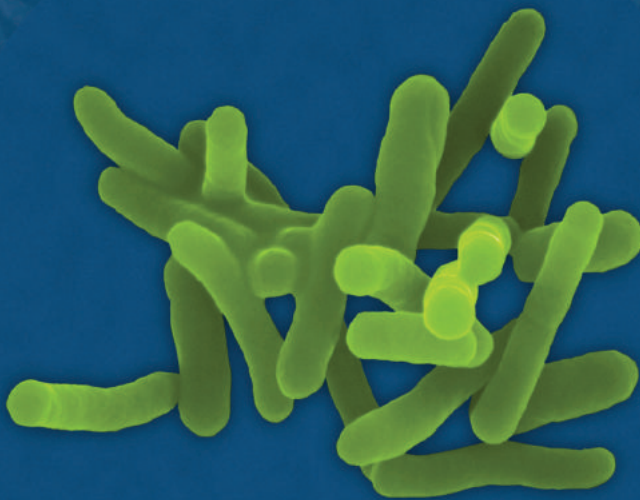




Microbiological risk assessment of viruses in foods Part 2: Prevention and intervention measures

Meeting report



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MICROBIOLOGICAL RISK
ASSESSMENT SERIES

Microbiological risk assessment of viruses in foods Part 2: Prevention and intervention measures

Meeting report

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Contributors

EXPERTS

Ingeborg Boxman, Wageningen Food Safety Research, Wageningen University and Research, the Kingdom of the Netherlands

Nigel Cook, Jorvik Food and Environmental Virology Ltd., the United Kingdom of Great Britain and Northern Ireland

Christophe Gantzer, Université de Lorraine, France

Miranda de Graaf, Department of Viroscience, Erasmus MC, the Kingdom of the Netherlands

Duncan Gitonga Ithinji, KALRO Veterinary Science Research Institute (KALRO-VSRI), Kenya

Lee-Ann Jaykus (emeritus), North Carolina State University, the United States of America

Tao Jiang, China National Center for Food Safety Risk Assessment, China

Leera Kittigul, Faculty of Public Health, Mahidol University, Thailand

Kalmia E. Kniel, University of Delaware, the United States of America

Catherine McLeod, Cawthron Institute, New Zealand

Nada M. Melhem, Faculty of Health Sciences, American University of Beirut, Lebanon

Xiang-Jin Meng, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, the United States of America

Neda Nasheri, Health Canada, Canada

Courage Kosi Setsoafia Saba, Department of Microbiology, Faculty of Biosciences, University for Development Studies, Ghana

Magnus Simonsson, European Union Reference Laboratory for Foodborne Viruses, Swedish Food Agency, Sweden

Fernando Rosado Spilki, Molecular Microbiology Laboratory, Institute of Health Sciences, Feevale University, Brazil

Jacqueline Williams-Woods, United States Food and Drug Administration, the United States of America

RESOURCE PERSONS

Sarah Cahill, Joint FAO/WHO Food Standards Programme, Italy

Donald W. Schaffner, Rutgers University, the United States of America

SECRETARIAT

Juliana De Oliveira Mota, WHO, Switzerland

Akio Hasegawa, WHO, Switzerland

Yves Oukouomi Lowe, FAO, Italy

Moez Sanaa, WHO, Switzerland

Kang Zhou, FAO, Italy

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Abbreviations

BMS	bivalve molluscan shellfish
CCFH	Codex Committee on Food Hygiene
EFSA	European Food Safety Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
HAV	hepatitis A virus
HEV	hepatitis E virus
HPP	high pressure processing
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
MNV	murine norovirus
NSSP	National Shellfish Sanitation Programme
PAA	peracetic acid
RTE	ready-to-eat
RT-PCR	reverse transcription polymerase chain reaction
RVA	Group A rotavirus
TV	Tulane virus
US FDA	United States Food and Drug Administration
WHO	World Health Organization

Declaration of interests

All participants completed a Declaration of Interests form in advance of the meeting.

Donald W. Schaffner was involved in consultancy work for the food industry. Therefore, while he was invited to participate in the meeting, he participated as a technical resource person and was excluded from the decision-making process regarding the final recommendations. The rest of the declared interests reported were not considered by FAO and WHO to have any interest that may be perceived as a potential conflict in light of the objectives of the meeting.

All the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting.

All the experts participated in their individual capacities and not as representatives of their countries, governments or organizations.

Executive summary

Meeting objectives

The Expert Committee reviewed the scientific literature published since the 2008 Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) report on foodborne viruses (FAO and WHO, 2008), relative to control measures to protect the food supply chain from contamination with foodborne viruses. The virus-commodity pairs chosen were those identified in Part 1 (food attribution, analytical methods and indicators) of this series of expert meetings. Specifically, during this Part 2 meeting, the Expert Committee: 1) reviewed the relevant scientific literature; 2) deliberated on the developments that have occurred to control foodborne viruses in the relevant food supply chains since the 2008 JEMRA report; and 3) identified the most promising approaches to protect further the food supply chain from virus contamination.

The Expert Committee decided that water intended for drinking was not within the scope of this committee. Water relevant to virus transmission was considered only for water used in food production, processing and preparation; used as an ingredient; and as a vehicle for food contamination, where water is not the final product that is consumed.

Conclusions

In the Part 1 Expert meeting, the virus-commodity combinations that ranked of highest priority were human norovirus and hepatitis A virus in shellfish, fresh and frozen produce, prepared and ready-to-eat (RTE) foods, and hepatitis E virus in pork and wild game. The Part 2 Expert meeting focused on these virus-commodity combinations and their associated contamination routes. Human fecal matter and vomit from infected individuals are the primary sources of contamination for norovirus and hepatitis A virus. Across the food supply chain, their primary contamination routes are fecally impacted waters, food handlers carrying foodborne viruses, and surfaces. Zoonotic hepatitis E virus is present in the meat, organ tissues, and excretions of infected swine and some game animals. Since that initial expert meeting report from 2008, awareness of the public health importance of these foodborne virus-commodity combinations has increased, resulting in additions or changes to some food supply chain management strategies and research initiatives. Prevention remains the cornerstone of control

of foodborne viruses. This is because these viruses are environmentally persistent and resistant to many treatments commonly used to inactivate foodborne pathogens. Effective inactivation methods continue to be necessary and are currently being evaluated.

Shellfish

Shellfish (bivalve molluscan shellfish) are contaminated with viruses primarily by fecally-impacted growing waters arising from community wastewater, septic tank failures, non-point source pollution, and discharge from boats and other recreational or commercial uses. Sanitary surveys are increasingly used to evaluate human fecal pollution status in shellfish growing areas and can be used to determine the conditions in which harvesting can occur safely. The use of male-specific coliphages to assist in evaluating the efficacy of depuration and relaying processes appears promising. The use of more effective tertiary wastewater treatment can reduce viral load in effluent but requires infrastructure investment. Climate change is anticipated to result in heavier rainfall in some locations, which may increase the likelihood of sewage overflows or runoff. Contaminated products are either discarded or diverted to processing (depuration, relaying, heat, or high pressure).

Fresh and frozen produce

Fresh and frozen produce is usually contaminated preharvest by sewage sludge, human fecally impacted source waters (e.g. used for irrigation, washing, pesticides, frost protection), and infected food handlers (pickers/packers). Frozen produce (particularly berries) predominates in foodborne virus outbreaks associated with produce, aided by the fact that freezing preserves virus infectivity and results in globally distributed products with extended shelf-life. In the last 16 years, prevention of virus introduction during production and processing has been included in textual refinements to Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and Good Hygiene Practices (GHPs). Specific production-related intervention strategies should focus on water source, location, method and timing of application. Emerging treatments of water (e.g. ozone, photocatalysis, ultraviolet and ultrafiltration) demonstrate potential, but require infrastructure investment. Biochar filtration is a relatively inexpensive method that shows promise for treating reused water.

Prepared and ready-to-eat (RTE) foods

In the case of norovirus and hepatitis A virus, prepared and RTE foods are usually contaminated through handling by infected food handlers. Prevention focuses on exclusion of infected food handlers from work, gloving, surface disinfection, and attention to personal hygiene, including handwashing. Facilities (handwashing and toilets) should be readily available and appropriately designed to encourage good personal hygiene. In many countries, national or regional policies guiding appropriate employee behaviours have been implemented in food service. This includes policies about the length of time infected food handlers should be excluded from work and mandates for glove use while preparing foods. Most countries actively promote handwashing; some specify when and how to wash. Nonetheless, compliance with such behaviours is often poor. In response to findings that noroviruses are shed and aerosolized in vomiting events, formalized guidelines for clean-up and disinfection after vomiting or defecation incidents in food service have been implemented.

Pork and wild game meat

Zoonotically transmitted hepatitis E virus enters the food chain by infection of pigs and wild game animals. Human exposure occurs by: consumption of raw or inadequately cooked meats and tissues derived from these animals (e.g. liver, intestine and muscle), direct contact with infected animals on farms and in slaughterhouses (surface cross-contamination), or use of untreated pig manures or runoff from farms. Recent studies have proposed that control measures should focus on prevention of animal infection at the preharvest phase (i.e. biosecurity measures and disinfection) and post-harvest interventions (i.e. preventing cross-contamination, virus inactivation by heat and avoiding the use of high-risk tissues in product formulations).

Intervention methods focused on virus inactivation

While most of the control measures discussed above are designed to prevent virus contamination, inactivation methods are also being investigated. It is important to note that several intrinsic (e.g. water activity, pH) and extrinsic (e.g. vacuum packaging, and storage temperature) parameters have minimal effect on virus inactivation. Novel food processes remain experimental. Accurate assessment of their efficacy is complicated by myriad factors (e.g. the inability to culture the many wild-type strains of these viruses *in vitro* from foods; the need to use cultivable surrogates, which often behave differently from wild-type viruses,

in laboratory-based studies; variability in matrix, virus, strain and location in food; lack of consistency between studies; and the absence of scale-up studies). Nonetheless, some of these methods are promising.

Shellfish: Depuration (<48 hours) does not always adequately remove and/or inactivate viruses from contaminated products. For diverted product, thermal processing at very high internal temperatures, 90 seconds at > 90 °C, can inactivate viruses, but this may result in an unacceptable product. Emerging data indicate that other time-temperature combinations can lead to the same outcome. High pressure processing can be effective for virus inactivation, although organoleptic properties may be impacted.

Fresh and frozen produce: Most fresh berries are not washed post-harvest. Washing other produce items (e.g. lettuce and green onions) with water alone removes < 1 log₁₀ foodborne viral pathogens; the addition of low concentrations of chlorine-based disinfectants (e.g. hypochlorite and chlorine dioxide) can boost efficacy but with regulatory and organoleptic concerns. For produce diverted to thermal processing, commercial sterilization (e.g. jams and jellies) should result in inactivation of virus. Standard juice pasteurization conditions should provide some inactivation, but longer times and/or higher temperatures may be needed to eliminate heat-resistant strains. Novel and emerging food-processing techniques have been investigated, but none yet have a strong body of evidence to justify their routine use.

Prepared and RTE foods: Chemically-based virus inactivation and removal in this commodity group focuses on surface disinfection and hand sanitation. For maximum efficacy, surfaces should be cleaned before disinfection. Surface disinfection guidelines for norovirus disinfection using free chlorine differ by country. Most commercial disinfectants and hand sanitizers, used under manufacturer recommended conditions, provide only partial inactivation of norovirus. There is significant variability in product performance based on active substance(s) and formulation.

Pork and wild game meat: In meat, hepatitis E virus is highly resistant to heat. For example, it was reported that it took 20 minutes in a pâté-like product to obtain the similar inactivation as was observed for a relatively pure virus suspension treated at the same temperature (70–72 °C) for 2 minutes. Omitting the use of high-risk contaminated tissues (liver or blood) in raw or undercooked pork products can also reduce transmission risk from foods.

Data gaps and future directions

Many data gaps and needs were identified, often commodity group specific. An overarching issue throughout is the limited ability to routinely cultivate wild-type foodborne viruses in the laboratory, which complicates the ability to validate interventions, compare studies and/or interpret monitoring data. Specific directions for future research and/or development include the following observations:

- Early identification of contamination hotspots (e.g. wastewater surveillance) may be a useful control tool.
- Technologies such as remote sensing (satellite imagery) and hydrographic dye studies could help to predict virus dispersion (i.e. how far viruses travel in waterways).
- The usefulness of indicator organisms to predict virus occurrence and infectivity could be better understood through appropriately designed studies with global representation, from which large, coordinated datasets are collected and analysed using robust statistical methods.
- Emerging scientific data should be used to develop surface disinfectant and hand sanitizer formulations with greater efficacy against environmentally stable viruses.
- Capacity building is critical, especially in low- to middle-income countries, particularly in education and training, sharing epidemiological and genomic sequencing data, and technology transfer.
- Vaccinations are an effective control measure but are not yet developed and/or not routinely implemented in policy globally.
- Risk assessments would be useful, particularly with consideration of region-specific practices, in terms of better understanding the relative value of alternative or combined intervention methods.
- Novel interventions are in development, but these should be validated using the relevant viruses before wide application in real-world prevention and control situations.



Introduction

1.1 BACKGROUND

Since viral agents associated with foodborne diseases began to be recognized as a hazard, efforts have been undertaken to mitigate their risk. These efforts have been challenging due to the unique nature of these microbes and the difficulties associated with *in vitro* cultivation. Consequently, for example, the effects of commonly used inactivation strategies (e.g. heat, disinfectants) have not been completely elucidated. Furthermore, although standard methods for detection of hepatitis A virus and human norovirus in common food vehicles (berries, leafy vegetables and shellfish) are now available, methods for other foods (e.g. dates) or other viruses (most prominently hepatitis E virus) still await standardization.

The 2008 JEMRA Report (FAO and WHO, 2008) briefly reviewed existing approaches for preventing and ameliorating virus contamination and recommended that strategies should be focused on priority virus-commodity combinations. The Codex Alimentarius foodborne virus guidelines published in 2012 (FAO and WHO, 2012) described several processes and practices which are effective at reducing virus load. These guidelines, however, were focused on norovirus and hepatitis A virus (HAV) and advanced no information on measures that could be used against hepatitis E virus (HEV), an emerging foodborne virus at that time.

Since 2008, there has been further research into foodborne virus control and advancements in methodology mediating the acquisition of new data that were not available when the JEMRA 2008 Report and Codex foodborne virus guidelines were published. The current report brings up to date the overview of interventions for the prevention of foodborne virus contamination and for inactivation in instances in which contamination occurs.

1.2 OBJECTIVES

Based on the JEMRA meeting and its report (FAO and WHO, 2008), the Codex Alimentarius Commission established the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food* (CXG 79-2012) (FAO and WHO, 2012). The primary purpose of these guidelines was to provide direction on how to prevent or minimize the presence of human enteric viruses in foods, most specifically hepatitis A virus (HAV) and norovirus. This guideline is applicable to all foods – with a focus on ready-to-eat food, from primary production through to consumption – for the control of human enteric viruses. It also contains an annex on the control of HAV and human norovirus in bivalve molluscs (Annex I) and an annex on the control of HAV and human norovirus in fresh produce (Annex II). These annexes provide additional recommendations for control of these viruses in those specific commodities. With several emerging issues associated with foodborne viruses and recent scientific developments, the Codex Committee on Food Hygiene (CCFH) requested that JEMRA provide scientific advice to inform the review of the guidelines at its 53rd session in 2022, especially on the following areas (items 1–5) (FAO and WHO, 2022):

- 1) an up-to-date review of the foodborne viruses and relevant food commodities of highest public health significance;
- 2) a review of the scientific evidence on prevention and intervention measures and their efficacy;
- 3) a review of the analytical methods for relevant enteric viruses in food commodities;
- 4) a review of scientific evidence on the potential utility of viral indicators or other indicators; and
- 5) a review of the various risk assessment models with a view towards constructing more applicable models for wide use among member countries, including a simplified risk calculator.

To meet the request of the CCFH, the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) convened the Part 1 meeting to work on the food attribution, analytical methods and indicators of viruses in foods (items 1, 3 and 4) in September 2023 (FAO and WHO, 2024). The goal of the Part 2 meeting was to gather and evaluate recent data, evidence and scientific opinions on the topic of prevention and intervention measures and the efficacy of interventions in the food continuum for foodborne viruses (item 2) based on the outcomes of the Part 1 meeting.

1.3 SCOPE

The virus-commodity pairs for which prevention and intervention measures are described in this report are those which were identified in Part 1 (food attribution, analytical methods and indicators) of this series of expert meetings as being of highest priority with regard to risk. These combinations are human norovirus and hepatitis A virus in shellfish, fresh and frozen produce, and in prepared and ready-to-eat (RTE) foods; and hepatitis E virus in pork and wild game. Water relevant to virus transmission was considered only for water used in food production, processing and preparation; used as an ingredient; and as a vehicle for food contamination, each where water is not the final product that is consumed.

In the scope of this report, the following definitions have been adopted:

- 1) **Virus control:** prevention, removal and/or inactivation of virus contamination of food
- 2) **Intervention in viral contamination:** action(s) that lead to virus control
- 3) **Prevention of viral contamination:** action(s) intended to stop the entry of a virus into the food supply chain
- 4) **Virus removal:** physical action(s) resulting in the reduction or elimination of virus particles from food(s) or surface(s)
- 5) **Virus inactivation:** a process, treatment, condition or circumstance that causes the loss of infectivity of a virus in food(s) or along the food supply chain

1.4 LITERATURE REVIEW

A review of scientific studies was done to gather updated information since the last JEMRA meeting (FAO and WHO, 2008), creating a list of resources that offer scientific data on how to prevent and remove/inactivate virus contamination in foods. Scientific articles were selected from two databases (Web of Science and PubMed), and as there was a need to consider studies published in languages other than English; data from member countries and expert opinions were also relied upon.

