

Foodborne viruses: Detection, risk assessment, and control options in food processing

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Abstract :

In a recent report by risk assessment experts on the identification of food safety priorities using the Delphi technique, foodborne viruses were recognized among the top rated food safety priorities and have become a greater concern to the food industry over the past few years. Food safety experts agreed that control measures for viruses throughout the food chain are required. However, much still needs to be understood with regard to the effectiveness of these controls and how to properly validate their performance, whether it is personal hygiene of food handlers or the effects of processing of at risk foods or the interpretation and action required on positive virus test result. This manuscript provides a

description of foodborne viruses and their characteristics, their responses to stress and technologies developed for viral detection and control. In addition, the gaps in knowledge and understanding, and future perspectives on the application of viral detection and control strategies for the food industry, along with suggestions on how the food industry could implement effective control strategies for viruses in foods. The current state of the science on epidemiology, public health burden, risk assessment and management options for viruses in food processing environments will be highlighted in this review.

Highlights

► Foodborne virus outbreaks carry both a heavy public health and economic burden ► Reliable detection of viruses in food matrixes remains a challenge ► Current process validations are hampered by difficulty in cultivating viruses. ► Classical approaches to risk assessment are possible, if the appropriate methodologies are developed. ► Risk assessments are based on general principles and research is needed to support these assessments. ► Research effort, needs to be undertaken to understand the ecology, behaviour and transmission of foodborne viruses from the farm and to the consumer.

Keywords : Virus, Detection, Risk assessment, Food, Processing technologies

1. Introduction and Background

1.1 Introduction

Foodborne disease is a significant contributor to the global disease burden (Table 1). Outbreaks and illnesses caused by foodborne microbial pathogens place a heavy burden on health, not only through illness but also through the costs associated with measures taken to reduce the impacts on populations. In today's world with its global reach, the potential for the spread of foodborne illness across country and continental barriers is immense. Worldwide, Norovirus (NoV) is the leading agent of acute gastroenteritis (Table 1), causing about 1 in 5 cases in developed countries (CDC 2016). In countries where rotavirus vaccines are implemented, NoV has surpassed rotaviruses as the most common cause of childhood gastroenteritis requiring medical attention (Payne et al. 2013).

The Centers for Disease Control and Prevention (CDC) conducted detailed analyses of gastroenteritis outbreaks in the US between 2009-2012 and 48% or 1008 of the 2098 foodborne illness outbreaks reported were due to NoV (Hall et al. 2014). Restaurants were the most common setting for these outbreaks with the majority of these attributed to infected food handlers (70%). It is interesting to note that of the 324 outbreaks where a food item was identified only 67 outbreaks reported contamination linked to a single category of food (Hall et al. 2014). The most common categories of food linked to outbreaks were leafy greens, fresh fruit and shellfish. However, any food can be implicated in outbreaks. Contaminated raw ingredients or fresh produce can be sourced from very distant locations and used as ingredients in a wide variety of foods, thereby increasing the potential for spread of infection and impact of illness across the food industry. In 2012, frozen berries –

specifically strawberries – were implicated in large-scale outbreaks of NoV and Hepatitis A virus (HAV). During a 2-month span in 2012, approximately 11,000 people in Germany were affected by NoV gastroenteritis. Epidemiological investigations found that frozen strawberries imported from China were the vehicle of contamination (Mäde et al. 2013) while HAV in frozen mixed berries from various countries (Canada, Bulgaria, Serbia and Poland) was linked to an increase in cases in Northern Italy (Rizzo et al. 2013).

Foodborne illness also carries a high economic burden and it is estimated to cost the US economy between \$55.5 and \$93.2 billion per year (Scharff 2015). In the Western World, comprehensive analyses are available for the health impacts of foodborne viral disease such as the study by Hoffmann et al. (2012) based on 2011 data in the US. In this study, five pathogens, nontyphoidal *Salmonella enterica*, *Campylobacter* spp., *Listeria monocytogenes*, *Toxoplasma gondii*, and NoV, accounted for approximately 90% of the total quality-adjusted life years (QALYs) with NoV alone contributing 5,000 lost QALYs. This translates into a cost of approximately \$2 billion per year due to NoV (Hoffmann et al. 2012), while studies in the Netherlands reported the costs of NoV and HAV illnesses in 2012 to be around €106 million and €900,000, respectively (Mangen et al., 2013 and 2015).

Consequently, foodborne viruses are recognized among the top food safety priorities in a recent report by risk assessment experts who applied the Delphi technique (Rowe and Bolger 2016). Thus, over the past few years foodborne viruses have become a greater concern to both the food industry and regulatory bodies. It is only recently that infections caused by foodborne viruses have started to be routinely

monitored in surveillance systems and this is only performed in some industrialized countries.

In addition, the development of standard or accredited detection methods, such as the International Standards Organization (ISO) standard for HAV and NoV detection using real-time polymerase chain reaction (PCR) (International Standards Organization 2013, 2017), have allowed an increasing number of NoV or HAV infections to be definitively linked to contaminated food consumption.

While PCR detection is useful, it has also led to questions throughout the food industry about the interpretation of a positive test result in foods, as there is little information linking the presence of genomes to virus infectivity. However, given a virus' main route of transmission, its presence typically suggests that fecal contamination has occurred somewhere along the supply chain from farm to fork. This has left regulators and industry alike wondering how best to respond and react to positive findings (Stals et al. 2013). The recent NoV infectivity assay developed by Ettayebi et al. (2016) will by no means be employed on a routine basis, but the assay gives the possibility to determine the threshold of NoV genome copies that may pose a health threat. All stakeholders in the food industry agree that control measures for viruses throughout the food chain are required. However, much still needs to be understood with regards to the effectiveness of these controls and proper validation of their performance, whether it is the personal hygiene of food handlers, processing on of at risk foods or the interpretation and action on a positive test result in a virus testing program (ACMSF 2015; EFSA 2011).

The review will provide a general overview of foodborne viruses and their characteristics, responses to changes in environmental conditions, as well as a critical discussion on efficacy of technologies to control viral hazards. Technologies are summarized to provide insights into their mechanism of action for controlling viral hazards. Finally, a perspective on the application of science and technology for the industry is discussed.

In this respect, the information presented can be a useful resource for food safety decision making and provide guidance which will allow the industry to adopt more effective control measures for viruses in food processing.

2. Foodborne Viruses – Occurrence and Risks

2.1 Description of foodborne viruses

Viruses are obligate intracellular parasites that require susceptible host cells for propagation and host infection. The extracellular infectious particle or virion is, from a structural point of view, very simple, consisting of a nucleic acid, either single stranded (ss) or double stranded (ds) DNA or RNA, surrounded by a protein coat. The presence or absence of an envelope, a lipid bilayer derived from host cell membranes and viral proteins, viruses are classified as enveloped or non-enveloped. Based on their size and shape, nucleotide composition and structure of the genome, as well as mode of replication, viruses are distributed into families, a few of which are grouped into orders (King et al. 2012).

A large number of different viruses may be found in the human gastrointestinal tract causing a wide variety of diseases (Table 2). Although any virus able to cause

disease after ingestion could be potentially considered foodborne and/or waterborne, in practice most reported viral foodborne illnesses are gastroenteritis or hepatitis, caused by human NoV and HAV, respectively. However, other viral agents such as enteroviruses, sapoviruses, rotaviruses, astroviruses, adenoviruses, and Hepatitis E virus (HEV) have been implicated in food- and/or water-borne transmission of illness. Extremely high numbers of viruses may be shed in stools of patients suffering from gastroenteritis (inflammation of the gastrointestinal tract) or hepatitis, who may excrete up to 10^{13} and 10^{10} virus particles, respectively, per gram of stool (Costafreda et al. 2006; Ozawa et al. 2007; Caballero et al. 2013). The symptoms of viral gastroenteritis include nausea, vomiting and abdominal pain, and occasionally fever and headache (Arness et al. 2000). While bacterial gastroenteritis agents are usually responsible for the most severe cases, viruses such as NoV, are responsible for the largest number of cases (Hall et al. 2014).

Hepatitis can result in a serious debilitating condition progressing from illness with fever, headache, nausea and malaise to vomiting, diarrhea, abdominal pain and jaundice. Globally, HAV accounts for about 50% of the total hepatitis cases and although usually self-limiting, it may incapacitate patients for several months and even evolve to fulminant cases leading to death or emergency liver transplantation (O'Grady 1992), with a 2.7% mortality rate in adults over the age of 50.

HEV occurs much less frequently in developed countries than HAV but has a higher mortality rate, particularly in pregnant women where it can reach 25% in infections caused by genotypes 1 and 2 (Kumar et al. 2004). In Asia, the Middle East and Africa, HEV infection is principally the result of a waterborne transmission, mostly

associated with genotypes 1 and 2 (Wong et al. 1980). In contrast, in industrialized countries, infection is zoonotically spread, primarily from swine where seropositivity for genotypes 3 and 4 in animals older than six months is nearly 100% (Ruggeri et al. 2013).

Besides HEV, other important human foodborne viral pathogens may emerge from a zoonotic source. For example, in Malaysia in 1998, an outbreak of severe febrile encephalitis with high mortality rate was reported in humans. This was caused by Nipah virus and transmitted through consumption of contaminated pig meat (EFSA 2011). Another rare example of foodborne viral zoonosis is tick-borne encephalitis that can be transmitted by unpasteurized milk and cheese from dairy animals infected by the etiological agent, a flavivirus (Kríz et al. 2009).

2.2 Epidemiology of foodborne viruses

When outside of their hosts, viruses are merely inert particles, and their associated risk greatly depends on the ability to maintain their infectivity. Factors affecting virus persistence in the environment and food have been previously described (EFSA 2011; Sánchez and Bosch 2016) and decontamination technologies employing a number of these factors to reduce infectious virus numbers in food products will be discussed.

Virus contamination of food products can occur either at pre-harvest or post-harvest (Pintó and Bosch 2008). Foods at risk of contamination at the pre-harvest stage, essentially resulting from environmental pollution, include bivalve mollusks, particularly oysters, clams and mussels, salad crops, such as lettuce, green onions

and other leafy greens, and soft fruits, such as raspberries and strawberries. Improper food-handling through poor hygienic practices is responsible for the majority of post-harvest contamination, mostly involving ready-to-eat foods like sandwiches, cold cuts and pastries. Many outbreaks have been caused by infected workers harvesting the crop, or by food handlers in restaurant and home settings and been linked to salad crops and soft fruits.

2.3 How are foodborne viruses spread?

Foodborne virus infections are predominantly transmitted via the fecal-oral route through ingestion of contaminated food and/or water, or through a secondary route of infection and/or by person-to-person contact. Human sewage/feces, infected food handlers and animals (and their waste) harboring zoonotic viruses have been previously identified as major transmission routes (FAO and WHO 2008). Zoonoses and zoonotic infections caused e.g. by HEV can occur via contact with live animals and through contaminated parts of animals used as food, e.g. meat, organs, milk, eggs (EFSA 2017).

