborne outbreak of norovirus (NoV) relating to frozen strawberries from China – around 11,000 cases were reported (Bernard et al., 2014).
- In 2013 around 1,400 cases of hepatitis A virus (HAV) were reported from 12 EU countries, including the island of Ireland (IOI). The mostly likely source was frozen Bulgarian blackberries and/or Polish redcurrants (EFSA, 2014d; Fitzgerald et al., 2014).
- In 2016 412 people in Denmark contracted NoV from lettuce produced in France (Muller et al., 2016a; Muller et al., 2016b).

A recent review of berry-related outbreaks and detections in the EU identified 32 events between 1983 and 2013, of which 27 were related to NoV, involving over 15,000 cases (Tavoschi et al., 2015)

Many countries and agencies, including the European Commission (EC) and the European Food Safety Authority (EFSA), are investigating control options to reduce the burden of illness relating to foodborne viruses in foods, particularly in fresh produce and shellfish (EFSA, 2011, 2014a, 2014b, 2014c). It is clearly necessary that more information is required on the occurrence of viruses in produce and contamination pathways in order to develop control strategies that minimise risk to consumers.

Given the increasing recognition of fresh produce in transmitting viruses and the 2013 hepatitis A (HAV) berry outbreak on the IOI, it is pertinent to review current knowledge about viral contamination of fresh produce on the IOI. There should be a particular focus on current methods of detection for viruses in produce, and epidemiology and prevalence studies.

For the purposes of this review, the Codex definition of fresh produce has been applied and thus the review applies to "*fresh fruit and vegetables grown in the field (with or without cover) or in protected facilities (hydroponic systems or greenhouses)*" (Codex, 2012), and includes frozen products

3 Project aim and objectives

The objectives of this review are to

- Evaluate the literature regarding recent trends in foodborne viruses, including studies on detection methods, epidemiology and the prevalence of viruses in foods.
- Survey outbreaks of disease associated with NoV and HAV from fresh produce on the IOI and overseas.
- Examine approaches to monitoring and surveillance of NoV and HAV on the IOI and overseas.
- Identify data gaps and uncertainties and make recommendations on the most critical data gaps that could be addressed to assist in reducing the human health impacts of Nov and HAV.

4 Methodological approach

Review of current literature

Systematic literature searches were undertaken to collate (that is, collect and combine) information on NoV gastroenteritis (inflammation of the stomach and small intestine) and HAV illness outbreaks related to fresh produce, and on occurrence and prevalence surveys of fresh produce for these two viruses. Searches began with a structured electronic search using the Google Scholar and PubMed search engines. Searches for outbreaks commenced with the string:

Illness AND outbreak AND raspberries AND norovirus OR norwalk OR calicivirus OR hepatitis OR HAV OR "hepatitis A"

The search was then repeated for each fresh produce commodity identified as being produced or imported into the IOI (Table 2 Table 3). Searches for prevalence studies used the string:

occurrence OR prevalence, AND produce, AND norovirus OR norwalk OR calicivirus OR hepatitis OR HAV OR "hepatitis A"

No geographical or time limits were applied to the searches. Initially the titles and abstracts of citations identified were reviewed for relevance to this project. For searches that resulted in large numbers of "hits", references were sorted using the "relevance" function and the titles and abstracts of the first 160 publications identified were reviewed. Non-English language studies were not included. Additional papers were accessed using the reference list of reviewed publications.

Publications identified using this approach were read in full and relevant details were organised into tables. For papers describing illness outbreaks, details collated included the produce type involved, country impacted, country of produce origin, number of cases implicated and the nature of the evidence gathered to link foods to the outbreaks (Table 4, Table 5 and Table 6). For papers describing prevalence studies, details collated included sampling locations, commodities tested, sampling country, number of samples analysed, number of positive samples and virus concentration ranges (Table 9, Table 10, Table 11 and Table 12).

Additional literature searches were also conducted using Google Scholar and PubMed to identify publications on four topics:

- Methods of analysis for produce
- Contamination pathways and sources for viruses in fresh produce
- Survival of viruses on produce
- Inactivation (death) of viruses following processing.

Format of the report

This report has been presented in the format of a risk assessment (RA), which is a structured approach used to ensure that sound science forms the basis of standards, guidelines and recommendations on food safety. The approach taken follows that recommended by Codex in "*Principles and Guidelines for the Conduct of Microbiological Risk Assessment*" (Codex Alimentarius Commission Guidelines 30 [CAC/GL-30], 1999). Consistent with these guidelines, this report includes sections on

- Hazard identification (Section 5)
- Hazard characterisation (section 6)
- Exposure assessment (section 7)
- Risk characterisation (section 8)

Definitions for these and other core terms are provided in the Glossary.

5 Hazard identification

Most viruses are small in size, around 20 to 400 nm, and require living cells to replicate (reproduce themselves), thus do not multiply in the foods of concern (Anonymous, 2008). Their structures are diverse, with many viruses simply comprising a non-enveloped protein coat and a genome (genetic material). Viruses with protein coats that are enveloped by a membrane, for example the influenza or "flu" virus, tend to be more sensitive to heat, acids and drying, than non-enveloped viruses (for example, NoV). Some viruses have single-stranded RNA genomes (for example, NoV and HAV). Others have double-stranded RNA genomes (for example, rotaviruses) or DNA genomes (for example, adenoviruses).

Notably, the foodborne viruses are predominantly non-enveloped viruses and thus are relatively hardy in comparison with bacteria. They can persist for long periods in the environment and in foods, and are more resistant to common processing methods, for example pasteurisation or heat treatment.

Viruses are transmitted from person to person in various ways, including inhalation of aerosolised viral particles (that is, microscopic particles suspended in the air), sexual intercourse, contact with blood particles and from infected animals ("zoonotic transmission", as in the case of hepatitis E Virus [HEV]). Enteric (intestinal) viruses can also be transmitted through the faecal–oral route. They trigger a variety of illnesses in humans, including paralysis (from poliovirus), myocarditis (inflammation of the heart muscle, for example from Coxsackievirus) and – more frequently – gastroenteritis, for example from NoV, rotavirus (RV), sapovirus, astrovirus, adenoviruses (AdV), or hepatitis A or E. Most viral illness outbreaks are caused by person–to–person transmission, with estimates of the proportion of illnesses attributed to food transmission varying.

Viruses of concern

Given the apparent global increase in foodborne viral outbreaks, in 2007 the World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations (FAO) convened an expert meeting to provide advice on food commodities of concern. The meeting identified viruses commonly causing foodborne illness as

- Norvirus (NoV)
- Hepatitis A virus (HAV)
- Group A human rotavirus (RV).

Emerging viruses of concern were identified as

- Hepatitis E virus (HEV)
- Nipah virus (NiV)

- Highly pathogenic avian influenza virus (HPAI) (bird flu)
- Severe acute respiratory syndrome–causing coronavirus (SARS–CoV).

Food commodity information was also reviewed and several combinations were considered to be of higher risk, including NoV and HAV in fresh produce and in shellfish (Anonymous, 2008).

In 2007, mandatory EU-wide reporting of foodborne outbreaks to the EFSA was initiated. In the EU in 2014 there were 5,251 foodborne illness outbreaks (both weak and strong evidence outbreaks) reported by 26 member states. Foodborne viruses were implicated in 1,072 of the outbreaks, involving 11,740 cases, 2,486 hospitalisations and two deaths. Norovirus was the most commonly implicated virus, accounting for 97.6% of the illness cases. Hepatitis A virus was the next most commonly implicated virus, and was responsible for 1.8% of cases. Flavivirus (FV) was attributed to 16 cases and rotavirus to seven cases (EFSA, 2015).

Another source of information on viruses of concern is the EU Rapid Alert System for Food and Feed (RASFF), which contains notifications from countries on food safety issues. From 1998 to August 2016 there were 245 alert notifications involving foodborne viruses, of which 81% involved NoV (or suspected NoV) and 19% involved HAV*.

Collectively this information shows that while a variety of viruses have been linked to foodborne illness outbreaks, only a few are regularly implicated in illnesses, namely NoV and HAV (Table 1), and this review therefore principally focusses on risk related to NoV and HAV in fresh produce. There is limited evidence of risk from produce and the so-called “emerging viruses” such as RV, FV, HEV, SARS and avian influenza (Table 1) which are either rarely implicated or have not been implicated in foodborne illness to date. While these viruses are not the focus of this review, it is important for risk managers to be aware of the potential for emergent issues relating to their presence in the food supply chain. The potential risk from these viruses is discussed further by the EFSA (2011).

* Estimate based on data accessed from RASSF portal in September 2016: http://ec.europa.eu/food/safety/rasff_en)

Table 1: Viruses transmitted by food; adapted from Le Guyader et al. (2012)

Family	Genus	Common name (examples)	Type of illness	Frequency of food transmission	Foods normally involved
Adenoviridae	*Adenovirus*	Types 40–41	Gastroenteritis (inflammation of the stomach and small intestine)	Rare	
Astroviridae	*Astrovirus*		Gastroenteritis	Rare	
Caliciviridae	*Norovirus*		Gastroenteritis	Frequent	Shellfish and fresh produce
	Sapovirus		Gastroenteritis	Uncommon	Shellfish
Coronaviridae	*Coronavirus*	SARS	Common cold, pneumonia (inflammation of air sacs in the lungs), enteric (intestinal) disease	Suspected to be zoonotic (able to pass from animals to humans)	
Flaviviridae	*Flavivirus*		Fever, vomiting, fatigue, pain in neck and back, encephalitis (inflammation of the brain)	Rare	Cow, sheep and goat milk
Hepeviridae	*Hepevirus*	Hepatitis E virus	Hepatitis (liver disease)	Uncommon	Pig meat
Orthomyxiviridae	*Influenza A*	H5N1	Influenza (“flu”)	Unreported	Bird meat
Paramyxoviridae	*Henipavirus*	Nipah virus	Influenza-like illness, febrile encephalitis (inflammation of the brain caused by a high fever)	Rare	
Picornaviridae	*Kobuvirus*	Aichi virus	Gastroenteritis	Uncommon	Shellfish
	Enterovirus		Diverse syndromes	Uncommon	Shellfish
	Hepatovirus	Hepatitis A virus	Hepatitis	Frequent	Shellfish and fresh produce
Reoviridae	*Rotavirus*		Gastroenteritis	Rare	

Blue shading indicates viruses frequently implicated in foodborne outbreaks.

Norovirus

"Norovirus" is a genus (a subfamily) within the family Caliciviridae (Table 1), and they comprise a group of viruses that primarily cause gastroenteritis. In Ireland there are between 1,000 and 2,000 notifications (outbreaks and cases) of NoV disease annually (Health Protection Surveillance Centre [HPSC], 2016). This probably represents a small fraction of the actual cases, as under-reporting is widely acknowledged, with one UK estimate showing 288 cases in the community for every case reported (Tam et al., 2012b). In the UK (including Northern Ireland), NoV is the most common cause of infectious intestinal disease and is estimated to be responsible for three million cases annually (Tam et al., 2012a; Tam et al., 2012b).

Transmission of NoV mostly occurs directly from person to person, with estimates of foodborne spread varying – one recent study suggests that around 14% of all outbreaks are attributed to food (Verhoef et al., 2015). In 2004–2005 a prospective study on NoV outbreaks in Ireland and NI was undertaken; it showed that of 152 outbreaks in Ireland, 99.3% involved person–to–person transmission, and 0.7% involved foodborne spread. Similarly, in NI over the same period 109 of 110 outbreaks were attributed to person–to–person spread. However, identifying foodborne outbreaks is not always easy and this may explain why some variability is observed between countries in the proportion of outbreaks attributed to foods (Verhoef et al., 2015). Identification of contaminated foods as a cause of outbreaks is particularly challenging when consumers share meals, and is made even more difficult by a lack of analytical capability in some countries.

Noroviruses are divided into seven genogroups (GI to GVII) based on variations in the capsid (the cell's shell) proteins. Noroviruses infect a variety of animals, including humans (genogroups GI, GII and GIV), pigs (GII), cattle and sheep (GIII), dogs (GIV, GVI and GVII) and mice (GV) (de Graaf et al., 2016). While different strains have been detected in various animals, and despite the high tendency for recombination shown for human NoV (meaning that genes exchanged by "parent" viruses result in genetically different or diverse "offspring", or progeny), zoonotic transmission has not yet been identified (although some human NoVs have been detected in animal faeces).

Genogroups are further divided into genotypes, of which there are over 40. GII.4 strains are the cause of recent global epidemics and are responsible for most outbreaks in the community. However, a new emerging strain, GII.17, seems to be becoming more prevalent throughout the world (Zhang et al., 2015). Of interest, there is an unexpectedly high proportion of GI strains responsible for shellfish-related outbreaks of NoV. This is considered to be related to specific ligands (molecules that bind to other molecules) present in oyster tissues that facilitate accumulation and retention of these strains (Le Guyader et al., 2012). More generally, a higher proportion of GI strains are transmitted through food and water, when compared with general community outbreaks (de Graaf et al., 2016; Lysén et al., 2009; Verhoef et al., 2015).

Noroviruses are small (27 to 32 nm), non-enveloped, icosahedral-shaped (twenty-sided) particles that are assembled from 90 dimers (molecules that are made up of two other identical, simpler molecules) of

capsid viral protein 1 (VP1) and several copies of VP2. The "P2" subdomain of VPI is variable; this is the region that is responsible for ligand and antibody recognition and presumably for receptor binding. ("Receptor binding" is a process in which certain protein molecules – receptor cells – receive a chemical signal that causes a change or response in the cell.) The genome consists of single-stranded, positive-sense RNA (meaning it can be translated into protein in the host cell) that is around 7.6 kbases in length.

Noroviruses are very robust, persist for long periods in the environment and are resistant to many common food production processes. They are among the most infectious pathogens (just a few particles may induce pathogenesis, or disease) and they are the first viral agents for which a "genetic sensitivity" has been demonstrated. Indeed, for infection to occur the virus needs to bind to particular polymorphic glycans (polysaccharides) of the histo-blood group type and, due to their genetic diversity, they can infect all humans (Le Pendu et al., 2014).

Norovirus was not able to be cultured for a long time. Recently a culture method based on enteroids – mini organs, or "organoids", created in the laboratory from intestinal cells – has been developed but this cannot be used routinely yet. Therefore, NoVs are detected in foods using genomic detection methods (Section 7 Detection methods). The lack of a culture method has meant that studies on food processing techniques that aim to inactivate NoV generally use culturable surrogate viruses such as murine (mouse) NoV, feline (cat) calicivirus (FCV) and Tulane virus (TV) to estimate infectivity.

Hepatitis A virus

Hepatitis A virus belongs to the genus Hepatovirus, which is within the Picornaviridae family (Table 1). Person–to–person transmission of HAV is the most common vehicle; however, foodborne infections do occur periodically. Hepatitis A occurrence in Ireland has declined from 16 per 100,000 people in 1989, to 0.77 per 100,000 in 2015 (HPSC, 2016); and "*from 2004 to the end of 2012 no foodborne outbreaks due to HAV were reported in Ireland* (reviewed in Fitzgerald et al. [2014])."