The records from Web of Science (n = 8 507) and PubMed (n = 6 683) were added into the literature review software DistillerSR. The function “Duplication Detection” was used by comparing the Title, Author and Abstract. The “Smart

Quarantine” feature in Distiller was used to remove these duplicates, resulting in 12 317 publications which were used to establish the working database for the meeting. The search was carried out on 3 January 2024. The keywords used for searching the literature are detailed in Annex 1.1.

The database was further refined using a two-step process for relevance screening and resulted in 593 articles that were prepared in a dataset for the experts’ reference. The details of this procedure were presented to the experts for their further review and are included in Annex 1.2. Some of the experts were also asked to collect data from their regions, which were shared before the meeting. The experts performed a further review of these publications and added additional outbreak, monitoring and surveillance data. This database is not included in this report but served to facilitate the discussion to support the expert opinions during the meeting.

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Transmission of viruses through the food chain

Human norovirus and hepatitis A virus contaminate foods extrinsically (i.e. they do not occur naturally in at-risk foodstuffs). They originate in the digestive tracts and other organs of human beings (Howley, Knipe and Enquist, 2023) and are transmitted mainly via fecal matter from infected persons, entering the food supply by various routes of contamination. Confirming the 2008 JEMRA Report, the September 2023 Part 1 meeting on food attribution, analytical methods and indicators iterated that these transmission routes are predominantly through water contaminated by human waste (e.g. sewage overflow, septic tank leakage) and/or via the hands of infected food handlers who do not adhere to good hand hygienic practices, either due to negligence or the lack of appropriate on-site sanitation facilities. Contact with virus-contaminated surfaces is another potential mean by which foods can become contaminated, but its relative importance is not well characterized.

Water is used in primary production for irrigation and often as a vehicle for the application of fungicides or pesticides. If the water used for these purposes is contaminated by human fecal material, it could act as a vehicle for contaminating food crops. Enteric virus contamination of irrigation water has been observed in several studies (Kokkinos *et al.*, 2012; Maunula *et al.*, 2013). There is also a remote possibility that raw, untreated human waste (e.g. “night soil”) may be being used for fertilization of crops intended for human consumption, or defecation in growing fields may be a source of viral contamination of foods. Water is also used post-harvest for washing, and although outbreaks from wash water contamination appear rare, this is a possible source of contamination (Oinam, 2008; Kawa *et al.*, 2019; Akash *et al.*, 2022).

When contaminated during primary production, virus risks are elevated for products that are subsequently consumed raw or only minimally processed. Extensive laboratory and outbreak data demonstrate that infectious human norovirus and hepatitis A virus can remain on contaminated foods for weeks or longer and are especially persistent if the foodstuff is frozen (Cook, Knight and Richards, 2016; Cook *et al.*, 2018).

The second major source of food contamination with human norovirus and hepatitis A virus is the hands of infected food handlers. This can happen at the harvesting or processing phases, such as during handpicking and packing, and more frequently during food preparation. The contaminated food can then be consumed without any sort of subsequent step that might inactivate the viruses. Hands become contaminated with these viruses when food handlers practice poor personal hygiene (e.g. inadequate or absent handwashing) due to inherent human behaviours or the absence of adequate toilet facilities and supplies. Human norovirus is also shed in the vomitus of infected individuals which can sometimes serve as a source of virus contamination, particularly through public vomiting events occurring close to food storage or preparation (Todd, 2013). Again, since these viruses are environmentally persistent, contaminated surfaces may occasionally serve as a vehicle of cross-contamination to foods.

Hepatitis E virus genotypes 3 and 4 are found naturally in various animals, especially domestic pigs and certain wild game such as boar and deer. These animals normally show no signs of infection. The virus is present in elevated concentrations in organs such as the liver, and when infected animals are slaughtered, their products (e.g. liver pate or raw-pork sausage) can harbour infectious virus (Spahr *et al.*, 2018). Moreover, if animals are viraemic at the time of slaughter, their muscle tissue may also become contaminated. If these products, even if used as an ingredient in other foods (Pavio *et al.*, 2017), are consumed raw or insufficiently treated to inactivate hepatitis E virus, they can pose a risk of infection to the consumer (EFSA BIOHAZ Panel, 2017).

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3

Current state of control measures by commodity group

3.1 SHELLFISH

3.1.1 Relative risk by exposure route (risk assessment)

Bivalve molluscan shellfish (BMS) (e.g. oysters, mussels, clams, and cockles) can act as vehicles for transmission of human norovirus and hepatitis A virus; there is little evidence of shellfish-related human disease outbreaks involving other viruses (Le Guyader *et al.*, 2008; Said *et al.*, 2009). These enteric viruses can accumulate in the digestive diverticula of shellfish as they feed in waters impacted by human sewage. Ingestion of raw or undercooked shellfish products can lead to illness in humans (Le Guyader *et al.*, 2006, Rasmussen *et al.*, 2016, Park *et al.*, 2018). Mitigation strategies such as depuration or relaying exist to reduce or eliminate potential enteric virus hazards linked with consumption of raw or undercooked shellfish. Bivalve molluscan shellfish present a relatively high risk because: i) they can bioaccumulate (and concentrate) viruses from contaminated waters; ii) they are often grown in coastal waters where there is potential for human fecal contamination; and iii) they are often consumed raw or lightly cooked (Gyawali *et al.*, 2019). Because human sewage-contaminated water can harbour multiple strains of enteric viruses, there is an increased chance of becoming contaminated with multiple viral strains, which in turn increases the chance of virus recombination events in infected individuals (Ludwig-Begall, Mauroy and Thiry, 2018; Ollivier *et al.*, 2022). Since most enteric viruses are environmentally stable, they may withstand wastewater treatment methods and contaminate shellfish harvested for human consumption. The efficiency of wastewater treatment is generally assessed based on the removal/

inactivation of fecal indicator bacteria, such as fecal coliforms. However, since the relationship between the presence and levels of fecal indicator bacteria and enteric viruses is uncertain, their measurements cannot definitively be used as a proxy for enteric virus contamination in shellfish. Microbial source tracking can be used to identify sources of human fecal contamination and may help to guide shellfish management (Harwood *et al.*, 2014)

The routes of shellfish contamination are dependent on their proximity to the pollution source, but the major contributors include community wastewater (e.g. treatment plants and sewerage, especially when treated sewage becomes contaminated with untreated sewage due to treatment undercapacity during heavy rainfall events); leaking septic tanks; runoff; discharges from recreational/small boats and harvesting vessels, and commercial cruise or freight ships; and recreational use (e.g. swimming). In less populated countries like New Zealand, harvesting areas can be more remote, and these are often affected by septic tanks and boats. Meanwhile, in low- to middle-income countries, there may be an absence of sewage treatment facilities or poor functionality. In many countries, wastewater treatment has improved over time, but climate change is anticipated to result in heavy rainfall that may result in the release of raw wastewater into water used for marine farming. Enteric viruses can move throughout the environment sometimes by attaching to particulates in groundwater, seawater, rivers and estuaries (Wyn-Jones and Sellwood, 2001). Deficiencies in treatment and distribution of contaminated water contribute to continued molluscan shellfish enteric virus outbreaks. Secondary treatment of wastewater, which is the most common treatment method, reduces human noroviruses by only 1–2 log₁₀. This means that the potential for norovirus contamination of seawater should continue to be considered when choosing production areas.

3.1.2 Contamination prevention practices

In the United States of America, the National Shellfish Sanitation Programme (NSSP) and its Model Ordinance provide guidance for the safe and sanitary control of the growing, processing and shipping of BMS for human consumption (US FDA, 2019). Controls to minimize the potential impact of human fecal material on the sanitary quality of shellfish are instituted through comprehensive sanitary surveys. These surveys are based upon the identification of pollution sources, their magnitude, and their potential impact on the shellfish growing area. After conducting the sanitary survey, growing areas are classified to determine the conditions under which harvesting can occur safely, as well as when to ensure the closure of growing areas due to elevated contamination risk. Currently, the use of hydrographic dye studies provides additional measures to minimize the impact of wastewater contamination of shellfish growing areas (Goblick *et al.*, 2011; Campos *et al.*, 2017).

Most high-income countries, such as those within the European Union (EU), have introduced regulations about the management of BMS harvested from production waters based on the level of bacterial indicators (*E. coli*), which classifies sites as: safe (A sites); further purification needed (B sites); or further processing needed (e.g. relaying, high pressure, ionizing radiation, thermal processing/commercial sterilization, and so on [C sites]) (Gyawali *et al.*, 2019). Despite these regulations, EU baseline surveys of human norovirus contamination in commercial oysters, tested using the ISO 15216 method, show a prevalence of viral nucleic acid as high as 34.5 percent [IC: 30.1–39.1 percent] in production areas during the 2016–2018 winter periods (EFSA, 2019). As high as 68.7 percent of samples tested positive for human norovirus RNA in a survey from various dispatch centres of the United Kingdom of Great Britain and Northern Ireland (Lowther *et al.*, 2018).

3.2 FRESH/FROZEN PRODUCE

3.2.1 Relative risk by exposure route (risk assessment)

Frozen berries were identified as a food commodity of elevated public health risk for foodborne transmission of human norovirus and hepatitis A virus (FAO and WHO, 2024), and while not ranked in the top three at-risk foods, fresh berries were also of concern. This is consistent with the 2008 report (FAO and WHO, 2008). When outbreaks occur, or berry samples test positive for viral RNA during prevalence or surveillance studies, root cause analysis is rarely done; therefore, the ultimate source of contamination is often unknown. That being said, numerous routes of contamination exist, the most important being water impacted by human fecal pollution, and the hands of infected food handlers. The former is more likely to occur during primary production, the latter during harvest and processing. The use of raw sewage as fertilizer cannot be ruled out as a potential contamination source, especially on very small farms in low and middle-income countries (Oinam, 2008; Kawa *et al.*, 2019; Akash *et al.*, 2022). Collectively, existing risk assessments suggest that large, widely dispersed contamination events associated with fecally impacted water coming into contact with berries shortly before harvesting are likely drivers of large outbreaks (Miranda and Schaffner, 2019). From a simulation model, this was also the only scenario that could explain the large number of illnesses in the human norovirus outbreak that occurred in Germany in 2012 associated with frozen strawberries (Miranda and Schaffner, 2018).

3.2.2 Contamination prevention practices

Fresh and frozen berries are valuable to the human diet for their flavour and nutritional benefits (Miotti *et al.*, 2024). Yet, numerous outbreaks of viral disease associated with their consumption have occurred worldwide, as reviewed by Bozkurt *et al.* (2021). Since fresh and frozen berries are predominately consumed without thermal processing, contaminated berries can present a health hazard. Potential exists for inactivation using non-thermal processing methods, though most are not yet commercialized and may be difficult to apply to meet industrial needs. Due to the several contamination routes and general lack of contamination source attribution, prevention is the key to control, which means diligent attention to GAPs and GHPs. Berries can become contaminated with enteric viruses through contaminated agricultural water that may be used for irrigation, fertilization, frost prevention, and pesticide application. Post-harvest wash water can also be contaminated. Contamination of water by human fecal waste or sewage leaks is a growing concern worldwide and often related to weak and aging infrastructure. Unfortunately, these issues are often difficult to pinpoint. Contamination can also occur through application of human fecally contaminated soil and soil amendments. Berries can be contaminated by infected persons (e.g. pickers or harvesters from endemic areas); and berries grown in areas where sanitation is inadequate or unavailable can increase the risk of contamination. Surfaces and fomites used in pre- and post-harvest activities (tables, conveyors, belts, containers, processing environments) may also become contaminated with human fecal material or vomitus, resulting in cross-contamination of berries. All of these potential contamination sources would be considered relevant in managing prevention, emphasizing the necessity of safe water, safe soil, good personal hygiene and clean processing environments.

3.3 READY-TO-EAT/PREPARED FOODS

The combined designation ready-to-eat (RTE)/prepared foods has been used to reflect the fact that in some regions of the world it is common to refer to these foods as “ready-to-eat” while in other regions, “prepared foods” is more common. In either case, this term refers to foods that require some degree of handling by a food service worker during preparation and are not intended to receive additional treatment that might inactivate viruses prior to consumption (i.e. they are ready-to-eat immediately). Both terms are also used interchangeably in the 2008 report (FAO and WHO, 2008).

3.3.1 Relative risk by exposure route

The 2008 report stated that infected food handlers were believed to be the most common sources of human norovirus and hepatitis A virus contamination in RTE/prepared foods. Although specific attribution literature is scant, Hall *et al.* (2012) identified that the most common commodity group for foodborne human norovirus outbreaks in the United States of America was ready-to-eat foods, usually prepared in restaurants, other commercial venues, and institutional settings, and most often contaminated by an infected food handler. Unfortunately, there is very little information about how different foods, especially ready-to-eat foods, contribute to HAV transmission worldwide (Di Cola *et al.*, 2021), even though HAV outbreaks linked to restaurant foods sporadically occur. In the 2008 report, the risk of zoonotic HEV was not considered high enough for ranking purposes. A 2014 rapid qualitative risk assessment (Appuhamy *et al.*, 2014) concluded that there was a low risk of HEV transmission via food handlers in general, although that risk could be elevated if highly susceptible individuals were consuming foods prepared by an HEV-infected food handler who had recently returned from an endemic country. The 2008 report also stated that the relative contribution of hand versus surface contamination (occurring due to a vomiting or fecal contamination event and resulting in cross-contamination of food during food preparation) remained to be established for human norovirus and hepatitis A virus. Given the analysis of Hall *et al.* (2012), it is likely that hands are more important than surfaces for RTE food contamination. Risk assessments have informed our understanding of human norovirus transmission in food handling settings and the relative efficacy of select mitigation strategies including surface cleaning and sanitizing, hand hygiene, gloving, and the exclusion or restriction of ill food workers (Fanaselle *et al.*, 2022; Duret *et al.*, 2017).

3.3.2 Contamination prevention practices

Duret *et al.* (2017) evaluated 21 different food handling scenarios and concluded that the prevention strategies studied would not completely prevent human norovirus contamination of food when a symptomatic employee was present in the food establishment. These authors concluded that full compliance with a recommendation of exclusion of symptomatic food employees from work would reduce mean illnesses by 75 percent from the baseline representing current practices. Conversely, when there was no compliance with exclusion from work, mean illnesses were 226 percent above the baseline value. The same study suggested that efficient handwashing, frequent handwashing with gloving compliance, and elimination of contact between hands, faucets, and door handles in restrooms (high-touch surfaces) would reduce the mean number of infected

patrons to 58 percent, 62 percent and 75 percent of the baseline, respectively. Based on this risk assessment, exclusion of ill food workers would be the most efficacious contamination prevention strategy.

The Fanaselle *et al.* (2022) study used the same model to evaluate 60 different control scenarios. These authors concluded that hand hygiene compliance and ill food employee exclusion strategies had the largest impact on the number of consumer norovirus illnesses, while surface cleaning in food establishments had a relatively small impact in reducing disease burden. Hand washing compliance after using the restroom strongly impacted the predicted number of illnesses. Again, exclusion policies were important: 94 percent compliance with exclusion of ill food employees was predicted to decrease ill consumers to 71 percent of baseline. Interestingly, restriction of ill food employees (by assigning them duties not associated with direct food contact, but still allowing them to remain at work) was not effective for reducing human norovirus illness.

Individuals infected with HAV often shed the virus prior to showing symptoms, making exclusion of ill workers difficult. For this reason, prevention strategies for HAV transmission through prepared/RTE foods have focused on immunization in some parts of the world, particularly in higher income countries where the disease has low endemicity and therefore much of the population is still susceptible. In early work, Jacobs *et al.* (2000) used a decision analytic framework to evaluate the cost effectiveness of vaccinating food service workers against hepatitis A at age 20, finding that this policy, as applied to 100 000 food service workers, would cost USD 8.1 million and reduce the costs of hepatitis A treatment, public health intervention, and work loss by USD 3.0 million, USD 2.3 million, and USD 3.1 million, respectively. They concluded that the cost-benefit ratio of this policy would exceed the generally accepted standard of cost effectiveness. Meltzer *et al.* (2001), using a Monte Carlo simulation approach, concluded that vaccination of restaurant workers would not be cost-effective from either restaurant or societal perspectives. However, a more recent article (Roberts, 2017) refutes these conclusions, commenting that there may be several reasons why these older analyses are no longer valid, including reduced vaccination costs, who might pay, who might already be vaccinated, as well as increasing costs of post-exposure prophylaxis and lawsuits. The hepatitis A virus vaccine has been available in the United States of America since 1996 and has been recommended as part of the childhood immunization package since 2006 (Fiore, Wasley and Bell, 2006). Recommended use worldwide occurs on a country-by-country basis. Although many public health agencies have recommended hepatitis A vaccination for all food service employees, many companies do not adhere to this recommendation, and there is no mandatory or voluntary programme in place at the current time in the United States of America. The situation is much more complex in low- and

middle-income countries where the disease has higher endemicity, so a greater proportion of the population may have acquired immunity by early to mid-adulthood.

Inactivation of viruses in RTE/prepared foods has been investigated (Roh *et al.*, 2020; Tang *et al.*, 2018; Bozkurt, D'Souza and Davidson, 2015) but is not practical given the very nature of RTE/prepared foods, which are intended to be delivered directly to the customer for consumption without heating or further processing. Inactivation of viruses on fomites and food handlers' hands, which can be a source of contamination of RTE/prepared foods, is discussed elsewhere (see sections 4.3 and 4.4).