Sewage treatment may not completely remove or inactivate viruses and removal efficiency of sewage treatment is dependent on viral load (Okoh et al. 2010; Pouillot et al. 2015). Murine Norovirus (MNV), often used as a surrogate for NoV in persistence studies, and HAV have been found to survive in certain types of manure and biosolids for more than 60 days (Wei et al. 2010). Thus, the use of contaminated sludge and/or irrigation water on agricultural products in the field is an important route of viral transmission (de Keuckelaere et al. 2015). Proximities of latrines to sources of irrigation water, or even lack of latrines in growing areas have been

identified as risk factors for viral transmission (Taylor 2013; Li et al. 2015). Water polluted with human sewage has been recognized as a mode of viral transmission, where contamination can take place at various stages in the food chain (FAO and WHO 2008) including contamination of bivalve mollusks by direct contact with human sewage in their breeding areas. Irrigation water and water used to dilute agrochemicals and fertilizers poses a risk for pre-harvest contamination of fresh produce while water used for the washing process may become a vehicle for further transmission through the processing of contaminated batches (Verhaelen et al. 2013).

Water-related diseases are not only associated with waters used for drinking purposes and agriculture, such as crop irrigation, but also with those used for food processing, leading to foodborne illness outbreaks (Wheeler et al. 2005; Widdowson et al. 2005). While infected persons shed high numbers of viruses in their stools, NoV may also be transmitted through vomit, which can lead to longer lasting contamination of the respective environment thereby causing a series of illnesses which may last up to several weeks (Lopmann et al. 2012). Another important factor in viral transmission is the shedding of viral particles before and after onset of symptoms and by asymptomatic carriers who appear to be healthy but are able to transmit viruses through food handling and/or by contaminating surfaces where food was handled (EFSA 2011).

Gaps in our understanding of viruses and their behavior

Our understanding of viruses and their behavior has evolved slowly and is hampered mainly by difficulties in both detection and quantification of infectious virus particles. Reliable detection of viruses in food matrices remains a challenge, not only due to non-optimal tedious isolation and detection methods, but also due to the low level of viral contamination and the heterogeneous distribution of viral particles in foods (Mäde et al. 2013). Additionally, the presence or absence of bacterial fecal indicators in food, such as *E. coli*, has proven to be unreliable to indicate presence of enteric viruses (Borchardt et al. 2003; Pintó et al. 2009; Galović et al. 2016). In the absence of reliable indicators, the presence of viruses in food is detected using methods which are currently based on detection of viral nucleic acids that do not indicate viral infectivity (Li et al. 2015). This creates issues in interpreting results for risk assessments as it is difficult to correlate viral nucleic acid detection to likelihood of causing disease.

The NoV infective dose, or the point at which 50% of the population would become ill when exposed to the virus, is difficult to determine. However, current estimates suggest an infective dose in a range between 15 and 1300 genome copies or 1-10 virus particles (Teunis et al. 2008; Atmar et al. 2014). The figure is further supported by studies on oyster-related outbreaks where very low virus concentrations were linked to probability of infections with NoV (Thebault et al. 2013). Similarly, the risk of infection due to HAV in shellfish has been investigated using outbreaks and the vehicles which caused them. Pintó et al. (2009) studied if the number of viral particles (viral nucleic acids) with genome copies of 10-100 genomic copies/g could be correlated with risk of infection. However, it is uncertain if recovery of genome copies during sample processing was 100%, or if there is a fixed relationship

between genome copies and infectious units (Pintó et al. 2009). Based on these studies it is inferred that low doses of either NoV or HAV are capable of causing disease in humans.

Another factor to be considered is viral persistence and stability in different environments, such as on wet or dry surfaces in food processing facilities, or in different food matrices. In fresh produce for example, foodborne viruses were found to survive longer than the shelf-life of the products (Li et al. 2015) and in shellfish, enteric viruses are known to persist for several weeks or months (Drouaz et al. 2015). Survival of enteric viruses has been demonstrated on different household and industrial surfaces where HAV was found to be more resistant to desiccation than other enteric viruses (Abad et al. 1994). Finally, transfer rates have been studied experimentally, identifying variables that have a major influence on transmission as reviewed by Li et al. (2015). The transfer rates for MNV were shown to decrease after drying or after multiple transfers (Tuladhar et al. 2013). While this information is useful as an approximation for survival of HuNoV, it also points to one of the major gaps in understanding virus behavior, where there are limitations in working with and culturing a number of important pathogenic foodborne viruses. The reliance on surrogates, such as MNV, in survival and transmission studies and the reliance on outbreak data to determine infective dose, create uncertainty in risk assessment studies for viruses. However, this may change in the near future with the successful culturing of a number of enteric viruses.

In summary, there are current data gaps in the understanding of foodborne viruses and their behavior. The gaps relate to the unknown relationship between genome

copies and infective virus particles, the use of surrogates to mimic the behaviour of foodborne viruses in industrial settings as well as in laboratory studies, and knowledge about the infective dose of different viruses and virus strains including HEV and their characteristics and persistence in different food matrices e.g. low moisture foods; current prevalence and levels of viruses in agricultural products; the effect of food processing techniques on viral infectivity/inactivation in particular with consumer trends towards minimally processed foods and use of non-thermal technologies; efficacy of commonly used disinfectants on viruses; and, impact of global trade on the emergence of new virus strains or variants through mechanisms contributing to virus variability (recombination, reassortment, mutation, etc).

3. Methods of Detection

The majority of methods currently used for the detection of foodborne viruses are based on PCR. These methods focusing on NoV and HAV with others under development are more sensitive and require shorter times for analysis than cell culture-based methods. The advantages and disadvantages of available methods for detection of human enteric viruses in food are described in Table 3 with more details on specific methods outlined in the section below.

3.1 ISO/CEN method

An ISO technical specification (International Standards Organization 2013; International Standards Organization 2017) for standardized quantitative and qualitative RT-qPCR detection of NoV and HAV in food matrices including bivalve mollusks, leafy green vegetables, berries, food surfaces and bottled water describes matrix specific protocols for virus extraction and a common RNA extraction method

based on capsid disruption using a chaotropic reagent followed by adsorption of RNA to silica particles.

As virus detection in food matrices is challenging due to physical and chemical properties of the food, the ISO method includes certain criteria intended to prevent false-negative interpretation or underestimation of virus quantity. A virus process control is added to measure the efficiency of virus extraction. The inhibition of target amplification is evaluated by adding an RNA control, e.g. mengovirus, to the RT-qPCR reaction.

However, simplification of the standard, i.e. virus elution and concentration from various matrices which allow a high recovery, needs to be addressed. Direct extraction of RNA from berry surfaces by immersion into lysis buffer was efficient in detecting some NoV surrogates on artificially contaminated berries (Perrin *et al.* 2015). A further step towards complete validation, however, requires demonstrated detection of viral pathogens in naturally contaminated samples and comparison of performance between laboratories. The major issue when analyzing food matrices is the difficulty of detecting low levels of virus due to limited sample size, and the availability of the ISO method should not hinder method improvements or optimization.

3.2 Quantification and confirmation

Quantification of virus represents an advance in outbreak investigations and routine monitoring as it can provide data to develop acceptance levels in food commodities and development of quantitative risk assessments (Pintó, 2009). Quantification by RT-qPCR can be done by using a standard curve generated from known amounts of

the target sequence represented by synthetic or *in vitro* transcribed RNA or DNA (Costafreda et al. 2006; da Silva et al. 2007; Gentry et al. 2009; Le Guyader et al. 2009; Hata et al. 2011). Regardless of the method used, the most critical step is the reverse transcription (RT) reaction, with ssRNA being the optimal choice as external amplification control (Costafreda et al. 2006). However, the production and quantification of standard materials by individual laboratories may lead to differences between standard curve intercepts and thus induce inter-laboratory variation in quantification. This suggests the use certified standard reagents may reduce variation.

Inter-laboratory (comparative) studies and the use of various reagents and qRT-PCR systems for quantification of low levels of viruses (e.g. <100 genome copies/g) can lead to result variability e.g. different Ct values obtained by various laboratories (CEFAS 2011; CEFAS 2012).

Importantly, viruses are often unevenly distributed in a batch of food, making it necessary to test replicates or a pool of samples to obtain the most reliable qualitative or quantitative results (Le Guyader et al. 2010; Müller et al. 2015). Presently, there are no regulatory microbiological criteria (e.g. standards, guidelines or specifications) applied relating to viruses. Most food companies and authorities mainly ask for qualitative results as part of production hygiene testing or outbreak investigations (Müller et al. 2015). For confirmation of a positive qRT-PCR signal and to assist epidemiological studies, systematic typing of strains linked to disease outbreaks and surveillance of viruses in food commodities is recommended (EFSA 2011). As the short (~100 bp) amplicon from standard RT-qPCRs is not suitable for strain typing, current protocols include conventional RT-PCRs targeting a longer and

more variable region for sequencing (Mattison et al. 2009; Siebenga et al. 2009; Pérez-Sautu et al. 2011; Vinjé et al. 2004). As strains may cluster differently depending on the regions used for phylogeny, sequencing regions should preferably include potential recombination sites (Vinjé et al. 2004; Symes et al. 2007; Mattison et al. 2009; Siebenga et al. 2009; Bull and White 2011). However, as repeatedly reported from outbreak investigations, it is difficult to obtain a useful sequence from positive RT-qPCR food samples (Sarvikivi et al. 2012). This may be due to a lack of recognition by the conventional primers, simultaneous amplification of multiple strains, the amount of virus being below the detection limit for conventional RT-PCR or extraction of insufficiently pure RNA to get amplification suitable for sequencing. All of these reasons may explain a Belgian, French and Canadian screening study where only 34.6% of positive samples, were confirmed by systematic typing using RT-PCR and sequencing (Baert et al. 2011).

3.3 Molecular virus detection from intact virus capsids.

Viral genomes detected by RT-qPCR do not necessarily represent infectious particles, and these molecular detection assays need to be refined to better predict infectivity of the viruses. As viruses need an intact capsid to be infective, studies have been performed to achieve detection of RNA only from these intact viral particles. RNase or propidium monoazide treatments may be used, as successfully demonstrated on HAV subjected to thermal inactivation (Topping et al. 2009; Sánchez et al. 2012). However, such approaches have to be adapted depending on the virus and treatment applied (Escudero-Abarca et al. 2014a). In addition, suppression of inactivated virus signals may not be complete, which may lead to an overestimation of infectious viruses (Moreno et al. 2015). Since the methods rely on

the ability of propidium monoazide and RNase to penetrate damaged or destroyed capsids, viruses inactivated by interventions or processes that do not reduce or destroy capsid integrity, e.g. those targeting nucleic acids directly, cannot be studied by such approaches.