Hepatitis A outbreaks related to food are less common than norovirus (EFSA, 2015). This may be partially related to patchy global prevalence of HAV. Hepatitis A is endemic (commonly occurs) in under-developed countries. Infections result in life-long immunity; generally children become infected early in life and therefore serious infections in adults are rare. In contrast, in developed countries such as the IOI, HAV prevalence is low due to hygiene practices of a relatively higher standard, leaving adults more susceptible to infection. Vaccination may also reduce viral shedding (the release of the next generation of viruses) and subsequent contamination and infections (Chironna et al., 2012).

While there is only a single serotype, or strain, of HAV (indicating low antigenic variability), the genome is variable with several genotypes and subgenotypes. The VPIX2A junction of the genome is widely used for genotyping and using this region results in six genotypes. Genotypes I, II, and III infect humans but the others are of simian (monkey or ape) origin. Genotypes I and II contain two subgenotypes each. Different genotypes are prevalent throughout the world, thus sequencing the genome can help to

identify the source or country from which the virus originates. ("Sequencing" means identifying the exact order of the nucleotides – the building blocks of nucleic acid – in a DNA molecule.)

Similar to NoV, HAV has a single-stranded RNA genome of around 7.4 kbases and is of icosahedral shape with a diameter of around 27 to 32 nm. It is also considered to be very stable and persistent in the environment and can resist standard processes applied to foods to inactivate commonly found bacteria. Current advice for foods at risk of HAV contamination (such as oysters from polluted areas, or berries imported from HAV-endemic countries) is for heat treatment that exceeds 90 °C for 90 seconds or more.

Again similar to NoV, HAV is commonly detected in foods using PCR-based methods (Section 7 Detection methods). Some HAV strains have been isolated and shown to produce cytopathic effects (structural changes in host cells, caused by infection) in monkey kidney cell lines, which allows detection of some strains using quantitative plaque assays (Cromeans et al., 1987; Nasser and Metcalf, 1987). (Plaque assays are used to measure the concentration of a virus present, highly accurately.) This has enabled evaluations of the infectivity of HAV following certain food processing treatments to be undertaken, including heating, high pressure processing and so on (Kingsley et al., 2005).

Fresh produce types of potential concern

Table 2 and Table 3 show the volume (tonnes) of fresh fruit and vegetable commodities produced on the IOI and imported in 2015. The data presented probably underestimates the amount of produce available for consumption because imports into and exports from NI are not included, as it was not possible to source these data.

Regarding fruits (Table 2) a significant quantity of apples is produced on the IOI (mainly NI), along with a variety of different berries, particularly blueberries and strawberries. Most other fruits, particularly tropical fruits such as melons, mangoes and pineapples, are imported from both EU and non-EU countries. Most vegetables consumed (Table 3) are produced on the IOI; however, there is also a variety of imports, with large quantities of potatoes and tomatoes imported.

This data shows the type and volume of fresh produce available for consumption on the IOI and thus represents the list of produce that could potentially act as vectors (means of transport) for foodborne viruses. Sections 6 and 7 provide details on produce commodities that have been linked to virus outbreaks and in which viruses have been detected.

Table 2: Volume of fresh fruit commodities produced in and imported (minus exports) into the island of Ireland in 2015

Fruit type	Tonnes produced
Apples	53,239
Soft fruits[b]	13,457
Blueberries	9,615
Strawberries	7,166
Raspberries	311
Rhubarb	297
Blackcurrants	224
Blackberries	30
Fruit type	Tonnes imported[a] (minus exports)
Bananas	79,426
Citrus fruits[c]	59,117
Apples	56,159
Pears and quinces	45,294
Grapes	17,606
Melons	14,985
Soft fruits[d]	9,632
Avocadoes and mangoes	6,492
Frozen berries[e]	4,349
Fresh berries[f]	3,087
Pineapples	2,162
Coconuts	1,098
Dates	323
Figs	225

Sources: Department of Agriculture, Food and the Marine (ROI); Department of Agriculture and Rural Development (NI); Central Statistics Office (ROI); Anonymous (2016)

[a]Volumes of fruit imports (minus exports) into Northern Ireland are not included, as HM Revenue and Customs declined to provide the statistics under section 21 of the Freedom of Information Act

[b]"Soft fruits" here includes cranberries, gooseberries, loganberries and tayberries

[c]"Citrus fruits" includes grapefruits, lemons, limes, mandarins and oranges

[d]"Soft fruits" here includes apricots, cherries, peaches and plums

[e]"Frozen berries" includes blackberries, blackcurrants, raspberries and strawberries

[f]"Fresh berries" includes blackberries, raspberries and strawberries

Table 3: Volume of fresh vegetable commodities available for consumption (production plus imports[a] minus exports) on the island of Ireland in 2015

Vegetable type	Tonnes available for consumption on the IOI	Production	Imports	Exports
Potatoes	517,292	347,700	179,237	9,645
Herbs	176,000	176,000		
Carrots and turnips	112,284	79,102	34,235	1,053
Brassicas[b]	66,387	41,910	25,069	592
Tomatoes	51,589	4,427	48,242	1,080
Mushrooms	43,425	72,213	3,767	32,555
Swedes	19,570	19,570		
Peas	12,851		13,005	154
Parsnips	12,478	12,478		
Garlic and leeks	8,823	4,912	4,023	112
Cucumbers	8,019	1,832	6,297	110
Lettuce	6,930	7,369	446	885
Sweet potatoes	4,964		4,970	6
Onions and shallots	4,250	3,695	2,647	2,092
Beans	4,238		4,311	73
Sweetcorn	3,414		3,509	95
Celery	2,623	2,623		
Scallions (spring or green onions)	857	857		
Spinach and kale	725	725		
Parsley	282	282		
Courgettes	191	191		

Sources: Department of Agriculture, Food and the Marine (Ireland); Department of Agriculture and Rural Development (NI);

Central Statistics Office (Ireland) (Anonymous, 2016)

[a]Volumes of vegetable imports into and exports from Northern Ireland are not included, as HM Revenue and Customs declined to provide the statistics under section 21 of the Freedom of Information Act

[b]"Brassicas" includes broccoli, Brussels sprouts, cabbage, calabrese and cauliflower

6 Hazard characterisation

Norovirus

Norovirus, also known as “winter vomiting disease”, causes acute gastroenteritis. The incubation period is between 12 and 72 hours and illnesses typically last for two or three days. Diarrhoea is the most common symptom, along with vomiting, abdominal cramps, fever, watery diarrhoea, headaches, chills and myalgia (muscle pain) (de Graaf et al., 2016). Projectile vomiting is common and is hypothesised to contribute to transmission of the virus through aerosolisation and general environmental dispersal. Large quantities of virus are also excreted in stools, with around 10^8 genome copies per gram of faeces, and up to 10^{11} in some cases (Atmar et al., 2008). Excretion of virus in the faeces continues after symptoms subside for up to three or four weeks, further contributing to the spread of the virus.

Histo-blood group antigens (HBGAs) are carbohydrate molecules (“glycans”) located on a variety of gastrointestinal, blood and epithelial (lining) cells, and also in saliva. Histo-blood group antigens play a role as receptors of many different bacteria and viruses, and prior to infection NoV binds to these glycans (Le Pendu et al, 2014). Certain strains of NoV bind to particular HBGAs, and variations in the expression of HBGAs between humans results in host susceptibility towards particular strains (Tan et al., 2009). In other words, the same strain of NoV cannot infect everybody but everybody is susceptible to at least one strain.

Immunity to NoV following infection was initially considered to be short term (less than two years); however, recent studies suggest that protection may be longer term (four to eight years) than the early studies indicated (Simmons et al., 2013). Immunity may play a role in reducing the severity of disease, the duration of viral shedding and transmission (Lopman et al., 2016).

The infectious dose of NoV is very low. The median infectious dose (ID_{50}) of a GI.1 strain has been evaluated in two studies, which found that it was: (1) between 18 and 1,015 genome copies (Teunis et al., 2008); and (2) approximately 1,320 genome copies for secretor-positive persons who were blood type O or A (Atmar et al., 2014). (“Secretors” leak their blood-type antigens into body fluids such as saliva and semen; “non-secretors” have a gene that blocks this process.) The same low infectious dose has also been shown for some GII strains (Kirby et al., 2014). Thebault et al. (2013) estimated the ID_{50} for both GI and GII NoV in oysters, and values ranged between 1.60 and 7.51 genome copies per oyster consumed. The low infectious dose of NoV, coupled with the large quantities of virus shed in faeces and its high resistance to sewage treatment (Sano et al., 2016), explains its high prevalence in the community and transmission through food.

Hepatitis A virus

Hepatitis A virus causes an acute illness that, similar to other types of hepatitis, is typified by fever, malaise (general discomfort), anorexia (loss of appetite), nausea, abdominal discomfort, dark urine and jaundice (yellowing of the skin or eyes). The incubation period is between 15 and 50 days, and illness usually lasts less than two months. The disease is more severe in adults than young children, making outbreaks more problematic in developed countries with non-immune adults (Pintó et al., 2010).

Viral replication occurs in the liver. Virions (complete infectious viruses, free of host cells) reach the gastrointestinal tract in bile and are then excreted in faeces (Cuthbert, 2001). The main transmission route is faecal–oral; however, other forms of transmission can occur, including by the parental route and through sexual practices (Pintó et al., 2010). Up to 10^{11} genome copies/g faeces have been detected in patients who have shed the virus for up to six weeks (EFSA, 2011). Thus, food primarily becomes contaminated with HAV through poor hygienic practices. Both symptomatic and asymptomatic (symptomless) carriers shed virus and, of high concern to food production, excretion of virus begins before the onset of symptoms. The infectivity of HAV is not known but has been assumed to be around 10 to 100 virus particles (EFSA, 2011).

Epidemiological data

Fresh produce was responsible for a significant proportion of the NoV foodborne outbreaks in the EU, with 14.5% and 6.6% of outbreaks related to vegetable and fruit consumption, respectively (EFSA, 2015). Fruits and vegetables were also implicated in 70 (29%) of the 245 RASFF alert notifications pertaining to viruses between 1998 and 2016. Produce types implicated included raspberries, blackberries, blueberries, strawberries, berry and fruit mixes, dates and lettuce[2].

Callejón et al. (2015) undertook a review to identify foodborne outbreaks in the USA and EU between 2004 and 2012 in which fresh fruit and vegetables were implicated. In addition to the scientific literature, epidemiological databases in the USA and EU were scrutinised. Norovirus was found to be the main pathogen responsible for outbreaks in both the US (59%) and EU (53%). Outbreaks in the US were predominantly associated with consumption of salads and NoV outbreaks in the EU were mainly linked to berries (of which a significant proportion were noted to be caused by raspberries).

An overview of published NoV and HAV outbreaks that have been associated with various types of fresh produce is provided here, including the number of cases, country involved and level of evidence gathered during outbreak investigations.

[2]Data accessed from the RASFF portal: http://ec.europa.eu/food/safety/rasff_en

International produce outbreaks

Table 4, Table 5 and

Table 6 show outbreaks published in the scientific literature that have been linked to vegetables, berries and “fruit other than berries”, respectively. The strength of evidence linking foods to illness outbreaks varies between investigations and does not always unarguably prove that the food is the cause of the outbreak. For EU member states, the EFSA requires that foodborne outbreaks are categorised into two groups: those that have “strong evidence” implicating a food vehicle, and those supported by “weak evidence”.

Evidence linking foods to outbreaks includes epidemiological, microbiological, environmental and product tracing investigations. Strong epidemiological evidence implies a statistically significant association between illness cases and food, and strong microbiological evidence involves identification of the causative agent (the virus or strain of virus) in cases and in the food in question (EFSA, 2016a). To assist in interpreting the certainty with which a food was linked to an illness outbreak, Table 4, Table 5 and

Table 6 also summarise the nature of evidence that was gathered during the investigation.

Regarding outbreaks linked to vegetables (Table 4), HAV has caused illnesses in the USA from the consumption of scallions (green or spring onions) imported from Mexico on a number of occasions. The evidence linking scallions with the outbreaks was relatively strong for two of the outbreaks (Amon et al., 2005; Wheeler et al., 2005). Sun-dried (semi-dried) tomatoes were implicated in multiple outbreaks of HAV in Australia, France, the Netherlands and the UK. The number of cases impacted ranged from seven to 562 per outbreak, and the underpinning evidence linking cases to tomatoes was strong in several of the outbreaks (Donnan et al., 2012; Gallot et al., 2011). Lettuce (salad) is linked to NoV and HAV illnesses in a variety of European countries and the USA. The supporting evidence was strong in several of these outbreaks, including a recent outbreak of NoV involving lettuce exported from France to Denmark that caused illness in 412 consumers (Muller et al., 2016a; Muller et al., 2016b).

Celery has rarely been linked to viral outbreaks (Pebody et al., 1998; Sivapalasingam et al., 2004; Warner et al., 1991). However, one instance of celery contamination occurred during meal preparation due to sewage contamination in the kitchen (Warner et al., 1991); it did not involve contamination during primary production. Carrots were reported to be implicated in one outbreak in a review paper but no details on the outbreak are available; however, it was noted that the carrots were served alongside celery (Sivapalasingam et al., 2004).

Global outbreaks of both NoV and HAV have been associated with a variety of different berry types (Table 5). The majority of reported outbreaks have involved frozen berries (18 of 22 outbreaks). Raspberries were implicated in over 50 per cent of the outbreaks, while the next most common berry type implicated was strawberries (3 of 22 outbreaks). In some instances mixed berries were linked to outbreaks (Montaño-

Remacha et al., 2014; Rizzo et al., 2013), and in one outbreak berry cake mix was the suspected vector (Guzman-Herrador et al., 2014; Guzman-Herrador et al., 2015). Berry outbreaks affected consumers in North America, Europe and Australasia, with berries originating from a number of countries including China, Poland, Serbia, Bulgaria, Bosnia and Mexico. The number of cases implicated ranged between five and 11,000 per outbreak.

Several other types of fruit produce have also been linked to viral outbreaks (

Table 6), including pomegranate seeds, which were associated with HAV outbreaks in Canada and the USA. The level of evidence described (which included descriptive epidemiology, product tracing and, in one outbreak, HAV being detected in pomegranates) indicated that the association between illness and pomegranate consumption was strong (Collier et al., 2014; Swinkels et al., 2014). Multiple outbreaks (more than seven cases) of NoV attributed to cantaloupe and watermelon consumption in the USA are described in two review papers (Bowen et al., 2006; Walsh et al., 2014); underpinning epidemiological evidence was not described. An HAV outbreak involving 351 cases occurred in Egypt and was strongly linked to the consumption of fresh orange juice (Frank et al., 2007). Several other outbreaks have involved orange juice, and in one instance fresh grapefruit was implicated (Eisenstein et al., 1963; Hooper et al., 1977).