3.4 PORK AND GAME

3.4.1 Relative risk by exposure route (risk assessment)

The two genotypes of hepatitis E virus (HEV-3 and HEV-4) which are recognized as responsible for foodborne disease can infect pigs and other animals, especially game such as wild boar and deer. Exposure routes include consumption of contaminated animal products; direct zoonotic transmission; and potential cross-contamination (e.g. knives used for cutting meat which are subsequently used on other foodstuffs without cleaning). Virus has been detected in consumable products from HEV-infected animals, with particularly high loads in liver, blood, bile, intestine, intestinal content, and muscle (De Sabato *et al.*, 2020; Salines *et al.*, 2019; García *et al.*, 2020). A significant proportion of pigs, for example 6 percent in the United States of America and 16 percent in the Kingdom of the Netherlands, were found to be viraemic at slaughter age (5–6 months) (Boxman *et al.*, 2022, Sooryanarain *et al.*, 2020). Detection of HEV RNA in the sera of infected animals was associated with detection of HEV RNA in liver and caecum content (Boxman *et al.*, 2022). This poses a food safety risk, as liver is often not well cooked; pork products made from viraemic pigs may contain residual virus-contaminated blood; pork may be contaminated during dressing of the carcass; and contaminated blood may be used as an ingredient.

Inclusion, without adequate thermal processing, of HEV-contaminated viscera such as liver and blood as ingredients for the preparation of RTE or minimally-processed food, constitutes an important exposure risk (Boxman *et al.*, 2017; Bigoraj, Paszkiewicz and Rzeżutka, 2021). HEV-3, and to lesser extent HEV-4, RNA has been detected in some pork products from the retail market, including liver, liverwurst, liver pate, (raw) pork sausages, and dried hams (Heldt *et al.*, 2016; Boxman *et al.*, 2019; Wang *et al.*, 2021; Feagins *et al.*, 2007; Locus *et al.*, 2023;

Boxman *et al.*, 2020; Moor *et al.*, 2018); viral RNA has also been detected in porcine blood products which could be used as ingredients in food (Boxman *et al.*, 2017). Cases of hepatitis E have been reported or associated with consumption of processed pork products such as sausages (Colson *et al.*, 2010; Said *et al.*, 2014; Moal, Gerolami and Colson, 2012; Pavio, Merbah and Thébault, 2014) or consumption of HEV-contaminated raw or undercooked pork or game meats (Rodríguez-Lázaro, Hernandez and Cook, 2018; Yin *et al.*, 2019; Tei *et al.*, 2003; Akpoigbe *et al.*, 2023).

Zoonotic transmission can also occur through the direct exposure of individuals via the feces and urine of HEV-infected animals on farms or at slaughterhouses. Seroprevalence studies of anti-HEV IgG have been carried out in different populations with special attention to occupational exposure and eating habits (De Schryver *et al.*, 2015). Individuals working in slaughterhouses, hunters, forestry workers, pig farmers, swine veterinarians, and workers who were exposed to septic tanks are reported to be at elevated risk of HEV infection (Chaussade *et al.*, 2013; Meng *et al.*, 2002; Faber *et al.*, 2018; Mughini-Gras *et al.*, 2017; Lange *et al.*, 2017; Acosta *et al.*, 2022; Tulen *et al.*, 2019; Mrzljak *et al.*, 2021; Geng *et al.*, 2019; Carpentier *et al.*, 2012).

HEV-3 has been detected in some game such as deer, wild boars and hare, as well as in game meats (Arnaboldi *et al.*, 2021; Tsokana *et al.*, 2020; Serracca *et al.*, 2015; Mendoza *et al.*, 2022) and cases of hepatitis E have been reported in individuals who consumed contaminated deer or wild boar meats (Tei *et al.*, 2003; Takahashi *et al.*, 2004; Rivero-Juarez *et al.*, 2017). Wild boars may also serve as a source of infection for domestic pigs (Schlosser *et al.*, 2015). Other possible transmission routes, though less well documented than the above, include surface cross-contamination of different animal tissues through contaminated shared equipment in slaughterhouses, and pig manure application in soil as a fertilizer without prior virus inactivation. The latter can lead to potential contamination of surface or coastal waters during heavy rainfalls and runoff events (Rutjes *et al.*, 2009; Dell, Meisinger and Beegle, 2011; Takuissu *et al.*, 2022) and concomitant contamination of produce (Brassard *et al.*, 2012; Maunula *et al.*, 2013) and shellfish (Mesquita *et al.*, 2016; Rivadulla *et al.*, 2019).

Regarding the vaccination against HEV, an HEV-1-based vaccine for human use is of limited availability and is currently licensed for use only in China and Pakistan (Zhong *et al.*, 2023; Zhu *et al.*, 2010), as the vaccine has not yet been prequalified by the World Health Organization (WHO) for use in endemics and outbreaks (WHO, 2015). The vaccine efficacy is reportedly 100 percent during the 12-month period after three doses (Zhu *et al.*, 2010) and is 83–87 percent over a 10-year period (Huang *et al.*, 2024). The vaccine-induced antibodies

against HEV persist in vaccinated individuals for at least 8.5 years (Huang *et al.*, 2024). There is a need to further evaluate the safety and efficacy profile of the available vaccine particularly in high-risk populations such as pregnant women, immunocompromised individuals, abattoir workers, and pig handlers to increase its global accessibility (WHO, 2015; Peron *et al.*, 2023). Since cases of foodborne hepatitis E are predominantly caused by HEV-3 and HEV-4, it would be important to develop a broadly protective vaccine against multiple genotypes of HEV, including HEV-3 and HEV-4.

3.4.2 Contamination prevention practices

Considering the many sources of foodborne hepatitis E virus exposure, most control measures focus on preventing hepatitis E virus infection in at-risk animals rather than removal or inactivation of the virus from food (EFSA BIOHAZ, 2011). Hepatitis E virus-specific interventions relative to animals (pig and game) are limited. Biosecurity measures such as disinfection of pig farm premises, hygienic measures for pig handlers, reducing the number of workers on farms, and the presence of a quarantine area have been studied for their ability to reduce the introduction of the hepatitis E virus from outside the farm or in-farm transmission of the virus among pigs (Dubbart *et al.*, 2024; Galipó *et al.*, 2023; Meester *et al.*, 2021). A higher risk of having hepatitis E virus-positive livers in slaughter-age pigs was reported to be associated with early slaughter, inadequate hygiene measures, and surface origin of drinking water (Walachowski *et al.*, 2014). In a case-control study in Dutch pig farms, it was reported that biosecurity measures such as using rubber and steel floor material in fattening stalls, cleaning pig driving boards weekly, and adequate fly control were effective measures to consider for hepatitis E virus control on infected pig farms (Meester *et al.*, 2023). Some farm management practices such as segregation of pigs by early age before decay of maternal antibodies and the use of an all-in/all-out swine production system can minimize hepatitis E virus transmission on the farm (Busby *et al.*, 2013). Unfortunately, measures to prevent hepatitis E virus infection in game animals are lacking.

Porcine liver, diaphragm, or blood (products) should be given a sufficient heat treatment to inactivate HEV, prior to being added to ready-to-eat food products, such as raw pork sausages, salami, or figatellu.

Monitoring of the infection status of pigs through serology- and PCR-based testing on farms (Ianiro *et al.*, 2021, 2023; Risalde *et al.*, 2020) or at the slaughterhouse (Boxman *et al.*, 2022; Patrizio *et al.*, 2023; Meester *et al.*, 2022; Sooryanarain *et al.*, 2020) can aid in developing targeted strategies to reduce HEV contamination during slaughter and pork processing. Monitoring studies of

hepatitis E virus contamination in foods, such as testing of animal liver and other tissues, and pork products (such as sausage and liver pate) (Boxman *et al.*, 2019; Anheyer-Behmenburg *et al.*, 2017; EFSA BIOHAZ, 2011) have been performed in some countries, as have been case-control studies to identify risk factors for hepatitis E virus infection (Smith *et al.*, 2021; Tulen *et al.*, 2019; Faber, Askar and Stark, 2018).

Measures to reduce fecal contamination of carcasses can also help control potential viral contamination on the surface of carcasses (EFSA BIOHAZ, 2011). Appropriate management and biosecurity measures can extend from the farm to include transport, slaughter, processing and storage to reduce the risk of hepatitis E contamination of pork products (Walachowski *et al.*, 2014; Holt *et al.*, 2016). An important prevention practice would be the exclusion of hepatitis E virus-contaminated viscera, such as liver and blood, that are not adequately thermally processed prior to use as ingredients for preparation of RTE or minimally processed food.

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Progress in intervention strategies across the supply chain

4.1 INFORMATION INFORMING INTERVENTIONS RELATIVE TO ENVIRONMENTAL SOURCES OF CONTAMINATION

4.1.1 Waters

Water sources, including irrigation and wash water, and wastewater, can significantly impact food safety by introducing foodborne viruses into the food chain. Indeed, water plays a critical role in food production, from growing water for shellfish production, to irrigation and post-harvest washing of berries, to water used in food processing and preparation. Source water is a term used to define the initial water supply used for various purposes such as irrigation, pesticide dilution, and washing of produce on the farm. In terms of microbiological safety, source waters can be ranked in decreasing order of safety as follows: groundwater, potable water, and surface water. Shellfish production relies on natural bodies of water such as oceans, estuaries, bays, and coastal areas as the source of water. Source water can become contaminated with foodborne viruses, which mostly originate from human fecal wastes (with the exception of zoonotic HEV-3 and HEV-4) through runoff, untreated manure, biosolids, wastewater discharges, wildlife contamination (zoonotic HEV-3 and HEV-4 only), and open defecation. Various mitigation strategies, including physical, chemical, and biological treatments, have

been studied for their effectiveness in reducing viral contamination and ensuring the safety of food products exposed to water during production and processing. These aspects are discussed below. Preventing source water contamination necessitates effective water quality management practices and monitoring programs (FAO and WHO, 2019; 2021).

4.1.2 Wastewater and manure

Viruses shed in the feces of infected individuals can be naturally present in wastewater, presenting a risk for foodborne transmission if untreated wastewater comes into contact with food, or with water used in food production or processing. Hence, effective management of wastewater is critical in protecting the food supply from viral contamination. Wastewater reclamation has long been practiced, particularly in arid regions, to utilize water resources more efficiently (EU *et al.*, 2015). However, there is a growing interest in wastewater reclamation even in water-abundant regions, due to water resources coming under increasing stress, particularly in areas with intensive agriculture.

Proper wastewater treatment is essential for the prevention of foodborne outbreaks. The wastewater treatment process consists of three main stages: primary, secondary, and tertiary water treatment. Various methods are employed for wastewater treatment, encompassing physical, biological, and chemical treatments, all of which can effectively reduce viral loads. Physical methods of wastewater treatment include settlement, slow sand filtration and membrane filtration technology. Membrane filtration employs various techniques such as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (Qiu *et al.*, 2015; Ren *et al.*, 2022).

Biological treatment methods include biochar (a variant of activated charcoal) and membrane bioreactors that utilize biological processes, such as activated sludge, to break down organic matter and reduce viral loads in wastewater (Plaza-Garrido *et al.*, 2023; Wu *et al.*, 2022; Gholipour *et al.*, 2022).

Examples of commonly used chemical wastewater disinfection methods are chlorination, hydrogen peroxide, ozonation, and sunlight-mediated disinfection. Additionally, advanced oxidation processes (AOPs) and nanomaterials, such as silver nanoparticles, are also utilized for chemical disinfection purposes (Rathore *et al.*, 2023; Gururani *et al.*, 2021; Ibrahim *et al.*, 2020; Lisboan *et al.*, 2018).

Replacement of old lagoon-based sewage treatment with membrane bioreactor sewage treatment plants has resulted in a notable reduction in human norovirus (RNA copies) in treated sewage and oysters (Schaeffer *et al.*, 2018). A study comparing natural wetlands to a treatment process combining UV, chlorination,

and some commercial products, for example, microsand-based filtration, showed reduced viral loads in wetlands, with a reduction of 3.9 log₁₀ genome copies for human norovirus GII and 2.8 log₁₀ genome copies for HAV. In contrast, the combination of UV, chlorination, and microsand-based filtration resulted in 1.9 and 2.5 log₁₀ genome copies reduction for human norovirus GII and human adenovirus, respectively (Gonzales-Gustavson *et al.*, 2019). Mathematical modelling has demonstrated that lagoons are generally more efficient than mechanical wastewater treatment plants (WWTPs) in reducing human norovirus RNA copy numbers. Additionally, when comparing disinfection methods, UV treatment is more effective for inactivating viruses than chlorine (Pouillot *et al.*, 2022).

It is important to note that relatively simple and basic interventions can have a significant impact in low resource countries. For example, sinking a borehole, extending water pipelines, constructing latrines, and promoting hygienic practices through education in a rural Kenyan community reduced diarrhoea and improved water quality, indicating a direct public health benefit (Wandera *et al.*, 2022).

Infectious hepatitis E virus can be present in pig manure and urine, and therefore the use of manure or manure slurry as fertilizer is a potential source of hepatitis E virus contamination of the environment (Kasorndorkbua *et al.*, 2005), which may in turn become a source of contamination for preharvest fresh produce. Pig manure treatment before soil application can reduce hepatitis E virus contamination (Meester *et al.*, 2021), and a psychrophilic anaerobic biodigestion process reportedly reduced more than 2 log₁₀ virus load in the processed swine manure (Souza *et al.*, 2020), although the virus concentration in final effluent remained high.

4.1.3 Irrigation water

Irrigation water can be a source of viral contamination for fresh produce. Human pathogenic viruses, including hepatitis E virus, have been found in irrigation water samples from leafy green vegetables and berry fruit production chains (Kokkinos *et al.*, 2017).

Intervention strategies to reduce the risk of viral contamination involve controlling which water source is used, its location, irrigation method(s) and timing of irrigation. Most importantly, the use of water that has been impacted by human sewage should be avoided. Irrigation of crops with water that may have been contaminated beyond the minimum acceptable limit by animal waste, runoff from livestock farms, biosolids, organic manure and so forth, which elevates the

risk of contamination with viruses like hepatitis E, as well as bacterial enteric pathogens, should always be discouraged. In some regions, water for irrigation has undergone treatment processes to help remove contaminants and pathogens. The efficacy of treatment is often specified in government regulations and may include requirements for specific treatment types, monitoring and testing to ensure the safety of water used for irrigation of human food crops (FAO and WHO 2019).

In some instances, wastewater may be reused for irrigation purposes, but only if pathogen levels have been reduced to a sufficient degree so as not to pose deleterious health effects to the exposed population. Recycled water used for irrigation must be treated to ensure the safety of recycled water (Review in Gerba Betancourt and Kitajima, 2017). It has been demonstrated that Tulane virus and murine norovirus can persist for long periods (months, even years) in environmental waters used for irrigation in agriculture, suggesting the same behaviour for human norovirus (Anderson-Coughlin *et al.*, 2023). Some technologies, as described below, have been evaluated for water reuse, although specific application to berries has not yet been investigated.

On-farm treatments utilize processes similar to conventional wastewater treatment but with variations (e.g. pollutant removal capacity) to suit site-specific characteristics and agricultural practices (Perez-Mercado *et al.*, 2019). Risk modelling suggests that disinfection with UV light may be necessary prior to using reclaimed water for irrigation, to reduce human norovirus concentrations to levels meeting public health standards. For resource-constrained regions, cost-effective methods like slow sand filtration and biochar filtration (Abdiyev *et al.*, 2023; Bonanomi *et al.*, 2020) can be adopted to reduce virus levels of irrigation water (Troldborg *et al.*, 2017).

Moreover, employing biochar filters to treat greywater (domestic wastewater from, e.g. baths, washing machines and so on) has shown promising results, effectively removing microorganisms. Consumption of onions irrigated with treated greywater demonstrated a median annual risk for rotavirus diarrhea of 4.96×10^{-4} to 4.37×10^{-3} , which is lower than the risk associated with onions irrigated with untreated greywater. Recycling treated greywater through biochar filtration may thus enhance the microbial safety of irrigated fresh produce (Dalahmeh *et al.*, 2016). Similarly, zero-valent iron, a type of metallic nanoparticle, has been used with sand filtration to remove viruses from irrigation water. This method demonstrated a 2–3 log₁₀ reduction in murine norovirus and Tulane virus, suggesting that zero-valent iron may have potential for decontaminating water used in crop irrigation and facilitating the reuse of treated processing water for safe food production (Shearer and Kniel, 2018).

In regions experiencing water scarcity, treatment of effluent from a wastewater treatment plant with chlorine dioxide may produce acceptable water for irrigation purposes. Studies on the effect of chlorine dioxide on human noroviruses and astroviruses showed that, while treatment did not significantly reduce the viral load in water, the viruses were not detected in the irrigated lettuce product (López-Gálvez *et al.*, 2018).

4.1.4 Processing wash water

Post-harvest washing is a critical step in the processing of fresh produce, sometimes including berries, to ensure their quality and safety. Process wash water needs to be maintained at appropriate microbial quality to prevent pathogen cross-contamination of the produce during washing, and it can be treated with chemical disinfectants to do this (Allende *et al.*, 2024). Numerous studies have shown that the efficacy of chemical disinfection is dependent on many factors, including concentration, exposure time, temperature, type of product treated, microorganism type, and organic load (Lin *et al.*, 2020). Commonly used chemical disinfectants for controlling microbial load in produce wash water include sodium hypochlorite (free chlorine), chlorine dioxide (ClO₂) and peroxyacetic acid (PAA). While not applied to berries, a study on inactivation of Tulane virus and human norovirus at recommended operational limits was performed for vegetable wash water. More than 8 log₁₀ human norovirus and 3.9 log₁₀ Tulane virus were inactivated within 1 min at 20 ppm free chlorine. For ClO₂ (3 ppm), similar reductions were observed after 1 min for Tulane virus and 5 min for human norovirus. Peroxyacetic acid treatment at 80 ppm for 5 min prevented human norovirus replication in the human intestinal enteroid (HIE) model but was less effective on Tulane virus (Allende *et al.*, 2024). Regulations on chemical disinfectants that can be used in wash water for fresh produce for human consumption may vary between countries, and use in berries can be challenging because of fruit fragility and the short shelf-life of fresh product.