Nucleic acid aptamers for the capture of some NoV strains have been proposed and ssDNA aptamers may be used as an alternative to antibodies (Escudero-Abarca et al., 2014b; Moore et al., 2015). Aptamers may be quite specific depending on their design. Hence, a large panel of different aptamers could be used to recognize different viral strains. Additionally, their ability to detect a specific three-dimensional capsid structure could be used to indicate the presence of complete viral particles. Other techniques such as phage nanoparticle reporters in lateral-flow assays seem to be promising (Hagström et al. 2015), or the use of artificial receptor ligands such as high affinity molecularly imprinted polymers (Altintas et al. 2015).

Based on NoV binding to histo-blood group antigen glycans, these glycans have been proposed as tools for the evaluation of capsid integrity (Dancho et al. 2012; Wang and Tian 2014). After treatment of NoV by chlorine, heat or ultra-violet (UV) radiation, selective binding of virus to glycans showed a three \log_{10} reduction in genome titers, thus demonstrating the capacity of the glycans to specifically target undamaged capsid (Wang and Tian 2014). This technique was also used for evaluating the effects of high hydrostatic pressure on MNV and Tulane virus (Li et al. 2015). The combination of pig mucin binding and RNase treatment reduced detection of damaged particles after different inactivation treatments (Karim et al. 2015; Afolayan et al. 2016).

3.4 Detection of infective viruses

Cell culture based methods can be used to detect some enteric viruses, using a series of concentration and purification steps to elute viruses from the food matrix taking special care to avoid reduction of virus infectivity and such methods were shown to be efficient for detection of some enteroviruses or HAV strains from environmental or food samples (Metcalf et al. 1995; Pintó et al. 2009). However, despite numerous attempts using monolayer or 3-D tissue structures of a variety of cell lines, no reproducible *in vitro* replication for NoV could be achieved (Duizer et al. 2004; Straube et al. 2011). Recently, the replication of a GII.4 Sydney NoV strain was achieved in B-cells in the presence of histo-blood group antigens expressing enteric bacteria (Jones et al. 2014, 2015). Human intestinal enteroids allowed cultivation of several strains of NoV showing an increase of up to 3 log₁₀ for some strains (Ettayebi et al. 2016). This enteroid system, already successfully applied in several laboratories, will help to identify, qualify and investigate correlations with appropriate surrogates that behave similarly to NoV, allowing the food industry to use these surrogates to evaluate the effectiveness of control strategies.

Cell culture based methods have been used to initially amplify viral nucleic acids, and remove inhibitors, prior to detection by RT-qPCR or qPCR depending on virus type. This integrated cell culture (ICC) (RT)-qPCR /qPCR assay shortens the time to detect infective virus particles and has been used to detect adenoviruses, astroviruses, enteroviruses and HAV (Chung et al. 1996; Abad et al. 1997; De Medici et al. 2001; Choo and Kim 2006). The method allowed infectivity analysis of viruses found in shellfish samples (Chironna et al. 2002; Croci et al. 2005) and detection of viruses that may not cause cytopathic changes in cell culture (e.g., HAV). The

number of samples that were positive by ICC-(RT)-qPCR was usually lower than those obtained by direct (RT)-qPCR due to the elimination of inactivated virus that may be detectable using molecular methods (De Medici et al. 2001) or possibly the inability of the cell line to support growth of some virus strains.

3.5 New technologies

Recent technical developments provide opportunities to improve the detection, quantification and identification of viruses in food matrices. Beside some technical improvements of quantification as provided by digital PCR, accuracy of PCR based technologies could be enhanced by improvement of enzymes, probe labelling and knowledge of viral genome sequences (Sedlak and Jerome 2013; Kishida et al. 2014). The application of next generation sequencing to viral genomes will not only contribute to viral identification but also provide new data that will improve primer and probe design for targeted PCR assays. In the near future, identification of the virome in clinical and environmental samples will also be helpful in analysis of food samples, as well as, improving knowledge on any relationships between bacterial and viral contamination (Kohl et al. 2015; Moore et al. 2015b; Newton et al. 2015).

4. Risk Assessment of Viruses in Foods

4.1 Risk Assessment

To assess risks associated with viruses and other hazards in the food chain and put in place appropriate control measures, the use of risk assessment techniques has been suggested by international bodies (Codex Alimentarius 1995; WTO 1995) and increasingly accepted by governments around the world as a basis for national legislation in relation to food safety (European Commission 2002; Dong et al. 2015).

There are two main approaches in performing a risk assessment, an epidemiological approach (top-down approach) starting from data on illness and moving towards the hazard in the product and a food chain approach (bottom-up approach) starting from the hazard in the product and moving towards an estimate of the probability of illness (Zwietering and Van Gerwen 2000). Risk assessments can also be quantitative, when models are used to link the different risk assessment components resulting in a numerical quantification of the risk or qualitative when no models are used (Nauta 2000). Finally, depending on the type of risk estimate, risk assessments may be deterministic (point estimates) or stochastic (probabilistic estimates incorporating the uncertainty and or variability associated with different types of input data) (Lammerding and Fazil 2000). The following sections provide an overview of existing top-down/bottom-up risk assessments focusing on viruses and discuss how risk assessment findings can be used to reduce the public health burden of food related viral illnesses.

4.2 Bottom-up risk assessments on viruses

Most published risk assessments consider enteric viruses present in water (irrigation or drinking water) while fewer studies have examined viruses present in food products. An overview of waterborne fresh produce risk assessments can be found in the publication by De Keuckelare et al. (2015) and an overview of bottom-up foodborne risk assessments can be seen in Table 4 of this paper. For irrigation water, most risk assessments deal with rotavirus and other human enteric viruses (de Keuckelaere et al. 2015) while for food a wide variety of viruses and products are considered. NoV or HAV are dealt with in several of these risk assessments (Bouwknegt et al. 2015; Pintó et al. 2009; Jacxsens et al. 2017; Kokkinos et al. 2015;

Masago et al. 2006; Sumner 2011) as the viruses seem to be most commonly transmitted through food and water (Koopmans and Duizer 2002; Lopman 2015). While avian influenza viruses are not necessarily pathogenic to humans their spread through various food commodities are also the focus of several risk assessments (Golden et al. 2009; Métras et al. 2009; Bauer et al. 2010; Sánchez-Vizcaíno et al. 2010) following the attention given to this illness as a pre-eminent zoonosis, although foodborne transmission remains controversial. Despite the lack of data on prevalence, concentration and dose-response modelling for foodborne viruses, it is often possible to perform a quantitative risk assessment, but assumptions need to be made. For instance, in the absence of a cell culture based method for detection, the concentration of viruses in samples are often estimated by RT-qPCR in number of genome copies or PCR-detectable genome units/g of product and sometimes in combination with the MPN test (Bouwknegt et al. 2015; Pintó et al. 2009; Masago et al. 2006). Similarly, feeding trial data from other viruses after applying correction factors (Pintó et al. 2009) or from a specific virus strain (Bouwknegt et al. 2015), or simply an assumption on a threshold dose (Müller et al. 2017), may form the basis of the dose response models. Alternatively, in the absence of a specific dose-response model, an estimation of the number of exposures may be the final step of the risk assessment process (Sarno et al. 2017). Overall, this shows that the lack of data is not necessarily a barrier to performing a quantitative risk assessment (Coleman and Marks 1999).

4.3 Top-down risk assessments on viruses

Epidemiology-based risk assessments may provide data on prevalence and concentration of specific viruses in specific food commodities from national (Pintó et

al. 2009; Franck et al. 2015), European (Da Silva et al. 2015) or global (Greig and Ravel 2009; Matthews et al. 2012; Havelaar et al. 2015; Kirk et al. 2015) surveillance and outbreak studies. The output of such studies can be used to assess the risk of viral infections through water and food, thereby offering valuable information to support decision makers in the development of proactive integrated monitoring and risk management strategies to control viral contamination of the food supply chains (Rodriguez-Lazaro et al. 2012). Different types of top down risk assessments are discussed below.

Disease burden studies assess the impact of viral infections on public health by providing estimates of their incidence in the population, sometimes in the form of a uniform metrics such as Disability Adjusted Life Years (DALYs) or QALYs (Havelaar et al. 2015). The use of uniform metrics such as DALYs is preferable when comparing the disease burden of viruses with other illnesses in the population and is, in fact, recommended by the World Health Organization as a means of comparing the impact of illnesses that differ in their incidence and severity (WHO 2007).

Risk ranking studies provide a risk score for different types of product-pathogen contributions and aim to identify high risk products for the transmission of specific pathogens (Sumner and Ross 2002; EFSA 2013; Da Silva et al. 2015). Source attribution studies have been conducted by analyzing foodborne (viral) illness and outbreak data to estimate the proportion of human cases of specific enteric (viral) diseases attributable to a specific food product. Although reported outbreaks are only partially representative, they provide a direct link between the pathogen, its source and each infected person (Greig and Ravel 2009). Information on source attribution

may result in actions of intensified surveillance such as those introduced for imported frozen strawberries from China in 2013-2014 (European Commission 2012) after a large NoV outbreak in Germany (Bernard et al. 2014). Other actions can include introducing interventions in the chain of production which was the case in Denmark where legislation was changed to make heat-treatment (100°C, 1 min) of frozen raspberries compulsory in professional catering establishments (Müller et al. 2015).

Risk factor studies have been conducted by examining global epidemiological trends in human NoV outbreaks by transmission route, season and setting. The results demonstrated that foodservice and winter outbreaks were significantly associated with higher attack rates (Verhoef et al. 2015). Foodborne and waterborne outbreaks were associated with multiple strains (GI+GII). Waterborne outbreaks were significantly associated with GI strains, while healthcare-related and winter outbreaks were associated with GII strains. These results identify important trends for epidemic NoV detection, prevention, and control (Matthews et al. 2012). In addition, a study was performed in Denmark to clarify routes of contamination (Franck et al. 2015). The authors reviewed and categorized 191 calicivirus (189 NoV and 2 sapovirus) outbreaks occurring in Denmark from 2005-2011 according to the source of contamination. The review revealed that in 51 (27%) outbreaks, contamination had occurred during production, with frozen berries, lettuce and oysters being the most commonly implicated food products. It was concluded that another 55 (29%) outbreaks had occurred after guests had contaminated the food at self-serve buffets. Contamination from food handlers took place during the preparation or serving of the food in 64 (34%) of the outbreaks of which 41 (64%) (one of five outbreaks) were caused by asymptomatic food handlers – who either had contact with ill household

members, or retrospectively were found to be in the incubation- or recovery period at the time of handling the food. Data from contamination studies show that more than 1000 virus particles may be transferred from fecally-contaminated fingers to foods, so inactivation of at least 3 log₁₀ would be required to inactivate these agents (Koopmans and Duizer, 2004) and emphasizes the importance of hygienic handling prior to processing. For such reasons, guidelines (Codex Alimentarius 2012) have been written to help food authorities and the industry to manage sick leaves in cases of ill food handlers, in order to limit the transmission of viruses through food handling operations.