The source of contamination for citrus fruit or fruit juice outbreaks appears to be food handlers at the point of sale or processing, rather than during growth or harvesting. Other fruits, such as grapes, mangoes, avocadoes and pineapples, have also been suspected vectors (

Table 6) but the level of evidence provided is relatively weak (epidemiology only), with no details available on the pineapple and avocado outbreaks (reviewed in Strawn et al. [2011]).

All outbreaks reported have occurred primarily in North America, Europe and Australasia. There are differences in the national processes and systems in identifying foodborne outbreaks; this may lead to significant bias in reporting globally. Additionally, only a small proportion of outbreaks identified would likely be published in the scientific literature. Coupled with general under-reporting, it is therefore likely that outbreaks presented in Table 4, Table 5 and

Table 6 only represent a fraction of the global incidents.

Produce outbreaks on the island of Ireland

Food has been reported to contribute to around one per cent of NoV outbreaks in Ireland and NI between 2004 and 2014 (P. Garvey, presentation at *safe*food seminar 2015; Kelly et al., 2008).

The review of the literature for NoV and HAV outbreaks related to fresh produce on the IOI (Table 5) only revealed one outbreak: the 2013 outbreak in which 21 people contracted HAV from frozen imported berries (Fitzgerald et al., 2014). The HAV strain involved was identical to other HAV outbreaks that happened at the same time in the EU, involving around 1,400 cases (EFSA, 2014d).

Under-reporting of foodborne outbreaks is an issue. For NoV, the duration of illness is relatively short – roughly 24 hours – and many people do not present themselves to medical practitioners. Generally, large outbreaks that occur in institutions are more likely to be reported and investigated, whereas sporadic (more isolated or scattered) cases may not be captured in the statistics. For HAV, the incubation period is

relatively long – around one month – and it is common that food vehicles are not identified due to the difficulty of remembering meals consumed over this time period, which also contributes to under-reporting.

There is a lack of laboratory capability regarding detection of NoV and HAV in fresh produce on the IOI (the Marine Institute have the capability to test NoV and HAV in shellfish). This makes it difficult to collate evidence implicating foods in outbreaks, and possibly also contributes to under-reporting.

Table 4: Published outbreaks of norovirus and hepatitis A virus associated with vegetables, 1988–2016

Virus	Produce type	Year	Number of cases reported	Country affected	Country of food origin	Nature of evidence	Source
HAV	Green onions (scallions **or** spring onions)	1998	43	USA	Mexico or USA	Analytical epidemiology evidence	Dentinger et al. (2001)
HAV	Green onions	2003	601	USA (Pennsylvania)	Mexico	Analytical and descriptive epidemiology evidence Product tracing investigations	Anonymous (2003) Wheeler et al. (2005)
HAV	Green onions	2003	422	USA (Tennessee, North Carolina and Georgia)	Mexico	Analytical and descriptive epidemiology evidence Product tracing investigations	Amon et al. (2005)
HAV	Semi-dried tomatoes	2009	562	Australia	nr	Analytical and descriptive epidemiology evidence Product tracing investigations Microbiological evidence	Donnan et al. (2012)
HAV	Semi-dried tomatoes	2009–2010	13	Netherlands	nr	Descriptive epidemiology evidence Analytical epidemiology evidence	Chi et al. (2014) Petrignani et al. (2010a) Petrignani et al. (2010b)
HAV	Semi-dried tomatoes	2010	59	France	Turkey	Analytical epidemiology evidence Product tracing investigations	Gallot et al. (2011)
HAV	Semi-dried tomatoes	2011	7	UK	nr	Descriptive epidemiology evidence	Carvalho et al. (2012)
HAV	Semi-dried tomatoes	2011	8	Netherlands	nr	Descriptive epidemiology evidence	Fournet et al. (2012)

Virus	Produce type	Year	Number of cases reported	Country affected	Country of food origin	Nature of evidence	Source
HAV	Lettuce	1988	202	USA	USA or Mexico	Analytical epidemiology evidence	Rosenblum et al. (1990)
NoV	Celery	1988	1,002	USA	nr	Analytical epidemiology evidence Microbiological evidence	Warner et al. (1991)
HAV	Salad (possibly celery)	1996	30	Finland	nr	Analytical epidemiology evidence Microbiological evidence	Pebody et al. (1998)
HAV	Salad	1996–1997	30	Finland	nr	Analytical epidemiology evidence	Pebody et al. (1998)
HAV	Lettuce	2000–2001	54	Sweden	nr	Analytical epidemiology evidence	Nygård et al. (2001)
NoV	Lettuce (and/or soup)	2008–2009	19	Portugal	nr	Descriptive epidemiology evidence	Mesquita and Nascimento (2009)
NoV	Lettuce	2010	260	Denmark	France	Analytical and descriptive epidemiology evidence Microbiological evidence	Ethelberg et al. (2010)
NoV	Lettuce	2016	412	Denmark	France	Analytical epidemiology evidence Microbiological evidence	Muller et al. (2016a) Muller et al. (2016b)

nr = not reported

Table 5: Published outbreaks of norvirus and hepatitis A virus associated with berries, 1983–2016

Virus	Produce type	Year	Number of cases reported	Country affected	Country of food origin	Nature of evidence	Source
HAV	Frozen raspberries	1983	24	Scotland	Scotland	Descriptive epidemiology evidence	Reid and Robinson (1987)
HAV	Frozen raspberries	1988	5	Scotland	Scotland	Descriptive epidemiology evidence Product tracing investigations	Ramsay and Upton (1989)
NoV	Raspberries	1997	200	Canada	Bosnia	Analytical epidemiology evidence Microbiological evidence	Gaulin et al. (1998)
NoV	Frozen raspberries	1998	509	Finland	nr	Analytical epidemiology evidence	Pönkä et al. (1999a) Pönkä et al. (1999b)
HAV	Frozen strawberries	1990	28	USA	USA	Analytical epidemiology evidence	Niu et al. (1992)
HAV	Frozen strawberries	1997	258	USA	Mexico	Analytical epidemiology evidence Product tracing investigations	Hutin et al. (1999)
NoV	Frozen raspberries	2001	30	Sweden	nr	Microbiological evidence Descriptive epidemiology evidence	Le Guyader et al. (2004)
HAV	Blueberries	2002	81	New Zealand	New Zealand	Analytical epidemiology evidence Microbiological evidence	Calder et al. (2003)
NoV	Frozen raspberries	2005	1,000	Denmark	Poland	Descriptive and analytical epidemiology evidence	Korsager et al. (2005) Falkenhorst et al. (2005)
NoV	Frozen raspberries	2005	75	France	nr	Descriptive epidemiology evidence	Cotterelle et al. (2005)
NoV	Frozen blackberries	2005	241	Germany	nr	Analytical epidemiology evidence	Fell et al. (2007)
NoV	Raspberries	2006	43	Sweden	China	Descriptive epidemiology evidence Product tracing investigations	Hjertqvist et al. (2006)
NoV	Frozen raspberries	2009	200	Finland	Poland	Analytical epidemiology evidence Microbiological evidence	Maunula et al. (2009)
NoV	Frozen raspberries	2009	900	Finland	Poland	Analytical and descriptive epidemiology evidence Microbiological evidence	Sarvikivi et al. (2012)
NoV	Frozen raspberries	2010-2011	224	Denmark	Serbia	Analytical and descriptive epidemiology evidence Microbiological evidence	Muller et al. (2015)
NoV	Frozen strawberries	2012	11,000	Germany	China	Analytical epidemiology evidence Microbiological evidence	Bernard et al. (2014) Mäde et al. (2013)

Virus	Produce type	Year	Number of cases reported	Affected country	Country of food origin	Nature of evidence	Source
HAV[a]	Frozen berries	2013	21	Ireland	nr	Descriptive and analytical epidemiology evidence	Fitzgerald et al. (2014)
HAV[a]	Berry cake mix	2013–2014	33	Norway	nr	Descriptive epidemiology evidence Product tracing investigations Microbiological evidence	Guzman-Herrador et al. (2014) Guzman-Herrador et al. (2015)
[a]HAV	Frozen mixed berries	2013	352	Italy	Bulgaria, Canada, Poland, Serbia	Analytical epidemiology evidence Microbiological evidence	Montaño-Remacha et al. (2014) Rizzo et al. (2013)
[a]HAV	Frozen mixed berries (blackcurrants and redcurrants most common ingredients)	2013	1,589	EU multistate outbreak	Bulgaria and Poland suspected	Analytical and descriptive epidemiology evidence Microbiological evidence Product tracing investigations	Severi et al. (2015) Terio et al. (2015)
HAV	Frozen berries	2012–2013	103	Denmark, Finland, Norway and Sweden	nr	Descriptive and analytical epidemiology evidence	Lassen et al. (2013) Nordic Outbreak Investigation Team (2012)
NoV	Frozen raspberries	2013	85	Norway	nr	Analytical and descriptive epidemiology evidence	Einoder-Moreno et al. (2016)

nr = not reported

[a]Outbreaks part of common EU multistate outbreak of HAV

Table 6: Published outbreaks of norvirus and hepatitis A virus associated with fruits and other berries, 1973–2016

Virus	Produce type	Year	Number of cases reported	Country affected	Country of food origin	Nature of evidence	Source
HAV	Pomegranates	2013	165	USA	Turkey	Descriptive epidemiology evidence Product tracing investigations Microbiological evidence	Collier et al. (2014)
HAV	Pomegranates	2012	6	Canada	Egypt	Descriptive epidemiology evidence Product tracing investigations Microbiological evidence	Swinkels et al. (2014)
NoV	Cantaloupes	1973–2011	3[a]	USA	nr	nr	Walsh et al. (2014)
NoV	Cantaloupes	1999–2003	260[b]	USA	nr	nr	Bowen et al. (2006)
NoV	Watermelons	1973–2011	2[a]	USA	nr	nr	Walsh et al. (2014)
NoV	Grapes	2003	12	New Zealand	nr	Descriptive epidemiological evidence	Hill (2003)
HAV	Mangoes or strawberries	2012–2013	107	14 EU EFTA countries	Egypt	Analytical epidemiology evidence	Sane et al. (2015)
HAV	Avocadoes	2000	1[a]	USA	nr	nr	Strawn et al. (2011)
NoV	Avocadoes	2005–2006	4[a]	USA	nr	nr	Strawn et al. (2011)
HAV	Orange juice	2004	351	9 EU countries	Egypt	Descriptive and analytical epidemiology evidence	Frank et al. (2007) Frank et al. (2005)
NoV	Pineapples	1999 and 2001	2[a]	USA	nr	nr	Strawn et al. (2011)
HAV	Grapefruit	1974	133	USA	nr	Descriptive epidemiological evidence	Hooper et al. (1977)
HAV	Orange juice	1962	24	USA	nr	Descriptive and analytical epidemiology evidence	Eisenstein et al. (1963)

nr = not reported

[a]Number of outbreaks reported in review paper, number of cases not specified

[b]Number of cases in seven outbreaks

Viral risk from other food types

In the EU in 2014 NoV was the most commonly reported foodborne virus, accounting for 98% of viral outbreaks (EFSA, 2015). The distribution of NoV transmitted by various foods in the EU in 2014 is presented in Table 7. The most commonly implicated food vehicle group was "crustaceans, shellfish, molluscs and products thereof" (36.8%), followed by "mixed food" (22.4%), "vegetables and juices and other products thereof" (14.5%) and "buffet meals" (6.6%). Fresh produce (fruit and vegetables combined) accounted for 21.1% of the outbreaks. A small proportion of outbreaks were attributed to "meat" and "bakery products", for which contamination is likely to have occurred through food handlers. In contrast, the two commodities most frequently involved, shellfish and fresh produce, are generally consumed raw and contamination often occurs during primary production.

Table 7: Distribution of food vehicles in strong evidence outbreaks caused by norovirus in the EU, 2014; adapted from EFSA (2015)

Food vehicles	Percentage (%) of norovirus foodborne outbreaks
Meat	3.9
Bakery products	3.9
Other foods	5.3
Fruit and berries, fruit juices and other products thereof	6.6
Buffet meals	6.6
Vegetables, vegetable juices and other products thereof	14.5
Mixed food	22.4
Crustaceans, shellfish, molluscs and products thereof	36.8

Given the high proportion of viral outbreaks attributed to shellfish, significant focus has been placed on research and development initiatives for this food commodity. Analytical methods for detecting NoV and HAV in shellfish are well established and a standardised real-time RT–PCR method is available (Section 7 Detection methods). Prevalence studies on viruses in shellfish have been conducted in many countries around the world (Brake et al., 2014; Suffredini et al., 2008; Nishida et al., 2003; DePaola et al., 2007), and

viral occurrence is estimated to be high in some regions (Lowther et al., 2012; Constantini et al., 2006). These viral outbreaks and prevalence studies have prompted recommendations by EFSA that

"*... risk managers should consider establishing an acceptable limit for NoV in oysters to be harvested and placed on the market. NoV testing of oysters (standardized CEN method) should be used to verify compliance with the acceptable NoV limit established.*" (EFSA, 2012)

To ensure that suitable data are available to support development of a microbiological criterion for NoV, a survey is being conducted to determine the European prevalence of NoV-contaminated oysters in production areas and in batches of oysters at dispatch centres (EFSA, 2016b). The survey is being conducted over a two-year period between 2016 and 2018, after which the European Commission will consider the implementation of microbiological criteria for NoV in oysters.

7 Exposure assessment

Detection methods

1. A variety of methods have been developed for the detection of viruses in produce (Butot et al., 2007; Dubois et al., 2007; Fino and Kniel, 2008a; Guévremont et al., 2006; Pan et al., 2012; Papafragkou et al., 2008; Shan et al., 2005; Summa et al., 2012). The methods involve a similar series of steps:
    1. Collection of a representative sample
    2. Viral elution (separation by "washing" with solvent) from the matrix using a buffer (the "buffer" is the solvent)
    3. Viral concentration using a filtration- or precipitation-based approach
    4. Viral RNA extraction
    5. PCR-based detection. (No routine methods exist for culture of NoV or HAV).

While many methods have been developed, few have been used for routine surveillance, outbreak investigations or prevalence surveys. Table 8 provides an overview of selected methods that have been used in surveys of viral occurrence in produce.

Limited guidance exists regarding representative sampling strategies. Following a large-scale outbreak (approximately 11,000 cases) of NoV from frozen imported strawberries in Germany (Bernard et al., 2014), 11 samples were collected from the implicated lot of produce (with 28 subsamples analysed), which comprised 44 tons. Seven of the 11 samples were positive for NoV (Mäde et al., 2013). The authors noted that not all samples were positive, which could be related to low viral concentrations, the presence of PCR inhibitors in some berries, or heterogenous contamination (meaning it came from outside) of the lot. Sarvikivi et al. (2012) also found that only one of four raspberry samples from a batch implicated in a NoV outbreak was positive. Follow-up work demonstrated that the batch had originated from some 60 farms – if NoV contamination occurred at farm level the distribution within the batch would be very patchy due to the co-mingling of products.