Activated water systems are gaining interest for their potential to improve water quality through innovative treatments that enhance antimicrobial properties. Systems such as electrolysed water, plasma-activated water, micro-nano bubbles, plasma-bubble activated water, and hydrogen-rich water are considered safe and environmentally friendly options with potential antimicrobial activity. The effectiveness of activated water systems for post-harvest management, their impact on fresh produce (including colour and spoilage), and their ability to inactivate microorganisms have been reviewed (Malahlela *et al.*, 2024). This is currently an active area of research.

4.1.5 Soils

Soil, comprising inorganic particles and organic matter, constitutes the loose surface material covering most land and serves as a fundamental medium for plant growth, providing essential structural support, water and nutrients. To enhance soil fertility and increase crop productivity, fertilizers, manure and biosolids are commonly utilized. Biosolids are treated sewage sludge that has undergone additional processing to remove pathogens and reduce odor. However, due to their elevated microbial risks, biosolids must be treated further to reduce or eliminate potential pathogens before use (Al-Gheethi *et al.*, 2018). Current literature predominantly focuses on the mitigation of plant viruses rather than those impacting human health. While methodologies for detecting viruses in soil exist, there is limited literature on foodborne viral outbreaks in which soil was directly implicated as the source of contamination. However, when soil growth systems were challenged with artificially contaminated infectious Tulane virus, the virus could be recovered from all tissues of romaine lettuce plants, but not from the tissues of green onion and radices (Yang *et al.*, 2018).

Among the physical methods, heat treatments of soil with steam or by solarization may be promising due to their broad applicability and low ecological impact. The effectiveness of these methods can be further enhanced by incorporating exothermically reacting chemicals during steam treatment, and utilizing novel plastic films for solarization (Luvisi, Panattoni and Materazzi, 2015). A study with poliovirus type 1 and coxsackievirus in soil showed a direct correlation between virus persistence and temperature, soil type, and the liquid amendment in which viruses were suspended (Yeager and O'Brien, 1979). Enteric viruses can exhibit remarkable stability in soil over time, with murine norovirus displaying only marginal reductions in infectivity following a 28-day period (Wu *et al.*, 2023).

Moreover, the application of organic amendments, including alfalfa straw and biochar has demonstrated efficacy in reducing the incidence of a viral plant disease within soil ecosystems (Bonanomi *et al.*, 2020). The adverse effects of biochar, including changes in soil properties and impact on soil organisms, have been reviewed elsewhere (Brtnicky *et al.*, 2021). Numerous strategies for virus inactivation within amendments/biosolids have also been explored. For instance, lime stabilization (i.e. augmenting the soil pH to 12 for a minimum of 2 hours by adding calcium hydroxide or calcium oxide), produced significant reductions in several viral species. Fecal coliforms were undetectable following 2 hours of lime stabilization, demonstrating a 7- \log_{10} reduction. Adenovirus, bacteriophage MS-2 and rotavirus were below detectable concentrations following 2 hours of liming, demonstrating a 4- \log_{10} reduction of infectious particles (Bean *et al.*, 2007).

Poliovirus is also effectively inactivated with this method (Sattar *et al.*, 1976). Among various treatments of biological sludge, the lowest prevalence of viruses was reported for lime-stabilized sludge (Gholipour *et al.*, 2022). Pasteurization of liquid sewage sludge at 70 °C for 30 minutes has been approved for the production of class A biosolids under the US regulation 40 CFR Part 503 (ECFR, 1995). However, extensive metadata analysis suggests that this time–temperature combination may not be sufficient to achieve a 3- \log_{10} reduction of all pathogen groups, indicating the need for potentially longer or more intensive treatment specifications (Espinosa *et al.*, 2020).

4.2 INTERVENTIONS RELATIVE TO REMOVAL OF VIRUSES FROM FOODS (MOLLUSCAN SHELLFISH, A SPECIAL CASE)

Most interventions do not result in the actual removal of virus particles from a contaminated product, or if removal occurs, it is tangential with the use of a product or process that inactivates the viruses. Molluscan shellfish are an exception. While preharvest preventive interventions are preferable, postharvest interventions adapted to live oysters, such as depuration or relaying, are commonly used to decrease contaminant levels. Depuration and relaying have been used for over a century in many countries worldwide to reduce or eliminate pathogens in various BMS. Depuration involves placing the BMS in tanks with “clean” seawater (i.e. free from fecal pollution), which is then continuously disinfected, often using UV light radiation (60–80 mJ/cm²). Other water disinfection processes such as ozone, free chlorine or ionophores have also been described (McLeod *et al.*, 2017). Depuration must be performed under conditions allowing for the optimal filtration of BMS to promote the release of pathogens from their digestive tissues into the water. Throughout the process, some physico-chemical parameters of seawater must be monitored (i.e. dissolved oxygen, water flow, salinity, temperature, turbidity and pH). All depuration conditions are widely described in various documents in Europe and the United States of America (McLeod *et al.*, 2017; US FDA, 2023). The depuration time usually varies between 24 and 96 hours, depending on the initial level of bacterial indicator contamination in BMS. Under these conditions, bacteria (e.g. *E. coli*, *Salmonella* spp.) appear to be effectively eliminated. However, depuration under standard conditions is not considered effective for eliminating enteric viruses (Gyawali *et al.*, 2019; McLeod *et al.*, 2017; Pilotto Souza and Barardi, 2019; Hartard *et al.*, 2018). Viral foodborne outbreaks involving BMS have been described after 48–72 hours of

deuration (McLeod *et al.*, 2017). This shows that, at least for some deuration processes, it is possible that sufficient loads of infectious human norovirus remain in the BMS tissues to cause acute gastroenteritis in consumers (Pilotto, Souza and Barardi, 2019; Polo *et al.*, 2014a). Alternative measures, such as that introduced by the New Zealand Food Safety Authority, include the closure of harvest areas for at least 28 days after human sewage contamination events (Greening and McCoubrey, 2010; Lees, 2000).

Assessing the efficacy of deuration to eliminate pathogenic viruses from BMS is far from easy considering that detection of the viral genome by RT-qPCR is not proof of the presence of infectious virus (Gassilloud, Schwartzbrod and Gantzer, 2003). Nevertheless, the ISO 15216-1 (2017), which is the international standard detection method and relies upon endpoint detection using RT-qPCR, is becoming widely used in the European Union to monitor virus removal from BMS. Most publications report only a small reduction in viral genome copy number for deuration times less than 96 hours (Leduc *et al.*, 2020; Battistini *et al.*, 2021). Some studies demonstrate an increase of genome copy number during early stages of deuration (below 62 hours), probably due to changing compartmentalization of viruses within and around the shellfish digestive tissues (McMenemy, Kleczkowski and Taylor, 2023). Few studies describe deuration kinetics under commercial conditions (Ueki *et al.*, 2007; Savini *et al.*, 2009), but it can be assumed that the release of viral particles (in less 96 hours or less) is low ($< 1.0 \log_{10}$). Some studies demonstrate a greater release of human norovirus GII compared with GI (Younger *et al.*, 2020). Accumulation or removal of viral pathogens also differs from one shellfish species to another (Nappier *et al.*, 2010). For example, the removal of murine norovirus was higher in mussels compared to clams (Polo *et al.*, 2014a and b).

The time-temperature pairing during deuration is perhaps the most critical factor driving optimal removal of infectious human norovirus from the BMS (Rupnik *et al.*, 2021). The time to reach the first \log_{10} reduction (T_{90}) of the norovirus genome during deuration is estimated to be between 9.0 and 45.5 days (McLeod *et al.*, 2017). The virus can also be inactivated inside the BMS, as emphasized by Leduc *et al.* (2020). Viral inactivation inside BMS may be the main mechanism which explains the difference in behaviours between the survival of viral particles and the persistence of the corresponding viral genome observed in the BMS under the same experimental conditions (Leduc *et al.*, 2020; Hartard *et al.*, 2017). For example, Fajardo *et al.* (2014) isolated a bacterial strain from the digestive tissues of BMS with antiviral activity against murine norovirus or hepatitis A virus. Additionally, Provost *et al.* (2011) suggested that the ability to

resist the acidic pH of hemocytes may influence virus persistence in shellfish. Other studies hypothesized a release of viruses from the BMS into the seawater (Younger *et al.*, 2020; Souza *et al.*, 2013). Some authors describe virus removal as a two-phase process. The first phase is a relatively rapid removal of viruses directly related to the filtration rate of the BMS, and the second phase is related to a less efficient removal of those virus particles which resist depuration (Polo, Varela and Romalde, 2015).

Removal of viral genome is usually better at temperatures above 11 °C (Rupnik *et al.*, 2021; McMenemy, Kleczkowski and Taylor 2023). Conversely, the following parameters have been shown to have little or no impact on human norovirus release during the depuration process: salinity, algal feeding, light/dark, disturbance from pump vibration, citric acid, shellfish stress, presence of hydrolates at 1 percent, and coproducts of essential oil distillation (Battistini *et al.*, 2023; Younger *et al.*, 2020; Leduc *et al.*, 2020). In general, it appears that few factors that can be manipulated will result in improved depuration efficiency.

Relaying involves moving live BMS from their growing area to a natural area with cleaner waters for longer periods than used for depuration. Relaying, alone or in combination with depuration, is reported to be more successful than depuration alone for removing infectious human norovirus from BMS (McLeod *et al.*, 2017; Gyawali *et al.*, 2019). Despite the apparent effectiveness of relaying, some disadvantages still limit its universal deployment, including the lack of nearby clean water zones and excessive cost. Another problem is that the classification of the relaying zone is generally based on fecal indicator bacteria (*E. coli*), which do not always reflect the presence of human enteric viruses (Love *et al.*, 2010). The use of viral indicators, such as infectious male specific coliphages, to estimate the depuration/relaying efficiency of BMS, may provide an alternative approach to define more accurately the time required to eliminate viruses by depuration and relaying (Leduc *et al.*, 2020; Hartard *et al.*, 2017).

In summary, depuration is often insufficient, as evidenced by multiple outbreaks associated with depurated BMS (McLeod *et al.*, 2017). It is important to keep in mind that the effectiveness of depuration and relaying depends on multiple factors, notably the initial viral load in BMS, and the species of shellfish and the water temperature. Human noroviruses are the primary pathogen about which the effectiveness of depuration has been reported in the literature, and there are limited data on the efficacy of depuration on hepatitis A virus (but none on hepatitis E virus).

4.3 INTERVENTIONS RELATIVE TO INACTIVATION OF VIRUSES VIA FOOD-PROCESSING AND PRESERVATION METHODS

4.3.1 Traditional food preservation methods

Food preservation and processing are commonly done to reduce spoilage potential, increase shelf life, and assure food safety, all with a focus on maintaining nutritional and sensory qualities of the product(s). Food preservation methods can be applied during production, harvesting, processing, packaging, distribution, and preparation of foods. Most of these methods are designed and validated against bacteria, not viruses. Some of the commonly used conventional food preservation techniques include refrigeration, drying, freezing, and pasteurization.

Drying

Survival of enteric viruses in a dried state has been studied mostly on inanimate surfaces or fomites, and these data have been extensively reviewed (Alidjinou *et al.*, 2019; Yeargin *et al.*, 2016). The multistate outbreak of hepatitis A associated with the consumption of sun-dried tomatoes illustrated that if food is contaminated before drying, substantial numbers of viruses will remain infectious for extended periods of time after drying (Gallot *et al.*, 2011; Petrigani *et al.*, 2010).

Freezing

Freezing (≤ 0 °C) has little impact on non-enveloped virus infectivity, and studies show minimal reduction of enteric viruses and surrogates upon freezing and after extensive frozen storage (Richards *et al.*, 2012; Butot, Putallaz and Sanchez, 2008). In most instances, freezing preserves enteric viruses.

Refrigeration

Early studies on virus survival on fresh produce under refrigeration conditions were reviewed by Rzeżutka and Cook (2004); the information accruing from these studies indicated that viruses could retain infectivity for several days, probably from the point of purchase to the point of consumption, if the foods were stored refrigerated. Hepatitis A virus and Aichi virus decreased by approximately 4 \log_{10} and 5 \log_{10} respectively in cranberry-juice-based drinks at 4 °C over 21 days of storage, with infectious virus still being detectable at the end of that period (Sewlikar and Souza, 2017). A variety of infectivity reduction profiles was observed when several human norovirus surrogate viruses were inoculated

into orange and pomegranate juices, and milk which was stored at 4 °C for 21 days (Horm and D'Souza, 2011), with some viruses being eliminated and others persisting, depending on the food.

Thermal inactivation

Heat treatment and thermal processing methods such as pasteurization and canning have long been considered safe, effective and economical ways of reducing foodborne pathogens in various foods. However, heating may change organoleptic and nutritional features of food, and the use of adequate temperatures and time is important for its efficacy. Inner portions of the product need to reach the temperatures required for pathogen inactivation, including those for viruses. Cooking food to an internal temperature of 70 °C has been recommended by WHO in their “five keys to safer food” manual (WHO, 2012). Other governmental organizations and agencies have more detailed and food-specific recommendations for heating temperatures, often codified in federal regulations (Health Canada, 2023; CDC 2023a). Multiple studies have shown that human norovirus, HAV and HEV have relatively high heat stability, and the efficacy of heat treatment can vary depending on the virus and the food matrix (Bertrand *et al.*, 2012; Johne *et al.*, 2024; Bozkurt *et al.*, 2015). It has been stated that heating to the internal temperature of 70–72 °C for 2 min significantly reduces infectious titers, but often does not exceed 4 log₁₀-reduction of foodborne viruses (reviewed in Johne, Scholz and Falkenhagen, 2024). Heating to temperatures lower than 60 °C for ≤ 1 min rarely leads to more than 1 log₁₀ reduction of foodborne viruses in food matrices, but over 4 log₁₀ reduction can be achieved after 95 °C ≥ 1 min treatment (reviewed in Johne, Scholz and Falkenhagen, 2024).

Since the previous JEMRA meeting (FAO and WHO, 2008), more information has emerged on the thermal resistance of hepatitis E virus. Overall, hepatitis E virus has a high degree of thermal stability, similar to hepatitis A (Emerson, Arankalle and Purcell, 2005). Different combinations of time and temperature have been found to inactivate hepatitis E virus, but this depends on the type of food (Johne, Scholz and Falkenhagen, 2024; Stunnenberg *et al.*, 2023; Yunoki *et al.*, 2008; Schielke *et al.*, 2011). Interpretation of experimental results is complicated by issues in cultivating and accurately enumerating surviving virus, and protective properties of the suspending matrix. For example, a reduction of > 3.5 log₁₀ infectivity was achieved by heating a cell culture-adapted hepatitis E virus suspension at 80 °C for 1 min or at 70 °C for 2 min (Johne *et al.*, 2016; Johne, Scholz and Falkenhagen, 2024). In another study, it took 20 min at 71 °C to inactivate hepatitis E virus in a pate-like product as evaluated using the pig bioassay (Barnaud *et al.*, 2012), but as some groups of pigs shared pens in that

study, cross-contamination may have occurred, and 71 °C for 10 min was possibly adequate (Cook and van der Poel, 2015). In a different study, boiling or stir-frying hepatitis E virus-contaminated pig liver to an internal temperature of 71 °C for 5 minutes prevented the experimental infection in pigs; however, cooking to lower temperature (56 °C for 1 hour) did not prevent infection in all pigs (Feagins *et al.*, 2008). After reported illness due to consumption of undercooked figatelli, a delicacy containing raw pig liver, the product label was changed by French authorities to better advise consumers of the risks associated with consumption of uncooked pork (Ruetsch *et al.*, 2016) and hepatitis E virus (Colson *et al.*, 2010; Pavio, Merbah and Thébault, 2014).

4.3.2 Emerging processing methods for inactivation of viruses in foods

Various physical and/or chemical treatments have been investigated for improving food safety, and inactivation of enteric viruses is no exception. Non-thermal physical technologies with antiviral applications include ionizing radiation, UV irradiation and pulsed light, high hydrostatic pressure, and cold plasma. These have shown some ability to reduce viruses in a variety of foods, without significant impact on organoleptic properties. Likewise, chemical disinfection has been investigated, with a focus on chlorine-based and peracetic acid compounds. The inactivation efficacy of the physical and chemical technologies depends on the doses/concentrations used, contact or treatment times, the type of virus, and the food matrix. Cultivable surrogates of human norovirus, in place of human strains, have been used to evaluate the efficacy of inactivation treatments in many studies. Often, surrogates do not behave in the same manner as infectious human norovirus; they are often more sensitive (Richards, 2012; Cromeans *et al.*, 2014). Little has been done with hepatitis A virus since the 2008 JEMRA report. Effectiveness in virus inactivation when applied to the two critical food commodity classifications (i.e. BMS and berries) are described below for various treatments.

High hydrostatic pressure processing (HPP)

High hydrostatic pressure (HPP) treatment involves applying high isostatic pressure (300–600 MPa for 2–5 min) at chilled or mild process temperatures (< 45 °C) for a few seconds to over 20 min (Govaris and Pexara, 2021). Therefore, HPP is considered a non-thermal processing method. However, HPP can negatively affect the organoleptic properties of many foods, making its application limited to specific food types such as shellfish, meat and meat products, certain vegetable products and dairy (Lou *et al.*, 2016). It is generally believed that high pressure treatment denatures the viral capsid proteins and therefore prevents

infectious virions from attachment and internalization into host cells (Kingsley, 2013; Emmoth *et al.*, 2017). The efficacy of HPP against foodborne viruses is highly variable and dependent on the virus type and the matrix (Takahashi *et al.*, 2019) as well as pressure, treatment time, temperature, and particularly pH, with human norovirus being more resistant to pressure at lower pH values and higher temperatures (Yang *et al.*, 2022; Li, Chen and Kingsley, 2013; DiCaprio *et al.*, 2019). Food components such as salts, lipids, carbohydrates, and proteins play a protective role against inactivation of viruses by high pressure (Hirneisen *et al.*, 2012). Also, multiple studies have demonstrated variable results for the high-pressure treatment of human norovirus surrogates in the presence or absence of food matrices. This was expected as the capsid structure and physical properties for these surrogate viruses are distinct from human norovirus strains of genogroups I and II. Care should be taken in interpreting data since the surrogates are often more sensitive to high pressure treatment than are the human virus strains (Cromeans *et al.*, 2014).