4.4 Translating risk assessment into practice

Bottom-up and top-down risk assessments can help public health risk managers set priorities among different illnesses in the population or among different product-pathogen combinations and identify effective interventions for reducing the public health impact of foodborne viral illnesses. Identified interventions may vary depending on the type of risk assessment performed. Thus, food chain risk assessments provide more information on interventions targeted to processing/consumer practices. Epidemiological risk assessments facilitate interventions that can be deduced from studies about risk factors, implicated vehicles in outbreaks and high-risk product-pathogen combinations. A summary of the most important interventions for the control of viruses in the food chain could be setting adequate criteria for decimal reduction for viruses (may not be suitable for all foods) e.g. achieving a core temperature of 85-90°C for at least 1.5 min has been considered a virucidal treatment (CAC 2012). Implementing raw material/food production controls (oysters, berries, leafy greens) e.g. harvesting oysters and other

shellfish from non-contaminated areas, establishing an acceptable limit for NoV in oysters to be harvested and placed in the market, and testing of products for compliance to this acceptable limit (EFSA, 2012) are examples of these controls. Appropriate farm to fork implementation of food safety management systems (GAP, GHP, GMP) accompanied by suitable validation and verification procedures are primary mitigation options for reducing risk of NoV in berries and leafy greens (EFSA 2014a, EFSA 2014b). Improved/increased surveillance of high risk food commodities, e.g. soft fruits (European Commission 2012) and adequate hand hygiene and food handling education along with effective sanitation measures, strategies to manage ill workers, and provisions for a suitable period sickness/absence leave in the case of symptomatic food handlers or asymptomatic food handlers whose household members suffer from gastroenteritis (Franck et al. 2015) are options to manage risks.

5. Effect of Processing Technologies to Control Viruses

5.1 Introduction

Intrinsic and extrinsic factors of foods, food processing technologies and chemical based technologies could be used to control/inactivate enteric viruses from foods. While data from these control strategies focus on inactivating NoV, HAV and to a lesser extent, HEV (an emerging pathogen and where information is available), the gaps in knowledge or understanding the challenges faced by the food industry while validating and implementing viral control strategies need to be considered.

Validation of control strategies for viruses needs documented scientific evidence to demonstrate their effectiveness in reducing or eliminating viruses from foods

(National Advisory Committee on Microbiological Criteria for Foods 1998; Codex Alimentarius 2008). The replication assay recently developed for certain human NoV strains will allow more realistic evaluation and validation studies for viruses (Ettayebi et al. 2016). However, at present, the most common approach has been to use cultivable surrogate viruses such as FCV (Hoover and Kahn 1975), MNV (Karst et al. 2003), TuV (Farkas et al. 2008) and bacteriophages such as MS2 (Maillard et al. 1994; Shin and Sobsey 2003; Dawson et al. 2005) to mimic human NoV. Wild type HAV and HEV strains cannot be easily cultured in the laboratory. As alternative a cultivable laboratory adapted HAV HM-175 strain (Daemer et al. 1981) and a recently developed HEV cell culture method (Johne et al. 2016) are commonly used in studies. An ideal surrogate for human NoV should have similar biological, biochemical and biophysical characteristics as human NoV (Baker et al. 2012), and members of the same *Caliciviridae* family are logical surrogate choices. However, even enteric viruses within the same family could have different characteristics and the interpretation of the results from experiments using surrogates is challenging, because of differences in cultivation, detection and analytical methods. Moreover, variations in challenge study designs also complicates interpretation and comparison between studies.

5.2 Effects of intrinsic and extrinsic factors on viruses

Control strategies that rely on the intrinsic and extrinsic properties of foods e.g. pH, water activity (a_w), and refrigerated and frozen storage temperatures, have traditionally been used to keep foods microbiologically safe by inhibiting bacterial growth in foods. However, some of these control measures may not be directly

applicable to viruses since 'growth' is not a concern whereas 'survival' or maintaining infectivity is key.

Like many bacterial pathogens, viruses can remain relatively stable under refrigerated and frozen storage conditions (Mattison et al. 2007; Baert et al. 2008b; Huang et al. 2014; Mormann et al. 2015) with no reduction of MNV on spinach and spring onions over 6 months of frozen storage (Baert et al. 2008b) and $<1.2 \log_{10}$ reduction in strawberries (whole and puree) over 28 days frozen storage (Huang et al. 2014). The regulation of pH (by fermentation or addition of acid) and a_w levels (by drying or using solutes such as salt/sugar), combined with various storage conditions can have variable effects on different viruses (Table 5). MNV and TuV have demonstrated tolerance to a low pH (pH 2 for 1 h; Li et al. 2013), produced by lactic acid bacteria. Fermentation may produce antiviral properties and compounds could potentially be used as food additives (Al Kassaa et al. 2014), but the modes of action of these compounds are not well understood.

5.3 Antiviral food components and food packaging

Plant extracts have varied antimicrobial properties and have been used for raw and processed food preservation and to control transmission of enteric viruses (D'Souza 2014; Ryu et al. 2015). The inactivation of viruses treated with extracts from grape seeds, cranberries, mulberries, black raspberries and pomegranates using varying conditions including test substrate concentrations, temperatures and duration have been demonstrated (Table 6). Generally, the inactivation of both NoV surrogates and HAV was dependent on exposure time and test compound concentrations. The main effect of extracts from grape seeds on FCV, MNV and HAV seemed to be

reduced virus adsorption to cells (Su et al. 2011). A similar effect was reported for black raspberry seed extracts on FCV and MNV and with some indication of inhibition of MNV replication (Lee et al. 2016). Lemongrass oil, citral and allspice oil gave a time dependent reduction of MNV in PBS, resulting in 2.7, 3.0, and 3.4 log₁₀ reduction after 24 h, respectively. Spice oil is reported to affect the capsid and RNA directly, while lemongrass oil and citral appeared to reduce virus infectivity by coating the capsid (Gilling et al. 2014b).

Plant derived phenolic compounds, e.g. phenolic acids and flavonoids, showed antiviral effects against rotavirus and FCV (Matemu et al. 2011; Katayama et al. 2013). Chitosan, a positively charged polysaccharide composed of glucosamine and acetyl-glucosamine, has been shown to have antiviral effects on MNV, MS2 and FCV (Su et al. 2009; Davis et al. 2012, 2015). Grape seed and green tea extracts can be incorporated into edible chitosan films with a 5% grape seed extract reducing MNV titres by 4.0 log₁₀ after 3 h. Edible films enriched with green tea extracts (5 and 10%) were demonstrated to reduce MNV by 1.6 and 4.5 log₁₀ respectively (Amankwaah 2013).

The antiviral effects of various natural biochemicals were reviewed by Li et al. (2013). Saponin (1.0 µg/ml) had inhibitory effects on rotavirus by blocking attachment to host cells (Roner et al. 2010). An effect of citric acid was observed as binding of human NoVs to histo-blood group antigens (HBGA), which are considered as co-receptors for these viruses, was blocked (Hansman et al. 2012). Milk proteins may interfere with virus infection, e.g. lactoferrin blocks rotavirus (Wakabayashi et al. 2014), FCV and PV (McCann et al. 2003; McCall et al. 2011) entry into the cell.

Tryptic digest of lactoferrin or acylation and amidation of lactoferrin (Pan et al. 2007) and modification of other natural biochemicals may enhance antiviral properties and detailed in a review of antiviral properties of milk proteins and peptides by Pan et al. (2006). Essential oils (EO) containing terpenes, alcohols, aldehydes, and esters extracted from plants e.g. extract of *Hibiscus sabdariffa* showed 5.0 log₁₀ reduction of MNV and HAV (Joshi et al. 2015a). However, inactivation mechanisms remain unknown. A number of studies have reported the effect of EO and biochemicals on virus infectivity (Table 7) but despite the reports of efficacy demonstrated in in-vitro studies, there has been very limited application of these findings to date. One of the major hurdles in successful application is ensuring the antiviral compounds are present at the necessary virucidal concentrations wherever the viruses are present in a food. Due to the low infective dose of foodborne viruses, any intervention techniques acting alone would need to completely inactivate any viruses present in a food. In addition, there may be other factors present in foods that may interfere with antiviral effects.

5.4 Thermal processing

Thermal processing has remained one of the most effective strategy in inactivating foodborne viruses including human NoV, HAV and HEV. Temperatures $\geq 90^{\circ}\text{C}$ for more than 90 s are generally effective against enteric viruses, even in complex matrices such as shellfish (Codex Alimentarius 2012). A comprehensive review by Bozkurt et al. (2015) and equivalent time-temperature combinations of 90°C for 90 s in shellfish matrices by EFSA (2015) demonstrated the effectiveness of heat treatments on enteric viruses. In addition, human NoV GII.3 and GII.4 stool suspensions lost infectivity to stem cell derived human enteroids after 15 min at

60°C, which further demonstrated the effectiveness of heat as an inactivation strategy for enteric viruses (Ettayebi et al. 2016).

5.4.1 Effect of heat on viruses in liquids and food matrices with high water activity

It is widely accepted that boiling water (1 min minimum) effectively inactivates viruses ($> 4 \log_{10}$) e.g. enteroviruses, human rhinovirus (HRV), human NoV, HAV and HEV, (CDC 2009) (Table 8). At lower temperatures like those typically used for pasteurization, both HAV and MNV showed inactivation rates greater than $3.5 \log_{10}$ after 1 min at 72°C in water (Hewitt et al. 2009). Similarly, Hirneisen and Kniel (2013) reported heating at 70°C for 2 min inactivated MNV and TuV beyond the limit of detection and that NoV surrogates could behave similarly during heat treatment. D-values for NoV surrogates and HAV can vary depending on the heating system used (Arthur and Gibson 2015; Bozkurt et al. 2015) with MNV showing similar D-values at 72°C in cell culture medium, spinach and seafood, and HAV appeared to be better protected by the seafood matrix with D-values of 0.88 and 1.07 min at 72°C for HAV in cell culture medium and mussels, respectively, but no formal statistical comparison was reported (Bozkurt et al. 2014a, b, 2015). In contrast, there was no obvious protective effect from a matrix high in protein and fat (e.g. complex pet food) on inactivation of FCV (Haines et al. 2015).