These findings highlight the need to adopt appropriate sampling approaches, with suggestions that three subsamples (each analysed in duplicate) from each 10 kg box is considered reliable, and that analysing small numbers of samples may lead to false negative results (Mäde et al., 2013).

Analytical methods for the detection of foodborne viruses in produce have not yet been adopted on the IOI. Following the 2013 HAV outbreak related to frozen berries in Ireland, Fitzgerald et al. (2014) noted that "*the microbiological investigation was further complicated by limited food testing capability in Ireland*", which hampered the ability to microbiologically link outbreak cases to food samples. Indeed,

the lack of a validated standard method has historically limited regulatory and industry-based testing worldwide. In 2012, however, standard methods (ISO/TS 15216-1 and ISO/TS 15216-2) for the qualitative and quantitative detection of NoV and HAV in a variety of foods were published by the International Organization for Standardization (ISO) (ISO, 2012a, 2012b)

Table 8: Examples of methods used for viral recovery and detection in prevalence surveys of fruits and vegetables (Blue highlights show studies undertaken using the ISO/TS standard method for viral detection in soft fruits and salad vegetables)

Matrix	Elution	Concentration	Nucleic acid extraction	Polymerase chaine reaction (PCR)	Reference	Ref for PCR primers and probes
Variety of vegetables and fruits	3% beef extract, pH 8.5	Polyethylene glycol (PEG) precipitation	QIAamp® Viral RNA Mini Kit	Two-step RT–PCR	Allwood et al. (2004)	Vinje and Koopmans (1996)
Leafy greens and berries	Glycine 50mM, tris-HCl 100mM, 1% beef extract, pH 9 (pectinase included for berries)	PEG precipitation, chloroform/butanol	NucliSENS® miniMAG®	One-step real-time RT–PCR	Baert et al. (2011)	ISO (2012a, 2012b)
Raspberries, strawberries, tomatoes, cucumbers, leafy greens and fruit salads	Glycine 50mM, tris-HCl 100mM, 3% beef extract, pH 9.5, 150uL pectinix	Filtration, PEG precipitation, chloroform/butanol	QIAGEN® RNeasy® Mini Kit	Two-step real-time PCR	Baert et al. (2011) Stals et al. (2011)	ISO (2012a, 2012b)
Leafy greens	Phosphate-buffered saline (PBS)	Filtration (positive-charged Zeta Plus™), elution with 2.9% tryptose phosphate, 6% glycine, pH 9; concentration by Amicon® Ultra-15	QIAamp® Viral RNA Mini Kit	One-step real-time RT–PCR	Baert et al. (2011)	Kageyama et al. (2003)
Lettuce	Water	Ultracentrifugation	Not specified	RT–PCR	Hernández et al. (1997)	Hernández et al. (1997)
Onions	2.9% tryptose phosphate broth, 6% glycine, pH 9.0	Filtration (negatively-charged Millipore™ HA membrane filter) and elution with elution buffer	QIAGEN® RNeasy® Plant Mini Kit	One-step RT–PCR	Sahroni et al. (2011)	Trujillo et al. (2006)
Lettuce	Tris-glycine buffer, 1% beef extract, pH 9.5	PEG precipitation, chloroform/butanol	NucliSENS® miniMAG®	One-step real-time RT–PCR	Kokkinos et al. (2012)	Svraka et al. (2007) da Silva et al. (2007) Costafreda et al. (2006)
Raw vegetables	Glycine 50mM, tris 100mM, pH 9.5	PEG precipitation, centrifugation (Ultracel®-50K)	QIAamp® Viral RNA Mini Kit	Two-step RT–PCR and nested PCR	Cheong et al. (2009)	Kim et al. (2008)
Berries	Tris-glycine buffer, 1% beef extract, pH 9.5, 30 units pectinase	PEG precipitation, chloroform/butanol	QIAamp® Viral RNA Mini Kit	One-step RT–PCR and nested PCR	Terio et al. (2015)	Kingsley and Richards (2001) Normann et al. (1994) Robertson et al. (1992)
figs and dates	Tris-glycine buffer, 1% beef extract, pH 9.5	None	NucliSENS® miniMAG®	Two-step real-time RT–PCR	Boxman et al. (2012)	Costafreda et al. (2006)

The ISO/TS horizontal method involves various procedures for the liberation or elution of viruses from food surfaces, soft fruits and salad vegetables, bottled water and shellfish. For soft fruit and salad vegetables, the viral liberation procedure follows that published by Dubois et al. (2007) and involves elution using trisaminomethane (tris)- glycine buffer with 1% beef extract (pH 9.5) and polyethylene glycol (PEG) precipitation. Subsequently, a common RNA extraction method for all matrices is used, involving viral lysis (in which the cell walls are broken down) using guanidium thiocyanate (which denatures the protein) and then adsorption (in effect, adhesion) of RNA to silica. Separate real-time RT–PCR assays are then conducted for NoV GI, NoV GII and HAV using fluorescently labelled hydrolysis probes.

A large number of positive and negative controls are included at various steps of the method. These include process controls to ensure adequate recovery of the virus during extraction, and inhibition controls to check for potential matrix suppression effects. If the quantitative method is used, a series of controls and calibration curves based on quantified plasmid DNA (small DNA molecules inside cells but that are separate from the cell's own DNA) are incorporated and results can be reported as viral genome copies per gram of matrix.

There is a need for independently produced reliable nucleic acid controls to improve the concordance, or ease of cross-referencing, of results between separate laboratories. The ISO/TS method has been used in some of the recent prevalence surveys conducted on produce (Baert et al., 2011; Kokkinos et al., 2012; Terio et al., 2015). Its availability will result in further adoption of NoV and HAV testing of produce for general surveillance purposes and following outbreaks.

A drawback of PCR-based methods of detection is that they do not provide direct information on the infectivity of viruses that are detected and, because of this, it is difficult to predict whether a sample returning a positive result in a PCR assay poses a risk to human health. Knight et al. (2013) reviewed approaches for differentiating between infectious and non-infectious particles. Some additional steps to demonstrate capsid integrity (whether the cell wall is intact and the virus therefore active, or not) have been suggested. These include: the use of pig mucin (secretions from the stomach or intestines, for example, or saliva) to capture NoV (Tian et al., 2008a); pre-treatment of samples with ribonuclease (Rnase) or heat to degrade RNA from non-intact particles; and the use of propidium monoazide (PMA) treatments (a dye that preferentially binds to DNA exposed in dead cells, and so reveals them as inactive), as successfully demonstrated on HAV submitted to thermal (heat-based) inactivation (Sánchez et al., 2012). Such approaches hold promise for detection of infectious particles alone. However, their use as adjuncts (additions or supplements) to the standard ISO method is uncertain and would likely add significant cost (Knight et al., 2013).

Several approaches for the propagation of human NoV have recently been published, including one involving culture in B cells (white blood cells that secrete antibodies) with the aid of *Enterobacter cloacae* expressing HBGAs (Jones et al., 2014); however, this is yet to be reproduced in different laboratories. An assay using stem cell–derived human enteroids seems promising, as it allows the replication of several NoV strains, including both genogroups, and has been reproduced in at least five different laboratories (Estes, personal communication, 2016) (Ettayebi et al., 2016). This method will be useful for research investigating the infectivity

of NoV after various treatments, such as heat treatment, but it is unlikely to be amenable to routine use in a food testing laboratory (Ettayebi et al., 2016).

A useful adjunct for epidemiology-based investigations is the ability to accurately genotype viruses in both foods and clinical specimens; this allows confirmation of the same pathogen in patients and the suspected vector (the supposed source or means of transmission of the virus). Typically, this is performed by sequencing a genomic fragment, which confirms the presence of virus in a sample and provides information on the genotype. The ISO/TS PCR methods for NoV and HAV detection do not include standard procedures for sequencing of PCR-positive results (a "PCR-positive" result indicates a virus has been detected); because of this, sequencing protocols used by clinical and food laboratories can vary, including the genome regions targeted.

However, as sequence information derived from small genome regions may be misleading, particularly for new virus variants or recombinant strains (where viral "offspring" or progeny differ from their "parent" virus), some guidance on appropriate genoyping regions has been proposed to allow comparisons (Kroneman et al., 2013). Whole genome sequencing may be the preferred approach for the future (Fitzgerald et al., 2014).

Contamination pathways

Contamination of fresh produce with foodborne viruses can occur at any point in the supply chain, from production and processing through to point of sale. Hepatitis A virus and NoV are excreted in faeces; produce is susceptible to contamination if it comes into contact with human faeces or vomit, either directly or indirectly through contact with contaminated fomites. ("Fomites" are things that are likely to carry infection, such as surfaces, utensils and so on.) Recently a comprehensive review was undertaken to evaluate the fate of foodborne viruses in the supply chain for fresh produce that encompassed an appraisal of the contamination sources (Li et al., 2015), including

- Planting of virus-contaminated seeds
- Use of contaminated soil during production
- Use of contaminated water during production and processing
- Transmission by food handlers during harvesting
- Transmission during post-harvest handling
- Transmission by food handlers and consumers at point of sale.

Transmission through seeds and soil have not yet been specifically linked to foodborne outbreaks (Li et al., 2015); however, laboratory-based studies have shown that HAV and NoV surrogates remain infectious on alfalfa seeds for 50 days following germination (Wang et al., 2013). This suggests a need to ensure that germinating seeds are also subject to appropriate controls to prevent viral contamination. Soil used as a substrate (a growing medium) for produce could become contaminated through the inappropriate release of sewage into produce cultivation sites. While contaminated soil has not yet been attributed to a specific outbreak, Wei et al. (2010)

have demonstrated lettuce contamination following application of sludge and manure. Particular caution needs to be taken with the use of human excreta as fertilisers. Treatments should ensure adequate viral reductions.

The use of contaminated water during both production and processing represents a significant risk as it is used throughout the supply chain; because of this, water quality is considered a critical control point. Li et al. (2015) notes that the use of contaminated irrigation water has been linked to an HAV outbreak connected with frozen strawberries. In addition, irrigation water from a river was shown to be responsible for transferring a variety of viruses (including NoV) to strawberries in a field study (Brassard et al., 2012). Water is also commonly used as a base to apply fertilisers and other agri-chemicals, such as pesticides, and presents another contamination pathway, although outbreaks have not been firmly linked to this source yet.

Contaminated water could lead to contamination on the surface of produce from splashing or spraying but also to internalisation of viruses into the plant tissue by uptake through the plant's roots. Water used for irrigation, washing and so on can be sourced from a variety of reservoirs that vary in risk, including rainwater, deep or shallow groundwater wells, canals and rivers, and reclaimed wastewater. Water and soil can become contaminated through a variety of mechanisms, including poorly sited sewage discharge outlets, faulty or poorly designed on-site or municipal sewage management systems leading to inadequate treatment, sewage overflows following high rainfall events, and workers defecating or vomiting directly onto cultivation sites.

Outbreaks in which genotyping reveals the presence of multiple strains on produce are suggestive of contamination from sewage, rather than single infected food handlers. Consistent with this, the 2012 NoV gastroenteritis outbreak that affected some 11,000 strawberry consumers in Germany was suggested to be related to sewage contamination of the strawberries, as three different NoV genotypes were detected on the berries (Mäde et al., 2013).

Contamination of produce by food handlers at harvest, processing and point of sale also represents a significant risk. Transfer of viruses from hands to surfaces, utensils (for example, knives) and produce, and then to the hands of other workers, has been demonstrated (reviewed in (Li et al., 2015)). Thus the contamination of surfaces and produce by food handlers is self-perpetuating and may lead to further contamination events.

Several outbreaks have occurred in which food handlers at the harvest and processing level were implicated. Niu et al. (1992) concluded that contamination of strawberries with HAV was probably related to an infected picker, ultimately causing hepatitis in 28 people. Similarly, Calder et al. (2003) suggested that an outbreak of HAV in New Zealand associated with blueberries was caused by infected pickers. As part of the European-wide VITAL project, Kokkinos et al. (2012) undertook an analysis of the lettuce supply chain in Greece, Serbia and Poland and demonstrated that HAV and NoV were detected in toilet facilities and on harvesters' hands. It is notable that most outbreaks involving berries seem to implicate frozen berries, rather than fresh berries (Table 5). Post-harvest processing may involve individual quick-frozen berries, which are frozen then sorted by hand; this may create the opportunity for viruses to be introduced from the hands of workers who may be shedding viruses (including asymptomatic – symptomless – shedders).

For most produce outbreaks, source attribution studies are incomplete and so categorical information on how the produce become contaminated is not available. This is largely due to the complexity of the produce supply chain. During the large EU multistate outbreak (around 1,400 cases) of HAV between 2013 and 2014, EFSA was given a mandate to conduct trace-back investigations to identify the types of berries implicated and the production location; however, the investigation was inconclusive and could not identify a single source (although Bulgarian blackberries and Polish redcurrants were the most common ingredients). The investigation involved 6,227 transactions among 1,974 food operators – highlighting the complexity of the supply chain (EFSA, 2014d; Severi et al., 2015). This is also demonstrated by the trace-back investigations undertaken by Sarvikivi et al. (2012): in 2009 13 NoV outbreaks affecting around 900 people in Finland were found to be linked to frozen raspberries. Some NoV testing of batches was undertaken and one batch returned a positive NoV result. The wholesaler attempted to trace the batch to the farm and found that the batch had originated from 62 farms, making it impossible to determine how viruses were introduced into the supply chain.

In 2014 the EFSA undertook a series of recent scientific opinions on the risk posed by NoV in berries, leafy greens and tomatoes; they also concluded that risk factors were poorly documented in the literature (EFSA, 2014a, 2014b, 2014c). However, for each commodity they noted that the main risk factors were likely to include

1. Environmental factors (for example, rainfall) that increase transfer of NoV from sewage to irrigation water and fields
2. Use of sewage–contaminated water for irrigation or application of fungicides or pesticides
3. Contamination by pickers, food handlers and equipment.

Occurrence data for norovirus and hepatitis A virus in fresh produce (prevalence studies)

Currently there are no routine monitoring programmes in place for NoV and HAV in fresh produce on the IOI or in most other countries. This was perpetuated by the historical lack of an acceptable standardised testing approach for fruits and vegetables. Because of this, information on the general prevalence of viruses through the supply chain is limited, although such studies are becoming more common with the increasing adoption of the ISO/TS standard method, or comparable procedures. Tables 9 to 12 present summaries of the general prevalence (in non-outbreak situations) of NoV and HAV in fruits and vegetables reported in the literature to date. No prevalence studies are reported for NoV and HAV in produce on the IOI or the UK. (A recent study has been undertaken in the UK but is not yet published.)