Kingsley (2014), Yang *et al.* (2022), Lou *et al.* (2015a) and Sun *et al.* (2023) have extensively reviewed the data supporting the efficacy of HPP for virus inactivation in BMS. Since the previous JEMRA meeting (FAO and WHO, 2008), several studies have been done with the cultivable surrogates and molluscan shellfish. For example, murine norovirus infectivity decreased by a little over 1.5 log₁₀ after HPP treatment at 275 MPa for 5 min (Takahashi *et al.*, 2019), although Arcangeli *et al.* (2012) reported complete inactivation of the virus in Manila clams after HPP treatment with 500 MPa or higher doses for one min. Relative to human norovirus, Leon *et al.* (2011) fed human volunteers human norovirus (GI.1)-inoculated oysters that had undergone various HPP treatments, finding only the most rigorous treatment (600 MPa, 6 °C, 5 min) prevented infection in all challenged subjects. Other investigations confirmed that similar treatment regimens (450–600 MPa for 5 min) produced complete inactivation of human norovirus in BMS homogenate, as evaluated using a receptor binding method and a gnotobiotic pig model (Lou *et al.*, 2015b; Ye *et al.*, 2014). Imamura *et al.* (2018) found that HPP treatment (400 MPa for 5 min) reduced most human norovirus strains present in naturally contaminated cultured Japanese oysters to a level below the assay detection limit (Imamura *et al.*, 2018).

Recent studies have highlighted that human norovirus GII strains are more sensitive than are GI strains to HPP treatment in oysters, as complete inactivation (over 3 log₁₀) was achieved by treatment at 600 MPa at 20 °C for GII while similar treatment resulted in less than one log₁₀ reduction for GI human norovirus (Rachmadi *et al.*, 2024). Other studies have confirmed this trend (Tong *et al.*, 2023; Ye *et al.*, 2014). In separate studies, HPP treatment of human norovirus GI.1

at 600 MPa for 2 min in dry blueberries led to less than a one \log_{10} reduction (Li, Chen and Kingsley, 2013), and similar treatment of GII.4 in strawberry puree led to complete inactivation ($> 5 \log_{10}$) (DiCaprio *et al.*, 2019). As the experimental conditions in these studies were different, direct comparison cannot be made between the observations, although the results support differential resistance by human norovirus genotype.

High pressure processing may also be an effective inactivation treatment for enteric viruses in fresh produce. As is the case for shellfish, pressure, pH, moisture, temperature, food matrix, and virus strain all affect susceptibility to inactivation (Huang *et al.*, 2014; Li, Chen and Kingsley, 2013). For instance, Li, Chen and Kingsley (2013) found that a pressure treatment of 600 MPa for 2 min at various temperatures barely caused any reduction of Tulane virus or murine norovirus-1 on unwetted blueberries, although lower pressures (300–350 MPa) were effective if the product was suspended in buffer. Pressure at 400 MPa for 2 min at 4 °C inactivated murine norovirus -1, effecting a $> 5 \log_{10}$ reduction. Murine norovirus was more sensitive to HPP at neutral pH than at acidic pH and at 4 °C than at 20 °C in fresh produce (Lou *et al.*, 2011). Likewise, when applied to human norovirus-inoculated strawberry, blueberry, and raspberry purees, a treatment of ≥ 550 MPa for 2 min at 0 °C produced a $> 2.9 \log_{10}$ reduction of a GI.1 strain and a $> 4.0 \log_{10}$ reduction of a GII.4 strain. There were no notable organoleptic effects on the purees. However, when applied to cut fruit, \log_{10} inactivation was significantly lower. The GI human norovirus strain was more resistant to pressure than the GII strain, with greater \log_{10} reduction observed for blueberries (Huang *et al.*, 2016). Additional work is needed to validate this technology under commercially relevant conditions.

In fresh salsa, strawberry puree, sliced green onions, and shellfish artificially contaminated with hepatitis A virus, HPP treatment at 400 MPa for 5 min produced 2.5 to 4.3 \log_{10} reductions (reviewed by Sánchez, Aznar and Sánchez, 2015). In artificially contaminated pork pâté, however, a $< 0.5 \log_{10}$ reduction in hepatitis E virus was observed after treatment at 400 or 600 MPa for up to 5 min (Nasheri *et al.*, 2020). Based on current data, hepatitis E virus appears to be relatively resistant to HPP, and high pressure/long time combinations are likely to be necessary to achieve significant \log_{10} reductions in the food matrix (Nasheri *et al.*, 2020; Johne *et al.*, 2021).

Ionizing radiation

Irradiation is the non-thermal process of exposing food to ionizing radiation, such as gamma rays (from the radionuclides cobalt-60 or caesium-137), electron beams (e-beams), or X-rays, to inactivate pathogenic microorganisms in food

(Han *et al.*, 2023). Irradiation rays can inactivate viruses by damaging their genomes and also by releasing radicals that affect the integrity of the viral capsid (Liu and Shan 2023). Overall, irradiation is considered an effective and safe treatment for foodborne pathogen inactivation and control of food spoilage (Singh and Olabisi, 2017). However, due to negative consumer perception, and the fact that regulations for ionizing radiation may differ between countries, its commercial use is limited for foods. Also, irradiation can have a negative effect on the sensory properties of certain foods, and for this reason FAO and WHO have recommended that the dose of radiation for the sterilization of food products should not exceed 10 kGy (FAO and WHO, 2003). Gamma rays are highly penetrative and therefore the shadow effect barely impacts the efficacy of that treatment, while e-beams are less penetrative but are considered comparatively inexpensive and eco-friendly (DiCaprio *et al.*, 2016). Food matrix, pH and the virus type influence the efficacy of irradiation (Han *et al.*, 2023).

Electron-beam irradiation of murine norovirus on inoculated strawberries reduced virus infectivity by $< 1 \log_{10}$ at 6 kGy and $< 2 \log_{10}$ at 12 kGy (Sanglay *et al.*, 2011). An e-beam treatment at 3.9 kGy reduced Tulane virus in strawberries by $1.4 \log_{10}$, and 4.1 kGy reduced Tulane virus in lettuce by $1.3 \log_{10}$. Virus concentration dropped by $7 \log_{10}$ to undetectable levels, at target e-beam doses of 16 kGy or higher (Predmore *et al.*, 2015a). Gamma irradiation at 4 kGy was shown to reduce murine norovirus-1 and human adenovirus type 5 by $2 \log_{10}$ on fresh strawberries and raspberries (Pimenta, Margaça and Verde, 2019). In another study, however, a 20 kGy dose of gamma radiation was needed to achieve a $1.26 \log_{10}$ reduction in human norovirus genome copy number in a stool suspension, and that same dose affected the quality and texture of strawberries (Molina-Chavarria *et al.*, 2020). In general, gamma irradiation may be more effective than e-beam for inactivation of human norovirus and Tulane virus. However, both techniques require extremely high doses (often as high as 20 kGy), which exceeds the allowable limits for fresh produce (10 kGy) in countries that permit the use of ionizing radiation. These doses will also almost certainly impact product quality and shelf-life (DiCaprio *et al.*, 2016).

In an earlier study, Bidawid, Farber and Sattar (2000) demonstrated a one \log_{10} reduction in hepatitis A virus infectivity in lettuce and strawberries following radiation treatment at 2.72 and 2.97 kGy, respectively. In whole oysters, Praveen *et al.* (2013) reported that the e-beam dose required to reduce murine norovirus and hepatitis A virus titer by 90 percent (one \log_{10}) was 4.05 and 4.83 kGy. There is currently no information on the efficacy of ionizing radiation on hepatitis E virus in foods.

Ultraviolet radiation (UV) and pulsed light

Ultraviolet light (UV) is a form of electromagnetic radiation with wavelengths from 10 nm to 400 nm – shorter than that of visible light, but longer than X-rays. Based on wavelength, UV is divided into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm). UV-C has germicidal properties and has been used by the industry to inactivate pathogens as it has a safer use profile (Ezzatpanah *et al.*, 2022). UV-C can damage the viral genome and impact capsid integrity (Araud *et al.*, 2020; Tanaka *et al.*, 2018). UV-C, however, has low penetrability into foods, therefore its application is mostly limited to surfaces and certain liquids (reviewed in Pexara and Govaris, 2020). As with other inactivation strategies, the efficacy of UV-C treatment is influenced by the type of the virus, food matrix, its surface and composition (Nasheri *et al.*, 2021).

UV-C treatment at wavelengths ranging from 200–280 nm has been shown to reduce murine norovirus and hepatitis A virus on blueberries by 2–3 \log_{10} , which was greater than that on strawberries and raspberries ($< 2 \log_{10}$) (Butot *et al.*, 2018). Many ancillary factors affect the efficacy of UV inactivation of viruses on fresh produce, including poor penetration, surface topography of the product, and nucleic acid target type.

Pulsed light treatment is an emerging inactivation method that uses high-intensity white light at wavelengths of 200 to 1 100 nm for microseconds and may be suitable for delicate fruits and vegetables. Pulsed light reduced murine norovirus and hepatitis A virus by as much as 2 \log_{10} and has been shown to be effective in inactivating viruses on specific types of berries such as strawberries and raspberries (Jubinville *et al.*, 2022). These findings were confirmed more recently by Kim *et al.* (2024), with the added observation that the process worked quite well at inactivating murine norovirus-1 and hepatitis A virus inoculated on the surface of cranberries (3.5 \log_{10} reduction). Kingsley *et al.* (2018) found pulsed light to be ineffective in reducing Tulane virus from the surface of blueberries. Péloquin *et al.* (2023) showed that bacterial biofilms might be protective of norovirus surrogates inoculated on berries that were then treated by pulsed light. Further work is needed on pulsed light to better characterize inactivation profiles, address shadowing, and determine if these degrees of inactivation are sufficient for adequate control. The ease of commercialization also needs to be assessed. As is always the case for emerging technologies, the effect of the treatment on product quality and shelf-life must also be evaluated and considered prior to commercialization.

Ultraviolet (with and without pulsing) has also been investigated for its ability to inactivate foodborne viruses on surfaces. For instance, greater than a 2 \log_{10}

reduction of hepatitis A virus and murine norovirus was reported on stainless steel after treatment with pulsed UV-C at 254 nm (Park *et al.*, 2015; Corson *et al.*, 2024). Jean *et al.* (2011) treated murine norovirus-1 and hepatitis A virus-inoculated stainless steel and polyvinyl chloride surfaces with pulsed UV light, finding up to 5 log₁₀ inactivation in the absence of soil load, and 3 log₁₀ in its presence, even after only a 2-second treatment (see section 4.4.3. for further inactivation on surfaces).

Cold plasma

Cold plasma is a newer non-thermal technology proposed for use in food preservation and food safety. It is particularly effective in enzymatic inactivation, a driver of nutritional and sensory deterioration. The technology is safe and environmentally friendly and can be used to decontaminate food surfaces (review by Farooq *et al.* [2023]). A study of its virucidal effect demonstrated that the main factors influencing inactivation included the plasma generation power, exposure time and distance, plasma feed gas mixture, and the virus suspension medium. The reactive oxygen and nitrogen species produced by plasma may oxidize virus capsid proteins (Aboubakr *et al.*, 2015). Cold plasma treatment reduced Tulane virus titers up to 3.5 log₁₀ and murine norovirus titers > 5 log₁₀ on the surface of blueberries after treatment times at 120 and 90 s, respectively. Inactivation by cold plasma was effective on these virus surrogates under nonthermal conditions (Lacombe *et al.*, 2017). Although studies are limited, cold plasma may be a promising treatment approach and deserves further investigation.

Commercialization

Oxidizing chemical disinfectants, including free chlorine, sodium hypochlorite, chloride dioxide, peracetic acid, hydrogen peroxide, peroxyacids, and ozone, have been studied for their antiviral efficacy in berries. Free chlorine, for example, is able to damage viral capsids and genomes (Wigginton *et al.*, 2012). At a low level, the addition of chlorine to wash water used in post-harvesting processing is commonly thought to provide some virus inactivation, albeit perhaps minimal (< 1 log₁₀) at the concentrations and contact times generally approved (Cook, Knight and Richards, 2016; Cook *et al.*, 2018).

Inclusion of oxidizing disinfectants in produce wash waters has been comprehensively studied. Using the HIE system (Ettayebi *et al.*, 2016), Allende *et al.* (2024) demonstrated that infectious human norovirus could be inactivated in process wash waters intended for use with various fresh produce items. After benchtop experiments followed by mathematical modelling, they found that inactivation of > 8 log₁₀ human norovirus and 3.9 log₁₀ Tulane virus was achieved

in wash waters using 20 ppm free chlorine within 1 min, and by 3 ppm chlorine dioxide in 1 min (for Tulane virus) or 5 min (for human norovirus). Wash water containing 80 ppm peracetic acid reduced Tulane virus by about 2 log₁₀ although the virus could not be completely inactivated even after 20 minutes of contact time; in contrast, there was a complete loss of human norovirus infectivity after 5 min of this treatment (Allende *et al.*, 2024). It should be noted that these latter experiments were performed only on virus suspended in the water in which produce had been washed. Wash water performance when virus is present on the surface of the fruit is not well characterized but likely involves higher disinfectant concentrations and longer contact times.

An alternative to chlorine is chlorine dioxide. In a series of studies, investigators found some efficacy with gaseous chlorine dioxide (0.63 to 4.40 ppm-h/g) against Tulane virus-inoculated berries. For instance, at the lowest concentration tested, average reductions were 2.98, 3.40, 3.82, and 4.17 log₁₀ PFU/g for strawberries, raspberries, blueberries, and blackberries, respectively (Kingsley and Annous, 2019). A later study (Anous *et al.*, 2021) reported average log₁₀ reduction for strawberries, raspberries, blueberries and blackberries treated with 1.00 ppm-h/g, the lowest ClO₂ treatment tested, of 2.44, 2.49, 3.23, and 3.45 log₁₀, respectively. The highest treatment of 6.27 ppm-h/g produced an average log₁₀ reductions ranging from 3.20–4.42. However, Girard *et al.* (2016) found chlorine dioxide (50 ppm) to be relatively ineffective (< 1.0 log₁₀ inactivation) against murine norovirus-3 inoculated onto blueberries and strawberries.

Ozone is a strong oxidizing agent with less likelihood for production of toxic by-products. Predmore *et al.* (2015b) reported that treatment with 6 percent gaseous ozone for 40 min produced a > 3.3 log₁₀ PFU/g reduction in the titer of murine norovirus and Tulane virus on artificially contaminated strawberries. Of late, the combination of peroxide with organic acids (peroxy-acids) has gained attention, with varying results. At concentrations in the 80-ppm range and contact times of minutes, some have observed reductions as large as 3–4 log₁₀ of murine norovirus and/or Tulane virus surrogates on various berry products (Girard *et al.* 2016; Bouchard *et al.*, 2023), but others have shown poorer efficacy (Otiz-Sola *et al.*, 2020). Peroxyacids were not able to substantially reduce infectious titers of hepatitis A virus or hepatitis E virus and appear to be sensitive to organic matter (Bouchard *et al.*, 2023). Ortiz-Sola *et al.* (2022) recently examined combination treatments of sodium hypochlorite, water-assisted UV-C and/or PAA for inactivation of murine norovirus-1 on whole and fresh-cut strawberries, finding < 2 log₁₀ PFU inactivation.

Several studies have sought to compare a variety of oxidizing disinfectants as applied to foodborne virus inactivation on the surface of berries. In tests on

murine norovirus-3-inoculated strawberries and blueberries, Girard *et al.* (2016) compared peracetic acid (85 ppm, 1 min), sodium hypochlorite (50 ppm, 1 min), and chlorine dioxide (20 ppm, 1 min). The former two treatments reduced virus titers by as much as 3–4 log₁₀ (in some instances), but chlorine dioxide was less effective. Bouchard *et al.* (2023) found that on inoculated blueberries and strawberries, peracetic acid (80 ppm) reduced murine norovirus-1 titer by an average of 3.6–4.0 log₁₀ after 5 min, but a 0.5 min contact time was insufficient to inactivate the virus on either fruit. In that same study, peroxyacids reduced the infectivity of hepatitis A and E viruses on surfaces by < 2 log₁₀, regardless of concentration or exposure time.

At the current time, no one oxidizing disinfectant stands out as superior for reducing foodborne viruses on the surface of berries, and efficacy is highly impacted by concentration, contact time, and soil load. Furthermore, country- or region-specific regulatory restrictions may prove prohibitive to the use of some oxidizing disinfectants, as might the impact of the treatment(s) on shelf-life and quality of these fragile fruits.

There is little information on the effect of chemical disinfection on hepatitis E virus. Sodium hypochlorite at 1 000 ppm inactivated just under 3 log₁₀ of hepatitis E virus-1 suspended in water (Girones *et al.*, 2014). Hepatitis E virus-3 was relatively resistant to various alcohol-based commercial disinfectants, reducing infectivity by 1 log₁₀ or less, except for one product that contained phosphoric acid (Behrendt *et al.*, 2022). Some commercial disinfectant formulations, particularly those with active ingredients like quaternary ammonium compounds, glutaraldehyde or peracetic acid, achieved higher (between 2 and 3 log₁₀) reductions in hepatitis E virus-3 infectivity on stainless steel surfaces (Wißmann *et al.*, 2023). Further investigation is needed before making clear conclusions about the efficacy of commercial disinfectants on hepatitis E virus.

4.4 INTERVENTIONS FOR FOMITE-BASED SOURCES OF CONTAMINATION

4.4.1 Environmental persistence of viruses

Enteric viruses can persist for long periods of time on hard surfaces. Human noroviruses generally survive for at least 28 days with subtle differences in temperature and relative humidity, and in genogroup (Cook, Knight and Richards, 2016). Similar data exists for hepatitis E virus (Wolff, Günther and John, 2022).