Blanching, a widely used industrial process, of spinach at 80°C for 1 min reduced infectious MNV by at least $2.4 \log_{10}$ (Baert et al. 2008b). Steam blanching of various herbs at 95°C for 2.5 min showed inactivation of both HAV and FCV (Butot et al. 2009). Deboosere et al. (2010) developed a thermal inactivation model for HAV in red berries at different pH values and showed reduced pH led to faster inactivation in

the tested range of pH 2.5-3.3. Barnaud et al. (2012) showed that heating pork meat to an internal temperature of 71°C for 20 min was necessary to inactivate HEV and heating at 70°C for 2 min in buffer resulted in no detectable virus (>3.9-log decrease) using a cell culture based method (Johne et al. 2016). These result differences in lethal effects may due to the matrix used in thermal inactivation studies and is not uncommon.

5.4.2 Effect of heat on viruses in food matrices with low water activity

Significantly more time was needed to achieve a 2.0 log₁₀ inactivation of HAV in freeze-dried berries (20 min) compared to fresh herbs (2.5 min), which probably reflects the difference between dry and wet heat applications (Butot et al. 2009). In contrast, at a similar temperature (65.9°C), 20 h of dry heat applied to green onions was needed to reduce infectious HAV by >3.9 log₁₀ (Laird et al. 2011). Another study investigated the thermal inactivation of HAV in strawberry mashes supplemented with different sucrose concentrations showed D_{85°C} value obtained at 52°Brix of sucrose was approximately eight fold higher than at 28°Brix (Deboosere et al. 2004), demonstrating the protective effect of sugar on the thermal stability of HAV.

5.5 High pressure processing

The treatment of foods with high pressure processing (HPP) is based on compressing the food suspended in liquid and releasing pressure quickly (Barbosa-Canovas et al. 1998). Early HPP studies were conducted using FCV suspended in isotonic tissue culture medium and its inactivation after 5 min exposure to 275 MPa or more indicated applicability of HPP for inactivating human NoV (Kingsley et al. 2002). Also a pressure of 600 MPa at 6°C for 5 min was found to be sufficient to

completely inactivate NoV in oysters (Leon et al. 2011; CDC 2012). HAV and poliovirus (PV) are members of the Picornaviridae family but have differing susceptibilities; HAV can be inactivated by HPP while PV is resistant (Table 9).

HPP inactivation is strongly influenced by processing temperature, pH and salt concentration within the food, with higher efficiencies at an acidic pH and lower efficiencies at increasing salt concentrations (Kingsley and Chen 2009; D'Andrea et al. 2014). The dissociation and denaturation of proteins and inactivation of viruses by pressure are promoted by low temperatures (Weber 1993; Foguel et al. 1995; Gaspar et al. 1997; Bonafe et al. 1998; Tian et al. 2000; Kunugi and Tanaka 2002) possibly due exposure of nonpolar side chains to water at lower temperatures resulting in nonpolar interactions that are more affected by pressure and more compressible. However, the use of appropriate pressures, as shown in the volunteer study by Leon et al. (2011) and surrogates as concluded by Cromeans et al. (2014), demonstrating that TuV and MNV were appropriate surrogate viruses for HPP studies that mimic human NoV inactivation, are important factors.

As mentioned previously, the intrinsic properties can affect viral inactivation, as NaCl may act to stabilize viral capsid proteins thus requiring higher pressures for inactivation (Kingsley et al. 2002; Grove et al. 2009; Sánchez et al. 2011). Such observations may have important implications for future applications of HPP to shellfish and food products.

5.6 Ionizing radiation technologies

While irradiation is effective in preserving foods for the marketplace, its effectiveness against viruses is dependent on the size of the virus, the suspension medium, food product characteristics, and the exposure temperature (Patterson 1993; Farkas 1998). Most viruses are far more resistant to irradiation (Table 10) than vegetative bacteria, parasites, and fungi which may be due to their smaller size and even smaller genome size (often single-stranded RNA) (Farkas 1998). Two major irradiation technologies, gamma irradiation and electron beam (E-beam) that use high-energy electrons have been explored. A maximum absorbed dose allowed by the US Food and Drug Administration (FDA) is 4.0 kGy (FDA 2007), while in Europe the maximum allowed dose is 10.0 kGy (EFSA 2011). Doses permitted by international regulatory agencies vary depending on the type of food. However, the US FDA approved dose of 4 kGy is likely to achieve approximately 1.0 log₁₀ viral reduction and higher doses will be required to achieve higher viral reductions in most foods. Exposure to 8 kGy of gamma irradiation of a human NoV GII.3 and GII.4 stool suspension inactivates the viruses, as demonstrated using the stem cell derived human enteroids assay (Ettayebi et al. 2016). Considering work carried out using surrogates, MNV appears to be more resistant than TuV when treated with E-beam (Predmore et al. 2015).

5.7 Light based technologies

Light based technologies include UV light and high-intensity pulsed light (PL) (Table 11). Pulsed light involves electrical ionization of a xenon lamp to emit a broadband white light with a spectrum resembling that of sunlight (45% UV light).

The mechanism involved in antiviral activity of PL is probably disruption of viral structure that ultimately degrades viral proteins and RNA. PL at 12 J/cm² with 3-6 s exposure resulted in > 3.0 log₁₀ reduction of MNV in various liquids (Vimont et al. 2015). PL or UV may be used in combination with other control strategies (e.g. chlorine) resulting in synergistic benefits that could lead to increased UV induced viral genome damage (Rattanakul et al. 2015). However, the effectiveness of light based technologies is limited to certain types of liquids or surface decontamination. Various food characteristics such as turbidity of the liquid medium can affect UV or PL penetration and slower flow rates used to extend exposure times for better UV or PL efficacy may not be realistic. Successful application of this technology relies on the light reaching all the virus particles directly and if the viruses are present in cracks, crevices or openings in the surface of the food or surfaces, the viruses may be shielded from exposure to the light and will therefore survive.

5.8 Sanitizers used in produce processing

One of the main control strategies used by the produce industry is the use of chlorine in the form of sodium hypochlorite, calcium hypochlorite and hypochlorous acid from electrolyzed water. For fresh salad produce, such as salad leaves, peppers, carrots, cucumbers, the common industry practice is to wash in 30-40 ppm free chlorine at pH 6.8-7.1. Soft fruits such as strawberries and raspberries are typically exposed to a quick spray or 10 s immersion in 15-20 ppm free chlorine (Seymour 1999). Sodium hypochlorite with free chlorine levels (15-20 ppm for 1-2 min wash), resulted in reductions of 0.6 to 2.9 log₁₀ of viral surrogates (Casteel et al. 2008; Fraisse et al. 2011). Other sanitizers include hydrogen peroxide and ozone which are also strong oxidizing agents with examples of produce decontamination studies that included

product inoculation with a surrogate virus and an incubation step to mimic viral contamination of food products in the field are listed in Table 12.

During washing, water can act as a vehicle for virus cross contamination of fresh produce, and sanitizer in wash water reduces this risk (Holvoet et al. 2014). In addition to type and concentration of sanitizer, the efficacy of decontamination depends on the type of produce as well as the virus surrogate used, and method of inoculating the produce. With some produce types, the sanitizer may not penetrate cracks, crevices and openings and the protective waxy cuticle could act as a barrier while exudates from leafy green vegetables may allow viruses to attach and locate near pores or stomata thereby reducing sanitizer effectiveness due to reduced accessibility (Takeuchi and Frank 2000). Incorporating a surfactant to remove the waxy layer on certain fresh produce can increase the efficacy of the sanitizer (Predmore and Li 2011) and incorporating physical methods e.g. high power ultrasound can be used to dislodge viruses on the surface and improve sanitizer-produce interaction (Liu et al. 2009; Maks et al. 2009).

5.9 Challenges for validation

Food components and ingredients can have some antiviral properties and along with the intrinsic and extrinsic factors of foods, can play a role in controlling or reducing the viral load in foods. When combined with appropriate processing technologies, these factors can enhance the safety of susceptible foods by significantly reducing viral loads. In order to determine if processes applied to various food matrices are adequate, prevalence studies will be required to determine likely/worst case levels of

human enteric viruses in raw material from different geographical areas so that appropriate control measures could be designed and validated.

Validation is defined as “Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome” (Codex Alimentarius 2008) and the effectiveness of the control measure against viruses needs adequate evaluation and validation.

Currently used/applied food processing technologies can generally achieve approximately 1.0 log₁₀ to 3.0 log₁₀ reduction. However, the choice of surrogate and its preparation, treatment time, inoculation methods and time allowed for inoculum to attach to product and differences in analytical methods could have significant impact on observed reduction data (Knight et al. 2016). Hence, a standardized or harmonized method for evaluating decontamination strategies for foods would be very useful (Table 13). In the absence of a large scale and widely available cultivable human NoV assay, evaluation and validation of antivirals and processes are commonly performed using a cultivable surrogate. It is yet unclear if inactivation data obtained through the use of surrogates are representative for human NoV. Additionally, variations in surrogate inactivation levels have been documented. Even if inactivation of a surrogate and a human NoV strain is correlated, the resistance of other human NoV strains is unknown. A surrogate for HEV is also needed, as validation is currently not possible and inactivation is difficult to assess due to the need of an animal model (swine bioassay). However, using newly established cell culture methods, comparisons with surrogates should be possible (Ettayebi et al. 2016; Johne et al. 2016). Similarly, identified surrogates need to be cultured to high

titers for industry pilot-scale trials in order to establish process validity along with simple rapid methods for reliable detection and quantitation. The use of virus-like particles may be an alternate choice with the added bonus of enabling their use in scenarios where actual viruses cannot be introduced for safety reasons (Crawford et al. 1994; Bertolotti-Ciarlet et al. 2002). The NoV culture method (Ettayebi et al. 2016) is a significant advancement for NoV research. However, quantification of inactivation levels above 3.0 log₁₀ delivered by most processing technologies may be difficult to evaluate.

The use of processing technologies may improve the overall safety of the product but it cannot replace sound harvesting and manufacturing practices with regards to sanitation and hygiene. Incorporating additional preservation steps, such as thermal or high pressure processing, to an existing process should assist in destroying (or eliminating) viruses in many foods including seafood and minimally processed produce. Similarly, control strategies used to inactivate viruses in foods will require validation studies to confirm that the control strategies indeed work in controlling the viral hazard in the food of concern.

6. Discussion

Over the last 20 years, reports of foodborne illness outbreaks caused by viruses have been steadily increasing. Thus, foodborne viruses are a very serious threat to overall global health. While scientific information about viruses is increasing, and with the exception of a few industries such as shellfish and food service, there has been little guidance towards effective mitigation strategies and risk assessments provided for the industry. For risk assessors in industry and government, many

questions remain, and more work needs to be done on the prevalence of various foodborne viruses across commodities.