Norovirus has been detected in a variety of vegetable commodities sampled at retail level (supermarkets and farmers' or local markets) and during manufacture and distribution. These commodities include leafy greens (various lettuce species and spinach), red and green onions (scallions), cherry tomatoes and tomato slices, cucumber slices, parsley, cilantro (coriander), watercress, radish, leeks and purslane (Table 9). Detection rates in vegetables vary between studies and between countries markedly, with the range being between zero and 62.5%. For example, NoV was highly prevalent in leafy greens sampled from supermarkets in Canada, with 133 (21.0%) and 106 (16.5%) of 641 samples being positive for NoV GI and GII respectively (Baert et al., 2011); in

contrast, only two of 149 (1.3%) lettuce samples collected from supermarkets and farmers markets in Greece, Serbia and Poland were positive for NoV GI (Kokkinos et al., 2012).

Table 9: Occurrence of norovirus in vegetables

Sampling place	Produce type	Sampling country	Number of samples analysed	Number of samples norovirus detected	Virus concentration range	Reference
Retail outlets	Variety of produce (27 types)	USA	46	0	NA	Allwood et al. (2004)
Farms	Variety of vegetables	South Korea	30	1 (spinach)	Not quantified	Cheong et al. (2009)
Catering company	Leafy greens	Belgium	6	2	1.9 to 3.1 log genome copies/g GI	Baert et al. (2011)
Supermarket	Leafy greens	Canada	641	133	1.4 to 8.3 log genome copies/g GI	
Supermarket	Leafy greens	Canada	641	106	1.0 to 6.4 log genome copies/g GII	
Food companies	Leafy greens	France	6	2	3.0 to 3.5 log genome copies/g GI	
Food companies	Leafy greens	France	6	1	2.0 log genome copies/g GII	
Local markets	Red onions	Malaysia	30	1 (GI)	Not quantified	Sahroni et al. (2011)
Local markets	Green onions (scallions or spring onions)	Malaysia	30	4 (all GI)	Not quantified	
Manufacturers and distributors	Cherry tomatoes	Belgium	8	5	4.07 to 4.38 log genome copies/10 g GI	Stals et al. (2011) Baert et al. (2011) EFSA (2011)
Manufacturers and distributors	Cherry tomatoes	Belgium	8	4	3.91 to 5.04 log genome copies/10 g GII	
Catering company	Cucumber slices	Belgium	4	1 (GI)	Not recorded	
Catering company	Tomato slices	Belgium	4	0	NA	
Catering	Tomatoes	Turkey	95	1	Not quantified	Yilmaz et al. (2011)
Catering	Parsley	Turkey	92	0	NA	
Catering	Green onions (scallions or spring onions)	Turkey	93	1	Not quantified	
Catering	Lettuce and salad	Turkey	163	0	NA	
Supermarkets and farmers' markets	Lettuce	Greece, Serbia and Poland	149	2	5.0 to 6.0 PCR-detectable units/ 25 g	Kokkinos et al. (2012)
Supermarkets and farmers' markets	Lettuce	Greece, Serbia and Poland	126	1	10 PCR-detectable units/25 g	
Packinghouse	Cilantro (coriander)	Mexico	5	1	4.8×10^2 copies/g	

Packinghouse	Parsley	Mexico	2	1	3.6×10^2 copies/g	Felix-Valenzuela et al. (2012)
Packinghouse	Green onions (scallions or spring onions)	Mexico	18	4	2.1×10^2 to 5.7×10^3 copies/g	
Packinghouse	Lettuce	Mexico	3	0	NA	
Packinghouse	Cabbages	Mexico	2	0	NA	
Packinghouse	Jalapeno peppers	Mexico	2	0	NA	
Farms	Green onions (scallions or spring onions)	Egypt	144	49	5.6×10^2 genome copies/g GI (mean)	El-Senousy et al. (2013)
Farms	Watercress	Egypt	144	45	5.2×10^2 genome copies/g GI (mean)	
Farms	Radish	Egypt	144	37	1.7×10^2 genome copies/g GI (mean)	El-Senousy et al. (2013)
Farms	Leek	Egypt	144	30	5.9×10^2 genome copies/g GI (mean)	
Farms	Lettuce	Egypt	144	35	6.3×10^2 genome copies/g GI (mean)	
Manufacturers	Lettuce	France	210	26	Not recorded	Loutreul et al. (2014)
Supermarkets and markets	Spinach	Mexico	7	0	Not quantified	Parada-Fabián et al. (2016)
Supermarkets and markets	Parsley	Mexico	12	2	Not quantified	
Supermarkets and markets	Purslane	Mexico	11	1	Not quantified	

NA = Not applicable

Table 10: Occurrence of norovirus in fruits

Sampling place	Produce type	Sampling country	Number of samples analysed	Number of samples norovirus detected	Virus concentration range	Reference
Food companies	Raspberries and strawberries	France	150	3	2.4 to 5.0 log genome copies/g GI	Baert et al. (2011)
Food companies	Raspberries and strawberries	France	150	9	2.0 to 5.8 log genome copies/g GII	
Manufacturers and distributors	Raspberries	Belgium	10	3	2.45 to 3.20 log genome copies/10g GI	Stals et al. (2011)
Manufacturers and distributors	Raspberries	Belgium	10	3	3.05 to 3.70 log genome copies/10g GII	Baert et al. (2011)
Manufacturers and distributors	Strawberries	Belgium	20	4	2.29 to 4.10 log genome copies/10g GI	EFSA (2011)
Manufacturers and distributors	Strawberries	Belgium	20	3	3.05 to 3.77 log genome copies/10g GII	
Manufacturers and distributors	Fruit salad	Belgium	2	1	4.64 log genomic copies/10g GII	
Retail	Fresh raspberries	Czech Republic, Finland, Poland and Serbia	60	0	NA	Maunula et al. (2013)
Retail	Frozen raspberries	Czech Republic, Finland, Poland and Serbia	39	0	NA	
Retail	Fresh strawberries	Czech Republic, Finland, Poland and Serbia	21	0	NA	

Sampling place	Produce type	Sampling country	Number of samples analysed	Number of samples novirus detected	Virus concentration range	Reference
Manufacturers	Raspberries	France	162	27	Not recorded	Loutreul et al. (2014)
Manufacturers	Strawberries	France	32	4	Not recorded	
Manufacturers	Blackberries	France	2	1	Not recorded	
Manufacturers	Mixed berries	France	4	0	Not recorded	

NA = Not applicable

The prevalence of HAV has been studied in vegetables collected from packinghouses and retail outlets in Costa Rica, Greece, Serbia, Poland and Mexico. Hepatitis A viral RNA was detected in a variety of vegetables including lettuce, spinach, parsley, purslane, cilantro (coriander) and green onions (scallions or spring onions) (Table 11). It was also detected in two samples of lettuce (10% of those collected) from farmers' markets in Costa Rica (Hernández et al., 1997) but was not detected in lettuce heads (number [n]=149) collected as part of a recent study on the occurrence of HAV in Greece, Serbia and Poland (Kokkinos et al., 2012).

Studies reporting the general prevalence of HAV in fruits are scant (Table 12); however, Terio et al. (2015) analysed a variety of retail berry samples in Italy, in response to a multistate outbreak of HAV. Thirty samples of frozen redcurrants, mixed berries and strawberries were tested; one sample (redcurrant) was positive for HAV (Terio et al., 2015). A 2009/2010 surveillance survey of figs and dates in the Netherlands detected HAV RNA in one date sample out of 169 collected and tested. None of the 88 fig samples were positive. Trace-back investigations showed that three out of 14 date packages were contaminated and an HAV patient in the same period had eaten dates prior to becoming ill, although the strains in the date packages and patient were different (Boxman et al., 2012).

Comparison of detection rates across studies conducted is difficult, because different approaches are used for viral elution, concentration and detection in each study. Thus, method performance parameters such as the limit of detection (LOD, the definition of the smallest quantity above zero that it is possible to detect) are not consistent. Increasing adoption of the ISO/TS methods should improve comparability of studies in the future. The general lack of prevalence data and use of different methods prevents valid predictions on potential prevalence of NoV and HAV in produce on the IOI. However, it seems likely that detection rates on the IOI would be within the ranges reported in other studies to date (Table 9, Table 10, Table 11 and Table 12).

While the methods used in studies to date differ, all use PCR-based technology for detection and so do not provide information on the infectivity of viruses detected in samples. This means it is not possible to determine if positive samples in prevalence studies represented a risk to consumer health. However, the presence of viral RNA on produce indicates that human faeces has entered the supply chain at some point (especially considering that RNA is fragile when the capsid – the protein shell – is destroyed or damaged), thereby demonstrating a breakdown in good hygienic practice (GHP) .

The availability of the internation organization of standarization (ISO) standard method provides an opportunity for countries to implement a programme of continuous improvement in good hygienic practice and undertake sequential prevalence studies to objectively assess the viral detection rates and potential decreases in prevalence over a time as a result of enhanced safety through the supply chain.

Table 11: Occurrence of hepatitis A virus in vegetables

Sampling place	Commodity	Sampling country	Number of samples analysed	Number of samples hepatitis A virus detected	Virus concentration range	Reference
Farmers' markets	Lettuce	Costa Rica	20 (pooled samples of 5 lettuces)	2	Not quantified	Hernández et al. (1997)
Supermarkets and farmers' markets	Lettuce	Greece, Serbia and Poland	149	0	NA	Kokkinos et al. (2012)
Supermarkets and markets	Spinach	Mexico	7	2	Not quantified	Parada-Fabián et al. (2016)
Supermarkets and markets	Parsley	Mexico	12	1	Not quantified	
Supermarkets and markets	Purslane	Mexico	11	4	Not quantified	
Packinghouse	Cilantro (coriander)	Mexico	5	2	8.9×10^2 to 1.2×10^3 copies/g	Felix-Valenzuela et al. (2012)
Packinghouse	Parsley	Mexico	2	1	2.4×10^3 copies/g	
Packinghouse	Green onions (scallions or spring onions)	Mexico	18	4	2.8×10^2 to 1.3×10^3 copies/g	
Packinghouse	Lettuce	Mexico	3	0	NA	
Packinghouse	Cabbages	Mexico	2	0	NA	
Packinghouse	Jalapeno peppers	Mexico	2	0	NA	

NA = Not applicable

Table 12: Occurrence of hepatitis A virus in fruits

Sampling place	Commodity	Sampling country	Number of samples analysed	Number of samples hepatitis A virus detected	Virus concentration range	Reference
Grocery stores and markets	Frozen redcurrants, mixed berries, strawberries and blueberries	Italy	30	1	Not quantified	Terio et al. (2015)
Retail stores	Figs	Netherlands	88	0	NA	Boxman et al. (2012)
Retail stores	Dates	Netherlands	169	1	15 genome copies/30 g dates	Boxman et al. (2012)
Retail	Fresh raspberries	Czech Republic, Finland, Poland and Serbia	60	0	NA	Maunula et al. (2013)
Retail	Frozen raspberries	Czech Republic, Finland, Poland and Serbia	39	0	NA	
Retail	Fresh strawberries	Czech Republic, Finland, Poland and Serbia	21	0	NA	

NA = Not applicable

Virus survival in produce

Water is a key vector in the transmission of viruses to crops, particularly through irrigation and application of chemicals such as pesticides. The application of contaminated water can result in contamination on the exterior of produce, and internalised virus (the virus is taken up through the roots) (Chancellor et al., 2006; DiCaprio et al., 2015; Hirneisen and Kniel, 2013).

Norovirus and hepatitis A virus have been demonstrated to survive on the surface of various produce species for long periods. Dawson et al. (2005) found that MS2 phage, a culturable surrogate used for NoV, was reduced by up to 1 log[3] on tomatoes, cabbages, carrots, lettuce, parsley and peppers held at 4 °C for seven days. For lettuce held at 22 °C there was a 1-log reduction after seven days but minimal reductions were observed for tomatoes and parsley.

The persistence of NoV GI and GII, murine NoV (MNV – a culturable NoV surrogate) and AdV was investigated on raspberries and strawberries at temperatures between 4 °C and 21 °C (Verhaelen et al., 2012). Less than 0.5-log reductions were observed at 4 °C and 10 °C for raspberries, and at 4 °C for strawberries. At 21 °C, a 1.0-log reduction of culturable MNV on strawberries was observed after one day. However, with raspberries a 1.0-log reduction took three days (and only a 0.3-log reduction was observed for AdV). The study demonstrated greater viral persistence on raspberries compared with strawberries, helping to explain the larger number of outbreaks attributed to raspberries compared with other berry types (Table 5).

Inactivation rates (expressed as "D values" – the time for a 1-log reduction to occur) of eight, nineand 100 days were observed for HAV on the surface of cantaloupes, lettuce and bell peppers maintained at 18 to 36 °C in dry conditions (Stine et al., 2005). The authors noted that it would take around 822 days to reduce HAV by 99.9% in pre-harvest conditions. Similar survival studies also found that the ability of lettuce, fennel and carrots to adsorb HAV varied, with limited decreases in HAV in lettuce observed over nine days at 4 °C (Croci et al., 2002).

One-log reductions are likely to be inadequate to ensure the safety of produce: prevalence studies to date indicate that virus levels can be up to 6 log copies/g of produce (Table 9 to 12), and HAV has a low infectious dose. Thus, while data on virus survival on the surface of produce in "field" (real world) conditions are limited, the above studies indicate lengthy periods would be required for appropriate reductions – and that these would exceed the shelf life of the products.

The findings also suggest that survival on the surface of vegetables and fruits varies depending on the type of produce involved. Different virus types were also found to exhibit varying attachment patterns to

3 "Log reduction" is a mathematical term used to show the relative number of live microbes eliminated from a surface by disinfecting or cleaning. Each log (logarithm) reduction means lowering the number of microorganisms 10-fold, or by one decimal place.

lettuce (Vega et al., 2005), suggesting that special factors may be governing virus retention. The ability to remove NoVs from the surfaces of raspberries and lettuce by washing was found to vary, and the authors proposed that this may be related to differences in the types of ligand molecules that NoV binds to in each type of produce (Tian et al., 2011).

Virus localisation studies demonstrated that NoV-like particles aggregated (formed clusters) in and around the stomata (the pores) of lettuce and in minor veins (DiCaprio et al., 2015; Esseili et al., 2012). They were also found between the epidermal (exterior) cells and cell walls of both shoots and roots of green onions (scallions or spring onions) (DiCaprio et al., 2015). Immunofluorescence studies (using fluorescent DNA-binding dye) showed that NoVs bind to lettuce cell wall materials by using multiple carbohydrate structures (Esseili et al., 2012).

Recently, Gao et al. (2016) demonstrated that H-type HBGAs are found in lettuce tissue and that GII.4 NoV binds to the exposed fucose moiety. ("Moiety" refers to one of a pair of molecules, in this case of fucose, which is a carbohydrate – a sugar). This specific binding suggests that NoV will not be removed by simple washing procedures, a hypothesis confirmed by the studies of DiCaprio et al. (2015), who showed that chlorine washing was ineffective in removing NoV.