In studies conducted on stainless steel and blueberries at room temperature, HAV infectivity declined by less than one \log_{10} over 21 days (Leblanc *et al.*, 2019). Differences in viral reduction based on molecular detection and infectivity must be acknowledged, and studies are beginning to differentiate between the persistence of infectious virus and that of viral RNA (Trudel-Ferland, Jubinville and Jean, 2021). Recent infectivity studies on human norovirus persistence using HIE systems confirm earlier studies measuring genome copy numbers (Overbey *et al.*, 2021). The prolonged persistence of foodborne viruses on surfaces contributes to the risk of cross-contamination between surfaces, utensils, and between hands and food. In turn, this risk stresses the need for effective surface disinfectants, especially during outbreaks and incidents like vomiting.

4.4.2 Virus transferability

Virus transfer has been shown to depend greatly on the moisture of the fomites, hands, and foods involved, with an efficiency that may differ from what is observed in controlled laboratory experiments; transferability also varies by type of fomite and contact pressure (Verhaelen *et al.*, 2013; Tuladhar *et al.*, 2013). In laboratory studies, virus transfer has been documented to occur in both directions between fomites and foods and from gloved hands to foods (Stals, Uyttendaele and Baert, 2013; Verhaelen *et al.*, 2013). Studies have shown the potential for viruses to be transferred repeatedly, albeit usually less efficiently with each sequential tactile event, and sequential transfer was demonstrated in the preparation of a ready-to-eat food: In the process of making a sandwich, virus transfer between ingredients was demonstrated (Rönnqvist *et al.*, 2014). Successive transfer of viruses also occurred between contaminated utensils and food products (Wang *et al.*, 2013).

4.4.3 Efficacy of surface disinfection products and practices, including wiping

Evaluation and development of effective methods and products to inactivate foodborne viruses from surfaces remains an active area of research. Unfortunately, it is complicated by the inherent resistance of the viruses to many commonly used surface disinfectants, and also by the fact that virus cultivation is often difficult and study designs are not always comparable.

Often, testing antiviral efficacy of chemical and physical disinfection includes the use of cultivable surrogates and/or RT-qPCR assays. Earlier surrogates like feline calicivirus and bacteriophage MS-2 are now recognized as not ideal, as they often do not accurately mimic the behaviour of human noroviruses, tending to be

more sensitive to inactivation methods (Richards, 2012; Cromeans *et al.*, 2014). Nonetheless, the benchmark upon which licensing claims for anti-norovirus activity can be based for surface disinfectants, as per the US Environmental Protection Agency (EPA), is efficacy against feline calicivirus (US EPA, n.d.). The more recently used surrogates (murine norovirus type 1 and Tulane virus) behave in a manner more similar to human norovirus but are not necessarily perfect (Cromeans *et al.*, 2014; Richards, 2012). The reports of human norovirus propagation in HIE (Ettayebi *et al.*, 2016) and zebrafish larvae (Van Dycke *et al.*, 2019) offer hope for more relevant methods that evaluate human norovirus infectivity but have only had limited use for disinfection research purposes (e.g. Tan, Gong and Li, 2023; Costantini *et al.*, 2018) or to validate disinfection efficacy data obtained by other means (e.g. Escudero-Abarca *et al.*, 2022a). This is largely because the methods are non-quantitative in terms of characterization of log₁₀ reduction, expensive, laborious, and/or use very small inoculum volumes. In addition, studies are not coordinated, and standardized disinfection efficacy methods differ by region, making it difficult to compare results and perform meta-analyses. Often there are only a few studies evaluating the efficacy of a particular disinfectant or process. In many cases, an active ingredient is tested but not the entire commercial formulation, so synergistic effects cannot be teased apart. Additionally, there is a general lack of reference standard(s) with known efficacy against which new formulations can be compared. For these reasons, it can be difficult to draw conclusions about the efficacy of novel surface disinfection products and techniques.

The approaches that show the most promise and merit further study include copper, cold plasma, hydrogen peroxide, UV, and ozone. However, some of these may not be practical for particular venues (e.g. catering services vs factories) and needs (e.g. near instantaneous action) but may show potential for use when, for instance, residual activity is necessary (as might be the case for copper alloys). Table 1 summarizes a selection of studies on disinfectant efficiency, mostly done using the murine norovirus-1 and Tulane virus surrogates but also on RNase-RT-qPCR assays that can serve as a proxy for human norovirus infectivity. When selecting a disinfection approach, it is important to take into account the setting and circumstances under which the disinfection is to be undertaken. For example, outbreak vs non-outbreak settings; routine disinfection vs disinfection after an emetic or fecal contamination event; food-processing vs food service environment. Most products used in surface disinfection are sensitive to organic matter (Springthorpe and Sattar, 2005), making pre-cleaning essential, as already stated in the current Guidelines CAC/GL 79-2012 of Codex. Rarely, however, are cleaning methods rigorously defined. Recent research has, however, revealed the

relative importance of inactivation vs removal in disinfection (Faircloth *et al.*, 2022). In this study, 3 of the 4 products tested produced $\leq 0.5 \log_{10}$ reduction in Tulane virus and human norovirus by standard surface assay (without wiping), but inclusion of wiping with application of the disinfectant improved efficacy of the subsequent disinfection by 1.6 to 3.8 \log_{10} . High concentrations of virus (1.5–2.5 \log_{10}) could be recovered from the spent paper towels used for wiping. The study highlighted the importance of wiping but also suggested that repeated use of the spent wiping implement could result in cross-contamination if the disinfectant itself was ineffective.

Table 1 illustrates the high degree of variability in disinfection data. Oftentimes, this occurs between different studies evaluating the same virus–disinfectant pair. Overall, disinfection efficacy is impacted by active ingredient, product concentration/dilution, contact time, soil load, surface, and virus strain (for human norovirus). It is necessary to note that product formulation is important, and even a relatively ineffective active agent can perform better if formulated with synergistic ingredients. This was the case for a recent ethanol-based product (Escudero-Abarca *et al.*, 2022a). In general, product formulations relying on quaternary ammonium compounds as active ingredients produce minimal \log_{10} reductions in human norovirus (Gerba, 2015). In the United States of America, the standard recommendation for human norovirus surface inactivation is that promoted by the US Centers for Disease Control and Prevention (CDC), i.e. 1 000 ppm (0.1 percent) hypochlorite (bleach) for clean surfaces, and up to 5 000 ppm (0.5 percent) for dirty surfaces (CDC, 2023b). However, there is not a lot of scientific data supporting this recommendation, which is complicated by the lack of virus cultivability, the inadequacy of the surrogates, and strain-to-strain differences in disinfectant efficacy. Recent evidence suggests lower hypochlorite concentrations may be possible for clean surfaces, but even 5 000 ppm for 1 min may be insufficient for dirty ones (Escudero-Abarba *et al.*, 2022). It should be kept in mind that such high bleach concentrations may be prohibitive in many food applications due to concerns about safety, odour, corrosivity, and regulatory restrictions.

The data on inactivation of hepatitis E virus by surface disinfectants, either by active or commercial products, are too limited to make conclusions but suggest a similarly high degree of resistance as observed for the other enteric viruses.

Table 1. List of disinfectant for fomites

Treatment (concentration)	Virus or surrogate	Exposure time	Surface type	Type of test	Effect	Reference
alcohol-based commercial product	HuNoV GI.6; GI.4	30 s	Formica	RNase-RT-qPCR	> 3.5 log ₁₀	Faircloth et al., 2022
alcohol-based commercial product	TV	30 s	Formica	TV inf assay	> 3.5 log ₁₀	Faircloth et al., 2022
alcohol based PURELL® PSS	HuNoV GI.4	60 s	Stainless steel, 5% soil	RT-qPCR and HIE	2.5 log ₁₀ No infectivity remaining	Escudero-Abarca et al., 2022a
Ethanol 70%	MNV-1	30 s-1 min	Formica	Plaque assay	No inactivation of MNV	D'Souza and Su, 2010
Ethoxylated alcohols	HuNoV GI.4	10 min	Stainless steel	RNase-RT-qPCR	no effect	Girard et al., 2010
Sodium hypochlorite (3%) (=30 000 ppm)	HuNoV GI.4	5 min	Stainless steel	RNase-RT-qPCR	2 log ₁₀	Girard et al., 2010
Sodium hypochlorite (3%) (=30 000 ppm)	HuNoV GI.4	10 min	Stainless steel	RNase-RT-qPCR	3.5 log ₁₀	Girard et al., 2010
Sodium hypochlorite (5 000 ppm)	HuNoV GI.4	4 min	Stainless steel (fecally soil)	RT-qPCR	< 1 log ₁₀	Park and Sobsey, 2011
Sodium hypochlorite (5 000 ppm)	MNV-1	4 min	Stainless steel (fecally soil)	RT-qPCR	< 1 log ₁₀	Park and Sobsey, 2011

Treatment (concentration)	Virus or surrogate	Exposure time	Surface type	Type of test	Effect	Reference
Sodium hypochlorite (5 000 ppm)	MNV-1	3.2 min	Stainless steel (fecally soil)	Plaque assay	3 log ₁₀	Park and Sobsey, 2011
Sodium hypochlorite-based sanitizer (200 ppm)	TV	30 s	Formica	TV inf assay	≤ 0.5 log ₁₀	Faircloth et al., 2022
Sodium hypochlorite-based sanitizer (200 ppm)	HuNoV GI.6; GI.4	30 s	Formica	RNase-RT-qPCR	≤ 0.5 log ₁₀	Faircloth et al., 2022
Chlorine 200 ppm	MNV-1	18 s	Stainless steel	plaque assay	7 log ₁₀	Bolton et al., 2013
Hydrogen peroxide (liquid) (2.1%)	MNV-1	10 min	Stainless steel	Plaque assays	4 log ₁₀	Li et al., 2011
Peroxyacetic Acid (PAA) (100 ppm)	MNV-1	3 min	Stainless steel	Plaque assay	2 log ₁₀	Moon et al., 2021
Peroxyacetic Acid (PAA) (200 ppm)	MNV-1	3 min	Stainless steel	Plaque assay	4 log ₁₀	Moon et al., 2021
Peracetic (PAA) (50 mg/L)	MNV	5 min	Stainless steel or polyvinyl chloride with/without fouled condition	Plaque assay	3 log ₁₀	Vimont et al., 2015

Treatment (concentration)	Virus or surrogate	Exposure time	Surface type	Type of test	Effect	Reference
Peracetic acid or commercial peracetic-acid-based disinfectant (80 ppm)	MNV-1	0.5 min	Stainless steel	Plaque assay	3 log ₁₀	Bouchard <i>et al.</i> , 2023
Peracetic acid or commercial peracetic-acid-based disinfectant (1000 ppm)	HAV	1 min	Stainless steel	Plaque assay	< 1 log ₁₀	Bouchard <i>et al.</i> , 2023
Peracetic acid or commercial peracetic-acid-based disinfectant (1000 ppm)	HEV	5 min	Stainless steel	RT-PCR (culture)	< 0.6 log ₁₀	Bouchard <i>et al.</i> , 2023
Perpropionic (PPA) (50 mg/L)	MNV	5 min	Stainless steel or polyvinyl chloride with/without fouled condition	Plaque assay	3 log ₁₀	Vimont <i>et al.</i> , 2015
Per levulinic acid (PLA) (1000 ppm) ± 1% sodium dodecyl sulfate (PLAS)	MNV-1	5 min	Stainless steel	Plaque assay	1 log ₁₀ for PLA 2 log ₁₀ for PLAS	Bouchard <i>et al.</i> , 2023
Per levulinic acid (1000 ppm) ± 1% sodium dodecyl sulfate	HAV	1 min	Stainless steel	Plaque assay	< 1 log ₁₀	Bouchard <i>et al.</i> , 2023

Treatment (concentration)	Virus or surrogate	Exposure time	Surface type	Type of test	Effect	Reference
Per levulinic acid (1000 ppm) ± 1% sodium dodecyl sulfate	HEV	5 min	Stainless steel	RT-PCR (culture)	< 0.6 log ₁₀	Bouchard <i>et al.</i> , 2023
Levulinic acid (0.5%) + 0.5% SDS	MNV-1	5 min	Stainless steel	plaque assay	3.3 log ₁₀	Cannon <i>et al.</i> , 2012
Levulinic acid (5%) with SDS (2%), saturated wipe	MNV-1	18 s	Stainless steel	plaque assay	7 log ₁₀	Bolton <i>et al.</i> , 2013
SDS (2%) saturated wipe	MNV-1	18 s	Stainless steel	plaque assay	3.5 log ₁₀	Bolton <i>et al.</i> , 2013
Silver dihydrogen citrate	HuNoV GI.6; GI.4	15-1800 s	Stainless steel	RT-qPCR	< 4 log ₁₀	Manuel, Moore and Jaykus, 2017
AAS based sanitizer #	TV	30 s	Formica	TV inf assay	≤ 0.5 log ₁₀	Faircloth <i>et al.</i> , 2022
AAS based sanitizer #	HuNoV GI.6; GI.4	30 s	Formica	RNase-RT-qPCR	≤ 0.5 log ₁₀	Faircloth <i>et al.</i> , 2022
Glutaraldehyde (2%)	MNV-1	30 s-1 min	Formica	Plaque assay	6 log ₁₀	D'Souza and Su, 2010
QAC-based sanitizer (400 ppm) ##	HuNoV GI.6; GI.4	30 s	Formica	RNase-RT-qPCR	≤ 0.5 log ₁₀	Faircloth <i>et al.</i> , 2022
QAC-based sanitizer (400 ppm) ##	TV	30 s	Formica	TV inf assay	≤ 0.5 log ₁₀	Faircloth <i>et al.</i> , 2022

Treatment (concentration)	Virus or surrogate	Exposure time	Surface type	Type of test	Effect	Reference
Alpet D2 (QAC) ** saturated wipe	MNV-1	18 s	Stainless steel	plaque assay	3.5 log ₁₀	Bolton <i>et al.</i> , 2013
QAC	HuNoV GII	10 min	Stainless steel	RNase-RT-qPCR	no effect	Girard <i>et al.</i> , 2010
Trisodium phosphate (TSP) 5%	MNV-1	30 s	Formica	Plaque assay	> 6 log ₁₀ PFU	D'Souza and Su, 2010
Trisodium phosphate (TSP) 2%	MNV-1	30 s	Formica	Plaque assay	1 log ₁₀ MNV	D'Souza and Su, 2010
Trisodium phosphate (TSP) 1%	MNV-1	30 s	Formica	Plaque assay	No inactivation of MNV	D'Souza and Su, 2010
Neutral electrolyzed water pH 7	HuNoV GII.4	1-30 min	Stainless steel	RNase-RT-qPCR ; SDS-PAGE, TEM, HBGA-receptor binding	5 log ₁₀ by 250 ppm after 30 min. Loss of receptor binding at 5 ppm, 30 s Log reduction much affected by soil	Moorman, Montazeri and Jaykus, 2017
Neutral electrolyzed water pH 7	TV	1-30 min	Stainless steel	RNase-RT-qPCR ; SDS-PAGE, TEM, HBGA-receptor binding	4 log ₁₀ by 250 ppm after 30 min. Loss of receptor binding at 5 ppm, 30 s Log reduction much affected by soil	Moorman, Montazeri and Jaykus, 2017

Treatment (concentration)	Virus or surrogate	Exposure time	Surface type	Type of test	Effect	Reference
UV-C (260 nm wave length)	MNV-1	3 min (1800 mJ/cm ²)	Stainless steel	Plaque assay	< 1 log ₁₀	Moon <i>et al.</i> , 2021
UV-C (10-300 mW/cm ²)	HAV		Stainless steel	Plaque assay	HAV 0-2.6 log ₁₀	Park <i>et al.</i> , 2015
UV-C (10-300 mW/cm ²)	MNV-1		Stainless steel	Plaque assay	MNV-1 0-4.4 log ₁₀	Park <i>et al.</i> , 2015
pulsed ultraviolet (UV) light (200-1100 nm range)	HAV	2-3 s	Stainless steel	Plaque assay	Total reduction 5 log ₁₀	Jean <i>et al.</i> , 2011
pulsed ultraviolet (UV) light (200-1100 nm range)	MNV-1	2-3 s	Stainless steel	Plaque assay	Total reduction 5 log ₁₀	Jean <i>et al.</i> , 2011
Hydrogen peroxide (12.4 ml/m ³) surfactant-based product using fogging delivery	HuNoV G1.6		Stainless steel	RNase-RT-qPCR	G1.6: 2.5 log ₁₀ reduction genome copies G1.4: 2.7 log ₁₀ reduction genome copies	Montazeri <i>et al.</i> , 2017
Steam-ultrasound	HAV	0-5 s	Plastic, steel	Survival RT-qPCR	Survival: D values of HAV were 1.1-0.8 s on plastic, 1.4-0.8 s on steel: RT-qPCR: No clear trend of genome reduction was observed	Rajjuddin <i>et al.</i> , 2020

Treatment (concentration)	Virus or surrogate	Exposure time	Surface type	Type of test	Effect	Reference
Steam-ultrasound	MNV-1	0-5 s	Plastic, steel	Survival RT-qPCR	Survival: D values of MNV were 0.4-0.2 s on plastic, 0.9-0.7 s on steel: RT-qPCR: No clear trend of genome reduction was observed	Rajuddin <i>et al.</i> , 2020
Steam-ultrasound	MNV-1	3 s	Plastic	Plaque assay	3.7 log ₁₀	Schultz <i>et al.</i> , 2012
Hydrogen peroxide (vaporized, 2.5%)	MNV-1	10 min	Stainless steel	Plaque assays	< 1 log ₁₀	Li <i>et al.</i> , 2011
Chlorine Dioxide Gas 1-4 mg/litre	MNV-1	1-5 min	Stainless Steel Coupons	Plaque assay	2.5 mg/litre, 1 min = > 3 log ₁₀	Yeap <i>et al.</i> , 2016
Cold atmospheric pressure plasma (CAPP)	HuNoV GII.4	2 min	Petri dish	RNase-RT-qPCR	1 log ₁₀	Ahlfeld <i>et al.</i> , 2015

AAS = Acid and Anionic Surfactant, used at 1875ppm lactic acid and 700ppm dodecylbenzenesulfonic acid in Faircloth

QAC = Quaternary Ammonium Compound

** Alpet D2 = isopropanol-based quaternary ammonium compound

Sources: **Reference** column cites the corresponding studies. See References section for further details.