Due to on-going developments, it is difficult to have an overview of all viruses involved, related detection methods, underlying controls and risk assessment options. Therefore, the authors felt the need for a review focusing on understanding the limitations of existing control technologies and recommending potentially effective approaches for the future. In addition with the background on viral detection and behavior, it helps to facilitate discussions on control measures and their limitations. Attempts have been made to develop surrogate systems for viruses (e.g. bacteriophages or other model viruses). However, virus behavior is very type-specific and thus, there is a need to identify a large number of surrogates and improve detection methods to allow quantification following application of control measures. A recent review of NoV even suggested discontinuing all surrogate studies unless direct comparison between surrogate and NoV inactivation kinetics is established (Cook et al. 2016). The recent propagation system described for human NoV (Ettayebi et al. 2016) opens the possibility to develop more appropriate risk assessment models and recommendations for adequate processing technologies.

As detection methods improve and new ones are developed, the association of viruses with foodborne illness will only increase. In addition, there is potential for the detection of new and emerging viruses to be implicated in foodborne illness outbreaks. Furthermore, with the advancements in genomics and molecular microbiology, there is promise of continuous advancement in detection methods enabling not only improved phylogenetic characterization of viruses but also

enhancement of our ability to identify the geographic origins of food contamination (Hoffmann et al. 2016). The latter will help to improve food traceability to fully understand how and or where food becomes contaminated. However, with the development of new molecular methods and technologies for detection of viruses, as well as the implementation of metagenomic approaches, a better understanding or interpretation of a positive result is essential (Ceuppens et al. 2014).

Traditionally, processing technologies rely on the control of bacterial contaminants as a measure of their effectiveness. The relevance of viruses has become more evident in recent years, and therefore processing technologies are now also being assessed for their efficiency against viruses. Various studies have shown that some foodborne viruses are, in fact, more resistant than vegetative bacteria to certain control mechanisms and thus may not be inactivated at the same rate as bacteria (Bozkurt et al. 2014a, b). In addition, as the food industry increasingly moves towards milder thermal processes, as well as the use of non-thermal technologies, the likelihood of viruses surviving such treatments may increase.

This risk may be enhanced by the fact that we do not have reliable tools for validation of virus inactivation. Current validation approaches are hampered by the difficulty in cultivating viruses and by the unreliable surrogates that are currently available (see also Section 5.10).

A concerted research effort needs to be undertaken to understand the ecology, behavior and transmission of foodborne viruses from the farm and other potential sources, to the consumer. Such a research effort must not only focus on the in-depth

understanding of virus physiology and behavior, but also on the development of reliable and easy-to-use tools and technologies to detect, identify and model the fate of foodborne viruses. A portfolio of such optimized and standardized tools may allow scientists, industry professionals and regulators to develop appropriate risk assessment scenarios and process options for effective control of foodborne viruses.

In the overall context of foodborne viruses, it is necessary for all experts (academic, industry and regulatory) to harness the power of modern technology (e.g. Next Generation Sequencing, 'omics) to develop new paradigms in the study of viruses. The Food Industry will then be able to apply these learnings and tools to develop science-based, integrated food safety management systems, which guarantee transparency and safety to the consumer. Such an integrated system would encompass:

- (a) Primary production** – implementing best practices in agriculture and animal husbandry to ensure that viral (and other pathogen) contamination of raw materials is avoided;
- (b) Processing** – implementing robust decontamination technologies and validation tools to demonstrate the effectiveness of processes used including training and compliance of food handlers in good hygienic practices;
- (c) Consumer use** – implementing consumer-friendly guidelines based on sound science to ensure that foods do not become contaminated during use;
- (d) Surveillance and Monitoring** – implementing a robust surveillance and monitoring system that includes contamination incidents can increase trust in the food supply since data from surveillance networks are invaluable in understanding and predicting the spread of foodborne viruses.

It is important to assess viral hazards within food safety plans/management and include potential measures to control viruses taking current knowledge into account. The implementation of most control measures can be improved with a focus on training, supplier controls during processing and on intervention strategies in case of outbreaks (e.g. specific cleaning techniques). Training should focus on changing food handler and consumer habits, and creating a food safety culture, with awareness of effective hygiene measures (e.g. proper hand washing). Additionally, communication of gastrointestinal illness and how to contain the spread of infections e.g. by staying at home for a minimum number of days following gastrointestinal illness (currently 2-3 days according to a recommendation by Food Standards Agency UK), can help in preventing NoV transmission. Proper hand washing and strict compliance of hygienic measures are essential and still among the best control measures in preventing foodborne virus transmission by food handlers. In addition, when available, vaccination of food handlers e.g. HAV vaccination is recommended.

The rapid development of our understanding of foodborne viruses and their behavior in the last decade has enabled the application of risk assessment tools and assessing the effectiveness of food processing technologies for controlling viruses. However, some of the questions raised at the beginning remain unanswered, like the relationship between detected genome copies and infective virus particles. New knowledge has led to a more critical view, e.g. looking at equivalence in behavior when comparing target viruses and surrogates. New insights have raised more concern on whether usage of surrogates allows for any correlation with respect to the behavior of target viruses. The difficulty of cultivating viruses and reliable

methods for their detection at low levels are currently major factors to be addressed in order to allow further, more in depth research in all other areas. To make the best use of all data available, it is important that we explore the benefits of various risk assessment approaches to understand virus behavior. This insight can then be used to develop adequate control measures. In conclusion, effective tools and technologies to ensure control of viruses in the food chain can significantly reduce foodborne infections caused by viruses.

Acknowledgements and potential conflict of interest

This work was conducted by an expert group of the European branch of the International Life Science Institute (ILSI Europe). The expert group and this publication were coordinated and funded by the Microbiological Food Safety Task Force. Industry members of this task force/expert group are employees of food industry companies, and are listed on the ILSI Europe website at <http://ilsi.eu/task-forces/food-safety/microbiological-food-safety/>. All expert group members declare no potential conflicts of interest. The experts were not paid for the time spent on this work; however, the ILSI Europe Microbiological Food Safety Task Force offered to all non-industry members within the expert group support for travel and accommodation cost to attend meetings for discussing the manuscript, and a small compensatory sum (honorarium) with the option to decline. The expert group carried out the work, i.e. collecting/analysing data/information and writing the scientific paper separate to other activities of the task force. The research reported is the result of a scientific evaluation in line with ILSI Europe's framework to provide a precompetitive setting for public-private partnership (PPP). The opinions expressed herein and the conclusions of this publication are those of the authors and do not necessarily

represent the views, positions or policies of Campden BRI, IFREMER, ILSI Europe, Arla Foods, bioMérieux, Mondelēz Int., Nestlé, PepsiCo, Unilever, or any authors affiliation. ILSI Europe facilitated scientific meetings and coordinated the overall project management and administrative tasks relating to the completion of this work. In particular, the authors would like to thank Ms Lilou van Lieshout. Furthermore, the authors would like to acknowledge Prof. Marcel Zwietering for his critical reviews of the paper and contributions to the discussions during face-to-face meetings and Dr Alejandro Amezcua for taking over the official lead on behalf of Unilever from Dr Balkumar Marthi and his active involvement in the discussions. For further information about ILSI Europe, please email info@ilsieurope.be or call +32 2 771 00 14.

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Table 1. Contribution of Viruses to Global Burden of Foodborne Disease¹.

Diseases/Infections	Foodborne Illness (millions)	Percentage of Total Illnesses	Foodborne DALYs (millions)	Percentage of Total DALYs
Total Foodborne	600	-	33.0	-
Norovirus	120	20%	2.5	7.6%
Hepatitis A Virus	14	2%	1.4	4.2%

¹ Global burden of foodborne disease expressed as total number of illnesses and Disability Adjusted Life Years (DALYs). Percentages are calculated based on the Total Foodborne Disease Burden. Data from 2010. Adapted from WHO estimates of the global burden of foodborne diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015 (World Health Organization 2016)

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Table 2. Viruses documented to be found in the human gastrointestinal tract¹.

Genus	Genome	Popular name	Disease caused
<i>Enterovirus</i>	ssRNA	Poliovirus Coxsackie A, B virus Echovirus	Paralysis, meningitis, fever Herpangina, meningitis, fever, respiratory disease, hand-foot- and-mouth disease, myocarditis, heart anomalies, rash, pleurodynia, diabetes* Meningitis, fever, respiratory disease, rash, gastroenteritis
<i>Hepatovirus</i>	ssRNA	Hepatitis A virus	Hepatitis
<i>Kobuvirus</i>	ssRNA	Aichi virus	Gastroenteritis
<i>Parechovirus</i>	ssRNA	Human parechovirus	Respiratory disease, gastroenteritis, CNS infection
<i>Orthoreovirus</i>	segmented dsRNA	Human reovirus	Unknown
<i>Rotavirus</i>	segmented dsRNA	Human rotavirus	Gastroenteritis
<i>Norovirus</i>	ssRNA	Human norovirus	Gastroenteritis
<i>Sapovirus</i>	ssRNA	Human sapovirus	Gastroenteritis
<i>Hepevirus</i>	ssRNA	Hepatitis E virus	Hepatitis
<i>Mamastrovirus</i>	ssRNA	Human astrovirus	Gastroenteritis, CNS infection
<i>Flavivirus²</i>	ssRNA	Tick-borne encephalitis virus	Encephalitis, meningitis
<i>Coronavirus</i>	ssRNA	Human coronavirus	Gastroenteritis, respiratory disease, SARS, MERS
<i>Orthomyxovirus</i>	segmented ssRNA	Avian influenza virus	Influenza, respiratory disease
<i>Henipavirus</i>	ssRNA	Nipah virus, Hendra virus	Encephalitis, respiratory disease
<i>Parvovirus</i>	ssDNA	Human parvovirus	Gastroenteritis
<i>Mastadenovirus</i>	dsDNA	Human adenovirus	Gastroenteritis, respiratory disease, conjunctivitis
<i>Polyomavirus</i>	dsDNA	Polyomavirus	Progressive multifocal leukoencephalopathy, diseases of urinary tract
<i>Alphatorquevirus</i>	ssDNA	TT (Torque Teno) virus	Unknown, hepatitis*, respiratory disease* haematological Disorders*, cancer*

*uncertain whether the disease is caused by the virus.

¹Any virus in the gastrointestinal tract could potentially be transmitted via food.

²Has been found in food (milk) but not in gastrointestinal tract.

Table 3. Advantages and disadvantages of available methods for detection of human enteric viruses in food.