The binding of NoV to HBGAs in lettuce is comparable to the process by which NoV binds to oysters. Similar to produce, the recognition that NoV persisted for longer than bacteria in oysters when subjected to depuration in clean water led to the finding that oysters use specific ligands (HBGAs) to selectively accumulate NoV. ("Depuration" is a process to clear shellfish of biological impurities and grit; they are placed in clean water for a period of time.) Some NoV strains bind A-like HBGAs in the oyster digestive tissue, while other strains bind to a sialic acid ligand (Le Guyader et al., 2012; Maalouf et al., 2010, 2011; McLeod et al., 2009; Tian et al., 2008b).

Knowledge of the underlying biology on how NoV interacts and binds with foods may lead to advances in pre- and post-harvest treatments to reduce NoV and HAV, primarily through exploiting these specific interactions.

Effects of treatments used in food processing on viruses

The following provides an overview of the efficacy of common post-harvest produce treatments, such as washing, freezing and thermal (heat-based) processes, in removing NoV and HAV from fresh produce. The impact of some alternative technologies on viral persistence is also discussed, including modified atmosphere packaging (MAP), high pressure processing (HPP) and radiation. Given that there is no routine assay to measure infectious NoV, many studies use culturable surrogate viruses to evaluate the efficacy of treatments. For some treatments, surrogate viruses may be more readily inactivated than NoV, thus caution needs to be taken in extrapolating results.

Washing

A recent review found that washing with untreated water generally results in a 1-log decrease in the amount of virus detected and that the use of chlorine can improve reductions. Processors typically use between 50 and 200 parts per million (ppm) chlorine for contact times of one to two minutes; this generally leads to reductions of between 1 and 2 logs, with decreases varying depending on the type of produce and virus involved (Li et al., 2015).

Butot et al. (2008) investigated the impact of rinsing blueberries, raspberries and strawberries for 30 seconds in chlorinated (200 ppm) and non-treated water. Washing in non-treated water resulted in reductions of up to 1.5 log for HAV and NoV GI and GII. Washing with chlorinated water gave significant reductions of between 1.4 and 3.4 logs for NoV and HAV in blueberries and strawberries but only zero to 0.9 log for raspberries. The authors noted that the hair-like protrusions on raspberries may act to protect viruses against the chlorine treatment. Similarly, viruses have been found to persist for longer periods on raspberries than strawberries (Verhaelen et al., 2012).

Reductions in MS2 phage on a variety of produce types (including tomatoes, lettuce, scallions and strawberries) that were treated with 100 ppm free chlorine were between 0.30 and 2.14 logs (Dawson et al., 2005). The authors noted that MS2 phage is inactivated more rapidly in suspension (that is, mixed with liquid), with previous studies showing a 99% kill in 7 seconds with 0.6 ppm free chlorine. This may be in part due to the internalisation of some viruses within produce and their localisation in sites that are protected from the effects of chemical treatments, such as the stomata (the plant's pores).

Thus, the main purpose of chlorine and other such sanitisers is to reduce the microbial content of washing water and prevent the build-up of viruses and other pathogens. This point is important to recognise, as prevalence studies have shown that levels of virus in market-ready produce can be high, with over 6 logs of NoV reported in leafy greens and berry samples in some studies (Table 9 andTable 10). The application of chlorine and typical reductions on produce of between 1 and 2 logs may not be sufficient to reduce viral levels below an infectious dose.

Chlorine dioxide (ClO_2) presents an alternative to traditional chlorine-containing disinfectants. However, its effectiveness is also noted to be quite low, with concentrations of between 5 and 50 ppm having limited impact on HAV and NoV on raspberries and parsley (Butot et al., 2008).

Other sanitisers have also been investigated including peroxyacetic acid solutions (PAA), hydrogen peroxide, trisodium phosphate, ozone and surfactants. ("Surfactants" reduce the surface tension between two substances and are commonly used as detergents.) These show relatively modest reductions compared with potential contamination levels on produce (reviewed in Li et al., 2015). For example, MNV was reduced by no more than 1 log when onion bulbs and spinach were washed in water. Reductions increased to around 2.5 log with the addition of 20 ppm PAA for five minutes (Baert et al., 2008b). Following a five-minute exposure to ozone, FCV inoculated (meaning the virus was deliberately

introduced) onto lettuce and green onions (scallions or spring onions) was inactivated by 2.0 logs and MNV was reduced by 2.9 and 1.6 logs respectively (Hirneisen et al., 2011).

In summary, the application of sanitisers is unlikely to be completely effective in removing viruses from produce but is useful to ensure the safety of water used for washing processes.

Freezing

Freezing is a common process used for storing and transporting berries. Butot et al. (2008) investigated the reduction of HAV, NoV GI, NoV GII, FCV and RV in strawberries, raspberries, blueberries, parsley and basil. Freezing reduced viral levels by less than 1 log over 90 days, as determined by culture methods for HAV, FCV and RV, and by PCR for NoV. Levels of infectious HAV and RV remained the same throughout storage. Baert et al. (2008b) investigated the impact of freezing on the levels of infectious MNV in onions and spinach; no decrease was observed over six months. Thus, freezing has limited impact on the viability of NoV and HAV. Consistent with this, most illness outbreaks relating to berries are caused by frozen berries.

Freeze-dried berries may be used by the food industry in the manufacture of bakery goods, chocolate products, breakfast cereals and so on. Freeze-drying involves the freezing of fresh berries followed by placement of the berries in a reduced pressure environment. Butot et al. (2009) investigated the impact of freeze-drying on the NoV and HAV levels in blackberries, blueberries, raspberries and strawberries. Infectious HAV was reduced by freeze-drying by 1.4 to 2.4 logs. Norovirus RNA was found to decline by between 0.6 and 2.7 logs. While freeze-drying appears to reduce NoV and HAV, it is not likely to eliminate viruses from produce.

Thermal treatment

To ensure high quality produce, general recommendations suggest the storage of produce at 3 to 5 °C. As noted in the above paragraph, only very low levels of viral reduction are seen at these temperatures (Croci et al., 2002; Dawson et al., 2005; Shieh et al., 2009; Verhaelen et al., 2012), with inadequate reductions noted within the shelf-life of fresh produce. Mild temperatures also appear to be inadequate to eliminate NoV and HAV. Butot et al. (2008) investigated the impact of a warm water wash (43 °C) on NoV and HAV levels in blueberries, raspberries and strawberries – the reductions were no greater than washing with water at ambient temperature (room temperature, in this case 18 °C).

Few studies have been conducted to investigate the effect of milder temperatures associated with pasteurisation on NoV and HAV viability in produce. The impact of pasteurisation processes on MNV in raspberry puree were evaluated: a reduction of 2.8 log of MNV was observed when the puree was held at 75 °C for 15 seconds. The authors noted that infectious NoV would remain if high contamination loads were present (Baert et al., 2008a). Further studies (unpublished data reviewed in Li et al. (2015)) show a greater than 4.29-log reduction of MNV in raspberry puree held at 75 °C for 30 seconds.

The recent infectivity model developed for NoV in human enteroids indicated that after 15 minutes at 60 °C GII.3 and GII.4 NoV were inactivated (Ettayebi et al., 2016). However, it seems probable that pasteurisation will not completely eliminate all strains of NoV or HAV, considering early feeding studies by Dolin et al. (1972) in which NoV remained infectious following a 30-minute heat treatment at 60 °C. Further studies are needed to investigate the reduction of a wider range of enteric viruses during pasteurisation processes, including HAV, NoV and other culturable NoV surrogates (in addition to MNV).

Some produce types are subject to "blanching" prior to freezing (for example, spinach). Blanching involves temperatures between 75 °C and 100 °C and can be undertaken either by steaming or placement of produce in a hot water bath. Steam blanching of basil, mint, chives and parsley at 95 °C for two minutes resulted in reductions of between 2.4 and 4.0 logs for infectious HAV and FCV; and NoV RNA titres (the lowest concentration of antibodies that can still affect antigens, the infective agents, for example by making them clump together) were reduced by between 0.5 and 3.0 logs (Butot et al., 2009). Blanching of spinach leaves at 80 °C for one minute resulted in a decrease of around 2.4 logs of infectious MNV (Baert et al., 2008b).

Studies on the impact of high temperatures exceeding 75 °C on NoV and HAV in produce are limited. Cooking steps in which the food reaches of a temperature of 90 °C for 90 seconds are considered to be adequate to eliminate infectious virus; thus, advisories to boil imported frozen berries for one minute prior to consumption have been issued to reduce the impact of HAV outbreaks. Such treatments are known to be effective for shellfish (Hewitt and Greening, 2006). Consistent with this, the heating of freeze-dried berries (blackberries, blueberries, raspberries and strawberries) to 120 °C following the freeze-drying process was found to reduce the infectious HAV titre to undetectable levels (Butot et al., 2009).

Packaging

Modified atmosphere packaging (MAP) is frequently used for fresh cut lettuce. This involves using specific proportions of nitrogen, carbon dioxide and oxygen in sealed bags or containers to extend a food's shelf life primarily through reducing spoilage organisms. Bidawid et al. (2001) investigated the impact of MAP on the survival of HAV on lettuce. Hepatitis A virus was inoculated onto lettuce pieces and stored at room temperature and 4 °C under various gas mixtures. The results demonstrated that MAP did not affect HAV survival at 4 °C.

High pressure processing

High pressure processing (HPP) is a non-thermal (heatless) process that involves the use of pressures that are uniformly applied through a food to inactivate pathogens and spoilage bacteria. High pressure processing has been investigated as a process to inactivate foodborne viruses in a few types of fresh produce, including green onions (scallions), lettuce, blueberries and strawberry puree.

Kingsley et al. (2005) studied the inactivation of HAV in green onions (scallions) and strawberry puree exposed for five minutes to pressures ranging from 225 to 375 megapascals (MPa). Log reductions of infectious HAV were 4.32 and 4.75 for strawberry puree and green onions, respectively, when exposed to 375 MPa, and HAV concentrations following this treatment were below the LOD of the test (less than 0.5 log). For pressures less than or equal to 300 MPa, HAV reductions in strawberry puree ranged between 1.20 and 3.10 logs, and for green onions (scallions) reductions were between 0.28 and 1.42 logs. While treatment at 375 MPa was effective to inactivate HAV, the authors noted that the sensory qualities of the onions and strawberries were affected: the onions became flaccid (soft) and the strawberry surface was bleached.

Similar results were achieved by Lou et al. (2011), who demonstrated that infectious murine norovirus was reduced by more than 5 logs in fresh lettuce and strawberries that were pressurised at 400 MPa for two minutes. The quality of the lettuce and strawberries were also affected: lettuce became translucent (semi-transparent) and strawberries had considerable textural loss. The impact of HPP on TV and MNV in blueberries was investigated (Li et al., 2013). A treatment of 600 MPa for two minutes did not cause significant reductions of TV or MNV on dry blueberries. However, when blueberries were immersed in phosphate-buffered saline, inactivation of both viruses occurred at pressures less than or equal to 400 MPa.

In conclusion, HPP is effective in inactivating viruses if the pressure is sufficiently high; however, quality of the produce is compromised, which may be a barrier to industry uptake. Pressure levels, time and produce type impacts the effectiveness of HPP treatments in inactivating viruses. Different viruses also display varying resistance to HPP (Grove et al., 2008). Even different strains of the same virus type may react differently (Li et al., 2015). This means that so-called “surrogate” viruses may not always be representative of the non-culturable NoV. Ideally validation should be performed using the produce of interest with human NoV and HAV (Li et al., 2015).

Radiation

Ultraviolet (UV) light has been shown to have variable impacts on viruses on fresh produce. One study demonstrated a 4- to 5-log decrease in infectious HAV, FCV and Aichi virus on lettuce treated with 40 to 120 mW s/cm^2 (milliwatt-seconds per square centimetre); green onions (scallions) showed a 2.5- to 5.6-log reduction, whereas reductions between 1.9 and 2.6 logs were demonstrated for the three viruses on strawberries (Fino and Kniel, 2008b). Thus, the food matrix influences the efficacy of UV treatment. A key

drawback of UV treatment is that the light is absorbed by the surface of the food and does not penetrate into viruses that may be internalised.

Li et al. (2015) review the potential application of ionising radiation to fresh produce. Key points to consider are

1. Viruses are relatively hardy compared with bacteria and thus higher doses are required to inactivate them.
2. The doses that seem to be effective for viruses exceed those that are legally permitted.
3. Consumers in general are not accepting of irradiated foods
4. Sensory changes in irradiated foods may not be desirable.

Food consumption data (fresh produce)

Comprehensive data on the consumption of fresh produce on the IOI are available. The *North/South Ireland Food Consumption Survey* was undertaken between 1997 and 1999. It involved an investigation of food and beverage consumption in a representative sample (n=1,379) of 18- to 64-year-olds in the ROI and NI (Irish Universities Nutrition Alliance [IUNA], 2001). The survey provides data on average food group intakes (grams per day) for various fruit and vegetable commodities.

A more recent consumption survey was conducted between 2008 and 2010, which involved the collection of similar data for people over 18 years old in Ireland (IUNA, 2011). The European Food Safety Authority (EFSA) have also developed the *Comprehensive European Food Consumption Database*. This combines consumption data from each Member State and uses the data from the 2001 and 2011 IUNA surveys.

In 2013 the *Irish Food Portion Sizes Database* was published (Lyons and Giltinan, 2013). This database combines the data from four large national consumption surveys of children and adults in Ireland. The database provides median, 25th and 75th percentile portion weights for a wide variety of foods. These include strawberries, blueberries, "other berries", melons, tomatoes and lettuce – which are of high relevance to risk assessments (RA) of viruses in fresh produce. The available data would enable an RA of viruses in high-risk produce types on the IOI to be conducted in the future, although it would be a complex task as consumption levels vary for each produce type and for each age group assessed. The consumption surveys and portion size database can be accessed at http://www.iuna.net/

8 Risk characterisation

Data needs for risk assessment of foodborne viruses in fresh produce

Risk assessment is a tool that can be used to determine, either qualitatively or quantitatively, the likelihood of viral illness related to consumption of fresh produce commodities on the IOI. The availability of data on the IOI that could be used to support the RA of HAV and NoV in fresh produce is discussed.

Regarding the "hazard identification" component of risk assessment, NoV and HAV are identified in this review as the viruses of highest concern and should be the focus of future RA and risk management efforts. The evidence of risk from produce and the so-called "emerging viruses", such as RV, FV, HEV, SARS and avian influenza, is scant (Table 1).

This review identifies the produce commodities that are consumed on the IOI (Section 9) and their association with viral illnesses and detections globally (Sections 10 and 11). Collectively this information allows a qualitative appraisal of which produce types on the IOI may be of higher concern regarding viral contamination. Table 13 and Table 14 present produce types consumed on the IOI that have been associated with NoV and HAV outbreaks and detections.