4.5 INFORMATION ABOUT INTERVENTIONS RELATIVE TO INFECTED/ILL FOOD HANDLERS AND HUMAN HYGIENE

4.5.1 Exclusion

Given the large amount of virus shed by infected food handlers, exclusion of those workers from food production, processing and preparation is likely the best way of preventing foodborne viral disease transmission. For the retail food sector in the United States of America, some food workers report that they work while sick because they cannot afford to take time off (Clayton *et al.*, 2015). Foodborne illness rates declined after paid sick leave laws were introduced in some US states but increased in locations with laws that were less supportive (Hsuan *et al.*, 2017). When one large US national chain expanded paid sick leave during the COVID-19 pandemic, this reduced the share of employees working while sick (Schneider *et al.*, 2021). One-third of US restaurants' policies did not specifically address when ill food workers should be excluded from work (Norton *et al.*, 2015). Many restaurant managers report that they themselves had worked while ill, including some working while nauseous or with the "stomach flu" (Norton *et al.*, 2015). The previous JEMRA meeting (FAO and WHO, 2008) concluded that worker exclusion policies based on stool testing were not considered effective for reducing the risk of enteric disease, largely because rapid point-of-care tests were not readily available for viral gastroenteritis (Todd *et al.*, 2008). Such tests are still not widely available around the world. It is important to note that any exclusion policy would miss workers who are shedding the virus asymptotically, although there is some evidence that handwashing can be effective in controlling asymptomatic spread (Wensley *et al.*, 2023).

4.5.2 Compliance behaviours and design

Training and facility design are important in encouraging good behaviors. One comprehensive study reported that handwashing compliance was greatest among restaurant workers who had received food safety training, in restaurants with more than one hand sink, and in restaurants with a handwashing sink in workers' line of sight (Green *et al.*, 2007). Likewise, glove use was highest in chain restaurants and in restaurants with gloves available to workers in the food preparation areas. Handwashing and glove use were also correlated such that hand washing was less likely to occur when gloves were worn. As asymptomatic virus shedding can occur, food handlers should adhere to handwashing instructions at all times (see current Guidelines (CAC/GL 79-2012, quote 52) (FAO and WHO, 2012). The US Food and Drug Administration, in its recently updated

Employee Health and Personal Hygiene Handbook (US FDA, 2005), outlines the steps to be followed to prevent exposure to and/or spread of viral foodborne pathogens while handling food. These steps address risk management measures when workers develop foodborne disease symptoms while at work or at home. Similarly, promoting safe food handling has been extensively addressed by WHO (2012) as an inherent strategic goal of global food safety along the entire food chain. Guidelines and policies are needed to address the post-symptomatic phase among food-handlers, to reduce the likelihood of transmitting viral pathogens by carriers; this is especially important related to the duration of shedding virus, and returning to work. So far, such policies have not been universally developed or adopted.

While viral gastroenteritis commonly spreads via the fecal-oral route and through person-to-person contact (contaminated objects, foods or drinks), some outbreak investigations have implicated vomiting as a transmission risk, either by contamination of surfaces or through release of virus-laden aerosolized droplets (CDC, 2023c; Kirby, Streby and Moe, 2016; Petrignani *et al.*, 2015). Consequently, effective control measures should include knowledge about how to manage public vomiting events, and access to on-site vomit clean-up protocols/materials.

Food handling facilities should be designed and constructed in a manner that supports proper maintenance, cleaning and disinfection (FAO, 2023a). Prevention of food contamination by food handlers is a shared responsibility between the handlers and management. The maintenance of high operation standards relies on supervision, ongoing training and instruction of food handlers in addition to a conducive environment for the implementation of, and compliance with, safe handling of food (FAO, 2023b). This includes cleaning and disinfection of processing areas and surfaces, and access to washing sinks, among others. Appropriate procedures should be followed before and after food preparation.

Training programmes with innovative methods and tools (e.g. videos, infographics, online training or courses) are suggested to promote compliance with policies and guidelines. Knowledge-based training often fails to improve handwashing, while behaviour-based training is more effective in improving handwashing performance and frequency (Yu *et al.*, 2018). Consequently, knowledge-sharing models (taking knowledge into implementation/practice) are critical to promote behavioural changes (Yeargin *et al.*, 2021). Regular assessment of knowledge could be a measure to determine the effectiveness of training modules and their impact on daily operations. A survey of Dutch workers found gaps in education and training, and demonstrated that worker knowledge about human norovirus was low (Verhoef *et al.*, 2013). The survey also found that awareness of human norovirus was significantly higher among food handlers in institutional settings vs those in catering companies.

4.5.3 Handwashing

Documents such as the United States FDA Model Food Code advise on when to wash hands (US FDA, 2022) including nine different situations (ranging from “After using the toilet room” to “After touching bare human body parts...” and “Before donning gloves to initiate a task that involves working with food”). Compliance with many of these recommendations is notoriously poor (Boyce and Schaffner, 2021; CDC, 2024). One alternative to event-based handwashing is to wash hands on a regular schedule in response to a timer (George *et al.*, 2021), and while this is a simpler approach it might miss critical times when a handwash is needed (Tabish and Basch, 2020). Using a risk-based approach to prioritize handwashing events in food service has also been proposed (Clayton, Bolinger and Jaykus, 2018).

It has long been known that handwashing technique is important (Larson and Lusk, 1985), including the use of water and soap, washing interdigital spaces, attention to the area beneath fingernails, and mode of drying (Mardiko and von Lengerke, 2020; Lin *et al.*, 2003). Proper training is important for both foodservice (Yu *et al.*, 2018) and farm workers (Soon and Baines, 2012) although training methods and modalities may differ regionally.

Various technological innovations have been applied to handwashing, including automated mechanical methods (Sy *et al.*, 2020), badges and other systems (Marra and Edmond, 2012; Mondol and Stankovic, 2015), with much of the innovation occurring in healthcare where the margins are higher and the risks might be greater (Wang *et al.*, 2021). Challenges in implementing these technologies have also been identified (Conway, 2016). Any technological solution must be appropriate to where it is to be implemented; accordingly, a solution that works in a restaurant might not be feasible on a farm (PrinceGuerra *et al.*, 2020). Using literature and experimental data, a risk-based assessment of hand washing efficacy predicted that a touch-free system would lead to a small decrease in the likelihood of bacterial contamination of hands, when compared to conventional handwashing (Montville, Chen and Schaffner, 2002).

4.5.4 Gloving

Use of disposable gloves is emphasized in the US FDA Model Food Code and related documents (US FDA, 2022). Gloves can be made from a variety of materials, each with their own attributes and advantages (Michaels, 2004). While gloves are not without limitations, they can reduce risk of cross-contamination from bare hands (Montville, Chen and Schaffner, 2001), although evidence suggests that if not changed with appropriate frequency, some of their benefits

may be reduced (Lynch *et al.*, 2005). Research has shown that non-compliance with glove use recommendations can be improved to some degree by training (Rajagopal and Strohbehn, 2013). Glove use can help prevent microbial contamination, although regular glove changes, especially when damaged, are important in maintaining their effectiveness (Selvaraj *et al.*, 2023). Gloves should be removed by grabbing at the top of the cuff and peeling inside out (Michaels, 2004). When gloved hands have been in contact with potentially contaminated items (e.g. money, used napkins or paper towels, and so forth), donning new gloves is recommended before preparing food. Current guidance recommends washing hands before donning gloves (see current Guidelines CAC/GL 79-2012, quote 55 [FAO and WHO, 2012]).

It has been suggested that wearing gloves can give the wearer a false sense of security (Todd *et al.*, 2010), and some research shows that hand washing was less likely to occur with activities in which gloves were worn (Green *et al.*, 2007).

4.5.5 Hand sanitizers

It has been commonly claimed that alcohol-based hand sanitizers are not entirely effective against non-enveloped viruses, effecting only a $< 2-3 \log_{10}$ reduction (Foddai, Grant and Dean, 2016; Tuladhar *et al.*, 2015). While this was true historically, recent scientific studies have shown that it is possible to formulate products in such a way as to increase their anti-noroviral efficacy (Macinga *et al.*, 2008; Escudero-Abarca *et al.*, 2022b; Ettayebi *et al.*, 2022). These novel formulations can provide reductions exceeding $3 \log_{10}$, depending on the virus and the test method (Macinga *et al.*, 2008; Escudero-Abarca *et al.*, 2022b). However, this still might not be sufficient to be considered an elimination step.

Conventional wisdom has commonly claimed that hand sanitizers should never be used in food service except after a handwash (Foddai, Grant and Dean, 2016), but a growing research base shows this may not always be true for both bacteria and viruses (Boyce and Schaffner, 2021). Hand sanitizers might be especially useful in situations where water for handwashing is not readily available (De Aceituno *et al.*, 2015). The so-called Sani-Twice method, in which two sequential rounds of hand sanitizer use are performed in place of handwashing, has been examined in controlled *in vivo* studies (Edmonds *et al.*, 2010) and on farmworkers' hands (Prince-Guerra *et al.*, 2020) but not yet evaluated specifically for control of viral contamination on hands. Again though, this approach is considered an alternative to handwashing only in the absence of adequate facilities and supplies.

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Information gaps and future directions

Overarching themes

- The inability to routinely cultivate infectious wild-type foodborne viruses is the single most important impediment to advancing our ability to study and control them. In the interim, there is a need to develop rapid molecular-based detection methods that can better discriminate between infectious and non-infectious virus. Ideal infectivity assays would be relevant to all strains; produce quantitative results; and be widely available, inexpensive, and implementable in less resourced locations of the world.
- It is generally recognized that the cultivable virus surrogates used in disinfection and inactivation studies are often more susceptible than are the human strains. There is a need to adopt a more standardized approach to the use of surrogates, leaning toward those that are more relevant.
- Limited, largely regional quantitative risk assessments have been done regarding viral foodborne disease risks for shellfish, berries, and prepared foods. Risk assessment efforts should continue to expand with emphasis on hepatitis E virus, region-specific viral foodborne disease risks, and interpretation of public health risk within the confines of testing in foods and the relationship between detection and virus infectivity.
- Although important in designing effective intervention strategies, root cause analysis is rarely conducted after outbreaks or positive surveillance sampling. Resources should be made available so that root cause analysis can be more actively pursued across the food supply chain worldwide.

Sources of contamination

- Prevention remains the best course of action to protect the food supply from foodborne virus contamination. Prevention relies on adherence to GAPs, GMPs, GHPs, and so on. However, there is a general lack of information on the use and effectiveness of such programmes specifically for virus control. The key here is a better understanding of the effectiveness of identified control strategies, as well as an understanding of the behaviour and compliance rates of food workers.
- Waters contaminated with human material are a major source of virus contamination across the supply chain, and they have myriad sources (i.e. surface, ground, municipal, reuse, and so on). Water-specific information gaps that can provide a basis for future research initiatives include the following:
 - » The relative importance of various routes of human fecal contamination to agricultural production waters must be better characterized, particularly considering the impact of global climate change.
 - » There is insufficient knowledge about the frequency of wastewater overflow or instances of operating above capacity in relation to, (e.g. weather conditions). This information could significantly enhance sanitary surveys and inform decisions regarding the timing of closure for BMS growing areas. Establishment of early warning systems to alert harvesters to wastewater events could be a useful risk management tool.
 - » There is a need for identification and implementation of novel microbiological indicators which have proven predictive value to the risk of enteric viral contamination in waters.
 - » Improved and harmonized virological criteria for water/wastewater reuse must be established as water scarcity and catastrophic weather events become more common worldwide.
 - » Standardized tests for monitoring the virological safety of source, irrigation, and wash waters should be developed, which could then be used in food safety management systems to complement ISO 15216 methods for virus detection in foods.
- Infected food workers (e.g. pickers, packers, and food service handlers) practicing poor personal hygiene remain an important source of foodborne virus contamination. Areas of future research and risk management emphasis include the following:

- » Assuring universal access to adequate handwashing facilities in food production, processing and preparation is critical, so is assurance that workers are always engaged in good hand hygiene practices (compliance) when handling foods. This is likely to be more challenging for low- to middle-income countries.
- » Recent risk assessments identify the importance of exclusion of ill workers from food production, processing and preparation, but there are no standardized methods on how to do this, and often exclusion is not incentivized for the food worker. Policies and processes to facilitate exclusion are needed.
- » Effective vaccines for hepatitis A virus, which provide lifelong immunity, have been available for almost three decades, yet vaccination is not mandated and industry is hesitant to implement worker vaccination programmes. There is a need to better understand the impact of childhood vaccination recommendations on hepatitis A virus endemicity worldwide, and promote vaccination, particularly in low-to middle-income countries. As vaccines for other viruses (hepatitis E virus, human noroviruses) emerge, considerations for their use along the food supply chain will be necessary.

Fomites, vomiting and transmission

- There has been concern that human norovirus can be transmitted by saliva or through completely asymptomatic carriers, but data are scant.
- Contaminated surfaces can serve as a source of virus to foods, although the relative significance of this compared to waters and hands is unknown. Having a better understanding of the importance of fomites in transmission warrants further study.
- Human norovirus is shed in vomitus, and aerosolization of virus during vomiting events has been demonstrated, as well as deposition on surfaces which may then serve as transmission fomites. However, the importance of vomiting in foodborne transmission is unknown and should be further investigated.

Zoonotic transmission

- Hepatitis E virus, not yet prioritized in the previous JEMRA report (FAO and WHO, 2008), is a zoonotically transmitted foodborne virus about which there are many unanswered questions. With respect to sources of contamination and public health burden, there is a need for the following:

- » understanding the prevalence of foodborne human hepatitis E infection (HEV-3, HEV-4) and symptomatic illness, around the world;
- » monitoring the hepatitis E virus status (i.e. seroprevalence and virus carriage) in pigs at slaughter age, with coordination and data-sharing efforts among industries (e.g. producers, veterinarians, processors, and so forth) and governments, to develop targeted strategies to reduce viral contamination during slaughter and pork processing;
- » methods standardization should be pursued, along with inclusion of sequence and genotyping characterization of hepatitis E virus isolates from human, animal and environmental samples, for instance, through the global network initiative (Mulder *et al.*, 2019);
- » evaluation of the safety and efficacy profile of the regionally available human hepatitis E virus vaccines, particularly in high-risk populations including immunocompromised individuals, abattoir workers, and pig handlers is needed, as is increasing global accessibility; development of a hepatitis E virus vaccine for administration to postweaning pigs and evaluation of its effect on food safety. Virus-specific antivirals are still lacking; and
- » educational and outreach efforts to increase awareness about the risk of hepatitis E virus contamination in pork, game meats, and infection in humans. Target audiences would include food producers and processors, veterinarians, physicians, regulatory agencies, and public health authorities.

Removal and inactivation processes

- Although viruses present on or in solid foods (e.g. berries and BMS) are resistant to many traditional food-processing and preservation methods (e.g. freezing, refrigeration, pH, and water activity), the studies are dated and, in some instances, non-existent. For instance, current thermal processing guidelines for BMS rely on historical evidence of the absence of illness associated with products subjected to the relevant time/temperature combination (EFSA BIOHAZ, 2015). As better virus cultivation methods emerge, such studies might be repeated to provide more accurate information on \log_{10} inactivation.
- In BMS, depuration commonly applied (2 days) is not effective in eliminating enteric viruses, although the impact of extending depuration periods has been studied. In relaying, coliphages are often used as indicators of efficacy, but their correlation with human infection risk is unknown and should also be explored.

- Emerging food-processing technologies must be evaluated and validated before implementation using relevant viruses, in the appropriate food matrix, under conditions as close to commercial processing as possible, and with an eye towards harmonization of criteria to define an “effective” process. Included should be a characterization of the effects of the process on product, organoleptic, nutritional and shelf-life properties, as well as cost-benefit analysis. As it currently stands, the most promising emerging processes for produce (berries) and BMS are HPP and UV light, but some novel technologies (e.g. cold plasma) should also be further investigated.
- There are no standard methods to evaluate virus removal or inactivation methods. This makes it difficult to compare and draw definitive conclusions on efficacy among studies of existing or new methods. The field would benefit from a more harmonized approach.
- The risk of using pig blood or liver as raw ingredients for the manufacture of ready-to-eat pork products is unknown. In general, there are few studies evaluating the efficacy of either traditional or emerging methods to inactivate hepatitis E virus in pork products. To the extent possible, these studies should be initiated.

Hand and surface disinfection

- Standard methods to evaluate antiviral surface disinfection differ by region and study design. This makes it difficult to compare and draw definitive conclusions on disinfectant efficacy among studies. The field would benefit from a more harmonized approach.
- Virtually all commercially formulated surface disinfectants evaluated to date, even those marketed with anti-human norovirus claims (based on the feline calicivirus mode, in the United States of America), do not provide a 3- \log_{10} reduction in virus load when used following manufacturer’s instructions. This includes the quaternary ammonium compounds which are commonly used in food service. While data are limited, hepatitis E virus likely has disinfectant resistance similar to hepatitis A and human noroviruses. More studies are warranted.
- Commercial entities are developing novel surface disinfection formulations and products specifically directed towards human noroviruses. Continuing research and product development are needed to produce more efficacious disinfectants having anti-norovirus activity with acceptable safety and use profiles.