Method	Advantages (pros)	Disadvantages (cons)
ISO/CEN method	<ul style="list-style-type: none"> • Major viruses and food matrices are included • Increased confidence in the results due to use of controls and detailed description of how to interpret results; • International recognition of an ISO method increases implementation of a harmonised method in laboratories; • Introduces the possibility to compare and evaluate results from different laboratories; • Facilitates accreditation of laboratories for virus testing. 	<ul style="list-style-type: none"> • Improvements of the methods may be halted • Does not include methods for processed food matrices; • The high number of controls increases costs; • Commercial controls must be available; • May lead to non-detection of low levels of virus in some specific matrices; • Cannot distinguish between infectious and non-infectious particles; • Method complexity.
Quantification and confirmation	<ul style="list-style-type: none"> • Routine quantification provides data on baseline levels of viruses in food matrices and will inform implementation of acceptable levels; • Systematic confirmation of RT-qPCR results by sequencing provides information on virus strain epidemiology 	<ul style="list-style-type: none"> • Quantification by RT-qPCR is sensitive to inhibitors and has an unreliable accuracy for low levels of virus; • Confirmation of RT-qPCR positive results by sequencing is difficult due to low sensitivity; • Quantification and confirmation increase cost; • Time consuming.
Molecular virus detection from intact virus capsids	<ul style="list-style-type: none"> • Reduces overestimation of the number of infective virus particles. 	<ul style="list-style-type: none"> • A broad range of reagents needs to be developed; • Needs careful evaluation of protocols according to type of virus and matrices; • Infective and non-infective controls must be included; • Increases costs compared to standard PCR method.
Detection of infective viruses	<ul style="list-style-type: none"> • Allows detection of infectious viruses • ICC-RT-PCR <ul style="list-style-type: none"> ○ is more sensitive than cell culture alone; ○ detects infectious viruses that do not show cytopathogenic effect; ○ shortens the time for analysis compared to cell culture alone 	<ul style="list-style-type: none"> • Wild-type enteric viruses are generally difficult to cultivate; • A simple cultivation system for NoVs need to be optimized; • Cultivation increases the cost and time needed for diagnostics; • ICC-RT-PCR is not quantitative unless used as a Most Probable Number (MPN) test.
New technologies	<ul style="list-style-type: none"> • Digital PCR <ul style="list-style-type: none"> ○ is less sensitive to inhibitors in food matrices; ○ provides more accurate quantification 	<ul style="list-style-type: none"> • Increased costs and sample preparation; • Absence of standardized approach for next generation sequencing.

	independent of standard curves; <ul style="list-style-type: none">• Next generation sequencing can pick up emerging viruses and new virus strains .	
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Table 4. Overview of bottom-up risk assessments of viruses in food and drinking water.

Virus	Commodity	Year	Qualitative	Quantitative	Deterministic	Stochastic	Reference
Norovirus	frozen raspberries	2017		+		+	(Jacxsens et al. 2017)
Hepatitis E	raspberry purree						
Hepatitis E	swine liver and sausages	2017		+		+	(Sarno et al. 2017)
Hepatitis E	pork and wild boar products	2017		+		+	(Müller et al. 2017)
Ebola	cocoa beans	2016		+2			(Bergeron et al. 2016)
	palm oil						
	cashews						
Hepatitis A	clams, mussels	2015		+	+	-	(Polo et al. 2015)
Norovirus							
Norovirus	leafy green vegetable	2015		+		+	(Bouwknegt et al. 2015))
Norovirus	berry fruit	2015		+		+	
Hepatitis A	leafy green vegetable	2015		+		+	(Bouwknegt et al. 2015))
Hepatitis A	berry fruit	2015		+		+	(Bouwknegt et al. 2015))
Norovirus, hepatitis A	lettuce	2015		+		+	(Kokkinos et al. 2015)
Rotavirus, norovirus	street food salads	2014		+		+	(Barker et al. 2014)
Norovirus GI and GII	oysters	2013		+		+	(Thebault et al. 2013)
Hepatitis A	raw oysters	2012		+		+	(Thebault et al. 2013)
Norovirus	oysters	2012		+ ²			(Lowther et al. 2012)
Hepatitis A	prawns	2011	+				(Sumner 2011)
Avian influenza	poultry, shell eggs and egg products	2010		+		+	(Bauer et al. 2010)
Avian influenza	poultry	2010		+		+	(Sánchez-Vizcaíno et al. 2010)
¹ H5N1	poultry, wild birds?	2009	+				(Métras et al. 2009)
Hepatitis A	shellfish	2009		+	+		(Pintó et al. 2009)
H5N1	chicken	2009					(Golden et al. 2009)
Norovirus	drinking water	2006		+		+	(Masago et al. 2006)
Avian influenza	water	2005		+		+	(Schijven and Teunis 2006)
Avian influenza (H5 and H7)	poultry eggs	2004	+				(Sabirovic et al. 2004)
Norovirus, Hepatitis A	seafood	2002		+ ²	+		(Sumner and Ross 2002)

¹Highly Pathogenic Avian Influenza²Semi-quantitative

Table 5. Inactivation of viruses due to intrinsic and extrinsic properties of food.

Control measures	Matrix	Virus	Log ₁₀ reduction	Reference
Salt (2-20% w/v) neutral pH for 7 days at 4 & 20°C	Phosphate buffered (PBS) Saline	ECHO (Enteric Cytophatic Human Orphan Virus)	No reduction	(Straube et al. 2011)
Salt (6% w/v) neutral pH for 7 days at 4 & 20°C	PBS	FCV	2.2	(Straube et al. 2011)
10% Salt for 3 days at 10°C	Salted product oyster	MNV	0.4 0.6	(Park and Ha 2014)
Soy sauce containing 20, 15, 10, 5% salt for 5 days at 10°C	Preserved raw crab product in soy sauce	MNV	1.6 (20%) 1.4 (15%) 1.0 (10%) 0.6 (5%salt)	(Park and Ha 2015)
Soy sauce containing 20, 15, 10, 5% salt for 3 days at 10°C	Preserved raw crab product in soy sauce	MNV	1.0 (20%) 0.8 (15%) 0.5 (10%) 0.3 (5%salt)	(Park and Ha 2015)
pH 5.2 for 24 h at 22°C	Raw batter sausage	MNV	0.7	(Lange-Starke et al. 2014)
pH 3.2 (0.4% w/w DL-lactic acid) for 7 days at 4 & 20 °C	PBS	FCV ECHO	>6.0 (20°C), 2.0 (4°C) 0.3 (20°C), 0 (4°C)	(Straube et al. 2011)
pH 3.2 (0.4% w/w DL-lactic acid) for 3 h at 20°C	PBS	FCV	1.5	(Straube et al. 2011)
pH 2 for 1 h at 25°C	Cell culture media adjusted with HCl	MNV TuV	~0.0 0.4	(Li et al. 2013)
pH 10 for 1 h at 25°C	Cell culture media adjusted with NaOH	MNV TuV	~1.2 ~1.0	(Li et al. 2013)
Fermentation, 5% salt, 15 days, 18°C	Oyster	MNV FCV	1.6 3.0	(Seo et al. 2014)
Fermentation 20 days	Vegetable (dongchimi)	MNV FCV	1.5 4.2	(Lee et al. 2012)
<i>Lactococcus lactis</i> sp. <i>lactis</i> 24 h, 37°C	Bacterial Growth Medium Cell-Free Filtrate (BGMF) and Bacterial Cell Suspension (BCS)	FCV	1.3 (BGMF) 1.8 (BCS)	(Aboubakr et al. 2014)

Table 6. Antiviral effects of food components, food extracts and metal ions

Control measures	Matrix	Virus	Log ₁₀ reduction	Reference
Grape seed extract, 1-4 mg/ml, 24 h	Milk	MNV HAV	1.0	(Joshi et al. 2015b)
Grape seed extract, 1-2 mg/ml, 1 h	Apple juice	MNV HAV	5.0	
Grape seed extract, 0.25-1 mg/ml, 1 min	Lettuce	MNV HAV	0.0-0.3 0.7-1.3	(Su and D'Souza 2013a)
	Pepper	MNV HAV	0.0-0.8 0.7-1.3	
Grape seed extract, 0.5-2 mg/ml, 2 h	Cell culture medium	MNV HAV	0.8-1.7 1.8-3.2	(Su et al. 2011)
Grape seed extract, 2.5%, 3 h	Water	MNV	3.6	(Amankwaah 2013)
Cranberry juice, 50%, 1 h	Cell culture medium	MNV MS2	2.0-2.9 1.1	(Su et al. 2010)
Mulberry juice, 0.005%, 1 h	Cell culture medium	MNV	0.3	(Lee et al. 2014)
Black raspberry juice, 3 and 6%, 1 h	Cell culture medium	MNV	0.6-0.8	(Oh et al. 2012)
Pomegranate juice, 50%, 29 min	Cell culture medium	MNV MS2	0.8 0.2	(Su et al. 2011)
	PBS	MNV	0.0	
Orange juice, 21 days, 4°C			1.4	(Horm and D'Souza 2011)
Pomegranate juice, 21 days, 4°C			5.0	
Blend, 7 days				
Green tea extract, 2.5%, 3 h	Water	MNV	3.3	(Amankwaah 2013)
Acylated peptides from soybean 25 ug/ml, 1 h	Buffer	FCV	4.0	(Matemu et al. 2011)
Rutinosides of phenolic acids, 100-200 uM, 1 h	Cell culture medium	FCV	0.5-1.0	(Katayama et al. 2013)
Silver nano particles, 10 ⁷ -10 ⁹ particles/ml, different size, 1-6 h, 25°C	Water	MNV	0.5-6.0	(Park et al. 2014)
Silver-infused polylactide films, 0.1-1% wt, 24 h, 24°C	Buffer		2.0->4.4	(Martínez-Abad et al. 2013)
	Lettuce	FCV	>4.4	
	Paprika		0.0-1.0	
Biogenic silver nano particles, 5.4 mg/L, 30 min, 28 °C	Water	MNV	>4.7	(De Gusseme et al. 2010)
Chitosan, 0.7-1.5%, 3h, 37 °C	Water or acetic acid	MNV	0.1-1.0	(Davis et al. 2015)
		MS2	2.6-5.2	
		FCV	2.2-2.9	
Chitosan, 0.7%, 3h, 37 °C	Water	MNV	0.3	(Davis et al. 2012)
		MS2	2.4	
		FCV	0.2-3.4	
Chitosan, 0.7%, 3h, 37 °C	Water	MNV	0.0	(Su et al. 2009)
		MS2	1.4	
		FCV	2.8	

Table 7. The effects of biochemicals and essential oils (EO) on various viruses.