Table 13: Volume of fresh fruit commodities produced in and imported (minus exports)[a] into the IOI in 2015, and association with norovirus or hepatitis A virus illness outbreaks and contamination events

Blue shading shows products firmly (strong evidence) linked to outbreaks

Green shading shows products weakly associated with outbreaks

Orange shading shows products in which NoV or HAV has been detected but no outbreaks were recorded

Fruit type	Tonnes produced	Linked to viral illness outbreak (O) or contamination event (E)	Source
Apples	53,239	•	•
Soft fruits	13,457	•	•
Blueberries	9,615	Yes (O, E)	RASFF, literature
Strawberries	7,166	Yes (O, E)	RASFF, literature
Raspberries	311	Yes (O, E)	RASFF, literature

Fruit type	Tonnes produced	• Linked to viral illness outbreak (O) or contamination event (E)	• Source
Rhubarb	297	•	•
Blackcurrants	224	Yes (O)	Literature
Blackberries	30	Yes (O, E)	RASFF, literature
Bananas	79,426		
Citrus fruits[b]	59,117	Yes (O)	Literature
Apples	56,159		
Pears and quinces	45,294		
Grapes	17,606	Yes (O)	Literature
Melons	14,985	Yes (O)	Literature
Soft fruits[c]	9,632		
Avocadoes and mangoes	6,492	Yes (O)	Literature
Frozen berries[d]	4,349	Yes (O, E)	RASFF, literature
Fresh berries[e]	3,087	Yes (O, E)	RASFF, literature
Pineapples	2,162	Yes (O)	Literature
Coconuts	1,098		
Dates	323	Yes (E)	RASFF, literature
Figs	225		

[a]Volume of fruit imports into and exports from Northern Ireland are not included, as HM Revenue and Customs declined to provide the statistics under section 21 of the Freedom of Information Act

[b]"Citrus fruits" includes grapefruits, lemons, limes, mandarins and oranges

[c]"Soft fruits" includes apricots, cherries, peaches and plums

[d]"Frozen berries" includes blackberries, blackcurrants, raspberries and strawberries

[e]"Fresh berries" includes blackberries, raspberries and strawberries

Table 14: Volume of vegetable commodities available for consumption[a] (production plus imports minus exports) on the island of Ireland in 2015, and association with international norovirus or hepatitis A virus illness outbreaks and contamination events

Blue shading shows products firmly (strong evidence) linked to illness breaks

Green shading shows products weakly associated with illness outbreaks

Orange shading shows products in which NoV or HAV has been detected but no outbreaks were recorded

Produce type	Tonnes available for consumption in Ireland	Production	Imports	Exports	Linkage to viral illness outbreak (O) or contamination event (E)	Source	Routinely cooked prior to consumption
Potatoes	517,292	347,700	179,237	9,645			Yes
Herbs	176,000	176,000					Sometimes
Carrots and turnips	112,284	79,102	34,235	1,053	Yes (O, carrots)		Sometimes
Brassicas[b]	66,387	41,910	25,069	592			Yes
Tomatoes	51,589	4 427	48,242	1,080	Yes (O)	Literature	Sometimes
Mushrooms	43,425	72,213	3,767	32,555			Sometimes
Swedes	19,570	19,570					Yes
Peas	12,851		13,005	154			Yes
Cucumbers	8,019	1,832	6,297	110	Yes (E)	Literature	No
Lettuce	6,930	7,369	446	885	Yes (O)	RASFF, literature	No

Produce type	Tonnes available for consumption in Ireland	Production	Imports	Exports	Linkage to viral illness outbreak (O) or contamination event (E)	Source	Routinely cooked prior to consumption
Parsnips	12,478	12,478					Yes
Garlic and leeks	8,823	4,912	4,023	112	Yes (E, leeks)	Literature	Sometimes
Sweet potatoes	4,964		4,970	6			Yes
Onions and shallots	4,250	3,695	2,647	2,092	Yes (E, red onions)	Literature	Sometimes
Beans	4,238		4,311	73			Sometimes
Sweetcorn	3,414		3,509	95			Yes
Celery	2,623	2,623			Yes (O)	Literature	Sometimes
Scallions (spring or green onions)	857	857			Yes (O)	Literature	Sometimes
Spinach and kale	725	725			Yes (E)	Literature	Sometimes
Parsley	282	282			Yes (E)	Literature	Sometimes
Courgettes	191	191					Yes

[a]Volumes of fruit imports into and exports from Northern Ireland are not included, as HM Revenue and Customs declined to provide the statistics under section 21 of the Freedom of Information Act

[b]"Brassicas" includess broccoli, Brussels sprouts, cabbage, calabrese and cauliflower

Berries and melons are likely to pose a higher level of risk than other fruits regarding foodborne viruses on the IOI, as global NoV and HAV outbreaks have been strongly associated with consumption of these product types and significant volumes are consumed in Ireland (Table 13). Other fruits that are infrequently linked to illness (such as mangoes, avocadoes, grapes, citrus fruits and pineapples) likely pose a lower level of risk. Also of lower risk are foods that are not available for consumption on the IOI but have been associated with illness internationally, such as pomegranate arils (part of the seed) and fruits in which viruses have been detected but no illnesses reported (for example, dates) (Table 13).

Tomatoes, lettuce and scallions have been frequently linked to viral outbreaks and contamination events globally and are commonly consumed on the IOI. Therefore, these probably represent a higher risk than products rarely linked to outbreaks, such as celery and carrots (Table 14). Norovirus and hepatitis A virus have been detected in other vegetable commodities including cucumbers, leeks, red onions, parsley and spinach (Baert et al., 2011; Cheong et al., 2009; El-Senousy et al., 2013; Sahroni et al., 2011; Stals et al., 2011) but have not been linked to viral illness outbreaks to the authors' knowledge.

The higher-risk commodities identified in this review – berries, melons, tomatoes, lettuce and scallions – should form the focus of future risk assessment (RA) efforts on the IOI. While information exists on global virus outbreaks related to these produce types, very little data exists on illness outbreaks on the IOI. This could be because very few outbreaks occur but also could be related to a lack of sensitivity in the reporting system in place on the IOI. Notably, there is a lack of capability on the IOI to test foods that are implicated in outbreaks for viruses (with the exception of shellfish), which may hamper epidemiological investigations and contribute to under-reporting.

A critical component of RA is the exposure assessment, for which it is important to have data on the prevalence (the extent of occurrence) of NoV and HAV in produce. It is preferable to have quantitative occurrence data on viruses in produce at multiple points in the supply chain, including at production (including potential viral reservoirs such as irrigation water and harvesters' hands), processing (if applicable) and retail. Such data can assist the identification of vulnerable points in the supply chain at which viruses may be introduced. No data on the occurrence of NoV and HAV in fresh produce on the IOI exist at the moment.

Norovirus and HAV may be reduced to some extent by various treatment processes (Washing). If occurrence data through the supply chain is available, models can be developed to estimate risk reduction if virus levels are decreased at particular points in the supply chain. This can help to assess the efficacy of risk management controls. To enable models to be developed, a detailed supply chain map is needed for each high-risk commodity, showing common practices applied on the IOI (for example, irrigation, application of agrichemicals, washing and freezing).

Data on NoV and HAV prevalence in produce (particularly berries, tomatoes and melons) imported into the IOI would also be required for RA. Comprehensive sampling strategies would need to be developed

for sampling imported lots of commodities to minimise the risk of false negatives and ensure that the survey had enough power to report even what might be low levels of occurrence.

Consumption data, particularly portion size data, for the commodities of concern on the IOI are also required for exposure assessment and risk characterisation purposes. As outlined in Section 7 (Food consumption data (fresh produce), useful consumption data exists for the IOI, with the publication in 2013 of the *Irish Food Portion Sizes Database* (Lyons and Giltinan, 2013) and specific data for berries, tomatoes, melons and lettuce.

Conclusions on risk characterisation

There are significant data gaps that limit the characterisation of risk relating to NoV and HAV in fresh produce on the IOI, in particular a lack of information on viral illnesses relating to produce and viral occurrence through the produce supply chain.

In order to undertake a quantitative RA it is necessary to generate quantitative data on viral occurrence in produce on the IOI. In the past five years a standardised real-time PCR method for the detection of NoV and HAV in fresh produce has been published (ISO, 2012a, 2012b) and some EU countries have generated occurrence data and implemented improved surveillance measures following viral outbreaks (Section 11.3).

To evaluate risk relating to NoV and HAV in fresh produce in the IOI, it is first necessary to ensure that viral testing capability for produce is developed. For consistency with other EU member states, it is recommended that the ISO/TS method be implemented. Following this, data on the occurrence of HAV and NoV in high-risk produce (that is, berries, melons, tomatoes, lettuce and scallions) should be generated at key points through the supply chain.

Interpretation of the results of RT–PCR testing will need to be considered regarding the risk of infection to humans; however, the presence of viral RNA indicates that produce has been in contact with human faeces and thus there has been a breach in Good Hygiene Practices (GHP). Data generated from such an approach could be used in a quantitative RA framework and be used to implement specific management controls to reduce contamination in the future.

The EFSA notes that “there is a lack in knowledge on how much disease is caused by the viruses … and on how much of this disease can be attributed to foodborne spread” (EFSA, 2011). To understand what the potential burden of illness is, improved surveillance of viral outbreaks and occurrence in produce is necessary. Such measures would also assist in identifying the source of contamination and implementing controls.

9 Control options

Overview of fresh produce supply chain on the island of Ireland

Produce available on the IOI for consumption that has been strongly associated with NoV and HAV illness outbreaks includes (in order of highest volumes available to consumers) tomatoes, berries, melons, lettuce and scallions (green or spring onions) (section 8 Risk characterisation). Consistent with these findings, the EFSA recently ranked groups of food and pathogens: NoV and leafy greens eaten raw as salads, NoV and raspberries, and NoV and tomatoes, were in the five top-ranked groups (in order of decreasing risk) (EFSA, 2013).

Most tomatoes consumed in Ireland are imported, mainly from EU countries, notably Spain and the Netherlands. In contrast, lettuce (7,500 tonnes) and scallions (900 tonnes) are primarily produced on the IOI. Berry production in Ireland is significant; however, there are some imports (around 9,000 tonnes), mostly from the Netherlands and Egypt, and all melons are imported. Due to the climate in Ireland, tomatoes and lettuce are produced in protective structures such as greenhouses and tunnels, while scallions (green onions) are produced in fields. Regarding berry production, 42% (mainly strawberries) are produced in protective structures, while the balance are produced or collected outdoors (mainly blueberries).

The production processes, agricultural inputs and technologies used to produce these crops vary between operators and product types. However, common production activities that apply to most produce have been identified in a recent guidance note produced by the Food Safety Authority of Ireland (FSAI) (Figure 1). While there are differences in cultivation techniques used for protected and outdoor crops, the same risks exist regarding the potential introduction of foodborne viruses, particularly the need for clean and potable water (water that is fit for human consumption) throughout the supply chain, and scrupulous hygiene by food handlers.

Summary of existing control measures already in place

There is no specific EU, Irish or UK legislation to control viruses in fresh produce, and there are no regulations specifying microbiological criteria for viruses in produce (Anonymous, 2015). However, Regulation (EC) No. 852/2004 covers the production of primary products (including produce), and food business operators have a responsibility to ensure that their products are safe and protected against contamination.

In 2012, the Codex Committee on Food Hygiene produced *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food* (Codex, 2012). The guidelines apply to all foods, from primary production through to consumption, and seek to minimise viral contamination, particularly HAV and NoV; they include an annex on the "*Control of HAV and NoV in Fresh Produce*".

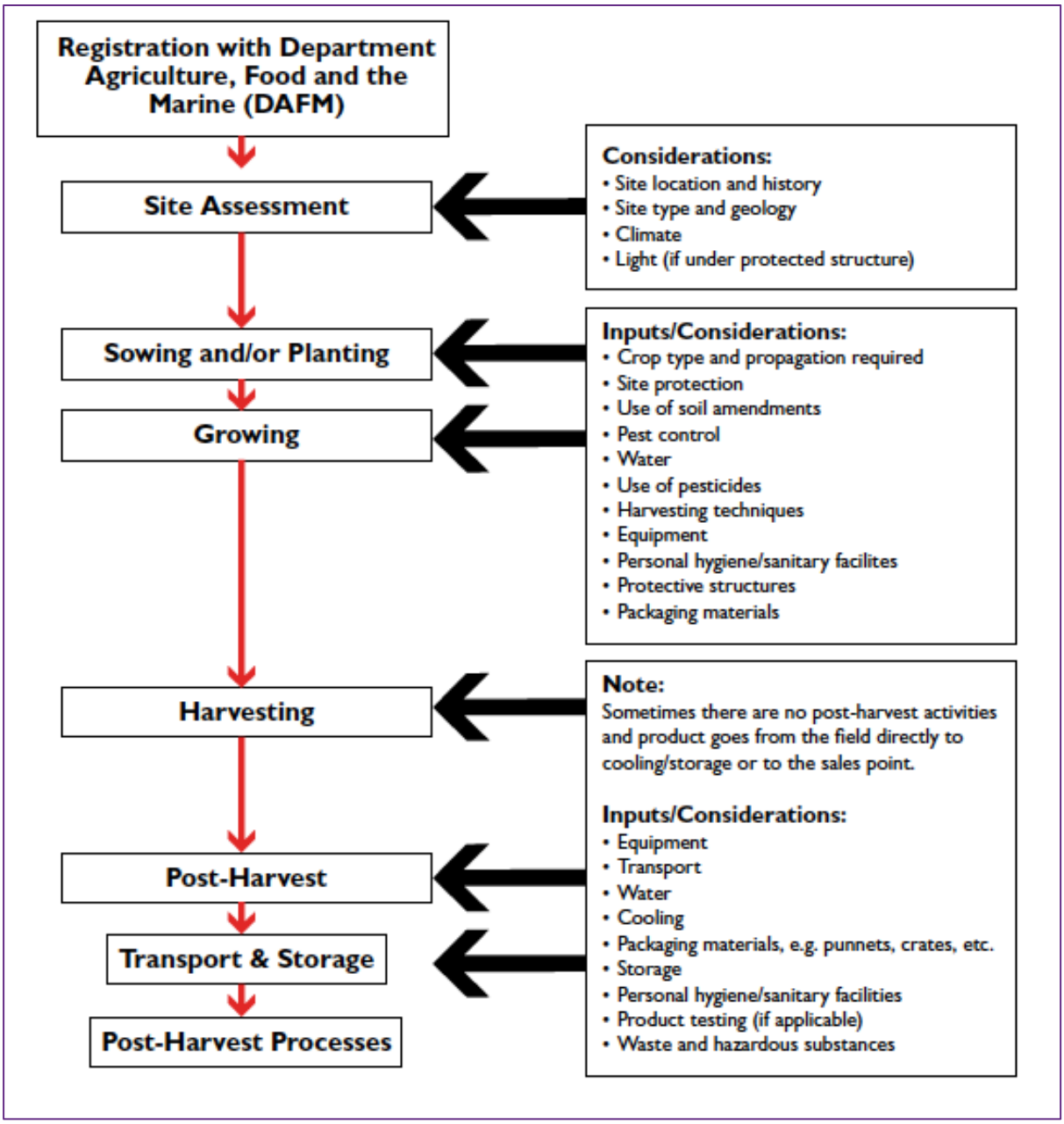
Topics cover hygienic production of food sources, handling, storage and transport, cleaning, personal hygiene, toilets and handwashing, general control programmes and consumer education.