- The importance of pre-cleaning/residual organic matter on disinfection efficacy is not well defined. Pre-cleaning is especially important when disinfecting after fecal and vomit contamination events, and this policy should be widely implemented across the food supply chain.
- As is the case for surface disinfectants, commercial hand sanitizers cannot be relied upon to completely inactivate foodborne viruses. Conventional wisdom has also claimed that hand sanitizers should never be used except after a handwash, but a growing research base shows this may not always be true. Improved hand sanitizers are needed, and their use in food production and preparation should be explored, especially in situations where water, supplies and/or facilities for handwashing are not readily available, or hands are not visibly soiled.

Food handlers/human hygiene

- Ill worker exclusion is likely the most effective means of controlling human norovirus transmission in food service. However, lack of knowledge, awareness, company policies, facilities, economy, and human behaviours make implementation difficult. More data are needed to understand the impact of worker exclusion policies, and how they may be revised and implemented with greater compliance.
- Alternatives to the “when to wash” guidelines included in many regulations and guidance documents are emerging. This might include event-based handwashing, scheduled handwashing, the use of a risk-based approach in prioritizing handwashing (Clayton *et al.*, 2018), and/or automated handwashing stations. More research is needed to investigate these options and codify them in regulations, as necessary.

Risk management and evaluation of measures taken

- Currently, there is a data gap between risk management decisions based on risk assessments, and the evaluation of the efficacy of management decisions, (i.e. whether the measures taken reduced the risk of foodborne viral disease). Regulations, recommendations and science-based interventions to mitigate the spread of foodborne viruses should underpin appropriate risk management measures. Evaluation of whether any measures taken were effective should be incorporated in each risk management plan.

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Conclusions

This report has reviewed the current state of the science regarding prevention, control and inactivation of foodborne viruses in the food supply chain. In several respects, the fundamental principles of these procedures remain the same as they were described in the 2008 Report (e.g. the application of good agricultural, manufacturing and hygiene practices throughout the food supply chain to prevent virus contamination, and the use of various approaches, physical [e.g. heat] and chemical [e.g. disinfectants] to inactivate and/or remove infectious viruses which have contaminated foods). Because the latter is difficult, efforts should predominantly focus on preventing virus introduction into foods.

Consistent with the September 2023 Part 1 meeting on food attribution, analytical methods and indicators, the commodity groups presenting the greatest risk of contamination and human disease are molluscan shellfish, fresh/frozen berries, RTE/prepared foods, and pork and game. For the latter commodity, foodborne virus transmission is predominantly zoonotic, and control measures are focused on prevention of infection of farmed animals and elimination of infectious virus primarily through heating of organ and muscle meats of pig or wild game origin. For shellfish, produce, and prepared foods, contamination by enteric viruses is most often associated with human fecal contamination, and possibly with vomitus in the case of human norovirus. In these cases, in order to institute appropriate controls, it is necessary to consider how any one particular commodity group might come into contact with human fecal matter. For all three groups, avoiding the use of fecally contaminated waters at primary production, processing, or food preparation is critical. Control measures on how to achieve this vary greatly by commodity group, (e.g. water quality standards and treatment strategies for shellfish; preventing inadvertent fecal contamination events in production [irrigation] waters for produce). Adherence to good hygienic practice by food

handlers at all stages in the food supply chain is essential. While various physical (e.g. heating, high pressure, ionizing radiation) and chemical (e.g. chlorine derivatives, novel disinfectants) methods have been investigated for the purpose of inactivating enteric viruses from foods, particularly shellfish and berries, none come to the forefront in terms of efficacy or practicality. Of particular concern is the rigour with which these treatments need to be applied to inactivate non-enveloped viruses (e.g. concentrations, contact times, and doses) in foods: If highly rigorous treatment conditions are required to produce adequate \log_{10} inactivation of viruses, unwarranted effects on the organoleptic qualities of the food can result.

Prevention and control findings over the last 15 years are numerous. For instance, it has now been confirmed that traditional depuration treatment may not be sufficient to result in complete elimination of enteric viruses from contaminated molluscan shellfish. While berries continue to emerge as important sources of outbreaks, there is limited knowledge about the root cause of contamination events, meaning that it is difficult to design targeted control strategies. The same can be said about the relative importance of fomites in the transmission of human noroviruses. Risk assessments have pointed out the importance of excluding infected food handlers from direct contact with foods, but how to do that effectively remains challenging, as is finding ways to ensure that good hand hygiene practices are adhered to rigorously by all, which might be addressed in national regulations. Finally, no single disinfectant process or product has emerged that has a high enough antiviral efficacy (usually $> 4 \log_{10}$) or qualifies for universal recommendation for any surface, or any product. For the time being, preventing contamination remains paramount, as the search for better ways to remove or inactivate enteric viruses from the food chain continues.



Annexes

Annex 1.1

The keywords used in the literature survey for outbreak, monitoring and surveillance data

The keywords used for searching the prevention and intervention measures of viruses in foods

Parameter	Search
1 Foods	avocado or bagel or bakery or bean or beef or berries or berry or beverage or bivalve or blackberry or blueberry or boar or bread or bulb or butter or cake or candy or cereal or chicken or cheese or chocolate or clams or cocoa or coffee or coleslaw or condiment or confectionery or cookie or crab or cracker or cranberry or cream or “cream powder” or cress or crustaceans or custard or dairy or date or deer or “delicatessen item” or dessert or dip or doughnut or echinoderms or “edible ice” or egg or fat or fig or fish or flour or flower or food or foodborne or fruit or fungi or game or gloves or “glove use” or grain or “green onion” or gum or “hand touched” or herb or honey or “ice cream” or “industrial water” or jam or jelly or juice or lamb or leafy or liver or “leafy green” or legume or lettuce or marmalade or meat or melon or milk or “milk powder” or molluscs or muffin or “municipal water” or mushroom or mussel or mustard or nectar or noodle or nut or offal or oil or oyster or pasta or pate or pie or pita or pome or pomegranate or pork or poultry or prawn or prepared or “prepared food” or “process water” or produce or pudding or pulse or raspberry or ready-to-eat or “RTE food” or roll or root or salad or “salad vegetable” or sauce or sausage or scallops or scone or seasoning or seaweed or seed or seeded or shellfish or sherbet or shrimp or “soft fruit” or solanaceous or sorbet or soup or soybean or spice or “spring onion” or sprouts or stem or stone or strawberry or sugar or sweet or syrup or “tap water” or tomato or tuber or turkey or vegetable or venison or vine or vinegar or “Virological quality” or “viral pollution” or “wash water” or “wash-water” or washing or “wash process” or “water quality” or “wash solution” or wild or “wild game” or yogurt
2 Hazards	norovirus or HuNoV or hNoV or “hepatitis A” or “hepatitis E” or rotavirus or sapovirus or enterovirus or astrovirus or “Enteric Adenovirus” or “human enteric virus” or “feline calicivirus” or calicivirus or “murine norovirus” or poliovirus or “tulane virus”

3 Intervention	<p>acid or alcohol or anthocyanidin or antimicrobial or antiviral or bacteriophage or biosecur or boil or catechin or chemical or chlorine or chill or chitosan or “chlorine dioxide” or citrate or “citric acid” or clean or cleaning or “cold atmospheric plasma” or contamination or control or cook or cool or copper or decreas or depuration or diminish or disinfect or efficacy or “electrolyzed water” or eliminat or “environmental hygiene” or eradic or “essential oil” or ethanol or exclude or extract or fabricat or filter or flavonoids or “food handler” or freez or “grapeseed extract” or H2O2 or “hand hygiene” or “hand sanitizer” or “hand washing” or heat or “high hydrostatic pressure” or “high pressure” or “hot water” or hygiene or hypochlorite or “hypochlorous acid” or “ill food worker” or immunis or immuniz or inactiv or inactiva or intervention or irradiat or isopropanol or mitigat or ozone or packaging or pasteuriz or Peracid or peroxide or Peroxyacetic acid or phage or phytocompounds or prevention or proanthocyanidin or “pulsed light” or “pulsed UV light” or purification or QAC or “quaternary ammonium compound” or reduc or reducing or reduction or relaying or remov or security or silver or soap or “sodium chlorate” or soil or “solar dry” or sun-dry or steam or storage or surfactant or train or treatment or trial or “trisodium phosphate” or UV or UVA or UVC or ultraviolet or ultrasound or vaccination or vaccine or wash or “water reuse”</p>
4	Title/Abstract (1 AND 2 AND 3)
5 Data base research	PubMed and Web of Science
6 Period covered	Since 2008 (when the previous MRA report on viruses was published)

Annex 1.2

Process the outcome in the Distiller

The records from databases were added into Distiller. The function “Duplication Detection” was used by comparing the Title, Author and Abstract. The relevance screening and confirmation screening were conducted.

A1.2.1 Relevance screening

Question	Options	Key definitions
<p>1. Does this citation describe research evaluating the efficacy and/or effectiveness) of prevention methods and/or interventions to control viruses in foods at any stage from primary production to consumption, including human behaviour in retail food settings or any stages?</p> <p>Selections 1-3 will pass the citation to the next review stage and the article will be procured.</p>	<ul style="list-style-type: none"> • Yes, primary research • Yes, systematic review/meta-analysis • Yes, risk assessment, risk profile, or other risk-based tool (e.g. cost-benefit analysis) • No (exclude) 	<p>Primary research is the collection of new data in a single study.</p> <p>Risk assessment is a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.</p> <p>Risk profile presents the current state of knowledge related to a food safety issue; it describes potential options that have been identified to date (if any), and the food safety policy context that will influence further possible actions. Other risk-based tools could include cost-benefit analyses, risk ranking, or risk prioritizations.</p> <p>Systematic review is a structured review of a clearly defined question with a transparent search strategy, relevant screening process, data extraction, risk-of-bias assessment and synthesis of results.</p> <p>Meta-analysis is a statistical technique that can be used on data collected in a systematic review.</p> <p>Exclude research on feral animals (e.g. feral pigs not produced for human consumption).</p>
<p>2. If no to the above, is the article a potentially relevant narrative literature review on the subject?</p> <p><i>(to be used for possible search verification)</i></p>	<ul style="list-style-type: none"> • Yes (check box if relevant) 	<p>Narrative reviews are expert-based reviews not using or reporting a structured or systematic approach (often they will not have a methods section).</p>

A1.2.2 Relevance confirmation

Question	Options	Key definitions
Relevance confirmation		
<p>Does this article investigate the efficacy and/or effectiveness of prevention methods or interventions to control viruses in foods at any stage from primary production to consumption?</p>	<ul style="list-style-type: none"> • Yes, prevention methods • Yes, intervention methods • Yes, both prevention and intervention • No, exclude • Other, specify (COMMENT) 	<p>Prevention refers to methods intended to prevent viruses from entering the food chain at any stage.</p> <p>Intervention refers to methods intended to inactivate and/or remove viruses from the food chain at any stage.</p>
Key article characteristics		
<p>Which viruses?</p>	<ul style="list-style-type: none"> • Norovirus • Hepatitis A virus • Hepatitis E virus • Rotavirus • Sapovirus • Astrovirus • Enterovirus • Enteric Adenovirus • Others: (COMMENT) • Not specific virus reported 	
<p>What food commodity is/ are investigated?</p>	<p>Specify: (COMMENT)</p>	<ul style="list-style-type: none"> • Prepared foods (foods that are ready to eat that have been handled by an individual other than the consumer) • Fresh and frozen berries • Shellfish • Pork • Wild game • And so forth

Question	Options	Key definitions
In what region was the study conducted?	<ul style="list-style-type: none"> • Africa: (COMMENT) • Asia and the Pacific: (COMMENT) • Europe and Central Asia: (COMMENT) • Latin America and the Caribbean: (COMMENT) • Near East and North Africa: (COMMENT) • North America: (COMMENT) • Not stated 	<p>Specify country name only (not subregions, states, provinces, and so on)</p> <p>According to the FAO regions</p>
Specify study design	<ul style="list-style-type: none"> • Experimental research: <ul style="list-style-type: none"> » Randomized controlled trial » Non-randomized controlled trial » Challenge trial » Quasi-experiment • Laboratory experiment • Observational research <ul style="list-style-type: none"> » Cohort study » Case-control study » Cross-sectional study » Environmental surveys » Behavioural studies (related to human behaviours) » Epidemiological analysis » Other • Genomic research • Systematic review: meta-analysis • Risk assessment, risk profile, cost-benefit analysis, or other risk-based tool 	<p>Observational study: This is the assignment of subjects into a treated group versus a control group outside the control of the investigator.</p> <p>Cross-sectional: This examines the relationship of a risk factor and outcome (disease) at a point in time on representative samples of the target population.</p> <p>Cohort study: Subjects with differing exposures to a suspected risk factor are observed through time for occurrence of an outcome.</p> <p>Case-control study: This compares exposure to the risk factor in subjects who have an outcome (the "cases") with subjects who do not have an outcome but are otherwise similar (the "controls") and are drawn from the same sampling frame.</p> <p>Other: These can be hybrid or other observational designs but not experiments evaluating the effectiveness of interventions. They can include environmental surveys or behavioural studies.</p> <p>Experimental study: Each subject is assigned to a treated group or a control group before the start of the treatment.</p> <p>Controlled trial: Subjects are allocated to intervention/comparison groups and evaluated for outcomes.</p> <p>Challenge trial: Subjects are artificially challenged or exposed to the disease agent and then allocated to the intervention groups for evaluation of the outcome.</p> <p>Quasi-experiment: Observations are made on a population before and after receiving an intervention.</p>

Question	Options	Key definitions
In what setting was the study carried out?	<ul style="list-style-type: none"> • Commercial/field conditions <ul style="list-style-type: none"> » Food-processing facility » Restaurant » Catering » Home • Research farm/pilot plant • Smallholder farm/abattoir conditions • Laboratory conditions (COMMENT*) <ul style="list-style-type: none"> » Surrogates » Human virus » Both » Not specified • Not reported 	<p>Please describe the following as much as possible if the article mentions it:</p> <ul style="list-style-type: none"> • Surrogate or human viruses were used in the study
Method to retrieve data:	<ul style="list-style-type: none"> • PCR • Viability assay (pretreatment+ PCR) • Binding assay • Culture assay • Other (COMMENT) 	
Does the article report any extractable data about intervention or prevention efficacy/effectiveness that could be used for possible meta-analysis?	<ul style="list-style-type: none"> • Yes • No, reason (COMMENT) 	
Type of data retrieved from article	<ul style="list-style-type: none"> • Qualitative data, specify: (COMMENT) • Quantitative data, specify: (COMMENT) 	<p>Please describe the following as much as possible if the article mentions it.</p> <ul style="list-style-type: none"> • Level + Unit of measure (Log10 reduction) • Virus is present or absent after the interventions (if applicable) • Loss of virus infectivity (proxy)

Question	Options	Key definitions
<p>What point in the food chain and category of intervention(s) or preventive controls is investigated in this article?</p>	<ul style="list-style-type: none"> ● Farm/farming plants <ul style="list-style-type: none"> » Biosecurity/management practices » Antivirals » Water treatment » Other (COMMENT) ● Transport to processing plants ● Processing: <ul style="list-style-type: none"> » Cleaning/disinfection of equipment/environments » High pressure treatment (High Hydrostatic Pressure) » Product washes » Antivirals » Thermal treatment » Standard processing procedures/good hygienic practices (GHPs) » Ionization/irradiation <ul style="list-style-type: none"> » Modified packaging » Other (COMMENT) ● Post-processing to consumer ● Biosecurity/management practices ● Antivirals ● Food/water acidification ● Other (COMMENT) ● Personal hygiene/human behaviours (all phases) ● Not applicable (as is experimental setting) 	<p>Biosecurity : This includes, but is not limited to, sanitation, biosafety, disinfection, hygiene and hygiene barriers, and the environment, and so on.</p> <p>Antivirals: antiviral effects of food components, food extracts and metal ions (e.g. Carvacrol, Acylated peptides, Silver nano particles, chitosan, essential oils, viral protease inhibitors, polymerase inhibitors and immunomodulator and so on)</p> <p>Water treatments: This is treatment which aims to remove contaminants from water that is used for production.</p> <p>High pressure treatment: This is a method of preserving and sterilizing food, in which a product is processed under very high pressure.</p> <p>Standard processing procedures/good hygiene practices (GHPs) refer to steps that prerequisite programmes, together with validated interventions (e.g. as part of a HACCP-based system), provide as a framework for the control of enteric viruses.</p> <p>Food/water acidification: This is the addition of organic acids, such as acetic acids to foods.</p> <p>Human behaviours (i.e. hand hygiene, restriction of ill workers, and so on) should be described if the article mentions them in the “Other”.</p>
<p>Efficiency of the intervention or prevention method</p>	<ul style="list-style-type: none"> ● The information is in the abstract, which is (COMMENT) ● Cannot tell from the abstract ● Other ways to reflect the efficiency (COMMENT) 	

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In response to a request from the 53rd Session of the Codex Committee on Food Hygiene (CCFH), the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) convened a meeting to review recent scientific developments, data, and evidence associated with foodborne viruses, specifically for prevention and intervention measures and the efficacy of interventions in the food systems continuum.

Since that initial expert meeting report from 2008, awareness of the public health importance of these foodborne virus-commodity combinations has increased, resulting in additions or changes to some food supply chain management strategies and research initiatives. Prevention remains the cornerstone of control of foodborne viruses. This is because these viruses are environmentally persistent and resistant to many treatments commonly used to inactivate foodborne pathogens. Effective inactivation methods continue to be needed and are continuously being evaluated.

Based on the above situation, the Expert Committee: 1) reviewed the relevant scientific literature; 2) deliberated on the developments that have occurred in control of foodborne viruses in the relevant food supply chains since the 2008 JEMRA report; and 3) identified the most promising approaches to further protect the food supply chain from virus contamination.

Agrifood Systems and Food Safety - Economic and Social Development
jemra@fao.org

<http://www.fao.org/food-safety>

Food and Agriculture Organization of the United Nations

Viale delle Terme di Caracalla
00153 Rome, Italy

Department of Nutrition and Food Safety
jemra@who.int

www.who.int/health-topics/food-safety/

World Health Organization

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