Control measures	Matrix	Virus	Log ₁₀ reduction	Reference
Oregano EO, 2%, 2 h, 37°C	Cell culture medium	MNV FCV	1.6 3.8	(Azizkhani et al. 2013)
Oregano EO, 4 %, 15 min-24 h, 24 °C	PBS	MNV	0.6	(Gilling et al. 2014a)
Oregano EO, 0.5-1% Zataria EO, 0.01-1% Thymol EO, 0.1-2% 2h, 37 °C	DMEM	HAV MNV HAV	0.1-0.4 0.0-0.4 0.1-2.5 0.0-0.2	(Sánchez and Aznar 2015)
Allspice EO Lemongrass EO 2-4%, 6-24 h, RT	PBS	MNV	0.7-3.4 0.7-2.7	(Gilling et al. 2014b)
Carvacrol, 0.5% Carvacrol, 1,0% 2h, 37°C	DMEM + 2% FCS	MNV HAV	6.0-7.0 1.0	(Sánchez et al. 2015)
Carvacrol, 0.5%, 15 min - 24h, 24°C	PBS	MNV	1.3-4.5	(Gilling et al. 2014a)
<i>Hibiscus sabdariffa</i> extract, 40-100 mg/ml, 24 h, 37°C	Deionised distilled water	MNV HAV	5.0 5.0	(Joshi et al. 2015a)
Flavonoids (four different), 0.5-1.0 mM, 2 h, 37°C	Cell culture medium	MNV FCV	0.0 0.0-5.0	(Su and D'Souza 2013b)
Flavonoids from sea grass, 20 ug/ml	Cell culture medium	HAV	>3.0	(Hamdy et al. 2012)
Proanthocyanidin (tannins), 0.1-5 mg/ml, 10 s	Water	FCV	0.1-3.0	(Iwasawa et al. 2009)

Table 8. Effect of thermal treatment on viruses in various matrices.

Control measure	Matrix	Virus	Log ₁₀ reduction	Reference
Rolling boil for 1 min minimum	Water	Enterovirus, HAV, NoV, Human Rhinovirus	>4.0	(CDC 2009)
72°C, 1 min	Water	MNV HAV	>3.5	(Hewitt et al. 2009)
71°C, 0.63 min	Milk	HAV	3.0	(Bidawid et al. 2000)
71°C, 7.09 min	Cream	HAV	3.0	(Bidawid et al. 2000)
79°C, 0.5 min	Petfood	FCV	>4.4	(Haines et al. 2015)
95°C, 2.5 min	Basil	FCV HAV	>4.0 >3.0	(Butot et al. 2009)
80°C, 1 min	Spinach	MNV	≥2.4	(Baert et al. 2008b)
75°C, 0.25 min	Raspberry puree	MNV	2.8	(Baert et al. 2008a)
80°C, 20 min	Freeze-dried berries	HAV	<2.0	(Butot et al. 2009)
65.9 °C, 20 h	Green onions	HAV	>3.9	(Laird et al. 2011)
85°C, 5 min	Strawberry mashes (52° Brix)	HAV	1.0	(Deboosere et al. 2004)
85°C, 1 min	Strawberry mashes (28° Brix)	HAV	1.0	(Deboosere et al. 2004)
60°C, 15 min	Stool	HuNoV	>5.0	(Ettayebi et al. 2016)

Table 9. High pressure effects on various viruses

Control Measure	Matrix	Virus	Log ₁₀ reduction	Reference
600 MPa, 5 min, 21°C	Cell culture medium	Aichivirus A846/88	0.0	(Kingsley et al. 2004)
275 MPa, 5 min, 22°C	Cell culture medium	FCV	7.0	(Kingsley et al. 2002)
375 MPa, 5 min, 22°C	Strawberry puree	HAV	4.3	(Kingsley et al. 2005)
	Sliced green onions		4.8	
400 MPa, 10 min, 25°C	Cell culture medium	Human cytomegalovirus	4.0	(Nakagami et al. 1992)
600 MPa, 5 min, 21°C	Cell culture medium	Human Parechovirus-1	4.6	(Kingsley et al. 2004)
400 MPa, 8 min, 22°C	Cell culture medium	Phage Φ	7.7	(Chen et al. 2004)
400 MPa, 20 min, 22°C	2% reduced fat milk		7.1	
600 MPa, 60 min, 20°C	Cell culture medium	Poliovirus	<1.0	(Wilkinson et al. 2001)
300 MPa, 2 min, 25°C	Cell culture medium	Rotavirus	8.0	(Khadre and Yousef 2002)
500 MPa, 5 min, 20°C	Cell culture medium	HAV	>3.5	(Grove et al. 2008)
300 MPa, 3 min, 20°C	Cell culture medium	FCV	>3.6	(Grove et al. 2008)
600 MPa, 5 min, 20°C	Cell culture medium	PV	0.0	(Grove et al. 2008)
600 MPa, 10 min, 13°C	Dry-cured ham	MS2	1.3	(Emmoth et al. 2016)

Table 10. Irradiation effects on viruses

Control Measure	Matrix	Virus	Log₁₀ reduction	Reference
4.05 kGy E-beam	Oysters	MNV	1.0	(Sanglay et al. 2011)
4.83 kGy E-beam	Oysters	HAV	1.0	(Sanglay et al. 2011)
2 kGy E-beam	PBS, DMEM	MNV	<1.0	(Praveen et al. 2013)
4-12 kGy E-beam	PBS DMEM	MNV	up to 6.4 up to 3.6	(Praveen et al. 2013)
4 kGy E-beam	Shredded	MNV	1.0	(Praveen et al. 2013)
12 kGy E-beam	cabbage		<3.0	
6 kGy E-beam	Diced	MNV	<1.0	(Praveen et al. 2013) ¹
12 kGy E-beam	strawberries		2.2	
16 kGy E-beam	Strawberry, lettuce	TuV	7.0	(Predmore et al. 2015)
Gamma irradiation	Stool	HuNoV	>5.0	(Ettayebi et al. 2016)
0.2 kGy gamma	Tap water, pH 7.6	Canine calicivirus FCV	2.4 1.6	(de Roda Husman et al. 2004)
2.84 kGy gamma	Oyster	PV	1.0	(Jung et al. 2009)
2.72 kGy gamma	Lettuce	HAV	1.0	(Bidawid et al. 2000)

Table 11. Effect of light based technologies on viruses

Control Measure	Matrix	Virus	Log₁₀ reduction	Reference
12 J/cm ² , 3-6 s, pulsed light	Various liquids	MNV	>3.0	(Vimont et al. 2015)
1.2 J/cm ² , UV + water	Blueberries	MNV	>4.3	(Liu et al. 2015)
1.2 J/cm ² UV	Blueberries	MNV	2.5	(Liu et al. 2015)
1.0 J/cm ²	PBS	Enveloped viruses	4.8	(Roberts and Hope 2003)
		Non-enveloped viruses	7.2	

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Table 12. Sanitisers used for produce washing and effects on viruses

Control measure	Matrix	Virus	Log ₁₀ Reduction	Reference
20 ppm free chlorine, 1 min	Strawberries	MS2	1.2	(Casteel et al. 2008)
	Cherry tomatoes		0.6	
	Head lettuce		1.1	
		0.7		
		HAV	1.4	
			1.0	
Potable water, 2 min and 0.5 min rinse	Iceberg lettuce perilla leaf	NoV	0.9-1.3	(Bae et al. 2011)
household detergent (0.1% conc.), 2 min and 0.5 min rinse	Iceberg lettuce perilla leaf	NoV	1.0-1.1	(Bae et al. 2011)
Sodium hypochlorite (15 ppm free chlorine), 2 min	Butter lettuce	HAV	1.9	(Fraisie et al. 2011)
		FCV	2.9	
		MNV	1.4	
peroxyacetic acid (POAA) based biocide (100 ppm), 2 min	Butter lettuce	HAV	0.7	(Fraisie et al. 2011)
		FCV	3.2	
		MNV	2.4	
Bubbles and ultrasound, 2 min	Butter lettuce	HAV	0.8	(Fraisie et al. 2011)
		FCV	0.5	
		MNV	1.2	
potable water, 0.42 min	Onions	MNV	0.4	(Baert et al. 2008b)
potable water, 2 min	Spinach	MNV	1.0	(Baert et al. 2008b)
6% gaseous ozone, 10-40 min	Strawberries	MNV	3.3	(Predmore et al. 2015)
		TuV	6.0	
25 ppm chlorine	Fresh-cut lettuce	MNV	1.7	(Liu et al. 2009)
100 ppm chlorine			2.3	
25 ppm chlorine + High Power Ultrasound (HPU)	Fresh-cut lettuce	MNV	2.7	(Liu et al. 2009)
			3.1	
100 ppm chlorine + HPU	Fresh-cut lettuce	MNV	2.5	(Liu et al. 2009)
			3.7	

Table 13. Highlights of using surrogates in processing technologies

Processing Technology	Possible Viral Inactivation Mechanism	Inactivation of Surrogates
Frozen and chilled storage	Instability of viral capsid	<ul style="list-style-type: none"> • Low reduction of most surrogates. • Viruses stable in most frozen or chilled conditions.
pH and water activity	Unknown, if any	<ul style="list-style-type: none"> • Low reduction of most surrogates, except FCV which is pH sensitive and thus not an appropriate surrogate for acidic matrices.
Antiviral food components and essential oils	Unknown, if any	<ul style="list-style-type: none"> • Viral inactivation is time and concentration dependent. • Some antivirals may require high concentrations resulting in limited food applications. • Inactivation levels can vary and dependent on retention of antiviral compounds activity.
Thermal processing	Disintegration of viral capsid	<ul style="list-style-type: none"> • High inactivation of most surrogates at 75°C in high water activity foods with times varying depending on matrix and surrogate chosen. • Low inactivation of most surrogates in low water activity foods. • Temperature for inactivation appears inversely proportional to water activity or moisture levels.
High pressure processing	Results in viral capsid instability and disintegration	<ul style="list-style-type: none"> • High inactivation of most surrogates between 400 and 600 MPa, except Poliovirus and Aichi virus which is HPP resistant and MS2 phage which appears more resistant than HAV. • Effective on high water activity foods. • Inactivation of viruses is inversely proportional to processing temperatures. However, inactivation of MS2 may be directly proportional to processing temperatures.
Irradiation	Unknown, if any	<ul style="list-style-type: none"> • Minor reduction of most surrogates at FDA approved dosages.
Light based technologies	Photochemical reactions may cause capsid instability	<ul style="list-style-type: none"> • High inactivation in clear liquids and on surfaces of most surrogates. • Low inactivation on complex food surfaces or turbid liquids or liquids containing particles. • Low penetration depth and reduced inactivation if viruses are in food matrices.
Sanitisers	Unknown, if any	<ul style="list-style-type: none"> • Low inactivation of most surrogates on fresh produce. • Chlorine still one of the effective sanitisers but efficacy affected by organic loads and not the choice sanitiser for some countries. • Some sanitisers may require additional rinse to reduce sanitiser concentrations to approved food contact levels.