Key points addressed in the guidelines include

- The recommendation to use only “clean” water for production
- That “*an assessment of possible human faecal contamination sources of the water (sanitary survey)*” should be undertaken
- That hand washing and toilets should be in close vicinity to areas where agricultural workers are working.

The EC project VITAL has produced a series of guidance notes for food business operators (FBOs) to use alongside the Codex guidelines to assist in preventing contamination of fresh produce.

Figure 1: Typical steps in primary food production in Ireland; reproduced with permission from FSAI (2016)



In 2014, the EFSA published a series of scientific opinions that included mitigation (harm reduction) options to reduce risks related to NoV in tomatoes, berries, leafy greens, bulb and stem vegetables, and carrots (EFSA, 2014a, 2014b, 2014c). The mitigation options include

- Compliance with pre-requisite (compulsory) programmes such as Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP)
- Careful selection of water sources for irrigation and pesticide application
- Avoiding the use or ingress (any unwanted introduction) of sewage-contaminated water

- Evaluation of production areas to identify sources of faecal pollution and consideration of interventions if necessary
- Hygiene training for persons involved in produce handling.

Adherence to the Codex guidelines and the EFSA recommendations, and hence GHP and GAP, should minimise the risk for viral contamination of fresh produce at production. The annual reports of Ireland's National Control Plan[4] provides an overview of compliance by primary producers to relevant legislation, including Regulation (EC) No 852/2004.

In 2014, 131 hygiene inspections of primary producers of fruit and vegetables were conducted. Ninety-three per cent of FBOs had minor non-compliances, 7% had serious non-compliances and four FBOs (3%) were closed as the non-compliances were deemed to pose a serious threat to public health. One hundred samples of water used for irrigation and washing were collected, of which 19 were non-compliant due to the presence of *Escherichia coli* (*E. coli*) or Enterococci, both of which can cause serious illnesses. The non-compliance by FBOs was considered to be due to a lack of knowledge and awareness of the potential risks.

In 2016, the FSAI produced Guidance Note 31, *Fresh Produce Safety in Primary Production in Ireland*. The guidance is not a legal document but is intended to assist FBOs to meet with legal obligations (FSAI, 2016). The guideline applies to all fresh produce produced in Ireland and covers GHP and GAP. It covers produce grown in fields and in protective structures, in addition to collection of produce from the wild. As noted in Section 7 Contamination pathways, the most significant issues regarding the virological safety of fresh produce pertain to the need for clean water throughout the supply chain (particularly for irrigation, washing, pesticide application and so on), and the hygiene of workers' hands (including pickers, processors and food handlers at retail level).

FSAI Guidance Note 31 addresses these key issues and includes information on the hygiene and health of food handlers, assessing site suitability, evaluating the vulnerability of water to contamination and assessing the suitability of water for use in production. A simple RA tool for growers is also incorporated to assess the risk of contamination arising from a combination of the water source, its intended use and potential contact with fresh produce (FSAI, 2016). Increasing the awareness of FBOs of FSAI Guidance Note 31 through targeted business extension and informative outreach programmes should assist in improving compliance with the hygiene regulations.

[4]The annual reports of the Ireland's National Control Plan are prepared by the Food Safety Authority of Ireland and the Department of Agriculture, Food and the Marine. The 2014 annual report can be viewed at:

https://www.fsai.ie/uploadedFiles/About_Us/service_contracts/national_control_plan/MANCP_Annual_Report_2014.pdf

Potential additional control options

Post-harvest controls

Regarding post-harvest controls for viruses, Codex notes that there are limited effective treatments available for viruses and so recommends that "*the control of NoV and HAV in fresh produce should focus on the prevention of contamination of fresh produce with human faecal material*" (Codex, 2012). Produce such as lettuce, berries, tomatoes and scallions are generally sold fresh (or frozen) and are minimally processed, if at all. Some processes, however, may involve washing (and could include chlorine) but this is unlikely to be completely effective in removing viruses (Cook and D'Agostino, 2013). Codex further notes that washing of produce is not a suitable method to eliminate viruses, and that chemical treatments, whilst effective for bacteria, may not be effective for viruses (Codex, 2012).

Vaccination

A significant quantity of berries, melons and tomatoes are imported into Ireland, with large quantities of tomatoes coming from Spain and berries from Egypt, as well as other non-specified EU and non-EU countries. The importation of foods from countries with high to intermediate levels of HAV infection may increase risk to consumers on the IOI, where HAV infection rates and immunity are low, thus leaving consumers more susceptible to infection.

To reduce transmission of HAV to foods, vaccination of food handlers, from primary production through to point of sale, could be an effective control (reviewed in Cook and D'Agostino, 2013). Universal childhood vaccination, and/or vaccination of consumers during outbreaks, may also reduce the distribution of the virus (Severi et al., 2015). Vaccination programmes are also expensive and the costs would need to be considered against the wider impacts on society arising from outbreaks.

Importation controls

The supply chain for some products, particularly berries, is complex; it can involve the importation of mixed batches that are sourced from intermediaries (agents or third parties) and ultimately are derived or sourced from a multitude of farms – for example, in excess of 60 farms (EFSA, 2014d; Sarvikivi et al., 2012). As discussed (Section 7 Contamination pathways), this can make trace-back investigations extremely challenging and in some cases it is not possible to determine at which point in the supply chain a hygiene failure has occurred. Determining where failures occur is critical to ensuring that hygiene improvements are instigated.

Food businesses (including importers) need to ensure that their products are sourced from suppliers (down to the farm level) who implement appropriate hygiene standards. A move towards importing products that are derived from fewer – but trustworthy – farms, for which information on their hygiene standards is known and available, may assist in guaranteeing a higher level of food safety for imported produce.

Consumer advisories

In addition to the main controls, which seek to minimise contamination of produce during production and processing, are consumer advisories and public health warnings. During the 2013 HAV outbreak related to berries, the FSAI issued precautionary advice to boil imported frozen berries for one minute prior to consumption (Fitzgerald et al., 2014). Similar approaches could be taken in the future if needed, particularly for berries, tomatoes and scallions, which can be cooked prior to consumption.

Monitoring and surveillance

There has been no routine or ongoing monitoring of NoV or HAV in produce in the EU member states. However, the ISO standard method does allow the quantitative determination of NoV and HAV in produce and could be used for verification of GAP and GHP (EFSA, 2014a, 2014b, 2014c). Thus, FBOs could consider the implementation of "in-house" virus criteria for produce – a similar approach has been taken in the shellfish sector in Scotland, whereby certain shellfish companies implement thresholds for NoV which, when exceeded, trigger production area closures.

Testing imported products for NoV and HAV may also provide assurances that GHP and GAP have been followed; after the 2012 NoV outbreak in Germany (Mäde et al., 2013), the EC authorised testing of Chinese strawberries imported into the EU (Commission Regulation (EU) No 323/2014). Similar approaches could be applied to produce imported from other destinations perceived as "high risk" (particularly those where HAV is endemic). However, as noted previously (Section 7 Detection methods), significant care would need to be taken with the sampling plan to avoid false negatives.

It is acknowledged that "you cannot test your way to food safety"; however, testing may play a role in verifying that primary measures to reduce possible faecal contamination of foods are working.

Recently, the European Commission (EC) requested the EFSA to consider the development of microbiological criteria for NoV in tomatoes, leafy greens, berries, and bulb and stem vegetables. The EFSA found that at this time there is insufficient data to provide a risk base for establishing such criteria for NoV (EFSA, 2014a, 2014b, 2014c). However, surveys of NoV at specific steps in the food chain for leafy greens, tomatoes and berries were recommended to support further deliberations on the introduction of an EU-wide criteria in the future. The shellfish sector are somewhat more advanced, with the EFSA recently commencing an EU-wide survey of NoV in production areas and depuration facilities, to support the implementation of microbiological criteria (EFSA, 2016b).

10 Uncertainty and data gaps

This review has identified a series of uncertainties and data or capability gaps.

- Import and export volumes of fresh produce are not readily available for NI; only data that are aggregated (combined) into broad categories encompassing multiple commodities are available. This means there is some uncertainty around the volume and types of produce available for consumption.
- There is no laboratory capability on the IOI for the analysis of NoV and HAV in fresh produce.
- There is considerable uncertainty regarding the proportion of viral outbreaks on the IOI that may be attributed to foodborne (and particularly fresh produce) transmission.
- There is no information on the occurrence of NoV and HAV in potentially high-risk produce commodities on the IOI.
- Regarding global viral outbreaks related to fresh produce, little firm data is available on the cause or source of the outbreaks. Most information is inferential.
- There is uncertainty regarding the reduction and elimination of NoV through commonly applied food processing techniques (such as thermal processes) due to the lack of an infectivity assay for NoV and the widespread use of surrogate viruses in such studies.
- There is limited information available on appropriate sampling approaches for the detection of viruses in soft fruits and salad vegetables.
- There is a lack of publicly available information on the supply chain of fresh produce on the IOI, including common production and processing steps.
- Awareness amongst primary producers of the potential food safety risks and the need to adhere to hygiene regulations and FSAI Guidance Note 31 could be improved.

11 Conclusions

It has become increasingly apparent that foodborne viruses are a significant cause of illness outbreaks globally and that food may influence virus distribution worldwide. Norovirus and hepatitis A virus are the most common viruses implicated, accounting for more than 99% of foodborne viral outbreaks in the EU in 2014.

Globally, NoV and HAV illness outbreaks have been linked to a variety of fresh produce commodities, but the main produce types implicated are berries, lettuces, tomatoes, melons and scallions, or green onions. Ireland is a primary producer of berries, lettuces and scallions, while the majority of melons and tomatoes consumed are imported. Improvements need to be made regarding the reporting of consumer complaints worldwide, and further epidemiological studies are needed, to enable more precise identification of food types that are implicated in viral outbreaks and evaluation of risk.

Significant progress on the development of molecular diagnostics for viruses in foods has been made, and in 2012 an ISO technical specification for the detection of NoV and HAV in a variety of foods, including salad vegetables and soft fruits, was published (ISO, 2012a, 2012b). Furthermore, effective and efficient commercial kits are available for NoV and HAV detection. The progression of this method in conjunction with enhanced epidemiological investigations and networks has led to improvements in surveillance of foods following virus outbreaks, resulting in the increasing recognition of produce as a vector. The method has also enabled some countries to evaluate viral occurrence in different produce types.

Currently there is no data on the occurrence of NoV in fresh produce on the IOI, and very limited data attributing viral outbreaks to fresh produce. These data gaps hamper the ability to characterise the risk of viral illness relating to fresh produce consumption. To facilitate risk assessment, a first critical step is to implement a test method for viruses in produce on the island of Ireland.

Prevalence studies have been conducted in a variety of other countries, and NoV and HAV have been detected and quantified in many produce types, including those not yet implicated in viral illness outbreaks (for example, dates, leeks, radishes and parsley). Thus, risk managers should be aware that a broader array of food types may be responsible for viral illness outbreaks than historically considered. Implementation of viral test methods for produce on the IOI would enhance surveillance of suspected foods, provide prevalence data to support risk assessment, and enable producers and importers to verify the efficacy of GHP and GAP. Such data would provide a scientific basis for future discussions with the EC and EFSA regarding the potential implementation of microbiological criteria for NoV in produce.

Uncertainty regarding the efficacy of post-harvest treatments (largely relating to the historical absence of a culture method for NoV), and the lack of processing for many product types, means it is critical that viruses are prevented from coming into contact with produce during production and

harvesting. This is primarily achieved through ensuring that water used during production is clean, and food handlers and pickers adhere to GHP.

In regards to these critical risk management steps, it is necessary to ensure that current guidelines are adopted by FBOs, including the recently produced FSAI Guidance Note No. 31 and the Codex Guidelines on control of viruses in foods. Training programmes for produce workers covering the role of foods in virus transmission, transmission pathways and details on the infectivity of NoV and HAV, are also essential and should be prioritised.

12 Recommendations

Eleven recommendations are made for the consideration of *safe*food and their members, including regulatory agencies, FBOs and science providers on the island of Ireland.

1. Norovirus and hepatitis A virus should be the priority in future work programmes involving viruses on the IOI.

2. Emerging viruses, such as rotavirus, feline calicivirus, hepatitis E virus, severe acute respiratory syndrome and avian influenza (bird flu), have the potential to cause foodborne illness, and risk managers should be aware of emergent issues relating to their presence in the food supply chain.

3. Emphasis should be placed on ensuring the safety of the foods most commonly consumed on the IOI that have been firmly associated with viral outbreaks internationally. This includes tomatoes, scallions, lettuce, melons and berries.

4. There is increasing evidence of the presence of NoV and HAV in a wider array of fruits and vegetables, such as dates, leeks, parsley and others. Stakeholders should be aware that “non-typical” food types may be responsible for viral illness outbreaks in the future.

5. It is recommended that the ISO/TS method for HAV and NoV in salad vegetables and soft fruits be implemented on the IOI.

6. Epidemiological investigations to identify foods that may be responsible for viral outbreaks on the IOI should be supported and reinforced.

7. Consideration should be given to the development of whole-genome sequencing techniques for HAV and NoV on the IOI to accurately type strains involved in outbreaks and assist in confirming linkages between foods and patients.

8. It is recommended that a prevalence survey of NoV and HAV in high-risk produce types be conducted on the IOI, using the ISO/TS standard method. Such a survey should involve a robust sampling plan in which samples are collected from vulnerable points in the supply chain of each product type included, at which contamination could be introduced.

9. Following the collection of baseline viral occurrence data, it is recommended that a risk assessment of viruses in fresh produce on the IOI be conducted to evaluate the burden of illness, and potential control options.

10. To enable modelling of viral reduction (as part of risk assessment) through commonly applied production and processing steps on the IOI, a detailed supply chain map is needed for each high-risk commodity, showing common practices applied (such as irrigation, application of agrichemicals, washing and freezing).

11. Training programmes that cover Good Agricultural Practice, Good Hygiene Practice, the role of foods in virus transmission, transmission pathways and the infectivity of NoV and HAV should be targeted at workers involved in the production of tomatoes, scallions, berries and lettuce.

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***safe*food:**
7 Eastgate Avenue, Eastgate, Little Island, Co. Cork
7 Ascaill an Gheata Thoir, An tOiléan Beag, Co. Chorcaí
7 Aistyett Avenue, Aistyett, Wee Isle, Co. Cork
Tel: +353 (0)21 230 4100 ***Fax:*** +353 (0)21 230 4111
Email: info@safefood.eu ***Web:*** www.safefood.eu

www.safefood.eu
HELPLINE
NI 0800 085 1683
ROI 1850 40 4567